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Shifeng Pan

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Major professor

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INTERSPECIFIC HYBRIDIZATION AND BACTERIAL BLIGHT (*XANTHOMONAS CAMPESTRIS PV. PELARGONII*) RESISTANCE STUDIES IN *PELARGONIUMS*

By

Shifeng Pan

A DISSERTATION

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ABSTRACT

INTERSPECIFIC HYBRIDIZATION AND BACTERIAL BLIGHT (*XANTHOMONAS CAMPESTRIS PV. PELARGONII*) RESISTANCE STUDIES IN *PELARGONIUMS*

By

Shifeng Pan

Chromosome numbers were determined for thirty-eight *Pelargonium* taxa. Nineteen of them have not been previously reported, and seven show different chromosome numbers from the ones reported previously. The chromosome number of 2n=8 was verified for *Pelargonium* (*P.*) species *P. elongatum*. For the crossability studies, two hundred and ninety-three interspecific cross combinations were performed among different species, hybrid cultivars and inbred lines. Twenty-eight of the crosses produced viable seeds, and 19 of them showed partial seed development. One interspecific hybrid plant was produced from an '**Inbred White**' (*P. x hortorum*) plant crossed with *P. grandiflorum*, one of the suggested ancestral species of the *P. x domesticum*. *P.peltatum* was found to be a bridge species crossing with both *P. cordifolium*, another proposed *P. x domesticum* ancestor, and *P. x hortorum* cultivars.

Twenty one *Pelargonium* species, cultivars and

interspecific hybrid plants were screened for bacterial blight and leaf spot (*Xanthomonas campestris pv. pelargonii*) resistance. There were significant differences for bacterial blight resistance among different genotypes studied. ***P. odoratissimum, P. cordifolium, P. cucullatum, P. grandiflorum, P. peltatum x P. cucullatum, P. grandiflorum x 'Tiny Tot', 'Tiny Tot' x 'Earliana', P. betulinum x P. cordifolium, P. grandiflorum x P. cucullatum, P. grandiflorum x P. betulinum, P. cucullatum x P. cordifolium*** and ***P. scabrum x P. seritrilotum*** all showed high resistance to the disease. A hybrid resulting from the cross of *'Inbred White'* (*P. x hortorum*) and *P. grandiflorum*, a proposed ancestral species of *P. x domesticum*, showed a high level of tolerance. However, its seed parent *'Inbred White'* was highly susceptible. This result indicates that the resistant gene(s) was transferred to the *P. x hortorum* from *P. grandiflorum*.

Characteristics of morphology and cytogenetics of the successfully produced interspecific hybrids were studied. Plant height for the cross *'Inbred White'* and *P. grandiflorum*, and the flower size in diameter for the cross *P. peltatum* and *P. cordifolium* exhibited hybrid vigor. All other studied characteristics were intermediate between the 2 parents. Variable chromosome numbers and abnormal meiotic divisions were also observed in the cytogenetical studies of the interspecific hybrids.

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CHAPTER 1

INTRODUCTION

I. Origin:

The genus *Pelargonium* belongs to the family *Geraniaceae*, which is subdivided into 5 tribes: *Geranieae, Biebersteiniceae, Wendtieae, Vivianeae* and *Dirachmeae*. The *Pelargonium* containing tribe *Geranieae*, is subdivided into 5 genera: *Geranium, Pelargonium, Erodium, Monsonia* and *Sarcocaulon*. There are 16 sub-genera in the genus *Pelargonium* (**Figure 1**) (Clifford, 1958; Van Der Walt, 1977; Van Der Walt and Vorster, 1981; Taylor, 1988). Moore (1971) divided cultivated Pelargonium into 11 groups.

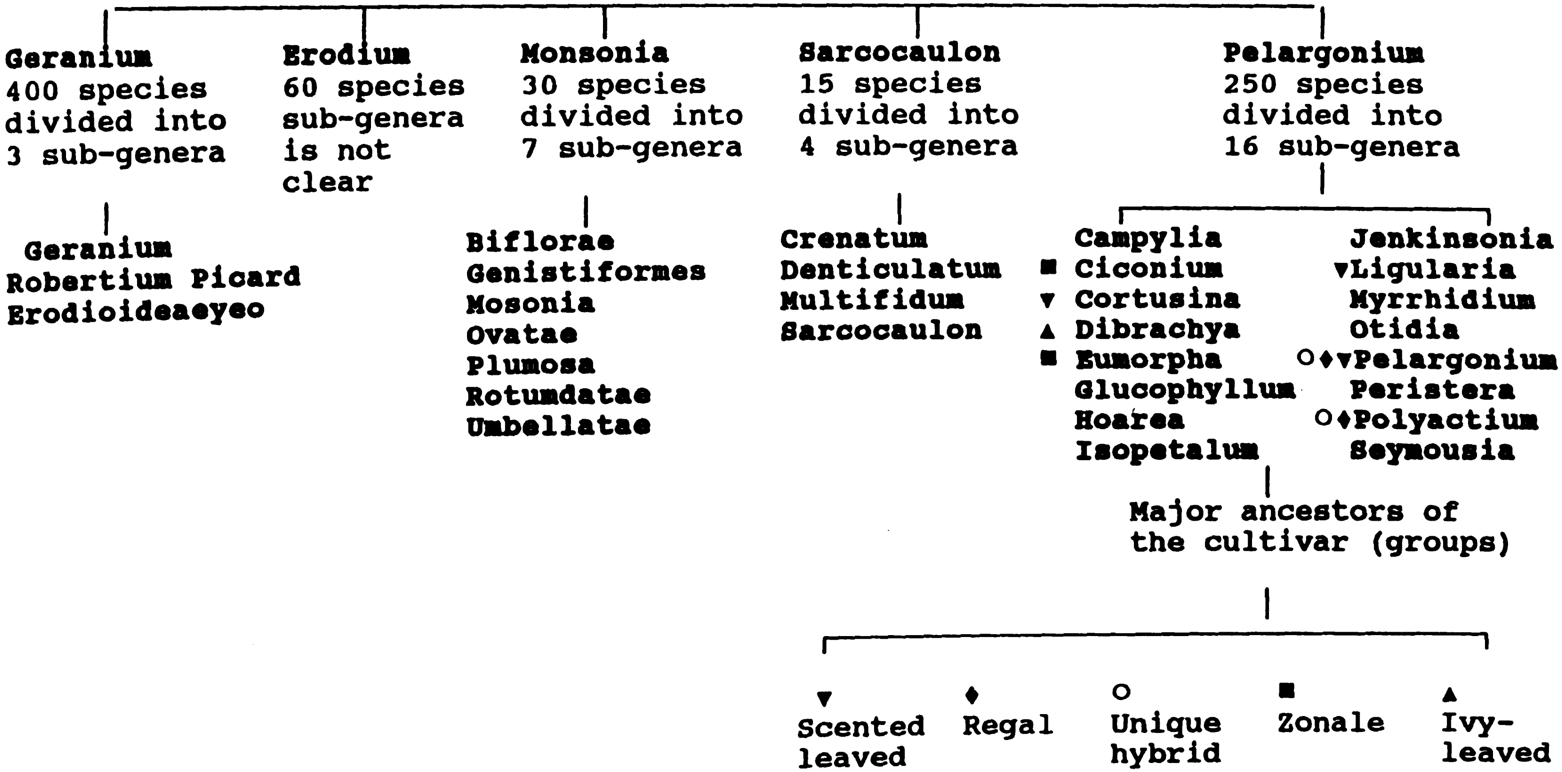
Pelargonium, containing about 280 species and a large number of hybrids and cultivars, is native to South Africa (Dewolf, 1983; Harney, 1966). It is believed that *Pelargonium*, probably *Pelargonium zonale* was introduced into Europe by the Dutch governor of the Cape Colony in 1609 (Clifford, 1958). Between 1800 and 1830, two groups of hybrids began to be distinguishable: 1) *Pelargonium X hortorum* (*P. X hortorum*), the zonale geranium, and 2) *Pelargonium X domesticum* (*P. X domesticum*), the Martha Washington or Regal geranium. A third group, the ivy-leaved

Pelargonium was not introduced into Europe until after 1850 (Dewolf, 1983).

P. X hortorum is considered to have evolved from the species *P. inquinans, P. zonale, P. hybridium, P. frutetorum,* and *P. scandens* with some possible contribution from *P. acetosum* and *P. stenopetalum* (Clifford, 1958). Harney (1976) from his chromatographic studies of the secondary biochemical constituents using crude alcoholic leaf extracts postulated that *P. zonale, P. inquinans, P. scandens* and *P. frutetorum* were the major contributors. Considering both morphological characters and biochemical markers, Harney (1976) suggested that *P. hybridum* might have contributed to *P X hortorum*, but *P. stenopetalum* was doubtful to have made any contribution.

P. X domesticum is considered by many to be the most beautiful *Pelargonium* and has evolved from numerous interspecific crosses within the genus. *Martha Washington Pelargonium, Lady Washington Pelargonium, Regal Pelargonium,* and *Show Pelargonium* are all common names used for this group, which has been in existence for many years. In the 1880s serious breeding began in Germany, France, and England (Taylor, 1988). Because the original crosses were not recorded or details lost (Clark, 1988), the ancestry of these plants is really not known. The most important contributing species are thought to be *P. cucullatum, P.*

Figure 1. Geraniaceae Family Tree
(From Taylor, J. 1988)



grandiflorum, P. angulosum, P. fulgidum, P. betulinum, P. capitatum, P. cordifolium and *P. ignescens* (Clifford, 1958; Taylor, 1988; Hanniford and Holcomb, 1982).

The initial flowering season is rather short (flower only once each spring) for most of the older *Regal Pelargoniums*, but continual improvements have extended the length of flowering period as well as outdoor Summer performance for some new cultivars (Taylor, 1988).

Special environmental factors are required for *P. X domesticum* for flower initiation and flower development. Temperature is the most important environmental factor affecting flowering (Crossley, 1968; Nilsen, 1975; Hackett and Kister, 1974). A four week temperature treatment at 10^{0}C is necessary to insure bud initiation in all cultivars (Hanniford and Holcomb, 1982). This group also responds to high irradiance of long days for flower development (Van De Veen and Meijer, 1959). Nilsen (1975) and Hackett and Kister (1974) also indicated that daylength is not important in flower initiation, but it can hasten flower development.

II. Cytology

Basic chromosome numbers of X=8, 9, 10, and 11 have been reported for the natural *Pelargonium* species (Daker, 1969; Darlington, 1955; Clifford, 1958). Species that *P. X hortorum* evolved from have a basic number of X=9, and are diploids (Clifford, 1958). Those species all belong to the section of *Ciconium.*

Very recently, a new basic chromosome number of X=4 for *Pelargonium* species *P. elongatum* has been reported by Gibby and Westfold (1983; 1986).

Reported diploid chromosome numbers for the genus *Pelargonium* are 2n=16, 17, 18, 20, 22, 35, 36, 40, 44, 45, 60, 81, 88 and 90. Since the chromosome number of 2n=8 was observed for *P. elongatum* (Gibby and Westfold) and verified in the present research, a new number of 2n=8 can be added. This lowest chromosome count raises the question that possibly all the currently cultivated commercial *Pelargonium* cultivars are really polyploids. The first polyploid *Pelargonium* cultivars were reported by Badr and Horn (1886) as described by Harney (1975). Breeding probably started first at the diploid level with horticulturists later unknowingly selecting tetraploid cultivars (Chow and Harney, 1971). Investigations by Clifford (1958) and Philippi (1961) indicated that tetraploid cultivars cannot be crossed with diploids. Badr and Horn (1971), however, determined that parthenogenetically formed seeds developed after crosses between parents with different ploidy levels.

Daker (1967; 1969) did a detailed cytological study of haploid cultivar 'Kleine liebling' (n=9). He found the shoot tip was haploid but the roots were either haploid, mixed haploid-diploid or diploid, and each of the 3 types of roots could be found on a single cutting. The analysis of microsporogenesis of the haploid plant showed the formation of a small number of bivalents. Trivalents and bivalents were found in the colchicine induced diploid. The bivalents found in the diploid closely resembled the ones found in the haploid. Daker (1967) suggests that these chromosomal associations in meiotic cells of the haploid and diploid plants indicate that there is interchromosomal homology within the genome. The chromosomal pairing at meiosis in the colchicine - induced diploid could be expected normal, but Daker (1967) found that it was very similar to that in the haploid, with a large proportion of univalents and pseudobivalents or non - chiasmata bivalents. Daker (1967) also found that in the haploid plants only a small proportion of the pollen mother cells progressed through a second division resulting in normal quartet formation, and he suggests that this abnormal meiosis is due to the accumulation of deleterious recessive genes which adversely affect meiosis. Therefore, he concluded that nonsynapsis of the chromosomes in the cultivar, whether haploid or diploid, is due to genes rather than lack of chromosome homology, and such genes could accumulate through lack of selective pressures over a long period of vegetative propagation.

Warburg (1938) proposed that the genus *Pelargonium* is more advanced morphologically than the genus *Geranium*, and that hybridization has played an important role in the formation of polyploids. *Pelargonium* shows the greatest level of interspecific hybridization, and many cultivars within the genus *Pelargonium* are polyploids. Both autotetraploids and allotetraploids have been found within the genus *Pelargonium* (Warburg, 1938).

Philippi (1961) reported irregular segregation of chromosomes in tetraploid cultivars during the first meiotic division. The irregular segregation resulted in pollen inviability and decreased fertility. In addition, he also observed 1, 2 or 3 chromosomes which were not arranged on the metaphase plate. Those chromosomes divided and were later found isolated in the plasma of the tetrad. Chromosomal configurations typical of autotetraploids were noted at meiosis in tetraploid cultivars studied. Philippi (1961) also found a negative correlation between the number of univalents which occurred per PMC and the fertility of the tetraploid cultivars. This association was not found in diploid cultivars.

III. Interspecific Hybridization

Both *P. X hortorum* and *P. X domesticum* groups are the results of interspecific crosses of species within the subgenera *Ciconium* and *Pelargonium* respectively. *P. X domesticum* and *P. X hortorum* appear to be genetically incompatible. No successful interspecific hybridization has been reported between *P. X hortorum* and *P. X domesticum*. Through the medium of the section *Dibrachya* a transfer of genetic material might be possible (Clifford, 1958). Sweet, as cited by Clifford (1958), noted that *P. X succulentum* could have resulted from the cross of *P. X domesticum* and *P. peltatum* (ivy-leaved Pelargonium). *P. peltatum* will cross with *P. x hortorum* (Clifford, 1958; Craig, 1971). Therefore, it might be possible that *P. peltatum* can be used as a bridge to cross with both *P. X hortorum* and *P. X domesticum*. Sweet also suggested that *P. X rigescens* might have resulted from a cross between *P. cordifolium* and a zoned species of *Ciconum*. The earliest interspecific hybrids were mentioned by Clifford (1958) but there was no data available on the cultivars that were used in the crosses (Craig, 1982). Knicely (1964) reported the results of controlled interspecific hybridization between and within *P. x domesticum* and *P. x hortorum* groups. He conducted 215 crosses and only 50 crosses within the 2 groups produced viable seeds. Thirty five crosses (between groups and within group) showed partial seed development, and the remaining 130 between group and within group crosses showed no indication of seed set. As described by Craig (1971), the crosses in Knicely's study, which only showed partial seed development, might indicate that embryos were formed but did not develop due to breakdown of the endosperm.

Protocols for rescue of zygotic embryos in *Pelargonium* have been developed (Adams, 1967; Kato and Tokumasu, 1983; Scemama & Raguin, 1990). Adams (1967) reported an embryo rescue method for diploid P. X hortorum. Kato and Tokumasu (1983) conducted interspecific hybridization between *P. X domesticum* and some other *scented-leaved Pelargonium* species using ovule culture technique, with the intention of introducing some desirable characters into *P. X domesticum* from the scented-leaved *Pelargonium*. They obtained potential ever-flowering and multiflorous type hybrid plants from crosses of *P. quercifolium* and *'Grand Slam'*, *'Priace Ruper'* and *'Strawberry Sundae'* respectively. Further improvement by using anther culture and backcrossing procedures with these hybrid plants were attempted, but no result was reported. They also found that tetraploid X tetraploid (2n=44) crosses generated a higher callus formation rate (80%) than did diploid (2n=22) X tetraploid crosses (3.1-6.5%).

IV. Bacterial Blight Resistance

The most serious disease of *Pelargonium* is a vascular wilt caused by the bacterium, *Xanthomonas campestris pv pelargonii* (Brown) Dye (*X. c. pelargonii*). The disease at first was called bacterial stem rot and leaf rot (Munnecke, 1954). Later Knauss and Tammen (1967) described it as bacterial blight, which is now the most often used name.

X. c. pelargonii is a gram-negative, aerobic and rod-shaped bacterium. It is catalase positive, hydrogen sulfide positive, oxidase negative, and has one polar flagellum. It produces a yellow extracellular polysaccharide slime called 'Xanthan' on nutrient agar (Smith et al., 1952). The bacterium can reduce nitrate, liquify gelatin, but does not produce indole. *X. c. pelargonii* is found only in association with plants or plant material, and it can be easily identified when isolated from infected plant tissue (Dye, 1980).

Pelargonium leaves infected with *X. c. pelargoniii* show 2 types of symptoms (Stephens et al.). One of the leaf symptoms is a wilting at the leaf margins. The infected areas of the leaf rapidly die and form "V" shaped, yellow lesions bounded by dark veins. The leaves then wilt, although the leaf petioles remain firm. The affected leaves may drop off immediately or may hang on the plant for a week or more (Nelson & Nichols, 1982). Another leaf symptom is the development of lesions which first appear as small water-soaked spots on the underside of leaves. These lesions enlarge, become well defined and slightly sunkin with a yellow halo, followed by a wilting and death of the leaf. Plants showing leaf lesions may develop the stem rot phase of bacterial blight. The stems turn gray to dull black and finally develop a dry rot (Horst & Nelson).

There are two main factors affecting symptom development. First, symptoms of the disease develop more rapidly under higher temperature conditions. Symptom expression has been found to increase as temperature was increased from 10 to 27°C during the first 5 weeks after inoculation (Kivilaan & Scheffer, 1958). This research also indicated that plants grown in a high-nitrogen program developed symptoms more rapidly than plants grown under a low-nitrogen level.

The bacteria enter the host plant generally through wounds or stomates. Histochemical observations of plants of both susceptible and resistant *Pelargonium* species inoculated with the bacteria through wounds in the stem showed that in the susceptible plants the bacteria initially spread through the plants through the xylem (Wainwright and Nelson, 1972). Then it entered the vascular cambium and rapidly moved to the phloem cortex and epidermis. In the resistant plants, the pathogen had limited proliferation in the xylem and did not spread out of the xylem. Wainwright and Nelson (1972) also observed differences in the levels of tannin-like materials between susceptible and resistant *Pelargonium* species, and suggested that the tannin-like materials may be responsible, in part, to differences in resistance to bacterial blight. In addition, they found tylosis formation and deposition of suberin-like material in plants which were infected with the bacterium which may play a role in restricting the spread of the bacterium.

Heavy losses of *Pelargonium X hortorum* to this disease has been reported yearly (Nelson & Nichols, 1982; Stephens & Tuinier, 1989). Due to the lack of bacterial blight resistance cultivars, the most effective control is to prevent the introduction of the bacteria into production areas. The strict sanitation procedures which have been found to reduce the introduction and spread of the disease are as follows: only use disease free (culture-indexed) plants; do not grow cutting *Pelargoniums* near *P. peltatum, P. X hortorum* or *P. X domesticum*; discard infected plants; sterilize planting containers, cutting knives and greenhouse tools; avoid overhead watering or misting as the bacterium spreads through splashing water droplets; and control white flies (Stephens et. al.).

There is no chemical available for controlling this disease. The culture-indexing procedure is very expensive and the plants can be lost later to bacterial infection. To really help control the bacterial blight disease in *P. X hortorum*, resistant cultivars are definitely needed. Useful resistance genotypes to bacteria blight have not been found in cutting-propagated *P. X hortorum* (Knauss & Tammen, 1967; Theiler, 1977), seed propagated *P. X hortorum* (Tuinier, 1985; Mojdehi & Singleton, 1990), or *ivy-leaved Pelargonium* (Tuinier, 1985). However, bacterial blight resistance has been observed in species from the *P. X domesticum* group such as *P. acerifolium, P. tomentosum, P. scarboroviae, P. scabrum, P. betulinum, P. grandiflorum, P. mullicaule*, and *P. hispidum* (Mojdeli & singleton, 1990; Knauss & Tammen, 1967), *P. cordifolium, P. grandiflorum, P. betulinum,* and

'Tiny Tot' (Dunbar and Stephens 1989).

If the transfer of this resistance from the *P. x domesticum* group into *P. x hortorum* through interspecific or bridge hybridization could be accomplished, it would be a mile stone in the development of bacterial blight resistant cultivars of *P. x hortorum*.

This research was conducted to see if such a transfer was possible and to provide further useful information for *Pelargonium* bacterial blight resistance breeding.

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CHAPTER 2

CHROMOSOME NUMBERS AND CROSSABILITY STUDIES IN *PELARGONIUMS*

Additional key words: Species, Pelargonium, Interspecific Hybridization, Chromosome number.

ABSTRACT

Chromosome numbers were determined for 38 *Pelargonium* species, cultivars and inbred lines. Nineteen of them have not been previously reported, and 7 show different chromosome numbers from the reported ones. The chromosome number of 2n=8 was verified for *Pelargonium elongatum*. Two hundred and ninety-three crosses were performed among different species, hybrid cultivars and inbred lines. Twenty-eight of them produced plump, normal appearing seeds, and 19 of them showed only partial seed development. One hybrid plant was produced from an inbred *P. x hortorum* plant crossed with *P. grandiflorum*, one of the suggested ancestral species of the *Regal pelargonium*. *P.peltatum* was found to be a bridge species crossing with both *P. cordifolium*, another proposed *P. x domesticum* ancestor, and *P. x hortorum* cultivars.

INTRODUCTION

Only a few interspecific hybridization studies have been reported in *Pelargonium* (Harney and Chow, 1971; Kato and Tokumasu, 1983; Knicely and Walker, 1966). Those researchers were attempting to study genetic relationships between different species , with the aim of bringing desired horticultural characteristics from one species into another, especially between the P. x hortorum and *P. x domesticum*. Bacterial blight *Xanthomonas campestris pv. pelargonii* (*X. c. pelargonii*) resistance has been reported in *P. x domesticum* (Craig, 1971; Dunbar and Stephens, 1989; Knauss and Tammen, 1967; Powell and Bunt, 1978; Stephens and Tuinier, 1989). But heavy losses of *P. x hortorum* commercial cultivars to bacterial blight caused by *X. c. pelargonii* are reported yearly (Ewart, 1982; Dunbar and Stephens, 1989). Interspecific hybridization could be valuable in transferring bacterial blight resistance from *P. x domesticum* into *P. x hortorum*, and also the ever flowering character from the latter into the former.

Chromosome numbers reported for *P. x hortorum* are 2n=17, 18, 35 and 36, and for *P. x domesticum* 2n=17, 18, 22, 35, 36, 42, 44 and 45 (Craig, 1971; Ewart, 1982; Knicely and Walker, 1966; Walker and Craig, 1961). The two groups have

no common progenitors (Ewart, 1982), but with the great variability and diverse ancestry within the genus, the possibility exists that species of one group could cross directly with species of the other groups or through a bridge species or plant (Knicely and Walker, 1966). However, no such success has been reported to date (Craig, 1971; Stephen and Tuinier, 1989). The lack of success may be due to chromosome number differences and the use of a narrow species germplasm base. The purpose of this research was to determine the chromosome numbers of *Pelargonium* species, hybrid cultivars and inbreds used in this study, and to study the crossabilities among different taxa.

MATERIALS AND METHODS

Pelargonium species:

Twenty-eight *Pelargonium* species (obtained mostly from South Africa) and hybrid cultivars were assembled for this study (Table 1). They were: *P. acetosume, P. acraem, P. betulinum, P. capitatum, P. citronellum, P. cordifolium, P. cucullatum, P. denticulatum, P. frutetorum, P. fulgidum, P. fruticosum, P. grandiflorum, P. hermanrifilium, P. hirtum, P. hispidum, P. inquinans, P. multicaule, P. peltatum, P. papilionaceum, P. reniforme, P. scabrum, P. seritrilobum, P. tetragarum, P. zonale, P. violavium, P. x domesticum cv 'Tiny Tot' and P. x domesticum cv 'Earliana'.* It has been possible to match most of those species with the color plates in the book "Pelargonium in South Africa" (Walt 1977) for proper name usage. Nine of the *Pelargonium* species were provided by PanAmerican Seed Inc. in West Chicago, IL. *P. violavium* and 'Earliana' were obtained from Merry Gardens in the United States. Two *P. x hortorum* inbred lines, 'Picotee' (MSU80-38A), a male sterile picotee colored plant, and 'Inbred White' (MSU87-8C), a white flower colored fertile plant from the MSU breeding program were also used.

Chromosome number determination:

Where possible both root tips and pollen mother cells (PMCs) were used for chromosome number determination for each species, cultivar and inbred line.

Vigorous roots were taken from the plants at 10:00 am, 1:00 pm and 3:00 pm. Cell division phases were found at all times. Root tips were put in cold water and stored in a 1°C refrigerator for 12 hrs, and then they were transferred to a fixative solution (absolute ethanol-chloroform-acetic acid 6-3-1) for 24 hrs. After washing in distilled water, the roots were put in 5N HCL for 10 minutes at room temperature, and then washed with distilled water. The root tips were smeared in a drop of two percent acetocarmine solution which was used for chromosome staining.

The right stage for PMC meiosis was found to be in the flower buds that were about 1-3mm long. The optimal sized flower buds were picked and fixed in Farmer's solution (ethanol-acetic acid 3:1) for 24 hrs at 1°C. Then they were transferred to 70% alcohol and stored in a refrigerator at a temperature of 1°C until needed. The same two percent acetocarmine solution used to stain root cells was employed in the PMC chromosome determination. Ten to twenty cells were counted from each species, hybrid cultivar or inbred line. A phase contrast Olympus system microscope (model BHS) set up with an automatic photomicrographic system (model PM-10ADS) was used to study the prepared material and take the desired photos.

Cross pollination procedures:

Low temperature is required for *P. x domesticum* and derived species to initiate flower buds (Hachett et al., 1974; Hanniford et al., 1982; Powell and Bunt 1978). This material was grown at a 10°C day and night temperature with 10 hours (6:00AM to 4:00PM) of high pressure sodium lighting for about 3 months. The material all flowered about 75 days later. Under these environmental conditions it was possible to keep the plants flowering for several months. A total of 293 cross combinations were tried among 38 species, cultivars and inbred lines. Three to 63 flowers were pollinated for each cross depending upon the number of flower available at time of pollination. Pollinations were done during the summers in a screened greenhouse. Standard pollination procedures were applied. Reciprocal crosses were tried when possible.

RESULTS AND DISCUSSION

Chromosome number determination:

Chromosome numbers determined for all the species, cultivars and inbreds used in this research are shown in **Table 1.** When compared with published counts (Chartejee and Sharma, 1970; Daker, 1969; Darlington and Wylie, 1955; Knicely and Walker, 1966; Moore, 1971; Gibby and Westfold, 1983), nineteen have not been reported, seven showed different chromosome numbers from the previous reports, and 4 showed the same basic chromosome number (ratio) but different ploidy levels. These differences in chromosome number may be due to different sources of the same species, chromosome lost or added during breeding, or incorrect identifications.

P. elongatum, section Eumorpha (Clifford, 1958, Taylor, 1988), had a 2n=8 chromosome number (**Figure 1**), which is a new basic number (x=4) for *Pelargonium* (Gibby and Westfold, 1986). This research verified the previous reports of Gibby and westfold (1986). This species is fertile with small, yellow tinted, white flowers. But it is difficult to use as a seed parent because of very early anther dehiscence in the flower bud. The discovery of this low genome number (X=4) indicates that the currently cultivated diploid *Pelargonium* varieties and cultivars are actually polyploids;

Table 1. Chromosome number determinations for the collected species, cultivars and inbreds used in this study.

Plant names	Chrom 2n No.	Reported 2n No.
'Tiny Tot'[z]	22	-
P.acetosum[y]	18	18
P.acraeum[y]	18	-
P.betulinum[y]	22	-
P.capitatum[y]	66	54, 66
P.citrorellum[y]	18	-
P.cordiforlium[y]	22	22
P.cucullatum[y]	22	22
P.denticulatum[y]	22	44
'Earliana'[x]	44	-
P.frutetorum[y]	18	18
P.fruticosum[y]	22	-
P.fulgidum[y]	44	22, 44
P.grandiflorum[y]	22	22
P.hermanrifilium[y]	18	-
P.hirtum[y]	44	-
P.hispidum[y]	44	-
P.inquinans[y]	18	18, 36
P.multicaule[y]	22	-
P.peltatum[y]	18	18, 36
P.papilionaceum[y]	28	44
P.reniforme[y]	22	-
P.scabrum[y]	22	36, 22
P.semitrilotum[y]	22	-
P.tetragarum[y]	22	22
P.violavium[x]	36	20
P.zonale[y]	18	18
'Inbred White'[w]	18	-
'Picotte'[w]	18	-
P.alchemilloides[v]	16	16, 18, 34, 36
P.coriandrifolium[v]	22	18, 22
P.elongatum[v]	8	8
P.mollicomum[v]	22	-
P.myrrhidifolium[v]	22	-
P.odoratissimum[v]	16	16
P.quinquelobatum[v]	26	-
P.ranunculiphyllum[v]	36	-
P.tongaense[v]	18	-

[z] obtained from Netherlands
[y] obtained from the Natioal Botanic Garden of South Africa
[x] obtained from Merry Gardens in U. S. A.
[w] Michigan State University breeding Lines
[v] provided by Pan American seed Inc., West Chicago, IL

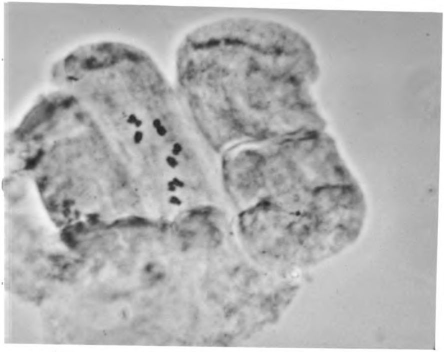

Figure 1. Somatic chromosome number of 2n=8 for *Pelargonium* species *P. elongatum* (2500X).

Table 2. Interspecific *Pelargonium* crosses which produced plump, normal appearing seed.

Cross	No. flowers pollinated	Fruit set %	No. seed obtained	Germination %
'Tiny Tot' x P.cucullatum	24	4.2	5	60.0
P.cucullatum x 'Tiny Tot'	27	17.8	24	58.3
P.acraeum x P.inquinans	14	53.0	32	87.5
P.frutetorum x P.inquinans	13	10.8	7	42.9
P. frutetorum x P.zonale	13	4.4	2	0.0
P.citrorellum x P.cucullatum	7	11.4	4	50.0
P.citrorellum x 'Tiny Tot'	4	50.0	10	100.0
P.citrorellum x P.zonale	8	7.5	3	0.0
P.cucullatum x P.cordifolium	20	26.0	26	61.5
P.cucullatum x P.hispidum	18	1.1	1	0.0
P.cucullatum x P.papilionaceum	12	3.4	2	0.0
P.cucullatum x P. tetragarum	9	8.9	4	25.0
P.scabrum x 'Tiny Tot'	10	24.0	12	50.0

Table 2 (cont'd).

Cross	No. flowers pollinated	Fruit set %	No. seed obtained	Germination %
P.scabrum x P.seritrilobum	3	40.0	6	83.3
P.scabrum x 'Earliana'	6	3.3	1	0.0
P.peltatum x P.cordifolium	42	1.4	3	33.3
P.peltatum x 'Picotte'	53	4.9	13	23.3
'Picotte' x P.peltatum	63	7.9	25	20.0
'Inbred White' x P.grandiflorum	13	0.5	1	100.0
'Inbred White' x P. peltatum	48	0.8	2	0.0
P.grandiflorum x 'Tiny Tot'	40	0.5	1	0.0
P.grandiflorum x P.cordifolium	10	6.0	3	100.0
P.grandiflorum x P.cucullatum	26	0.3	1	0.0
P.grandiflorum x P.betulinum	17	17.6	15	0.7
P.semitrilotum x 'Tiny Tot'	8	12.5	5	40.0
P.seritrilotum x P.cordifolium	6	13.3	4	50.0
P.tetragatum x P. scabrum	20	1.0	1	100.0

Table 3. Results of selfing the interspecific *Pelargonium* hybrids.

Hybrid plant	No. flowers pollinated	Seed set %	No. seed obtained
(*P. grandiflorum* X 'Tiny Tot)	35	0.0	0.0
(*P. peltatum x P. cordifolium*)	194	0.0	0.0
'Tiny Tot' x 'Eariana'	65	2.8	9.0
(*P. betulinum x P. cordifolium*)	14	0.0	0.0
(*P. cucullatum* x 'Tiny Tot')	237	2.3	27.0
(*P. cucullatum x P. tetragatum*)	151	5.6	42.0
('Inbred White' x *P. grandiflorum*)	125	1.1	2.0
(*P. seritrilotum x P. cordifolium*)	83	0.5	2.0
(*P. citrorellum x P. cucullatum*)	100	2.8	14.0
(*P. scabrum x P. seritrilotum*)	99	1.0	5.0
(*P. grandiflorum x P. cordifolium*)	94	0.0	0.0
(*P. grandiflorum x P. betulinum*)	47	0.0	0.0
(*P. citrorellum x* 'Tiny Tot')	136	1.3	9.0
(*P. cucullatum x P. cordifolium*)	25	16.0	22.0
('Tiny Tot' x *P. cucullatum*)	40	10.0	20.0
(*P. seritrilotum x* 'Tiny Tot')	65	0.0	0.0
(*P. scabrum* x 'Tiny Tot')	62	0.3	1.0

Table 4. Results of backcross interspecific *Pelargonium* hybrids.

Cross	No. flowers pollinated	Seed set %	No. seed obtained
(*P. peltatum* x *P. cordifolium*) x *P. peltatum*	84	0.5	2.0
('Inbred White' x *P. grandiflorum*) x *P. grandiflorum*	68	0.0	0.0
G2('Inbred White'x *P. grandiflorum*) x 'Inbred White'	53	0.0	0.0
('Inbred White'x *P. grandiflorum*) x (*P. peltatum* x *P. cordifolium*)	24	4.2	5.0
(*P. cucullatum* x 'Tiny Tot') x 'Tiny Tot'	80	0.6	2.0
(*P. scabrum* x 'Tiny tot') x 'Tiny Tot'	70	0.0	0.0
(*P. scabrum* x *P. seritrilotum*) x *P. scabrum*	12	8.3	5.0
(*P. scabrum* x *P. seritrilotum*) x *P. seritrilotum*	103	1.1	7.0
(*P. citrorellum* x 'Tiny Tot') x 'Tiny Tot '	70	5.4	19.0
(*P. citrorellum* x 'Tiny Tot') x *P. citrorellum*	8	0.0	0.0
(*P. grandiflorum* x *P. cordifolium*) x (*P. seritrilotum* x *P. cordifolium*)	70	4.9	17.0
(*P. seritrilotum* x *P. cordifolium*) x *P. cordifolium*	40	1.5	3.0
(*P. citrorellum* x *P. cucullatum*) x *P. cucullatum*	60	38.3	115.0
(*P. grandiflorum* x *P. betulinum*) x *P. betulinum*	40	0.0	0.0
('Tiny Tot' x *P. cucullatum*) x 'Tiny Tot'	63	7.9	25.0
(*P. seritrilotum* x 'Tiny Tot' x 'Tiny Tot'	43	0.0	0.0
(*P. seritrilotum* x 'Tiny Tot' x *P. seritrilotum*	40	0.0	0.0
('Tiny Tot' x 'Earliana') x 'Tiny Tot'	64	5.3	17.0

however, the most common chromosome numbers (2n=18, 22, 36 and 44) do not fit this basic number.

Crossability:

From the 293 cross combinations of the 38 species, cultivars and inbreds, twenty-eight produced seeds. This resulted in 17 groups of seedlings (**Table 2**). Nineteen of the 293 crosses showed only fruit elongation (**Table 5**).

P. x domesticum cv 'Tiny Tot' was successfully crossed with *P. cucullatum*, a species reported as one of the ancestors of *P. x domesticum* (Clifford, 1958; Craig, 1971; Ewart, 1982; Hanniford et al., 1982; Knicely and Walker, 1966). The seed set was lower (4.2%) when 'Tiny Tot' was used as seed parent as compared with that (17.8%) of the reciprocal cross (**Table 2**). The hybrid seedlings showed a maternal influence on plant height and compactness. Tiny Tot has a short, compact plant habit, and *P. cucullatum* has a relatively loose, taller plant habit. The hybrid plants are shorter and more compact when 'Tiny Tot' was used as seed parent, and looser and taller plants were produced when *P. cucullatum* was used as the seed parent (**Figures 2 and 3**). This phenomenon suggests a cytoplasmic effect. The hybrids could be further selfed and backcrossed to 'Tiny Tot'. In selfing, the seed set (10.0%) of ('Tiny Tot' X *P. cucullatum*) was higher than that (2.3%) of the reciprocal cross (**Table 3**). After backcrossing to 'Tiny Tot', 'Tiny Tot' X *P. cucullatum* produced a higher seed set than it's reciprocal hybrid (**Table 4**). These results suggest that 'Tiny Tot' is utilized most effectively as a female plant in interspecific cross.

Both *P. acareum* and *P. frutetorum*, which belong to the subgenus ciconum, hybridized successfully with *P.inquinans* - a proposed ancestral species of *P. x hortorum* (Craig, 1971; Ewart, 1982; Walker and Craig, 1961). *P. acareum* x *P. inquinans* had a seed set of 53% and seed germination of 87.5% (**Table 2**). *P. frutetorum* successfully crossed with *P. inquinans* producing a seed set of 10.8% and seed germination of 42.9%. This species also crossed with *P. zonale*, another proposed ancestral parent of *P. x hortorum*, but with a very low seed set (4.4%) and no seed germination. This data suggests that *P. acareum* has a closer genetic relationship with *P inquinans* than *P. frutetorum* does, and *P. frutetorum* has a closer relationship with *P. inquinans* than with *P. zonale*.

P. citrorellum hybridized with *P. cucullatum*, *P.x domesticum* cv 'Tiny Tot', and *P. zonale* with a seed set of 11.4%, 50% and 7.5% respectively. However, the hybrid seeds of *P. citrorellum* x *P. zonale* did not germinate (**Table 2**).

When *P. cucullatum* was crossed with 'Tiny Tot', *P. cordifolium*, *P. hispidum*, *P. papilionaceum* and *P. tetragarum*, it produced what looked like normal seed (**Table 2**), but the hybrid seeds of *P. cucullatum x P. hispidum* and *P. cucullatum x P. papilionaceum* did not germinate after

Figure 2. Plant types for the cross of *'Tiny Tot'* x *P. cucullatum* and their hybrid plant. Left: 'Tiny Tot'; Center: hybrid plant; Right: *P. cucullatum*

Figure 3. Plant types for the cross of *P. cucullatum* X *'Tiny Tot'* and their hybrid plant. Left: *P. cucullatum*; Center: hybrid plant; Right:'Tiny Tot'.

hand scarification and sowing.

The inviability of some of these interspecific seeds may be due to the incompatibility between the genomes of the parental species, and the incompatibility between the genotype of the hybrid zygote and the genotypes of the endosperm.

P. scabrum, a scented-leaved *Pelargonium*, was successfully crossed with *'Tiny Tot'*, and *P. seritrilotum* (**Table 2**), and the aromatic character did transfer to the hybrid seedlings. In this case the scented leaf character is probably dominant. *P. scabrum* is also reported to be very highly resistance to bacterial blight *X. c. pelargonii* (Dunbar and Stephens, 1989).

A *P. x hortorum* 'Inbred White' was found to be crossable with *P. x domesticum* species *P. grandiflorum*. One viable seed was obtained (**Table 2**). This plant had yellow tented leaves at the young seedling stage, but after 12 weeks became more vigorous with normal green foliage. *P. grandiflorum* is reported to be bacterial wilt resistant (Dunbar and Stephens, 1989), and present screenings (**Chapter 3**) confirm this information.

P. peltatum, an ivy - leaved *Pelargonium*, is reported to cross with *P. x hortorum* and to be rust resistant (Craig, 1971)). It is, however, highly susceptible to bacterial wilt (Craig, 1971; Knauss and Tammen, 1967; Wainwright and Nelson, 1972). Forty two flowers of *P. peltatum* were

Table 5. Interspecific *Pelargonium* crosses with only fruit elongation but no seed set.

Cross	No. flowers Pollinated
'Tiny Tot' x P.denticulatum	18
'Tiny Tot' x P.grandiflorum	8
'Tiny Tot' x P.inquinans	73
'Tiny Tot' x P.zonale	90
P.capitatum x 'Tiny Tot'	33
P.cordifolium x 'Tiny Tot'	19
P.cordifolium x P.frutetorum	21
P.fruticosum x P.inquinans	5
P.fruticosum x P.zonale	3
P.fulgidum x P.fruticosum	17
P.grandiflorum x P.fulgidum	12
P.inquinans x P.multicaule	12
P.zonale x P.grandiflorium	5
P.zonale x P. peltatum	16
P.peltatum x 'Tiny Tot'	20
P.peltatum x P.denticulatum	5
P.peltatum x P.inquinans	12
P.peltatum x P.scabrum	11
P.peltatum x P.violavium	5

Table 6. Results of crosses between chromosome numbers of various *Pelargonium* species.

Chromosome No.(2n) combination	No. cross attempted	No. cross producing seed
8 x 18	1	0
18 x 8	4	0
18 x 18	53	10
18 x 22	74	2
18 x 28	2	1
18 x 36	3	0
18 x 44	17	0
18 x 66	5	0
22 x 18	43	0
22 x 22	54	12
22 x 28	1	0
22 x 36	2	0
22 x 44	19	2
22 x 66	1	0
36 x 8	1	0
44 x 18	6	0
44 x 22	2	1
44 x 66	1	0
66 x 18	2	0
66 x 22	2	0
Total	293	28

pollinatedwith the pollen of *P. cordifolium*, and produced three hybrid seeds (seed set of 1.4%). This is the first successful report of a cross between the 2 species groups, and the first successful cross of *P. peltatum* and *P. cordifolium*. One of the seeds germinated and developed into a vigorous plant. The hybrid plant is more like *P. cordifolium* in leaf type, and like *P. peltatum* for plant type with multiple branched stem(s). The results from this research and previous studies (Craig, 1971), indicate that *P. peltatum* could be a usable bridge species between *P. x hortorum* and *P. x domesticum*.

One second generation (G_2) plant was obtained from 125 self pollinations of the hybrid plant 'Inbred White' X *P. grandiflorum* after the hybrid plant was moved from a 10^0C greenhouse to another 23^0C greenhouse. This G_2 plant is partially fertile. Twenty four flowers of this plant were pollinated with the pollen from the hybrid plant *P. peltatum* X *P. cordifolium*, and 5 seeds were obtained (**Table 4**). If these seeds can be germinated, this germplasm will possibly bring the gene(s) for bacterial blight resistance from the species *P. grandiflorum* and *P. cordifolium* together with *P. x hortorum* and *P. peltatum*. But more work still needs to be done in solving the self incompatibility problems of hybrid plants derived from the cross of *P. peltatum* and *P. cordifolium*, as well as the backcross incompatibility to both parents in the cross of 'Inbred White' X *P.*

grandiflorum. During this study, however, two seeds were obtained when this interspecific hybrid plant was selfed after being moved from a 10^{0}C greenhouse into one of 23^{0}C. This might indicate that a higher average growing temperature could possibly help break down the self incompatibiltity for this cross. If the bridge would prove to be successful, the bacterial blight resistance transferred from *P. x domesticum* to *P. x hortorum* would become more realistic. Other horticulturally desired characters, such as expanded flower coloration for *P. x hortorum*, and non-temperature controlled flower initiation for *P. x domesticum* might also be possible.

The nineteen crosses which showed only fruit elongation (**Table 5**) may have been due to early endosperm abortion, as well as the incompatible interaction of related embryo, endosperm and maternal tissues. Those crosses might prove to be successful by using embryo rescue to culture the early stage embryos as has been done by previous research (Kato and Tokumasu, 1983).

Crosses between chromosome numbers of various *Pelargonium* species showed that 18 x 18 and 22 x 22 were the most successful but other combinations were possible (Table 6).

SUMMARY

Chromosome numbers were determined for a total of 38 species, cultivars and inbreds. Nineteen of the taxa have not been reported previously, and 7 others had chromosome numbers differing from previous reports.

Due to the great variability and diverse ancestry that is found within *P. x hortorum* and *P. x domesticum* it has been suggested that successful crosses could be accomplished if a common progenitor could be found (Nicely and Walker, 1966). The successful cross between 'Inbred White' and *P. grandiflorum has supported this hypothesis.*

Besides the success of the direct cross of *P. x hortorum 'inbred white'* and *P. grandiflorum*(an ancestor to *P. x domesticum*), a possible bridge species (*P. peltatum*) has been found which can cross with both *P. x hortorum* and the *P. x domesticum* related species *P. cordifolium.*

One interspecific hybrid seedling of *P. x hortorum 'Inbred White'* x *P. grandiflorum* and one *P. peltatum* x *P. cordifolium* seedling were obtained. This is the first successful report on such crosses. Since *P. grandiflorum* and *P. cordifolium* have been found highly resistance to bacterial blight *X. c. pv. pelargonii* (Chapter 3), the success of these crosses has great possible value for

transferring the bacterial blight resistance to *P.* x *hortorum*.

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CHAPTER 3

BACTERIAL BLIGHT (*Xanthomonas campestris pv.pelargonii*) RESISTANCE IN *PELARGONIUMS*

Additional key words. Disease, Xanthomonas pelargonii, Resistance, Susceptible, Screening, Inoculation

ABSTRACT

Twenty one Pelargonium species, cultivars and hybrid plants were screened for bacterial blight (*Xanthomonas campestris pv. pelargonii*) resistance. The statistical results indicated that there were significant differences for bacterial blight resistance among different genotypes studied. *P. odoratissimum*, *P. cordifolium*, *P. cucullatum*, *P. grandiflorum*, *P. peltatum* x *P. cucullatum*, *P. grandiflorum* x *'Tiny Tot'*, *'Tiny Tot'* x *'Earliana'*, *P. betulinum* x *P. cordifolium*, *P. grandiflorum* x *P. cucullatum*, *P. grandiflorum* x *P. betulinum*, *P. cucullatum* x *P. cordifolium* and *P. scabrum* x *P. seritrilotum* showed high resistance to the disease. A hybrid resulting from the cross of *'Inbred White'* and *P. grandiflorum*, a proposed ancestral species of *P. x domesticum*, showed great tolerance. However, its seed parent *'Inbred White'* was

highly susceptible to this disease. This indicates that resistance gene(s) was transferred from P. *grandiflorum*, and that resistance is an inheritable character.

INTRODUCTION

Bacterial blight caused by *X. c. pelargonii* is recognized as the most serious disease in *Pelargonium X hortorum*, and heavy losses of *Pelargoniums* to this disease has been reported yearly in greenhouses (Nelson and Nichols, 1982; Stephens and Tuinier, 1989). The disease is common in cuttings and seedlings of *P X hortorum*, as well as in the ivy-leaved pelargoniums (*P. peltatum*). All commercial cultivars of *P. X hortorum* are highly susceptible (Stephens et al.), and no genotypes of this species group are known to posses useful resistance genes (Craig, 1971; Knauss and Tammen, 1967; Munnecke, 1954). There is also no chemical control available for this disease. Culture-indexing is used for disease control; but the procedure is expensive, and the culture-indexed plants are not resistant and can be lost to later infection. Most species and cultivars of *P.* x *domesticum* are, however, resistant to this disease (Knauss and Tammen, 1967), although there has been no report of successful resistant gene transfer from P. X *domesticum* to P. X *hortorum*.

A worthy objective would be to transfer resistance genes from *P. x domesticum* to *P. x hortorum*. This research was undertaken with the purposes of screening a broad base of *Pelargonium* germplasm for bacterial blight resistance, and providing useful breeding information directed toward the development of resistant cultivars of *P.* x *hortorum*.

MATERIALS AND METHODS

Plant Materials:

Pelargonium species *P. cordifolium, P. cucullatum, P. betulinum, P. scabrum, P. seritrilotum* and *P. peltatum* were obtained from the National Botanic Gardens of South Africa. *P. achemelloides, P. elongatum, P. mollicomum, P. odoratissimum, P. quinquelobatum P. ranunculiphyllum* and *P. tongaense* were obtained from the Pan-American Seed Company, West Chicago, IL. *P. X hortorum 'Inbred White'* is a Michigan State University (MSU) breeding line. All Plants were grown in a screened greenhouse, supplemented with ten hours (daily) of high pressure sodium lightings, and following recommended growing procedures. Plants were inoculated when they were three month old. After inoculation the plants were grown at a temperature of 24°C day and 22°C night to promote growth of the bacterial blight organism.

Inoculation:

Bacterial strain X-1 (from Kansas) provided by Dr. K. Dunbar in Dr. C. Stephens' laboratory of the Department of Botany and Plant Pathology of MSU was used. The strain was used because of its aggressive property (Dunbar and Stephens, 1989). The X-1 strain was stored in a saline solution (0.85% NaCl) for about one month at 4°C was streaked onto Difco nutrient agar in petri dishes and incubated at 23°C. After 3-5 days a single colony was transferred to 25 ml of modified Lederberg's complete broth (elimination of glucose, Dunbar and Stephens, 1989). The broth culture was incubated at 25°C on a rotary-shaker at 125 rpm for 2 days. Then the bacteria were pelleted by centrifugation at 900 g for 15 min. The pellets were suspended in water, and diluted to the desired concentration (10^7) of colony forming units (cfu) based on standard dilution plating and turbidimetric techniques (Dunbar and Stephens, 1989). Five plants (cuttings) of each species, cultivar and hybrid plant were sprayed to runoff using a hand sprayer, and placed in closed clear plastic bags for 3 days. Plants were randomly arranged on the bench, and grown at 24°C in the greenhouse to promote disease development. The control plants were sprayed with water. Three observations were taken at 21, 45 and 71 days respectively. The following numerical disease ratings were made on the percent of tissue blighted (yellowed and dried).

1 = ≤10% of the leaf tissue blighted;

2 = 11-30% of the leaf tissue blighted;

3 = 31-50% of the leaf tissue blighted;

4 = 51-70% of the leaf tissue blighted;

5 = ≥70% of the leaf tissue blighted;

6 = whole plant died.

Statistical Methods:

Experimental data were analyzed using MSTAT microcomputer software. The significance F test and the least significant differences (LSD) test were performed at the $\alpha=0.05$ level. Disease resistance means were calculated for 5 replications of 21 plant genotypes on the last 2 observations (45 days and 71 days). The disease ratings from the first observation (21 days) were not used since the disease symptoms were developing at that time.

RESULTS AND DISCUSSION

Disease ratings for each species, cultivar, breeding line and plant are listed in **Table 1**. There was a significant difference ($\alpha=0.05$) for the disease resistance among species from different groups.

P. x *hortorum 'Inbred White'*, *P. alchemilloides*, *P. elongatum*, *P. mollicomum*, *P. quinquelobatum*, *P. peltatum*, *P. ranumculiphyllum*, and *P. tongaense* were very susceptible to bacterial blight. The inoculation spots on the plants of this susceptible group spread quickly, and the inoculated leaves became yellowish within the first two weeks (Figure 1). Many of the plants were dead 45 days after inoculation (**Table 1**).

'Inbred White', a line from the MSU breeding program showed enlarging spots at 7 days after inoculation. About 45 percent of the leaf tissues was blighted in 21 days (Figure 2). At 45 days all the plants were entirely blighted, and the plants were dead by day 71. *P. grandiflorum*, a *P. x domesticum* ancestor, showed very tiny spots with a dark brown halo one week after inoculation. Plants developed a few small spots by 21 days, and the spotted leaves fell off at 45 days. At 71 days the plants no longer showed symptoms. The hybrid plant derived from the cross of the above two ('Inbred White' X *P. grandiflorum*) developed some spots at 21 days after inoculation. About 25 per cent of the leaf tissue of plants was blighted at 21 days, and remained about the same at 45 days. At 71 days about 30 per cent of the leaf tissue of the plants was blighted, but new shoots were developing (**Figure 4 & Figure 6**).

The hybrid plants of *P. cordifolium* (a *P. X domesticum* progenitor species) and *P.peltatum* (an ivy-leaved *Pelargonium*) and parent plants were also screened (**Table 1**). The pollen parent *P. cordifolium* developed very small spots with a dark brown halo around the centers at 21 days after inoculation (Figure 3). Less than 20 per cent of the leaf tissue of the plants was blighted at day 45. No new spots had developed at 71 days, and the plants started to produce new shoots. The seed parent *P. peltatum* had developed very large spots on the leaves by the end of the first week after

Table 1. *Xanthomonas campestris. pv. pelargonii* resistance ratings (1-best, 6-worst) for *Pelargonium* species, cultivars and hybrid plants

Items	Disease resistance rating means[z]	
Species[y]		
'Inbred White'	5.8	A
P. grandiflorum	1.2	FG
P. cordifolium	1.2	FG
P. cucullatum	1.5	EF
P. peltatum	5.7	A
P. alchemilloides	5.8	A
P. elongatum	6.0	A
P. mollicomum	4.3	B
P. odoratissimum	2.0	CD
P. quinquelobatum	4.3	B
P. ranunculiphyllum	5.8	A
P. tongaense	6.0	A
F1('Inbred Wh.' X *P.grandiflorum*)	2.2	C
F1(*P.peltatum* x *P.cordifolium*)	1.7	DE
F1(*P.grandiflorum* x *'Tiny Tot'*)	1.2	FG
F1(*'Tiny Tot'* X *'Earliana'*)	1.0	G
F1(*P.betulinum* X *P.cordifolium*)	1.2	FG
F1(*P.grandiflorum* X *P.cordifolium*)	1.7	DE
F1(*P.grandiflorum* X *P. betulinum*)	1.3	EFG
F1(*P.cucullatum* X *P.cordifolium*)	1.0	G
F1(*P.scabrum* X *P.seritrilotum*)	1.3	EFG
$LSD_{0.05}$	0.45	
Days post inoculation[x]		
21 days	1.6	B
45 days	3.2	A
71 days	3.3	A
$LSD_{0.05}$	0.15	

[z] means with the same letters are not significantly different, those with different letters are significantly different at α=0.05 by Least Significant Difference (LSD) test.
[y] means from 5 replications on the last 2 observation times (45 days and 71 days)
[x] means from 21 genotypes and 5 replications for each genotype

Table 2. *Xanthomonas campestris pv. pelargonii* resistance ratings (1-best, 6-worst) for *'Inbred White'*, *P. grandiflorum* and their hybrid plants, and for *'P. peltatum*, *P. cordifolium* and their hybrid plants.

Items	Disease resistance rating means[z]
Genotype[y]	
'Inbred White'	5.8 A
P. grandiflorum	1.2 C
'Inbred White' x *P. grandiflorum*	2.2 B
$LSD_{0.05}$	0.50
P. peltatum	5.7 A
P. cordifolium	1.0 C
P. peltatum x *P. cordifolium*	1.8 B
$LSD_{0.05}$	0.41

[z] means with the same letters are not significantly different, those with different letters are significantly different at α=0.05 by Least Significant Difference (LSD) test.

[y] means from 5 replications and last 2 observation times(45 days and 71 days)

Figure 1. Bacterial blight (*X. c. pelargonii*) resistance screening for 'Inbred White' showing enlarging inoculation spots and yellowish leaves at 10 days after inoculation.

Figure 2. Bacterial blight (*X. c. pelargonii*) resistance screening for 'Inbred White' showing about 45% of leaf tissues was blighted at 21 days post inoculation.

Figure 3. Bacterial blight *(X. c. pelargonii)* resistance screening for *P. cordiforlium* showing spots with the typical dark brown halo during the first three weeks after inoculation.

Figure 4. Bacterial blight (*X. c. pelargonii*) resistance screening for the 'Inbred White', *P. grandif.* and their hybrid plant (71 days post inoculation).
Left:'Inbred White, plant completely dead;
Center: hybrid plant, showed less symptoms;
Right: *P. grandiflorum*, no symptoms.

Figure 5. Bacterial blight (*X. c. pelargonii*) resistance screening for *P. peltatum* , *P. cordifolium* and their hybrid plant (71 days post inoculation). Left: *P. peltatum*, plant completely dead; Center: hybrid plant, showed less symptoms; Right: *P. cordifolium*, no symptoms.

Figure 6. Resistance of 'Inbred White', P. grandiflorum **and their hybrid to** *X. campestris pv. pelargonii.*

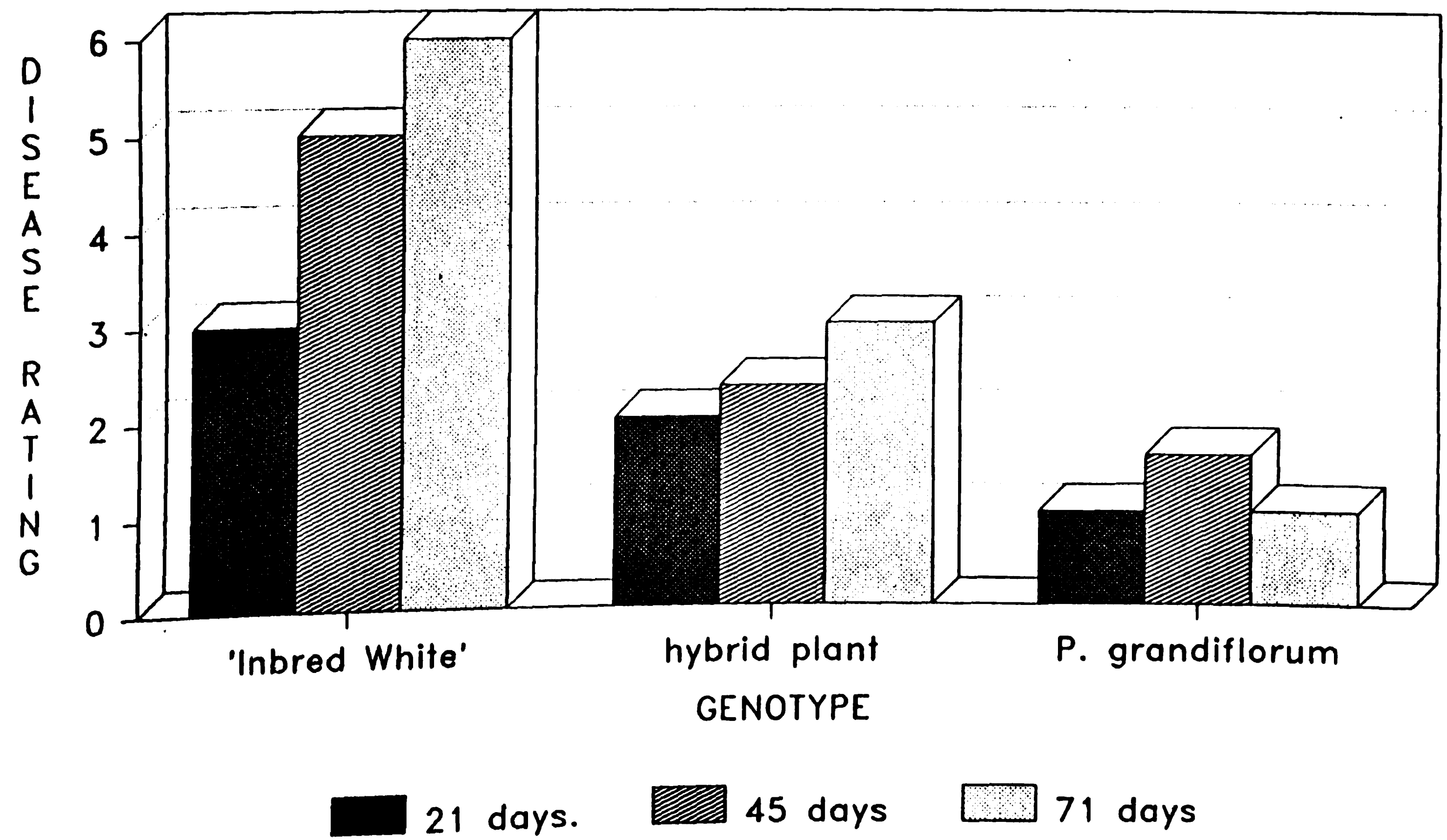
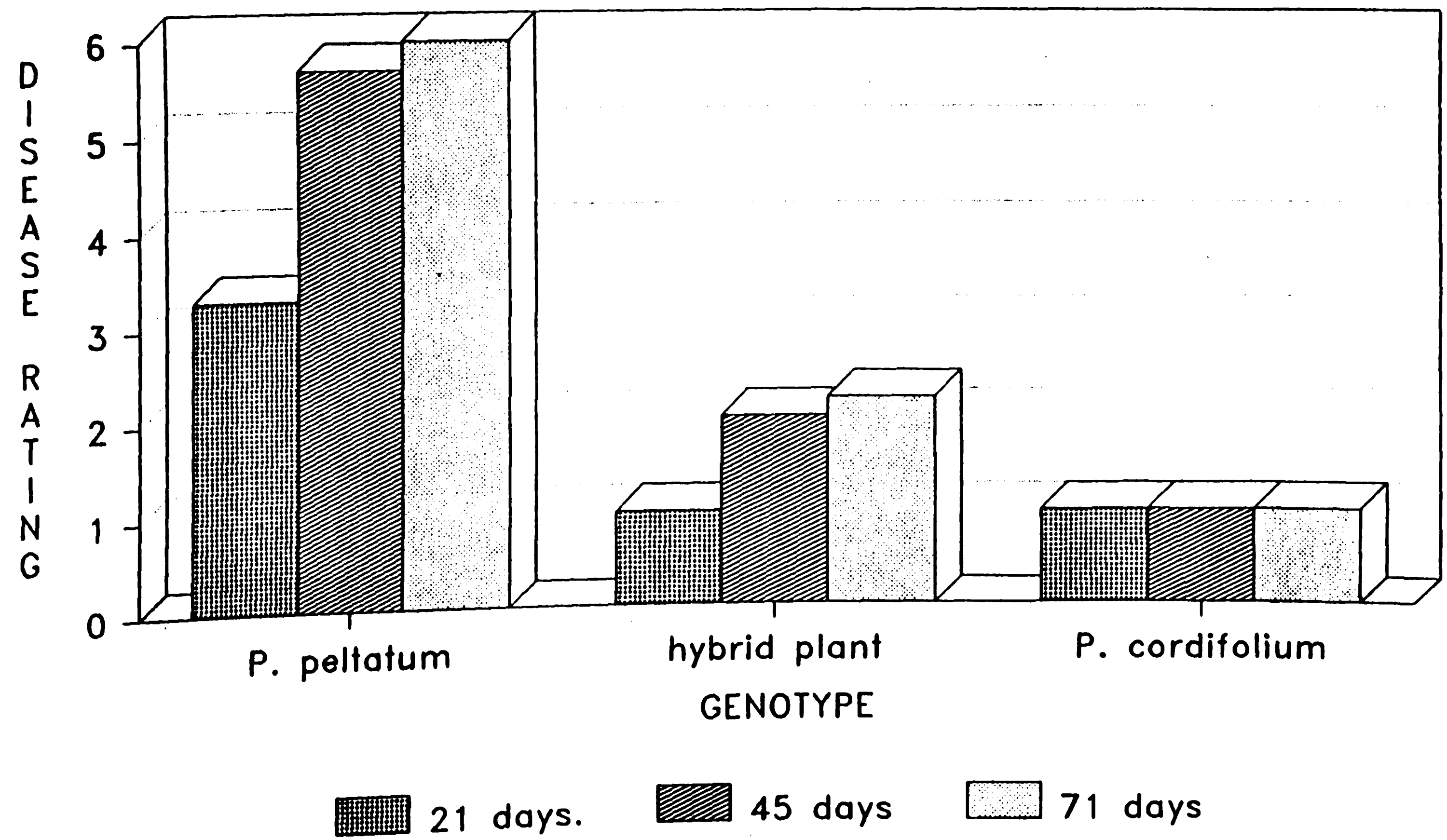


Figure 7.. Resistance of *P. peltatum*, *P. cordifolium* and their hybrid to X. *campestris pv. pelargonii.*



inoculation. After 21 days, fifty per cent of the leaf tissue of the plants were blighted. The plants were completely dead at 45 days post inoculation. In comparison, the derived hybrid plants developed only small spots on the leaves. They had less than 20 per cent of their leaf tissue blighted at day 45 and the level remained stable through day 71 (**Figure 5**).

The results (**Table 1**) also revealed that *P. cucullatum*, *P. odoratissimum*, *P. grandiflorum* X *'Tiny Tot'*, *'Tiny Tot'* X *'Earliana'*, *P. betulinum* X *P. cordifolium*, *P. grandiflorum* X *P. betulinum*, *P. cucullatum* X *P. cordifolium*, *P. scabrum* X *P. seritrilotum* all were highly resistant to the disease. These taxa had only tiny spots with the typical dark brown halo during the first two weeks after inoculation. The spots did not enlarge, although some of the infected leaves fell off the plants at about 30 days after inoculation.

The dark brown color that formed around the infected spots may be a defensive system in which the healthy cells around the infected area produced some secondary metabolic compounds, such as tannin like materials, lignin etc. (Wainwright and Nelson, 1972), forming colored flavonoids, and restricting the bacteria from spreading to the healthy cells (Asada, 1982, Sequeira et al., 1977). When the leaves of resistant plants are infected, there is probably some kind of growth regulator (such as abscissic acid etc.)

reaction (Stedan and Sequeira, 1970) at the base of the leaf petioles which causes infected leaves to fall off the plants and probably prevents the pathogen from spreading to the stem tissue. These are typical systems plants used to against disease attachment and maintain their normal physiological metabolism and growth (Sequeira et al., 1977).

The statistical results showed that there was no significant difference (a=0.05) between the observations taken at 45 days and 71 days (**Table 1**). Observations taken at 21 days and 45 days, and at 21 days and 71 days, however, showed a significant difference at the 0.05 significance level. This result indicates that 45 days post inoculation would be enough time to determine the differences for bacterial blight resistance among different species or genotypes. It would be more logical to use the average data took at the last 2 observations (45 days and 71 days) to conclude the differences for bacterial blight resistance among different species or genotypes since the disease symptoms were still developing at the time of the first observation (21 days).

The resistance for bacterial blight *X. c. pelargonii* was an inheritable trait; since the hybrid plants from both crosses of *'Inbred White'* X *P. grandiflorum* and *P. peltatum* X *P. cordifolium* showed resistance (**Table 1, Table 2, Figure 6 and Figure 7**).

SUMMARY

Among the one inbred, 11 *Pelargonium* species, and 9 interspecific hybrid plants screened, twelve showed a high level of resistance to the disease *X. c. pelargonii*, and eight were very susceptible. The resistance hybrid plants from *P. peltatum* X *P. cordifolium* and from '*Inbred White*' X *P. grandiflorum* indicates that the gene(s) for bacterial blight resistance was transferred into *P. x hortorum* and into *P. peltatum* germplasm from two bacterial blight resistance progenitor species of the *P. x domesticum* group. The statistical results showed that observations taken at 21 days after inoculation with *X. c. pelargonii* were significantly different (α=0.05) from those at 45 days and at 71 days, however, there was no significant difference between observations taken at 45 days and 71 days. Significant differences were also found for the bacterial blight resistance among different *Pelargonium* species or genotypes.

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CHAPTER 4

MORPHOLOGICAL, CYTOLOGICAL AND *IN VITRO* CULTURE STUDIES ON INTERSPECIFIC HYBRIDS OF *PELARGONIUM*

Additional Key Words. *Pelargonium*, Morphology, Cytology, In Vitro Culture, Interspecific Hybrids

ABSTRACT

The morphology and cytogenetics of interspecific hybrids were studied. Plant height for the cross '*Inbred White*' and *P. grandiflorum*, and flower size in diameter for the cross *P. peltatum* and *P. cordifolium* exhibited hybrid vigor. Other morphological characters such as leaf area (L x B), petiole length, flower color and stigma color were intermediate between the 2 parents. Variable chromosome numbers and abnormal meiotic divisions were also observed for the interspecific hybrids. Meiotic abnormalities such as chromosomes being out of the equatorial plane at early metaphase, and an unequal chromosome distribution to tetrads may have been responsible for the lower percentage pollen grain viability in the hybrid. '*Inbred White*', *P. grandiflorum* and their hybrid revealed significant genotypic variances on the ability to initiate shoots on 2 different culture mediums.

INTRODUCTION

Interspecific hybridization studies among the progenitor species of *P. x domesticum* and some breeding lines of *P. x hortorum* have already been described, where hybrid plants were recovered between '*Inbred White*'(a MSU breeding line) and *P. grandiflorum* (a *P. x domesticum* progenitor species), and between *P. peltatum* and *P. cordifolium* (Chapter 2). This material showed bacterial blight resistance (Chapter 3). The purpose of this paper is to report on morphological, cytological and *in vitro* culture studies of the interspecific hybrids.

MATERIALS AND METHODS

Hybrid plants obtained from the crosses between '*Inbred White*' and *P. grandiflorum* and between *P. peltatum* and *P. cordifolium* were evaluated and compared with the parents for morphological and other characteristics. Plant height, leaf area, petiole length and flower diameter were measured on mature plants. Fresh collected pollen grains from opened flowers were stained with 2 per cent acetocarmine for 1 hour to determine their viability using a light microscope. Round stained pollen grains were recorded as viable. About 1000 pollen grains were scored for fertility for each genotype.

To study mitosis, roots were taken from plants, put in cold water and stored in a 1°C refrigerator for 12 hrs, and then they were transferred to a fixative solution (absolute ethanol-chloroform-acetic acid 6-3-1) for 24 hrs. After washing in distilled water, the roots were put in 5N HCL for 10 minutes at room temperature, and then washed with distilled water. The root tips were smeared in a drop of two percent acetocarmine solution which was used for chromosome staining.

To study meiosis, anthers were collected at the optimal stage (which is about 1-3 mm long) from the hybrid and parent plants, and were fixed in freshly prepared ethyl alcohol—chloroform—glacial acetic acid (6-3-1) for 24 hours at 1°C. Then they were transferred to 70% ethyl alcohol. Anthers were smeared in 2% acetocarmine stain for chromosomal analysis.

Five plant leaves from '*Inbred White*', *P. grandiflorum* and their hybrid were collected and washed with a 0.01% (w/v) alconox detergent solution for *in vitro* culture studies. The leaves were rinsed for 3 minutes using tap water, and washed in 95% ethanol for 1 minute. The leaves were put in a 20% (v/v) commercial household bleach solution with 0.5 ml of tween 20 per liter for 20 minutes, and rinsed with sterile water 4 times. The surface-sterilized leaves were cut into ca. 1 cm^2 discs, and 5 leaf discs were placed in each plate under aceptic conditions.

The basic Murashige and Skoog (MS) medium (1962) was modified to produce 2 different culture mediums after Dunbar and Stephens (1989). The No.1 medium (MS-1) was the basic MS medium supplemented with 2.0mg/l zeatin, 1.9mg/l indole acetic acid (IAA), and 2.0% sucrose. This medium was adjusted to pH 5.8 with 1.0 N KOH and solidified with 0.9% agar. At 32 days, regenerated shoots were recorded in each culture plate. At 40 days the regenerated shoots were transferred to the same MS-1 medium except that IAA was eliminated and zeatin was reduced to one-tenth concentration to induce roots. The No.2 medium (MS-2) was the basic MS medium supplemented with 2.0mg/l naphthalene acetic acid (NAA), 2.0mg/l 6-benzylaminopurine (6-BAP), and 2.0% sucrose. This medium was also adjusted to pH 5.8 and solidified with 0.9% agar. Regenerated shoots were counted at 32 days, and at 40 days the shoots were transferred to the same MS-2 medium except that NAA was eliminated and 6-BAP was reduced to one-tenth to induce roots. All the cultures were maintained at 24°C with a 16 h photoperiod of 40 $\mu E/M^2S$ cool white fluorescent light.

Due to the tannin like materials released from the plant leaf discs of *P. grandiflorum* and the hybrid plant before the 32 day period, some leaf discs had to be transferred to fresh medium 2 to 3 times before regenerated shoots were observed.

A two factor factorial, completely randomized design, with 2 media and 3 genotypes as independent variables, was used for this in vitro culture study. There were 5 replications (plates) for each genotype, and 5 leaf discs in each plate.

RESULTS AND DISCUSSION

Morphology of the interspecific hybrids and their parents:

The morphological and other characteristics of '*Inbred White*', *P. grandiflorum* and their hybrid are listed in **Table 1**. The '*Inbred White*' parent is a moderately branched, soft wood, shrub shaped plant, and *P. grandiflorum* is an erect, more woody, shrub shaped plant. Their hybrid is an erect, soft wood, shrub shaped plant, and the hybrid plant was taller than either parent. The hybrid plant was intermediate in leaf area, petiole length and flower diameter. The flower petal and the stigma color of the hybrid was intermediate (pink), rather than being purplish like *P. grandiflorum* or white like '*Inbred White*'. Pollen stainability was very high in both parents but lower for the hybrid (**Table 1**).

Table 2 shows the morphological characters for *P. peltatum, P.cordifolium* and their hybrid. *P. peltatum* has a trailing, more woody plant habit; whereas, *P. cordifolium* is an aromatic plant that is well branched with a spreading habit. Their hybrid, however, has a compact shrub like plant habit that is well branched. The diameter of the flower of the hybrid is larger than either parent, The hybrid is intermediate in plant height, leaf area, and petiole length. Flower color for *P. peltatum* is pinkish - mauve with darker margins on the 2 upper petals; wherease, *P. cordifolium* is light pink with purple blotches in the 2 upper petals. Except for showing more purple pigments in the 2 upper petals, no other appreciable differences were observed among the hybrid plant for flower color. The stigma color for the hybrid and the 2 parents was all purple.

Pollen viability as recorded by percentage of acetocarmine staining was very high for *P. cordifolium*, relatively high for *P. peltatum*, and lower for the hybrid (**Table 2**). The lower pollen viability for the hybris is probably due to the variable chromosome numbers, and (or) abnormal meiotic division of hybrid plant.

Cytogenetical studies of the interspecific hybrids:

Cytological analysis of the parents *'Inbred White'* and *P. grandiflorum* revealed a normal chromosome division (**Table 1**). *'Inbred White'* has a chromosome number 2n=18, and *P. grandiflorum* has 2n=22 (**Figure 1 & 2**).

Variable mitotic chromosome numbers of 2n= 18, 19, and 20 were observed for the hybrid of 'Inbred White' x *P. grandiflorum* (**Figures 3, 4, and 5**). Irregular meiotic chromosome behavior was also observed in the hybrid. **Figures 6 and 7** show chromosome doublings that were ble I

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Table 1. Morphological, cytological and other characteristics of *'Inbred White'*, *P. grandiflorum* and their hybrid

Characters	*'Inbred White'*	*P.grandiflorum*	*'Inbred White' X P.grandiflorum*
Habit	moderately branched soft wood shrub like plant	erect, herbaceous, shrub like plant	erect, soft wood, shrub like plant
Plant height (cm)	20.4±0.39[z]	44.96±3.82	49.02±0.96
Leaf area (L x B) (cm)	2.42 x 3.36 ±0.11 ±0.17	5.40 x 7.96 ±0.21 ±0.34	4.30 x 6.54 ±0.16 ±0.21
Petiole length (cm)	3.44±0.19	2.66±0.24	3.18±0.23
Flower diameter (cm)	3.72±0.15	2.18±0.20	3.48±0.15
Flower color	white	purplish with darker streaks on two upper petals	white blush
Stigma color	white	purple	pink
Pollen stain-ability (%)	96.8±3.96	98.1±2.54	48.0±3.12
Phytotoxicity to in vitro cult. sterilization	relatively resistant	very suscept.	resistant
Tannin like materials released in in vitro cult. media	none	large amount	some
Chromosome No. (2n)	18	22	18, 19, & 20
Meiotic paring	normal	normal	unnormal

[z] mean±standard deviation

Table 2. Morphological and cytological characteristics of *P.peltatum*, *P. cordifolum* and their hybrid

Characters	*P.peltatum*	*P.cordifolum*	*P.peltatum* X *P.cordifolum*
Habit	climbing, herbaceous plant	spreading & branched, aromatic, shrub like plant	compact, well branched, shrub like plant
Plant height (cm)	long straggling shoots	93.94±1.26[z]	27.43±1.80
Leaf area (L x B) (cm)	3.46 x 4.20 ±0.17 ±0.21	5.22 x 4.72 ±0.25 ±0.23	2.80 x 3.60 ±0.07 ±0.07
Petiole length (cm)	3.34±0.21	6.18±0.24	3.52±0.15
Flower diameter (cm)	3.72±0.16	2.68±0.16	4.28±0.24
Flower color	pinkish mauve with darker margins on 2 upper petals	light pink with purple blotch on 2 upper petals	purple with darker purple blotches on 2 upper petals
Stigma color	purple	purple	purple
Pollen stain-ability (%)	83.50±4.16	97.80±2.75	46.20±8.34
Chromosome No. (2n)	18	22	20, 21, & 22

[z] mean±standard deviation

Figure 1. Meiotic chromosome number of 2n=18 for *P. X hortorum* *'Inbred White'* (x2500)

Figure 1. Meiotic chromosome number of 2n=18 for *P. X hortorum* '*Inbred White*' (x2500)

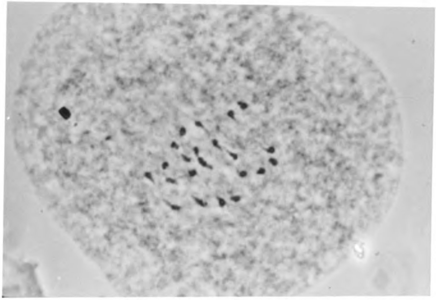

Figure 2. Meiotic chromosome number of 2n=22 for *Pelargonium* species *P. grandiflorum* (x2500)

Figure 3. Mitotic chromosome number of 2n=18 for the hybrid of '*Inbred White*' x *P. grandiflorum* (x2500)

Figure 4. Mitotic chromosome number of 2n=19 for the hybrid of *'Inbred White'* x *P. grandiflorum* (x2500)

Figure 5. Mitotic chromosome number of 2n=20 for the hybrid of *'Inbred White'* x *P. grandiflorum* (x2500)

Figure 6. Meiotic chromosome doubling for the hybrid plant of '*Inbred White*' x *P. grandiflorum* (x2500)

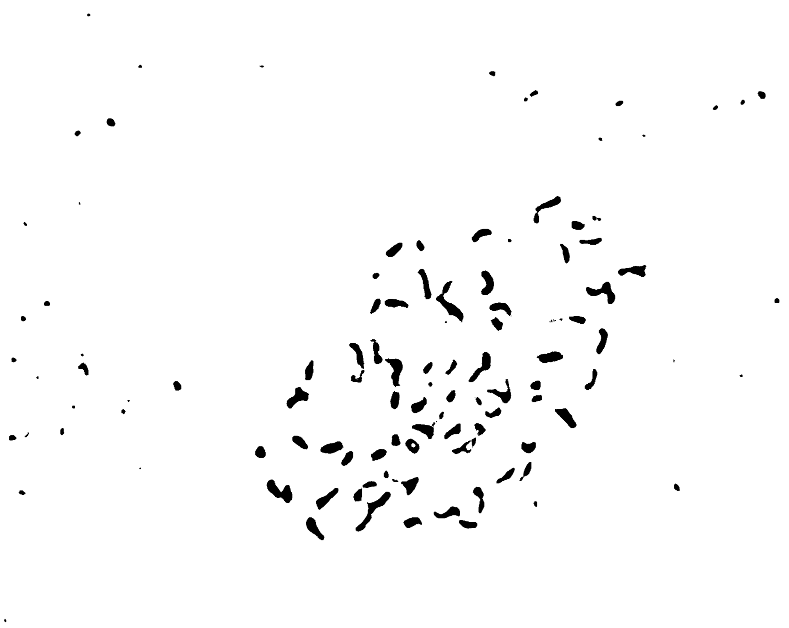

Figure 7. Meiotic chromosome doubling for the hybrid plant of '*Inbred White*' x *P. grandiflorum* (x2500)

Figure 8. Meiotic chromosome laying out of the equatorial plate at early metaphase I for the hybrid plant of '*Inbred White*'x *P. grandiflorum* (x2500)

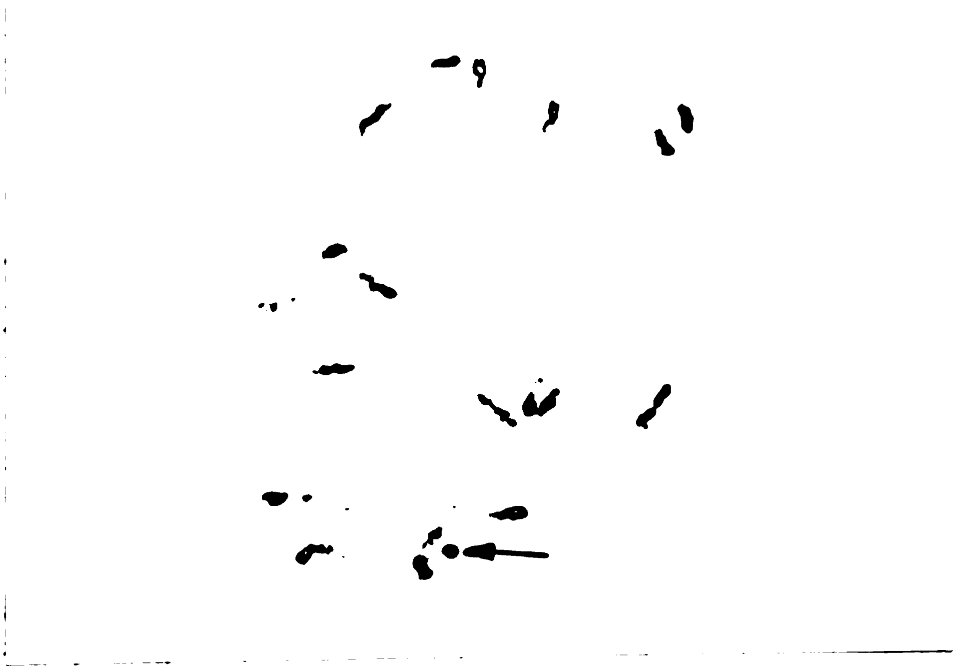

Figure 9. Meiotic chromosomes showing special end Attachments and a chromosome segment for the hybrid plant of *'Inbred White'* x *P. grandiflorum* (x2500)

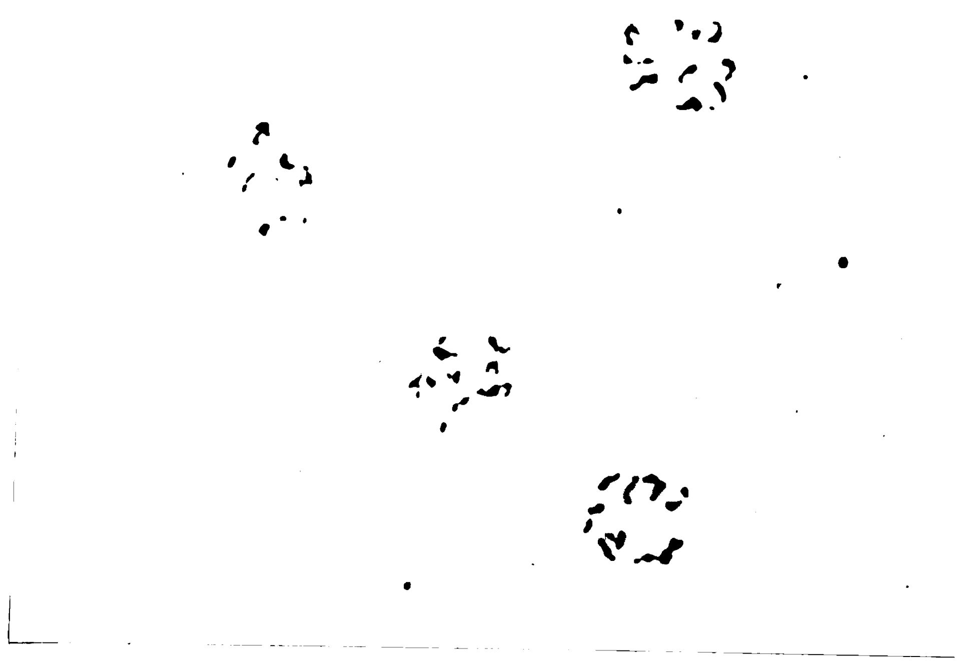

Figure 10. Unequal distribution of meiotic chromosomes during the tetrad stage from the hybrid plant of '*Inbred White*' x *P. grandiflorum*.
Top Right corner: n=10;
Bottom Left Corner: n=9;
Bottom Right corner: n=12.

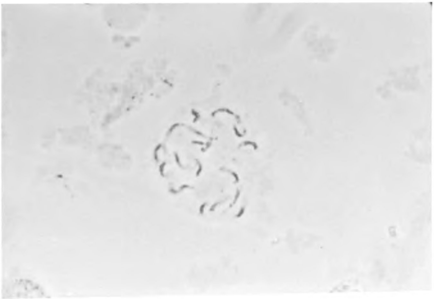

Figure 1. Meiotic chromosome number of 2n=18 for *P. X hortorum* '*Inbred White*' (x2500)

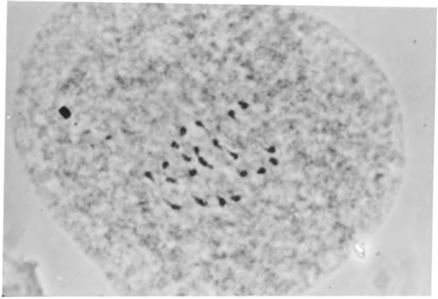

Figure 2. Meiotic chromosome number of 2n=22 for *Pelargonium* species *P. grandiflorum* (x2500)

Figure 3. Mitotic chromosome number of 2n=18 for the hybrid of *'Inbred White'* x *P. grandiflorum* (x2500)

Figure 4. Mitotic chromosome number of 2n=19 for the hybrid of *'Inbred White'* x *P. grandiflorum* (x2500)

Figure 5. Mitotic chromosome number of 2n=20 for the hybrid of '*Inbred White*' x *P. grandiflorum* (x2500)

Figure 6. Meiotic chromosome doubling for the hybrid plant of *'Inbred White'* x *P. grandiflorum* (x2500)

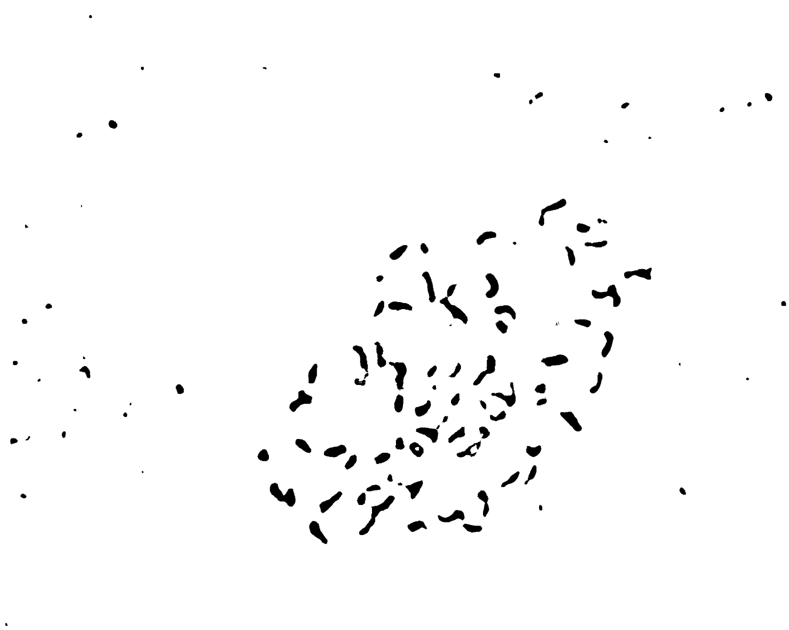

Figure 7. Meiotic chromosome doubling for the hybrid plant of *'Inbred White'* x *P. grandiflorum* (x2500)

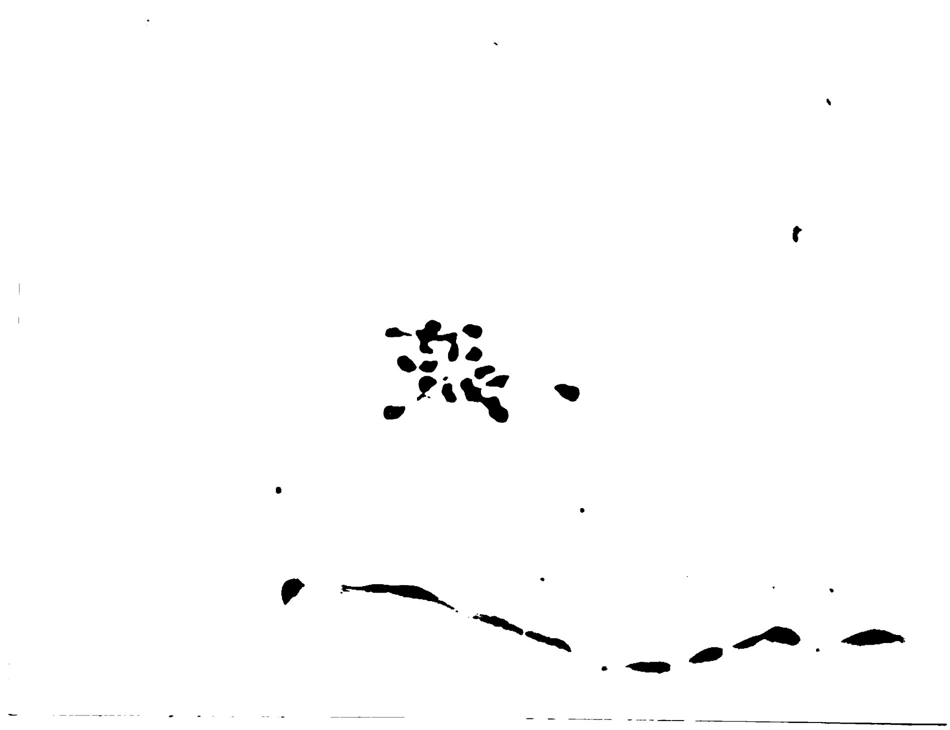

Figure 8. Meiotic chromosome laying out of the equatorial plate at early metaphase I for the hybrid plant of '*Inbred White*'x *P. grandiflorum* (x2500)

Figure 9. Meiotic chromosomes showing special end Attachments and a chromosome segment for the hybrid plant of *'Inbred White'* x *P. grandiflorum* (x2500)

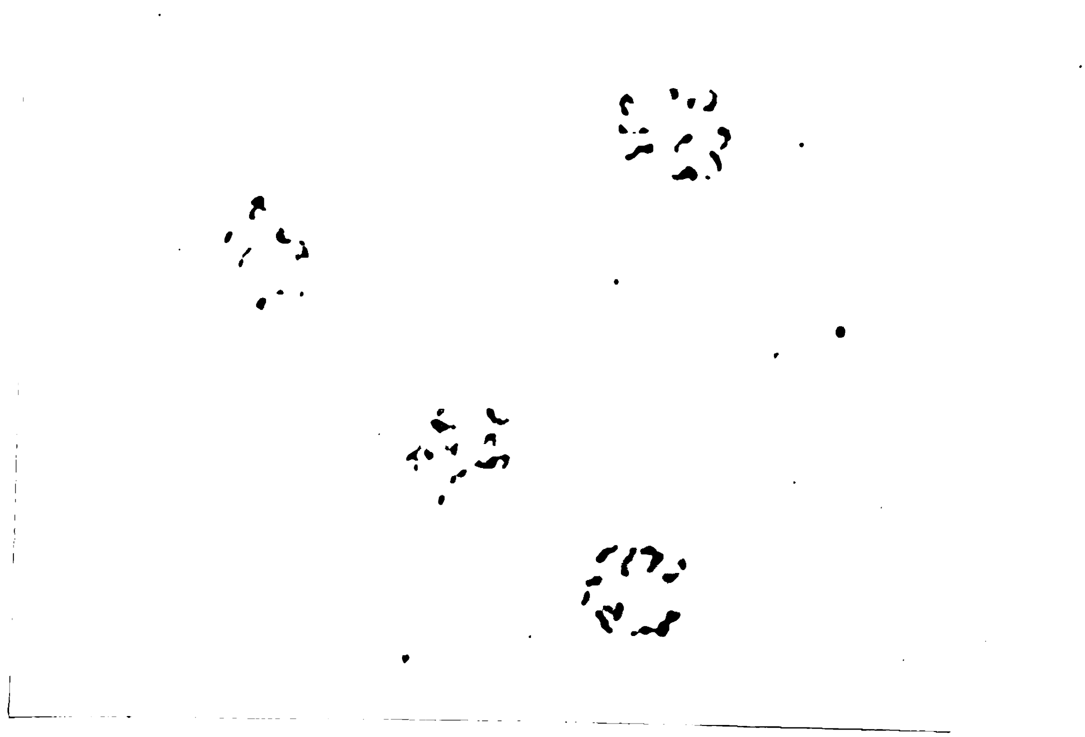

Figure 10. Unequal distribution of meiotic chromosomes during the tetrad stage from the hybrid plant of '*Inbred White*' x *P. grandiflorum*.
Top Right corner: n=10;
Bottom Left Corner: n=9;
Bottom Right corner: n=12.

observed. Which probably resulted from the formation of unreduced gametes from the 2 parents. Other meiotic abnormalities for the hybrid include chromosomes being found away from the equatorial plate at early metaphase (**Figure 8**), Chromosome attachments and chromosome fregment (**Figure 9**), and an unequal chromosome distribution in the tetrad (**Figure 10**).

The observations of chromosomes being found away from the equatorial plate at early metaphase, and the unequal distribution of chromosomes in tetrad formation could be responsible for the low level of pollen viability as determined by acetocarmine staining. The similar evidences have been found in the interspecific hybrids between Colocasia (L.) schoot and C. gigantea hook F. by Okada and Hambali (1989).

The meiotic chromosome numbers found in this study for *P. peltatum* is n=9 (2n=18), and for *P. cordifolium* n=11 (2n=22) (**Figures 10 and 11**). This is the same as reported by other researchers (Darlington and Walie, 1955; Knicely and Walter, 1966; Daker, 1969; Chartejee and Sharma, 1970; Moore, 1971). Variable mitotic chromosome numbers of 2n=20, 21 and 22 were also found in the hybrid of *P. peltatum* and *P. cordifolium* (**Figures 12, 13 and 14**). Except the normally expected chromosome number of 2n=20, the other aneuploid chromosome numbers (2n=21, a trisomic, and 2n=22, a tetrasomic) very possibly resulted from the partition of

Figure 11. Meiotic chromosome number of 2n=18 for *Pelargonium* species *P. peltatum* (x2500).

Figure 11. Meiotic chromosome number of 2n=18 for *Pelargonium* species *P. peltatum* (x2500).

Figure 12. Meiotic chromosome number of 2n=22 for *Pelargonium* species *P. cordifolium* (x2500).

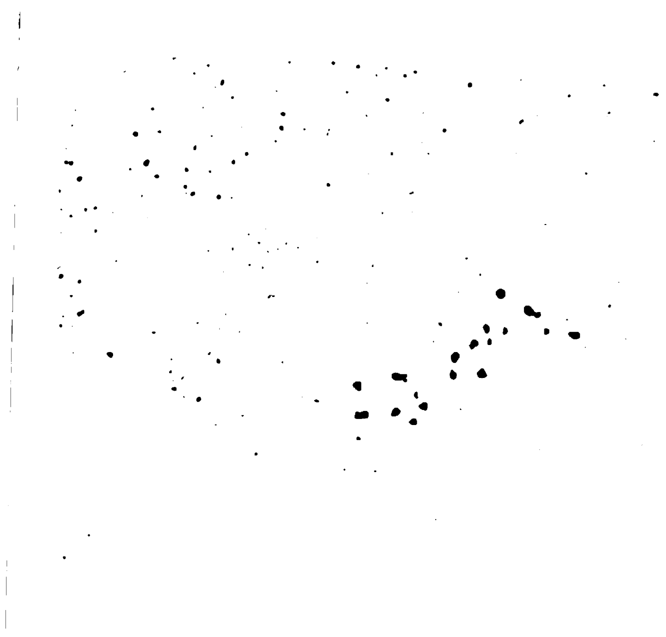

Figure 13. Mitotic chromosome number of 2n=20 from the hybrid plant of *P. peltatum* x *P. cordifolium* (x2500)

Figure 14. Mitotic chromosome number of 2n=21 from the hybrid plant of *P. peltatum* x *P. cordifolium* (x2500)

Figure 15. Mitotic chromosome number of 2n=22 from the hybrid plant of *P. peltatum* x *P. cordifolium* (x2500)

gametes containing an unbalanced chromosome number from the 2 parents, and lead to an abnormal spindle formation at the metaphase stage of mitosis.

***In vitro* culture studies for '*Inbred White*', *P. grandiflorum* and their hybrid plant:**

Genotype differences were observed in response to *in vitro* culture (**Table 3**). The leaf tissue of *P. grandiflorum* was very sensitive to the surface sterilization procedure, and the leaf samples showed considerable physical damage. '*Inbred White*' showed much less damage and their hybrid showed very little damaged.

Obvious differences were observed for tannin release from leaf discs after in vitro culture for only 7 days. Large amounts were released from *P. grandiflorum*, none from '*Inbred White*', and some from the hybrid (**Table 1**).

The two different culture media (MS-1 and MS-2) had significantly different effects (α=0.001) on shoot regeneration for all 3 plant genotypes (**Table 3**). *P. grandiflorum* produced a large amounts of shoots (18-110/plate) on both media. '*Inbred White*' produced very few shoots (0-0.5/plate). The hybrid of the above two, however, regenerated relatively a large amounts of shoots (2-24/plate). *P. grandiflorum* and the hybrid regenerated more shoots on MS-2 media than that on MS-1, but this was not the case for '*Inbred White*'. It performed poor for shoot regeneration and produced a large amount of callus on both media (Table 4). The results observed here support the conclution of Dunbar and Stephens (1989) that MS-2 was the best medium for *Regal Pelargonium* shoot regeneration. Another new culture medium need to be developed for 'Inbred White' for the regeneraion of a large number of shoots.

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Table 3. Mean number of shoots and shoot primordia regenerated from '*Inbred White*', *P. grandiflorum* and their hybrid plant on two different culture mediums (MS-1 and MS-2).

Treatment	No.shoot regenerated[z]
Medium	
MS-1	3.12
MS-2	8.42
F test	36.78***
Genotype	
'*Inbred White*'	0.02
P. grandiflorum	14.76
'*Inbred White*' X *P. grandiflorum*	5.05
$LSD_{0.05}$	2.80
F test	145.23***
Medium X Genotype	
F test	40.00***

[z] means from five replications with 5 plates in each replication.

*,** & *** means significant difference at 0.05, 0.01 and 0.001 level respectively.

Table 4. Total number of shoots and shoot primordia regenerated from '*Inbred White*', *P. grandiflorum* and their hybrid plant on two different culture mediums (MS-1 and MS-2).

	Total shoot number[z]	
	MS-1	MS-2
'Inbred White'[y]	1.0	0.0
P. grandiflorum	184.0	555.0
'Inbred White' x *P. grandiflorum*	49.0	77.0

[z] from 5 replications (plates), and 5 leaf discs in each plate.
[y] large amount of callues were produced on both MS-1 and MS-2 media.

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SUMMARY

♦ Chromosome numbers were counted for all 38 taxa in this research. The chromosome numbers for 19 of them had not been previously reported, 7 showed different chromosome numbers than those listed in previous reports, and 4 showed the same basic chromosome number as previous reports but different ploidy levels.

♦ A new chromosome number of 2n=8 was discovered.

♦ Two hundred and ninety three interspecific crosses were conducted among and within the two groups of *P. x hortorum* and *P. x domesticum*. Twenty eight of the crosses produced normal appearing seeds, which resulted in 17 groups of seedlings, and 19 crosses showed only fruit elongation.

♦ A *P. x hortorum* 'Inbred White' was found to be crossable with the *P. x domesticum* progenitor species *P. grandiflorum*. One plant was obtained from the cross.

♦ The ivy-leaved *Pelargonium*, *P. peltatum*, which has previously been reported to cross with *P. x hortorum* and to be *Pelargonium* rust resistant, was found to be crossable with *P. cordifolium*. Three hybrid seeds were produced, and one of the seeds germinated and developed into a vigorous plant.

♦ Twenty one *Pelargonium* genotypes were screened for bacterial blight resistance. Twelve showed a high level of resistance to the disease, and 8 were very susceptible. There were significant differences for bacterial blight resistance among the genotypes screened.

♦ Hybrid plants from both crosses of 'Inbred White' X *P. grandiflorum* and *P. peltatum* X *P. cordifolium* showed resistance. These hybrid plants could serve as an important source of bacterial blight resistance in *P. x hortorum* commercial cultivar development.

♦ Plant height for the cross of 'Inbred White' X *P. grandiflorum*, and the flower size for the cross of *P. peltatum* X *P. cordifolium* exhibited hybrid vigor. The leaf area, petiole length, flower color and stigma color of the hybrids from both crosses were intermediate to their parents.

♦ Variable mitotic chromosome numbers and abnormal meiotic divisions were observed for the interspecific hybrid of 'Inbred White' X *P. grandiflorum*. Variable somatic chromosome numbers were also observed for the interspecific hybrid plant of *P. peltatum* X *P. cordifolium*. These irregulations may be responsible for the low percentage of pollen grain viability.

♦ In vitro culture studies on 'Inbred White', *P. grandiflorum*, and their hybrid plant showed significant genotypic variances on the ability to initiate shoots on 2 different culture mediums. Differences in the amount of tannin-like material released from the leaf tissues of both parents and hybrid were also observed.

SUGGESTIONS FOR THE CONTINUATION OF THIS RESEARCH

♦ One second generation (G2) plant of the 'Inbred White' x *P. grandiflorum* has been obtained. This G2 plant is partially fertile. It produced 6 seeds after pollinated with the pollen from the plant of *P. peltatum x P. cordifolium*. It will be very important that further screening and selection be undertaken with this germplasm.

♦ A colchicine treatment is recommended to double the chromosome numbers for the hybrids obtained from the cross 'Inbred White' x *P. grandiflorum*, *P. peltatum x P. cordifolium*, and [G2('Inbred White' x *P. grandiflorum*) x (*P. peltatum x P. cordifolium*)]. If they can be successfully selfed and/or backcrossed to the parental lines, for further selection and improvement might be more attainable.

♦ A backcross [*P. peltatum x P. cordifolium*] x *P. peltatum* has produced 2 normal appearing seeds, if they can be germinated, there is the potential for the breeding of hanging-basket type bacterial blight resistance *Pelargonium* cultivars. This hybrid germplasm might also serve as bridge germplasm for future crosses between the *P. x hortorum* and *P. x domesticum* groups.

♦ **An average temperature change from a low of 10°C to a high of 23°C is suggested for future *Pelargonium* interspecific hybridization studies to help seed set.**

APPENDIX

Table A1. *Xanthomonas campestris pv. pelargonii* disease resistance ratings for *Pelargonium* species, cultivars and hybrid Plants (2nd screening).

Items	Disease resistance rating means[z]	
Genotype[y]		
'Inbred White X *P.grandiflorum*)	2.7	H
'Inbred White'	6.0	BC
F1 (P.peltatum x *P.cordifolium*)	1.7	IJ
'Tiny Tot'	1.3	JK
P. cucullatum	1.7	IJ
P. scabrum	1.0	K
P. seritrilotum	1.0	K
P. peltatum	5.7	A
P. citrorellum	1.3	JK
P. cordifolium	1.0	K
P. denticulatum	2.7	GH
P. grandiflorum	1.2	K
P. odoralissimum	2.1	I
P. grandiflorum x P. betulinum	1.8	I
'Tiny Tot' X 'Earliana'	1.0	K
P. betulinum X P. cordifolium	1.0	K
P. scabrum X P. seritrilosum	1.0	K
P. scabrum X 'Tiny Tot'	1.0	K
P. grandiflorum X P. cordifolium	1.0	K
P. citrorellum X 'Tiny Tot'	1.7	IJ
P. seritrilotum X P. cordifolium	1.0	K
P.x h.'picotee' X P. frutetorum	5.3	AB
P. tongaense X [(P.x h.'picotee' X P. frutetorum) X 'Picotee']	4.4	D
[(P.x h.'picotee' X P. frutetorum) X 'Picotee']	3.3	F
(P.x h.'picotee' X P. frutetorum) X P. elongatum	3.1	FG
P.x h.'Picotee' X P. acreaum	3.8	E
(P.x h.'Picotee' X P. acreaum) X P. elongatum	3.8	E

Table A1 (cont'd).

Items	Disease resistance rating means[z]	
G2(*P. zonale* X *P. frutetorum*)	5.0	BC
G2(*P. frutetorum* X *P. zonale*)	4.8	CD
G2[(*P.x h.*'picotee' X *P. frutetorum*) X *P. elongatum*]	3.8	E
(*P.x h.*'Picotee' X *P. acreaum*) X (*P.x h.*'picotee' X *P. frutetorum*)	4.9	BC
$LSD_{0.05}$	0.41	
F test	131.7**	
Days post inoculation[x]		
21 days	1.4	C
45 days	3.0	B
71 days	3.9	A
$LSD_{0.05}$	0.12	
F test	899.4**	

[z] means with the same letters are not significantly different, those with different letters are significantly different at α=0.05 by Least Significant Difference (LSD) test.

[y] means from 5 replications and last 2 observation times(45 days and 71 days)

[x] means from 21 genotypes and 5 replications for each genotype

** - significant difference at α=0.01 level

Table A2. *Xanthomonas campestris pv. pelargonii* disease resistance ratings for '*Inbred White*, *P. grandiflorum* and their hybrid plants, and for *P. peltatum*, *P. cordifolium* and their hybrid plants (2nd screening).

Genotype[y]	Disease resistance rating means[z]	
'*Inbred White*'	5.8	A
P. grandiflorum	1.3	C
'*Inbred White*' x *P. grandiflorum*	2.4	B
$LSD_{0.05}$	0.50	
F test	114.9**	
P. peltatum	5.0	A
P. cordifolium	1.0	C
P. peltatum x *P. cordifolium*	1.6	B
$LSD_{0.05}$	0.33	
F test	380.33**	

[z] means with the same letters are not significantly different, those with different letters are significantly different at α=0.05 by Least Significant Difference (LSD) test.

[y] means from 5 replications and last 2 observation times(45 days and 71 days)

** - significant difference at α=0.01 level

Table A3. *Pelargonium* interspecific crosses performed with no indication of seed development.

Cross	No. flowers pollinated
'Tiny Tot' x *P. acetosum*	23
'Tiny Tot' x *P. capitatum*	7
'Tiny Tot' x *P. cordifolium*	6
'Tiny Tot' x *P. fretetorum*	29
'Tiny Tot' x *P. fulgidum*	55
'Tiny Tot' x *P. multicaule*	10
'Tiny Tot' x *P. tetragarum*	9
'Tiny Tot' x *P. peltatum*	34
'Tiny Tot' x *P. betulinum*	14
'Tiny Tot' x 'Earliana'	26
'Tiny Tot' x *P. violavium*	11
'Tiny Tot' x (*P. frutetorum* x *P. zonale*)	10
P. acetosum x 'Tiny Tot'	21
P. acetosum x *P. cordifolium*	6
P. acetosum x *P. frutetorum*	11
P. acetosum x *P. fulgidum*	5
P. acetosum x *P. grandiflorum*	8
P. acetosum x *P. hispidum*	9
P. acetosum x *P. multicaule*	7
P. acetosum x *P. scabrum*	8
P. acetosum x *P. tetragatum*	9
P. acetosum x *P. peltatum*	9

Table A3 (cont'd).

P. acetosum x 'Earliana'	4
P. acetosum x 'Inbred White'	10
P. acraeum x 'Tiny Tot'	16
P. acraeum x *P.zonale*	6
P. acraeum x *P.peltatum*	8
P. acraeum x *P.elongatum*	5
P. acraeum x *P.mollicomum*	8
P. capitatum x P. zonale	13
P. capitatum x P. fulgidum	21
P. capitatum x P. betulinum	9
P. citrorellum x P. capitatum	9
P. citrorellum x P. cordifolium	6
P. citrorellum x P. frutetorum	4
P. citrorellum x 'Inbred White'	7
P. citrorellum x (*P. grandiflorum x* 'Tiny Tot')	10
P. cordifolium x P. grandiflorum	16
P. cordifolium x P. hermanrifilium	10
P. cordifolium x P. hispidum	8
P. cordifolium x P. inquinans	18
P. cordifolium x P. zonale	16

Table A3 (cont'd).

P. cordifolium x *P. scabrum*	18
P. cordifolium x *P. peltatum*	20
P. cucullatum x *P. zonale*	14
P. cucullatum x *P. peltatum*	24
P. cucullatum x ('Inbred White' x *P. grandiflorum*)	8
P. frutetorum x 'Tiny Tot'	41
P. frutetorum x *P. acraeum*	7
P. frutetorum x *P. capitatum*	4
P. frutetorum x *P. citrorellum*	16
P. frutetorum x *P. cordifolium*	22
P. frutetorum x *P. cucullatum*	7
P. frutetorum x *P. fulgidum*	8
P. frutetorum x *P. grandiflorum*	16
P. frutetorum x *P. hermanrifilium*	10
P. frutetorum x *P. hispidum*	10
P. frutetorum x *P. tetragarum*	18
P. fruticosum x *P. frutetorum*	6
P. fruticosum x *P. fulgidum*	30
P. fugidum x *P. capitatum*	13
P. fulgidum x *P. frutetorum*	15
P. fulgidum x *P. inquinans*	41

Table A3 (cont'd).

P. fulgidum x P. zonale	8
P. fulgidum x P. peltatum	6
P. grandiflorum x P. frutetorum	17
P. grandiflorum x P. fruticosum	8
P. grandiflorum x P. inquinans	22
P. grandiflorum x P. zonale	14
P. grandiflorum x P. tetragarum	36
P. inquinans x P. capitatum	32
P. inquinans x 'Tiny Tot'	33
P. inquinans x P. fulgidum	75
P. inquinans x P. grandiflorum	10
P. inquinans x P. peltatum	7
P. inquinans x P. betulinum	11
P. zonale x P. cordifolium	14
P. zonale x P. fulgidum	7
P. zonale x P. fruticosum	4
P. zonale x P. seritrilotum	6
P. zonale x P. denticulatum	7
P. zonale x P. capitatum	6
P. reniforme x P. grandiflorum	14
P. scabrum x P. acetosum	9
P. scabrum x *P. cordifolium*	3

Table A3 (cont'd).

P. scabrum x *P. frutetorum*	8
P. scabrum x *P. inquinans*	13
P. scabrum x *P. multicaule*	8
P. scabrum x *P. peltatum*	5
P. scabrum x *P. betulinum*	4
P. seritrilotum x P. grandiflorum	4
P. seritrilotum x P. fruticosum	6
P. seritrilotum x P. inquinans	4
P. tetragarum x 'Tiny Tot'	7
P. tetragarum x P. acetosum	9
P. tetragarum x P. acraeum	10
P. tetragarum x P. cordifolium	8
P. tetragarum x P. denticulatum	10
P. tetragarum x P. frutetorum	2
P. tetragarum x P. grandiflorum	8
P. tetragarum x P. hermanrifilium	8
P. tetragarum x P. inquinans	3
P. tetragarum x P. zonale	4
P. tetragarum x P. reniforme	11
P. tetragarum x P. scabrum	6
P. tetragarum x 'Inbred White'	6

Table A3 (cont'd).

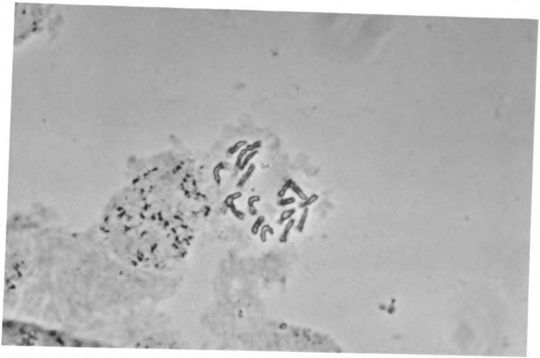
P. tetragarum x P. citrorellum	10
P. tetragarum x P. hermanrifilium	4
P. tetragarum x P. hirtum	4
P. tetragarum x P. hispidum	5
P. tetragarum x P. renifirme	5
P. tetragarum x 'Earliana'	5
P. tetragarum x (*P. cucullatum x P. tetragarum*)	14
P. peltatum x P. acetosum	15
P. peltatum x P. cucullatum	16
P. peltatum x P. grandiflorum	12
P. peltatum x P. hispidum	2
P. peltatum x P. zonale	12
P. peltatum x P. betulinum	8
P. peltatum x 'Earliana'	18
P. peltatum x 'Inbred White'	37
P. peltatum x P. tongaease	10
P. peltatum x ('Inbred White' x *P. grandiflorum*)	10
P. betulinum x 'Tiny Tot'	6
P. betulinum x P. frutetorum	16
P. tongaense x P. peltatum	21
P. tongaense x (P. grandiflorum x P. scabrum)	19

Table A3 (cont'd).

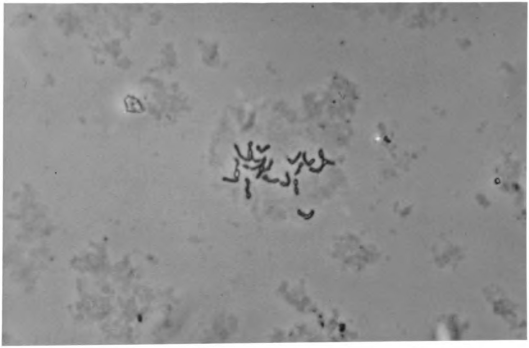
P. tongaense x *P. seirtrilotum*	20
P. quinquelobatum x P. zonale	3
P. quinquelobatum x *P. acraeum*	6
P. ranunculiphyllum x *P. elongatum*	4
'Picotee' x 'Tiny Tot'	40
'Picotee' x *P. acraeum*	5
'Picotee' x *P. capitatum*	158
'Picotee' x *P. cucullatum*	37
'Picotee' x *P. denticulatum*	9
'Picotee' x *P. fruticosum*	36
'Picotee' x *P. fulgidum*	122
'Picotee' x *P. grandiflorum*	28
'Picotee' x *P. multicaule*	10
'Picotee' x *P. scabrum*	39
'Picotee' x *P. seritrilotum*	5
'Picotee' x (*P. citrorellum* x *P. cucucllatum*)	13
'Picotee' x *P. elongatum*	21
'Picotee' x *P. mollicomum*	10
'Picotee' x *P. myrrhidifolium*	17
'Picotee' x *P. tongaense*	13
'Inbred White' x 'Tiny Tot'	27
'Inbred White' x *P. cordifolium*	11

Table A3 (cont'd).

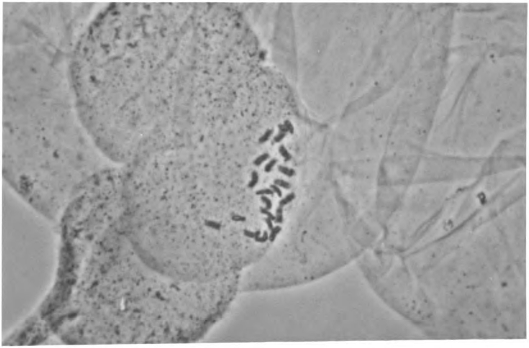
'Inbred White' x *P. fulgidum*	8
'Inbred White' x *P. myrrhidifolium*	7
'Inbred White' x *P. mollicomumm*	8
'Inbred White' x *P. tongaense*	15
'Inbred White' x *P. seritrilotum*	9
'Inbred White' x *(P. citrorellum x P. cucucllatum)*	12
'Inbred White' x *(P. grandiflorum x P. cordifolium)*	12
'Inbred White' x *(P. peltatum x P. cordifolium)*	35
'Inbred White' x ('Inbred White' x *P. grandiflorum*)	46
('Inbred White' x *P. grandiflorum*) x *P. grandiflorum*	75
('Inbred White' x *P. grandiflorum*) x *P. cucullatum*	17
('Inbred White' x *P. grandiflorum*) x *P. inquinans*	91
('Inbred White' x *P. grandiflorum*) x (*P. peltatum x P. cordifolium*)	49



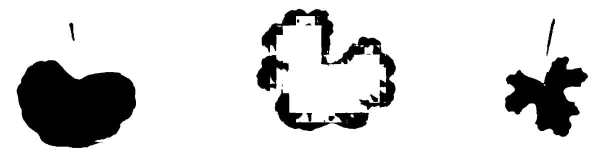
AF1. Mitotic chromosome number of 2n=16 for *P. alchemilloides* (2500X)



AF2. Mitotic chromosome number of 2n=18 for *P. acetosum* (2500X)



AF3. Mitotic chromosome number of 2n=22
for *P. tetragarum* (2500X)



AF4. Plant leave types for 'Inbred White', *P. grandiflorum* and their hybrid.
Left: 'Inbred White';
Center: hybrid;
Right: *P. grandiflorum*.

AF5. Flower types for 'Inbred White', *P. grandiflorum* and their hybrid.
Left: 'Inbred White';
Center: hybrid;
Right: *P. grandiflorum*.

AF6. Plant leaf types for *P. peltatum*, *P. cordifolium* and their hybrid.
Left: *P. peltatum*;
Center: hybrid;
Right: *P. cordifolium*.

AF7. Flower types for *P. peltatum*, *P. cordifolium* and their hybrid.
Left: *P. peltatum*;
Center: hybrid;
Right: *P. cordifolium*