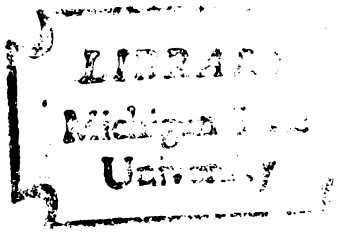

This is to certify that the

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Breeding and Genetic Studies in Zinnia elegans

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Lowell C. Ewart

Major professor

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THE INHERITANCE OF A MALE STERILE, APETALOUS INFLORESCENCE AND NARROW LEAF SHAPE IN *ZINNIA ELEGANS* JACQ.

By

Robin Kay Duffy

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THE INHERITANCE OF A MALE STERILE, APETALOUS INFLORESCENCE AND NARROW LEAF SHAPE IN *ZINNIA ELEGANS* JACQ.

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Four apetalous male sterile lines, M_1 - M_4, were selected and 3 fertile petaled lines, P_1 - P_3, were developed and selected. The 12 M x P cross combination F_2 backcross M:P ratios were evaluated by χ^2. A 3 gene recessive model has been hypothesized as a possible explanation for results obtained. In this model the genotype of the male sterile = $ms_1ms_1ms_2ms_2ms_3ms_3$. A variable number of recessive alleles were hypothesized to be present in the P lines.

Two narrow -N_1 and N_2 and 2 wide -W_1 and W_2 leaf lines were developed. Three crosses were made: N_2 x W_2, W_1 x N_2 and W_2 x N_1. Six generations: P_1, P_2, F_1, F_2, BC-P and BC-P_2 for each of the 3 crosses were evaluated on the basis of their mean leaf widths. A generation means analysis indicated additive gene effects were of significant importance in the control of leaf width while both dominance and epistasis had nonsignificant effects. High gains from selection on the basis of leaf shape are therefore indicated.

To my Parents

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INTRODUCTION

The genus Zinnia L. (1759) is a member of the family Compositae/Asteraceae, tribe Heliantheae. Named after its discoverer, Johann Gottfried Zinn, a professor of medicine at Goettingen, the genus consists of 17 annual and perennial species which are native to the southwest United States, Mexico and Central America, with one species, Zinnia peruviana, found in South America (16,26).

Torres originally divided the genus into 2 subgenera: Zinnia and Diplothrix, with the former divided into 2 sections: Zinnia and Mendezia (26). This was later expanded with the addition of the section Tragoceras to the subgenus Zinnia (20). Tragoceras was originally thought to be a separate genus, but upon investigation of supposed intergeneric hybrids, the high intergenomic homology indicated that separate genera were not justifiable (20). It is within the section Zinnia, that Z. elegans Jacq. (1793), the species responsible for the genera's popularity, is located. Most of the present day Z. elegans cultivars have been acquired by selection of genetic variation within this one species (16). There are 2 other Zinnia species which are also grown horticulturally, although their use has thus far been limited. One, Z. haageana Regel (1861), which is sometimes incorrectly referred to as Z. angustifolia sensu.DC., Z. mexicana Hort. ex Vilm., or Z. multiflora sensu HBK., is in the

section Zinnia. The other, the true Z. angustifolia HBK. (1820), at times incorrectly sited (2) and sold as Z. linearis Benth., is in the section Mendezia. These two species are different in growth form and habit in comparison to Z. elegans, having a tendency to be more fruticose and slender stemmed and possessing smaller single or double whorled inflorescences. Z. angustifolia is of extreme importance and interest; since it possesses disease resistance to Erysiphe cichracearum, powdery mildew, Alternaria zinniae, alternaria leaf spot and Xanthomonas nigromaculans F. sp. zinniae, bacterial leaf spot, desirable resistances lacking in Z. elegans (15,16). Past literature accounts state that the cross between Z. elegans and Z. angustifolia is unsuccessful (16). Recent evidence, however, shows this is not the case. At Michigan State University interspecific hybrids between these 2 species have been obtained with Z. angustifolia as the female parent. Other researchers have also recently reported the cross to be possible (15,18). As of yet, the fertility of the interspecific hybrid has not been determined, although there are reports that it is sterile (15,18). There are also reports of the reciprocal cross being possible, but only through embryo culture (25). However, the interspecific hybrids produced at MSU were obtained without the use of special cultural techniques. Z. angustifolia heads were emasculated and hand pollinated with pollen from Z. elegans. Seeds were harvested after the heads had dried down on the plant and sown in a sterile medium. Germination occurred from 8 to 16 days later. The success of this cross makes the achievement of disease resistance in Z. elegans a possibility.

Since 1796, with the introduction of Z. elegans to Europe and resultant varietal selection, the zinnia has become a popular garden flower. Goldsmith and Wilson (10) rank it as the third annual presently grown. A garden flower All-America Selection survey of 15 seed companies ranked the zinnia as first for units (seed packets) sold through seed displays and second for mail order packet sales (35). The 1981 Bedding Plants Incorporated survey of bedding plant growers, however, shows that the zinnia is only 1% of the total bedding plants grown for sale (34). Therefore, an obvious discrepancy between consumers' preference and the growers supply exists.

Horticulturally, the zinnia, by the very nature of its great diversity, has been broken down into a number of classes based on height, flower type and color. There are five height categories: (1) extra dwarf: not over 6 inches tall, (2) dwarf: from 6 to 12 inches tall, (3) medium: 18 to 24 inches tall, (4) tall: 24 to 30 inches tall and (5) giants: 30 to 36 inches and taller. Flower types may be broken down as follows: (1) single: heads possessing only 1 or 2 whorls of ray flowers, the remainder of the head being composed of disk flowers and (2) double: in which the head possesses 3 or more whorls of ray flowers, generally more whorls of ray than disk flowers. These, in turn, may be divided into: (1) dahlia flower types: in which the ray corollas are flattened and (2) cactus flower types: in which the ray corollas are twisted and curled. Flower size ranges from 3.5-4 cm on the extra dwarfs to 10-13 cm diameters on the giants. Flower color may be: (1) entire: with the ray corollas all one color, (2) bi- tri- or multicolored: with the

ray corollas divided into distinct color bands and (3) variegated: in which the ray corollas are multicolored, but there is no distinct color pattern, as in the Whirlygig and Peppermintstick cultivars. These two cultivars were obtained from a cross between Z. elegans and Z. haageana, the interspecific hybrid retaining most of the overall plant characteristics of Z. elegans, except the corolla colors. Bicolors also occur in certain F_1 hybrids depending on the genotypes used in the cross. This great diversity, plus sun and drought tolerance, long lasting bloom qualities and good cut flower keeping quality may provide a partial explanation for the zinnia's consumer popularity.

From 1972 to 1982 15 All-America winners were Z. elegans cultivars and all of these were F_1 hybrids, which may also account for some of the present-day popularity (1). As recently as 1963 and 1965, when Firecracker and Zenith Yellow respectively, were introduced, F_1 hybrids were being produced. The only open pollinated cultivars to become AAW were Old Mexico in 1962 and Persian Carpet in 1952, two Z. haageana cultivars. Old Mexico, a tetraploid, was produced by doubling the chromosome number of Persian Carpet with colchicine. Even with all the F_1 winners, most of the seed produced for packet sales is still derived from open pollinated sources (1). This may be explained by the high labor cost of producing F_1 hybrid seed, resulting in a higher cost per package than is usually thought feasible for packet seed sales.

Presently, the zinnia has limited bedding plant use when grown and marketed as a pack item or in 7.5-10 cm pots. What

production there is, is limited to dwarf varieties. This limited production may be due to the problems encountered by the growers, such as diseases, etiolation, weak growth and lack of flower production in the packs, any one of which would lead to an unsaleable plant. These problems, plus the expense of hybrid seed, makes most of the presently grown Z. elegans cultivars unsuitable for pack production.

Considering the consumer preference for the zinnia as a seed item, there has not been much published research on the zinnia as a potential bedding plant item or on the inheritance of horticulturally important traits. It appears that what genetic information is known is held in confidence by private industry (16). Much of the work on the genus overall is aimed toward disease resistance, a trait still lacking in Z. elegans cultivars (5,15,16). Cytogenetic investigations and evolutionary studies of some Zinnia species have been conducted (16,19,26,29,33). There are disagreements between the various investigators, however, on the base chromosome numbers of certain species. For example, Z. angustifolia is reported as having n=11 and n=12 (33,26,16). Differences here could be attributed to variable techniques, the presence of chromosome variation between collection sites or to classification problems arising from the species name corrections. Z. elegans is generally agreed to have n=12 (26,16,33). Z. haageana is also reported to have n=12, but it has been found that within the varieties presently cultivated this may be variable, with counts of n=10, 11 and 12 observed at MSU. These variations indicate a possible need to locate the

original germplasm sources if interspecific synthesis is to be pursued further. Cytotaxonomy, chromatographic and hybridization has also been carried out on some cespitose perennial species (27, 28,29,30).

Interspecific hybridization has also been conducted, involving 4 Zinnia species in the section Mendezia (17). Studies on induced polyploidy and cytotaxonomy have been carried out with Z. angustifolia in the hope of increasing its value as an ornamental crop. The results, however, were unsuccessful (2,22,28). Published genetics on Z. elegans is lacking, although several interspecific hybrids have been achieved. These are: Z. elegans x Z. haageana and Z. elegans x Z. angustifolia as mentioned earlier and Z. elegans x Z. peruviana via embryo culture (23).

Autotetrapolid induction of Z. elegans has been pursued to develop more vigorous cultivars (22). The results of this early study indicated limited success, although there are presently several excellent cultivars on the market which are autotetraploids. A very early study on the genetics of flower color inheritance in Z. elegans was included in a compilation by Paris, Haney and Wilson (21) on the interaction of genes for flower color. Although an excellent detailed study, the earliness of its occurrence limits its full potential since many of the present day flower colors did not exist at the time the study was conducted.

The purpose of this study was to investigate the inheritancd of horticulturally important traits in Z. elegans. The two traits

studied were a (1) male sterile, apetalous inflorescence and (2) narrow leaf shape. It is hoped that an understanding of these characters may improve F_1 hybrid seed production and also provide a zinnia ideotype suitable for bedding plant pack production. This thesis will be broken down into two chapters, the first covering the inheritance of a male sterile inflorescence and the second covering the inheritance of the narrow leaf shape.

ABSTRACT

INHERITANCE OF A MALE STERILE APETALOUS
IN ZINNIA ELEGANS

A study was conducted to determine the inheritance of a male sterile apetalous inflorescence in Zinnia elegans. Four apetalous male sterile lines, M_1 - M_4 were selected and 3 fertile, petaled lines, P_1 - P_3 were selected and developed. Select F_2, BC-M (backcross to the male sterile) and BC-P (backcross to ferile parent) generations from the 12 M x P cross combinations were evluated for their M:P segregation. All M:P ratios were evaluated by χ^2. M-P ratios of 1:63, 1:3 and 1:15, probability > 10%, were observed in the F_2 generations. M:P ratios of 1:7, 1:1 and 1:3, probability > 10%, were observed in the BC-M generation. All BC-P crosses resulted in 100% fertile, normal petaled plants. A 3 gene recessive model has been presented as an explanation for the male sterile results obtained. In this model the male sterile genotype is $ms_1ms_1ms_2ms_2ms_3ms_3$. A variable number of recessive alleles were hypothesized to be present in the P lines. The possibility that the apetalous and male sterile characters may be pleiotropic was suggested. Apetalous male sterile segregation from plants supposedly 100% petaled has been noticed in the consumers' garden. Identification of the control of the male sterile character and of the presence of recessive alleles in potential pollen parents is of vital

importance in the efficient production of nonsegregating F_1 hybrid seed.

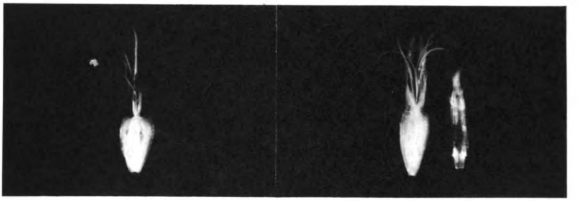
THE INHERITANCE OF A MALE STERILE APETALOUS INFLORESCENCE

Introduction

The discovery of an apetalous, male sterile inflorescence was a major breakthrough in F_1 hybrid seed production of zinnias (Fig. 1) (16). The male sterile inflorescence in Z. elegans has sometimes been referred to as "femina," since its head is supposedly entirely pistillate (22), which is not the case in Z. elegans. In the Z. elegans male sterile inflorescence, stamanoid production does occur, but no anther sacs or pollen grains are produced (Fig. 2). Therefore, herein, this inflorescence type will be referred to as male sterile, rather than "femina." Another unique characteristic associated with the male sterile trait is that the head is entirely apetalous. There have been isolated instances when petaloid formation has been seen, but this is a rare occurrence. Herein, the male sterile apetalous inflorescence will be referred to as male sterile and the derived lines as M_1, M_2, M_3 and M_4. The plants possessing a normal capitulum composed of perfect disk flowers and imperfect pistillate rays (with corollas), will be referred to as petaled and the derived parental lines as P_1, P_2 and P_3. Although there are a number of genetic and cytoplasmic male sterile mechanisms present in

Fig. 1. Male sterile, apetalous inflorescence in Z. elegans.

Fig. 2. Stamanoid structures in Z. elegans. Left, fertilized floret with extracarpellary extension removed, note withered stigma and style surrounded by 3 turgid stamanoid structures. Right unfertilized floret, note turgid pistil surrounded by 3 stamanoid structures, far right is chaff- a modified bract which subtends each floret.



floriculture crops (3,4,5,6,17), the male sterile, apetalous association is unique. The only other inflorescence of this type known occurs in the genus Tagetes, which is also in the Compositae family (31,32). It was the Tagetes inflorescence that resulted in the original coining of the term "femina." It does appear that, similar to Z. elegans, there is pistillate and stamanoid, rather than only pistillate production occurring in Tagetes. There are also reports that unlike Z. elegans occasional seed set occurs in Tagetes without known pollination having taken place (11,13). There could be several explanations for this unexpected seed set: (1) insect or wind pollinations from pollen-producing plants due to poor isolation, (2) occasional pollen production on the Tagetes male sterile or (3) apomixis of some form occurring, which has been reported in other members of the Compositae family (14). An apetalous inflorescence has also been reported in the Aster (7). Unfortunately, this inflorescence had perfect disk flowers, making it useless as a female parent in hybrid seed production.

No published work has been done on the investigation of this interesting and useful character. The reports available simply state that it is known to occur, and various personal communication sources concerning the possible genetic control, are in considerable disagreement (11,18). These disagreements come from breeders within the industry which at present are utilizing the male sterile. One author hypothesized that a fertility restoring gene exists to maintain the line (16). Two personal communications from separate seed companies

concerning the number of genes controlling this character hypothesize that: (1) it is controlled by 2 genes (18) and (2) the male sterile only occurs when there are 2 recessive alleles present at 2 loci (11). Preliminary testcross results on the lines used here indicated a different mechanism. Apparently there were indeed 2 genes and 2 loci involved but expression was due to duplicate recessice epistasis. Therefore, an investigation of this character was conducted to clarify which genotype(s) are most suitable for efficient F_1 hybrid production.

The male sterile lines all appeared to be similar anatomically. There are, however, differences in the number of stamanoid structures produced, their final development and as mentioned earlier, in the occurrence of occasional petaloid structure formation. Therefore, male steriles from 4 different cultivar sources were chosen and crosses to 3 different petaled cultivar sources made.

This inheritance study was conducted on (1) the male sterile characters; (2) possible linkage or pleiotropic associations between the male sterile and apetalous character and (3) identification of desirable genotypes for F_1 hybrid seed production.

Materials and Methods

Parental selections. During the fall of 1977 selections for leaf shape (an additional study) and male sterile types were made among open pollinated plants of 16 cultivars. The petaled selections were self-pollinated, the seed sown and further selections made in the spring of 1980. The selected male steriles were maintained and

further increased by vegetative propagation. The petaled lines were selfed and their progeny evaluated for additional male sterile segregation. Final selfing was completed the winter of 80-81 and 3 homozygous derived lines: P_1, P_2 and P_3 were selected for petaled inflorescences. This evaluation was on 142 progeny for each parent. These progeny were grown in AC 4/8 flats. Six plants from each derived P line were then selected as parents for the study. Four male steriles: M_1, M_2, M_3 and M_4 were selected following the initial sowing. Seed sources are as follows: M_1 and P_1 were selected from the same open pollinated source: Wild Cherry, an F_1 hybrid of the Fruit Bowl series; M_2 and P_3 were selected from the same open pollinated source: Peter Pan Plum; M_3 was selected from an open pollinated dwarf experimental line; M_4 was selected from open pollinated plants of an experimental semi-dwarf orchid line and P_2 was selected from open pollinated plants of the F_1 line Tangerine, a member of the Fruit Bowl series.

Crossing scheme. Each M line was crossed with each P line resulting in 12 F_1 pedigrees. The F_1's were selfed to produce the F_2 generation and also backcrossed to both parents. The P lines were also selfed. Final evaluation was made on the sowing of the P parent, F_1, F_2, BC-M (backcross to the male sterile parent) and on select BC-P (backcross to the fertile parent) crosses. Seed used was taken from both the ray and disk flowers. Plants were grown in screened greenhouses to prevent insect pollinations. Greenhouse temperatures ranged from a high of 43°C during the day to 21°C at night from May

September and 32°C day to 21°C night in October to April. Individual heads from each M line and emasculated heads from each P line were left unpollinated and checked for seed set. In either case, no seeds were produced. For the BC-P crosses, all disk flowers were removed from the inflorescence with a fine tip tweezers. The tweezers were sterilized in 70% alcohol. Crosses were made with camel hair brushes which were also sterilized in 70% alcohol.

Seed collection and sowing of the F_2, BC-M and BC-P generations. From preliminary studies, it was found that seed could either be dried on the flower head or removed from the head before drying. Seed could be collected 3 weeks after pollination, placed in a seed dryer at 47.5°C temperatures for 48 hours and sown. After 8 days 92% germination of the trial crosses was achieved, with first seedling emergence seen 2 days following seed sowing. It was also found that cutting the achenes outer wall, presumably to allow ease of water entry and of seedling emergence, improved germination percentages and decreased germination time in seed collected before complete drydown on the head.

The procedure followed for seed collection was that all seeds were placed in the dryer for 48 hours still attached to the flower head. Following drying and seed separation, the distal portion of the achene of each seed was cut and peeled back slightly before sowing. Seeds were sown in AC 4/8 flats in sterile VSP mix, covered with vermiculite, misted in with water, and given a Banrot R drench of

1 tsp/gallon of water. Seedling emergence was recorded 2-20 days following sowing. Seeds were sown from October 2-19, 1981. Temperatures were set at 27°C day and 21°C night, but fluctuated from a high of 38°C to a low of 21°C during the duration of the experiment. Lights were on throughout the entire experiment. For the first 2 weeks lights were on for 16 hours and off for 8 hours. For the remainder of the experiment, plants were under fluorescent lights which were on for 12 hours and off for 12 hours. At all times plants were watered with warm (22°C) water. Plants were fed 150 ppm of 20-20-20 every third watering.

Statistical analysis. Inflorescence data for the F_2, BC-M and BC-P generations were analyzed using χ^2 (24). Where

$$\chi^2 = \Sigma \frac{(O - E)^2}{E}$$

Results and Discussion

The segregation results obtained in the F_2, BC-M and BC-P generations indicate that control of the male sterile characters is different from that of the previously proposed 2 gene recessive model or 2 gene duplicate recessive epistatic model (11,18). The χ^2's of the F_2 and BC-M segregations indicate three, rather than two genes (Tables 1-4), shown by the significant 1:63 ratios obtained in some of the crosses. The occurrence of the 1:3 and 1:15 ratios indicates that the genotype of the P parents were not homozygous dominant for all 3 loci, but rather they had variable numbers of

TABLE 1. Chi-square results for the F_2 and BC-M_1 generations in crosses with M_1.

Cross	F_2			BC-M_1			BC-P^z
	1:3	1:15	1:63	1:1	1:3	1:7	
	χ^2	χ^2	χ^2	χ^2	χ^2	χ^2	
$M_1 \times P_1$							
1	1.88**	x	x	.54**	x	x	
2	x	.002**	3.89	x	.13**	x	
$M_1 \times P_2$							
1	x	.004**	x	x	1.97**	x	
2	x	1.48**	.07**	x	.82**	x	
3	x	x	.01**	x	2.19*	x	
4	x	3.36*	x	x	3.94	x	
5	x	.22**	x	x	.18**	x	
6	x	1.36**	.08**	x	1.52**	x	
7	x	.36**	x	x	.03**	x	
8	x	1.19**	x	x	.68**	x	
9	x	.02**	x	x	.14**	x	
10	1.55**	x	x	.67**	3.55*	x	
11	2.35**	x	x	-	-	-	
12	1.59**	.88**	x	-	-	-	
13	x	.02**	x	-	-	-	

TABLE 1. Continued

Cross	F_2			$BC\text{-}M_1$			$BC\text{-}P^z$
	1:3	1:15	1:63	1:1	1:3	1:7	
	χ^2	χ^2	χ^2	χ^2	χ^2	χ^2	
$M_1 \times P_3$							
1	.7**	x	x	x	.35**	x	
2	.41**	x	x	1.21**	x	x	
3	2.11**	x	x	1.39**	x	x	
4	.10**	x	x	-	-	-	
5	3.63*	x	x	1.05**	x	x	
6	.24**	x	x	-	-	-	

* P > .05 that deviations are due to chance (1 df, .05=3.84).

** P > .10 that deviations are due to chance (1 df, .10=2.17).

x P < .05 that deviations are due to chance.

z All backcrosses to the P parent resulted in 100% fertile, petaled plants.

\- Backcrosses corresponding to these F_2s not sown.

TABLE 2. Chi-square results for the F_2 and BC-M_2 generations in crosses with M_2.

Cross	F_2			BC-M_2			BC-P^z
	1:3	1:15	1:63	1:1	1:3	1:7	
	χ^2	χ^2	χ^2	χ^2	χ^2	χ^2	
$M_2 \times P_1$							
1^{ry}	x	.99**	2.3**	2.78**	.03**	.83**	
2^r	x	.86**	.20**	0	0	0	
$M_2 \times P_2$							
1^y	0	0	0	x	2.73*	.93**	
2^y	0	0	0	x	1.78**	.19**	
3^y	0	0	0	x	1.33**	.45**	
$M_2 \times P_3$							
1^{ry}	1**	x	x	3.23*	.025**	2.66*	
2	.05**	x	x	.13**	x	x	
3^{ry}	x	.12**	1.30**	x	3.34*	x	
4^y	.98**	x	x	x	3.34*	x	
5^r	0**	2.4**	x	0	0	0	

*: P > .05 that deviations are due to chance (1df, .05 = 3.84).

**: P > .10 that deviations are due to chance (1df, .10 = 2.17).

x: P < .05 that deviations are due to chance.

z: All backcrosses to the P parents resulted in 100% fertile, petaled plants.

r,y: F_2 and BC-M population sizes respectively were < 30.

0: Plants not in flower.

TABLE 3. Chi-square results for the F_2 and BC-M_3 generations in crosses with M_3.

Cross	F_2			BC-M_3			BC-P^z
	1:3	1:15	1:63	1:1	1:3	1:7	
	χ^2	χ^2	χ^2	χ^2	χ^2	χ^2	
$M_3 \times P_2$							
1	0**	x	x	-	-	-	
2^r	.66**	x	x	-	-	-	
3^r	.08**	x	x	-	-	-	
$M_3 \times P_3$							
$1^{r,y}$	.11**	x	x	.2**	3.07*	x	
2	2.62**	3.32*	x	1.8**	x	x	

*: P > .05 that deviations are due to chance (1df, .05 = 3.84).

**: P > .10 that deviations are due to chance (1df, .10 = 2.17).

x: P < .05 that deviations are due to chance.

z: All backcrosses to the P parents resulted in 100% fertile, petaled plants.

0: Plants not in flower.

-: Seeds from respective cross not sown.

TABLE 4. Chi-square results for the F_2 and BC-M_4 generations in crosses with M_4.

	F_2			BC-M_4			
	1:3	1:15	1:63	1:1	1:3	1:7	BC-P^z
	χ^2	χ^2	χ^2	χ^2	χ^2	χ^2	
M_4 x P_1							
1[r]	0**	.55**	x	-	-	-	
M_4 x P_2							
1	x	.45**	x	-	-	-	
2	x	.03**	3.73*	-	-	-	
3	x	0**	x	0	0	0	
4	x	.25**	2.43*	-	-	-	
5	x	.03**	3.73*	0	0	0	
6	2.33*	3.89	x	-	-	-	
M_4 x P_3							
1	0	x	x	-	-	-	
2[y]	2.79*	x	x	.05**	x	x	
3[r]	.004**	x	x	-	-	-	
4[r]	.004**	x	x	0	0	0	
5[r]	.39**	x	x	-	-	-	

*: P > .05 that deviations are due to chance (1df, .05 = 3.84).

**: P > .10 that deviations are due to chance (1df, .10 = 2.17).

x: P < .05 that deviations are due to chance.

z: All backcrosses to the P parents resulted in 100% fertile, petaled plants.

r,y: F_2 and BC-M population sizes respectively were < 30.

0: Plants not in flower.

recessive alleles. The data also indicates that the individual P lines differend in the number of recessive alleles present within the line. The consistent 1:3 and 1:1 F_2 and BC-M ratios, respectively, obtained in crosses with P_3 which had large population sizes reflect this genetic situation. P_2 appears to have a variable number of recessive alleles present, as indicated by the 1:3, 1:15 and 1:63 segregations which occurred. Although fewer crosses with P_1 were made, it seems that here, too, one or more loci have recessive alleles present. The data suggest that the male sterile genotype is the same in M_1 - M_4, as evidenced by their consistent behavior when crossed with the same P line, but that the P genotype is variable. A genotypic model is hypothesized (Table 5), which may explain these results.

That so few of the significant values for the 1:63 ratio appear may be partially explained by the fact that P_2 was a very weak line. Cuttings were taken to insure that the line was maintained. However, 2 plants were lost quite early in the study and by the end of the crossing period, all but one plant had died and that one was not very healthy. These plants were also lost before any crosses other than those to some of the M_1 were made. If by chance one of the P_2 plants which was lost early in the study was homozygous dominant at all 3 loci the number of F_2 generations segregating in the 1:63 would be limited and only manifested in crosses with M_1. Some of the largest F_2 population sizes were highly significant for the 1:63 ratio in crosses with M_1 and it is

felt that the low number of 1:63 ratios obtained does not negate the possibility of 3 gene control.

If, however, one were to eliminate the 1:63 segregations on the basis of supposed chance occurrence, then a 2 gene double recessive model may fit into an explanation of how the male sterile character may be controlled. Here male sterile expression would be caused by 2 homozygous recessive alleles at 2 loci. It can be seen that if one loci in the fertile parent was heterozygous, with the other homozygous dominant, a 1:3 and 1:1 F_2 and BC-M ratio, respectively, would occur. If this were true, the breeding practices necessary in the 3 gene model would still need to be followed for the 2 gene model. It would be even more important to maintain pure fertile parents in this case. With the 2 gene model, if a heterozygous genotype was present in the pollen parent, a 1:3 ratio would occur in the F_1 generation, undesirable in a breeding program designed to produce 100% petaled plants. The fact that only 2 genes are involved in this model makes the occurrence of a heterozygous condition and resultant segregation a greater possibility. This also adds further support to the 3 gene model, since segregations in marketed seed do not occur with the frequency of a 2 gene model or consistently in a 3:1 or 1:1 ratio size, but much closer to the 1:7 ratio, as would be expected with 3 genes. Therefore, in order to tentatively explain the occurrence of all ratios, a 3 gene, rather than a 2 gene, model which is suggested.

The simplest type of genetic explanation possible to be inclusive of all results has been suggested. This is a 3 gene model,

TABLE 5. Hypothesized genotypic model for the male sterile and fertile parents with expected segregation ratios in the F_2 and BC-M generations.

Genotype			Ratio M:P	
M		P	F_2	BC-M
$ms_1ms_1ms_2ms_2ms_3ms_3$	x	$Ms_1Ms_1Ms_2Ms_2Ms_3Ms_3$	1:63	1:7
	x	$Ms_1MS_1Ms_2Ms_2ms_3ms_3$	1:15	1:3
	x	$Ms_1Ms_1ms_2ms_2ms_3ms_3$	1:3	1:1
	x	$Ms_1Ms_1Ms_2ms_2Ms_3ms_3$	1:63	1:7
		or	1:3	1:1
	x	$Ms_1Ms_1Ms_2Ms_2Ms_3ms_3$	1:63	1:7
		or	1:15	1:3
	x	$Ms_1ms_1Ms_2ms_2Ms_3ms_3$*	all possible F_2 and BC-M ratios	

*F_1 segregates in a 1:7 ratio, M:P.

with the male sterile character only expressed when all 3 loci are homozygous recessive. The fact that 6 fertile parents were used in the initial cross does not allow for conclusive results, but this does appear to be a feasible explanation of the results obtained. With the 6 fertile parents used for each P line, there existed the possibility of different genotypes involved in the initial crosses, even though all were phenotypically similar and there was no segregation of male sterile characters in previous selfings. It does appear that this was the case. With a 3 gene model there is also the fact that irrespective of the number of inbred generations, if one loci is homozygous dominant, the male sterile character will not be seen. It is also easy to visualize a single locus or possibly even 2 loci becoming fixed in the recessive character. This may explain why one seed company individual reported only seeing the 1:15 segregation ratio when the male sterile was crossed with open pollinated varieties rather than those which had been involved in F_1 hybrid production or which were themselves hybrids. Here if the F_1 had been crossed with a male sterile larger ratios would occur. Population sizes were also such that a definite yes/no statement concerning the results cannot be made, but 3 genes are strongly evidenced.

Within the limits of the plant populations observed there is also an indication that the control of the apetalous character may be pleiotropic. A petaled sterile or apetalous fertile plant was not observed in any population grown. This does not negate the

possibility of it being a tightly linked character but puts forth further support that it may be pleiotropic.

If it is considered that the genotype of the male sterile is of one type, then it is not this genotype, but the fertile genotype which must be considered. This is very important to a breeding program; as it would have a major influence on present breeding practices. Test crosses should be performed to observe F_2 segregation as an indication of the fertile genotype. It is also very possible that once desirable genotypes of both the fertile and male sterile types are developed that parental maintenance by vegetative cuttings or tissue culture means would be the most practical method. The ease at which all stages of vegetative growth, rooted, from woody stem material to very succulent juvenile growth, indicates that this would not be a difficult method to incorporate. If tissue culture methods were developed, this would be an even greater benefit in that large numbers of plants could be rapidly propagated. In comparison to tissue culture, the rooting of vegetative cuttings is archaic, but desirable over the backcross, and rogueing methods presently utilized.

From the model, the genotype with the most potential problems is the one which is heterozygous at all 3 loci. Here a 1:7 segregation ratio occurs in the F_1 generation. Caution must be taken in that pollen parents should probably never be selected from an apetalous x petaled F_1 combination, as it is here that the potential for the heterozygous condition is the greatest.

The male sterile is a necessary phenotype for hybrid seed production in Z. elegans. The 3 gene model will require a revision of some of the present breeding practices. Evidence of this is the segregations occurring in marketed seed. Pollen parents and progeny should be backcrossed and testcrossed to observe potential segregations before used in hybrid combinations. The identification of the genetic control of this character is of major importance to the seed industry. This model explains some of the segregation patterns observed in seed presently marketed and the different segregation ratios obtained in crosses with different pollen parents. Further research into this character and that of potential pollen parents would be desirable. It would be beneficial to identify pollen parents homozygous dominant at all 3 loci so that assurance is made that no unwanted segregations will occur. The exact identity of the genetic control of the male sterile character is also important for seed production and maintenance of this character. It would be desirable to have tissue culture methods developed and available to maintain the male sterile breeding stock.

ABSTRACT

INHERITANCE OF A NARROW LEAF SHAPE IN ZINNIA ELEGANS

An inheritance study was conducted on the narrow leaf shape in Z. elegans. Two narrow (N_1 and N_2) and 2 wide (W_1 and W_2) leaf lines were developed. Parental classification of narrow and wide was based on mean leaf widths calculated from measurements taken at the widest portion of the leaf. The first 8 leaves above the cotyledons were measured at a standardized flower bud stage. Three crosses were made: $N_2 \times W_2$, $W_1 \times N_2$ and $W_2 \times N_1$. Six generations: P_1, P_2, F_1, F_2, BC-P_1 and BC-P_2, for each of the 3 crosses were classified on the basis of mean leaf widths. For each of the crosses, plants were grown in a randomized complete block design, 20 blocks per cross. A generation means analysis was performed and additive gene effects were of significant importance in the control of leaf width, while both dominance and epistasis had nonsignificant effects. High gains from selection on the basis of leaf shape are therefore indicated. The development of narrow leaf Z. elegans lines for bedding plant pack production is desirable. The narrow leaf shape provides better light penetration and air circulation within the flat allowing greater branch development, shorter plant heights and flowering in the pack. The narrow leaf shape makes it possible to overcome the present problems of etiolation, yellowing of leaves, weak

growth, disease susceptability and lack of flower production associsted with most of the zinnias presently marketed as bedding plants.

THE INHERITANCE OF A NARROW LEAF SHAPE IN *ZINNIA ELEGANS*

Introduction

Z. elegans is a favorite garden plant when grown from seed, but has not yet been adapted to greenhouse production as a bedding plant. Growers state that with all the cultural problems encountered, coupled with the high cost of seed, most zinnia cultivars do not make a quality, saleable bedding plant pack item. Thus, there is need for a zinnia which will perform well when grown in pack production, as well as in the garden. A plant type with a narrow leaf would meet these criteria.

Such a narrow leaf zinnia ideotype was first discovered segregating from the normal wide leaf type of the cultivar Wild Cherry of the Fruit Bowl series (Fig. 1) (7). The exact origin of this narrow leaf type is not known, but there are several hypotheses as to the origin (Fig. 2): (1) directly from a cross with *Z. haageana*, (2) from *Z. angustifolia*, either by a direct cross or with *Z. haageana* acting as a bridge between the two, or (3) from a mutation in *Z. elegans*. Although the narrow leaf character has been available for a number of years, up until now, it has not been utilized. This is evident by the fact that there are no pure line narrow leaf cultivars presently available on the market.

Fig. 1. Left narrow leaf zinnia segregant; right wide leaf cultivar commonly seen.

Fig. 2. Top Z. elegans narrow and wide leaf types. Lower leaf two Z. angustifolia leaf type examples: cultivars Classic and "linearis." Lower right Z. haageana leaf type example: cultivar Marginata.

Zinnia elegans

Narrow Wide

Zinnia angustifolia **Zinnia haageana**

Classic "linearis" Marginata

The Z. elegans wide leaf cultivars available at the present time may be characterized by the following ideotype: leaves widely cordate (maximum 7 cm at widest portion) and entire, arranged in a decussate sessile manner on the stem, with an overall tendency to droop, overlapping lower leaf axiles when final size has been attained; one main stem with few branches and a more upright growth habit. In contrast, the narrow leaf ideotype may be characterized as follows: leaves narrowly saggitate (maximum 3 cm at widest portion), with occasional widely separated serrations near the leaf apex, arranged in a decussate sessile manner on the stem, held perpendicular to the stem; several main stems with much branch development and forming a more cespitose form of growth. In comparing the 2 ideotypes, it appears that the additional light penetration made available to the narrow leaf type allows the development of lateral branches. Lateral buds may be seen on the wide leaf type but these do not develop. The leaves on the wide leaf type may completely cover the lateral buds 2 leaf tiers below, thus preventing development of lateral branches. Along with greater branch development in the narrow leaf type, there also appears to be an overall reduction in height of the plants in comparison to their wide leaf counterparts. Observations of breeding lines differing only in leaf width showed the narrow leaf types were several inches shorter than their wide leaf counterparts. Along with the greater amount of light penetration provided by the narrow leaf type, there is also a greater amount of air circulation provided by the narrow leaf type. This is of considerable importance in the physical prevention of disease problems during growth in

plastic bedding plant flats. The problems with moisture build-up and high humidity conditions which lead to diseases such as powdery mildew, may be reduced by this increased air circulation. The presence of these beneficial characters identifies the narrow leaf shape as a desirable trait to investigate.

This study was therefore undertaken to determine the inheritance of the narrow leaf shape in Z. elegans. Hopefully such information can be used to produce a zinnia ideotype suitable for bedding plant pack production.

Materials and Methods

Parental selections. During the fall of 1977 selections for leaf shape and male sterile segregation (an additional study) were made among open pollinated plants of 16 cultivars. The narrow and wide selections were self pollinated, the seed sown and further selections made in the spring of 1980. These selections were selfed and their progeny evaluated for significant deviations from the narrow or wide character. Final selfing was completed the winter of 80-81. Two homozygous narrow: N_1 and N_2 and two homozygous wide: W_1 and W_2 lines were selected. This evaluation was on visual observation of 64 progeny for each individual line. Six plants were selected out of each population as parents for each of the 4 lines. These 24 plants were analyzed statistically using Bartlett's test for homogeneity (24) and the 4 lines were not homogeneous. This may have been due to the low leaf width variance in the N_1 line, a possible indication that this line had undergone more inbreeding than the

others. Since the 4 lines were not homogeneous, it was necessary to calculate an effective degrees of freedom (Table 1). Comparisons were based on mean leaf widths of each population: $\bar{N}_1 = 2.6$, $\bar{N}_2 = 2.22$, $\bar{W}_1 = 4.3$ and $\bar{W}_2 = 4.6$. As indicated N_1 and N_2 were not significantly different from each other and W_1 and W_2 were not significantly different from each other, but in all N and W combinations there were significant differences. Lines were tested using measurements taken at the widest portion of the 5th leaf pair on all plants at anthesis of the first flower. The seed sources were as follows: N_1 was selected from open pollinated plants of Wild Cherry, an F_1 hybrid of the Fruit Bowl series; N_2 was selected from open pollinated plants of the F_1 hybrid Yellow Sun; W_1 was selected from the open pollinated source Peter Pan Plum and; W_2 was selected from open pollinated plants of an experimental F_1 semi-dwarf orchid line.

Crossing scheme. Four crosses were made: $N_1 \times W_1$, $N_2 \times W_2$, $W_1 \times N_2$ and $W_2 \times N_2$. Seed set and development of $N_1 \times W_1$ was very slow and percent viable seed were very low. The F_1 of this cross was extremely double and had very few disk flowers for pollen production.

A generations means analysis was used to determine the type of gene effects controlling leaf character. Six generations were developed and used in the study: P_1, P_2, F_1, F_2, BC-N (backcross to the narrow parent) and BC-W (backcross to the wide parent). For both backcrosses all disk flowers were removed from the female parents and the F_1 generation plants used as pollen parents. The F_1 was produced in a similar manner, P_1 having all disk flowers removed with

TABLE 1. Comparison of parental populations based on mean leaf width.

Parents**	Effective Degrees of Freedom	t calculated	t tabular .05
W_1 vs W_2	17	1.40 ns	2.11
W_1 vs N_1	20	13.57*	2.086
W_1 vs N_2	20	12.46*	2.086
N_1 vs N_2	18	1.75 ns	2.101
N_1 vs W_2	14	10.01*	2.145
N_2 vs W_2	15	10.48*	2.131

*: significant at the 5% probability level.

**: $\overline{W}_1 = 4.3$

$\overline{W}_2 = 4.6$

$\overline{N}_1 = 2.6$

$\overline{N}_2 = 2.2$

ns: not significant

pollen supplied by P_2. For the P_1, P_2 and F_2 generations seeds were collected from both disk and ray flowers. Plants were grown in screened greenhouses at temperatures of 28°C day to 21°C night October to April and from a maximum 43°C to 21°C night May to September. Individual emasculated heads were left unpollinated and checked for seed development. No seeds were produced on any unpollinated head. Disk flowers were removed from the heads with a fine tip tweezers. The tweezers were sterilized in 70% alcohol. Crosses were made with camel hair brushes.

Seed collection and sowing. The same procedure was followed for seed collection and sowing as outlined in the previous chapter. Seedling emergence occurred 2-14 days after sowing. The 6 generations of the cross $N_2 \times W_2$ were sown 10/2, $W_1 \times N_2$, 10/9 and $W_1 \times N_1$, 10/15. Plants were under lights for the duration of the experiment. The first 2 weeks lights were on for 16 hours and off for 8 hours. For the remainder of the experiment, lights were on 12 hours and off 12 hours. At all times the plants were watered with warm 22°C water. Plants were fed 150 ppm 20-20-20 every third watering.

Experimental design and statistical analysis. For each cross plants were grown in a randomized complete block design. There were 3 P_1, 3 P_2, 3 F_1, 12 F_2, 7 BC-N and 4 BC-W replications per block. Twenty blocks were grown for each cross. Within each block replications were randomized as was the assignment of block order. To reduce border effects for the plants on the outer edges of the

blocks flats containing plants used in the male study were placed around the outer edges of the blocks.

Leaf length and width measurements were taken when the flower buds had reached 3.2 mm in size. The widest portion of the leaf was recorded. Length and width measurements were taken on the first 3 leaf pairs above the cotyledon and width measurements only were taken on the 4th leaf pair. Data were evaluated using a generations means analysis (9). A generation means analysis supplies information on the type of gene effects involved in the expression of the character under analysis. The estimates of the six parameters were calculated as follows:

$$m = \overline{F}_2$$

$$a = \overline{BCP}_1 - \overline{BCP}_2$$

$$d = -1/2\ \overline{P}_1 - 1/2\ \overline{P}_2 + \overline{F}_1 - 4\overline{F}_2 + 2\ \overline{BCP}_1 + 2\ \overline{BCP}_2$$

$$aa = -4\overline{F}_2 + 2\ \overline{BCP}, + 2\ \overline{BCP}_2$$

$$ad = -1/2\ \overline{P}_1 + 1/2\ \overline{P}_2 + \overline{BCP}_1 - \overline{BCP}_2$$

$$dd = \overline{P}_1 + \overline{P}_2 + 2\overline{F}_1 + 4\overline{F}_2 - 2\overline{BCP}, - 2\overline{BCP}_2$$

Frequency distributions for each generation were also plotted. An analysis of the variance was calculated on a block, entry basis since there was an unequal number of generation replications per block.

Results and Discussion

The mean leaf widths of the 6 generations for each of the 3 crosses are shown in Table 2. In each of the 3 crosses the female

TABLE 2. Mean leaf width (cm) of parents and crosses for leaf width comparisons.

Crosses	Populations					
	P_1[r]	P_2[z]	F_1	F_2	BC-P_1	BC-P_2
$N_2 \times W_2$	2.56	3.99	4.04	3.65	3.31	4.06
$W_1 \times N_2$	3.61	2.80	3.81	3.45	3.69	3.32
$W_2 \times N_1$	3.68	2.45	3.69	3.59	3.88	3.15

r: P_1 is the female parent in the cross.

z: P_2 is the male parent in the cross.

parent was designated as P_1, resulting in negative values for the gene effects (Table 3) when the narrow leaf parent was P_1. The negative value is due to the definitions of the various gene effects and has no effect on the value's significance or nonsignificance. In each cross the mean of the F_1 and that of the BC-W exceeds that of the wide leaf parent, indicating a heterotic effect in the direction of the wide leaf.

The estimates of the 6 parameters for gene effects using the means (Table 2) are shown (Table 3). Throughout this analysis the assumptions are: (1) 2 alleles per locus, (2) most of the positive alleles in one parent and negative alleles in the other parent, (3) no linkage, (4) environmental and genotypic effects were additive and (5) no trigenic or higher interactions. The results (Table 3) show that for each of the 3 crosses the gene

TABLE 3. Mean estimates of the six gene effects in the 3 Zinnia elegans leaf width crosses.

Crosses	Gene Effects					
	m	a	d	aa	ad	dd
$N_2 \times W_2$	3.65**	-.75**	.89	1.49	-.04	-.24
$W_1 \times N_2$	3.45**	.37$^+$	.85	.24	-.04	-.23
$W_2 \times N_1$	3.59**	.73**	-.13	-.31	.12	-.23

+, *, **: Significant at the 10%, 5%, and 1% levels respectively.

effects important in the expression of leaf width are additive, as indicated by their significant values. The negative sign for the a value in the cross $N_2 \times W_2$ is due to the definition of a, as mentioned earlier. If the wide leaf parent had been considered as P_1 in this cross, the value would have been positive. It has no influence on the significance of a, it just indicates that the $\overline{P}_1$ value was less than $\overline{P}_2$. Since a is defined as $\overline{BC\text{-}P_1} - \overline{BC\text{-}P_2}$, it shows that additive effects are indeed at work. Still considering the narrow leaf parent as P_1, in the $BC\text{-}P_1$ there is a greater number of narrow modifiers causing the leaf width to decrease and approach that of the narrow leaf parent. The nonsignificant dominance and epistatic values indicate these are of minor importance in expression of the leaf width character. Dominance estimates were close to being significant at the 10% level, however, indicating that possibly not all of the modifiers effecting leaf width have an additive effect,

but some may be expressing dominance. The overall expression of the narrow leaf character versus wide appears to be controlled as a pseudo recessive/dominant character respectively in regard to characteristic shape. The exceeding of the wide leaf parent mean and the approach of the wide leaf parent leaf shape by the F_1 is evidence of this. The great number of modifying genes apparently present, however, effect the leaf width in a quantitative manner, causing a degree of variation within each characteristic narrow and wide group. It is important that the overall control of these modifiers and the subsequent leaf shape is due to additive gene effects. Having a character of interest under the control of additive effects is important to a breeding program in that gains from selection can only be realized when selecting traits controlled by additive or additive x additive gene effects. In contrast, selection of a trait influence by dominant gene effects would be hindered since the heterogenous condition is masked.

The frequency distributions (Figs. 3-8) characterize leaf shape as a multigenic character. The F_2 distributions (Figs. 4, 6, 8) all approach that of a normal curve, indicating the presence of modifying genes. Leaf width is also greatly influenced by the environment. Comparisons of the P_1 distribution (Fig. 3) with the P_2 distribution (Fig. 5) and the P_2 distribution (Fig. 3) with the P_1 distribution (Fig. 7) are those of the same parent. However, the distributions, although of similar overall shape, have different mean values. Narrow leaf parent N_2 had a more variable distribution

Fig. 3. Frequency distribution of mean leaf width in cm of the P_1, P_2 and F_1 generations for the cross $N_2 \times W_2$.

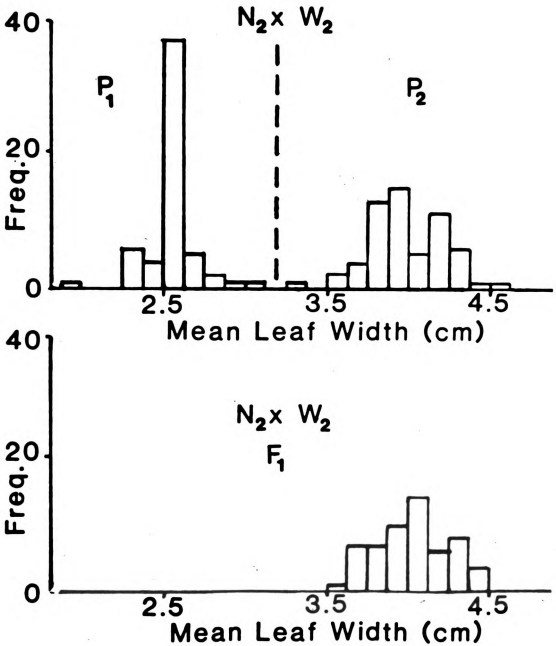

40
20
0
Freq.
N2 x W2
P1
P2
2.5
3.5
4.5
Mean Leaf Width (cm)
40
20
0
Freq.
N2 x W2
F1
2.5
3.5
4.5
Mean Leaf Width (cm)

Fig. 4. Frequency distributions of the mean leaf width in cm of the F_2, BC-P_1 and BC-P_2 generations for the cross N_2 x W_2.

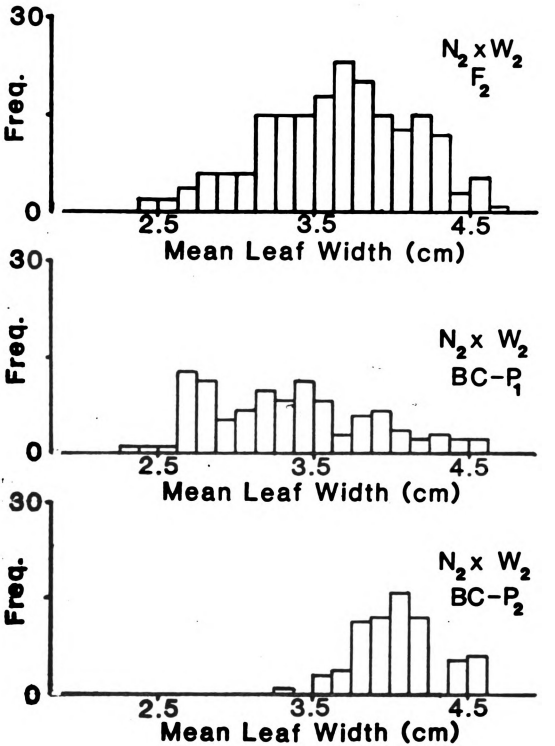

30
Freq.
0
$N_2 \times W_2$
F_2
2.5
3.5
4.5
Mean Leaf Width (cm)
30
Freq.
0
$N_2 \times W_2$
$BC-P_1$
2.5
3.5
4.5
Mean Leaf Width (cm)
30
Freq.
0
$N_2 \times W_2$
$BC-P_2$
2.5
3.5
4.5
Mean Leaf Width (cm)

Fig. 5. Frequency distributions of the mean leaf width in cm of the P_1, P_2, and F_1 generations for the cross $W_1 \times N_2$.

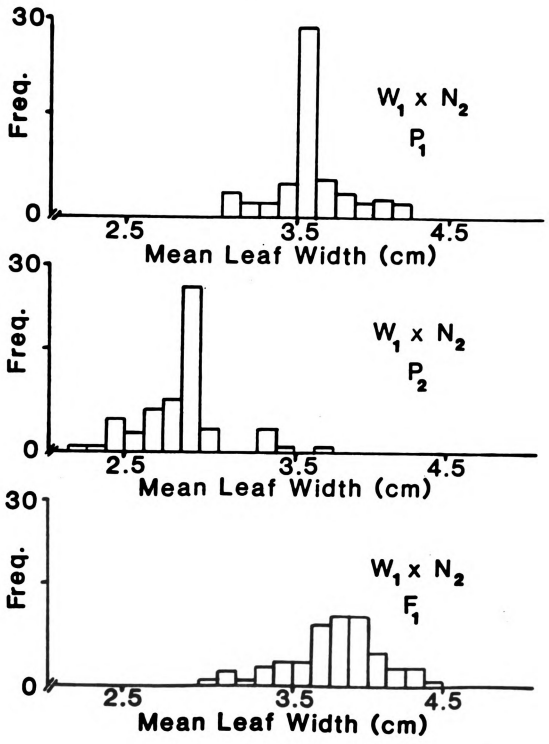
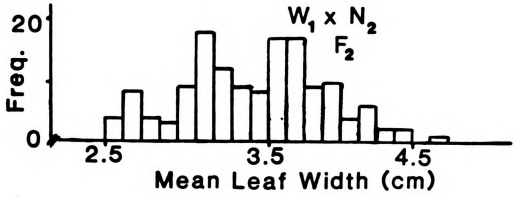
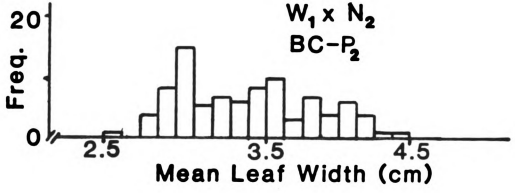

30
Freq.
0
2.5
3.5
4.5
Mean Leaf Width (cm)
W1 x N2
P1
30
Freq.
0
2.5
3.5
4.5
Mean Leaf Width (cm)
W1 x N2
P2
30
Freq.
0
2.5
3.5
4.5
Mean Leaf Width (cm)
W1 x N2
F1

Fig. 6. Frequency distributions of the mean leaf width in cm of the F_2, BC-P_2 and BC-P_1 generations for the cross $W_1 \times N_2$.


20
Freq.
0
W1 x N2
F2
2.5
3.5
4.5
Mean Leaf Width (cm)


20
Freq.
0
W1 x N2
BC-P2
2.5
3.5
4.5
Mean Leaf Width (cm)

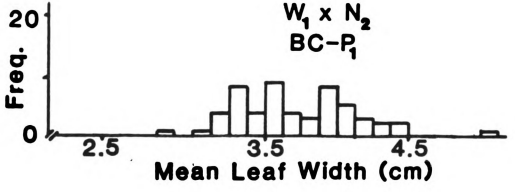
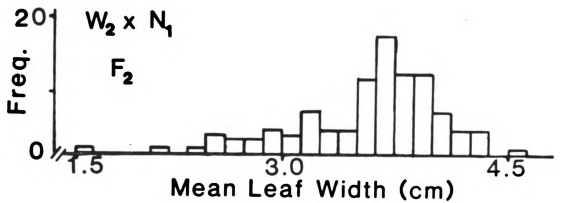
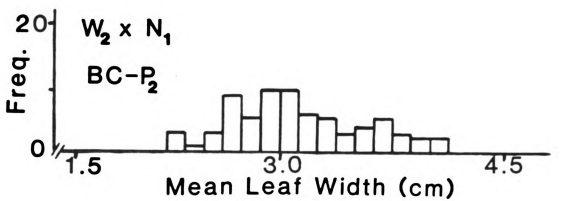

20
Freq.
0
W1 x N2
BC-P1
2.5
3.5
4.5
Mean Leaf Width (cm)

Fig. 7. Frequency distributions of the mean leaf width in cm of the P_1, P_2 and F_1 generations for the cross $W_2 \times N_1$.


20
Freq.
0
W2 x N1
F2
1.5
3.0
4.5
Mean Leaf Width (cm)


20
Freq.
0
W2 x N1
BC-P2
1.5
3.0
4.5
Mean Leaf Width (cm)

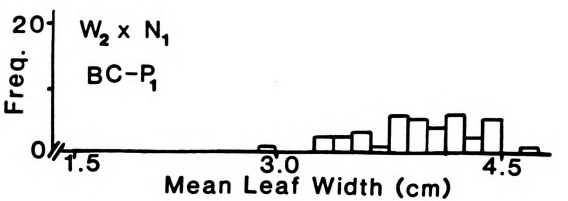
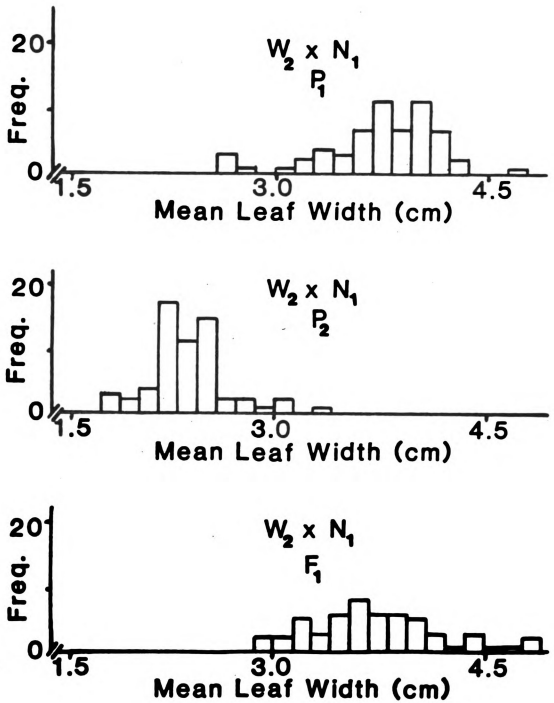

20
Freq.
0
W2 x N1
BC-P1
1.5
3.0
4.5
Mean Leaf Width (cm)

Fig. 8. Frequency distributions of the mean leaf width in cm of the F_2, BC-P_2 and BC-P_1 generations for the cross $W_2 \times N_1$.


20
Freq.
0
W2 x N1
P1
1.5
3.0
4.5
Mean Leaf Width (cm)
20
Freq.
0
W2 x N1
P2
1.5
3.0
4.5
Mean Leaf Width (cm)
20
Freq.
0
W2 x N1
F1
1.5
3.0
4.5
Mean Leaf Width (cm)

with more values into the wide range in the $W_1 \times N_2$ cross than in the $N_2 \times W_2$ cross. When measuring the individual pairs the 2 leaves in a pair were seldom the same. Environmental influence was also evidenced by within block variation. In comparing the 3 P_1s or the $3P_2$s or the 3 F_1s, each group assumed to be homozygous, there was considerable variation. The frequency distributions of the F_1s, F_2s and the backcrosses indicate that not all the effects are additive. The presence of slight transgressive segregation at the wide end of the F_2 curves and the exceeding of the mean of the wide leaf parent by the F_1 and BC-W generations indicates that not all genes involved are influencing the character additively. Effects due to dominance may be slightly greater than estimated simply due to the nature of this analysis. A generation means analysis has a tendency to cancel out positive and negative characters since it is based on mean calculations which resulted from summing across a number of loci. This becomes a problem when number 1 in the list of assumptions is violated. The different levels in vigor of the generations must also be considered here as possible influences on the analysis. This is especially true for cross $W_2 \times N_1$ in which there were 247 missing values, the greatest number being in the F_2 generation. However, this generation had the largest number sown per block; therefore, the frequency distribution is probably representative. The $W_2 \times N_1$ F_2 mean is also comparable to those of the other 2 crosses.

The fact that leaf width is largely controlled by additive gene effects is very important in a breeding program designed to

incorporate this trait. By direct phenotypic selection of the narrow leaf characteristic shape, pure lines can be attained. Selection for this character within male sterile lines should also be feasible. Such lines could lead to the production of narrow leaf F_1 hybrids. The male sterile narrow leaf lines could also be developed by backcrossing and selecting from within the F_2. Once production of narrow leaf F_1 seed is possible, this could be marketed for bedding plant use. The narrow leaf inbreds also perform well in the field so no changes need to be made in field seed production in present programs. Open pollinated production could also be set up in the field for the packet seed industry. Field production performance is important from 2 views; first, the breeder wants a plant which can be grown under field conditions as most of the seed production is outdoors in temperate climates and second the consumer wants a plant which will survive in the home garden.

Interestingly, the narrow leaf character has not been utilized previously. The ease of phenotypic selection due to the narrow leaf characteristic shape results in an easily acquired trait. Possibly the fact that the trait is controlled by additive effects and that the narrow leaf character is recessive by nature, may explain the lack of exploitation. In the industry, selection is geared toward easily recognizable and obtainable traits. The number of wide leaf types far outnumber the few narrow leaf types which may appear. Therefore, the germplasm containing the narrow leaf modifiers would be more difficult to initially obtain

and the option of developing narrow leaf lines would be limited. There is also the possibility that the narrow leaf character was eliminated due to the difference in leaf shape, although there would be no justifiable grounds for this beside breeders' preference. Narrow leaf zinnia plants perform well in the garden, as seen in previous summer trials, have a well-developed vigorous plant form and growth habit, flower continually throughout the summer and have exactly the same bloom sizes, types and colors of the wide leaf types. No surveys have been done on consumer preference, but there is no reason to believe that this form would be undesirable from the consumers' point fo view.

It is felt that with development of pure narrow leaf lines in both the fertile and the male sterile inflorescence types that very successful F_1 hybrids and open pollinated cultivars could be established as both bedding plants and packet seed items. Further research into narrow leaf lines with greater disease resistance is also desirable. Here plants which are physically and physiologically resistant to diseases could be developed, adding to their success potential.

APPENDIX

TABLE A-1. Observed and expected results in the F_2 generation for crosses with M_1.

Cross	Total	Observed		Expected		
		M	P	1:3	1:15	1:63
$M_1 \times P_1$						
1	85	16	70	21.5/64.5	5.4/80.6	1.30/84.7
2	35	2	33	8.8/26.3	2.2/32.8	.55/34.5
$M_1 \times P_2$						
1	62	4	58	15.5/46.5	3.9/58.1	.97/61.0
2	49	1	48	12.3/36.8	3.1/45.9	.77/48.2
3	70	1	69	17.5/52/5	4.4/65.6	1.10/68.9
4	69	8	61	17.3/51.8	4.3/64.7	1.10/67.9
5	51	4	47	12.8/38.3	3.2/47.8	.80/50.2
6	47	1	46	11.8/35.2	2.9/44.1	.70/46.3
7	48	4	44	12.0/36.0	3.0/45.0	.80/47.3
8	38	4	34	9.5/28.5	2.4/35.6	.60/37.4
9	85	5	80	21.3/61.8	5.3/79.7	1.30/83.7
10	42	7	35	10.5/31.5	2.6/39.4	.70/41.3
11	46	7	39	11.5/34.5	2.9/43.1	.70/45.3
12	17	2	15	4.3/12.8	1.1/15.9	.30/16.7
13	35	2	33	8.8/26.3	2.2/32.8	.50/34.5
$M_1 \times P_3$						
1	60	18	42	15.0/45.0	3.8/56.3	.9/59.1
2	52	11	41	13.0/39.0	3.3/48.8	.8/51.2
3	57	19	38	14.3/42.8	3.6/53.4	.9/56.1
4	52	12	40	13.0/3.9	3.3/48.8	.8/51.2
5	18	8	10	4.5/13.5	1.1/16.9	.3/17.7
6	50	11	39	12.5/37.5	3.1/46.9	.8/49.2

TABLE A-2. Observed and expected results for the BC-M_1 generation for backcrosses with M_1.

Cross	Total	Observed		Expected		
		M	P	1:1	1:3	1:7
$M_1 \times P_1$						
1	91	42	49	45.5/45.5	22.5/68.3	11.4/79.6
2	55	16	39	27.5/27.5	13.8/41.3	6.9/48.1
$M_1 \times P_2$						
1	61	20	41	30.5/30.5	15.3/45.8	7.6/53.4
2	49	15	34	24.5/24.5	12.3/36.8	6.3/42.9
3	67	22	45	33.5/33.5	16.8/50.3	8.4/58.6
4	71	25	46	35.5/35.5	17.8/53.3	8.9/62.1
5	47	13	34	23.5/23.5	11.8/35.3	5.9/41.1
6	56	18	38	28.0/28.0	14.0/42.0	7.0/49.0
7	42	11	31	21.0/21.0	10.5/31.5	5.3/36.8
8	53	16	37	26.5/26.5	13.3/39.8	6.6/46.4
9	21	6	15	10.5/10.5	5.3/15.8	2.6/18.4
10	24	10	14	12.0/12.0	6.0/18.0	3.0/21.0
$M_1 \times P_3$						
1	60	17	43	30.0/30.0	15.0/45.0	7.5/52.5
2	67	29	38	33.5/33.5	16.8/50.3	8.4/58.6
3	72	41	31	36.0/36.0	18.0/54.0	9.0/63.0
4	--	--	--	--	--	--
5	34	14	20	17.0/17.0	8.5/25.5	4.3/29.8
6	--	--	--	--	--	--

TABLE A-3. Observed and expected results in the F_2 generation for crosses with M_2.

Cross	Total	Observed		Expected		
		M	P	1:3	1:15	1:63
$M_2 \times P_1$						
1	15	0	15	3.8/11.3	.94/14.1	.23/14.8
2	13	0	13	3.3/ 9.8	.81/12.2	.20/12.8
$M_2 \times P_3$						
1	27	9	8	6.8/20.3	1.70/25.3	.42/26.6
2	45	11	34	11.3/33.8	2.80/42.9	.70/44.3
3	22	1	21	5.5/16.5	1.40/20.6	.34/21.7
4	34	11	23	8.5/25.5	2.12/31.9	.53/33.5
5	4	1	3	1.0/3.0	.25/ 3.8	.06/ 3.9

TABLE A-4. Observed and expected results in the BC-M_2 generation for backcrosses with M_2.

BC-M_2	Total	Observed		Expected		
		M	P	1:1	1:3	1:7
$M_2 \times P_1$						
1	9	2	7	4.5/ 4.5	2.3/ 6.8	1.10/ 7.9
$M_2 \times P_2$						
1	15	1	14	7.5/ 7.5	3.8/11.3	1.90/13.1
2	12	1	11	6.0/ 6.0	3.0/ 9.0	1.50/10.5
3	10	2	8	5.0/ 5.0	2.5/ 7.5	1.30/ 8.8
$M_2 \times P_3$						
1	15	4	11	7.5/ 7.5	3.8/11.3	1.90/13.1
2	30	14	16	15.0/15.0	7.5/22.5	3.80/26.3
3	29	3	26	14.5/14.5	7.3/21.8	.45/28.5
4	19	5	14	9.5/ 9.5	4.8/14.3	2.40/16.6

TABLE A-5. Observed and expected results in the F_2 generation for crosses with M_3.

Cross	Total	Observed		Expected		
		M	P	1:3	1:15	1:63
$M_3 \times P_2$						
1	36	9	27	9.0/27.0	2.30/33.8	.56/35.4
2	15	3	12	3.8/11.3	.94/14.1	.23/14.8
3	18	5	13	4.5/13.5	1.10/16.9	.28/17.7
$M_3 \times P_3$						
1	25	7	18	6.3/18.8	1.60/23.4	.39/24.6
2	37	5	32	19.3/27.8	2.30/34.7	.58/36.4

TABLE A-6. Observed and expected results in the BC-M_3 generation for backcrosses with M_3.

BC-M	Total	Observed		Expected		
		M	P	1:1	1:3	1:4
$M_3 \times P_3$						
1	5	3	2	2.5/2.5	1.3/3.8	.63/4.4
2	5	4	1	2.5/2.5	1.3/3.8	.63/4.4

TABLE A-7. Observed and expected results in the F_2 generation for crosses with M_4.

Cross	Total	Observed		Expected		
		M	P	1:3	1:15	1:63
$M_4 \times P_1$						
1	12	3	9	3.0/ 9.0	.75/11.3	.19/11.8
$M_4 \times P_2$						
1	33	3	30	8.3/24.8	2.10/30.9	.52/32.5
2	36	2	34	9.0/27.0	2.30/33.8	.56/35.4
3	32	2	30	8.0/24.0	2.00/30.0	.50/31.5
4	45	2	43	11.3/33.8	2.80/42.2	.70/44.3
5	36	2	34	9.0/27.0	2.30/33.8	.56/35.4
6	45	6	39	11.3/33.8	2.80/42.2	.70/44.3
$M_4 \times P_3$						
1	44	11	33	11.0/33.0	2.3/41.3	.69/43.3
2	43	6	37	10.6/32.3	2.7/40.3	.67/42.3
3	21	5	16	5.3/15.8	1.3/19.7	.33/20.7
4	21	5	16	5.3/15.8	1.3/19.7	.33/20.7
5	19	6	13	4.8/14.3	1.2/17.8	.30/18.7

TABLE A-8. Observed and expected results in the BC-M generation for backcrosses with M_4.

$BC\text{-}M_4$	Total	Observed		Expected		
		M	P	1:1	1:3	1:7
$M_4 \times P_3$						
2	19	9	10	9.5/9.5	4.8/14.3	2.8/16.6

TABLE A-9. Analysis of the variance for leaf width cross $N_2 \times W_2$.

Source of Variation		df	SS	MS	F Calculated
Blocks		19	4.94	.26	1.75 ns
Entries		31	124.44	4.01	27.37**
Among generations	5		114.02	22.80	155.45**
Within generations	26		10.42	.40	2.73**
Error		499	73.19	.15	
TOTAL		549[z]	202.52		

z: 90 missing plots

**: significant at the 1% level, ns: not significant

TABLE A-10. Analysis of the variance for leaf width cross $W_1 \times N_2$.

Sources of Variation		df	SS	MS	F Calculated
Blocks		19	9.96	1.87	3.55**
Entries		31	58.02	8.35	12.65**
Among generations	5		41.76	.63	56.46**
With generations	26		16.27	.15	4.23**
Error		420	62.11		
TOTAL		470[z]	130.10	.52	

z: 169 missing plots

**: significant at the 1% level.

TABLE A-11. Analysis of the variance for leaf width cross $N_1 \times W_2$.

Sources of Variation		df	SS	MS	F Calculated
Blocks		19	26.19	1.38	10.11**
Entries		31	109.33	3.53	25.90**
Among generations	5		98.85	19.77	145.05**
Within generations	26		10.48	.40	2.95**
Error		342	46.61	.14	
TOTAL		392[z]	182.12		

z: 247 missing plots

**: significant at the 1% level.

TABLE A-12. Variances* of the 6 generation means for the 3 crosses for leaf width analysis.

Cross	Generation					
	P_1	P_2	F_1	F_2	BC-P_1	BC-P_2
$N_2 \times W_2$	.049	.049	.049	.021	.021(N)	.037(W)
$W_1 \times N_2$	.049	.049	.049	.021	.037(W)	.021(N)
$W_2 \times N_2$	.045	.045	.045	.011	.019(N)	.034(W)

*: Variance calculated as MS error/replications, rounded up to 3 places.

TABLE A-13. Variance of the 6 gene effects and t-test for significance of gene effects in the 3 crosses for leaf shape analysis.

Gene Effect	Crosses					
	$N_2 \times W_2$		$W_1 \times N_2$		$W_2 \times N_1$	
	δ^2	t^z	δ^2	t	δ^2	t
m	.012[r]	33.005**	.012	31.036**	.011	33.691**
a	.058	-3.114**	.058	1.536 +	.053	3.174**
d	.499	1.337	.504	1.200	.464	-.185
aa	1.506	1.218	.403	.371	.396	-.487
ad	.082	.122	.083	-.127	.076	.442
dd	1.411	.199	1.423	.200	1.310	-.202

z: $t = n/\delta n$

r: all numbers rounded up to 3 places.

**, *, +: significant at the 1%, 5%, and 10% level respectively.

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