

Backcross Hybrids of *Zinnia angustifolia* and *Z. violacea*: Embryology, Morphology, and Fertility

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Abstract. True-breeding lines of *Zinnia marylandica* Spooner, Stimart and Boyle [allotetraploids of *Z. angustifolia* H.B.K. and *Z. violacea* Cav. ($2n = 46$)] were reciprocally backcrossed with diploid and autotetraploid forms of *Z. angustifolia* ($2n = 22$ or 44) and *Z. violacea* ($2n = 24$ or 48). In most cases, backcrosses were more successful with *Z. angustifolia* and *Z. violacea* as autotetraploids than as diploids. Seed-generated, backcross (BC_1) families were obtained by crossing *Z. marylandica* (as female) with autotetraploid *Z. angustifolia* or autotetraploid *Z. violacea*. BC_1 plants were phenotypically intermediate between the two parental lines for most morphological characters. Crosses between *Z. marylandica* and autotetraploid *Z. angustifolia* yielded BC_1 plants with 33% stainable pollen, whereas crosses between *Z. marylandica* and autotetraploid *Z. violacea* yielded BC_1 plants that produced malformed, poorly-stained pollen. No embryos were observed in capitula collected from field-grown BC_1 plants. BC_1 hybrids of *Z. marylandica* and autotetraploid *Z. violacea* produced larger capitula and more ray florets than *Z. marylandica*, and exhibited novel combinations of floral pigments not observed in *Z. marylandica* ray florets. BC_1 hybrids of *Z. marylandica* and *Z. violacea* have commercial potential as seed-propagated, bedding plants.

The common zinnia [*Zinnia violacea* (McVaugh, 1984); formerly *Z. elegans* Jacq. (Tomes, 1963)] is a popular bedding plant and cut flower that exhibits considerable phenotypic diversity for plant habit and ray floret color and morphology. Commercial cultivars of *Z. violacea*, however, are subject to attack by three major pathogens: *Alternaria zinniae* Pape (alternaria blight), *Erysiphe cichoracearum* DC. ex Merat (powdery mildew), and *Xanthomonas campestris* pv. *zinniae* Hopkins and Dowson (bacterial leaf and flower spot) (Andersen, 1971; Jones and Strider, 1979; Lipschutz, 1965; Tomes, 1963). These pathogens incite moderate to severe epiphytotic within *Z. violacea* plantings, resulting in plant losses and/or decreased ornamental value. The narrow-leaved zinnia [*Z. angustifolia* (McVaugh, 1984)] is highly resistant to these pathogens (Andersen, 1971; Jones and Strider, 1979; Lipschutz, 1965; Torres, 1963), and is a promising source of disease resistance for *Zinnia* breeding programs.

Crosses between *Z. angustifolia* ($2n = 2x = 22$) and *Z. violacea* ($2n = 2x = 24$) yield infertile allodiploids ($2n = 2x = 23$) (Boyle and Stimart, 1982; Terry-Lewandowski et al., 1984). Colchicine-induced allotetraploids ($2n = 4x = 46$) exhibit preferential pairing of homologous chromosomes and infrequent pairing of homoeologous chromosomes, and, as a consequence, are partially fertile and breed true from seed (Terry-Lewandowski et al., 1984). Allotetraploids of *Z. angustifolia* and *Z. violacea*, collectively known as *Z. marylandica* (Spooner et al., 1991), exhibit high levels of resistance to *A. zinniae* and *E. cichoracearum* and moderate to high levels of resistance to *X. campestris* pv. *Zinniae* (Terry-Lewandowski and Stimart, 1983).

To obtain the expression of new characters, it would be desirable to backcross *Z. marylandica* to the parental species. The objectives of this study were to determine the pathways of backcrossing *Z. marylandica* to *Z. angustifolia* and *Z. violacea*, and to examine the morphology and fertility of the BC_1 plants and uniformity within BC_1 families.

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Materials and Methods

Plant material. Table 1 lists the accessions of *Z. angustifolia*, *Z. violacea*, and *Z. marylandica* that were used for study. Autotetraploid clones of *Z. angustifolia* 'Linearis White' were obtained by treating lateral buds with colchicine (Boyle and Stimart, 1982); the clones were propagated asexually and intercrossed to create line A88C. Two plants of *Z. violacea* 'State Fair' ($2n = 4x = 48$) were crossed reciprocally and their progeny sib-crossed for two generations; the resulting line (V86C) was true-breeding for yellow ray florets and exhibited more phenotypic uniformity than the original cultivar. *Zinnia marylandica* line M1C was produced by crossing *Z. angustifolia* 'Linearis White' (as female) with *Z. violacea* 'Thumbelina Mini-Pink'; line M2C was produced by crossing *Z. angustifolia* 'Linearis White' (as female) with *Z. violacea* 'Crimson Monarch'. Procedures used for interspecific hybridization were described previously (Boyle and Stimart, 1982). One interspecific hybrid seedling in each F_1 population was clonally propagated by rooting terminal shoot cuttings. Fertile allotetraploid shoots were recovered after treating lateral buds with colchicine (Boyle and Stimart, 1982). Seeds were harvested from selfed florets, and were bulked. Lines M1C and M2C bred true-to-type in subsequent

Table 1. *Zinnia* accessions used as parental material, and their chromosome numbers, genomic formulas, and sources.

Taxon	Cultivar or line	Chromosome		Source ²
		no. ($2n$)	Genomic formula ¹	
<i>Z. angustifolia</i>	'Linearis White'	22	AA	SI
	A88C	44	AAAA	MA
<i>Z. violacea</i>	'Crimson Monarch'	24	VV	BO
	'Orange King'	24	VV	BO
	'Thumbelina Mini-Pink'	24	VV	BO
	V86C	48	VVVV	MA
<i>Z. marylandica</i>	M1C	46	AAVV	MA
	M2C	46	AAVV	MA

¹A = *Z. angustifolia* ($n = 11$); V = *Z. violacea* ($n = 12$).

²BO = Bodger Seeds, Ltd., Lompoc, Calif.; MA = Univ. of Mass, Amherst; SI = Sutton and Sons (India) Pvt. Ltd., Calcutta, India.

generations and were maintained by selfing. Henceforth, the parental material will be referred to by their genomic formulas instead of their chromosome numbers (Table 1).

Growing conditions. Plants were grown in glasshouses (Expts. 1, 4, and 5) or in an outdoor plot adjacent to the glasshouse range (Expts. 2 and 3). Temperature setpoints were 18/22C (heat/vent) during glasshouse experiments; actual glasshouse temperatures ranged from 16C minimum to maximum of about 40C for short durations during the summer. Plants were grown under natural daylengths (NDs) when the natural photoperiod was >14 h, and were otherwise grown under artificial long days (LDs) provided by supplementing ND with about 3 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-2}$ incandescent irradiation from 1600 to 2200 HR. Plants were fertilized at each irrigation with 20N4.3P-16.6K at 200 mg N/liter.

BC₁ embryo development (Expt. 1). Line M 1 C was crossed reciprocally with 'Linearis White', A88C, 'Orange King', and V86C. Emasculation and pollination procedures were reported previously (Boyle and Stimart, 1982). Ovaries were removed from florets at 14 days after pollination and were dissected under a stereomicroscope ($\times 32$). Presence or absence of an embryo, and if present, the state of embryo development (normal or abnormal) were recorded for each floret. Embryos were classified as abnormal if aborted or rudimentary (abnormally small). Embryos that were desiccated or necrotic were defined as aborted. The procedures described by Boyle et al. (1987) were used for identifying rudimentary embryos.

Morphology of BC₁ plants (Expt. 2). Four-week-old seedlings of A88C, V86C, M1C, and two BC₁ families (female M1C \times male V86C and female M 1 C \times male A88C) were planted in an outdoor plot on 2 July 1988. Entries were arranged in a randomized complete block design with two blocks and one plot per block. Each plot consisted of 20 seedlings (four rows with five seedlings per row), with plants spaced on 30-cm centers. Data were collected on plant height (from the soil surface to upper surface of the primary capitulum), leaf area (mean of two leaves collected from the second node below the primary capitulum), petal area of ray florets (mean of four fully expanded petals on the primary capitulum), number of ray florets (primary capitulum), and diameter of the primary capitulum. Leaf and ray petal areas were measured on a leaf area meter (LI-3000A; LI-COR, Lincoln, Neb.) Data were recorded on 2 and 4 Sept. 1988. To minimize border effects, data were collected on six plants in the inner two rows of each plot. One variable (number of ray florets per capitulum) was transformed [$\log_{10}(x + 1)$] due to variance heterogeneity. Means and standard deviations were calculated for each variable. Data were analyzed by the analysis of variance (ANOVA) procedure (SAS Inst., Cary, N. C.)

Fertility of BC₁ plants (Expt. 3). Flowering plants of A88C, V86C, M1C, and the two BC₁ families were harvested from the border rows of the field plot described in Expt. 2. Flowering plants of 'Orange King' and 'Linearis White' were harvested from an adjacent plot. Stems with mature capitula were trimmed to 15 cm, placed in water-filled containers, and brought to the laboratory. Pollen samples were obtained the following day. For each entry, pollen was collected from a minimum of six capitula, and then bulked to produce a composite pollen sample. Pollen viability was estimated by staining in 0.05% (w/v) aniline blue in lactophenol (Arlington and La Cour, 1942). Intensely stained grains were scored as viable, whereas unstained or lightly stained grains were scored as inviable.

Senesced capitula were harvested from plants of the two BC₁ families and line M1C that were growing in the field plot described earlier. Florets were removed from capitula and the ovaries were

dissected under a stereomicroscope ($\times 32$) to determine if embryos were present. About 50 capitula were examined for each entry.

Flower color of BC₁ plants (Expt. 4). Ray floret color for *Z. angustifolia* (Linearis White' and A88C), *Z. violacea* ('Crimson Monarch', 'Orange King', 'Thumbelina Mini-Pink', and V86C), *Z. marylandica* (M1C and M2C), and three BC₁ families was characterized qualitatively with the *R.H.S. Colour Chart* (Royal Horticultural Society, 1986). Fully expanded petals from glasshouse-grown plants were used to obtain single or interpolated values best describing color phenotype. Nominal descriptions of ray floret color were obtained from Kelly and Judd's (1976) dictionary of color names. Presence or absence of vacuolar pigments and carotenoid-containing chromoplasts was determined using visible light microscopy (Boyle and Stimart, 1989). A minimum of 10 petals were examined for each entry.

BC₁ seed emergence and within-family uniformity (Expt. 5). BC₁ seed was produced by crossing lines M1C and M2C (as maternal parents) with V86C. At harvest, shrivelled and light-weight seeds were retained in the seed lots to obtain unbiased estimates of seedling emergence. Seeds (480 per BC₁ family) were sown in a peat-based germination medium that was maintained at 23C by bottom heat. The number of emerged seedlings was recorded at 14 days after sowing, and percent seed emergence was calculated for each BC₁ family.

Five-week-old BC₁ seedlings were transplanted into a 1.1 \times 14.0 m glasshouse ground bed on 12 Jan. 1987. The two BC₁ families were arranged in a randomized complete block design, with two blocks and one plot per block. Each plot consisted of 105 plants (15 rows with seven plants per row), and plants were spaced on 18-cm centers. Data were collected on 65 plants in the inner 13 rows of each plot. Plant height, leaf length, and width (maximum dimensions of one leaf collected from the second node below the primary capitulum), diameter of the primary capitulum, ray petal length and width (mean of two fully expanded petals on the primary capitulum), number of ray florets (primary capitulum), and days to flowering from seed sowing were recorded. The mean and coefficient of variation were calculated for each variable. A two-tailed F test was used to test for equal variances for the two BC₁ families.

Results and Discussion

Experiment 1. Generally, backcrosses with *Z. marylandica* were more successful with *Z. angustifolia* and *Z. violacea* as autotetraploids than as diploids (Table 2). For crosses between *Z. angustifolia* (AA) and *Z. marylandica*, the percentage of florets with embryos was significantly greater with *Z. angustifolia* as the maternal parent as compared to the reciprocal cross. However, most embryos from crosses between *Z. angustifolia* (AA) and *Z. marylandica* were developmentally abnormal, regardless of crossing direction. Crosses between *Z. angustifolia* (AAAA) and *Z. marylandica* yielded similar results in both directions, with 64%–65% of pollinated florets containing embryos and 9%–10% abnormal embryos. Reciprocal crosses between *Z. violacea* (VV) and *Z. marylandica* yielded few embryos, regardless of crossing direction, and 22% of the embryos exhibited abnormalities. When *Z. violacea* (VVVV) was backcrossed with *Z. marylandica*, embryo yields were more than 8-fold higher and the percentage of abnormal embryos was lower with *Z. marylandica* as maternal parent rather than as pollen parent.

Bernström (1953) reported that few BC₁ progeny were obtained when allotetraploid *Lamium intermedium* (= *L. hybridum* Vill.) was crossed reciprocally with its diploid ancestors, i.e., *L.*

Table 2. Percent florets with embryos and percent abnormal embryos for backcrosses of allotetraploid *Zinnia marylandica* (AAVV) with diploid and autotetraploid forms of *Z. angustifolia* and *Z. violacea*.

Cross (female x male)	Florets pollinated (no.)	Florets with embryos (%)	Abnormal embryos (%) ²
<i>Z. marylandica</i> x <i>Z. angustifolia</i> (AA) ¹	300	3	100
<i>Z. angustifolia</i> (AA) x <i>Z. marylandica</i>	302	28	78
Reciprocal differences		***	NS
<i>Z. marylandica</i> x <i>Z. angustifolia</i> (AAAA)	308	65	9
<i>Z. angustifolia</i> (AAAA) x <i>Z. marylandica</i>	304	64	10
Reciprocal differences		NS	NS
<i>Z. marylandica</i> x <i>Z. violacea</i> (VV)	288	0	---
<i>Z. violacea</i> (VV) x <i>Z. marylandica</i>	258	7	22
Reciprocal differences		***	---
<i>Z. marylandica</i> x <i>Z. violacea</i> (VVVV)	233	69	1
<i>Z. violacea</i> (VVVV) x <i>Z. marylandica</i>	268	8	45
Reciprocal differences		***	***

¹Number of aborted or rudimentary embryos/total number of embryos (x100).

²A = *Z. angustifolia* (n = 11); V = *Z. violacea* (n = 12).

NS,*** Nonsignificant or significant at P < 0.001, respectively, by heterogeneity χ^2 test.

Table 3. Expected chromosome numbers for embryos and endosperm obtained from backcrossing allotetraploid *Zinnia marylandica* (AAVV) with diploid and autotetraploid forms of the parental species *Z. angustifolia* and *Z. violacea*.

Cross (female x male)	Chromosome no.	
	Embryo (2n)	Endosperm (3n)
<i>Z. marylandica</i> x <i>Z. angustifolia</i> (AA) ¹	34	57
<i>Z. angustifolia</i> (AA) x <i>Z. marylandica</i>	34	45
<i>Z. marylandica</i> x <i>Z. angustifolia</i> (AAAA)	45	68
<i>Z. angustifolia</i> (AAAA) x <i>Z. marylandica</i>	45	67
<i>Z. marylandica</i> x <i>Z. violacea</i> (VV)	35	58
<i>Z. violacea</i> (VV) x <i>Z. marylandica</i>	35	47
<i>Z. marylandica</i> x <i>Z. violacea</i> (VVVV)	47	70
<i>Z. violacea</i> (VVVV) x <i>Z. marylandica</i>	47	71

¹A = *Z. angustifolia* (n = 11); V = *Z. violacea* (n = 12).

amplexicaule L. and *L. purpureum* L. In contrast, numerous BC₁ hybrids were obtained when autotetraploid *L. amplexicaule* was crossed reciprocally with *L. intermedium*, and when autotetraploid *L. purpureum* was used as a pollen parent in crosses with *L. intermedium*. Similar results were obtained in the current experiments: backcrosses with allotetraploid *Z. marylandica* were more successful when the parental species were autotetraploids rather than diploids (Table 2). Increasing the ploidy level of the parental material appears to be an effective technique for circumventing barriers in backcrosses with allopolyploids.

Zinnia produces a monosporic, eight-nucleate embryo sac of the *Polygonum* type (Maheswari Devi, 1963; Pullaiah, 1981). Double fertilization in *Polygonum*-type embryo sacs yields endosperm nuclei with two sets of maternal chromosomes and one set of paternal chromosomes, and zygotic nuclei with one set of maternal chromosomes and one set of paternal chromosomes. Since *Z. marylandica* and the parental species differ in chromosome number, reciprocal crosses would thus result in zygotes with identical chromosome numbers and endosperms with different chromosome numbers (Table 3). As a consequence, reciprocal crosses will exhibit differences in the ratios of chromosome

numbers in the embryo, endosperm, and maternal tissue, and in gene dosage (Hadley and Openshaw, 1980; Stebbins, 1958). Reciprocal differences in the percentage of florets with embryos and the percentage of abnormal embryos may have resulted from differences in gene dosage and/or chromosome numbers (Table 2).

When attempting to cross two species differing in chromosome number, it is often recommended to utilize the species with the greater number of chromosomes as the maternal parent (Hadley and Openshaw, 1980; Stebbins, 1958). For three pairs of *Zinnia* backcrosses, however, higher yields of embryos were obtained when the species with fewer chromosomes was used as the maternal parent, and, for the fourth pair [*Z. marylandica* x *Z. angustifolia* (AAAA)], there was no reciprocal difference in the percentage of florets with embryos (Table 2). These results demonstrate the importance of performing reciprocal crosses to maximize the probability of success in interspecific hybridization programs.

Experiment 2. Crosses between *Z. marylandica* MIC and *Z. angustifolia* A88C yielded BC₁ plants that were intermediate between the two parents in capitulum diameter, resembled A88C in ray petal area, resembled MIC in number of ray florets, and exceeded both parents with respect to plant height and leaf area (Table 4). BC₁ progeny of MIC and *Z. violacea* V86C were intermediate between the two parents for all morphological characters that were examined. The coefficients of variation for the BC₁ families were similar to the parental lines for most of the morphological characters.

Zinnia capitula that consist primarily or entirely of ray florets (double flowers) have the greatest ornamental value, whereas capitula with ray florets in a single whorl (single flowers) or a few whorls (semi-double flowers) have less ornamental value. Most modern-day cultivars of *Z. violacea* yield a high percentage of plants with double- and semi-double flowers. Cultivars of *Z. angustifolia*, however, are single-flowered. Boyle and Stimart (1982) reported that *Z. angustifolia* x *Z. violacea* allodiploids (AV) resembled the *Z. angustifolia* parent (AA) with respect to numbers of ray florets, even when the *Z. violacea* parent (VV) produced double flowers (> 100 ray florets per capitulum). In the current study, BC₁ progeny of *Z. marylandica* and autotetraploid *Z. violacea* produced significantly more ray florets than *Z. marylandica*, but only half as many ray florets as autotetraploid *Z.*

Table 4. Morphological characters of *Zinnia angustifolia* (Z.a.) A88C, *Z. marylandica* (Z.m.) M1C, *Z. violacea* (Z.v.) V86C, and two BC₁ families.

Accession	Genomic formula ²	Plant ht (cm)		Leaf area (cm ²)		Ray petal area (cm ²)		Ray petals (no.)		Capitulum diam (cm)	
		Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
<i>Z. angustifolia</i> A88C	AAAA	36.1 e ¹	17.6	8.6 e	12.0	1.6 d	11.0	10.1 d	14.3	4.4 e	8.6
BC ₁ : <i>Z.m.</i> M1C x <i>Z.a.</i> A88C	AAAV	52.0 c	6.4	22.0 c	16.0	1.8 d	14.4	13.0 c	8.0	4.9 d	6.4
<i>Z. marylandica</i> M1C	AAVV	43.7 d	15.5	15.7 d	10.5	2.6 c	5.4	12.4 c	7.3	6.0 c	3.6
BC ₁ : <i>Z.m.</i> M1C x <i>Z.v.</i> V86C	AVVV	73.7 b	4.1	53.1 b	14.0	3.4 b	28.1	16.4 b	13.9	7.8 b	9.9
<i>Z. violacea</i> V86C	VVVV	77.9 a	5.4	81.1 a	12.9	5.1 a	11.2	32.2 a	46.1	11.1 a	4.7

¹A = *Z. angustifolia* (n = 11); V = *Z. violacea* (n = 12). Genomic formulas for the two BC₁ families are expected values and not based on actual chromosome counts.

²Mean separation within columns by Duncan's multiple range test, P = 0.05.

Table 5. Stainability of pollen for *Zinnia angustifolia* (Z.a.) 'Linearis White' and A88C, *Z. violacea* (Z.v.) 'Orange King' and V86C, allotetraploid *Z. marylandica* (Z.m.) line M2C, and two BC₁ families.

Accession	Genomic formula ²	Pollen grains examined	Stainable pollen (%) ¹
		(no.)	
<i>Z.a.</i> 'Linearis White'	AA	1122	87.0
<i>Z.a.</i> A88C	AAAA	973	63.6
BC ₁ : <i>Z.m.</i> M1C x <i>Z.a.</i> A88C	AAAV	1329	33.5
<i>Z.m.</i> M1C	AAVV	912	53.1
BC ₁ : <i>Z.m.</i> M1C x <i>Z.v.</i> V86C	AVVV	988	0
<i>Z.v.</i> 'Orange King'	VV	1481	95.3
<i>Z.v.</i> V86C	VVVV	1036	27.2

¹A = *Z. angustifolia* (n = 11); V = *Z. violacea* (n = 12). Genomic formulas for the two BC₁ families are expected values and not based on actual chromosome counts.

²Y, Stainability evaluated using aniline blue in lactophenol.

violacea (Table 4); these BC₁ plants were generated using *Z. violacea* cultivars with either semi-double (V86C) or double (Thumbelina Mini-Pink' and 'Crimson Giant') flowers, and a *Z. angustifolia* cultivar ('Linearis White') with single flowers. These results suggest that the *Z. angustifolia* genome exerts a dominant effect on the floral morphology of *Z. marylandica*, limiting the number of ray florets in capitula of F₁ and BC₁ hybrids.

Experiment 3. Pollen stainability varied substantially among the seven entries (Table 5). *Zinnia marylandica* M1C yielded about 50% stainable pollen. BC₁ progeny of M1C and *Z. violacea* V86C produced malformed pollen grains that were either unstained or lightly stained, whereas BC₁ progeny of M1C and *Z. angustifolia* A88C yielded about 33% darkly-stained pollen grains. Autotetraploid plants of *Z. violacea* (V86C) and *Z. angustifolia* (A88C) produced fewer stainable pollen grains than their diploid counterparts.

Field-grown plants of M1C yielded 30 to 75 plump seeds per capitulum. No embryos, however, were observed in capitula collected from any field-grown BC₁ plants.

Bumblebees (*Bombus* spp.) and honeybees (*Apis mellifera*) were frequently observed on and near *Zinnia* capitula of field-grown plants. These insects spent less time on flowers of the BC₁ plants compared to flowers of *Z. angustifolia*, *Z. marylandica*, and *Z. violacea*. Social bees such as bumblebees and honeybees search for flowers that yield the greatest quantities of pollen and nectar (Richards, 1986; Widrechner and Senechal, 1992). Brief visitation times on BC₁ plants may be due to insufficient pollen and/or nectar, which may have been partially responsible for lack of seed production by field-grown BC₁ plants.

Experiment 4. Ray florets of *Z. angustifolia* 'Linearis White' and A88C were devoid of chromoplasts and vacuolar pigmentation (Table 6). Pigmentation of *Z. violacea* ray florets consisted of chromoplasts in the cytoplasm, pink to red flavonoids in the vacuoles, or both. Vacuolar pigmentation was present and chromoplasts were absent in ray florets of *Z. marylandica* lines M1C and M2C, and BC₁ hybrids obtained from crossing *Z. marylandica* M1C with either *Z. angustifolia* A88C or *Z. violacea* V86C. BC₁ progeny obtained from crossing *Z. marylandica* M2C with *Z. violacea* V86C produced both vacuolar pigmentation and chromoplasts in their ray florets, yielding a distinctive petal color (deep yellowish pink) not found in other BC₁ hybrids.

Diversity of ray floret color in *Z. violacea* is due primarily to variation in the amount of carotenoids and flavonoids that accumulate in epidermal cells (Boyle and Stimart, 1989). Carotenoids are necessary to achieve vivid red ('Crimson Monarch'), strong orange (Orange King'), and brilliant yellow (V86C) floral colors in *Z. violacea* (Table 6). Carotenoids are not present in ray florets of *Z. angustifolia*, nor are they found in allodiploid (AV) or allotetraploid (AAVV) hybrids of *Z. angustifolia* and *Z. violacea* (Table 6; Boyle and Stimart, 1989), thus limiting the range of floral colors that are possible within these taxa. BC₁ progeny of *Z. marylandica* and *Z. violacea* V86C exhibited differences in floral pigmentation, with carotenoids absent in BC₁ plants derived from *Z. marylandica* M1C but present in BC₁ plants derived from *Z. marylandica* M2C (Table 6). These differences are likely due to gene dosage effects. Presence of carotenoids in *Z. violacea* ray florets is controlled by a recessive gene (*ca*) (Boyle and Stimart, 1988). BC₁ progeny of *Z. marylandica* M2C and *Z. violacea* V86C contained three *Z. violacea* genomes (AVVV), all of which coded for presence of ray floret carotenoids (*ca*); two genomes were from V86C (*ca ca*) and one genome came from 'Crimson Monarch' (*ca ca*) via *Z. marylandica* (*ca ca*). BC₁ progeny of *Z. marylandica* M1C and *Z. violacea* V86C, however, contained only two *Z. violacea* genomes coding for presence of ray floret carotenoids, both of which came from V86C (*ca ca*); the third *Z. violacea* genome came from 'Thumbelina Mini-Pink' (*Ca*) via *Z. marylandica* (*Ca ca*). Presence of carotenoids in BC₁ hybrids of *Z. marylandica* and *Z. violacea* will likely extend the range of ray floret color beyond that observed within lines of *Z. marylandica*.

Experiment 5. Percent seed emergence was 49% for the cross M1C × V86C, but was significantly higher (57%) for the cross M2C × V86C (P < 0.05). BC₁ plants of M1C × V86C were less variable in plant height, leaf dimensions, capitulum diameter, and ray petal length compared to BC₁ plants of M2C × V86C (Table 7). The two BC₁ families displayed similar variances for ray petal width, ray petal number, and days to flowering.

Relatively uniform BC₁ families were generated in this study

Table 6. Color and pigment location in ray floret petals of *Zinnia angustifolia* (Z.a.), *Z. violacea* (Z.v.), *Z. marylandica* (Z.m.), and BC₁ interspecific hybrids.

Accession	Genomic formula ^z	Ray floret color ^y	RHS no. ^x	Pigment location	
				Plastid ^w	Vacuole ^v
Z.a. 'Linearis White'	AA	White	155A	-	-
Z.a. A88C	AAAA	White	155A	-	-
Z.v. 'Crimson Monarch'	VV	Vivid red	45A	+	+++
Z.v. 'Orange King'	VV	Strong orange	28B	+	++
Z.v. 'Thumbelina Mini-Pink'	VV	Strong purplish pink	61D	-	++
Z.v. V86C	VVVV	Brilliant yellow	13A	+	-
Z.m. M1C	AAVV	Vivid purplish red	58B	-	++
Z.m. M2C	AAVV	Moderate purplish red	52A	-	++
BC ₁ : Z.m. M1C x Z.a. A88C	AAAV	Pale pink	62D	-	+
BC ₁ : Z.m. M1C x Z.v. V86C	AVVV	Deep purplish pink	62A	-	++
BC ₁ : Z.m. M2C x Z.v. V86C	AVVV	Deep yellowish pink	43C	+	++

^zA = *Z. angustifolia* (n = 11); V = *Z. violacea* (n = 12). Genomic formulas for the BC₁ families are expected values and not based on actual chromosome counts.

^yColor names from Kelly and Judd's (1976) color standards.

^xRoyal Horticultural Society (RHS) Colour Chart numbers based on comparisons with fully expanded petals.

^wPresence (+) or absence (-) of chromoplasts in upper epidermal cells.

^vIntense (+++), moderate (++) or weak (+) pigmentation in upper epidermal cells, or pigmentation absent (-).

(Tables 4 and 7), and were attainable due to the true-breeding, allotetraploid constitution of *Z. marylandica* (Terry-Lewandowski et al., 1984). Greater phenotypic uniformity maybe possible by utilizing elite, highly uniform inbred lines of *Z. angustifolia* and *Z. violacea* for BC₁ seed production. An effective method for preventing selfing in the maternal parent is also necessary for producing uniform BC₁ families. In the current experiments, selfing was prevented by emasculating capitula of *Z. marylandica* before crossing (Boyle and Stimart, 1982). This procedure is laborious and not practical for producing large quantities of BC₁ seed. Commercial firms produce F₁ hybrid seed of *Z. violacea* by using apetalous, male-sterile (femina) plants as maternal parents (Reimann-Philipp, 1983). Attempts to transfer the femina trait from *Z. violacea* to *Z. marylandica* have been unsuccessful, probably due to recessiveness of this character in the presence of the *Z. angustifolia* genome (T.H. Boyle, unpublished data). An alternative method for producing BC₁ seed would be to use clonally propagated, self-incompatible plants as maternal parents, as has been done for producing F₁ seed in several other Composite species (Reimann-Philipp, 1983). Sporophytic self-incompatibility (SI) is present in *Z. angustifolia* and *Z. violacea* (Boyle and Stimart, 1986; Samaha and Boyle, 1989), and is expressed in some *Z. marylandica* clones (T.H. Boyle, unpublished data).

These experiments demonstrate that seed-generated BC₁ prog-

eny can be produced from crossing *Z. marylandica* to autotetraploid forms of *Z. angustifolia* and *Z. violacea*. BC₁ plants from crosses between autotetraploid *Z. angustifolia* and *Z. marylandica* yielded smaller, less showy capitula than those of *Z. marylandica* (Table 4). In contrast, BC₁ plants obtained from crossing *Z. marylandica* with autotetraploid *Z. violacea* were highly ornamental, with larger capitula, more ray florets, and a greater diversity of floral pigments than *Z. marylandica* (Tables 4 and 6). These plants may have commercial potential not only for their ornamental value, but also for their infertility, therefore providing a high degree of proprietary protection for seed producers. Further exploitation of *Z. marylandica* germplasm is likely to yield additional material with ornamental value.

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Table 7. Influence of *Z. marylandica* genotype on morphology, flowering, and uniformity of two BC₁ families.

Statistic	Plant	Ray florets						
		Leaves		Petal		Capitulum		
		ht (cm)	Length (cm)	Width (cm)	length (cm)	width (cm)	No.	diam (cm)
<i>BC₁: Z. marylandica M1C x Z. violacea V86C</i>								
Mean	65.4	11.4	8.5	2.9	1.9	17.9	7.4	96.3
SD	7.0	0.9	1.0	0.3	0.2	2.1	0.8	4.1
<i>BC₁: Z. marylandica M2C x Z. violacea V86C</i>								
Mean	73.7	13.7	9.1	3.7	1.9	17.7	8.9	104.8
SD	9.8	1.3	1.3	0.4	0.3	1.9	1.1	4.3
F test ^z	***	***	**	**	NS	NS	**	NS

^zTwo-tailed F test for equal variances.

^{ns}, **, ***Nonsignificant or significant at $P < 0.01$ or 0.001 , respectively.

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