Multiple Resistance in Induced Amphiploids of *Zinnia elegans* and *Z. angustifolia* to Three Major Pathogens

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ABSTRACT

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Inoculations with *Erysiphe cichoracearum, Alternaria zinniae*, and *Xanthomonas campestris* pv. *zinniae* were performed on colchicine-induced amphiploids of *Zinnia elegans* and *Z. angustifolia* to determine whether the newly derived germ plasm was resistant to three major pathogens of the popular garden ornamental *Z. elegans*. Experimental results demonstrated that the amphiploids possess high levels of resistance to *E. cichoracearum* (causal agent of powdery mildew) and *A. zinniae* (causal agent of Alternaria blight) and moderate to high levels of resistance to *X. campestris* pv. *zinniae* (causal agent of bacterial leaf and flower spot). These amphiploids will be valuable intermediaries in the transfer of multiple genes for disease resistance from *Z. angustifolia* to *Z. elegans*.

Zinnia elegans Jacq. (2n = 24) (11,12) is a widely cultivated garden ornamental prized for its great diversity in plant form and flower color. A recent decline in popularity has been reported, presumably because of recurring epiphytotics of powdery mildew caused by *Erysiphe cichoracearum* DC. ex Merat (L. Drewlow, Bodger Seeds, Ltd., Lompoc, CA, *personal communication*). Two additional diseases that have threatened commercial seed production are Alternaria blight caused by *Alternaria zinniae* Pape (4) and bacterial leaf and flower spot caused by *Xanthomonas campestris* pv. *zinniae* Hopkins & Dowson (syn. *X. nigromaculans* f. sp. *zinniae* Hopkins & Dowson) (10).

Resistance to the three pathogens (4,10,12) has been identified in a less frequently grown species, *Z. angustifolia* HBK (2n = 22) (6,11). Interspecific hybrids between these species may serve as intermediaries in the transfer of genes for disease resistance from *Z. angustifolia* to *Z. elegans*. Boyle and Stimart (1) successfully recovered a limited number of hybrids derived from reciprocal matings and restored partial fertility in the F_1 by treatment with colchicine. Based on a preliminary screening test, they reported that *Z. angustifolia* and all F_1 hybrids appeared resistant to infection by *E. cichoracearum*. Concurrent cytological investigations in our laboratory have demonstrated that the colchicine-induced amphiploids (2n = 46) formed predominantly bivalents at metaphase I as a result of preferential pairing of homologous chromosomes. Consequently, they bred true to their intermediate condition, with little or no segregation in subsequent generations (11). This study was initiated to screen derived amphiploids for resistance or tolerance to the three major pathogens of *Z. elegans*: *E. cichoracearum, A. zinniae,* and *X. campestris* pv. *zinniae*.

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MATERIALS AND METHODS

Two sterile F_1 hybrids of species reciprocal parentage were selected to provide advanced generations for plant disease studies (1). Fertility was restored in hybrids of *Z. angustifolia* × *Z. elegans* 'Cherry Ruffles' (F_1-CR) and *Z. elegans* 'Whirligig' × *Z. angustifolia* (F_1-W) by treating axillary buds with 0.1% aqueous colchicine plus Dupont Spreader-Sticker (one drop per 20 ml) for 5 days after shoot-tip removal. Fertile shoots with stainable pollen and seed set were designated as the C_0 generation. Subsequent generations of amphiploids (C_1, C_2, and C_3) were advanced by self-pollination and designated as described previously, ie, C_2-CR, and C_2-W, respectively. Seedlings were transplanted to 10-cm plastic pots in a steam-treated medium of equal parts soil:sand:sphagnum peat when the first pair of true leaves had fully expanded.

All parents, F_1 hybrids, and colchicine-induced amphiploids were maintained in the greenhouse under a 14-hr-day continuation regime supplemented by incandescent light (4 $\mu E/m^2/sec$) and at temperatures ranging from 27 to 18 C (day/night). Plants were fertilized weekly with 20-8.7-16.6 of NPK at 236 ppm nitrogen (N). With the exception of one outdoor study in the summer of 1982, all experiments were performed in the greenhouse during November through March of 1981 and 1982.

Two generations of each amphiploid (C_1-CR, C_2-CR, C_1-W, and C_2-W) were screened in a completely randomized experimental design for resistance to *E. cichoracearum* using artificial inoculum. Included were 780 plants each of C_1-CR and C_1-W. C_2-CR and C_2-W were each represented by six families with 48 individuals per family. The pathogen was maintained on infected *Z. elegans* plants growing in the inoculation house. Inoculations were performed by shaking heavily infected plants above 11-wk-old test plants at 3-day intervals for 9 days. Leaves and ray florets were scored 2 wk after the first inoculations as either resistant or susceptible.

A field plot was established to evaluate C_2 and C_3 generations for resistance to *E. cichoracearum*. Parents and induced-amphiploids were planted in a randomized complete block design. Each subplot consisted of 24 parents (*Z. angustifolia* and either *Z. elegans* 'Whirligig' or 'Cherry Ruffles') and 40 amphiploids (C_2-CR, C_2-W, C_3-CR, or C_3-W) replicated over two blocks. Plants were allowed to become naturally inoculated with airborne conidia from nearby infected plants. Evaluations were conducted in early November 1982, when the susceptible *Z. elegans* parents had become severely diseased. Leaves and ray florets were rated as either resistant or susceptible.

Amphiploids were screened for resistance to *A. zinniae* after artificial inoculation in the greenhouse. The two parental *Z. elegans* cultivars, *Z. angustifolia*, C_2-CR, and C_2-W were arranged in a randomized complete block experimental design with six plants per plant type replicated over eight blocks on a single bench. A single conidium from an isolate collected in the field was used to generate the inoculum. Diseased leaves were surface-disinfested for 5 min in 0.5% sodium hypochlorite supplemented with two drops of Tween 20 per 500 ml of solution. Leaf sections (5 mm^2) were placed on 4% potato-dextrose agar and incubated under 12 hr/day of cool-white fluorescent irradiation (42 $\mu E/m^2/sec$) at 26 C. Hyphal tips from the advancing edge of the mycelium were transferred to modified V-8 juice agar (5) without

$CaCO_3$ and incubated as described previously. Sporulation was induced after 10 days of incubation by scraping the mycelium and allowing the plates to air-dry throughout one additional light-dark photoperiodic cycle.

The conidial and mycelial inoculum was prepared by homogenizing the dried agar cultures in sterile deionized water and straining the suspension through a 200-μm mesh screen. The final inoculum concentration was adjusted at 1×10^4 conidia per milliliter with sterile deionized water using a hemacytometer counting chamber.

Ten-week-old flowering plants were inoculated with *A. zinniae* by spraying the conidial suspension onto the upper and lower surfaces of leaves and flowers until runoff. Control plants were sprayed with sterile deionized water. After inoculation, plants were placed in a moist chamber for 24 hr with intermittent misting at 2-hr intervals and subsequently transferred to the greenhouse with day/night temperatures of about 27 and 21 C, respectively.

Disease severity was evaluated 12 days after inoculation according to the following scale: 1 = resistant (no lesions on stems, leaves, or flowers); 2 = hypersensitive (necrotic flecking on stems, leaves, and ray petals); and 3 = susceptible (severe infection of stems, leaves, and ray petals resulting in deformation or death of leaves and shoot tips).

Reaction of C_2-CR and C_2-W to *X. campestris* pv. *zinniae* was evaluated in the greenhouse using an isolate furnished by D. L. Strider of North Carolina State University. Pathogenicity was confirmed on known susceptible *Z. elegans* cultivars (3). The experimental design was similar to that described for the *A. zinniae* screening test.

Bacterial cultures were grown in nutrient broth at 28 C for 48 hr. A Petroff-Hausser counting chamber was used to adjust the final inoculum concentration to 2×10^8 cells per milliliter in sterile deionized water (3).

Seven-week-old plants were inoculated with the bacterial suspension by spraying the upper and lower surfaces of leaves and flowers until runoff. Control plants were sprayed with sterile deionized water. All plants were placed in the moist chamber 3 hr before and 24 hr after inoculation, with intermittent misting at 2-hr intervals (3).

Disease severity was evaluated 3 wk after inoculation by counting necrotic spots with yellow halos on two leaves at the third node from the base of each plant, according to the following scale: 0 = no lesions; 1 = 1–2 lesions per leaf; 2 = 3–6 lesions; 3 = 7–20 lesions; and 4 = more than 21 lesions per leaf. To standardize severity scores with respect to leaf area among genotypes, values obtained from *Z. elegans* 'Cherry Ruffles' and 'Whirligig' leaves were divided by two because their leaf areas were twice as large as the amphiploids.

RESULTS

Inoculation with *E. cichoracearum* in the greenhouse resulted in severe infection and sometimes death of *Z. elegans* cultivars. In contrast, *Z. angustifolia*, C_1-CR, and C_2-CR remained free of infection. Intermediate in response to both parents were C_1-W and C_2-W, which showed resistance on leaves and susceptibility on ray florets. Based on conidial germination, however, signs of the pathogen on these amphiploids were restricted to senescing petal tissue and characterized by nonsporulating colonies with dark-colored hyphae.

Field-grown plants responded to natural inoculation with *E. cichoracearum* in the same manner as that observed in the greenhouse. One slight difference was noted on entries of C_2-W and C_3-W in which the petal tissue surrounding the infection site became red; the mycelium, however, was again nonsporulating and dark-colored.

The destructive potential of *A. zinniae* on the parental *Z. elegans* cultivars became apparent after greenhouse inoculations (Table 1). These entries sustained severe infections characterized by extensive necrosis, stem-girdling, and tip dieback; a few plants died. Most of the amphiploids were classified as hypersensitive because the disease failed to develop beyond visible flecking on stems, leaves, and flowers. *Z. angustifolia* displayed mixed reactions of hypersensitivity (44%) and resistance (56%); in the latter case, no lesions were visible after inoculation with *A. zinniae*. Isolation from necrotic tissues of hypersensitive and susceptible plants confirmed presence of the pathogen.

Inoculation with *X. campestris* pv. *zinniae* resulted in extensive disease development on *Z. elegans* 'Cherry Ruffles' and 'Whirligig.' The adjusted number of lesions per leaf on these cultivars varied from seven to more than 21 (Table 2). *Z. angustifolia* was highly resistant and remained free of symptoms after inoculation. Both amphiploids were intermediate in response to *X. campestris* pv. *zinniae*, but C_2-CR was more resistant than C_2-W.

Throughout all screening experiments, the hybrid polyploids were highly uniform in disease response. This uniformity was especially evident where genotypic variation was expected within C_2 families in response to artificial inoculation with *E. cichoracearum*; segregation for susceptibility and resistance was not detected in these populations.

DISCUSSION

The colchicine-induced amphiploids examined in this study possess high levels of resistance to *E. cichoracearum* and *A. zinniae* and moderate to high levels of resistance to *X. campestris* pv. *zinniae*. Although *E. cichoracearum* became established on ray florets of *Z. elegans* 'Whirligig' × *Z. angustifolia* amphiploids, these hybrid polyploids should be classified as tolerant because infection was restricted to senescing petal tissue and sporulation was suppressed. Both amphiploids showed a hypersensitive response to *A. zinniae*. The difference

Table 1. Response of *Zinnia elegans*, *Z. angustifolia*, and induced amphiploids to artificial inoculation with *Alternaria zinniae*

Parent or amphiploid	Response[a]		
	Resistant	Hypersensitive	Susceptible
Z. elegans 'Cherry Ruffles'	0	0	100
Z. elegans 'Whirligig'	0	0	100
C_2-CR[b]	6	94	0
C_2-W[c]	15	85	0
Z. angustifolia	56	44	0

[a] Numbers represent percentage of entries in each response group, based on disease severity index: resistant = no lesions; hypersensitive = visible flecking on stems, leaves, and ray petals; and susceptible = severe infections resulting in extensive necrosis, stem girdling, and tip dieback.
[b] *Z. angustifolia* × *Z. elegans* 'Cherry Ruffles' (C_2 generation).
[c] *Z. elegans* 'Whirligig' × *Z. angustifolia* (C_2 generation).

Table 2. Response of *Zinnia elegans*, *Z. angustifolia*, and induced amphiploids to artificial inoculation with *Xanthomonas campestris* pv. *zinniae*

Parent or amphiploid	DSI[a]	Resistant entries[b] (%)
Z. elegans 'Cherry Ruffles'	3.45[c]	0
Z. elegans 'Whirligig'	3.37	0
C_2-CR[d]	0.42	89
C_2-W[e]	2.15	23
Z. angustifolia	0.00	100

[a] DSI = disease severity index, which is the calculated average of the disease severity scores from 96 observations: 0 = no lesions, 1 = 1–2 lesions per leaf, 2 = 3–6 lesions, 3 = 7–20 lesions, and 4 = more than 21 lesions per leaf.
[b] Percentage of all plants with disease severity scores of 0 or 1.
[c] Values obtained from *Z. elegans* 'Cherry Ruffles' and 'Whirligig' leaves were divided by two because their leaf areas were twice as large as the amphiploids.
[d] *Z. angustifolia* × *Z. elegans* 'Cherry Ruffles' (C_2 generation).
[e] *Z. elegans* 'Whirligig' × *Z. angustifolia* (C_2 generation).

between hypersensitivity and resistance, however, diminished rapidly as plant growth continued after inoculation; disease development was effectively arrested at the initial stages of infection. Whereas both amphiploids displayed an intermediate response to *X. campestris* pv. *zinniae*, levels of resistance were considerably different. The C_2-CR amphiploid developed far fewer lesions than C_2-W, which may be classified as only moderately resistant. It is evident that this germ plasm will be extremely valuable in the gene transmission for disease resistance from *Z. angustifolia* to cultivars of *Z. elegans*.

The lack of variability in disease response to *E. cichoracearum* in all advanced generations of colchicine-induced amphiploids is attributed to suppression of pairing between homologous chromosomes. This phenomenon of genetic control of pairing has previously been demonstrated in wheat (2,8) and barley (7). The hybrid polyploids form predominantly bivalents at metaphase I and therefore do not segregate for intermediate characteristics (11). Their cytological and genetic behavior parallels that of a fully homozygous diploid; they attain instant homozygosity and stabilization of characters upon chromosome doubling through colchicine treatment (9).

Reciprocal differences of amphiploids in response to *X. campestris* pv. *zinniae* and *E. cichoracearum* may be a reflection of genotypic variation in the parental *Z. elegans* cultivars, maternal effects, or polygenic inheritance. The first proposal bears the least relevance because *Z. elegans* 'Cherry Ruffles' and 'Whirligig' are equally susceptible to both pathogens. Alternatively, the differences may be due to maternal effects in which genes for resistance to bacterial leaf and flower spot and powdery mildew are cytoplasmically inherited. Under these circumstances, amphiploids of *Z. angustifolia* × *Z. elegans* 'Cherry Ruffles' would be expected to be more resistant than amphiploids of the reciprocal cross, *Z. elegans* 'Whirligig' × *Z. angustifolia*. A third possibility is that resistance may be polygenically controlled and segregation for these genes results in a differential expression of resistance and susceptibility in derived amphiploids. Additional studies are suggested to determine the actual cause of reciprocal differences in disease response to these two pathogens.

Successful hybridization between *Z. angustifolia* and *Z. elegans* has pioneered the incorporation of multiple genes for disease resistance into cultivated forms of *Z. elegans*. The full potential of induced amphiploids may be exploited in serving as a genetic bridge between the distantly related species through backcrossing to *Z. elegans*. Additional work is being undertaken in this laboratory to determine the mode of inheritance of resistance to *E. cichoracearum* in interspecific hybrids and induced amphiploids of *Z. elegans* and *Z. angustifolia*.

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