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Influence of *Zinnia angustifolia* HBK genotype on embryonic and vegetative development of *Z. angustifolia* × *Z. elegans* Jacq. interspecific hybrids*

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Summary. Interspecific crosses between *Zinnia angustifolia* clones (maternal parents) and *Z. elegans* lines (paternal parents) were performed to investigate postzygotic barriers among *Z. angustifolia* × *Z. elegans* hybrids and to determine influence of parental genotype on embryonic and vegetative development of interspecific hybrids. Variation in percentage of emerged seedlings (PES) and percentage of morphologically normal hybrids (PNH) was attributable to *Z. angustifolia* clones with minor or no effect attributable to *Z. elegans* lines. Heterogeneity in PES values among *Z. angustifolia* clones was due to differences in amount of hybrid embryo breakdown and ungerminable seed. Cytological observations of normal and abnormal interspecific hybrids revealed similar chromosome numbers (2n = 23) but indicated a low mitotic index for abnormal hybrids. Genetic analysis of PES and PNH suggested control by multiple genes inherited from the *Z. angustifolia* genome. Adequate sampling of the *Z. angustifolia* gene pool would permit exploitation of genetic variability present within the species and allow improvements in PES and PNH for interspecific hybrids.

Key words: Clonal selection – Interspecific hybridization – *Zinnia angustifolia* – *Zinnia elegans* – Postzygotic barriers

Introduction

Improvement of cultivated plants may depend on exploitation of genetic variation in related species when cultivated or wild gene pools of the crop have been exhausted. Theoretically, related species comprise the richest and most diverse gene pools, but transfer and incorporation of alien genes is often hampered by reproductive barriers (Zohary 1973). Lack of crossability, hybrid inviability, hybrid sterility, and disharmonious interactions betwen genomes commonly occur in interspecific crosses and are manifestations of evolutionary divergence.

Zinnia angustifolia HBK (formerly *Z. linearis* Benth.) and *Z. elegans* Jacq. are species grown as ornamentals, with *Z. elegans* the most commonly cultivated. Recent interest in *Z. angustifolia* has developed due to its genetic resistance to alternaria blight (incited by *Alternaria zinniae* Pape), bacterial leaf and flower spot (incited by *Xanthomonas campestris* pv. *zinniae* Hopkins and Dowson) and powdery mildew (incited by *Erysiphe cichoracearum* DC. ex Merat) (Torres 1963; Lipschutz 1965; Andersen 1971; Jones and Strider 1979). These 3 pathogens are capable of eliciting severe epiphytotics within stands of *Z. elegans*. Interspecific hybridization would provide an expanded gene pool potentially useful for transfer of disease resistance from *Z. angustifolia* to *Z. elegans*.

Sexual hybridization between *Z. angustifolia* and *Z. elegans* has been reported as successful when *Z. angustifolia* was the maternal parent (Boyle and Stimart 1982). Phytopathological studies (Terry-Lewandowski and Stimart 1983) demonstrated that colchicine-induced amphiploids exhibited high levels of resistance to the 3 pathogens. However, embryo abortion and abnormal morphological development of some *Z. angustifolia* × *Z. elegans* F_1 hybrids were found to limit the number of hybrids which could be used for further breeding (Boyle and Stimart 1982). Morphologically abnormal hybrids were characterized by reduced leaf size and internode length, absence of flowers and slow growth, compared to normal hybrids. It was not determined whether parental genotypes affected growth and development of interspecific hybrids.

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The objectives of this study were to characterize post-zygotic barriers among *Z. angustifolia* × *Z. elegans* hybrids and to determine the influence of parental genotype on embryonic and vegetative development of interspecific hybrids.

Materials and methods

Five lines of *Z. elegans* and 12 clones of *Z. angustifolia* were used as parental material (Table 1). Lines of *Z. elegans* were derived from partially inbred commercial cultivars that were sib-crossed for 2 additional generations to increase uniformity. Seedlings of *Z. angustifolia* were randomly selected from 2 commercial cultivars and cloned by rooting terminal shoot cuttings. Six seedlings were cloned from 1 cultivar (hereafter designated as AO clones) and 6 seedlings from the other cultivar (hereafter designated as AW clones).

Emasculation and crossing procedures were reported previously (Boyle and Stimart 1982). Techniques used in seed germination, photoperiod regulation of plant growth and culture of plant material were similar to those reported previously (Boyle and Stimart 1983; Boyle et al. 1986). Greenhouse temperatures were maintained at a minimum of 15.5°/18 °C (night/day). Four experiments were conducted.

1 Interspecific hybrid seed emergence and plant development

Crosses between 12 *Z. angustifolia* clones (maternal parents) and 5 *Z. elegans* lines (paternal parents) were initiated to determine the influence of parental genotype on seedling emergence and morphological development of interspecific hybrids. The number of emerged seedlings was recorded for each cross prior to transplanting (12–18 days after seed sowing). Days to flowering, flower diameter, leaf width and length, number of nodes on the main axis below the first inflorescence, number of ray petals, and plant height were recorded as described previously (Boyle and Stimart 1982, 1983) (data not presented). Data were used as criteria in distinguishing interspecific hybrids as either phenotypically normal or abnormal (Boyle and Stimart 1982). For each maternal clone and pollinator line, the percentage of emerged seedlings (PES) and percentage of normal hybrids (PNH) were calculated.

Heterogeneity chi-square (H χ^2) analyses were performed separately for *Z. angustifolia* clones and *Z. elegans* lines to determine parental species influence on PES and PNH.

2 Chromosome numbers of parents and interspecific hybrids

Mitotic chromosome analysis was conducted on *Z. angustifolia* clones and *Z. elegans* lines used in the first experiment, and

Table 1. Seedling emergence and normal hybrids from *Z. angustifolia* × *Z. elegans* crosses. Percentage values for *Z. angustifolia* clones are means from crosses with 5 *Z. elegans* lines; values for *Z. elegans* lines are means from crosses with 12 *Z. angustifolia* clones

Clone or line	Florets pollinated	% emerged seedlings	H χ^2	% normal hybrids[a]	H χ^2
Z. angustifolia					
AO1	448	37.5		40.6	
AO2	310	28.7		0	
AO3	373	28.4		47.2	
AO4	188	24.5		41.3	
AO5	299	11.0		0	
AO6	254	27.0		5.6	
AO clones			62.1***[b]		96.9***
AW1	427	26.0		0	
AW2	604	51.1		44.7	
AW4	233	39.9		2.2	
AW5	377	21.0		1.3	
AW7	197	3.0		0	
AW9	156	9.0		42.9	
AW clones			254.9***		158.4***
All cones			335.3***		255.3***
Z. elegans					
'Canary Bird'	875	27.9		24.6	
'Crimson Monarch'	879	27.1		27.3	
'Enchantress'	473	24.7		25.6	
'Orange King'	963	33.4		29.2	
'Purity'	679	26.5		19.6	
All lines			17.2**		6.1 NS

[a] No. normal hybrids/No. emerged seedlings (× 100)

[b] Differences significant at 1% (**) or 0.1% (***) level or not significant (NS) by heterogeneity (H) χ^2 test

normal and abnormal interspecific hybrids. Excised roots tips from rooted terminal shoot cuttings were pretreated for 4 h in 0.1% aqueous colchicine at 2 °C and fixed in Carnoy's fluid (6:3:1 of 95% ethanol, chloroform and glacial acetic acid, respectively) for 24 h or longer. Root tips were hydrolyzed in 1N HCl at 60 °C for 15 min, stained with Feulgen for 1 h, and squashed in 1% acetocarmine. A minimum of 10 counts was made on intact, well-spread cells of each clone, line, and hybrid.

3 Interspecific hybrid embryogenesis

Crosses between *Z. angustifolia* clones AW1, AW2, and AW9 (maternal parents) and *Z. elegans* 'Orange King' were performed to determine if barriers to hybrid embryogenesis were present. Florets were fixed in Carnoy's fluid at 3, 14, and 28 (seed maturity) days after pollinations. Techniques used in embryological observations were reported previously (Boyle and Stimart 1986). Presence or absence of an embryo, and if present, embryo length and state of development (aborted or healthy) were recorded for each dissected floret. Embryos were classified as abnormal if aborted or rudimentary in length. Embryos were defined as rudimentary if embryo length was below the lower tail of the distributed values for embryo length plotted in histograms for each cross. This criterion was considered an unbiased estimate of abnormally small embryos.

For each time period, an analysis of variance was performed on embryo length data. Heterogeneity χ^2 analyses for the percentage of abnormal embryos were calculated for each *Z. angustifolia* clone and each time period.

4 Genetic analysis of PES and PNH

Two populations were generated from reciprocal crosses between *Z. angustifolia* clones AW1 and AW2, and 9 F_1 progenies from each population were selected randomly from among seedlings. The 2 parental and 18 F_1 progeny clones were crossed (as maternal parents) with *Z. elegans* 'Canary Bird' and 'Orange King'. Seedling emergence data were collected for each cross and PES was calculated as described previously.

Interspecific families derived from crossing the 2 parental and 18 F_1 progeny clones with *Z. elegans* 'Orange King' were transplanted to 1.2 × 14.6 m ground beds. A randomized complete block design with 2 blocks was employed with 49 plants per plot (7 rows with 7 plants per row) for each interspecific family. Data were collected on 35 plants in the inner 5 rows to minimize border effects. Days to flowering, fresh weight of aerial parts, plant height, and number of nodes were recorded, and data were used to distinguish normal and abnormal hybrids for calculation of PNH for each cross.

Heterogeneity χ^2 analyses were performed on PES and PNH data and partitioned to separate parental, F_1 progeny and reciprocal F_1 population effects of *Z. angustifolia* clones.

Results

1 Interspecific hybrid seed emergence and plant development

Significant differences in PES were observed between *Z. angustifolia* clones used in interspecific crosses (Table 1). Values exhibited continuous variation between 3.0% and 51.0% among the 12 *Z. angustifolia* clones, with greater variation among AW clones compared to AO clones. Significant differences in PES were observed also between *Z. elegans* lines used as paternal parents, but the range in values was less than among *Z. angustifolia* clones.

Heterogeneity among *Z. angustifolia* clones was highly significant for PNH (Table 1). Among the AO and AW clones, PNH values exhibited discontinuous variation, ranging from 0% to 5.6% and from 40.6% to 47.2%. Differences in PNH observed between *Z. elegans* lines were not significant.

2 Chromosome numbers of parents and interspecific hybrids

Mitotic chromosome counts of the 12 *Z. angustifolia* clones and 5 *Z. elegans* lines used as parents (Table 1) indicated somatic chromosome numbers of $2n=22$ and $2n=24$, respectively. Cytological observations of normal and abnormal interspecific hybrids indicated $2n=23$, as previously reported (Terry-Lewandowski et al. 1984), and did not reveal irregularities due to polyploidy or aneuploidy among abnormal hybrid cells. However, a low mitotic index in root tips of abnormal interspecific hybrids allowed only 5 confirmed counts for this group.

3 Interspecific hybrid embryogenesis

The percentage of *Z. angustifolia* florets with embryos following interspecific pollinations was similar for all clones (Table 2). Minor but significant differences in embryo length between *Z. angustifolia* clones were present at 3 and 14 days, but not at 28 days.

The percentage of abnormal embryos increased between 3 and 28 days after pollination for all *Z. angustifolia* clones. However, the increase observed between 3 and 28 days was not significant for AW1 (H $\chi^2=3.9$; $P>0.10$) or AW2 (H $\chi^2=4.5$; $P>0.10$), but was significant for clone AW9 (H $\chi^2=106.8$; $P<0.001$), with a 10-fold increase occurring between 3 and 14 days, and a 2-fold increase between 14 and 28 days (Table 2). Significant heterogeneity existed among clones at 14 and 28 days with respect to percentage of abnormal embryos, but AW1 and AW2 were not significantly different at either 14 (H $\chi^2=0.1$; $P>0.70$) or 28 (H $\chi^2=0.3$; $P>0.50$) days. Over 95% of abnormal embryos observed at 28 days were either partially or fully necrotic, and less than 5% were classified as rudimentary (1.8 mm or less in length).

Table 2. Pollinated florets with embryos, embryo length and abnormal embryo development in crosses of *Zinnia angustifolia* (*Z. a.*) clones with *Z. elegans* (*Z. e.*) 'Orange King'

Cross	Florets pollinated	% florets with embryos	Embryo length[a]	% abnormal embryos[b]
	3 days after pollination			
Z. a. AW1 × *Z. e.* 'Orange King'	97	70.1	24.9 b[d]	4.4
Z. a. AW2 × *Z. e.* 'Orange King'	100	70.0	34.6 a	2.9
Z. a. AW9 × *Z. e.* 'Orange King'	90	80.0	34.8 a	2.8
Significance[c]	–	–	**	NS
	14 days after pollination			
Z. a. AW1 × *Z. e.* 'Orange King'	286	66.8	2.0 b	10.5
Z. a. AW2 × *Z. e.* 'Orange King'	294	70.7	2.0 b	11.1
Z. a. AW9 × *Z. e.* 'Orange King'	300	80.7	2.2 a	31.9
Significance	–	–	**	**
	28 days after pollination			
Z. a. AW1 × *Z. e.* 'Orange King'	211	71.1	2.2	13.3
Z. a. AW2 × *Z. e.* 'Orange King'	175	71.1	2.2	11.3
Z. a. AW9 × *Z. e.* 'Orange King'	270	70.7	2.2	67.0
Significance	–	–	NS	***

[a] Embryo length in μm at 3 days and in mm at 14 and 28 days
[b] No. of rudimentary or aborted embryos/total no. of embryos (× 100)
[c] Differences significant at 1% (**) or 0.1% (***) level or not significant (NS) by F test (for embryo length) or heterogeneity χ^2 test (for % abnormal embryos)
[d] Mean separation within columns by Duncan's multiple range test, 5% level

4 Genetic analysis of PES and PNH

The PES was not affected significantly by the *Z. elegans* line used as a paternal parent for AW1, AW2, and most F_1 clones (Table 3). No significant trend was apparent among 7 F_1 clones for which PES was significantly affected by the *Z. elegans* pollinator. Among the parental clones, PES was greater for AW2 than for AW1 in crosses with both pollinators (Tables 3 and 4). Among crosses with 'Orange King', PES was lowest for AW1; in crosses with 'Canary Bird', PES was low for AW1 but not significantly different from AW1/AW2-1 (H $\chi^2 = 3.5$; $P > 0.05$), AW1/AW2-3 (H $\chi^2 = 0.1$; $P > 0.70$), AW1/AW2-7 (H $\chi^2 = 0.8$; $P > 0.30$), and AW2/AW1-5 (H $\chi^2 = 2.5$; $P > 0.10$). Clones AW1/AW2-6 and AW2/AW1-9 yielded the highest PES in crosses with both pollinators, and these values were significantly greater than for AW2 in crosses with 'Orange King' (H $\chi^2 = 13.6$; $P < 0.001$ and H $\chi^2 = 37.7$; $P < 0.001$, respectively) and 'Canary Bird' (H $\chi^2 = 12.5$; $P < 0.001$ and H $\chi^2 = 16.9$; $P < 0.001$, respectively).

In separate analyses of pollinators 'Canary Bird' and 'Orange King', and in combined analysis, heterogeneity among all 20 clones, the 18 F_1 progeny and the reciprocal F_1 populations, was highly significant for PES values (Table 4). Although significant, differences in mean PES for the reciprocal F_1 populations were only 3.5% and 5.6% for pollinators 'Orange King' and 'Canary Bird', respectively. Variances of PES values for reciprocal F_1 populations were not significantly different for either 'Canary Bird' ($F_{8,8} = 1.19$; $P > 0.10$) or 'Orange King' ($F_{8,8} = 1.51$; $P > 0.10$) as pollinators.

Block differences in PNH were not significant among progenies derived from crosses with the 20 *Z. angustifolia* clones and 'Orange King'. The PNH for AW1 did not differ from AW2/AW1-4, but AW1 was significantly less than the other 17 F_1 clones and AW2 (Table 5). Clone AW2 did not differ from AW1/AW2-6, but was significantly higher than the other 17 F_1 clones.

Significant heterogeneity for PNH existed among all 20 *Z. angustifolia* clones and among the 18 F_1 clones, but not between the reciprocal F_1 populations (Table 6). The difference in mean PNH for the reciprocal F_1 populations was 4.4%. The variance of PNH values for the 9 AW1/AW2 F_1 clones was not different significantly from the variance of the 9 AW2/AW1 F_1 clones ($F_{8,8} = 1.29$; $P > 0.10$).

The coefficient of determination (r^2) for PES values on PNH values was 0.19, indicating that only 19% of the total variation in PNH was explained by the linear function of the independent variable PES (Fig. 1).

Table 3. Effect of *Zinnia angustifolia* clones crossed with *Z. elegans* 'Canary Bird' and 'Orange King' on percentage of emerged seedlings

Z. angustifolia clone	*Z. elegans* pollinator				'Canary Bird' versus 'Orange King' (H χ^2)
	'Canary Bird'		'Orange King'		
	Florets pollinated	% emerged seedlings	Florets pollinated	% emerged seedlings	
AW1	72	27.8	559	23.6	0.01 NS[a]
AW2	119	51.3	569	53.9	0.54 NS
F_1–AW1×AW2					
1	116	41.4	409	32.3	4.12*
2	345	50.1	800	44.6	2.37 NS
3	88	28.4	685	41.5	6.10*
4	196	55.6	754	48.0	3.47 NS
5	84	51.2	331	51.7	0.01 NS
6	154	72.1	771	63.9	4.54*
7	239	33.5	591	36.7	1.17 NS
8	127	43.3	255	45.5	0.16 NS
9	184	54.3	389	44.0	5.68 NS
Σ	1,533		4,985		
$\bar{X}$		48.6		46.0	2.77 NS
F_1–AW2×AW1					
1	157	51.6	492	54.3	0.25 NS
2	197	55.3	768	49.1	2.19 NS
3	264	48.9	578	28.9	30.51***
4	75	57.3	195	51.3	0.48 NS
5	183	38.2	720	47.5	5.64*
6	187	70.6	598	53.0	16.35***
7	131	57.2	721	41.3	12.74***
8	204	43.1	691	51.1	2.91 NS
9	150	75.3	672	70.8	2.01 NS
Σ	1,548		5,435		
$\bar{X}$		54.2		49.5	11.22***

[a] Differences significant at 5% (*) or 0.1% (***) level or not significant (NS) by heterogeneity (H) χ^2 test

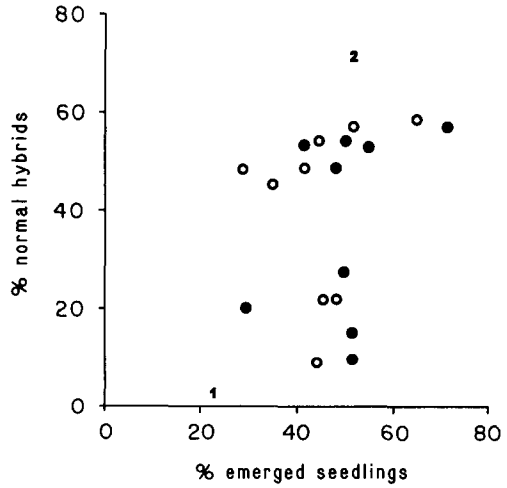

Fig. 1. Relationship of percentages of emerged seedlings and normal hybrids for interspecific hybrids of *Zinnia angustifolia* clones AW1 (**1**), AW2 (**2**), and F_1 progeny of AW1×AW2 (○) or AW2×AW1 (●) used as metenal parents in crosses with *Z. elegans* 'Orange King'. Coefficient of determination (r^2) for % normal hybrids versus % emerged seedlings = 0.19

Discussion

Percentage of emerged seedlings (PES)

At least 2 mechanisms contributed to differences in PES observed among interspecific progeny of the *Z. angustifolia* clones. The first mechanism, hybrid embryo breakdown, was expressed by all 3 clones to different degrees and was the major barrier to hybrid seedling emergence for AW9 (Table 2). Hybrid embryo breakdown was observed as early as 3 days after pollination and continued up to 28 days after pollination. Evidence for the second barrier among *Z. angustifolia*×*Z. elegans* hybrids can be deduced by comparing values for the percentage of pollinated florets with embryos, percentage of abnormal embryos at 28 days, and PES values for clones AW1 and AW2 (Tables 1 and 2): differences between AW1 and AW2 for the first 2 variables were not significant whereas PES of AW2 was nearly twice that of AW1. This second barrier ap-

Table 4. Heterogeneity (H) χ^2 tests for percentage of emerged hybrid seedlings from *Zinnia angustifolia* clones crossed with *Z. elegans* 'Canary Bird' and 'Orange King'

Comparison	df	*Z. elegans* pollinator		Combined analysis
		'Canary Bird'	'Orange King'	
Between clones	19	185.6***[a]	566.1***	670.6***
Between parents	1	10.1**	109.3***	119.2***
Between F_1 progeny	17	169.5***	425.0***	510.7***
Between reciprocal F_1 populations	1	9.9**	11.1***	19.1***

[a] Differences significant at 1% (**) or 0.1% (***) level

Table 5. Effect of *Zinnia angustifolia* clones crossed with *Z. elegans* 'Orange King' on percentage of normal hybrids

Z. angustifolia clone	% normal hybrids[a]	Clonal comparison	
		AW1	AW2
AW1	2.9	–	70.49***[b]
AW2	71.4	70.49**	–
F_1–AW1×AW2			
1	48.6	38.29***	7.62**
2	14.2	5.83*	46.67***
3	48.6	38.29***	7.62**
4	22.8	12.50***	33.12***
5	55.7	47.22***	3.87*
6	58.6	51.05***	2.54 NS
7	45.7	34.96***	9.54**
8	22.8	12.50***	33.12***
9	54.2	45.36***	4.41*
$\bar{X}$	41.9	–	–
F_1–AW2×AW1			
1	54.2	45.36***	4.41*
2	27.2	16.19***	27.46***
3	20.0	10.16**	37.30***
4	10.0	2.97 NS	54.72***
5	47.1	36.61***	8.55**
6	52.8	43.54***	5.13*
7	54.2	45.36***	4.41*
8	15.7	6.87**	44.19***
9	55.7	47.22***	3.87*
$\bar{X}$	37.5	–	–

[a] Pooled data from 2 blocks
[b] Differences significant at 5% (*), 1% (**) or 0.1% (***) level or not significant (NS) by heterogeneity χ^2 test

Table 6. Heterogeneity (H) χ^2 tests for the percentage of normal hybrids from *Z. angustifolia* clones crossed with *Z. elegans* 'Orange King'

Comparison	df	H χ^2
Blocks	1	2.1 NS[a]
Between clones	14	216.4***
Between parents	1	38.3***
Between F_1 progeny	17	150.1***
Between reciprocal F_1 populations	1	2.7 NS

[a] Difference significant at the 0.1% (***) level or not significant (NS) by heterogeneity (H) χ^2 test

parently acts after seed has attained full size. It was not determined whether unemerged seed was dormant and eventually acquired the ability to germinate or if seed was inviable and incapable of germination.

Genetic control of PES was mainly attributable to *Z. angustifolia*, although interaction of maternal and paternal parents observed among some F_1 hybrid clones (Table 3) suggests a low degree of influence by *Z. elegans*. This variation may have been due to greenhouse environmental effects: *Z. angustifolia* clones were pollinated within a few hours of pollen dehiscence (Boyle and Stimart 1982), but daily changes in greenhouse temperatures may have influenced pollen viability or seed set.

The cytoplasmic background did not markedly influence the mean nor variance in PES values observed among reciprocal F_1 populations of *Z. angustifolia* clones, although reciprocal differences were statistically significant (Table 4). Hence, no interaction between nuclear genes and cytoplasmic factors was apparent. Assuming uniparental inheritance of plasmagenes, these results indicate control of PES by nuclear genes present in *Z. angustifolia*.

The number of genes controlling PES cannot be determined with certainty from this study, since limitations in the number of *Z. angustifolia* clones that could be used as maternal parents resricted sample size. For an estimate, it is necessary to define *Z. angustifolia* genotypes based on the number of emerged progeny. For "all-or-none" traits such as PES with 2 phenotypic classes, i.e., emerged and nonemerged seedlings, "all" must be defined in relative rather than absolute terms. The mean percentage of florets with embryos following interspecific pollinations was 69.4% and 74.1% for clones AW1 and AW2, respectively (calculated from Table 2). These values represent the normal seed set, and PES values for interspecific hybrids approaching these levels would indicate no inhibition of seedling emergence, or "all", and constitute the upper range of PES values that could be obtained. Based on data in Table 1, the lower range of PES values is near zero. Both parental clones AW1 and AW2 have PES values significantly lower than the upper range and therefore

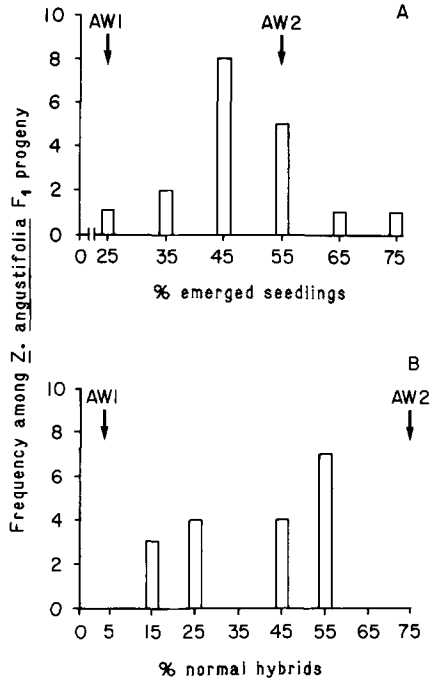

Fig. 2. Effect of *Zinnia angustifolia* clones on percentage of emerged seedlings (PES) (**A**) and percentage of normal hybrids (PNH) (**B**) for interspecific hybrids of *Z. angustifolia* × *Z. elegans* 'Orange King'. Bars represent the number of *Z. angustifolia* F_1 progeny clones producing PES and PNH values in each designated class interval. Arrows indicate PES and PNH values for parental clones AW1 and AW2. Class interval is 10%

appear to have genes inhibiting seedling emergence. In contrast, PES for the clone AW2/AW1-9 falls within the upper range for crosses with both *Z. elegans* pollinator lines; this recombinant genotype appears to possess no genes inhibiting seedling emergence. Examination of PES values of other F_1 clones indicates a high degree of dominance for higher PES values and continuous variation over the range (Fig. 2). Based on these results, it appears that multiple genes control PES. Genetic analysis of a large number of F_1 progeny clones is necessary to substantiate this hypothesis.

Percentage of normal hybrids (PNH)

Abnormal *Z. angustifolia* × *Z. elegans* hybrids expressed a syndrome of morphological and developmental aberrations reflecting gross physiological disturbances, as reported previously (Boyle and Stimart 1982). Hybrids with aberrant morphology were also obtained following interspecific crosses in *Gilia* (Grant and Grant 1954), *Gossypium* (Hutchinson 1946; Stephens 1946, 1950), *Papaver* (McNaughton and Harper 1959), *Vigna* (Chen et al. 1983), and other *Zinnia* species (Torres 1963, 1964). Cytological analysis of normal and abnormal *Z. angustifolia* × *Z. elegans* hybrids revealed that these disturbances were controlled genetically rather than chromosomally. Stebbins (1965) proposed that genes controlling plant organ shape and genes governing the number and arrangement of plant parts exert their action by affecting first, the tempo and distribution of mitosis, and second, the timing of transition from mitosis-determined to be enlargement-determined state. The low number of mitotic cells observed in root tips of abnormal hybrids suggests physiological disturbances may involve irregularities in timing of mitotic cell cycle processes. This would account for slow growth and low biomass accumulation for abnormal hybrids relative to normal hybrids. Investigations of relative length of the mitotic cycle and S-phase duration are required to verify this hypothesis.

Genetic control of PNH was due to *Z. angustifolia* with no significant effect from the *Z. elegans* pollinator line. As with PES, no interaction of nuclear genes and cytoplasmic factors was apparent, as evidenced by lack of a significant difference between the reciprocal F_1 populations (Table 6). Thus, control of PNH appears to involve certain genes in the nuclear genome of *Z. angustifolia* that are expressed when combined in the same nucleus with the genome of *Z. elegans.*

As with PES, the number of genes controlling PNH can only be estimated. The upper limit of PNH values indicating no inhibition of growth and development would be 100%, and the lower limit based on data in Table 1 is 0%. The PNH value for clone AW2 was 71.4% (Table 5), which was close to 75%. This latter value could be obtained in the simplest case by assuming 2 independent recessive genes at heterozygous loci: gametes with *ab* genotype would inhibit development of their interspecific progeny whereas *AB, Ab* and *aB* genotypes would not affect development. Clone AW1 exhibits PNH values at the extreme lower limit and must contain additional or unique genes controlling PNH. Clustering of PNH values among F_1 progeny clones was observed (Fig. 2); PNH values are bimodally distributed and not equidistant from either parent. Few F_1 progeny clones were recovered with PNH values close to either parental clone (Table 5), although this may reflect the small sample size of *Z. angustifolia* clones which were tested. Based on these data, genetic control of PNH is apparently by multiple genes, with individual genes having additive but unequal effects. The low correlation between PES and PNH (Fig. 1) suggests that genetic control of PNH is independent of PES.

A significant environmental component of the observed phenotypic variation in PNH values was evident, based on comparisons of PNH values from Tables 1 and 5 for clones AW1 (0% and 2.9%, re-

spectively) and AW2 (44.7% and 71.4%, respectively). This contrasts sharply with observed phenotypic variation for PES, which suggests little effect due to environment (compare Tables 1 and 3). Growth and morphological development of the interspecific hybrids may have been affected by seasonal variations in photoperiod, photosynthetic photon flux density and temperature, as has been previously reported for *Z. elegans* (Boyle and Stimart 1983; Boyle et al. 1986).

Intraspecific role of genes controlling PES and PNH

The present study indicates that *Z. angustifolia* and *Z. elegans* are reproductively isolated due to hybrid inviability among some F_1 hybrids and suggests that hybrid inviability is due to expression of genes inherited from the *Z. angustifolia* genome. There was no evidence to indicate that genes controlling PES and PNH affected seedling emergence or morphological development within *Z. angustifolia* populations; hence, the intraspecific role of these genes controlling PES and PNH in interspecific hybrids remains unknown.

Role of Zinnia elegans

The lines of *Z. elegans* used as pollinators either had little or no effect on expression of seedling emergence or vegetative development after emergence. However, it cannot be concluded from this study as to whether genes controlling development of interspecific hybrids are present within this species. Uniformity of response among *Z. elegans* genotypes suggests either: 1) homozygosity for genes controlling barriers; or 2) absence of controlling genes. Sampling and testing of diverse *Z. elegans* genotypes would be expected to resolve this question.

Practical conclusions

The genetic variability present within the *Z. angustifolia* gene pool, with regard to its effects on seedling emergence and development of interspecific progeny, indicates that selection can be practiced among interspecific families, based on performance of the maternal parent, to increase PES and modify F_1 hybrid growth and development. A similar scheme was reported by Chen et al. (1983): selection of superior-performing *Vigna umbellata* hybrids for use as pollinators was found to increase viable seed produced from the cross, *V. radiata* × *V. umbellata*. A prerequisite for *Zinnia* hybrid improvement is adequate sampling of the *Z. angustifolia* gene pool. Interspecific hybrids can then be selected within families derived from interspecific crosses utilizing superior-performing *Z. angustifolia* clones as parents. Colchicine treatment and subsequent restoration of fertility in induced amphidiploids (Boyle and Stimart 1982; Terry-Lewandowski et al. 1984) provides source material for introduction of genes for disease resistance into *Z. elegans* (Terry-Lewandowski and Stimart 1983) or for development of commercial hybrid cultivars with more decorative plant characteristics than currently present in either species.

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