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**SELF-INCOMPATIBILITY IN ZINNIA ANGUSTIFOLIA HBK (COMPOSITAE):
I. TECHNIQUES FOR ASSESSING AND QUANTIFYING
SELF-INCOMPATIBILITY; AND
II. GENETICS**

A Thesis Presented

by

RAYMOND R. SAMAHA

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

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Department of Plant and Soil Sciences

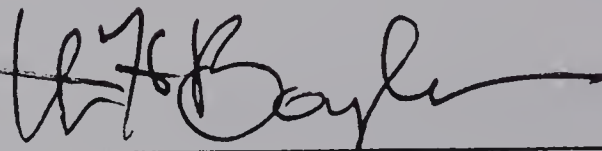
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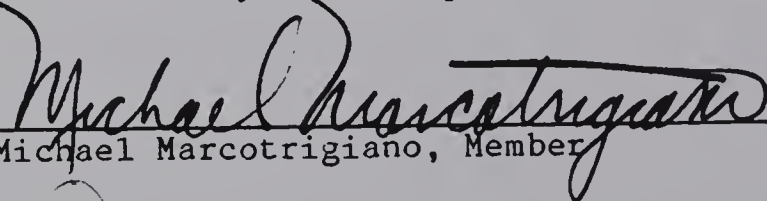
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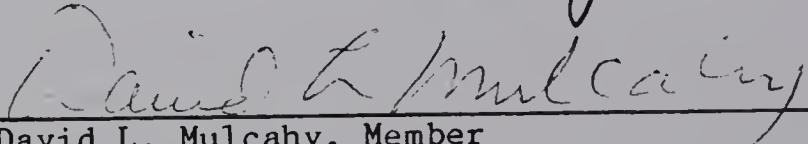
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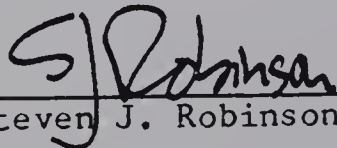
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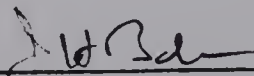
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A Raymond, Angélique et Nada

" Il est parait-il des terres brulées
donnant plus de blé qu'un meilleur avril."

J. Brel

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ABSTRACT

SELF-INCOMPATIBILITY IN ZINNIA ANGUSTIFOLIA HBK (COMPOSITAE):

I. TECHNIQUES FOR ASSESSING AND QUANTIFYING

SELF-INCOMPATIBILITY; AND

II. GENETICS.

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Embryo set, normal light microscopy, and incident-light fluorescence microscopy were used to assess self-incompatibility (SI) in Zinnia angustifolia HBK. Percentage of florets with embryos 21 days following self- or incompatible cross-pollinations ranged from 0 to 9.9%, whereas compatible crosses yielded 55.5 to 87.1% florets with embryos. For the second technique, pollinated pistils were stained with aniline blue in lactophenol and viewed with normal light microscopy; pollen load and number of germinated grains were significantly higher for compatible compared to incompatible crosses, and both variables were positively correlated ($r = 0.89 - 0.96$) to % embryos observed 21 days following crosses. For the third technique, pollinated pistils were stained using decolorized aniline blue and viewed with incident-light fluorescence microscopy; among compatible crosses, pollen load was higher and little

or no callose was observed in stigmatic papillae, whereas for incompatible crosses, pollen load was low and callose lenticules were deposited in stigmatic papillae. Callose fluorescence was quantified on pollinated pistils using a photomultiplier connected to an epifluorescence microscope. Mean callose fluorescence intensity ranged from 47.9% to 62.6% for incompatible and from 6.4% to 9.9% for compatible crosses, and was negatively correlated ($r = -0.95$) with % embryos observed 21 days following crosses. Microscopical techniques permit rapid assessment of SI and may be used routinely when each observed or measured parameter is highly correlated to the incompatibility response. In a second series of experiments, the genetics of SI in Z. angustifolia was studied. Two Z. angustifolia clones were used as parental material, and 14 F₁ clones were generated, intercrossed in a full diallel, and reciprocally backcrossed to both parents. Both F₂ and backcross progeny were generated and tested for incompatibility responses. Zinnia angustifolia expressed a one-locus sporophytic SI system with a linear dominance series of S-alleles in both pistil and pollen.

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CHAPTER 1

REVIEW OF LITERATURE

Introduction

Self-incompatibility (SI) is a genetically induced mechanism whereby a hermaphroditic seed plant producing functional male and female gametes is unable to generate zygotes after self pollination (Lundqvist, 1964). The SI system is based on the capacity of the pistil to reject a pollen grain or tube that is carrying the same incompatibility allele (de Nettancourt, 1977).

Self-incompatibility is also defined to include incompatible matings between plants with identical incompatibility alleles, and the rejection reactions following incompatible cross pollinations are indistinguishable from those following self pollinations. The responses following incompatible matings suggest the SI phenomenon involves a cell-cell recognition system, and thus recognition factors are prerequisites for rejection reactions. It appears that the SI recognition process involved is very similar to the immune system found in vertebrates: both systems depend on an interaction between membrane- or wall-bound molecules and extracellular molecules (Knox and Clarke, 1984). However, plants carrying an SI system will reject "self" genotypes whereas immune systems will reject "foreign" genotypes (Heslop-Harrison, 1975).

Classification of SI

Self-incompatibility can be classified according to the stage at which the male gametophyte receives the necessary information for the determination of the incompatibility phenotype (de Nettancourt, 1977). Two distinct systems have been discerned: gametophytic and sporophytic. In gametophytic self-incompatibility (GSI) systems, the incompatibility phenotype of pollen is determined by the individual microspore genotype, while in sporophytic self-incompatibility (SSI) systems the incompatibility phenotype of pollen is determined by the pollen-generating parent (sporophyte). Hence, pollen produced by a heterozygous diploid plant expressing GSI will carry two incompatibility phenotypes (1:1), whereas all pollen produced by a plant expressing SSI will carry the same incompatibility phenotype. This basic difference between the genetic control of SSI and GSI reflects differences in the timing of expression for gene(s) controlling incompatibility (de Nettancourt, 1977).

Families known to display GSI are the Solanaceae, Rosaceae, and Liliaceae; families expressing SSI are the Compositae and Cruciferae (Heslop-Harrison, 1975).

One of the main dissimilarities between GSI and SSI systems is exhibited in stigma morphology. Most genera with GSI systems have "wet" stigmas with smooth surfaces (or low papillae) bearing copious exudates at maturity (Heslop-Harrison et al., 1975). These secretions accumulate over time and form a viscous coating where pollen grains attach and germinate. In contrast, "dry" stigmas occur in families characterized

by a SSI system and in the Gramineae (two-loci GSI); "dry" stigmas have well-developed papillae and lack copious exudate at maturity. Among the Cruciferae and Compositae species, papillae are characterized by an outer hydrated proteinaceous pellicle and an inner cuticle (Mattsson et al., 1974). The cuticle is comprised of short radially directed rods with discontinuities which provide for water movement from protoplasts of papillae to the pellicle.

A strong relationship has been found between pollen cytology and SI systems (Brewbaker, 1957). In general, pollen is binucleate (one vegetative and one generative cell nucleus) and relatively long-lived in species exhibiting GSI, whereas pollen is trinucleate (one vegetative cell nucleus and two gametic nuclei), does not easily germinate in vitro, and is relatively short-lived in species displaying SSI. An exception to this generalization is the Gramineae where a GSI system is present and pollen is trinucleate (Heslop-Harrison, 1975).

Self-incompatibility can also be classified according to the site of inhibition, i.e., where the rejection reaction occurs: the stigma, style or ovary (de Nettancourt, 1977). A strong relationship exists also between pollen cytology and the site of inhibition, with stigmatic inhibition correlated with trinucleate pollen, and stylar (or ovarian) inhibition with binucleate pollen (Brewbaker, 1957). In the SSI systems exhibited by Compositae and Cruciferae, the papillate stigmatic surface forms the barrier site during incompatible pollinations (Heslop-Harrison et al., 1975).

A third criterion for classification of SI systems is based on the number of genetic loci and allelic interactions involved in the

determination of the SI phenotype (de Nettancourt, 1977). A polyallelic series can occur at one, two or (rarely) several loci in GSI systems. Among SSI systems, one-locus polyallelic control mechanisms are most common. However, multigenic SSI systems have been proposed for the crucifers Capsella grandiflora (Riley, 1936), Cardamine pratensis (Correns, 1912) and Eruca sativa (Verma and Lewis, 1977), and the composite Helianthus annuus (Habura, 1957; Shchori, 1969). Independent gene action or various dominance relationships can occur in the pollen and pistil of species expressing SSI, whereas independent gene action occurs in both pistils and pollen of GSI systems.

By convention, the genetic locus controlling monofactorial SI systems is designated as "S". In species exhibiting a polyallelic series, each allele is designated by an S, followed by a number (S₁, S₂...) (de Nettancourt, 1977).

**Pollen and pistil behavior following compatible
and incompatible crosses among species expressing SSI**

Following a compatible cross, the pollen-pistil interaction proceeds through successive stages: a pollen grain lands on the stigmatic surface and becomes attached; the grain hydrates and pollen exine-held fractions are emitted and flow over the pellicle surface; the grain germinates to produce a pollen tube which penetrates the stigmatic surface; and the pollen tube grows through the style until it penetrates the female gametophyte where it discharges its two male gametes (Heslop-Harrison et al., 1975). Following hydration and germination of Helianthus annuus pollen, pollen tubes penetrated the cuticle at the base of papillae,

passed through the cell wall, and entered the transmitting tissue where they made a 90° turn and grew down through the intercellular matrix of the transmitting tissue to the ovule (Vithanage and Knox, 1977a). The early events following an incompatible cross are not different from a compatible cross. However, subsequent to hydration the pollen grains may: 1) fail to germinate; 2) begin to germinate but produce a short, malformed pollen tube occluded with callose (a polysaccharide composed of 1,3 beta-glucans); or 3) produce a pollen tube that will grow in an abnormal manner, fail to penetrate the stigmatic surface, and deposit callose lenticules (adcrustations) in the pollen grain and/or tube (Dickinson and Lewis, 1973b; Heslop-Harrison, 1975).

Among SSI systems, stigmatic contact with an incompatible grain is followed by rapid deposition of callose lenticules in papillae (Dumas and Knox, 1983). The rejection reactions are very specific and occur between one pollen grain and an adjacent papilla (or papillae). They do not diffuse throughout the whole stigma (Heslop-Harrison, 1975). Callose production was detected following incompatible matings for the composites Cosmos bipinnatus (Howlett et al., 1975) and Helianthus annuus (Vithanage and Knox, 1977a,b), and many crucifers including Brassica oleracea (Kerhoas et al., 1983), Iberis sempervirens (Heslop-Harrison et al., 1974), and Raphanus campestris (Dickinson and Lewis, 1973). However, little or no callose was observed following compatible crosses among these specie

The incompatibility determinants

Since the incompatibility specificity of pollen is determined by the diploid sporophyte in SSI systems, it is logical to conclude that recognition substances are synthesized prior to anthesis. Pandey (1958, 1960) proposed that for SSI systems the S-alleles are expressed at the latest after meiosis but before the cleavage of the microspore mother cell cytoplasm. In an alternative hypothesis, Heslop-Harrison (1967, 1968a) and Knox et al. (1970) suggested that incompatibility substances in SSI systems are not synthesized by the meiocyte but rather by adjacent sporophytic tissue, the tapetum. Substances synthesized by the tapetum are held in cisternae of the endoplasmic reticulum, released upon tapetal degeneration, and accumulate in the outer (exine) layer of the pollen wall (Dickinson and Lewis, 1973; Heslop-Harrison, 1975; Heslop-Harrison et al., 1973). The pollen exine in composites is sculptured in such a way as to form cavities (tectum) which are admirably adapted to receive and hold externally-derived materials (Heslop-Harrison, 1968b). Among nontectate grains, such as those in the Cruciferae, tapetally-derived proteins are deposited in depressions on the exine surface (Dickinson and Lewis, 1973).

Exine-held proteins are capable of diffusing freely to the stigmatic surface within seconds after pollen grain hydration (Heslop-Harrison, 1975). In the Cruciferae, exine-held proteins bind to the pellicle within 10-15 minutes after pollination and thereafter cannot be removed easily by leaching (Heslop-Harrison et al., 1974).

It is known that tapetally-derived, wall-held proteins carry the incompatibility specificity, and evidence for this has come use of the

"pollen-printing" and other techniques (Heslop-Harrison et al., 1974). In pollen-printing, pollen grains are placed in contact with a hydrated agar film thus permitting wall-held materials to diffuse out into the film. The proteins may be identified by staining, enzymatic activity, or immunofluorescence (Heslop-Harrison et al. 1973). Agar films containing exine-held (sporophytic) diffusates from Iberis semperflorens pollen stimulated callose deposition when films were placed on a stigma of a plant whose genotype was incompatible with the pollen parent (Heslop-Harrison et al., 1974). Using alternative techniques, Dickinson and Lewis (1973) extracted pollen wall-held (tryphine) materials by centrifugation and stimulated production of callose by applying the extracts to stigmas of plants incompatible with plants from which the tryphine was derived.

Biochemical characterization of pollen wall fractions for Cosmos bipinnatus (Compositae) (Howlett et al., 1975) and Iberis semperflorens (Cruciferae) (Heslop-Harrison et al., 1974) was performed using thin-layer gel chromatography, polyacrylamide gel electrophoresis, and gradient diffusion tests against rabbit antibodies prepared against total leachates. The principal constituents identified were proteins (or glycoproteins) with a molecular weight in the range of 10-40 kilodaltons for Cosmos and 10-20 kilodaltons for Iberis. Disappointingly, attempts to detect differences in the pollen proteins of SSI species that might reflect incompatibility genotypes have been unsuccessful to date, and thus the pollen determinants of incompatibility are still elusive.

On the stigma, the proteinaceous pellicle constitutes the receptor site for pollen grains and their associated pollen wall diffusates (Heslop-Harrison et al., 1975; Mattsson et al., 1974; Vithanage and Knox, 1977a). The cuticle has discontinuities which offer communication between the pellicle surface and protoplasts of the papillae, and water needed for hydration of the pollen grains travels through these discontinuities (Mattsson et al., 1974, Heslop-Harrison et al., 1975). Proteins within the pellicle are apparently synthesized in microbodies in the cortical cytoplasm of the papillae (Heslop-Harrison et al., 1975). Recently, however, the accepted view that the superficial pellicle is the only component of the papillae active in the SI response (Mattsson et al., 1974) was challenged by results obtained by electron microscopy (EM) which suggested that the "pellicle and underlying cuticle would act together as a sealing layer through which the pollen grain would gain access to the molecules" (Dickinson, 1985). Further results from a new EM technique involving dry fixation (Elleman and Dickinson, 1985) have shown the papillar cell wall contains a palisade layer of subcuticular vesicles, thus supporting the "glandular" model for papillar structure advanced by Dickinson (1985).

In contrast to the inconclusive results obtained with pollen SI determinants, S-allele specific glycoproteins have been identified from stigmas of Brassica oleracea (Nasrallah and Wallace, 1967; Nishio and Hinata, 1977). A breakthrough was achieved by Nasrallah et al. (1985) when they cloned a cDNA sequence representing an abundant style-specific mRNA in Brassica oleracea which encoded S-allele specific glycoproteins.

Although S-allele-specific glycoproteins have been detected in stigma extracts by various methods (Dickinson and Roberts, 1985), their role in the SI reaction has not yet been elucidated.

The role of callose in SSI systems

Callose is an uncolored, gelatinous, amorphous, isotropic polysaccharide composed of 1,3 beta-glucans or a combination of 1,3 and 1,4 beta-linked glucans (Dumas and Knox, 1983; Herth et al., 1974; Vithanage et al., 1980). Production of callose is a response to the S-gene expression: it occurs in stigmatic (papillae) cells and pollen tubes following incompatible matings in SSI systems, but is not present (or occurs in low levels) in compatible matings (Dickinson and Lewis, 1975; Heslop-Harrison 1974, 1975; Heslop-Harrison et al., 1973; Howlett et al., 1975). Heslop-Harrison (1967) and Heslop-Harrison et al. (1973, 1974, 1975) showed that pollen proteins held in the interbacular cavities of the exine elicit the callose production. As discussed previously, these pollen wall proteins originate from the tapetum and are released when pollen is transferred to the stigmatic surface. It has been proposed that callose is synthesized by cytoplasmic vesicles during formation of the wall investing the tetrad of microspores and that control over the callose synthesis may be exerted by surface receptors in the pellicle (Dickinson, 1970). These receptors probably control the incompatibility reaction (Kerhoas et al., 1983) and also cause callose production (Dumas and Knox, 1983).

Callose is hypothesized to play both active and passive roles in the incompatibility reaction (see review by Dumas and Knox, 1983):

1) callose synthesis utilizes carbohydrates and other substrates that would otherwise be used for pollen tube growth (Currier, 1957; Sedgley, 1977); 2) callose accumulation in incompatible pollen tubes may be due to changes in the concentration of wall-synthesizing and wall-degrading enzymes responsible for pollen tube growth (Reynolds and Dashek, 1976; Dumas and Knox, 1983); and 3) callose protects against dehydration (Barskaya and Balina, 1971; Vithanage et al., 1980) and thus may form a barrier preventing hydration of incompatible pollen or penetration of pollen tubes (Heslop-Harrison, 1975). Callose has a remarkable water binding capacity: 84% of its dry weight may be lost on lyophilization (Vithanage et al., 1980).

The genetics of SI in the Compositae

A considerable amount of experimental data has been collected on the Compositae which is of major interest in these studies. Several species of the Compositae have been shown to possess an SSI system. Sporophytic SI systems with polyallelic control at a single locus were first described for Crepis foetida (Hughes and Babcock, 1950) and Parthenium argentatum (Gerstel, 1950). Monofactorial-polyallelic SI systems have also been reported for Ageratum houstonianum (Stephens et al., 1982), Calotis cuneifolia (Davidson and Stace, 1986), Carthamus flavescens (Imrie and Knowles, 1971), Cichorium intybus (Eenink, 1981b), Chrysanthemum carinatum (Jain and Gupta, 1960), Chrysanthemum cinerariaefolium (Brewer, 1974), Cosmos bipinnatus (Crowe, 1954), and Senecio cruentus (Barkley, 1968). Polyallelic control at more than one locus was proposed for Helianthus annuus (Habura, 1957; Shchori, 1969)

and Chrysanthemum morifolium (Zagorski et al., 1983). The presence of at least three genes exerting control over the SI system in the latter species is easily explained by the fact that C. morifolium is hexaploid (Zagorski et al., 1983). In contrast, H. annuus is diploid and the postulated two-loci SI system contrasts with SI systems reported for other diploid composites.

Dominance of S-alleles in pollen and independence (expression of both S-alleles) in the pistil are the most common allelic relationships reported among species characterized by SSI systems (de Nettancourt, 1977). Among the Compositae, independence of alleles in the pistil was reported for Carthamus flavescens (Imrie and Knowles, 1971), Chrysanthemum carinatum (Jain and Gupta, 1960), Chrysanthemum cinerariaefolium (Brewer, 1974), Crepis foetida (Hughes and Babcock, 1950), and Parthenium argentatum (Gerstel, 1950); however, both dominance and independence of alleles in the pistil were hypothesized for Cichorium intybus (Eenink, 1981) and Cosmos bipinnatus (Crowe, 1954), and a linear dominance series with no independence was reported for Ageratum houstonianum (Stephens et al., 1982). In all SI genetic studies reported for Compositae species, either dominance and independence of S-alleles or a linear dominance series were found for allelic relationships in the pollen.

SI in the genus Zinnia (Compositae - Heliantheae tribe)

Limited information on SI has been published for the genus Zinnia. Zinnia pistils have papillate stigmas with little or no secretory exudates at maturity and trinucleate pollen (Pullaiah, 1981), and these

characteristics are typical of species exhibiting a SSI system (Heslop-Harrison, 1975). Self-pollination tests of most of the 17 Zinnia species showed that SI is common within the genus. Torres (1962) studied the subgenus Diplothrix and found 5 among 6 species in the subgenus to be completely SI, including Z. acerosa, Z. anomala, Z. citrea, Z. grandiflora, and Z. juniperifolia. The sixth species, Z. oligantha, was regarded as strongly SI (Torres, 1964). Later, Olorode (1970) determined 4 of the 8 species assigned to the subgenus Zinnia (section Mendezia) were SI; these species were Z. angustifolia, Z. gregii, Z. leucoglossa, and Z. littoralis. It was also suggested that Z. elegans (subgenus Zinnia, section Zinnia) was SI after difficulties in inbreeding were encountered (Pollard, 1939). Among Zinnia species, only Z. peruviana in subgenus Zinnia section Zinnia has been reported as self-compatible (Pollard, 1939; Torres, 1963).

Self-incompatibility in the aforementioned studies among Zinnia species was determined by seed set comparisons and pollen fertility was estimated by pollen stainability. The mode of inheritance of SI was not reported for any of the SI Zinnia species.

Recent evidence supports previous work indicating SSI in Z. angustifolia and Z. elegans. Callose was produced in pollen tubes and stigmatic papillae of both species in response to self pollinations (Boyle, 1986; Boyle and Stimart, 1986a,b). In Z. angustifolia, the percentage of florets with embryos 14 days following self-pollination ranged from 0.8 to 24.6%. In contrast, 68.1 to 80.6% of florets pollinated with compatible pollen contained embryos (Boyle and Stimart, 1986a,b). However, no studies have determined whether a

monofactorial-polyallelic SSI system exists in Z. angustifolia or Z. elegans.

Assessment of SI systems

There are a number of means of assessing whether SI systems are present following controlled crosses: 1) presence (or absence) of embryos or mature seed (Frankel and Galun, 1977); 2) pollen germination and/or tube cytology (Gerstel and Riner, 1950; Lewis, 1979); and 3) stigmatic callose responses, which are specific to SSI systems (Dumas and Knox, 1983; Heslop-Harrison et al., 1973, 1974; Kerhoas et al., 1983; Vithanage and Knox, 1977a,b; Vithanage et al., 1980).

Embryo and seed counting is the most common procedure used in assessment of incompatibility systems in the Compositae (Barkley, 1968; Davidson and Stace, 1986; Drewlow et al., 1973; Eenink, 1981b; Imrie and Knowles, 1971; Jain and Gupta, 1960; Stephens et al., 1982; Zagorski et al., 1983). The procedure demands little in terms of equipment and permits qualification (presence or absence) and quantification (relative strength of S-alleles) of SI systems. However, there are three disadvantages to the procedure: 1) the criterion for deciding whether a cross is compatible or incompatible may be arbitrary when percent seed set (or embryos observed) forms a continuum of classes in a frequency histogram rather than a discrete (or bimodal) distribution; 2) it may require an extended period of time (up to 60 days) before incompatibility can be assessed; and 3) its reliability may be confounded by post-zygotic lethal factors causing embryo abortion or apomixis (haploid or diploid parthenogenesis).

Cytoplasmic stains have been used for assessment of SI systems among numerous Compositae species, including Cosmos bipinnatus (Crowe, 1954), Crepis foetida (Hughes and Babcock, 1950), and Parthenium argentatum (Gerstel 1950). Pollen germination and tube cytology can be observed with the use of various stains, e.g., with acid fuchsin-light green in lactophenol (Lewis, 1979), or aniline blue in lactophenol (Rawlins, 1933). Pollinated pistils (fixed or fresh) are stained and viewed at high light intensity under normal light microscopy; the cytoplasm of viable, incompatible pollen grains will stain pink (with acid fuchsin-light green) or blue (with aniline blue), whereas compatible pollen will be unstained (devoid of cytoplasm) with empty pollen tubes penetrating the stigma (Lewis, 1979). Another stain is acetocarmine and basic fuchsin (Chandler, 1931); pollen tube cytoplasm will be dark red and surrounding tissue lightly stained. Cytoplasmic staining methods allow a more rapid assessment of SI compared to embryo or seed counts, but they may not provide a clear distinction between compatible and incompatible pollinations (Eenink, 1981a). In addition, cytoplasmic stains result in general staining of the entire style, whereas SI responses specific to SSI systems have localized reactions, often between a single stigmatic papilla and a pollen grain (Heslop-Harrison, 1975).

A third means of assessing SI is based on callose production. The most widely used technique for determination of callose in SI research is the decolorized aniline blue fluorescence (ABF) method. Aniline blue is a triarylmethane dye containing several minor impurities, among which is the fluorochrome which complexes with callose and other cell wall

polysaccharides (Eschrich and Currier, 1964). There are 2 ABF protocols currently in use: 1) pistils are stained with 0.005 or 0.01% aniline blue prepared in 0.15M K_2HPO_4 (pH approx. 8.2) (Currier, 1957); and 2) pistils are stained in 0.05 or 0.1% aniline blue prepared in 0.1M K_3PO_4 (pH approx. 12.4) (Martin, 1959; du Crehu, 1968).

Among Compositae species, the ABF method has been used for the study of SI in Calotis cuneifolia (Davidson and Stace, 1986), Chrysanthemum cinerariaefolium (Brewer, 1974), Cosmos bipinnatus (Knox, 1973), Helianthus annuus (Vithanage and Knox, 1977a,b), and Zinnia species (Boyle and Stimart, 1986a,b).

The advantages of the ABF method are: 1) the specificity of callose stigmatic responses to SSI systems; 2) the rapidity of callose production, which has been reported to appear within two minutes of treatment of Brassica oleracea stigmas with self-pollen diffusates (Kerhoas et al., 1983); and 3) the high intensity of callose fluorescence that is easily visualized using epifluorescence microscopy. The disadvantages of the ABF method are: 1) callose production is variable, i.e., it is dependent on the strength of the SI alleles in incompatible sporophytes (Boyle and Stimart, 1986b; Boyle, 1986; Kerhoas et al., 1983), 2) it has been reported to occur following some compatible matings in Brassica (Ockendon, 1986); and 3) the microscopical equipment needed for analysis is costly.

Although callose can also be identified using normal light microscopy by staining with diachrome resorcin blue (Eschrich and Currier, 1961) or lacmoid-martius yellow (Nebel, 1931), the ABF method

appears to be the best procedure. With the ABF method, callose fluoresces bright yellow against a dark background.

CHAPTER 2

TECHNIQUES FOR ASSESSING AND QUANTIFYING SELF-INCOMPATIBILITY

IN ZINNIA ANGUSTIFOLIA HBK (COMPOSITAE).

Introduction

Self-incompatibility (SI) is a genetically controlled mechanism preventing fertilization following self-pollination or matings between plants with identical incompatibility phenotypes. Plant breeders confronted with SI may be interested in: 1) introducing incompatibility into cultivars or breeding lines from either other lines or wild relatives; 2) eliminating incompatibility permanently from breeding material; 3) regulating the strength of incompatibility by either genetic or physiological means; or 4) utilizing incompatibility for hybrid seed production (Frankel and Galun, 1977). Knowledge of the genetics of incompatibility and identification of breeding line genotypes are prerequisites to efficient exploitation or elimination of incompatibility. Hence, techniques used to assess the presence (or absence) of SI and the relative strength of S-alleles are critical for genetic studies of SI systems.

Many species of the Compositae are economically important and thus significant to plant breeders. Three methods are used currently for assessment of SI systems among Compositae species: 1) embryo or seed counts; 2) pollen tube behavior observed on stigmas or within styles, using normal light microscopy and cytoplasmic stains such as acid fuchsin-light green (Lewis, 1977) or aniline blue in lactophenol (Darlington and La Cour, 1942); and 3) callose production in stigmatic

papillae and pollen tubes, using incident-light fluorescence microscopy and decolorized aniline blue as a stain (Dumas and Knox, 1983; Heslop-Harrison et al., 1973). Although callose deposition in stigmatic papillae and pollen tubes has been proposed by Heslop-Harrison et al. (1973, 1974) as a bioassay for SSI systems in the Cruciferae and Compositae, limited data has been published to support routine use of this procedure. Additionally, no quantitative measurements of the fluorescence emitted from stigmatic callose following compatible and incompatible crosses have been reported.

Zinnia (Heliantheae tribe) is a New World genus comprised of 17 species (Torres, 1963), 11 of which have been reported as SI (Olorode, 1970; Pollard, 1939; Torres, 1962, 1963, 1964). Olorode (1970) performed selfing tests on Z. angustifolia HBK and, using seed count data, concluded the species was SI. Boyle and Stimart (1986a,b), utilizing incident-light fluorescence microscopy (ABF method), embryological observations, and seedling emergence data, confirmed the presence of SI in Z. angustifolia. Production of callose in stigmatic papillae of Z. angustifolia following self-pollinations suggested sporophytic control of SI (Boyle and Stimart, 1986a,b).

The objectives of the following study were to: 1) compare 4 procedures, i.e., embryo set, aniline blue in lactophenol as a cytoplasmic stain with normal light microscopy, the ABF method, and quantification of the callose fluorescence via photometry, in assessing the SI system in Z. angustifolia; and 2) determine the limitations and strengths of each procedure.

Materials and Methods

All experiments were performed at the University of Massachusetts, Amherst, MA (42° 22.5' N latitude). Three seedlings of Z. angustifolia were used as parental material. Seedlings were selected at random from 2 commercial cultivars and asexually propagated (cloned) by rooting terminal shoot cuttings under intermittent mist. Previous studies indicated the 3 clones (A01, AW1, and AW2) produced functional pollen and ovules (Boyle, 1986). Clones A01 and AW2 were reciprocally cross-compatible and highly SI, with self-pollinations resulting in less than 2% of florets containing embryos (Boyle and Stimart, 1986b). Clone AW1 was reciprocally cross-compatible with A01 and AW2, and partially self-compatible, with 22-29% of self-pollinated florets containing embryos (Boyle and Stimart, 1986b; Boyle et al., 1987).

An F₁ population was generated using A01 as the maternal parent and AW2 as the paternal parent, and 14 seedlings (designated as A1 through A14) were selected randomly and cloned as described previously. Preliminary crosses indicated the 14 F₁ clones produced functional pollen and ovules.

Techniques used in culture of plant material were similar to those reported by Boyle and Stimart (1983). Photoperiod regulation of plant growth during fall, winter and early spring was provided by supplemental incandescent irradiation from 1600 to 2200 HR (12 $\mu\text{mol s}^{-1} \text{m}^{-2}$). Greenhouse temperatures were maintained at a minimum of 18°/20°C (night/day).

Inflorescences were emasculated by removing hermaphroditic disk florets 1-3 days prior to anthesis, and each inflorescence was covered

with a glassine bag following emasculation. Pollinations were performed 1-3 days after emasculation by transferring pollen from disk florets to expanded stigmas of the female parent using a fine-tip paint brush; inflorescences were rebagged after pollination. The exact amount of pollen applied to each stigma could not be controlled, but pollinations were equalized by covering the receptive area with excess pollen. Four experiments were conducted.

Embryological observations

Three F_1 clones (A6, A10, and A13) were: 1) self-pollinated; 2) backcrossed (as females) to both parents; and 3) outcrossed (as females) to clone AW1. Clones A10 and A13 were also crossed (as females) with 1-2 additional F_1 clones. A minimum of 100 florets were pollinated for each self or cross.

Embryological observations were performed 21 days after pollinations by excising ovules and removing the embryo sac under a dissecting microscope (25X). Preliminary experiments demonstrated embryos at 21 days were approximately 2 mm in length and easier to distinguish compared to 14 days after pollination (Boyle and Stimart, 1986a,b). Presence or absence of an embryo was recorded for each dissected floret. Percent embryo set was calculated for each self and cross.

Cytoplasmic staining using aniline blue in lactophenol

Clones A6, A10, and A13 were selfed and outcrossed to a compatible Z. angustifolia clone (compatibility verified by results in Expt. 1). Twenty-four hr following pollinations, styles were removed and fixed in

Carnoy's fluid (6:3:1 of 95% ethanol, chloroform and glacial acetic acid, respectively) for 24 hr or longer. Styles were washed in distilled water, softened for 5-6 hr in 8N NaOH, rewashed in distilled water, and cleared overnight in 86% lactic acid. Material was washed again in distilled water and stained with 0.01% aniline blue in lactophenol (Darlington and La Cour, 1942) for 20-30 min. Styles were mounted in a drop of lactic acid and viewed under high light intensity using normal light microscopy.

Counts were made on the number of: 1) germinated pollen grains devoid of cytoplasm (unstained); 2) germinated pollen grains full of cytoplasm (stained); 3) ungerminated pollen full of cytoplasm (stained); and 4) pollen grains adhering to the stigma (pollen load). For each self and cross pollination, pollen on 1 stylar branch of 10 styles was counted.

For each F_1 clone, heterogeneity chi-square tests were performed to compare number of pollen grains observed in the first 3 classes (described above) for incompatible versus compatible crosses. The analysis of variance (ANOVA) procedure was used to compare pollen load for incompatible versus compatible crosses for each F_1 clone.

Experiments using acid fuchsin-light green as a cytoplasmic stain (Lewis, 1979) gave results similar to aniline blue in lactophenol. However, the resolution obtained with aniline blue was superior and thus the latter stain was utilized for assessment of SI.

Aniline blue fluorescence (ABF) method

The F₁ clones used in Expt. 2 were again selfed, backcrossed to both parents, and outcrossed to a compatible clone. In addition, the following crosses were performed: AW1 selfed, AW2 selfed, AW1xAW2 and AW2xA01; these crosses were previously studied by Boyle and Stimart (1986b) using the ABF method and served as controls. Twenty-four hr following pollinations, styles were removed from florets, fixed, and softened as described previously in Expt. 2. Material was then cleared for 30-60 min in 86% lactic acid, washed with distilled water, and finally with 0.025M Trisglycine buffer (pH 8.4) to decrease quenching of fluorescence (Mulcahy and Mulcahy, 1987). Styles were stained with a 0.1% solution of aniline blue dissolved in 0.1M K₃PO₄ (du Crehu, 1968; Martin, 1959) for a minimum of 1-2 hr. Callose resolution was optimum in styles stored at 2°C for 24 hr or more in 0.1% aniline blue solution (Boyle and Stimart, 1986b). Styles were mounted in a drop of 0.1% aniline blue and covered with a cover slip, but not squashed. Incident-light fluorescence microscopy was performed using a Zeiss microscope equipped with a 100W high pressure Hg lamp and filter set for UV-violet light excitation (exciter filter BP 395-425, dichromatic beam splitter FT 425, and barrier filter LP 450).

Observations using the ABF method were: 1) pollen load, estimated by counts of pollen on 1 stylar branch for 8 styles; 2) number of germinated pollen grains, i.e., the presence or absence of pollen tubes, estimated by counts on ca 8 styles; 3) average length of pollen tubes, measured on 15 random pollen grains using an ocular micrometer at 200X; 4) degree of inhibition of pollen tubes attempting penetration of

stigmatic surface and pollen tube behavior (i.e., whether pollen tubes growth was normal or aberrant), with measurements performed on 8 styles; and 5) relative intensity of callose fluorescence in pollen tubes and stigmatic papillae, with measurements on 8 styles.

Pollen tube lengths were measured using the ABF procedure rather than aniline blue in lactophenol due to superior resolution of individual tubes with the former procedure. Pollen load and pollen tube length data were analyzed statistically by the ANOVA procedure.

Quantitative determination of callose fluorescence

The F_1 clones used in Expt. 2 were self-pollinated and outcrossed to compatible clones. In order to determine the response of the parental clones, the following pollinations were also performed: A01 selfed, AW2 selfed, and AW2xA01. Pollinated styles were fixed, softened, cleared, and stained as described previously under Expt. 3.

Quantification of callose fluorescence was obtained with a photomultiplier connected to a Zeiss Standard microscope equipped for incident-light fluorescence microscopy. Measurements were compiled by a computer using the Zeiss manual photometry program (MPP2) software package. A pinhole of 0.1 mm diameter and 10x objective were used for all measurements, and a regular white business card (Linen finish, Butter Paper Co., Holyoke, MA) was used as a reference standard to set the 100% value for the MPP2 program. Preliminary studies indicated that a white business card was superior to other materials tested and showed minimal photodegradation (quenching) and minimal variation in fluorescence intensity over time.

For incompatible crosses, the fluorescence intensity of 10 contact points between pollen grains and stigmatic papillae (i.e., where callose lenticules accumulated) was measured on 5 styles. For compatible crosses, the fluorescence intensity of 10 contact points between individual pollen tubes and adjacent papillae was measured on 5 styles.

Fluorescence intensity data were analyzed as a completely randomized design. Five stigmas (replications) were pollinated and 10 measurements (sampling units) per stigma were performed for each treatment (compatible or incompatible mating), with 2 treatments for each F_1 clone and 3 for parental clones. A hierarchical classification was used to separate treatment effects (incompatible and compatible crosses), experimental error (variation among measurements of different stigmas of the same cross), and sampling error (variation among measurements on the same stigma) (Steel and Torrie, 1980). Data was subjected to logarithmic transformation prior to statistical analysis (ANOVA) due to significant differences between variances for compatible and incompatible crosses.

Simple correlations (r values) were calculated between % embryos and: 1) pollen load (Expts. 2 and 3); 2) number of germinated pollen grains (Expt. 2); 3) pollen tube length (Expt. 3); and 4) fluorescence intensity (Expt. 4) (Steel and Torrie, 1980).

Results

Embryological observations

Percentage of florets with embryos following self- or incompatible cross-pollinations ranged from 0 to 9.9% for the 3 F₁ clones, and from 0.8 to 1.7% for the parental clones A01 and AW2 (Table 1). In contrast, 24.6% of florets contained embryos following self-pollination of clone AW1. Percentage of florets with embryos following compatible matings was higher than for incompatible pollinations and ranged from 55.5 to 87.1% for the 3 F₁ clones, and from 63.4 to 85.7% for the parental clones.

Cytoplasmic staining using aniline blue in lactophenol

The number of germinated pollen grains devoid of cytoplasm (unstained) was low for both incompatible and compatible crosses, with 0-0.3 grains per stylar branch observed for incompatible crosses and 1.3-2.8 per stylar branch for compatible crosses (Table 2; Fig.1a and b). More germinated pollen with cytoplasm (stained) was present than germinated, unstained pollen: 6.1-12 and 11.6-32 germinated and stained pollen grains were observed per stylar branch for incompatible and compatible crosses, respectively. The total number of germinated grains (stained and unstained) per stylar branch was positively correlated ($r = 0.89$) to % embryos observed following crosses (Expt. 1 data).

Number of ungerminated pollen grains with cytoplasm was 5.7-12.1 per stylar branch for incompatible crosses and 11.7-25.6 per stylar branch for compatible crosses (Table 2). For all 3 F₁ clones, heterogeneity χ^2

tests for compatible versus incompatible crosses were significant ($P < 0.05$), indicating major differences in the frequencies of pollen grains observed in the 3 classes for compatible and incompatible matings.

For the 3 F_1 clones, pollen load was significantly higher following compatible crosses compared to incompatible crosses (Table 3; Fig. 2a and b). Pollen load ranged from 18.8 to 27.5 per stylar branch for incompatible crosses and from 46.8 to 96.7 per stylar branch for compatible crosses (Table 3 and Fig. 2). A high positive correlation ($r = 0.96$) was observed between pollen load and % embryos observed following crosses (Expt. 1).

Microscopical observations revealed some incompatible pollen tubes ruptured at the apices and released cytoplasm onto the stigmatic surface (Fig. 1a). It was also noted that pollen tube behavior was easily observed when pistils stained with aniline blue in lactophenol were viewed under incident-light fluorescence using blue light excitation (same as Expt. 3). Using this protocol, pollen grains and pollen tubes appeared dark blue on a yellow background (the stigma), and ruptured pollen tubes with released cytoplasm were clearly observed with this technique (Fig. 2c). Unfortunately, pollen tubes in the stylar transmitting tissue were not observable under either normal light or incident-light fluorescence microscopy with aniline blue in lactophenol as a stain (Fig. 1 and 2).

Aniline blue fluorescence (ABF) method

Generally, pollen germination was low (5-15%) for incompatible crosses and high (>70%) for compatible crosses. However, pollen germination was intermediate (40-60%) for both the incompatible cross A10xA01 and the compatible cross AW1xAW2 (Table 4).

Pollen tube growth following compatible crosses was normal, except for a few abnormal pollen tubes which were detected among some crosses (Table 4). Normal pollen tubes were not associated with stigmatic callose production and were readily observed penetrating the stigmatic surface (Fig.3a). Compatible pollen tubes ranged from 30.5-38.9u in length and were significantly longer than incompatible tubes ($P < 0.001$), with the exception of the incompatible cross AW1 selfed and compatible cross AW2xA01 for which differences in tube lengths were not significant (Table 5). Pollen tube length was highly correlated ($r = 0.94$) with % embryos observed following crosses (Expt. 1 data).

Pollen tube growth following incompatible crosses was typically abnormal (Table 4). Abnormal pollen tubes were characterized by their short length and stubby apices, or by distorted and/or twisted growth, and were most often associated with stigmatic callose production (Fig.3b). The length of incompatible pollen tubes ranged from 23.4-28.2u (Table 5). Rarely, abnormal pollen tubes were observed which appeared to penetrate the stigmatic papillae. In some incompatible crosses, a few normal pollen tubes were observed, but their numbers were insignificant compared to the number of abnormal pollen tubes.

Fluorescence of callose in pollen tubes of incompatible crosses was mostly intense, but varied considerably for some crosses (Table 4). For

compatible crosses, callose fluorescence in pollen tubes was characteristically variable and ranged from very low to intense. Callose was not detected in compatible pollen tubes which had entered the stylar transmitting tissue, as reported previously by Boyle and Stimart (1986a,b).

Stigmatic callose fluorescence was a localized response involving 1 or a few papillae (Fig.3c and d). Stigmatic responses following incompatible crosses were characterized by many callose lenticules exhibiting intense fluorescence and some with intermediate fluorescence. For two crosses (A13xAW2 and AW2xAW2), however, the number of callose lenticules was variable with some styles having fewer lenticules than others. Callose lenticules were either not present in papillae or few in number following most compatible matings (Table 4). For some compatible crosses, some variation between styles was apparent, with the number of callose lenticules varying from few to many and the lenticules usually intense in fluorescence.

Pollinations were made very carefully in order to avoid any production of wound callose in stigmatic papillae (Kerhoas et al., 1983). However, some wound callose was still apparent on some stigmas, but it was relatively easy to differentiate from callose produced due to presence of incompatible pollen: wound callose was mostly diffuse, encompassed many papillae, and fluoresced faintly, whereas callose elicited by SI responses was restricted to few papillae and generally fluoresced intensely.

Pollen loads for incompatible crosses were low and ranged from 12.1 to 42.6 grains per stylar branch (Table 5). Pollen loads for compatible

crosses were significantly higher than for incompatible crosses ($P < 0.001$), and ranged from 49.3 to 92.1 grains per stylar branch.

Quantitative determination of callose fluorescence

Mean fluorescence intensity for incompatible crosses was significantly higher than compatible crosses ($P < 0.01-0.001$), and ranged from 47.90% to 62.64% for incompatible and from 6.42% to 9.94% for compatible crosses (Table 6). A high negative correlation was observed ($r = -0.95$) between mean fluorescence intensity and % embryos observed following crosses (Expt. 1). F-tests for experimental error (using sampling error mean square as a divisor) were significant for all ANOVAs (Table 7), indicating substantial variation in fluorescence was present among stigmas receiving the same pollen source.

Discussion

Selection of appropriate methodology for assessment of SI systems is based on many factors, including applicability, complexity, economics, and reliability of technique. Embryo or seed counting demands little in terms of equipment and permits qualification (presence or absence) and quantification (relative strength of S-alleles) of SI systems. However, there are disadvantages to the procedure: 1) the criterion for deciding whether a cross is compatible or incompatible may be arbitrary when percent seed set (or embryos observed) forms a continuum of classes in a frequency histogram rather than a discrete (bimodal) distribution; 2) an extended period of time may be required following pollination before incompatibility can be assessed; and 3) the reliability may be

diminished by suboptimal environmental conditions, apomixis (haploid or diploid parthenogenesis), or post-zygotic lethal factors causing embryo abortion. Despite its limitations, embryo or seed counting is the most common procedure used in assessment of SI systems among Compositae species (Barkley, 1968; Davidson and Stace, 1986; Drewlow et al., 1973; Eenink, 1981b; Imrie and Knowles, 1971; Jain and Gupta, 1960; Stephens et al., 1982; Zagorski et al., 1983). Embryological observations in the present study yielded results similar to those reported by Boyle and Stimart (1986a,b) and revealed discrete differences between compatible and incompatible matings for Z. angustifolia, with certain cross-pollinations consistently failing to produce embryos. These responses are thus correlated positively with S-gene expression. Although embryological observations require considerable involvement with emasculation, pollination, and dissection, the data obtained is mostly unambiguous and provides reference data for comparing other methods used for assessment of SI.

Cytoplasmic staining methods allow a more rapid analysis of SI compared to embryo or seed counts, and have been used for assessment of SI systems among the Compositae species Cosmos bipinnatus (Crowe, 1954), Crepis foetida (Hughes and Babcock, 1950), Parthenium argentatum (Gerstel 1950), and Senecio vulgaris (Warren et al., 1988). The disadvantages associated with cytoplasmic staining methods (and microscopical methods in general) are that: 1) they may not always provide a clear distinction between compatible and incompatible pollinations, as reported for Cichorium intybus (Eenink, 1981a); and 2) the floral morphology of some species may technically prohibit the emasculation

required for controlled pollinations unless male sterile lines are available.

In Crepis foetida (Hughes and Babcock, 1950) and Parthenium argentatum (Gerstel and Riner, 1950), differences in pollen germination were observed between compatible and incompatible crosses, and this criterion was used to determine compatibility. Other studies, e.g. in Cichorium intybus (Eenink, 1981a) and in Brassica oleracea (Ockendon, 1972), were unable to distinguish incompatible and compatible matings based on the frequency of germinated pollen grains. In the present study, compatible and incompatible matings were correlated to the ratios of pollen classified as germinated and unstained, germinated and stained, and ungerminated and stained (Table 2). Germinated and unstained pollen grains were thought initially to be a specific characteristic of compatible crosses, but were difficult to identify and infrequently observed. However, the total number of germinated grains (unstained and stained) was 2-3 times greater following compatible matings as compared to incompatible matings (Table 2), and was correlated positively with % embryos observed following crosses ($r = 0.89$). Thus, the number of germinated grains is perhaps a better parameter for assessment of SI for Z. angustifolia.

Pollen load was quantified readily and considerably higher in compatible crosses compared to incompatible crosses (Table 3 and Fig.2a and b). For incompatible crosses, many pollen grains were dislodged from the stigma due to the staining procedure which involved multiple changes in solutions. This was due to the differential anchorage of compatible versus incompatible pollen: incompatible pollen germinates

less than compatible pollen and it does not normally penetrate the stigmatic surface. In addition, pollen load was highly correlated with % embryos observed ($r = 0.96$). Similar results were also reported for Crepis foetida (Hughes and Babcock, 1950), Parthenium argentatum (Gerstel and Riner, 1950), and Senecio vulgaris (Warren et al. 1987), and pollen load was used as the main parameter for assessment of SI among C. foetida and S. vulgaris. Considering that classification of pollen grains (Table 2) can be a very tedious and relatively time-consuming task, these results suggest that the best procedure for assessing SI in Z. angustifolia using cytoplasmic stains would be to use pollen load as the major criterion, followed by the number of germinated grains, and finally the occurrence of burst pollen tubes. The decision on whether a cross is compatible should not be based solely on 1 criterion when cytoplasmic stains are utilized, but rather should consider all three parameters of the incompatibility reaction.

The ABF method has been suggested as a phenotypic bioassay for incompatibility among SSI systems, with callose deposition in stigmatic papillae positively correlated to rejection phenomena (Heslop-Harrison et al., 1973; Dumas and Knox, 1983). The method provides numerous advantages over seed (embryo) counts and cytoplasmic staining procedures: 1) callose stigmatic responses are specific to SSI systems (Heslop-Harrison et al., 1973, 1974); 2) callose synthesis is rapid following incompatible matings, and has been reported to appear within 2 minutes of treatment of Brassica oleracea stigmas with self-pollen diffusates (Kerhoas et al., 1983); and 3) callose fluorescence is intense following incompatible matings, thus permitting easy

visualization using incident-light fluorescence microscopy. The primary disadvantage of the ABF method is that callose fluorescence is variable, i.e., it may be dependent on the strength of the SI alleles (Boyle and Stimart, 1986b; Kerhoas et al., 1983) and has also been reported to occur following some compatible matings in Brassica (Ockendon, 1986).

Among Compositae species, the ABF method has been used for the study of SI in Calotis cuneifolia (Davidson and Stace, 1986), Chrysanthemum cinerariaefolium (Brewer, 1974; Brewer and Parlevliet, 1969), Cosmos bipinnatus (Heslop-Harrison et al., 1973; Howlett et al., 1975; Knox, 1973), Helianthus annuus (Vithanage and Knox, 1977a,b), and Zinnia species (Boyle and Stimart, 1986b); among these species, however, it has been used as the sole method for SI assessment only for C.

cinerariaefolium. It should be noted that in the Compositae very few or no callose plugs are produced in compatible pollen tubes (Boyle and Stimart, 1986a,b; Eenink, 1981a; de Jong and Kho, 1982; Knox, 1973; Vithanage and Knox, 1977), a phenomenon which inhibits or prevents pollen tube identification in stylar tissue. Hence, the ABF method is restricted to assessing stigmatic responses for most Compositae species.

Observations using the ABF method with Z. angustifolia indicate incompatible crosses are generally associated with: 1) low pollen loads; 2) poor pollen germination; 3) intermediate to extensive amounts of stigmatic callose lenticules which generally fluoresce brightly; 4) abnormal behavior of pollen tubes; and 5) intense to intermediate fluorescence in pollen tubes. Compatible crosses are associated with: 1) high pollen loads; 2) high pollen germination, 3) no or very few callose lenticules on the stigma; 4) normal behavior of pollen tubes,

and 4) fluorescence in pollen tubes ranging from none to intermediate. The two most easily distinguished criteria separating compatible and incompatible matings were pollen load and fluorescence of the callose lenticules in stigmatic papillae.

Compatible pollen tubes were longer in comparison to incompatible tubes (Table 5), but pollen tube lengths may have been distorted by the coverslip and this may have decreased the accuracy of the measurements. Although pollen tube length was highly correlated ($r = 0.94$) with % embryos, we did not feel that this parameter was important for assessment of SI with the ABF method.

Fluorescence in pollen tubes was generally intense in incompatible crosses, but varied considerably. Variation in pollen tube fluorescence may have been due to differences in the relative strength of the corresponding S-alleles, with weak alleles producing less callose fluorescence, as has been reported for Brassica oleracea (Kerhoas et al., 1983) and suggested by Boyle and Stimart (1986b) for Z. angustifolia. Dickinson (1985), however, observed that Brassica oleracea genotypes with weaker S-alleles generated pollen tubes that were often thicker and contained more callose than among genotypes with stronger S-alleles. Whatever the reason, the variation in callose fluorescence within pollen tubes indicates this parameter is less reliable than stigmatic callose responses for assessment of SI for Z. angustifolia. Variability in callose deposition within pollen tubes was also reported by Dumas and Knox (1983) and Ockendon (1985), who noted that some callose may be found in compatible pollen tubes.

As observed using aniline blue in lactophenol, pollen loads following compatible matings were significantly higher than for incompatible matings (Table 5), and a high positive correlation ($r = 0.89$) was observed between pollen load and % embryos. Thus, pollen load appears to be an easily distinguished and reliable predictor of SI responses for Z. angustifolia. However, incompatible pollen loads varied considerably (Table 5), and these observations indicate the variable nature of adhesion of incompatible pollen to stigmatic papillae. Use of this parameter for assessment of SI responses in other species would require preliminary tests to determine if discrete differences existed between pollen loads for incompatible and compatible matings. Although minor variations were apparent, results of this study indicate stigmatic callose responses provide a convenient assay for incompatibility in Z. angustifolia, as reported previously (Boyle and Stimart, 1986a,b). Numerous callose lenticules were observed in stigmatic papillae following most incompatible crosses (Fig.3d), with fluorescence ranging from intermediate to intense. Variation in stigmatic callose fluorescence among incompatible crosses was attributable mainly to the number of lenticules present in papillae, and this variation may have been due to the amount of incompatible pollen each stigma received. A few lenticules with intense or faint fluorescence were observed on some styles receiving compatible pollen, and this may have been due to contamination from incompatible pollen or to variation inherent in the system, as suggested by Ockendon (1985). Quantitative analysis of callose fluorescence via photometry corroborated results of visual assessment and indicated highly

significant differences in % intensity between compatible and incompatible crosses. However, variation in intensity within compatible and incompatible crosses was also significant, and this was due mainly to the quenching of the fluorescence that occurs following exposure to UV. Quenching of callose fluorescence is an inherent feature of the ABF method, with incompatible crosses producing more stigmatic callose and exhibiting a greater degree of quenching compared to compatible crosses which produce very little callose and demonstrate correspondingly less quenching.

Quantification of callose fluorescence via photometry will require additional experimentation before it can be used routinely as a diagnostic tool for assessment of SI. The business card proved to be a useful reference standard for our purposes, but other researchers might want to rely on other more accurate standards which do not exhibit any quenching. However, quenching of fluorescence remains the major variable in the photometric quantification of callose fluorescence. Quenching was minimized in these experiments by blocking the UV beam after each measurement and by rapidly taking the measurements. Despite these precautions, variation in the fluorescent intensity within each cross was still evident. We may conclude that the photometric technique appears very promising and may prove very useful in assessment of incompatibility and S-allele strength.

In summary, use of microscopical methods for assessment of SI is feasible if each observed (or measured) parameter is highly correlated to the incompatibility responses. We stress the importance of keeping all parameters associated with the incompatibility reaction in mind when

trying to decide whether a cross is compatible or not. One should not try to identify a specific aspect of the incompatibility reaction but rather look at the overall response that occurs.

Table 1. Embryos observed 21 days following self- and cross-pollinations of 8 *Z. angustifolia* clones

Cross ♀x♂	Status	Florets pollinated	% Florets with embryos	Compatibility ^a
A6xA6	self	118	5.9	Inc
A6xA01	backcross	153	58.8	Comp
A6xAW2	backcross	107	0.0	Inc
A6xAW1	outcross	100	62.0	Comp
A10xA10	self	113	7.1	Inc
A10xA4	sibcross	101	86.1	Comp
A10xA9	sibcross	124	7.3	Inc
A10xA01	backcross	121	9.9	Inc
A10xAW2	backcross	118	72.9	Comp
A10xAW1	outcross	127	74.0	Comp
A13xA13	self	119	4.2	Inc
A13xA10	sibcross	109	87.1	Comp
A13xA01	backcross	117	55.5	Comp
A13xAW2	backcross	134	2.2	Inc
A13xAW1	outcross	136	64.0	Comp
AW1xAW1 ^b	self	171	24.6	Inc?
AW2xAW2 ^b	self	242	0.8	Inc
A01xA01 ^b	self	230	1.7	Inc
AW1xAW2	outcross	119	85.7	Comp
AW2xA01	outcross	110	63.4	Comp

^a Comp= compatible pollination; Inc= incompatible pollination

^b Embryological observations performed at 14 days after pollination.
Data from: Boyle, T.H., Stimart, D.P. (1986) Theor. Appl. Genet.
73:305-315

Table 2. Pollen characteristics determined using the cytoplasmic stain aniline blue in lactophenol and light microscopy. Pollen grains classified as: germinated and unstained (devoid of cytoplasm); germinated and stained (full of cytoplasm); or ungerminated and stained. Heterogeneity (H) X² values for ratios of pollen in the 3 classes for compatible versus incompatible pollinations

Cross ♀x♂	Compatibility ^a	Mean values ^b			HX ²
		Germinated and unstained	Germinated and stained	Ungerminated and stained	
A6xA6	Inc	0.0	6.1	8.7	
A6xAW1	Comp	1.3	11.6	11.7	10.77 ^{**c}
A10xA9	Inc	0.3	9.9	12.1	
A10xA4	Comp	1.8	31.2	25.6	8.22 [*]
A13xA13	Inc	0.0	12.0	5.7	
A13xA10	Comp	2.8	32.0	22.1	13.33 ^{**}

^a Comp= compatible pollination; Inc= incompatible pollination.

Compatibility determined by embryo counts

^b Pollen counted on 1 stylar arm of 10 pollinated pistils

^c Differences significant at 5% (*) or 1% (**) level by heterogeneity X² test

Table 3. Pollen load for 3 *Z. angustifolia* clones following compatible or incompatible pollinations. Pollinated pistils stained with aniline blue in lactophenol and viewed under normal light microscopy

Cross ♀x♂	Compatibility ^a	Mean pollen load ^b	Significance ^c
A6xA6	Inc	18.8	
A6xAW1	Comp	46.8	10.35**
A10xA9	Inc	27.5	
A10xA4	Comp	90.8	95.30***
A13xA13	Inc	19.9	
A13xA10	Comp	96.7	31.77***

^a Comp= compatible pollination; Inc= incompatible pollination. Compatibility determined by embryo counts

^b Pollen load counted on one stylar branch of 10 pollinated pistils

^c Differences significant at 1% (**) or 0.1% (***) level by F-test

Table 4. Pollen germination, pollen tube growth, and intensity of callose fluorescence in pollen tubes and stigmatic papillae, as determined by the aniline blue fluorescence (ABF) method. Numbers represent the most common responses observed for each cross

Cross (♀x♂)	Compatibility ^a	Pollen		Fluorescence	
		Germ. ^b	Tube growth ^c	Pollen tube ^d	Papillae ^e
A6xA6	Inc	1	4	1	2
A6xA01	Inc	3	1	4	5
A6xAW2	Comp	1	3	1	2
A6xAW1	Comp	3	1	4	4
A10xA10	Inc	1	4	1,2	2
A10xA01	Inc	2	4	1	1
A10xAW2	Comp	3	1	3	6
A10xAW1	Comp	3	2	4	4,5
A13xA13	Inc	1	3	1	1
A13xA01	Comp	3	2	4	4,5
A13xAW2	Inc	1	4	1	3
A13xAW1	Comp	3	2	4	5
AW1xAW1	Inc	1	3	1,4	1
AW2xAW2	Inc	1	3	1,4	3
AW1xAW2	Comp	2	1	3,4	6
AW2xA01	Comp	3	1	2,3	6

^a Comp= compatible cross; Inc= incompatible cross. Compatibility determined by embryo counts

^b 1 = little germination (5-15%); 2 = intermediate germination (40-60%); 3 = high germination (>70%)

^c 1 = normal; 2 = normal with a few abnormal tubes; 3 = abnormal with a few normal tubes; 4 = abnormal tubes

^d 1 = intense fluorescence; 2 = intermediate fluorescence; 3 = no fluorescence; 4 = variable fluorescence (intense to none)

^e 1 = many callose lenticles, intense fluorescence; 2 = many callose lenticles, intense and some intermediate fluorescence; 3 = many callose lenticles on some styles, few on others, intense fluorescence; 4 = little callose lenticles on few styles, intense fluorescence; 5 = little callose lenticles on few styles, faint fluorescence; 6 = no callose

Table 5. Pollen load and pollen tube length for compatible and incompatible crosses among 5 *Z. angustifolia* clones. Data determined using the aniline blue fluorescence (ABF) method

Cross (♀x♂)	Compatibility ^a	Pollen load ^b		Pollen tube length (u) ^c	
		Mean	Significanced ^d	Mean	Significance
A6xA6	Inc	12.1a ^e		26.2a	
A6xAW2	Inc	12.5a		24.2a	
A6xAW1	Comp	49.3b		36.9b	
A6xA01	Comp	52.8b	10.08***	37.3b	16.74***
A10xA10	Inc	20.4a		24.6a	
A10xA01	Inc	19.9a		24.2a	
A10xAW1	Comp	76.8b		36.5b	
A10xAW2	Comp	58.0b	15.66***	38.9b	28.32***
A13xA13	Inc	19.6a		25.0a	
A13xAW2	Inc	30.1a		24.2a	
A13xAW1	Comp	71.1b		35.3b	
A13xA01	Comp	79.6b	17.10***	33.7b	16.60***
AW2xAW2	Inc	42.6a		23.4a	
AW1xAW1	Inc	20.1a		28.2b	
AW2xA01	Comp	83.1b		30.5b	
AW1xAW2	Comp	92.1b	11.16***	35.3c	12.00***

^a Comp= compatible cross; Inc= incompatible cross. Compatibility determined by embryo counts

^b Pollen counted on 1 stylar arm from 8 pollinated pistils

^c Pollen tubes measured on 15 pollen grains per cross

^d Differences significant at 0.1% (***) level by F-test

^e Mean separation within columns by Duncan's multiple range test, 5% level

Table 6. Fluorescence intensity for compatible and incompatible crosses for 5 Zinnia angustifolia clones. Fluorescence intensity was measured using a Zeiss photomultiplier and manual photometry program

Cross (♀x♂)	Compatibility ^a	Fluorescence intensity(Z) ^b	Significance ^c
A13xA13	Inc	62.64	
A13xA10	Comp	9.36	565.76***
A10xA9	Inc	52.49	
A10xA4	Comp	9.94	780.85***
A6xA6	Inc	53.49	
A6xAW1	Comp	6.42	991.50***
AW2xAW2	Inc	62.10	
A01xA01	Inc	47.90	
AW2xA01	Comp	8.70	456.18***

^a Comp= compatible cross; Inc= incompatible cross.

Compatibility determined by embryo counts

^b Mean of 10 measurements on 5 styles

^c Analysis of variance performed on log-transformed data; differences significant at 0.1% (***) level by F-test

Table 7. Analysis of variance for the effect of compatible and incompatible crosses on intensity of callose fluorescence in pollen tubes and stigmatic papillae. Two crosses (1 compatible, 1 incompatible), 5 replications (stigmas), and 10 samples (measurements) per stigma performed for each F₁ clone. Three crosses (1 compatible, 2 incompatible) performed for parental clones

Clones	Source of variation	df	Sums of squares ^a	Mean squares	Computed F _b
Parents (AO1 & AW2)	Crosses	2	114.33	57.163	456.18***
	Experimental error	12	1.51	0.13	5.38***
	Sampling error	135	3.15	0.02	
A13	Crosses	1	92.81	92.81	565.76***
	Experimental error	8	1.31	0.16	4.77***
	Sampling error	90	3.10	0.03	
A10	Crosses	1	69.50	69.50	780.85***
	Experimental error	8	0.71	0.09	2.96**
	Sampling error	90	2.71	0.03	
A6	Crosses	1	112.89	112.89	991.49***
	Experimental error	8	0.91	0.11	3.63**
	Sampling error	90	2.82	0.03	

^a Analysis performed on log-transformed data

^b Significant at the 1% (**) or 0.1% (***) level

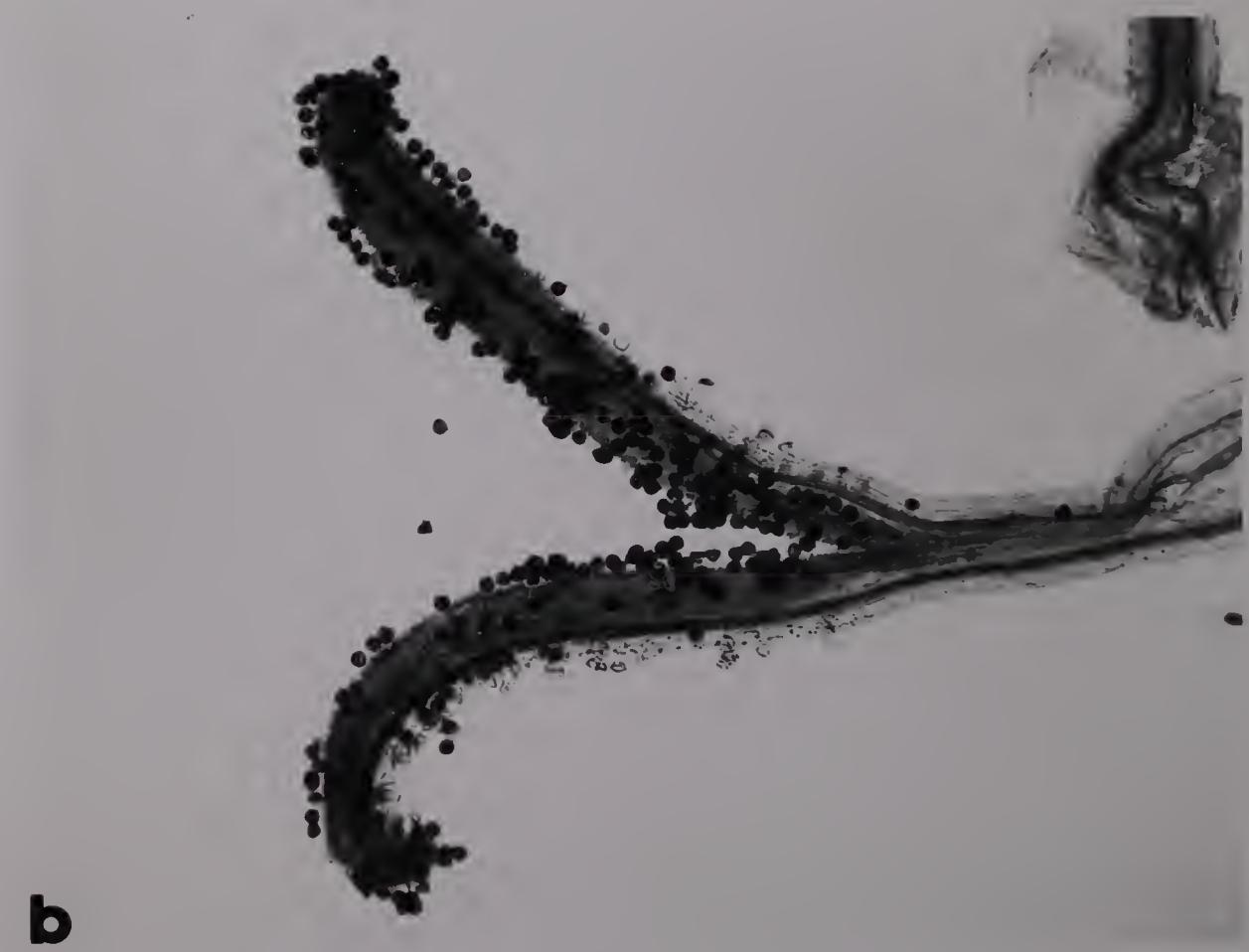
Fig. 1 a-b. Incompatible and compatible crosses of Zinnia angustifolia stained with aniline blue in lactophenol and viewed using normal light microscopy. **a.** incompatible mating (A13xA13) with stained pollen and burst pollen tubes (arrows); **b.** compatible mating (A13xA10) with stained (right, arrow) and unstained (left, arrow) germinated pollen.



Fig. 2 a-b. Incompatible and compatible crosses of Zinnia angustifolia stained with aniline blue in lactophenol and viewed using normal light microscopy. **a.** incompatible mating (A10xA9) with few adhering pollen grains; **b.** compatible mating (A13xA10) with many adhering pollen grains.



a



b

Fig. 2 c. Compatible cross of Zinnia angustifolia stained with aniline blue in lactophenol and viewed using UV epifluorescence microscopy. Compatible mating (A10xA4) with germinated pollen.

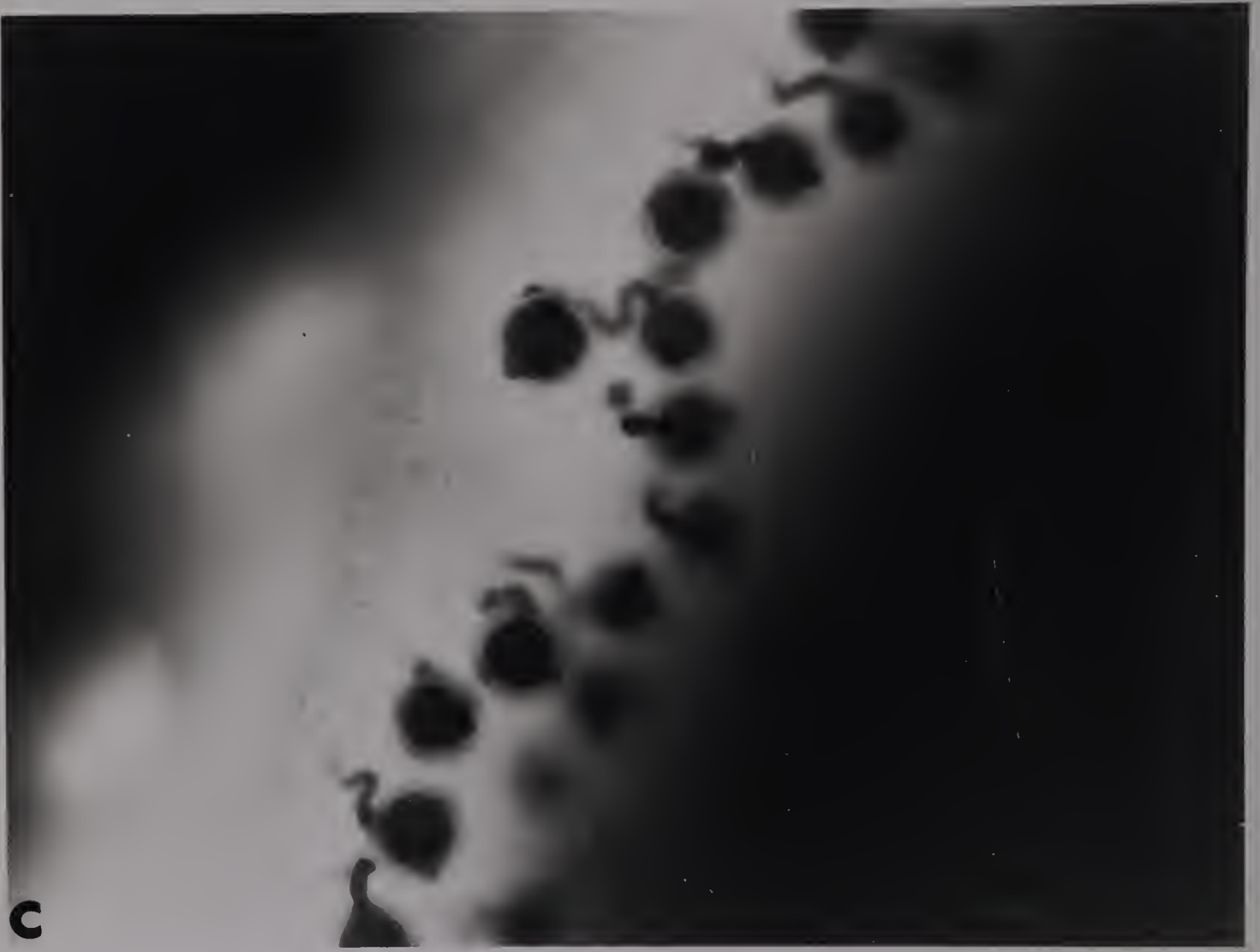


Fig. 3 a-b. Incompatible and compatible crosses of Zinnia angustifolia stained with decolorized aniline blue and viewed using UV epifluorescence microscopy. **a.** compatible mating (A10xAW1) with normal pollen tube growth; **b.** incompatible mating (A13xA13) with abnormal pollen tube growth and callose lenticules in adjacent papillae.

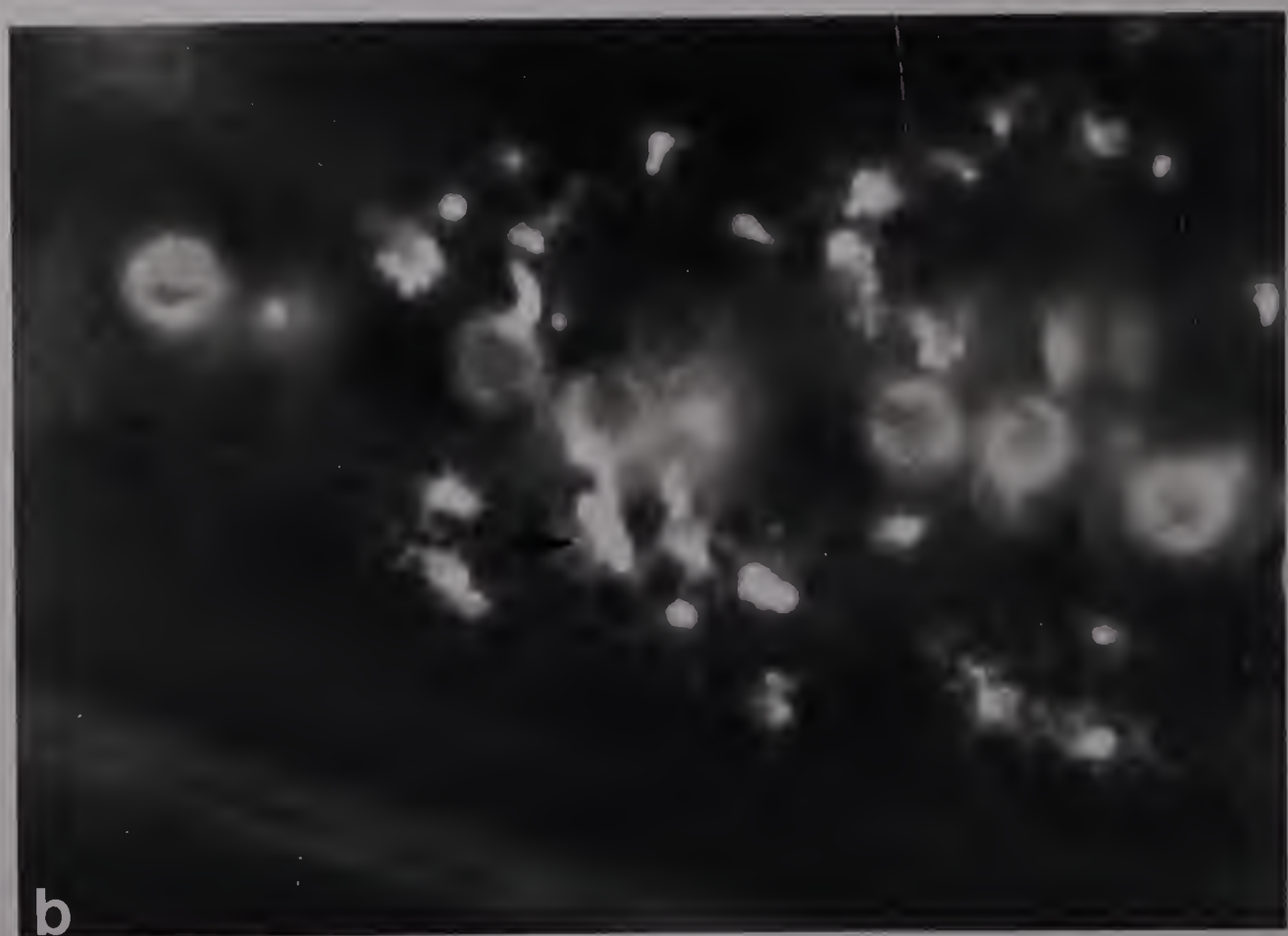
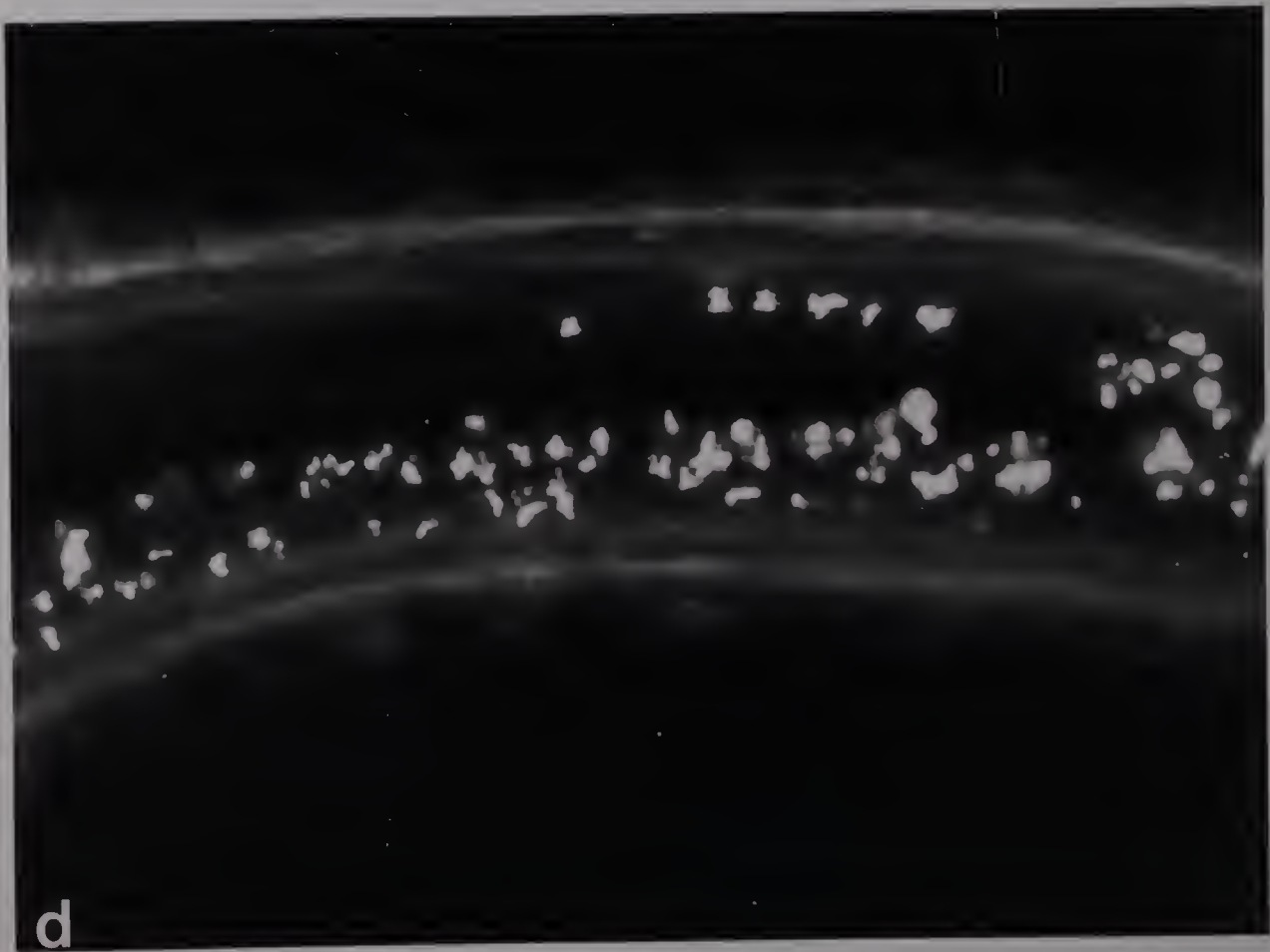
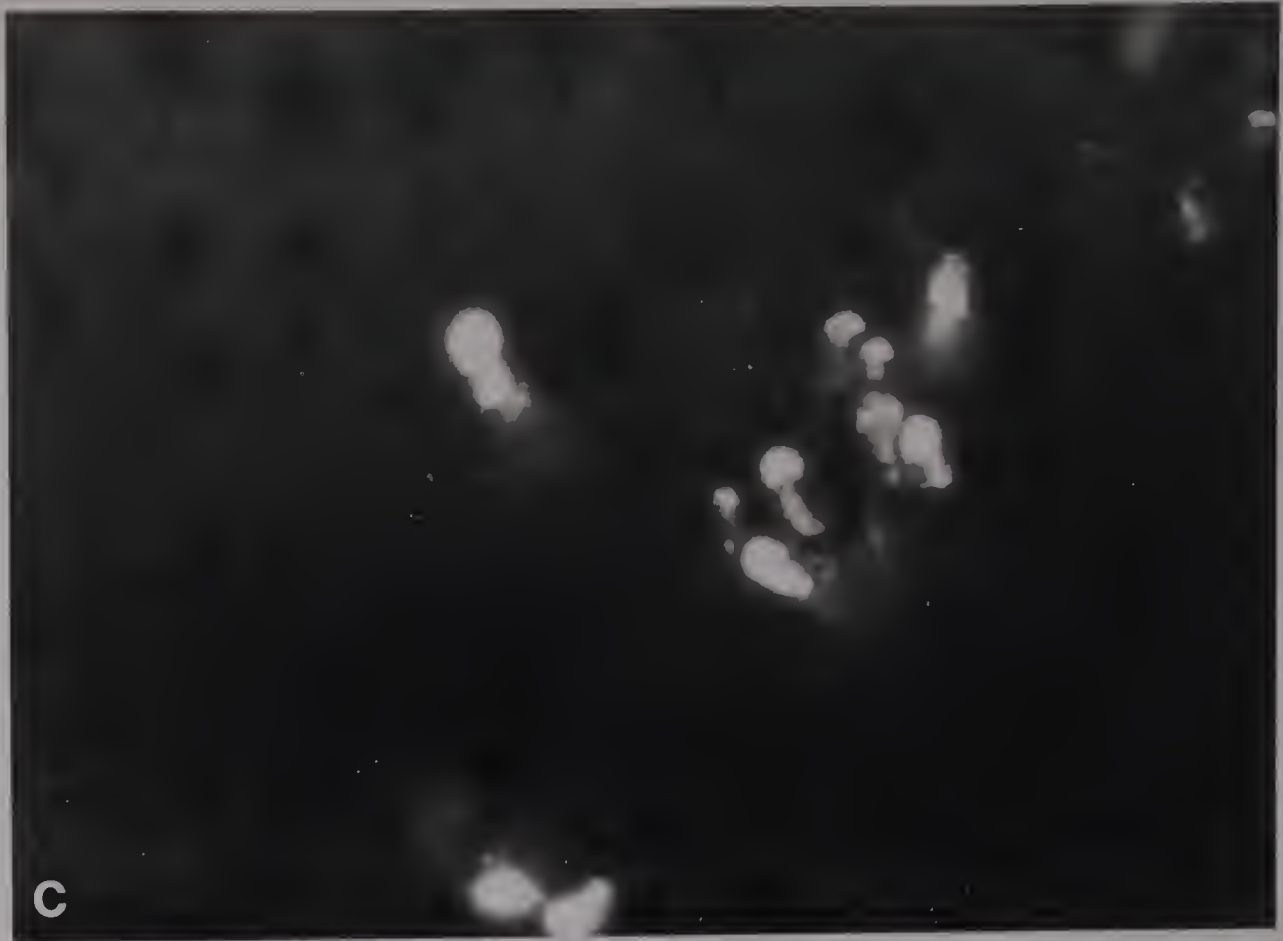


Fig. 3 c-d. Incompatible crosses of Zinnia angustifolia stained with decolorized aniline blue and viewed using UV epifluorescence microscopy.
c. incompatible mating (AW2xAW2) with callose in papillae;
d. incompatible mating (A11xA14) with low pollen load and callose lenticules in papillae representing former sites of pollen attachment.



CHAPTER 3

GENETICS OF SELF-INCOMPATIBILITY IN ZINNIA ANGUSTIFOLIA

Introduction

Self-incompatibility (SI) is defined as the physiological inability of an organism possessing both functional male and female gametes to be self-fertilized (Hughes and Babcock, 1950). The SI system is based on the capacity of the pistil to reject a pollen grain (or tube) expressing an identical incompatibility phenotype, and incompatible pollen may be derived either from the same ramet, different ramets, or different genets. Sporophytic self-incompatibility (SSI) is determined by the genotype of the sporophytic parent plant producing the pollen grain, and sporophytic control of SI has been determined for many Compositae species (Gerstel, 1950; Hughes and Babcock, 1950; Crowe, 1954; Habura, 1957; Jain and Gupta, 1960; Barkley, 1968; Brewer and Parlevliet, 1969; Shchori, 1969; Imrie and Knowles, 1971; Eenink, 1981b; Stephens et al., 1982; Zagorski et al., 1983; Davidson and Stace, 1986).

Zinnia (Compositae, tribe Heliantheae) is comprised of approximately 17 species, all native to the New World (Torres, 1963). Self-pollination tests of most of the 17 Zinnia species have indicated SI is common within the genus (Pollard, 1939; Torres, 1962, 1963; Olorode, 1970). Zinnia angustifolia HBK (formerly Z. linearis Benth. [Torres, 1963]) has been shown to be SI, based on selfing tests (Olorode, 1970) and microscopical observations (Boyle and Stimart, 1986a,b). However, no studies on the inheritance of SI in Zinnia have been reported.

All Compositae species except Helianthus annuus (Habura, 1957; Shchori, 1969) and Chrysanthemum morifolium (Zagorski et al., 1983) have been reported as having a monofactorial, polyallelic system controlling SI. In these two species, a multifactorial, polyallelic system has been hypothesized. The presence of 3 loci controlling the SI system in C. morifolium is easily explained due to its hexaploid constitution; in contrast H. annuus is diploid. Since Zinnia and Helianthus are both in the Heliantheae tribe, genetic analysis of SI in Z. angustifolia would provide evidence indicating whether multifactorial control of SI is widespread in the Heliantheae tribe or restricted in distribution. Additionally, determination of S-allele genotypes for Z. angustifolia clones and their F₁ progenies would be useful in future investigations of interspecific incompatibility with Z. elegans (Boyle and Stimart, 1986a,b). The objectives of the following study were to characterize the SI system and S-allele relationships in Z. angustifolia.

Materials and Methods

General procedures

All experiments were performed at the University of Massachusetts, Amherst, MA (42° 22.5' N latitude). Techniques used in seed germination and culture of plant material were similar to those previously reported (Boyle and Stimart, 1983, 1986b). Plant development was controlled during fall, winter, and early spring by regulating photoperiod with supplementary incandescent irradiation from 1600 to 2200 HR (12 $\mu\text{mol s}^{-1}$

m⁻²). Greenhouse temperatures were maintained at a minimum of 18°/20°C (night/day).

Inflorescences were emasculated by removing hermaphroditic disk florets 1-3 days prior to anthesis, and each inflorescence was subsequently covered with a glassine bag. Pollinations were performed 1-3 days after emasculation by transferring pollen from disk florets to receptive stigmas of the female parent using a fine-tip paint brush; inflorescences were re-covered following pollinations.

Description of plant material

Two plants of Z. angustifolia (A01 and AW2) were used as parental material. Plants were selected at random among seedlings of 2 commercial cultivars and asexually propagated (cloned) by rooting terminal shoot cuttings under intermittent mist. Previous studies indicated A01 and AW2 produced functional pollen and ovules, were reciprocally cross-compatible, and were highly SI, with self-pollinations resulting in less than 2% of florets producing embryos (Boyle and Stimart, 1986a,b).

An F₁ population was generated using A01 as the maternal parent and AW2 as the paternal parent, and 14 seedlings (designated A1 through A14) were selected randomly and cloned as previously described. The 14 full-sibs were intercrossed in a full diallel and reciprocally backcrossed to both A01 and AW2. Preliminary crosses indicated all 14 F₁ seedlings produced functional pollen and ovules.

Three full-sib F₂ families were generated from cross-compatible F₁ siblings, and 15 seedlings from each F₂ family were crossed (as maternal

parents) to both F_1 parents, A01, and AW2. Four backcross (BC_1) families were produced from crosses between F_1 s and compatible parental clones, and 15 seedlings from each BC_1 family were crossed (as maternal parents) to the F_1 parent and the recurrent BC parent (A01 or AW2).

Assessment of self-incompatibility

Embryological observations were performed 21 days after pollinations by excising ovules and removing the embryo sac under a dissecting microscope (25X). Presence or absence of an embryo was recorded for each dissected floret, and the percentage of florets with embryos was calculated for each cross. Approximately 35-40 florets (from 4-8 inflorescences) were pollinated for each cross in the F_1 diallel and among F_2 and BC_1 progenies.

Frequency histograms were used for determining whether crosses were compatible or incompatible (Stephens et al., 1982). Histograms were constructed from percentage data (% florets with embryos) calculated among F_1 , F_2 , and BC_1 progenies. Data were grouped into classes using a class interval of 5% and plotted against the number of crosses comprising the class interval. The cut-off values separating incompatible from compatible crosses was based on the distribution of classes in the histograms.

For 6 BC_1 progeny, embryological data were supplemented with microscopical observations in order to determine the compatibility-incompatibility status. Twenty-four hr following pollinations, styles were removed from florets and fixed in Carnoy's fluid (6:3:1 of 95% ethanol, chloroform and glacial acetic acid) for 24 hr or longer.

Styles were washed in distilled water, softened for 5-6 hr in 8N NaOH, rewashed in distilled water, and cleared for 30-60 min in 86% lactic acid. Material was washed again in distilled water, rinsed in 0.025M Trisglycine buffer (pH 8.4) to reduce fluorescence quenching (Mulcahy and Mulcahy, 1987), and stained using 0.01% aniline blue in 0.1M K_3PO_4 (Martin, 1958) for a minimum of 1 hr or longer. Incident-light fluorescence microscopy was performed using a Zeiss Standard microscope equipped with a 100W high pressure Hg lamp and filter set for UV-violet excitation. The compatibility-incompatibility status was based on observations of pollen load, pollen germination, and presence/absence of callose in pollen tubes and stigmatic papillae. A minimum of 5 stigmas (collected from 2 inflorescences) were observed for each cross.

Development and testing of SI models

The F_1 and backcross data were arranged in a diallel matrix, grouping all clones with similar compatibility patterns. Crosses were designated as "+" if compatible and "-" if incompatible. The diallel matrix was generated using the Self Incompatibility Genetic Modeling Application Systems-1 (SIGMAS-1) software package (Liedl and Anderson, 1985).

In order to establish a working hypothesis for the genetics of SI for Z. angustifolia, 312 SI models were developed for comparison with the data (see Appendix). All models assumed monogenic control of SI and presence of either 2,3 or 4 S-alleles. Models were constructed with all possible combinations of independent and dominant gene action of S-alleles in pollen and stigma. In total, 12 gametophytic SI models

(assuming independent action of S-alleles in pollen and pistil) and 300 sporophytic SI models (assuming independence and/or dominance of S-alleles in pollen and/or pistil) were compared with the P generation (A01xAW2), F₁ diallel, and BC data for Z. angustifolia to determine whether a gametophytic or sporophytic SI system was present (see appendix for models).

Fourteen sporophytic SI models (assuming dominance of S-alleles in pollen and pistil) were selected as possibilities based on agreement of model results with the P generation, F₁ diallel, and BC data for Z. angustifolia. One model was selected from among the 14, based on additional data derived from the F₂ and BC₁ families. Chi-square tests were used to determine the goodness-of-fit between observed results and expected values of F₂ and BC₁ data (Steel and Torrie, 1980).

Results and Discussion

Two intra-incompatible but reciprocally compatible classes were obtained from the F₁ full-sib diallel (Tables 8 and 9). Among the backcrosses, 1 class (A1, A2, A4, A6, A8, and A13) was reciprocally cross-compatible with A01 and reciprocally cross-incompatible with AW2. In contrast, the second class (A3, A5, A7, A9, A10, A11, A12, and A14) was reciprocally cross-compatible with AW2, but reciprocally cross-incompatible with A01. The F₁ frequency histogram for percentage of florets with embryos was bimodally distributed, with incompatible crosses yielding 15% or fewer embryos, whereas compatible crosses yielded 50% or more embryos (Fig. 4).

The 3 F_2 families exhibited 2, 3, or 4 compatibility classes in crosses to the F_1 and P generation (A01 and AW2) clones used as pollen parents (Tables 10-12). The F_2 family derived from A2xA5 segregated into 2 compatibility patterns, with 13 seedlings compatible with A5 and A01 but incompatible with A2 and AW2, and 2 seedlings incompatible with all 4 pollen testers (Table 10). The 15 F_2 seedlings obtained from A11xA4 produced 3 compatibility patterns: 9 seedlings were compatible with A11 and A01 and incompatible with A4 and AW2; 5 were compatible with A4 and AW2 and incompatible with A11 and A01; and 1 was compatible with all 4 pollen testers (Table 11). Four compatibility patterns were observed among the 15 F_2 seedlings derived from A12xA13: 8 seedlings were compatible with A12 and A01 and incompatible with A13 and AW2; 5 were compatible with A13 and AW2 and incompatible with A12 and A01; 1 was compatible with A13, A01, and AW2 and incompatible with A12; and 1 was compatible with A12 and AW2 but incompatible with A13 and A01 (Table 12). The F_2 frequency histogram for percentage of florets with embryos was also bimodally distributed, with incompatible crosses yielding 20% or fewer embryos whereas compatible crosses yielded 45% or more embryos (Fig. 5).

Among the 4 BC_1 progenies, 3 or 4 compatibility classes were exhibited within each family. Three patterns were observed among the 15 BC_1 progeny derived from A2xA01: compatible with the F_1 parent (A2) and incompatible with the recurrent BC parent (A01); compatible with the recurrent BC parent and incompatible with the F_1 parent; and compatible with both parents (Table 13). The 15 BC_1 progeny of A4xA01 displayed 4 compatibility patterns, with 14 seedlings exhibiting 3 patterns

identical to those observed among BC₁-A2xAO1 progeny, and additionally, 1 seedling which was incompatible with both parents (Table 14). Similarly, the 15 BC₁ progeny of A5xAW2 segregated into 4 compatibility patterns identical to those demonstrated by the BC₁-A4xAO1 progeny (Table 15). Three compatibility patterns were observed among the 15 BC₁-A12xAW2 progeny: compatible with the F₁ parent and incompatible with the recurrent BC parent; compatible with the recurrent BC parent and incompatible with the F₁ parent; and incompatible with both parents (Table 16). The compatibility patterns for the F₂ and BC₁ families are summarized in Table 17. The BC₁ frequency histogram for the percentage of florets with embryos also exhibited a bimodal distribution, with crosses yielding 15% embryos or fewer considered incompatible, and those yielding 40% or more considered compatible (Fig. 6). Six BC₁ seedlings, however, yielded between 25% and 40% florets with embryos in crosses with the F₁ and/or recurrent BC parents. Among these seedlings, 5 were determined to be compatible and 1 was incompatible, based on microscopical observations using the aniline blue fluorescence (ABF) method.

The 312 SI models (1 S-locus with 2,3, or 4 alleles) were tested against the data from Z. angustifolia, and in order to be acceptable, the models had to satisfy the following requirements: 1) reciprocal cross-compatibility between the parental clones; 2) 2 inter-compatible and intra-incompatible classes among the F₁ progeny; 3) among the backcrosses, all individuals in one F₁ class were compatible with one parent, whereas all individuals in the other F₁ class were compatible with the other parent; 4) no reciprocal differences among the backcrosses; 5) 2, 3, or 4 compatibility patterns within each F₂ family;

and 6) 3 or 4 compatibility patterns within each BC₁ family. The 12 gametophytic and 12 sporophytic SI models based on independent gene action were tested against the above criteria and failed to satisfy 1 or more of the requirements (Tables 18 and 19).

Among the 288 sporophytic SI models with allelic dominance in pollen and/or pistil, 14 satisfied the first 4 criteria but either failed or only partially satisfied the latter 2 criteria (Table 20). Only 1 among the 14 models approximated the compatibility patterns observed among as the F₂ and BC₁ progenies. However, in order for this model to be valid, some compatibility patterns had to be eliminated. This included 8 F₂ and BC₁ progeny that were incompatible with all pollen testers and 2 F₂ progeny displaying compatibility patterns which were obvious deviations from the main patterns (Table 17).

The model chosen as a working hypothesis was based on a single-locus sporophytic system operating with 3 alleles and following a linear dominance series, where S₁ is dominant to S₂ and S₃, and S₂ is dominant to S₃ in both pollen and stigma. According to this hypothesis, the parental clones would have the following genotypes: 1) A01 is homozygous and S₂₂; and 2) AW2 is heterozygous and S₁₃ (Table 21). The full-sib diallel consisted of 2 classes, with 1 class being S₁₂ and the other S₂₃ (Table 21). The model was tested by performing chi-square tests of S-genotype ratios from the F₂ and BC₁ families (Table 22). The observed S-genotype ratios were significantly different from the expected values among only 1 of the 3 F₂ families (A2xA5); in this cross, only S₁-genotypes were generated, whereas equivalent numbers of S₂-genotypes were expected. A similar deviation was reported previously by Stephens

et al. (1982) in their study of SI in the composite Ageratum houstonianum. The A2xA5 population size was too small to account for the deficient genotype, and it is possible that contamination may have occurred during the F₁ seed production phase which produced a surplus of S₁- genotypes. Among the 4 BC₁ families, the observed S-genotype ratios did not significantly differ from the expected values (Table 22).

As stated previously, the hypothesized model did not fully explain the SI responses of Z. angustifolia and required elimination of "aberrant" compatibility patterns in order to be tested, and the presence of these compatibility patterns requires an explanation. It should be emphasized that: 1) none of the 14 sporophytic models could account for the "aberrant" compatibility patterns evident among F₂ and BC₁ progeny; and 2) only 10 among a total of 105 F₂ and BC₁ progeny (9%) differed from the expected compatibility patterns. Unexpected compatibility classes were also discovered among BC₁ progeny of Parthenium argentatum (Gerstel, 1950).

A number of hypotheses may be advanced to account for the "aberrant" compatibility patterns. One hypothesis is the presence of female sterility among F₂ and BC₁ progeny, possibly due to inbreeding depression. Although parental and F₁ clones were tested and found to be male and female fertile, F₂ and BC₁ progeny were not tested due to time restrictions. Female sterility is a likely explanation among those progeny which were incompatible with all pollen testers, since 7 out of 8 of these progeny did not produce embryos (Tables 10, 14, 15, and 16); in contrast, incompatible crosses among most F₂ and BC₁ progeny produced some embryos. A second hypothesis would be contamination from self (or

foreign) pollen during production of F₂ and BC₁ seed. Although the emasculation and pollination technique was stringent, it is possible that contamination could have occurred, and this may account for some of the compatibility patterns evident among the F₂ and BC₁ progeny. A third hypothesis is the presence of novel S-allele relationships, i.e., interactions or mutual weakening (de Nettancourt, 1977). The occurrence of S-allele interactions or mutual weakening would be expected to generate an excess of progeny expressing compatibility patterns in which many or all crosses were ompatible. However, the data indicate most of the "aberrant" compatibility patterns are due to incompatibility with all pollen testers, and thus novel S-allele relationships are unlikely to occur among the Z. angustifolia plant material utilized in this study. A fourth hypothesis is that the SI reaction is modified by gene(s) independent of the S-locus, as previously reported for Brassica campestris (Hinata and Okazaki, 1986), although the data does not provide any evidence of additional gene(s) modifying the SI response in Z. angustifolia. A fifth hypothesis is that the SI system of Z. angustifolia is controlled by a multigenic sporophytic SI system with some degree of dominance. Among species displaying sporophytic SI systems, multigenic (2-loci) SI systems have been proposed for the crucifers Capsella grandiflora (Riley, 1936), Cardamine pratensis (Correns, 1912), and Eruca sativa (Verma and Lewis, 1977), and the composite Helianthus annuus (Habura, 1957; Shchori, 1969). However, the data obtained for Z. angustifolia do not support a 2-loci model: 1) 2 compatibility classes were observed in the F₁ diallel, whereas many compatibility classes are normally obtained among species expressing 2-

loci systems (de Nettancourt, 1977); and 2) the existence of a 2-loci sporophytic SI system for which 1 locus is homozygous for an S-allele is not possible, since all crosses would be incompatible due to matching of incompatibility determinants. Thus, the data suggests the "aberrant" incompatibility patterns are likely due to female sterility and/or contamination rather than novel S-allele relationships, presence of modifier genes, or additional loci controlling the SI response.

In addition to Z. angustifolia, the inheritance of SI has been elucidated for 2 other species in the Heliantheae tribe: Cosmos bipinnatus (Crowe, 1954) and Helianthus annuus (Habura, 1957; Shchori, 1969). It is evident that the SI system in Zinnia is more similar to the one hypothesized for Cosmos, i.e., a monofactorial sporophytic SI system with dominance and independence in both pollen and style, compared to Helianthus, which was postulated to be a 2-loci SI system. It is noteworthy to add that the base chromosome number (x) for Cosmos and Zinnia is 12, with C. bipinnatus and Z. angustifolia $2n = 24$, whereas the genus Helianthus is $x = 17$ and H. annuus is $2n = 34$ (Darlington and Wylie, 1956; Torres, 1963). These differences may reflect an evolutionary divergence within the Heliantheae and an amphiploid constitution for Helianthus, and this would explain the 2 loci controlling the SI response. Additional research is necessary to substantiate this hypothesis.

In conclusion, a gametophytic or sporophytic SI system based on independent gene action in pollen and pistil is not likely to operate for Z. angustifolia, as all 24 models failed to satisfy the required criteria generated from the F_1 full sib diallel. The agreement of data

for Z. angustifolia and predicted values for the tested model support the conclusion that SI in Z. angustifolia is a one-locus sporophytic system expressing a linear dominance series in both pollen and stigma.

Table 8. Percent florets with embryos observed in the full sib diallel and reciprocal backcrosses to both parents (A01 and AW2)

♀	♂	A1	A2	A4	A6	A8	A13	A3	A5	A7	A9	A10	A11	A12	A14	A01	AW2
A1		4	0	0	0	0	0	74	67	63	86	84	85	81	93	52	6
A2		0	3	3	0	3	0	76	86	92	88	78	91	94	97	67	3
A4		0	0	9	0	6	0	77	77	87	78	92	84	81	76	84	4
A6		0	0	0	6	0	0	64	71	54	85	61	58	69	79	56	0
A8		3	0	6	3	8	0	86	91	77	85	84	76	82	84	71	1
A13		6	3	0	0	0	4	74	83	89	80	87	76	76	84	56	2
A3		97	71	94	85	87	81	8	0	3	3	6	5	2	0	5	73
A5		63	89	86	70	81	90	0	10	6	0	0	0	7	0	10	74
A7		79	94	76	82	62	66	0	0	8	0	0	0	0	0	1	66
A9		94	82	74	73	86	82	3	2	4	3	3	0	3	0	10	67
A10		77	79	86	77	74	90	0	0	0	7	7	6	0	3	10	73
A11		81	78	93	91	67	88	0	0	6	8	3	11	7	0	10	81
A12		82	81	83	77	81	77	0	0	0	0	0	0	5	0	7	61
A14		82	77	85	84	95	89	2	0	5	3	0	0	2	12	5	79
A01		83	89	77	78	91	85	0	5	0	10	3	0	5	3	8	73
AW2		0	0	0	5	0	0	57	79	61	63	67	93	63	70	64	3

Table 9. Compatibility patterns in the full sib diallel and reciprocal backcrosses to both parents.

♀	♂ A1	A2	A4	A6	A8	A13	A3	A5	A7	A9	A10	A11	A12	A14	A01	AW2
A1	-ab-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-
A2	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-
A4	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-
A6	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-
A8	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-
A13	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-
A3	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+
A5	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+
A7	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+
A9	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+
A10	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+
A11	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+
A12	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+
A14	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+
A01	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+
AW2	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-

a +: compatible; -: incompatible

b Compatible crosses yielded 52% or more florets with embryos and incompatible crosses 12% or fewer

Table 10. Percent embryo set and compatibility patterns in the F₂ progeny A2xA5, tested with both its parents and A01 and AW2

A2xA5 ♀	♂ A2	♂ A5	♂ A01	♂ AW2
	Z Florets w/ embryos	Z Florets w/ embryos	Z Florets w/ embryos	Z Florets w/ embryos
1	8	58	57	0
2	5	81	86	0
3	9	50	50	0
4	0	59	62	0
5	0	50	59	0
6	20	59	57	13
7	0	0	0	0
8	3	74	52	2
9	0	0	0	0
10	17	95	52	6
11	0	81	65	0
12	3	53	48	5
13	9	52	67	0
14	4	58	65	0
15	0	50	52	0

^a +: compatible; -: incompatible

Table 11. Percent embryo set and compatibility patterns in the F₂ progeny A11xA4, tested with both its parents and A01 and AW2

A11xA4 ♀	♂ A11		♂ A4		♂ A01		♂ AW2	
	Z Florets w/ embryos	Comp	Z Florets w/ embryos	Comp	Z Florets w/ embryos	Comp	Z Florets w/ embryos	Comp
1	56	+ ^a	71	+	71	+	76	+
2	56	+	5	-	57	+	2	-
3	50	+	9	-	60	+	8	-
4	49	+	17	-	60	+	8	-
5	62	+	6	-	75	+	7	-
6	53	+	0	-	55	+	5	-
7	14	-	65	+	0	-	67	+
8	17	-	67	+	0	-	52	+
9	85	+	16	-	81	+	0	-
10	0	-	58	+	3	-	60	+
11	70	+	12	-	68	+	0	-
12	63	+	10	-	69	+	0	-
13	65	+	0	-	68	+	0	-
14	16	-	80	+	8	-	70	+
15	9	-	61	+	17	-	70	+

a +: compatible; -: incompatible

Table 12. Percent embryo set and compatibility patterns in the F₂ progeny A12xA13, tested with both its parents and A01 and AW2

A12xA13 ♀	♂ A12		♂ A13		♂ A01		♂ AW2	
	Σ Florets w/ embryos	Comp	Σ Florets w/ embryos	Comp	Σ Florets w/ embryos	Comp	Σ Florets w/ embryos	Comp
1	71	+ ^a	5	-	50	+	0	-
2	73	+	5	-	60	+	2	-
3	55	+	13	-	12	-	57	+
4	73	+	5	-	51	+	0	-
5	15	-	60	+	3	-	71	+
6	65	+	2	-	53	+	0	-
7	69	+	12	-	67	+	3	-
8	71	+	13	-	68	+	0	-
9	65	+	0	-	51	+	0	-
10	17	-	57	+	9	-	52	+
11	11	-	90	+	11	-	88	+
12	15	-	57	+	51	+	73	+
13	3	-	56	+	2	-	69	+
14	13	-	70	+	17	-	71	+
15	58	+	3	-	68	+	3	-

^a +: compatible; -: incompatible

Table 13. Percent embryo set and compatibility patterns in the backcross progeny A2xA01, tested with both its parents

A2xA01 ♀	♂ A2		♂ A01	
	% Florets w/ embryos	Comp	% Florets w/ embryos	Comp
1	12	- ^a	88	+
2	63	+	9	-
3	0	-	77	+
4	61	+	14	-
5	68	+	12	-
6	61	+	4	-
7	51	+	43	+
8	14	-	69	+
9	11	-	64	+
10	11	-	50	+
11	5	-	57	+
12	73	+	17	-
13	52	+	42	+
14	16	-	64	+
15	10	-	66	+

^a +: compatible; -: incompatible

Table 14. Percent embryo set and compatibility patterns in the backcross progeny A4xA01, tested with both its parents

A4xA01 ♀	♂ A4		♂ A01	
	Z Florets w/ embryos	Comp	Z Florets w/ embryos	Comp
1	72	+ ^a	7	-
2	72	+	5	-
3	75	+	9	-
4	80	+	11	-
5	16	-	84	+
6	80	+	6	-
7	17	-	6	-
8	9	-	80	+
9	50	+	5	-
10	86	+	4	-
11	87	+	45	+
12	63	+	37	+
13	28	-	55	+
14	3	-	32	+
15	69	+	5	-

^a +: compatible; -: incompatible

Table 15. Percent embryo set and compatibility patterns in the backcross progeny A5xAW2, tested with both its parents

A5xAW2 ♀	♂ A5		♂ AW2	
	% Florets w/ embryos	Comp	% Florets w/ embryos	Comp
1	0	- ^a	0	-
2	51	+	0	-
3	68	+	6	-
4	57	+	65	+
5	57	+	6	-
6	57	+	0	-
7	49	+	51	+
8	9	-	53	+
9	7	-	2	-
10	53	+	60	+
11	52	+	67	+
12	54	+	2	-
13	56	+	2	-
14	50	+	5	-
15	56	+	9	-

^a +: compatible; -: incompatible

Table 16. Percent embryo set and compatibility patterns in the backcross progeny A12xAW2, tested with both its parents

A12xAW2 ♀	♂ A12		♂ AW2	
	% Florets w/ embryos	Comp	% Florets w/ embryos	Comp
1	55	+ ^a	0	-
2	61	+	0	-
3	0	-	0	-
4	62	+	6	-
5	14	-	50	+
6	9	-	81	+
7	53	+	9	-
8	7	-	61	+
9	63	+	4	-
10	0	-	0	-
11	50	+	0	-
12	55	+	6	-
13	0	-	0	-
14	11	-	79	+
15	75	+	0	-

^a +: compatible; -: incompatible

Table 17. Compatibility patterns for F₂ and backcross (BC₁) progenies of *Z. angustifolia*. Compatibility patterns derived from crosses between F₂ and backcross progenies (as ♀) with F₁ and parental (AW2 and A01) clones (as ♂)

Cross	No. of progeny	Pollen source			
		F ₁	A01	AW2	
♀x♂					
F₂s					
A12xA13	8	+ ^a	-	+	-
	5	-	+	-	+
	1 ^b	-	+	+	+
	1 ^b	+	-	-	+
A2xA5	13	-	+	+	-
	2 ^b	-	-	-	-
A11xA4	9	+	-	+	-
	5	-	+	-	+
	1	+	+	+	+
BCs					
A12xAW2	8	+			-
	4	-			+
	3 ^b	-			-
A4xA01	8	+		-	
	4	-		+	
	2	+		+	
	1 ^b	-		-	
A5xAW2	8	+			-
	1	-			+
	4	+			+
	2 ^b	-			-
A2xA01	8	-		+	
	5	+		-	
	2	+		+	

^a +: compatible; -: incompatible

^b aberrant compatibility classes

Table 18. Twelve gametophytic self-incompatibility models, and results of *Z. angustifolia* parental crosses, full sib diallel, and backcrosses

Model	No. of alleles	Parents	No. of F ₁ compatibility classes	Backcrosses	
		Genotypes ♀ x ♂	Cross compatibility	Compatibility of F ₁ progeny	Reciprocal differences
1	2	S11 S22	Comp ^a	Some inc. ^b	Yes
2	2	S12 S11	Inc	-	-
3	2	S12 S22	Inc	-	-
4	3	S12 S13	Comp	Some inc. ^c	No
5	3	S12 S23	Comp	Some inc. ^c	No
6	3	S13 S23	Comp	Some inc. ^c	No
7	3	S12 S33	Comp	-	Yes
8	3	S13 S22	Comp	-	Yes
9	3	S23 S11	Comp	-	Yes
10	4	S12 S34	Comp	All comp.	No
11	4	S13 S24	Comp	All comp.	No
12	4	S14 S23	Comp	All comp.	No
Z. ang.	-	-	Comp	Class 1 comp. with parent 1 Class 2 comp. with parent 2	No

^a Inc = unilaterally incompatible; Comp = compatible

^b Class 1 compatible with both parents as male and incompatible with both parents as female

^c Class 1 compatible with both parents and class 2 compatible with one parent and incompatible with the other parent

Table 19. Twelve sporophytic self-incompatibility models for independent gene action in pollen and pistil, and results of Z. angustifolia parental crosses, full sib diallel, and backcrosses

Model	No. of alleles	Parents	No. of F ₁ compatibility classes	Backcrosses	
		Genotypes ♀ x ♂	Gross compatibility	Compatibility of F ₁ progeny	Reciprocal differences
1	2	S11 S22	Comp ^a	-	No
2	2	S12 S11	Inc	-	-
3	2	S12 S22	Inc	-	-
4	3	S12 S13	Inc	-	-
5	3	S12 S23	Inc	-	-
6	3	S13 S23	Inc	-	-
7	3	S12 S33	Comp	-	-
8	3	S13 S22	Comp	All inc.	No
9	3	S23 S11	Comp	All inc.	No
10	4	S12 S34	Comp	All inc.	No
11	4	S13 S24	Comp	All inc.	No
12	4	S14 S23	Comp	All inc.	No
<u>Z. ang.</u>	-	-	Comp	Class 1 comp. with parent 1 Class 2 comp. with parent 2	No

^a Inc = incompatible; Comp = compatible

Table 20. Fourteen sporophytic self-incompatibility models for dominant or dominant and independent gene action in pollen and/or pistil

Model	No. of alleles	Parents	Allelic relationships	
		Genotypes	Pollen	
		♀ x ♂	Pistil	
		compatibility		
1	2	S12 S22	S1 > S2	S1 > S2
2	3	S12 S23	S1 > S2 > S3	S1 > S2 = S3
3	3	S12 S23	S1 > S2 > S3	S1 > S2 > S3
4	3	S13 S22	S1 > S2 > S3	S1 > S2 = S3
5	3	S13 S22	S1 > S2 > S3	S1 > S2 > S3
6	3	S12 S23	S1 > S2 = S3	S1 > S2 = S3
7	3	S13 S23	S1 > S2 = S3	S1 > S2 = S3
8	3	S12 S23	S1 > S2 = S3	S1 > S2 > S3
9	3	S12 S33	S1 > S2 = S3	S1 > S2 = S3
10	3	S13 S22	S1 > S2 = S3	S1 > S2 = S3
11	3	S13 S22	S1 > S2 = S3	S1 > S2 > S3
12	4	S12 S34	S1 > S2 = S3 = S4	S1 > S2 = S3 = S4
13	4	S13 S24	S1 > S2 = S3 = S4	S1 > S2 = S3 = S4
14	4	S14 S23	S1 > S2 = S3 = S4	S1 > S2 = S3 = S4

^a Inc = incompatible; Comp = compatible

Table 21. Hypothesized S-allele genotypes in the F₁ diallel and backcrosses. Model based on single-locus sporophytic self incompatibility system with a linear dominance series (S₁>S₂>S₃) in both pollen and stigma

		F ₁			Parents	
		o	o S ₁₂	S ₂₃	A01 S ₂₂	AW2 S ₁₃
F ₁	S ₁₂		- ^a	+	+	-
	S ₂₃		+	-	-	+
Parents	A01	S ₂₂	+	-	-	+
	AW2	S ₁₃	-	+	+	-

^a +: compatible; -: incompatible

Table 22. Chi-square tests of S-genotype ratios in F₂ and backcross (BC₁) progenies

Cross	Genotype	Observed		Expected		P		
		S ₁₋	S ₂₋	S ₁₋	S ₂₋			
♀x♂	♀x♂	S ₁₋	S ₂₋	S ₁₋	S ₂₋			
F₂s								
A11xA4	S ₂₃ xS ₁₂	9	5	7	7	0.30-0.20		
A2xA5	S ₁₂ xS ₂₃	13	0	6.5	6.5	>0.001		
A12xA13	S ₂₃ xS ₁₂	8	5	6.5	6.5	0.50-0.30		
BCs								
		S ₁₋	S ₂₃	S ₃₃	S ₁₋	S ₂₃	S ₃₃	
A12xAW2	S ₂₃ xS ₁₃	8	4	0	6	3	3	0.20-0.10
A5xAW2	S ₂₃ xS ₁₃	8	1	4	6.5	3.25	3.25	0.50-0.30
		S ₁₂	S ₂₂		S ₁₂	S ₂₂		
A4xA01	S ₁₂ xS ₂₂	4	8		6	6		0.30-0.20
A2xA01	S ₁₂ xS ₂₂	8	5		6.5	6.5		0.50-0.30

Fig. 4. Frequency histogram of percent embryo set from the full-sib diallel.

FULL SIB DIALLEL



Fig. 5. Frequency histogram of percent embryo set from F₂ progenies.

F₂ PROGENIES

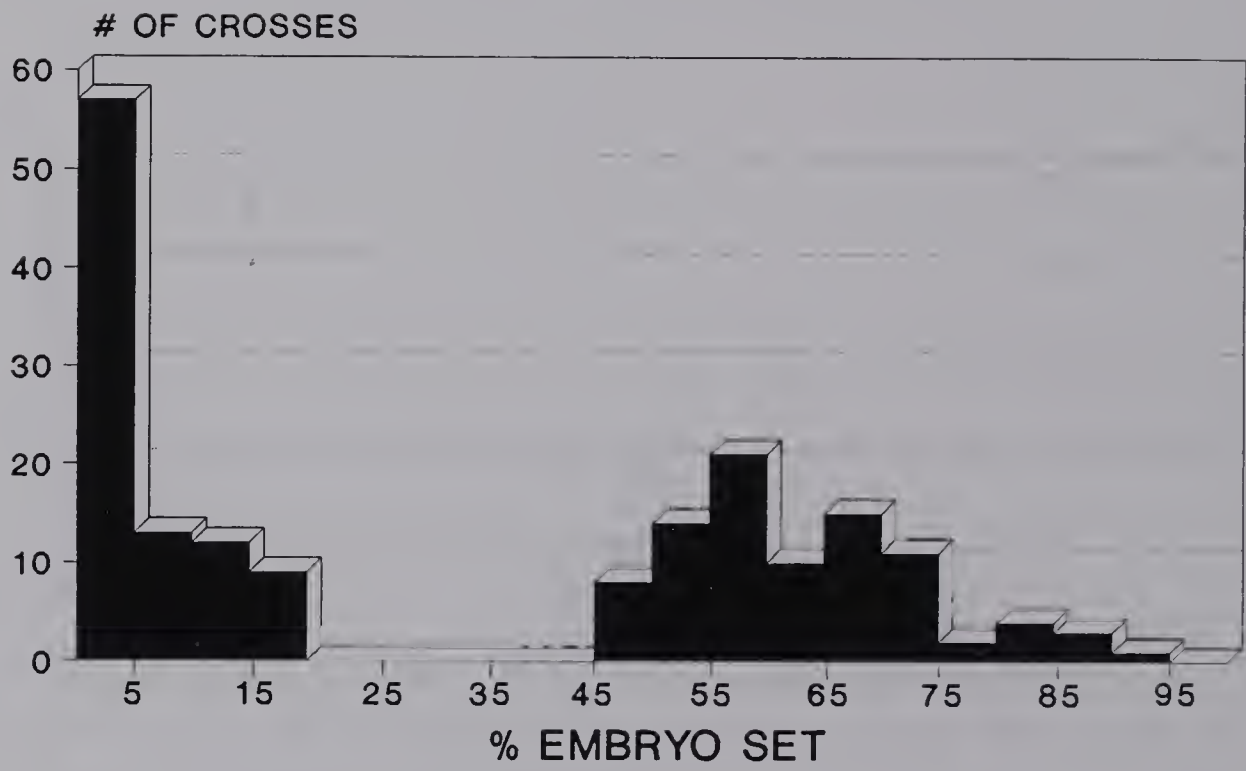
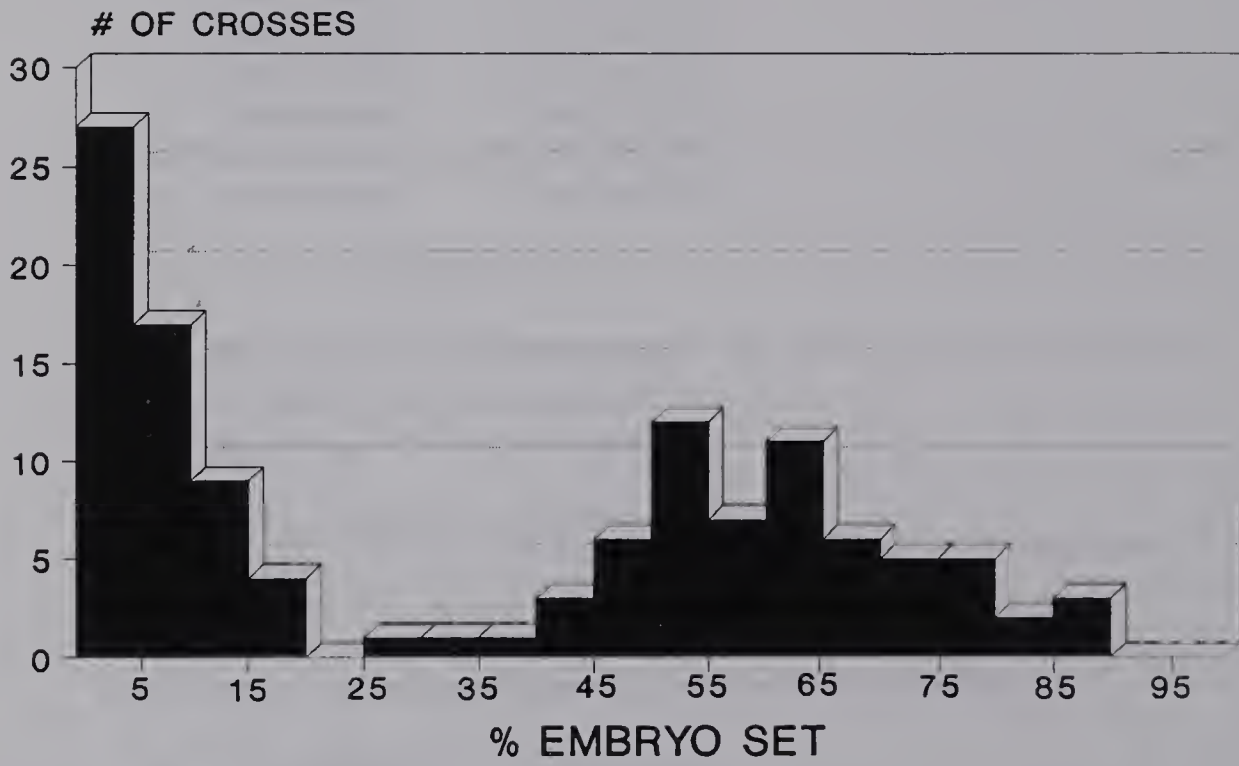


Fig. 6. Frequency histogram of percent embryo set from backcross progenies.

BACKCROSS PROGENIES



APPENDIX

Theoretical sporophytic and gametophytic self-incompatibility (SI) models based on number of alleles, allelic relationships, and parental genotypes.

I. Four alleles:

A. Sporophytic SI system:

1) Allelic relationships in pollen and stigma:

- a) independence: $S_1=S_2=S_3=S_4$
- b) dominance: $S_1>S_2=S_3=S_4$
- c) dominance: $S_1>S_2>S_3=S_4$
- d) dominance: $S_1>S_2=S_3>S_4$
- e) dominance: $S_1>S_2>S_3>S_4$
- f) dominance: $S_1=S_2>S_3>S_4$
- g) dominance: $S_1=S_2>S_3=S_4$
- h) dominance: $S_1=S_2=S_3>S_4$

2) Genotype possibilities:

- a) $S_{12} \times S_{34}$ and reciprocal
- b) $S_{13} \times S_{24}$ and reciprocal
- c) $S_{23} \times S_{14}$ and reciprocal

3) Possible models: $8 \times 8 \times 3 = 192$

B. Gametophytic SI system:

1) Allelic relationships in pollen and stigma:

- a) independence: $S_1=S_2=S_3=S_4$

2) Genotype possibilities:

- a) same as for sporophytic system.

3) Possible models: $1 \times 1 \times 3 = 3$

II. Three alleles:

A. Sporophytic SI system:

1) Allelic relationships in pollen and stigma:

- a) independence: $S_1=S_2=S_3$
- b) dominance: $S_1>S_2=S_3$
- c) dominance: $S_1=S_2>S_3$
- d) dominance: $S_1>S_2>S_3$

2) Genotype possibilities:

a) one allele in common:

- i) $S_{12} \times S_{13}$
- ii) $S_{12} \times S_{23}$
- iii) $S_{13} \times S_{23}$

b) one homozygote:

- i) $S_{12} \times S_{33}$
- ii) $S_{13} \times S_{22}$
- iii) $S_{23} \times S_{14}$

3) Possible models: $4 \times 4 \times 6 = 96$

B. Gametophytic SI systems:

1) Allelic relationships:

- a) independence: $S_1=S_2=S_3$

2) Genotype possibilities:

- a) same as for sporophytic system.

3) Possible models: $1 \times 1 \times 6 = 6$

III. Two alleles:

A. Sporophytic SI systems:

1) Allelic relationships in pollen and stigma:

- a) independence: $S_1=S_2$
- b) dominance: $S_1>S_2$

2) Genotype possibilities:

- a) $S_{11} \times S_{22}$
- b) $S_{12} \times S_{11}$
- c) $S_{12} \times S_{22}$

3) Possible models: $2 \times 2 \times 3 = 12$

B. Gametophytic SI systems:

1) Allelic relationships in pollen and stigma:

- a) independence: $S_1=S_2$

2) Genotype possibilities:

- a) $S_{11} \times S_{22}$
- b) $S_{12} \times S_{11}$
- c) $S_{12} \times S_{22}$

3) Possible models: $1 \times 3 = 3$

REFERENCES

- Aist, J.R. (1976) Papillae and related wound plugs of plant cells. *Ann. Rev. Phytopathol.* 14:145-163.
- Ascher, P.D. (1976) Self-incompatibility systems in floriculture crops. *Acta Hort.* 63:205-215.
- Barkley, T.M. (1968) Observations on the compatibility system in the florists' cineraria Senecio cruentus DC. (Compositae). *Phyton* 25:135-140.
- Barskaya, E.I., Balina, N.V. (1971) The role of callose in plant anthers. *Sov. Plant Physiol.* 18:605-610.
- Boyle, T.H. (1986) Gene expression in Zinnia angustifolia HBK, Z. elegans Jacq. and their interspecific hybrids. 1. Prezygotic barriers. 2. Postzygotic barriers. 3. Ray floret color. Ph.D. Dissertation, University of Maryland, College Park.
- Boyle, T.H., Stimart, D.P. (1983) Developmental responses of Zinnia to photoperiod. *J. Am. Soc. Hort. Sci.* 108:1053-1059.
- Boyle, T.H., Stimart, D.P. (1986a) Incompatibility relationships in intra- and interspecific crosses of Zinnia elegans Jacq. and Z. angustifolia HBK (Compositae). In: Mulcahy, D.L., Mulcahy, B.G., Ottaviano, E. (eds.). *Biotechnology and ecology of pollen*. Springer-Verlag, New York. pp. 265-270.
- Boyle, T.H., Stimart, D.P. (1986b) Self-incompatibility and interspecific incompatibility: relationships in intra- and interspecific crosses of Zinnia elegans Jacq and Z. angustifolia HBK (Compositae). *Theor. Appl. Genet.* 73:305-315.
- Boyle, T.H., Stimart, D.P. (1988) Inheritance of ray floret color in Zinnia angustifolia HBK and Z. elegans Jacq. *J. Hered.* 79:289-293
- Boyle, T.H., Stimart, D.P., Baughan, G.R. (1987) Influence of Zinnia angustifolia HBK genotype on embryonic and vegetative development of Z. angustifolia x Z. elegans Jacq. interspecific hybrids. *Theor. Appl. Genet.* 73:716-723.
- Brewbaker, J.L. (1957) Pollen cytology and incompatibility systems in plants. *J. Hered.* 48:217-277.
- Brewer, J.G. (1974) Incompatibility relationship in pyrethrum (Chrysanthemum cinerariaefolium Vis.). *Euphytica* 23:45-47.
- Brewer, J.G., Parlevliet, J.E. (1969) Incompatibility as a new method for identification of pyrethrum clones. *Euphytica* 18:320-325.

- Chandler, C. (1931) A method for staining pollen tubes within the pistil. *Stain Tech.* 6:25-26.
- Correns, C. (1912) Selbsterilitat und individualstoffe. *Festschr. d. mat.-nat. Gesell. zur 84. Versamml. Deut. Naturforsch. Artze, Munster i. W.*, 1-32.
- Crehu du, G. (1968) Early testing of pollen stigma compatibility relationships in Brassica oleracea by fluorescence. In: Dixon, G.E. (ed.) *Proc. Brassica Meeting of Eucarpia Hort. Sect., Wellesbourne, England.* pp. 34-36.
- Crowe, L.K. (1954) Incompatibility in Cosmos bipinnatus. *Heredity* 8:1-11.
- Currier, H.B. (1957) Callose substance in plant cells. *Amer. J. Bot.* 44:478-482.
- Darlington, C.D., La Cour, L.F. (1942) *The handling of chromosomes.* The Macmillan Company, New York.
- Darlington, C.D., A.P. Wylie. (1956) *Chromosome atlas of flowering plants.* The Macmillan Company, New York.
- Davidson, J.K., Stace, H.M. (1986) Genetics of self-incompatibility in Calotis cuneifolia. *J. Hered.* 77:471-472.
- Dickinson, H.G. (1970) Ultrastructural aspects of primexine formation in the microspore tetrad of Lilium longiflorum. *Cytobiologie* 1:437-449.
- Dickinson, H.G. (1985) The cytophysiological basis of the sporophytically controlled self-incompatibility mechanism operating in Brassica. In: Sussex, I., Ellingboe, A., Crouch, M., Malmberg, R. (eds.). *Plant cell/cell interactions. Current communications in molecular biology.* Cold Spring Harbor Laboratory, New York. pp. 7-14.
- Dickinson, H.G., Lewis, D. (1973a) The formation of the tryphine coating the pollen grains of Raphanus, and its properties relating to the self-incompatibility system. *Proc. Roy. Soc. Lond. B* 184:149-165.
- Dickinson, H.G., Lewis, D. (1973b) Cytochemical and ultrastructural differences between intraspecific compatible and incompatible pollinations in Raphanus. *Proc. Roy. Soc. Lond. B* 183:21-38.
- Dickinson, H.G., Roberts, I.N. (1985) Cell surface receptors in the pollen-stigma interaction of Brassica oleracea. In: Garrod, D.R., Chadwick, D.M. (eds.). *Receptors in plants and slime moulds.* Company of Biologists, Cambridge, England. p. 267.

- Drewlow, L.W., Ascher, P.D., Widmer, R.E. (1973) Genetic studies of self incompatibility in the garden chrysanthemum, Chrysanthemum morifolium Ramat. Theor. Appl. Genet. 43:1-5.
- Dumas, C., Knox, R.B. (1983) Callose and determination of pistil viability and incompatibility. Theor. Appl. Genet. 67:1-10.
- Eenink, A.H. (1981a) Compatibility and incompatibility in witloof-chicory (Cichorium intybus L.). 1. The influence of temperature and plant age on pollen germination and seed production. Euphytica 30:71-76.
- Eenink, A.H. (1981b) Compatibility and incompatibility in witloof-chicory (Cichorium intybus L.). 2. The incompatibility system. Euphytica 30:77-85.
- Elleman, C.J., Dickinson, H.G. (1985) Pollen stigma interactions in Brassica. IV. Structural reorganization in the pollen grains during hydration. J. Cell Sci. 80:141-159
- Eschrich, W., Currier, H.B. (1964) Identification of callose by its diachrome and fluorochrome reaction. Stain Tech. 39:303-307.
- Frankel, R., Galun, E. (1977) Pollination mechanisms, reproduction and plant breeding. Springer-Verlag, Berlin, Heidelberg, New York.
- Gerstel, D.U. (1950) Self-incompatibility studies in guayule. II. Inheritance. Genetics 35:482-506.
- Gerstel, D.U., Riner, M.E. (1950) Self-incompatibility studies in guayule. I. Pollen tube behavior. J. Hered. 41:49-55.
- Habura, E.C.H. (1957) Parasterilitat bei Sonnenblumen. Z. Pflanzenzuchtung 37:280-298.
- Herth, W., Franke, W.W., Bittinger, H., Kuppel, A., Keilich, G. (1974) Alkali resistant fibrils of beta-1,3 and beta-1,4 glucans: structural polysaccharides in the pollen tube wall of Lilium longiflorum. Cytobiology 9:344-367.
- Heslop-Harrison, J. (1967) Ribosome sites and S gene actions. Nature (London). 218:90-91.
- Heslop-Harrison, J. (1968a) Tapetal origin of pollen coat substances in Lilium. New Phytol. 67:779-786.
- Heslop-Harrison, J. (1968b) Pollen wall development. Science 161:230-237.
- Heslop-Harrison, J. (1975) Incompatibility and the pollen stigma interaction. Ann. Rev. Plant Phys. 26:403-425.

- Heslop-Harrison, J., Heslop-Harrison, Y., Barber, J. (1975) The stigma surface in incompatibility responses. *Proc. Royal Soc. Lond. B.* 188:287-297.
- Heslop-Harrison, J., Heslop-Harrison, Y., Knox, R.B. (1973) The callose rejection reaction: a new bioassay for incompatibility in Cruciferae and Compositae. *Incompatibility Newsletter* 3:75-76.
- Heslop-Harrison, J., Knox, R.B., Heslop-Harrison, Y. (1974) Pollen wall proteins: exine held fraction associated with the incompatibility response in Cruciferae. *Theor. Appl. Genet.* 44:133-137.
- Heslop-Harrison, J., Heslop-Harrison, Y., Knox, R.B., Howlett, B. (1973) Pollen wall proteins: gametophytic and sporophytic fractions in the pollen walls of the Malvaceae. *Ann. Bot.* 37:403-412
- Heslop-Harrison, J., Knox, R.B., Heslop-Harrison, Y., Mattsson, O. (1975) Pollen wall proteins: emission and role in incompatibility responses. In: Duckett, J.G., Racey, P.A. (eds.). *The biology of the male gamete.* Academic Press, London. pp. 189-202.
- Hinata, K., Okazaki, K. (1986) Role of stigma in the expression of self-incompatibility in crucifers in view of genetic analysis. In: Mulcahy, D.L., Mulcahy, B.G., Ottaviano, E (Eds.). *Biotechnology and ecology of pollen.* Springer-Verlag, New York. pp. 185-190.
- Howlett, B.J., Knox, R.B., Paxton, J.D., Heslop-Harrison, J. (1975) Pollen wall proteins: physicochemical characterization and role in self-incompatibility in Cosmos bipinnatus. *Proc. Roy. Soc. Lond. B* 188:167-182.
- Hughes, M.B., Babcock, E.B. (1950) Self-incompatibility in Crepis foetida L. subsp. rhoedifolia (Bieb.). *Schinz. et. Keller. Genetics* 35:570-588.
- Imrie, B.C., Knowles, P.F. (1971) Genetic studies of self-incompatibility in Carthamus flavescens Spreng. *Crop Sci.* 11:6-9.
- Jain, H.K., Gupta, S.B. (1960) Genetic nature of self-incompatibility in annual chrysanthemum. *Experientia* 16:364-365.
- Kerhoas, C., Knox, R.B., Dumas, C. (1983) Specificity of the callose response in stigmas of Brassica. *Ann. Bot.* 52:597-602.
- Knox, R.B. (1973) Pollen wall proteins: pollen-stigma interactions in ragweed and Cosmos (Compositae). *J. Cell Sci.* 12:421-443.
- Knox, R.B., Heslop-Harrison, J., Reed, C. (1970) Localization of antigens associated with the pollen grain wall by immunofluorescence. *Nature* 225:1066-1068.

- Liedl, B.E., Anderson, N.O. (1986) SIGMAS-1: a software program for self-incompatibility genetic modeling. *J. Hered.* 79:480.
- Lewis, D. (1979) Sexual incompatibility in plants. *Studies in biology* no. 110, Edward Arnold, London.
- Lundqvist, A. (1964) The nature of the two loci incompatibility system in grasses. IV. Interaction between the loci in relation to pseudo-compatibility in Festuca pratensis Huds. *Hereditas* 52:221-234.
- Martin, F.W. (1959) Staining and observing pollen tubes in the style by means of fluorescence. *Stain Tech.* 34:125-128.
- Mattsson, O., Knox, R.B., Heslop-Harrison, J., Heslop-Harrison, Y. (1974) Protein pellicle of stigmatic papillae as a probable recognition site in incompatibility reactions. *Nature* 247:298-300.
- Mulcahy, G.B., Mulcahy, D.L. (1987) Induced pollen tube directionality. *Amer. J. Bot.* 74(9):1458-1459.
- Nasrallah, M.E., Wallace, D.M. (1967) Immunogenetics of self-incompatibility in Brassica oleracea L.. *Heredity* 22:519-527.
- Nasrallah, J.B., Kao, T.H., Goldberg, M.L., Nasrallah, M.E. (1985) The cellular interaction of self-incompatibility and its control by the S locus of Brassica. In: Sussex, I., Ellingboe, A., Crouch, M., Malmberg, R. (eds.). *Plant cell/cell interactions. Current communications in molecular biology.* Cold Spring Harbor Laboratory. pp. 31-33.
- Nasrallah, J.B., Kao, T.H., Goldberg, M.L., Nasrallah, M.E. (1985) A cDNA clone encoding an S-locus specific glycoprotein from Brassica oleracea. *Nature* 318:263-267.
- Nebel, B.R. (1931) Lacmoid-martius yellow for staining pollen tubes in the style. *Stain Tech.* 6:27-29.
- Nettancourt, D. de (1977) *Incompatibility in angiosperms.* Springer Verlag, Berlin, Heidelberg, New York.
- Nishio, T., Hinata, K. (1977) Analysis of S specific proteins in stigmas of Brassica oleracea L. by isoelectric focusing. *Heredity* 38:391-396.
- Ockendon, D.J. (1985) Genetics and physiology of self-incompatibility in Brassica. In: Sussex I., Ellingboe A., Crouch M., Malmberg R. (eds.). *Plant cell/cell interactions. Current communications in molecular biology.* Cold Spring Harbor Laboratory, New York. pp. 1-6.

- Olorode, O. (1970) The evolutionary implications of interspecific hybridization among four species of Zinnia sect. Mendezia (Compositae). *Brittonia* 22:207-216.
- Pandey, K.K. (1958) Time of S-allele action. *Nature* 181:1220-1221.
- Pollard, L.H. (1939) The inheritance of some flower and plant characters in Zinnia. Ph.D. dissertation, University of California.
- Pullaiah, T. (1981) Studies in the embryology of Heliantheae (Compositae). *Plt. Syst. Evol.* 137:203-214.
- Rawlins, T.E. (1933) *Phytopathological and botanical research methods.* John Wiley and Sons, New York.
- Reynolds, J.D., Dashek, W.V. (1976) Cytochemical analysis of callose localization in Lilium longiflorum pollen tubes. *Ann. Bot.* 40:409-416.
- Riley, H.P. (1936) The genetics and physiology of self-sterility in the genus Capsella. *Genetics* 21:24-39.
- Sedgley, M. (1979) Structural changes in the pollinated and unpollinated avocado stigma and style. *J. Cell Sci.* 38:49-60.
- Shchori, Y. (1969) Self incompatibility studies in sunflower. *Res. Rep. Hebrew Univ., Jerusalem.* Cited in: *Sci. Agric.* 1:549.
- Steel, R.G.D., Torrie, J.H. (1980) *Principles and procedures of statistics, second edition.* McGraw-Hill Book Company, New York.
- Stephens, L.C., Ascher, P.D., Widmer RE (1982) Genetics of self-incompatibility in diploid Ageratum houstonianum Mill. *Theor. Appl. Genet.* 63:387-394.
- Torres, A.M. (1962) Cytotaxonomy of cepitose zinnias. *Amer. J. Bot.* 49:1033-1037.
- Torres, A.M. (1964) Hybridization studies in perennial zinnias. *Amer. J. Bot.* 51:567-573.
- Torres, A.M. (1963) Taxonomy of Zinnia. *Brittonia* 15:1-25.
- Verma, S.C., Lewis, D. (1977) Multigenic sporophytic system of incompatibility in Eruca sativa (Cruciferae). In: Malik, C.P. (ed.). *Advances in plant reproductive physiology.* Kalyani Publisher, New Delhi, India. pp. 180-188.
- Vithanage, H.I.M.V., Knox, R.B. (1977a) Development and cytochemistry of stigma surface and response to self and foreign pollination in Helianthus annuus. *Phytomorphology* 27:168-179.

- Vithanage, H.I.M.V., Knox, R.B. (1977b) Callose: its nature and involvement in self-incompatibility responses in Sunflower, Helianthus annuus, and the grasses Lolium perenne and Secale cereale. Incompatibility Newsletter no.8:34-35.
- Vithanage, H.I.M.V., Gleeson, P.A., Clarke, A.E. (1980) The nature of the callose produced during self pollination in Secale cereale. Planta 148:498-509.
- Warren J.M., Crawford, T.J., Oxford, G.S. (1988) Inhibition of self pollen germination in Senecio vulgaris L. Heredity 60:33-38.
- Zagorski, J.S., Ascher, P.D., Widmer R.E. (1983) Multigenic self-incompatibility in hexaploid Chrysanthemum. Euphytica 32:1-7.

