CYTOLOGICAL ANALYSIS OF SELF-INCOMPATIBILITY IN BERMUDAGRASS

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CYTOLOGICAL ANALYSIS OF SELF-INCOMPATIBILITY

IN BERMUDAGRASS

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CHAPTER I

INTRODUCTION

Bermudagrass, Cynodon dactylon (L.) Pers., is a warm-season, sod-forming, perennial species widely distributed throughout the world between latitudes 35 degrees north and south. It's center of diversity is generally considered to be Africa from which it was first introduced into the New World, primarily for use as a pasture grass. Tremendous variation in the species has allowed the breeding of highly adaptable cultivars with performance superior to unselected germplasm for both forage and turf use. Bermudagrass is now one of the most extensively used warm-season grasses in the southern United States.

Most of the improved commercial bermudagrass cultivars developed to date are vegetatively-propagated single plants selected from F_1 progeny populations, or discovered as naturally occurring variants. Until recently seed-propagated bermudagrass was limited to a strain naturalized in the Desert Southwest and referred to as "Arizona Common." Seed production of this strain has traditionally been in a small geographic area along the Colorado river in Arizona and California. Use of the strain for forage or turf is

susceptibility to leaf-spotting disease. While vegetative propagation is an efficient, effective means of establishing new stands of bermudagrass, seed propagation would be more practical and inexpensive under certain circumstances. Consequently, current objectives of many programs in bermudagrass breeding include the development of seed-propagated cultivars.

Bermudagrass is a naturally cross-pollinated species with ploidy levels from diploid (2n=2x=18) to hexaploid (2n=6x=54). Most of the cultivated forms of bermudagrass in the United States are tetraploids except for triploids intentionally produced by crossing diploid and tetraploid parents. The basic fertility expressed as % seed set, of bermudagrass varies widely, but generally is low with plants producing few or no seed. The low fertility of most germplasm has been attributed to meiotic irregularities (Harlan and de Wet, 1969) and the presence of a self-incompatibility mechanism which enforces outcrossing (Burton and Hart, 1967). Although self-incompatibility has been well documented in bermudagrass (Burton, 1965; Kneebone, 1967; Burton and Hart, 1967; Richardson, Taliaferro, and Ahring, 1978), little information is available on its cytological or genetic basis. Burton and Hart (1967) suggested a compatibility relationship of the diploid personate type of multiple oppositional factors based on the cross and self seed set of several clones, but their hypothesis has not been confirmed. This research was

conducted to characterize cytologically the self-incompatibility response in bermudagrass relative to pollen germination and the rate and extent of pollen tube growth.

CHAPTER II

REVIEW OF LITERATURE

Self-incompatibility in Flowering Plants

Although self-incompatibility has been simply defined as "the hindrance of fertilization" (Lewis, 1949) or "an inherited ability of a flower to reject its own pollen" (Ebert et al, 1989). It is recognized technically as a genetically controlled mechanism conditioned by "S" (self-incompatibility) genes at one or more loci, which prevent fertilization between gametes produced by the same plant and between certain individual plants of the same species (Cornish et al, 1988). Self-incompatibility functions as a breeding and evolutionary mechanism by preventing inbreeding and promoting outcrossing within a plant species. It was recognized by Darwin (1877) who related its importance in the evolution of flowering plants in his summation of studies on such species as Lythrum, Oxalis, and Polygonum.

Self-incompatibility is widely distributed among the flowering plants. East (1940) estimated that it occurrs in at least 3000 species. This estimation was later extended by Darlington and Mather (1949) to include one-half of the flowering plants, and by Brewbaker (1959) who estimated its occurrence in at least 71 families and 250 of their included

600 genera. D. de Nettancourt (1977) reported that self-incompatibility in cultivated plants is less rigidly controlled than in the wild and related such findings to the counteraction by pseudo-incompatibility. Psuedo-incompatibility is a reduction in the effectiveness of the incompatibility system due to the loss of alleles during domestication thus allowing partial inbreeding in some cultivated species. He stated that pseudo-incompatibility results from the sensitivity of the incompatibility reaction and of the self-screening effectiveness to environmental conditions. Rowlands (1964) attributed pseudo-incompatibility in cultivated species to the early domestication and imposition of selection criteria during the domestication process. In effect, the emphasis on traits selected for during domestication may have reduced the number of genotypes possessing the incompatibility trait. Although the literature indicates that self-incompatibility occurs less frequently in cultivated crops than in wild relatives, some of the most economically important species possess various degrees of incompatibility and therefore they are the most intensively studied (Table I).

Classification of Self-Incompatibility Systems

Systems of self-incompatibility have been classified as heteromorphic and homomorphic. Heteromorphic self-incompatible species produce flowers morphologically

TABLE I

DISTRIBUTION OF SELF-INCOMPATIBILITY AMONG ECONOMICALLY IMPORTANT PLANT SPECIES

Genus	Reference
Brassica	Thompson, 1957
Festuca	Lundqvist, 1961
Helianthus	Duvick, 1966
Medicago	Duvick, 1966
Hordeum	Lundqvist, 1962
Nicotiana	East and Mangelsdorf, 1925
Secale	Lundqvist, 1956
Solanum	Pandey, 1962 Pushkarnath, 1942

distinct for style length and anther level. Darwin (1880) first described the phenomenon as "heterostyled." Heteromorphic types are further classified as either distylic or tristylic types. This extension in classification depends on the morphology of the styles and anthers in relation to their position to each other. Species which are distylic have two types of floral architecture (short style + long filament and long style + short filament) segregating in populations of the species. Species which are tristylic have three distinct groups characterized by long, mid, and short styled flowers, each flower bearing anthers at two different heights which do not correspond to the level of its stigma (deNettancourt, 1977). The biology and genetics of heteromorphic types have been described for several species; for example, Linum (Ghosh and Shivanna, 1980) and Primula (Shivanna et al., 1981).

Homomorphic incompatibility occurs in more plant species than the heteromorphic type (East, 1940). In plants having homomorphic self-incompatibility, there are no gross differences in floral organization to distinguish the infertile mating types. The trait is genetic in nature and not readily identifiable without analyses of fertility or cytological studies. Homomorphic incompatibility systems have been subdivided into two distinct groups: gametophytic and sporophytic. Gametophytic systems are indicated when the phenotype of the pollen, the male gametophyte, is determined by its own haploid S genotype. Cytological

evidence in such families as Solanaceae and Rosaceae (de Nettancourt, 1977) shows that both the compatible and incompatible pollen grains hydrate and germinate normally on the stigma surface. As the pollen tubes grow through the transmitting tract of the style, callose is deposited in the walls and as plugs. In incompatible pollen types the callose is typically irregularly deposited, the walls thicken, and the tip may burst. Heslop-Harrison and Heslop-Harrison (1982) have found exceptions to this behavior in their study of self-incompatibility in Secale cereale. Their cytological study showed the pollen germinating, but the tubes arresting either at the stigma surface or very soon after penetration. Genetic control of gametophytic incompatibility systems is highly variable among species with evidence of one-, two-, three-, and four-locus systems with a varying number of alleles. The basis for genetic control by a two-locus system in the grasses was established by Lundqvist (1956) and Hayman (1956). Lundqvist (1964) also calculated that 84 different specificities exist in a population of Festuca pratensis with six alleles at the S-locus and 14 alleles at the Z-locus.

The pollen behavior in sporophytic systems, in contrast, is determined by the diploid S genotype of the pollen producing plant, the sporophyte. Cytologically, the sporophytic and gametophytic systems are similar in that pollen tube growth is arrested early in germination, usually on the stigma surface. In some instances, the pollen fails

to germinate. In these situations the callose is normally deposited both in the tip of the emerging pollen tube and at the site of contact on the papilla cell (Roberts et al, 1984).

The gametophytic system has been documented to occur in nearly half of the families of the flowering plants as compared to the observations that only six families possess the sporophytic type (Charlesworth, 1988). The control of sporophytic systems can either be diallelic as found in the system of Capsella (Riley, 1932, 1935) or polyallelic by a single locus as in Cruciferae (Bateman, 1955). Although such subdivisions exist they are not absolute because at least six families have been shown to display both heteromorphic and homomorphic systems of self-incompatibility (Brewbaker, 1959, and Vuilleumier, 1967).

Nature of the Self-Incompatibility Reaction

In order to explain the phenomenon of self-incompatibility in flowering plants, three contrasting models, the oppositional, complementary, and heterosis, have been proposed. The oppositional model, proposed by East (1926) is based on the premise that growth of incompatible pollen tubes is actively inhibited by specific molecules. Cornish et al. (1988) reviewed recent literature describing the reaction as operable when the constituents of the male gametophyte and the pistil interact, or combine to produce a

substance, and the process of the interaction, or characteristics of the product, adversely affect the development of the male gametophyte.

Lewis (1964) proposed the dimer hypothesis to explain the oppositional incompatibility reaction. The hypothesis is based on the presence of different dimer peptides in the pollen and style coded by the different S-alleles. Following self-pollination, a tetramer is formed which directly or indirectly inhibits the growth of the pollen tube. Van der Donk's (1975) hypothesis discounts the direct involvement of identical S-alleles, and suggests specific components of the style activate genes coding a substance that is essential for tube growth, and that pollen-specific molecules inhibit this process.

The proponents of the complementary model, Bateman (1952), and Kroes (1973), attribute the incompatibility reaction to a failure of the germinating pollen grains to receive essential factors such as nutrients, conditions, and stimuli necessary for tube penetration and growth. According to the this model, such factors result from the tubes' inability to utilize these complexes and this deficiency is genetically determined. Further explanation assumes that every S-allele corresponds to the absence of one specific enzyme in the pollen that is required for mobilization of a nutrient held immobilized in the stylar tissue by a stylar product of the identical allele.

The most recent model proposed was by Mulcahy and

Mulcahy (1983) for gametophytically self-incompatible species. Their heterosis model is a new concept of complementation to explain the discrepancies in the complementary model. The model is based on the assumption that dissimilar alleles in the pollen and style will cause a heterotic interaction, therefore increasing the rate of pollen tube growth. A reduction in growth occurs if the style is homozygous for deleterious recessive alleles and the pollen carries the same allele. The tube growth rate is determined by the sum of the pollen-style interactions, and incompatibility is due to pollen tube growth being too slow to effect fertilization. Although the concept accounts for the shortcomings in the other models it has been strongly criticized by Lawrence et al (1985) on the basis it is distinctly similar to the oppositional model.

Of the three models the oppositional is still the most widely accepted one. It is supported by data from experiments employing diverse procedures to characterize the incompatibility reaction. These include a series of disruptive treatments on the mature style tissue including: gamma radiation (Cresti et al., 1977), exposure to high temperatures (Ascher and Peloquin, 1966; de Nettancourt, 1971), and inhibitors of RNA and protein synthesis (Kovaleva et al., 1978). These treatments have been shown to minimize the self-incompatibility reaction, thus suggesting that tube arrest occurs in the mature stylar tissue. In addition to this evidence, Mau et al. (1986) overcame the self-

incompatibility barrier by selfing immature flowers, while Ascher and Peloquin (1966) found that the strength of the incompatibility reaction declines with aging of the flower. The evidence suggests that active opposition to tube growth can be disrupted or avoided thus allowing normal fertilization.

None of the models account for all the genetic and cytological observations. Many of the researchers cited above have proposed complex biochemical bases for their particular theory. While these theoretical explanations provide the framework for determining the nature of self-incompatibility systems, the genetic and physiological basis governing the process are yet to be elucidated.

Molecular Studies of Self-Incompatibility

Since 1985, there has been a surge of research interest in self-incompatibility, specifically the application of molecular techniques to study the nature and control of the S gene. Most of the molecular studies have been with the model-plant systems of Nicotiana alata, representing a gametophytic system, and Brassica oleracea, representing the sporophytic type. Recent research has determined the presence of stylar proteins associated with the inhibition of pollen tube growth. These stylar glycoproteins have been correlated with individual S alleles (Nasrallah, 1979, Cornish et al., 1988). The S alleles have been the target of the current molecular research on self-incompatibility

systems. Anderson et al (1986) reported the cloning of a DNA segment encoding the N. alata glycoprotein. This was accomplished by isolating the glycoprotein and obtaining the N-terminal amino acid sequence in order to prepare oligonucleotide probes (Anderson et al, 1986). The oligonucleotides probes were used to screen a cDNA library prepared from polyA+ RNA from mature styles. This method detected a cDNA sequence complementary to the original oligonucleotides. Since the recovery, this particular sequence has been used in comparison studies with other genotypes and species by utilizing northern analysis, Southern analysis and in situ hybridization histochemical analysis of the pistil (Cornish et al., 1987). Similar studies have been conducted in a wide range of species but few are as advanced as those in N. alata and B. oleracea.

Bermudagrass Breeding and Genetics

Bermudagrass, Cynodon dactylon (L.) Pers., is a genetically diverse species used extensively in the southern United States for turf, forage, and erosion control. The culture of bermudagrass is restricted to the southern half of the U.S. due to its lack of winterhardiness. The earliest record concerning the introduction of the species into the country is a diary dated 1751 (Burton and Hanna, 1985). As early as 1807, it was considered one of the most important grasses of the South (Mease, 1807).

Bermudagrass has a basic chromosome number of x=9 with

diploid (2n=18) and tetraploid (4n=36) forms occurring naturally. Tetraploid bermudagrasses are most prevalent; they usually have normal diploid meiosis (Forbes and Burton, 1963).

Hybridization has led to the development of many improved forage cultivars of bermudagrass since its introduction. One of the first improved cultivars was 'Coastal' which was an F_1 hybrid between 'Tift' (discovered by J. L. Stephens in an old cotton patch near Tifton, Georgia) and an African accession (Burton, 1954). It was released in 1943 by the Georgia Coastal Plain Station (Burton, 1943). 'Coastal' yielded well with sufficient protein content for beef production under fertilized conditions. Digestibility, however, was consistently low in 'Coastal' and attempts to overcome this shortcoming led to the subsequent development of 'Coastcross I' (Coastal x PI 255445) by Burton, Hart, and Lowery (1967). The improvement in digestibility increased animal performance by 30% over 'Coastal' (Chapman et al. 1972). Since the success of these early releases several more vegetatively propagated varieties have been released with improvement in winterhardiness, yield, disease resistance, and forage quality.

As bermudagrass was introduced increasingly northward in the United States, winterhardiness became the restricting factor in production. 'Midland' ('Coastal' x a cold-hardy common from Indiana) was released by the Oklahoma

Agricultural Experiment Station; it exhibits an increased winterhardiness over 'Coastal' (Harlan et al. 1954). It lacked the disease resistance and yield of 'Coastal' but it did not winterkill in the colder climate. 'Tifton 44', an F_1 hybrid between Coastal and a common bermudagrass from Berlin, Germany was released by Burton and Monson (1978). It is also more winter-hardy than 'Coastal' but produces 19% better daily gains (Heath et al., 1985). Another release by the Oklahoma Agricultural Experiment Station, 'Hardie', produced 6% more dry matter that was 6% more digestible than 'Midland' while maintaining excellent winterhardiness (Taliaferro and Richardson, 1980). This selection for increased winterhardiness has enabled bermudagrass to be introduced successly as far north as the central regions of Kansas and Missouri.

The improved cultivars of turf-type bermudagrasses are predominantly F_1 hybrids resulting from interspecific crosses of C. dactylon and C. transvaalensis. 'Tiffine', 'Tifgreen', and 'Tifway' cultivars are examples (Burton, 1965). Finer texture and increased shoot density are characteristic of these cultivars because of the use of C. transvaalensis germplasm. Other cultivars similarly developed are 'Midway' and 'Midiron' by the Kansas Agricultural Experiment Station, 'Texturf 10' and 'Texturf 1F' by the Texas Agricultural Experiment Station, and 'Midlawn' and 'Midfield' jointly released by the Oklahoma and Kansas Agricultural Experiment Stations in 1991.

'Sunturf', a variety with excellent cold and drought tolerance (Huffine, 1957), is a direct increase of a single plant taxonomically designated as C. magennisii. This species is thought to have resulted from natural hybridization of C. dactylon and C. transvaalensis.

In recent years two seeded-type bermudagrasses have been released and gained excellent acceptance by users. 'Guymon', released in 1982 by the Oklahoma Agricultural Experiment Station and the USDA-ARS, is a synthetic cultivar derived from the interpollination of two winter-hardy, self-incompatible clonal accessions (Taliaferro et al., 1982). It is a general-purpose bermudagrass used for forage, pasture, turf, and soil-stabilization. 'NumMex Sahara', developed by the New Mexico Agricultural Experiment Station, is an Arizona common selection with excellent seed production. Its cold tolerance is about the same as common and therefore is limited to southern regions of the United States.

Several hybridization methods have been described for controlled pollination of bermudagrass. Burton (1948) reported controlled hybrids could be produced by carefully removing the exserted, undehisced anthers if plants were maintained at 45°F in a dark room. Once emasculated, the plants would be brought into the light and pollinated. Burton later modified the technique using a mist chamber to allow the anthers to exsert but not dehisce thus allowing their removal by shaking the culm or using tweezers. The

plants were allowed to dry and then pollinated. Richardson (1958) reported a method for manually emasculating grass florets by hand without needing to control environmental conditions. Richardson considered the technique an exacting task, but thought that perseverance on the part of the breeder would be rewarded with success.

Burton (1965) reported that bermudagrass culms, ready to flower in 24 hours, will shed pollen and set seed if cut close to the ground and placed immediately in bottles of tap water. These bottles could be isolated or combined with other genotypes to produce selfs, crosses, or polycrosses. His technique of crossing was based on the assumption that the clones used exhibited a high degree of self-incompatibility allowing the breeder to retrieve hybrids rather than selfs. The mutual pollination technique saves labor in the production of new hybrids for evaluation, but should not be used in genetic studies unless completely self-incompatible genotypes are used.

The technique described above led Burton and Hart (1967) to attempt a procedure based on the same principles except under field conditions. The experiment was to ascertain if self-incompatibility does exist in Cynodon dactylon clones and if it could be used for commercial F_1 seed production in Arizona. Their results indicated that many clones of bermudagrass are highly self-incompatible and the most unrelated plants are cross-compatible. Forage tests indicated that the F_1 hybrid populations produced by

this technique will yield as well as or better than parental clones. In summary, Burton and Hart concluded the usefulness of the procedure would only be realized if enough seed was produced to be economically profitable. In Arizona, seed yields of their materials were considered insufficient to compete with other land uses.

Richardson et al. (1978) evaluated the self- and cross-fertility of eight winter-hardy bermudagrass clones and their self- and cross-fertilized progeny. Their research indicated significant variability among the bermudagrass genotypes for both self- and cross-fertility which is similar to results by Kneebone (1967), Burton and Hart (1967), and Harlan and de Wet (1969). The evidence from the different studies clearly indicate a strong self-incompatibility mechanism in the species.

In this study, five bermudagrass clonal plants were selected on the basis of their variability in self- and cross-pollinated seed set, as well as their use as a turf or forage grass. The five clones were self- and cross-pollinated under controlled conditions to examine the cytological mechanism by which self-incompatibility functions. Conclusions were based on cytological observations of pollen germination and the rate and extent of pollen tube growth.

CHAPTER III

MATERIALS AND METHODS

Field and greenhouse studies were conducted at the Agronomy Research Station, Stillwater, Oklahoma in 1989 and 1990. Laboratory procedures were conducted at the U.S. Department of Agriculture facilities in Stillwater and also at the Department of Agronomy's grass cytogenetics and tissue culture laboratory on the campus of Oklahoma State University, Stillwater, Oklahoma.

Five accessions of bermudagrass were selected based on their self- and cross-fertility under field conditions. Self-fertility was measured by randomly selecting 10 immature inflorescences. Pollinating bags were placed over the inflorescences to prevent cross-pollination. Once the inflorescences flowered and the seed reached maturity, the bags were collected and the percentage of florets setting seed was determined. Cross-fertility was measured by collecting 10 inflorescences that were open-pollinated in the field and determining percentage of seed set.

Five accessions were chosen from several holding nurseries at the Agronomy Reserach Station. SS-3 and SS-13 are forage-type selections made by Bill Richardson during a bermuda seed set study in 1971. Both accessions resulted

from a cross of Guymon and 9958. The 70-3 and 72-6 were turf-type selections from F_1 progeny of the cross of 44-2 and 53-3. The Zebra accession is a variegated F_1 seedling selected from the original F_1 variegated plant (Johnston and Taliaferro, 1975).

The five accessions were transplanted into the greenhouse from field plots when flowering began in late spring. To determine the amount of pollen germination and pollen tube growth following self-pollination, inflorescences of the five selected genotypes were first emasculated then pollinated with pollen collected from field plots of the same accession. Inflorescences were emasculated using a modification of the Richardson (1953) and the aid of a humidity chamber. The top and bottom 10% of the florets of each raceme were removed to reduce the variability in maturity of the florets collected. Following controlled pollination 24 hours after emasculation, 6 to 10 spikelets were collected at different time intervals. The spikelets were fixed in FAA and stored in 70% alcohol. Pistils were dissected from the florets and prepared for examination by fluorescence microscopy using a modification of Kho and Baer's (1968) technique. The pistils were removed from the 70% alcohol solution and rinsed with distilled water. The pistils were transferred into a 0.1% aniline blue solution for 1 hour. The stained pistils were placed on a slide with a drop of glycerine and a coverslip pressed on gently. They were examined with an Olympus BH2

photomicroscope with a fluorescence attachment. Pollen germination was determined by counting germinated and non-germinated pollen grains on the stigmas. Rate and extent of pollen tube growth into the pistil was recorded and photographed. Photographs were taken with Kodak Ektachrome 400 ASA slide film and developed by push-processing to 800 ASA.

For comparison to self-pollination, controlled reciprocal cross-pollinations were made between 70-3 x 72-6, SS-3 x SS-13, and Zebra x SS-3 accessions. The parents in each cross were used as females with similar frequency for comparison. The pistillate parent was emasculated and bagged to prevent pollen contamination from selfing and to control time of pollination. Pistils were examined with a dissecting scope to identify pollen contamination and any tissue damage which may have occurred during emasculation. Florets with contamination or damage were removed from the inflorescence. Eighteen to twenty-four hours after emasculation the stigmas of the pistillate parent were dusted with pollen from another accession. Following pollination 6 to 10 florets were removed at 2.5, 5.0, 7.5, 10 and 24 hour time-intervals. These florets were prepared as described above for examination by fluorescence microscopy.

CHAPTER IV

RESULTS AND DISCUSSION

Self-pollinations

Self-fertility in the five accessions exhibited varying levels of a seed set from 3.6% in SS-3 to 87.7% in Zebra (Table II). All five accessions had pollen germination of at least 80% within the first 2.5 hours of self-pollination (Table III). Pollen tubes elongated and quickly entered the stigmatic tissue with no detectable inhibition. The only accession which had pollen tubes extended beyond the stigmatic tissue was Zebra; its pollen tubes were detected in the micropyle of the ovule within the first 2.5 hours. At 5.0 hours post-pollination, the Zebra accession continued to have a remarkable rate of tube growth as compared to the other four accessions. Only two other accessions, 72-6 and 70-3, had pollen tubes penetrate and grow into the stylar tissue. At the 7.5 hour time interval, slow tube growth continued for all accessions except Zebra. Tubes of the other accessions which reached the style continued to elongate slowly and a few had entered the ovary. At 10.0 hours 70-3, 72-6, SS-3, and SS-13 had reached the micropylar region with most tubes still remaining in the stigmatic and stylar tissue (Figure 1). Zebra pollen tubes

TABLE II

COMPARISON OF THE SELF- AND CROSS-FERTILITY OF THE FIVE BERMUDAGRASS ACCESSIONS

Accession	Percent Seed Set	
	Self	Cross
70-3	20.4	84.3
72-6	11.8	77.8
SS-3	3.6	65.4
SS-13	4.5	39.5
Zebra	87.7	91.2

TABLE III

POLLEN GERMINATION AND POLLEN TUBE GROWTH OF FIVE ACCESSIONS OF BERMUDAGRASS WHEN SELF-POLLINATED

Time after pollination (hours)	Pistils observed (number)	Pollen grains observed (number)	Pollen germ. (%)	Tubes to:		
				style (number)	ovary (number)	micropyle (number)
70-3						
2.5	42	352	80	0	0	0
5.0	41	401	82	1	0	0
7.5	46	382	86	13	6	4
10.0	39	481	83	26	14	9
24.0	47	445	85	39	33	19
Total	187	2061	84	79	53	32
72-6						
2.5	39	468	84	0	0	0
5.0	51	496	89	3	1	0
7.5	57	684	88	3	2	0
10.0	29	332	91	8	5	4
24.0	43	533	91	17	15	10
Total	219	2503	88	31	23	14
SS-3						
2.5	21	452	80	0	0	0
5.0	27	522	81	0	0	0
7.5	25	515	86	5	1	0
10.0	38	661	85	4	2	0
24.0	46	765	88	7	3	1
Total	157	2915	84	16	6	1

TABLE III (Continued)

Time after pollination (hours)	Pistils observed (number)	Pollen grains observed (number)	Pollen germ. (%)	Tubes to:		
				style (number)	ovary (number)	micropyle (number)
SS-13						
2.5	23	584	80	0	0	0
5.0	25	610	78	0	0	0
7.5	33	781	80	6	3	3
10.0	27	652	83	5	4	2
24.0	32	605	84	17	9	4
Total	140	3232	81	28	16	9
Zebra						
2.5	21	752	92	22	10	6
5.0	25	828	94	42	33	9
7.5	26	894	93	68	51	21
10.0	32	961	95	140	83	43
24.0	19	681	91	221	195	78
Total	123	4116	93	493	372	143

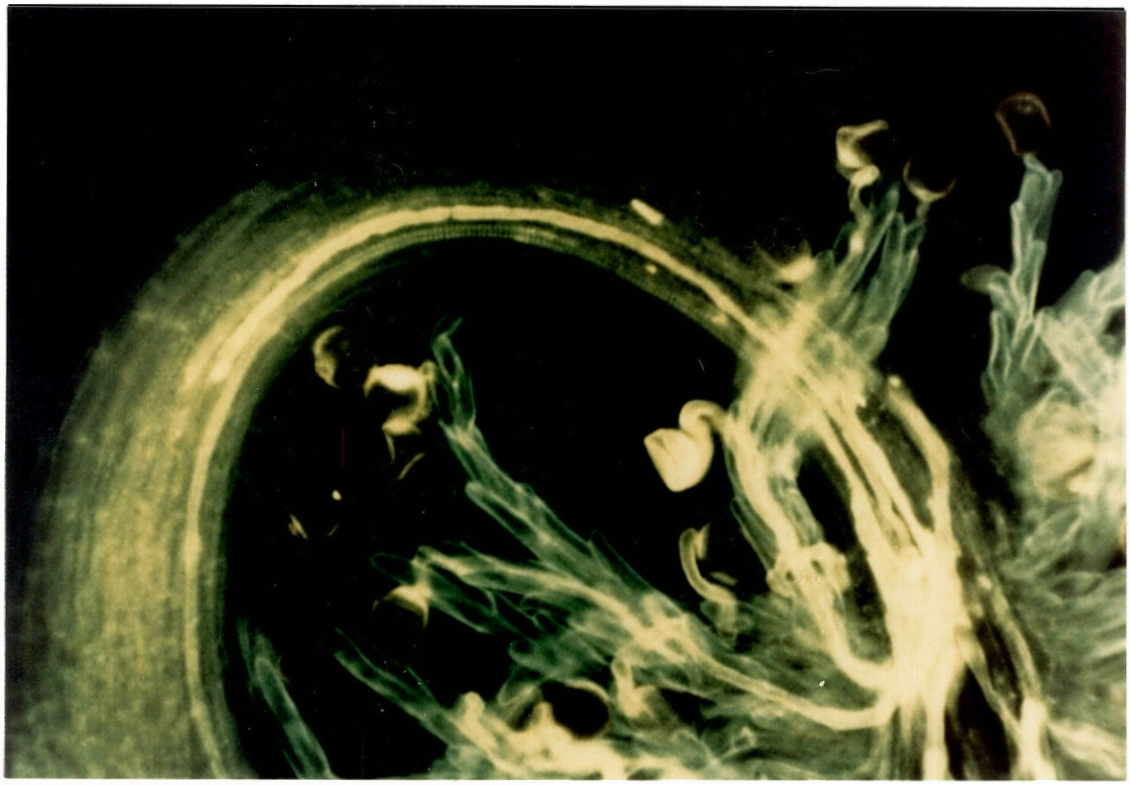

Figure 1. Pollen tube at the base of the stigma 24 hours after a self-pollination of plant SS-13.

continued to grow through the stylar tissue uninhibited and counting the tubes became difficult with as many as 15 tubes present at one time. Pollen tubes of Zebra could be traced to the entrance of the micropyle in most cases. By the 24 hour interval, the pollen tubes of Zebra which had not reached the micropyle ceased growing and a noticeable degeneration in stigmatic tissue was present. Less than 0.6% of their pollen tubes of the four other accessions reached the micropylar region when all time intervals were totaled. Stigmatic tissue of these accessions did not appear to be degenerating as in the Zebra, particularly in pistils which failed to have a pollen tube reach the micropyle. Over 3% of the observed pollen tubes of Zebra reached the micropyle, with many remaining in the ovary following what appeared to be successful fertilizations.

The results indicate inhibition of pollen tubes in both rate and extent of growth occurred in accessions 70-3, 72-6, SS-3, and SS-13 with no apparent effect due to pollen germination. Zebra pollen tube growth exhibited no inhibition and a much greater frequency of pollen tubes reached the micropyle than the other four accessions.

Cross-pollinations

Pollen germination within the first 2.5 hours was similar to that following self-pollination (Table IV). Pollen germination remained above 80% with the Zebra x SS-3 cross having a mean germination rate of 89% over all time intervals. Within the first 2.5 hours, all three cross-pollinations had pollen tubes which reached the micropyle. At the 5.0, 7.5, and 10.0 hour intervals more pollen tubes were identified as reaching the region of the micropyle with no apparent inhibition as observed in the self-pollinations of the same accessions (Figure 2). More than 8% of the total number of pollen grains counted in the cross of 70-3 x 72-6 reached the micropyle, with at least 5% in the two remaining crosses.

Only small differences between self- and cross-pollinations were found in pollen germination due to source. The mean percentage of germination of the cross of two accessions was observed to be approximately equal to average of the two means of the parents when self-pollinated thus indicating germination was not directly contributing to self-incompatibility. The rate and extent of pollen tube growth greatly differed between self- and cross-pollinations involving 70-3, 72-6, SS-3, and SS-13. In contrast there was little difference in rate or extent of pollen tube growth for Zebra due to pollen source. Some tubes of cross-pollinated grains generally reached the micropylar region within 2.5 hours whereas at least 5.0 or more hours were

TABLE IV

POLLEN GERMINATION AND POLLEN TUBE GROWTH OF FIVE ACCESSIONS OF BERMUDAGRASS WHEN CROSS-POLLINATED

Time after pollination (hours)	Pistils observed (number)	Pollen grains observed (number)	Pollen germ. (%)	Tubes to:		
				style (number)	ovary (number)	micropyle (number)
70-3 x 72-6						
2.5	43	642	83	37	11	6
5.0	49	694	87	65	31	29
7.5	53	703	85	86	41	72
10.0	52	651	86	128	68	67
24.0	46	664	89	195	161	98
Total	243	3364	86	511	312	272
SS-3 x SS-13						
2.5	37	651	85	20	11	2
5.0	29	589	79	56	35	23
7.5	33	723	83	88	50	41
10.0	22	532	83	91	53	44
24.0	41	810	85	131	94	71
Total	162	3305	83	386	243	181
Zebra x SS-3						
2.5	26	786	84	25	16	4
5.0	22	707	89	51	31	15
7.5	31	843	87	73	43	27
10.0	29	796	92	151	99	57
24.0	26	743	93	210	191	91
Total	134	3870	89	511	380	194

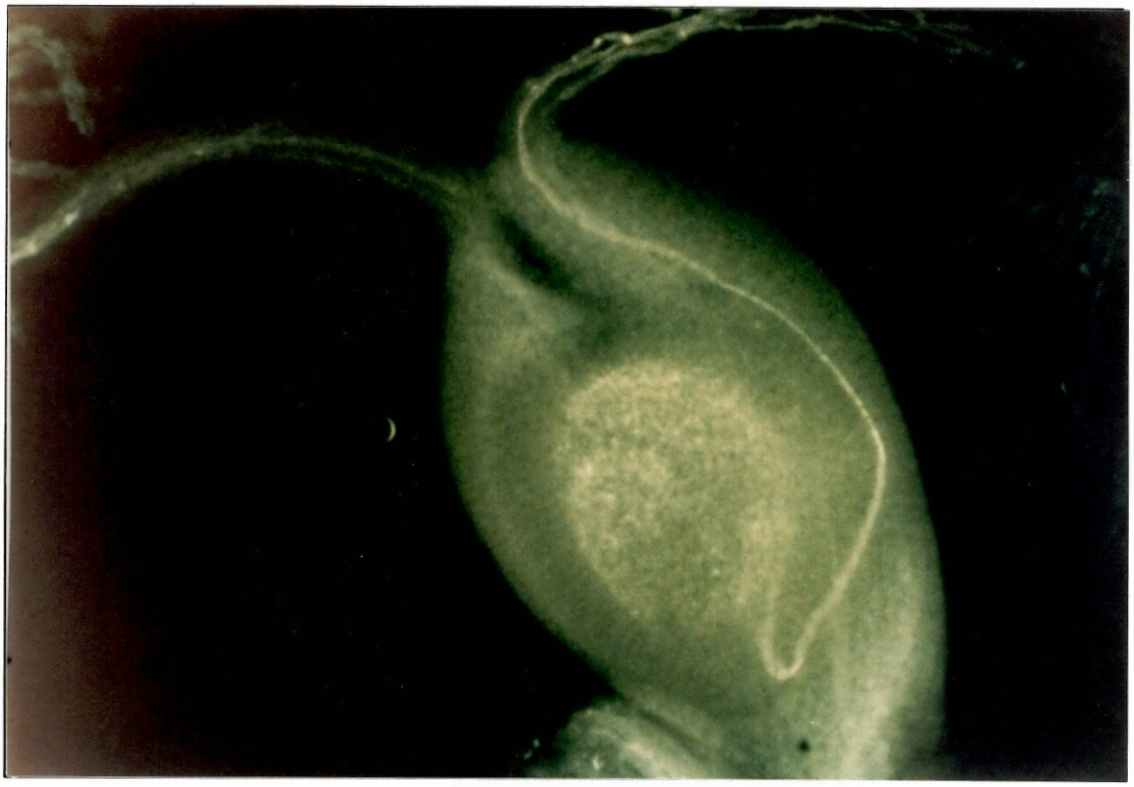

Figure 2. Pollen tube at the micropyle 5 hours after cross-pollination of plants SS-13 x SS-3.

required for this occurrence in self-pollinations of the five accessions except for Zebra. There were no significant differences found among genotypes with respect to maternal effects on pollen germination or initial tube penetration.

CHAPTER V

SUMMARY AND CONCLUSIONS

Findings of this study confirm the reports of Burton (1965), Kneebone (1967), and Richardson, Ahring and Taliaferro (1978) that a self-incompatibility mechanism is present in bermudagrass. The results suggest that the self-incompatibility mechanism previously reported in bermudagrass occurs in the stigmatic and stylar tissues. The extent and rate of pollen tube growth was substantially inhibited following self-pollinations of accessions which were previously identified under field conditions to exhibit self-sterility. The Zebra accession can be considered self-compatible while the other four accessions have varying levels of self-incompatibility. There were no cross-incompatibilities identified in the three reciprocal crosses performed in the experiment. Pollen source did not significantly affect pollen germination which suggests that it has no direct effect on self-incompatibility in bermudagrass.

The differences in the levels of self-fertility identified under field conditions as well as the research conducted in this experiment suggests self-incompatibility in bermudagrass is controlled by several alleles. The SS-3

accession exhibited 3.6% self-fertility in the field with only .03% of the pollen tubes reaching the micropyle following controlled self-pollination in the greenhouse. In contrast, the 70-3 accession had 20.4% self-fertility in the field with 1.5% of the pollen tubes reaching the micropyle. The results observed from the differences in self-fertility and pollen tube growth due to genotype indicate that more than one loci with multiple alleles may control the system of self-incompatibility in bermudagrass. Genetic studies will be needed to determine the actual number of loci and alleles controlling the mechansim. These studies would require the development of a large population of plants which would be intercrossed and techniques similar to the ones used in the study would have to be used.

The self-incompatibility system in bermudagrass resembles the self-incompatibility systems of other species determined to be gametophytically controlled (Dickinson and Lawson, 1975; Heslop-Harrison and Heslop-Harrison, 1982). The pattern observed in the inhibition of pollen tubes in the bermudagrass system appears to be gametophytically controlled in the sense that the behavior of the pollen is determined by its own genotype and not by the pollen parent. Some pollen tubes were able to grow into the ovary and eventually reach the micropyle. The model of a sporophytic system predicts that because the pollen parent controls pollen tube growth, no tubes should have reached the micropyle and inhibition of pollen tube germination and

growth should occur on or just inside the stigmatic tissue.

In summary, the results observed in this cytogenetic study will aid in the breeding of better bermudagrass cultivars. The cytological identification of the self-incompatibility system in bermudagrass will increase the breeder's understanding of the reproductive processes in the grass and assist in modifying current breeding procedures to develop more efficiently new and improved cultivars.

BIBLIOGRAPHY

Ascher, P. D., and S. J. Peloquin. 1966. Influence of temperature on incompatible and compatible pollen tube growth in Lilium longiflorum. Can. J. Genet. Cytol. 8:661-664.

Anderson, M. A., E. C. Cornish, S. -L. Mau, E. G. Williams, and R. Hoggart. 1986. Cloning of cDNA for a stylar glycoprotein associated with expression of self-incompatibility in Nicotiana alata. Nature 321:38-44.

Bateman, A. J. 1952. Self-incompatibility systems in angiosperms. I. Theory. Heredity 6:285-310.

Bateman, A. J. 1955. Self-incompatibility systems in angiosperms. III. Cruciferae. Heredity 9:52-68.

Brewbaker, J. L. 1959. Biology of the angiosperm pollen grain. Ind. J. Plant Breed. 19:121-133.

Burton, G. W. 1943. Coastal bermudagrass. Ga. Coastal Plains Exp. Sta. Cir., 10:12.

Burton, G. W. 1948. Artificial fog facilitates Paspalum emasculation. Agron. J. 40:281-282.

Burton, G. W. 1965. Breeding better bermudagrasses. Proc. 9th Int. Grassland Congr. 9:93-96.

Burton, G. W. and W. H. Devane. 1954. Ga. Agr. Exp. Sta. Bull. NS2.

Burton, G. W., and W. W. Hanna. 1985. Bermudagrass. In Heath, Barnes, and Metcalfe (Ed.), Forages, 4th ed. (p 247). Ames: Iowa State University Press.

Burton, G. W., and R. H. Hart. 1967. Use of self-incompatibility to produce seed propagated F_1 bermudagrass hybrids. Crop Sci 7:524-527.

Burton, G. W., R. H. Hart, and R. S. Lowery. 1967. Improving forage quality in bermudagrass by breeding. Crop Sci. 7:329-332.

Burton, G. W. and W. G. Monson. 1978. Registration of Tifton 44 bermudagrass. Crop Sci. 18:911.

Chapman, H. D., W. H. Marchant, P. R. Utley, R. E. Hellwig, and W. G. Monson. J. Anim. Sci. 34:373-78.

Charlesworth, D. 1988. Evolution of homomorphic sporophytic self-incompatibility. Heredity 60:445-453.

Cornish, E. C., M. A. Anderson, and A. E. Clarke. 1988. Molecular aspects of fertilization. Ann. Rev. Cell Biol. 4:229-255.

Cornish, E. C., J. M. Pettitt, I. Bonig, and A. E. Clarke. 1987. Developmentally controlled expression of a gene associated with self-incompatibility in Nicotiana alata. Nature 326:99-102.

Cresti, M., F. Ciampolini, and E. Pacini. 1977. Ultrastructural aspects of pollen tube growth inhibition after gamma irradiation in Lycopersicon peruvianum. Theor. Appl. Genet. 49:297-303.

Darlington, C. D. and K. Mather. 1949. The Elements of Genetics. Allen and Unwin Ltd.

Darwin, C. 1877. The Different Forms of Flowers on Plants of the Same Species. John Murray, London.

Darwin, C. 1880. The Different Forms of Flowers on Plants of the Same Species. (2nd ed.). John Murray, London.

Dickinson, H. G. and J. Lawson. 1975. Pollen tube growth in the stigma of Oenothera organensis following compatible and incompatible intraspecific pollinations. Proc. Roy. Soc. Lond. B. 188:327-344.

Donk, J. A. W. van der. 1975. Recognition and gene expression during the incompatibility reaction in Petunia hybrida L. Mol. Gen. Genet. 141:305-316.

Duvick, D. N. 1966. Influence of Morphology and Sterility on Breeding Methodology. Plant Breeding Symp., Iowa State Univ., Frey, K. J. (ed.).

East, E. M. 1926. The physiology of self-sterility in plants. J. Gen. Physiol. 8:403-416.

East, E. M. 1940. The distribution of self-sterility in flowering plants. Proc. Am. Phil. Soc. 82:449-518.

East, E. M. and A. J. Mangelsdorf. 1925. A new interpretation of the hereditary behavior of self-sterile plants. Proc. Nat. Acad. Sci. (Wash) 11:166-171.

Ebert R., M. A. Anderson, R. Bernatzky, M. Altschuler, and

A. E. Clarke. 1987. Genetic polymorphism of self-incompatibility in flowering plants. Cell 56:255-262.

Forbes, I. and G. W. Burton. 1963. Chromosome numbers and meiosis in some Cynodon species and hybrids. Crop Sci. 3:75-79.

Ghosh, S. and K. R. Shivanna. 1980. Pollen-pistil interaction in Linum grandiflorum. Planta 149:257-261

Harlan, J. R., G. W. Burton, and W. C. Elder. 1954. Okla. Agr. Exp. Sta. Bull. B-416.

Harlan, J. R., and J. M. J. de Wet. 1969. Sources of Variation in Cynodon dactylon (L.) Pers. Crop Sci 9:774-778.

Hayman, D. L. 1956. The genetic control of incompatibility in Phalaris coerulescens. Aust. J. Biol. Sci. 9:321.

Heslop-Harrison, J. and Y. Heslop-Harrison. 1982. The pollen-stigma interaction in the grasses. 4. An interpretation of the self-incompatibility response. Acta Bot. Neerl. 31:429-439.

Huffine, W. W. 1957. Sunturf Bermuda, a new grass for Oklahoma lawns. Oklahoma A&M College Exp. Sta. Bull. B-494.

Johnston, R. A. and C. M. Taliaferro. 1975. Effects of temperature and light intensity on the expression of a variegated leaf pattern in bermudagrass. Crop Science 15:445-447.

Kho, Y. O. and J. Baer. 1968. Observing pollen tubes by means of fluorescence. Euphytica 17:298-303.

Kneebone, W. R. 1967. Breeding seeded bermudagrass; unique Arizona research problem. Progressive Agriculture in Arizona 14(4): 4-5.

Kovaleva, L. V., E. L. Milyaeva, and M. K. H. Chuilukhyan. 1978. Overcoming self-incompatibility by inhibitors of nucleic acid and protein metabolism. Phytomorphology 28:445-449.

Kroes, H. W. 1973. An enzyme theory of self-incompatibility. Incompat. Newsletter Assoc. EURATOM-ITAL., Wageningen 2:5-14.

Lawrence, M. J., D. F. Marshall, V. E. Curtis, and C. H. Fearon. 1985. Gametophytic self-incompatibility re-examined: a reply. Heredity 54:131-138.

Lewis, D. 1949. Structure of the incompatibility gene. II. Induced mutation rate. Heredity 3:339-355.

Lewis, D. 1964. A protein dimer hypothesis on incompatibility. Proc. 11th Internat. Congr. Genet. The Hague, 1964. In: Genetics Today, S. J. Geerts (ed.) 3:656-663.

Lundqvist, A. 1956. Self-incompatibility in rye. I. Genetic control in the diploid. Hereditas 42:293-348.

Lundqvist, A. 1961. A rapid method for the analysis of incompatibilities in grasses. Hereditas 47:705-707.

Lundqvist, A. 1962. Self-incompatibility in diploid Hordeum bulbosum L. Hereditas 48:138-152.

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.

Mau, S. -L., E. G. Williams, A. Atkinson, M. A. Anderson, E. C. Cornish, B. Grego, R. J. Simpson, A. Kheyer-Pour, and A. E. Clarke. 1986. Style proteins of a wild tomato (Lycopersicon peruvianum) associated with expression of self-incompatibility. Planta 169:184-191.

Mease, J. 1807. A Geological Account of the United States, Comprehending a Short Description of Their Animal, Vegetable and Mineral Productions. Philadelphia, Birch and Small.

Mulcahy, D. L., and G. B. Mulcahy. 1983. Gametophytic self-incompatibility re-examined. Science N. Y. 220:1247-1251.

Nasrallah, M. E. 1979. Self-incompatibility antigens and S-gene expression in Brassica. Heredity 43:259-263.

Nettancourt, D. de. 1977. Incompatibility in Angiosperms. Springer-Verlag, Berlin-Heidelberg-New York.

Pandey, K. K. 1962. Interspecific incompatibility in Solanum species. Am. J. Botan. 49:874-882.

Pushkarnath. 1942. Studies on sterility in potatoes. IV. Genetics of self- and cross-incompatibilities. Ind. J. Genet. 2:11.

Richardson, W. L. 1958. A technique of emasculating small grass florets. Indian Journal of Genetics and Plant

Breeding 18:69-73.

Richardson, W. L., C. M. Taliaferro, and R. M. Ahring. 1978. Fertility of eight bermudagrass clones and open-pollinated progeny from them. Crop Sci 18:332-334.

Riley, H. P. 1932. Self-sterility in Shepherd's purse. Genetics 17:231-295.

Riley, H. P. 1935. Self-sterility and self-fertility in species of the Genus Nemesia. Am. J. Botan. 22:889-894.

Roberts, I. N., A. D. Stead, D. J. Ockendon, and H. G. Dickinson. 1984. Pollen-stigma interactions in Brassica oleraceae. I. Ultrastructure and physiology of the stigmatic papilla cells. J. Cell Sci. 66:241-253.

Rowlands, D. G. 1964. Self-incompatibility in sexually propagated cultivated plants. Euphytica 13:157-162.

Shivanna, K. R., J. Heslop-Harrison, and Y. Heslop-Harrison. 1981. Heterostyly in Primula. 2. Sites of pollen inhibition and effects of pistil components on compatible and incompatible pollen tube growth. Protoplasma 107:319-337.

Taliaferro, C. M. and W. L. Richardson. 1980. Crop Sci. 20:413.

Taliaferro, C. M., R. M. Ahring, and W. L. Richardson. 1983. Registration of Guymon bermudagrass. Crop Sci. 23:1219.

Thompson, K. F. 1957. Self-incompatibility in marrow-stem kale, Brassica oleracea var. acephala. I. Demonstration of sporophytic system. J. Genet. 55:45-60.

Vuilleumier, B. S. 1967. The origin and evolutionary development of heterostyly in the angiosperms. Evolution 21:210-226.

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