MEGASPOROGENESIS, EMBRYO SAC DEVELOPMENT, AND ESTIMATES OF PHENOTYPIC VARIABILITY IN EASTERN GAMAGRASS

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CHAPTER I

INTRODUCTION

Eastern gamagrass (Tripsacum dactyloides L.) is a warm season perennial plant that grows in clumps varying from one to four feet in diameter. It grows over the entire eastern half of the United States and has been found on favorable sites as far west as Colorado. It grows especially well in low areas where there is supplemental run-in water and deeper soils. In lowlands, it usually borders sloughgrass and switchgrass. In prairie meadows, it is associated with big bluestem (27). Eastern gamagrass spreads by rough, thick, and knotty rhizomes with short joints. The period of seed formation starts in June or July and ends in September (27, 28).

Eastern gamagrass is very palatable and nutritious and is eaten by all classes of livestock. Cattle are particularly fond of this species. Because it is highly palatable, it has been killed out by close grazing on most rangeland (27). Because of its height and leafiness, this grass produces a vast amount of forage and is a very productive hay crop (27). In one case in Texas, one animal unit grazed 2.5 acres on a yearlong basis. In this instance, the gamagrass produced 180 pounds of marketable beef per acre (28).

There have been no programs undertaken to genetically improve eastern gamagrass. Before an effective breeding program can be initiated, however, the reproductive behavior of the plant material of

interest must be studied to see if there are abnormalities such as sterility and apomixis. In addition, an estimate of the genetic variability between the plants would aid the plant breeder in selection. This study was conducted to obtain this basic genetic information. Megasporogenesis and embryo sac development were studied so that any abnormalities in the reproductive process could be identified. In addition, the variability of the plants was studied in conjunction with an analysis of the correlations of selected agronomic characters.

CHAPTER II

REVIEW OF LITERATURE

Megasporogenesis and Embryo Sac Development

In general, one cell of the nucellus, usually in the hypodermal layer, differentiates into an archesporial cell which becomes the megaspore mother cell (4, 11, 12, 23, 25). However, in some cases, it may divide to form a primary parietal cell and a primary sporogenous cell. The primary sporogenous cell becomes the megaspore mother cell (25). The megaspore mother cell, in most instances, arises at about the time that the integuments begin to differentiate or a while before this (4, 6, 12, 26, 40). It is usually characterized by being larger than the other cells as well as having denser cytoplasm and a more prominent nucleus (25). Artschwager and McGuire (4) found that in Sorghum vulgare Pers., the megaspore mother cell is at first polygonal being wider at its micropylar end. It has a large nucleus and dense cytoplasm. Later, it increases in size and becomes oblong. Cooper (12) found a similar megaspore mother cell in Euchlaena mexicana Schrad. which is twice as long as it is wide. In Digitaria Heister, Carmichael (11) noticed that the megaspore mother cell becomes elongated and lies across almost the whole length of the nucellus. In this genus, nucellar cells at the chalazal end of the megaspore mother cell become hypertrophied and are quite conspicuous because of enlargement of the

nucleus and densely staining cytoplasm. The nucleus is at the micropylar end of the megaspore mother cell in most cases.

The megaspore mother cell divides by meiosis to give at first a dyad and then a tetrad of megaspores. In most cases, both divisions are transverse giving a linear tetrad. Sometimes, as in Digitaria, there is only a triad formed because of the failure of one of the dyad cells to divide (11, 25).

In most species of Gramineae, only one of the four megaspores is functional (3, 5, 6, 11, 12, 15, 23, 24, 25, 34, 40). However, Mohamed and Gould (26) found that in Bouteloua Lag., all four megaspores participate in embryo sac development (the Adoxa type). When there is one functional megaspore, it is usually the chalazal member of the tetrad. However, Anderson (3) noted that in Poa L., the micropylar member would occasionally function. Cooper (12) found that in Zea mays L., the megaspore mother cell increases in size during the heterotypic prophase and by diakinesis, it is three times as long as it is wide. It is unequally divided by a cell plate at the end of the first meiotic division, the chalazal cell being twice as long as the micropylar cell. The second division also is unequal, especially in the chalazal daughter cell. As a result, the chalazal cell is much larger than the other three which soon disintegrate. In Digitaria, Carmichael (11) observed that the degenerated megaspores leave a line of heavy staining material from the functional megaspore to the micropyle. These two successive divisions change the diploidy of the megaspore mother cell into the haploidy of the megaspore. This completes the process of megasporogenesis (32).

The primary megaspore is the progenitor of the embryo sac. At first, it is accompanied by increased vacuolation with a large vacuole being formed on either side of the nucleus in the long axis of the cell (23, 25). In Digitaria (11) and Pennisetum ciliare (L.) Link. (6, 34), the megaspore enlarges greatly. In Euchlaena, Cooper (12) found that the cytoplasm of the primary megaspore becomes highly vacuolate in the chalazal end, and later this vacuolation is replaced by one large vacuole.

The primary megaspore divides three times by mitosis to give a mature embryo sac with eight nuclei. At the first division, a large vacuole develops between the two daughter nuclei which are referred to as the primary micropylar nucleus and the primary chalazal nucleus. Most of the cytoplasm is aggregated around these two nuclei (23, 24, 25, 32). In addition, the young embryo sac enlarges considerably (32). In Pennisetum ciliare (6), it is long and narrow with the two nuclei at opposite ends. The primary chalazal nucleus and the primary micropylar nucleus each divide two more times, and these divisions are accompanied by increased vacuolation (23, 24, 25, 32). Artschwager and McGuire (4) found that the chalazal nucleus divides perpendicular to the nucleus in the micropylar end and parallel to the long axis of the embryo sac. They found that a large vacuole separates the groups of two. The final division gives four nuclei in each group (23, 24, 25, 32). The two quartets of nuclei differentiate to produce the nuclei found in the mature embryo sac. The micropylar quartet forms an egg, two synergids, and a polar nucleus. The chalazal nuclei form three antipodals and a polar nucleus. As differentiation progresses, the two polar nuclei move to the center, thus forming a mature embryo sac

that is 8-nucleate and 7-celled (24, 25, 32). This embryo sac enlarges by digesting the adjacent nucellar tissue (4). Cooper (12) found that in Euchlaena mexicana, the 8-nucleate embryo sac is three times longer and three times wider than the functional megaspore was before it divided.

In general, the mature egg cell and the synergids are pyriform-shaped. The egg cell has its nucleus and cytoplasm in the lower end of the cell, while the synergids have their nuclei and cytoplasm toward the top of the cell. There is also a filiform apparatus associated with the synergids (24). Artschwager and McGuire (4) found that in Sorghum vulgare, the egg becomes more spherical as it develops and is less distinct than the antipodals. Kiesselbach (21) noted that in Zea mays, the egg is larger than the synergids. In most species, the synergids are ephemeral structures which may disintegrate before or slightly after fertilization (4, 5, 6, 21, 24, 34).

The antipodals are usually short-lived but can increase in size and number in such species as Euchlaena mexicana and Zea mays in which there may be a proliferation of twenty-five or thirty or more cells (12, 24, 40). Artschwager and McGuire (4) found fairly long-lived antipodals in Sorghum vulgare which do not completely disappear until the endosperm has reached the distal end of the inner integument. In this species, they also appear to be coenocytic. Taliaferro (34) and Bashaw (6) observed that in Pennisetum ciliare, the antipodals proliferate but are digested by the endosperm and the embryo after they develop. In Paspalum dilatatum Poir, the antipodals were observed by Bashaw and Holt (5) to divide several additional times, thus forming a compact oval cluster in the chalazal end of the embryo

sac. In Digitaria, a proliferation of six to nine antipodals was observed by Carmichael (11). Farquharson (15) found from six to sixteen antipodals in Tripsacum L. with ten to twelve being common.

Artschwager and McGuire (4) reported that in Sorghum vulgare, the polar nuclei are in close proximity to the egg with the adjacent sides flattened. Similarly in Zea mays, Weatherwax (40) observed that the two polar nuclei lie near the egg and are close to each other but do not fuse until after fertilization. However, in most species, the two polar nuclei lie in the middle of the embryo sac (25). In Zea mays, the embryo sac will remain in this condition for about two weeks if it is not fertilized (21).

There are various patterns of pollen tube growth. When pollination occurs, usually only one pollen tube nucleus reaches the micropyle. In Zea mays, it grows between the nucellar cells and releases two sperm cells into the embryo sac (21). Artschwager and McGuire (4) noted that in some instances in Sorghum vulgare, two pollen tubes may discharge their sperm cells after penetration into the embryo sac. Farquharson (15) found that in Tripsacum, the pollen tube usually grows between the ovary wall and the outer integument prior to reaching the micropyle. Cooper (12) observed that the pollen tube goes between the synergids in Zea mays and Euchlaena mexicana.

Shortly after the release of the sperm cells into the embryo sac, there is double fertilization (4, 21). After fertilization has been accomplished, there exists haploid antipodals, a diploid embryo, and triploid endosperm (21). In sexual species, the primary endosperm nucleus almost always divides before the zygote does (8, 25). In fact, in Tripsacum, it divides immediately after fertilization in most

cases (15). Artschwager and McGuire (4), working with Sorghum vulgare, observed that when the primary endosperm nucleus divides, the two daughter nuclei separate and become located on opposite sides of the egg. They noted four or more nuclei present in the endosperm before the zygote divided. Cooper (12) found that in Zea mays and Euchlaena mexicana, between four and eight nuclei make up the endosperm before the zygote divides. In Tripsacum, Farquharson (15) noted that there may be from eight to thirty-two free endosperm nuclei present before the zygote undergoes division. In general, the first and usually some of the succeeding divisions of the endosperm are not accompanied by wall formation and the nuclei remain free. The nuclei may remain free or be separated by walls in later divisions (25). Kiesselbach (21) noted that in Zea mays, there is a central vacuole during the early divisions with free endosperm surrounding it. Later, he found wall development. Farquharson (15) observed that the endosperm is peripheral and that there is cell wall formation on the third day after fertilization in Tripsacum. Likewise, Artschwager and McGuire (4) observed peripheral endosperm in Sorghum vulgare.

Unlike the endosperm, there are few, if any, free embryo nuclei. In the species of Gramineae, the embryo may develop by regular or irregular patterns of cell division (25). In Zea mays, Kiesselbach (21) found that the endosperm develops much more rapidly than the proembryo in the earlier stages. Farquharson (15) noted that in Tripsacum, the zygote may not divide at all until two days after pollination. She found that the embryo is pear-shaped for the first six to eight days until it shows its first asymmetric differences. Cooper (12) had difficulty in observing the details of the zygote

nucleus because of all the starch grains present in the cytoplasm. Johansen (20) sums up this process of development after fertilization as embryogeny.

Up to this point, only normal megasporogenesis, embryo sac development, and embryogeny have been discussed. Apomixis and polyembryony are deviations from this normal pattern.

In apomictically reproducing plants, normal sexual development is circumvented and nuclear or cellular fusion is not involved. The two broad groupings of this phenomenon are vegetative reproduction and agamospermy. Vegetative reproduction involves plant parts other than the seed and will not be treated here. In agamospermy, the embryo can either directly develop from the diploid ovular tissue (adventitious embryony) or from a diploid gametophyte (gametophytic apomixis). Gametophytic apomixis can be broken down into two types which are diplospory and apospory. In diplospory, the apomictic embryo sac develops from the archesporial cell, but regular meiotic chromosome reduction is missing. Apospory, on the other hand, involves the formation of a diploid gametophyte from cells of the integument or the nucellus. Apospory is the most prevalent type of apomixis described in the reviewed literature. After the embryo sac is formed, the embryo may develop by parthenogenesis (division of the egg cell) or apogamety (the division of one of the other cells) (33).

Bashaw and Holt (5) and Bashaw (6), working with Paspalum dilatatum, found a number of nucellar cells differentiating at the functional megaspore stage and subsequently encroaching upon the megaspore. In most instances, all four megaspores of the linear tetrad degenerate, but occasionally, the chalazal member develops to the

2-nucleate stage, and very rarely a mature sexual embryo sac is formed. Many of the ovules that were collected before pollination had proembryos, but no endosperm was evident, which suggests probable pseudogamous development (pollination required for endosperm development). Fisher, et al. (17), working with Pennisetum ciliare and Cenchrus setigerus Vahl., observed that the normal sequence proceeds to the 4-nucleate stage. At this time much abnormal enlargement of the nucellar cells surrounding the gametophytic cavity is evidenced. These nucellar cells form structures resembling embryo sacs which finally crowd the sexual embryo sac completely out. Proembryos were found in some of these embryo sacs, and in some cases, twin embryos were found. It was not possible to establish the origin of the endosperm that was present.

In Bouteloua, Mohamed and Gould (26) found that the megaspore mother cell does not function at all and that a nucellar cell develops into a normal looking 8-nucleate embryo sac. Some of the mature embryo sacs have only three nuclei, the synergids and antipodals not being present.

Grazi and Akerberg (18) found that in Poa, usually only one aposporous nucellar cell differentiates and is usually located in the chalazal part of the nucellus. The chalazal megaspore rarely grows to the 1-nucleate embryo sac stage but is usually crowded out before then by the aposporous cell which grows toward the micropyle and forms an 8-nucleate embryo sac similar to that found in Bouteloua. The antipodal cells in this apomictic embryo sac are abnormally large. Akerberg (1) found that, at times, two aposporous cells differentiate. In another paper (2), he said that it seems that pollination is necessary for the development of the ovule in apomictic, as well as sexual

strains of Poa pratensis L.

Weatherwax (40) and Farquharson (14, 15, 16) found evidence suggesting apomictic reproduction in Tripsacum dactyloides. Brown and Emery (9) proposed that in this species, the megaspore mother cell divides mitotically and that the egg or a synergid begins to form an embryo before the sexual triple fusion. A 5 n endosperm lends further proof of a diploid embryo sac. The embryo sac is probably 8-nucleate, although only five nuclei are observable when the embryo sac is mature. The antipodals are seen in young embryo sacs, but they evidently disintegrate before maturity.

Although, as described above, there are at times no visible differences between apomictic and sexual embryo sacs, there are several general differences between the two in most species of the Gramineae. If all of the mature embryo sacs are 4-nucleate instead of 8-nucleate, apomixis is probably present. In addition, if a proembryo and endosperm are present without the accompanying presence of antipodals, there is strong evidence for this phenomenon (9). Saran (31) proposes that sexual embryo sacs possess antipodal cells and two polar nuclei, whereas aposporous embryo sacs lack these structures.

Sexuality and apomixis obviously have a genetic basis. This proposal is supported by work done by Bashaw (6) and Taliaferro and Bashaw (35). Bashaw (6) found that about 1 out of 16 plants of Pennisetum ciliare are apomicts when heterozygous plants are crossed. Over a period of several years, Taliaferro and Bashaw (35) found that this ratio was probably closer to 13 sexual to 3 apomictic. They also found that if a sexual female and an apomictic male were crossed, a 5:3 ratio of sexual to apomictic progeny was obtained. From these

data, they hypothesized that the sexual plant is AaBb where B controls sexuality and has an epistatic effect on A which controls apomixis. These ratios suggest the proposed digenic control.

In addition to apomixis, or in conjunction with it, polyembryony can be expressed, which is the condition in which a seed may produce more than one embryo. There are several ways in which this can occur. First, more than one egg cell may be formed in the embryo sac. Secondly, the zygote or a young embryo may divide. A third possibility is the development of more than one embryo sac. This can arise from a mitotic division of a megaspore mother cell giving two megaspore mother cells, or the two megaspore mother cells may develop from separate nucellar cells. In addition, apospory may be present. Fourth, an embryo can develop directly from the cells of the nucellus without the formation of an embryo sac. This is the type of apomixis described earlier which was termed adventitious embryony. Lastly, other cells of the embryo sac besides the egg may develop into embryos. Synergids are the most likely to do this, but polar nuclei and antipodals have been observed to form embryos. This condition is termed apogamy or apogamety (15, 33).

Polyembryony occurs in several members of the Gramineae. Bashaw (6) found more than one embryo in over 30 per cent of the ovules that he examined from Pennisetum ciliare. Akerberg (1) found that polyembryony seems to be quite common in apomictic species of Poa pratensis. Tripsacum shows the highest percentage of polyembryony of all genera of the Gramineae. In fact, seeds with multiple embryos have been found in almost all tetraploid collections of Tripsacum (15). Weatherwax (40) found that over 50 per cent of the seeds have more than

one embryo. Farquharson (14) found that triple embryos are frequent in this genus. Embryos are usually contained in one embryo sac, but occasionally two embryo sacs are present. Farquharson (15) noted that in Tripsacum, two megaspore mother cells are usually formed from separate nucellar cells. Adventitious embryony has not been observed in Tripsacum. The extra embryos that Farquharson noted looked like they may develop from synergids because of the position they took in the embryo sacs. She stated that multiple embryos have never been found in diploid Tripsacum plants.

Sometimes embryo sacs may have more or less nuclei than normal. This may be in conjunction with apomixis or independent of it. When embryo sacs have less than the normal eight nuclei, it usually is caused by early degeneration of the antipodals. Sometimes they may be present but may be inconspicuous. Misdivision, however, can give a genuine reduction of nuclei. When more than the normal quota of nuclei are present, it may have arisen from fusion of two embryo sacs, migration of nuclei into the embryo sac, or the division of some of the eight normal nuclei (25). Carmichael (11) observed several abnormal embryo sacs in Digitaria. In one, there was only one large nucleus. Several had more than the normal complement of eight nuclei. One embryo sac was devoid of the egg apparatus but had one nucleus in the position of the polars in addition to the antipodals. Farquharson (15) noted more than the normal complement of polar nuclei in many embryo sacs of Tripsacum. Most likely there had been a division of the polar nuclei, but a combination of two embryo sacs and the disintegration of some of the nuclei could have caused this also.

Phenotypic Variability

A study was undertaken by Dhindsa and SIinkard (13) with 30 strains of Russian wildrye (Elymus juncus) from the United States and Canada. The purpose of their study was to determine the phenotypic variability of several agronomic traits and how closely they were correlated with one another. From these results, it could be determined if there was enough variability for a concentrated breeding program. Vegetative vigor, heading date, plant height, seed yield, fertility, seed size, and shoot length were analyzed. The means and coefficients of variation were calculated for each and considerable variability was shown. Highly significant correlations were obtained for vigor score with heading date and plant height, plant height with seed yield and date of heading, seed yield with fertility and seed size, and shoot length with seed size. Correlations that were significant were obtained for vigor with seed yield, and shoot length with seed yield. The authors postulated that if they selected for one of the seven characters, there would be no detrimental effect on the other traits. Selection for shorter plant height or earlier maturity, however, might have a detrimental effect on vegetative vigor and seed yield. In addition, the variability appeared to be great enough to warrant a selection program.

In other studies that show the importance of correlated factors, both shoot length and seed size were found to be positively correlated with seedling vigor. Black (7) found that in Trifolium subterraneum L., leaf area, as well as dry weight, is influenced by the embryo weight. Regressions between embryo weight and leaf area, as well as embryo

weight and dry weight, were significant when plants were harvested anywhere from emergence to the time when the twelfth trifoliate leaf had unfolded. Rogler (29) found that in crested wheatgrass (Agropyron cristatum (L.) Beauv.), there is a high positive correlation between size of seed and seedling emergence when the seed is planted at deeper levels. Kneebone and Cremer (22) observed that in buffalograss (Buchloe dactyloides (Nutt.) Engelm.), sideoats grama (Bouteloua curtipendula (Michx.) Torr.), switchgrass (Panicum virgatum L.), sand bluestem (Andropogon hallii Hack.), and Indiangrass (Sorghastrum nutans (L.) Nash.), larger seeds give more vigorous plants. Tossell (39) found a similar relationship in smooth bromegrass (Bromus inermis Leyss.).

Rutger, et al. (30) observed correlations among important characteristics in barley (Hordeum vulgare L.) including heading date, head erectness, height, lodging resistance, shatter resistance, yield, spikes/ft^2, bushel weight, kernel plumpness, barley color, and a number of malt quality factors. A positive correlation of lodging resistance and head erectness was significant at the 1 per cent level, showing that selection against head erectness might eliminate types that tend to lodge. Kernel color was correlated with kernel plumpness at the 1 per cent level. Blue aleurone tended to be exhibited in plump kernels while white aleurone was seen in thin kernels. In addition, yield and bushel weight appeared to be positively correlated at the 5 per cent level of significance. They also concluded that yield has no extremely large correlations with any of the other traits, even though in several instances, the significance level was surpassed. Thus, in a relatively large population, it would be possible to select for most of the factors without affecting yield.

Carlson and Moll (10) conducted a study on phenotypic and genotypic variation and correlation in 171 strains of orchardgrass (Dactylis glomerata L.). Factors that were included in the experiment were spring vigor, summer recovery, fall vigor, leafiness, maturity, panicle number, incidence of rust, incidence of leaf streak, and incidence of purple leaf spot. In general, the 171 strains showed quite a lot of variability. The correlations also varied in magnitude and significance levels among the different strains. As a rule, the genetic correlations were of greater magnitude than the phenotypic correlations. A very important observation was that a large proportion of the phenotypic variation, both in the strains and among them was genotypic for each trait.

CHAPTER III

METHODS AND MATERIALS

Megasporogenesis and Embryo Sac Development

The plants used in this study consisted of clonal lines grown both in the greenhouse and in the field. Six clonal lines were cytologically studied, namely Oklahoma accessions 11744, 11757, 11672, 11668, 11670, and 11669. Clonal lines 11744 and 11757 were collected in Florida. The other four lines were collected in Texas, Oklahoma, and Kansas.

Acetocarmine squash preparations were made of the young meristematic leaves of all six clonal lines. Successful chromosome counts were made on only one line, 11744, which was found to have 72 chromosomes. This is the tetraploid chromosome number (38). Tantravahi (37) found that both diploid and tetraploid types of Tripsacum dactyloides occur in the United States. The tetraploid plants are found along the Atlantic and Gulf coasts, and the rest of the plants found in the United States are diploids. If this is true, the other clonal lines, with the possible exception of 11757, are probably diploids because of their origin in the Great Plains.

Specimens for the study of megasporogenesis and embryo sac development were usually collected in the morning. The female spikes were collected at stages varying from those with extremely young florets to those having florets with the stigmas fully extended. In addition to these collections, heads of each strain were bagged after excising the

male inflorescence. The bagged female inflorescences were subsequently collected at various intervals.

The female spikes were placed in FAA (formalin--acetic acid--alcohol) (19) soon after they were collected to insure proper killing and preservation of the material. The plant material could be effectively stored in this solution for a month or more with no evident deterioration.

After the spikes had been left in the killing fluid for a minimum of twelve hours, the ovaries were dissected from the florets and placed in erythrosin dye. They were then placed in a vacuum chamber to remove as much air as possible. Upon completion of this step, they were dehydrated by using the tertiary butyl alcohol series (19). After completing the dehydration procedure, the ovaries were embedded in blocks of Tissuemat (Fisher Scientific Company).

A rotary microtome was used for sectioning the ovaries with the sections being 20 microns thick. After sectioning, the portion of the paraffin ribbon containing the embryo sac was mounted on a slide. Haupt's adhesive (19) was used for affixing the paraffin ribbon to the slide. In addition, a 4 per cent formalin solution (19) was used for floating purposes. These slides were placed in a slide warmer overnight and then were stained by using the safranin-O-fast green staining procedure (19). After staining, the slides were made permanent by using cover slips and Permount (Fisher Scientific Company).

After a period of 48 hours, the slides could be microscopically examined. The stage of development, as well as structures occurring in each embryo sac, was recorded. Any unusual nuclei or other

abnormalities were also recorded. Photomicrographs of representative embryo sacs were taken using a Polaroid camera.

Phenotypic Variability

The phenotypic variability study was conducted on several clonal lines growing in the field including Oklahoma accessions 11668, 11669, 11670, 11671, 11672, 11673, 11745, 11746, and 11820. All of these clones were collected in Kansas, Oklahoma, and Texas. In addition to these clonal lines, an open-pollinated population was analyzed.

Characters analyzed were plant height, leaf width, number of seed stalks, height of seed stalks, plant spread, leafiness, number of racemes per head, number of seeds per raceme, and green weight.

Measurements were made on a random sample of 10 plants from each clonal line and a random sample of 60 plants from the open-pollinated population. Plant spread and leafiness measurements were obtained subjectively using a rating scale going from 1 to 9. In the case of leafiness, "1" represents very few leaves and "9" represents an abundant amount of foliage. Likewise, in the case of plant spread, "1" represents upright growth while "9" represents more prostrate growth.

The data were then statistically analyzed using an IBM 360 computer. The mean, standard deviation, and range for each agronomic character were calculated for each clonal line and for the open-pollinated population. A correlation analysis was conducted on the open-pollinated population to give all possible two-factor phenotypic correlation estimates for the selected characters. To determine correlation estimates in the nine clonal lines, an analysis of variance and covariance was used. This gave estimates for both phenotypic and

genetic correlations. The estimates for the genetic correlations were extrapolated to the open-pollinated population. To check the reliability of the estimates of the genetic correlations, their variances were calculated by using the formula presented by Tallis (36).

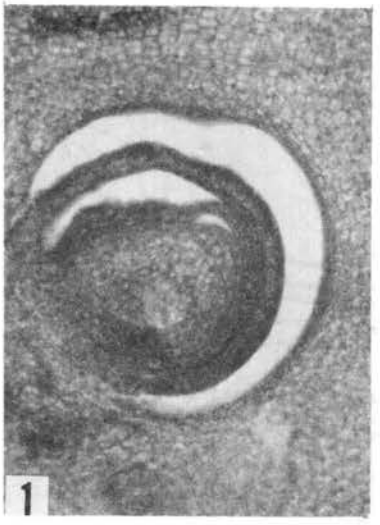
CHAPTER IV

RESULTS AND DISCUSSION

Megasporogenesis and Embryo Sac Development

Megasporogenesis in clonal lines 11757, 11668, 11669, 11670, and 11672 of eastern gamagrass is similar to the monosporic process described previously (3, 12, 15, 23, 24, 25, 34, 40). The archesporial cell, which later becomes the megaspore mother cell, arises from the hypodermal layer of the nucellus (Fig. 1). It increases in size and divides meiotically to form a linear tetrad of megaspores (Fig. 2). The chalazal member of the tetrad becomes the primary megaspore (Fig. 3) while the three spores nearest the micropyle degenerate.

Likewise, embryo sac development proceeds in the classical manner with the functional megaspore undergoing three successive divisions to form respectively a 2-, 4-, and 8-nucleate embryo sac (23, 24, 25, 32). At the 2-nucleate stage (Fig. 4), the embryo sac has enlarged considerably, and the nuclei occupy positions in opposite ends of the embryo sac. At the 4-nucleate stage (Fig. 5), the embryo sac widens with the two nuclei in each pair being close to each other. These nuclei divide again to give a chalazal quartet and a micropylar quartet (Fig. 6). The nuclei of each quartet are in close proximity to each other. Three of the chalazal nuclei form antipodals, while the other one forms a polar nucleus. Likewise, the micropylar quartet contributes a polar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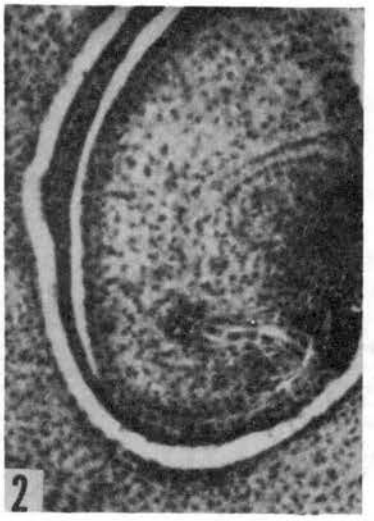
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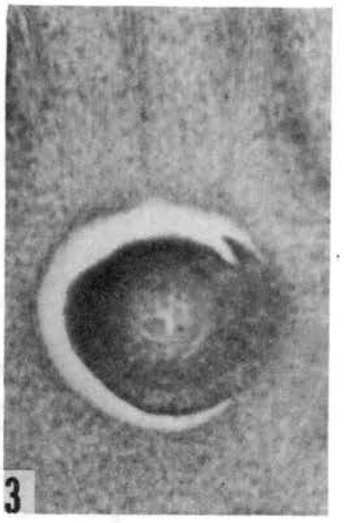
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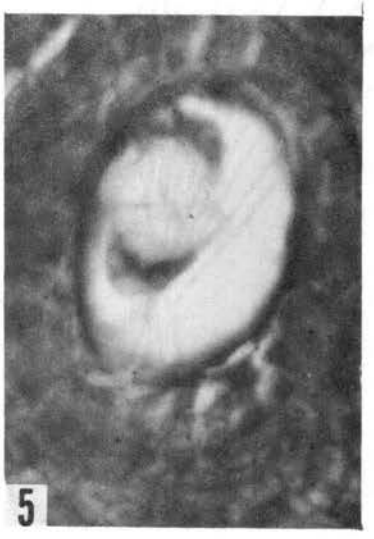
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6


7

nucleus, as well as an egg and two synergids (Fig. 7). The antipodals usually divide several times to give a proliferation of cells in the chalazal end, which is similar to the phenomenon that Farquharson (15) reported in Tripsacum. These cells are long-lived and do not completely disappear until the endosperm has digested them. The same situation was described in Pennisetum ciliare (6, 34). Generally, in the mature embryo sac, the polar nuclei are close to each other and are near the egg nucleus. In comparison to other nuclei in the embryo sac, they are very large and deep staining. The egg nucleus is much smaller and is sometimes hard to distinguish. The synergids are about the same size as the egg and are situated on either side of the egg toward the micropyle. They are usually short-lived and are not found in most mature embryo sacs. Less than 4 per cent of the mature embryo sacs analyzed in this study had synergid nuclei. The mature embryo sac is usually formed while the floral structures are still in the early boot stage.

After double fertilization, the endosperm may develop first, or the embryo may precede it in development. In most cases, the proembryo accompanied by two polar nuclei is seen in embryo sacs which have been fertilized (Fig. 8, 9). However, in many cases, free endosperm nuclei can be seen without the presence of an egg or a proembryo. This may be an abnormality in development; however, a more plausible explanation is the possibility that much dark staining material may cover the egg or young zygote at this time preventing detection. In general, the endosperm develops after the proembryo has completed a number of divisions. Figure 10 shows free endosperm and a proembryo, and Figure 11 shows cellular endosperm and an embryo embedded in it.

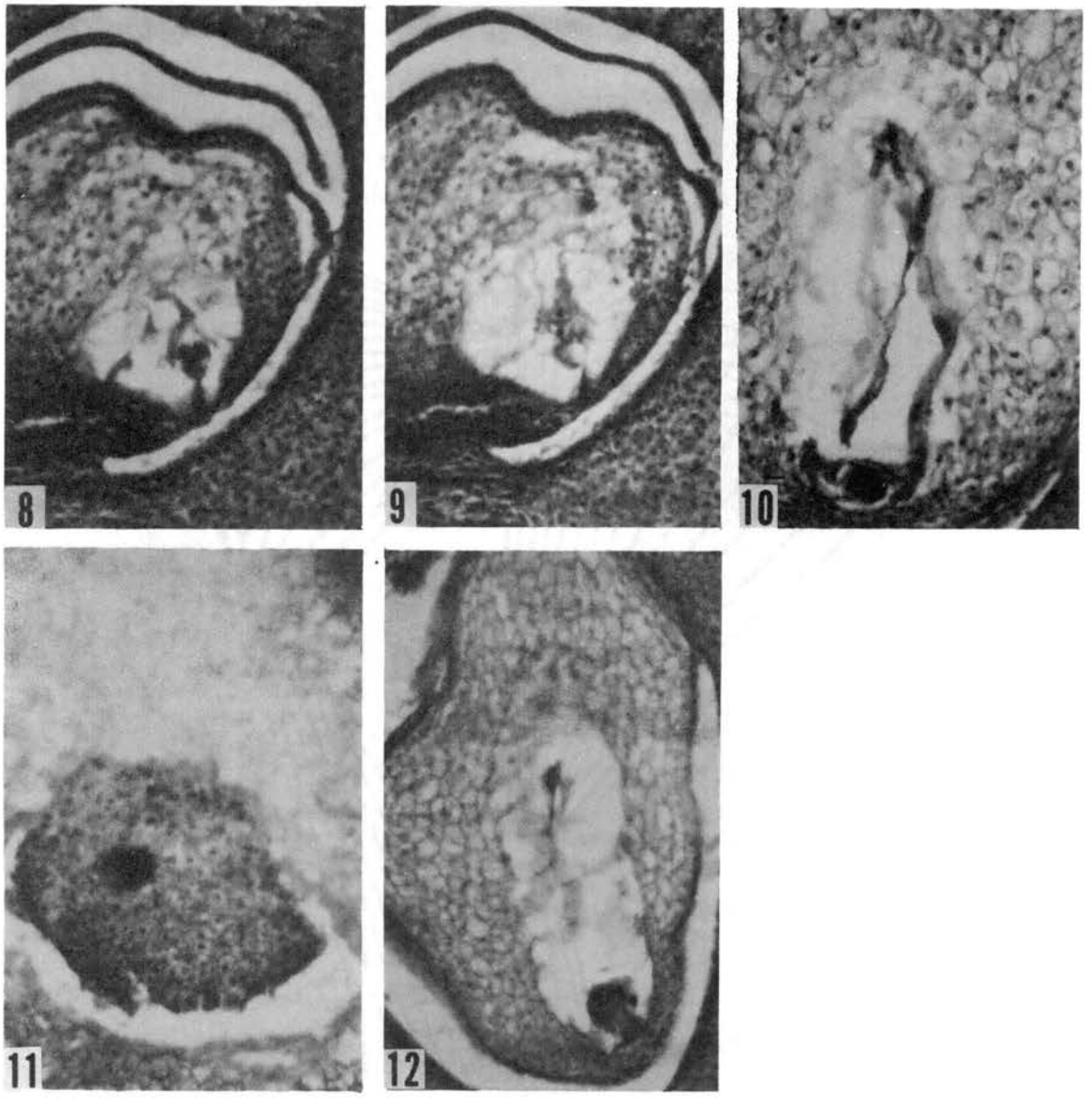

Figure 8-9. Successive Sections of an Embryo Sac Showing Proembryo and Two Polar Nuclei

Figure 10. Embryo Sac With Proembryo and Free Endosperm

Figure 11. Embryo and Cellular Endosperm

Figure 12. Apomictic Embryo Sac With Proembryo and Two Polar Nuclei

In contrast to what was found in this study, Farquharson (15) observed that in Tripsacum, the endosperm nuclei always divide first, thus producing several free endosperm nuclei before the zygote ever divides. In the present study, the endosperm was found to have many free nuclei before wall formation took place. It is peripheral at first producing a large vacuole in the middle of the embryo sac similar to that found in an earlier study on Tripsacum (15). In the present study, proembryos and endosperm were evident in some normal embryo sacs from flowers collected at first emergence of the stigmas, thus showing that the stigmas are receptive at a young stage.

Of the four clones in the bagging study, one, 11744, showed much adventitious embryonic development. Clonal lines 11670, 11669, and 11757 were observed with 99, 32, and 25 mature embryo sacs being examined respectively. There was no embryo development in any of these embryo sacs. On the other hand, of the 47 embryo sacs of 11744 that were examined, 31 (66%) showed proembryo development. However, there was no endosperm detected in any of these embryo sacs, which suggests pseudogamy (the necessity of fertilization for endosperm development). Figure 12 shows a typical embryo sac with an adventitious embryo. In clonal line 11744, the embryo sacs were similar in appearance to those of the other clones, and there were also observable linear tetrads of spores during megasporogenesis. Thus, the primary megaspore probably develops by the normal meiotic divisions, and would then form a reduced embryo sac. In a reduced embryo sac, the adventitious embryo would probably develop from a restitution nucleus. A combination of the egg and a synergid could produce such a nucleus. In clonal line 11744, there were also many extra nuclei occurring in the embryo sacs. These

could be responsible for embryo development, but it is rather unlikely because of the regular position of the proembryos in the micropylar part of the embryo sacs. There is a possibility that the apomictic embryo sacs may develop from unreduced megaspores. In this case, one nucleus, which may be the egg, a synergid or an extra nucleus directly develops into a proembryo. A similar situation was discussed earlier (9). Farquharson (15) found adventitious proembryos in similar positions in Tripsacum embryo sacs.

In clonal line 11744, proembryos are observed at the boot stage and form faster than those of the other clones with the possible exception of 11672. When the stigmas are half extended, both 11672 and 11744 have noticeably more proembryo development than do the other clones. When the stigmas are fully extended, 11744 still shows more proembryo development. On the other hand, endosperm development is very limited in this line, and there is no definite endosperm formed until the stigmas are fully extended. In fact, very few embryo sacs had any observable endosperm. None of the embryo sacs in the bagging study had endosperm. Unlike the results of a previous study (14), there were no multiple embryos found.

As mentioned above, another deviation from normal development is the presence of extra nuclei in some of the mature embryo sacs. Clones 11672, 11670, 11669, and 11668 had less than 5 per cent extra nuclei. On the other hand, clones 11744 and 11757 had many more extra nuclei with 33 and 13 per cent of the embryo sacs containing extra nuclei respectively. In most of the cases, the extra nucleus or nuclei is a "polar-type" and is usually in close proximity to the other polar nuclei. Farquharson (15) observed similar extra polar nuclei. In

some cases, smaller nuclei are clustered with the polar nuclei, which are particularly noticeable in 11744. There are other extra nuclei which cannot be classified as a particular type and occur in various locations in the embryo sacs.

Another phenomenon that was observed was that all six clonal lines have some florets with two ovaries instead of the normal single ovary. An exact count was not obtained, but the 11757 line has a much larger number of this variant type than do the other clones.

Phenotypic Variability

In general, there is much variability among the clonal lines and the open-pollinated population.

Table I shows that plant height varies much between the different clonal lines. Clonal line 11673 has the shortest plants with a mean of 65.7 cm, and 11746 is the tallest with a mean of 95.9 cm. The open-pollinated population exhibits much variability with regard to this character with the plants varying from 61 to 114 cm. It is more variable than the individual clonal lines as indicated by the standard deviations and the ranges.

Table II shows that leaf width varies much among the means of the clonal lines. Clonal line 11671 has the narrowest leaves (mean of 1.52 cm), and clonal line 11820 has the widest leaves (mean of 2.88 cm). The open-pollinated population shows noticeable variation with the leaf width ranging from 1.44 to 2.30 cm. However, it is not significantly more variable than the clonal lines, as shown by the ranges and standard deviations.

The number of seed stalks per plant is an extremely variable

TABLE I

VARIABILITY OF PLANT HEIGHT IN NINE CLONAL LINES AND AN OPEN-POLLINATED POPULATION OF EASTERN GAMAGRASS

Line	No. of Plants	Mean (cm)	Range (cm)	Std. Dev. (cm)
11673	10	65.7	56 - 74	5.56
11672	10	94.1	81 - 102	5.64
11671	10	81.6	69 - 97	9.38
11670	10	67.6	61 - 74	4.50
11669	10	93.0	81 - 112	9.63
11668	10	82.0	76 - 94	5.37
11745	10	88.9	79 - 99	6.85
11746	10	95.9	81 - 104	7.78
11820	10	93.6	79 - 102	7.18
O. P. Pop.	60	83.0	61 - 114	11.90

$LSD_{.05} = 6.3$

TABLE II

VARIABILITY OF LEAF WIDTH IN NINE CLONAL LINES AND AN OPEN-POLLINATED POPULATION OF EASTERN GAMAGRASS

Line	No. of Plants	Mean (cm)	Range (cm)	Std. Dev. (cm)
11673	10	1.70	1.40 - 2.14	0.22
11672	10	1.98	1.78 - 2.30	0.19
11671	10	1.52	1.18 - 1.64	0.14
11670	10	1.57	1.36 - 1.76	0.14
11669	10	1.87	1.62 - 2.14	0.18
11668	10	1.71	1.50 - 1.96	0.13
11745	10	2.68	2.28 - 2.98	0.30
11746	10	1.64	1.44 - 1.84	0.14
11820	10	2.88	2.66 - 3.16	0.19
O. P. Pop.	60	1.83	1.44 - 2.30	0.21

$LSD_{.05} = 0.17$

characteristic (Table III). It ranges from a mean of 16.6 in line 11745 to 55.1 in line 11672. Values ranging from 11 to 86 seed stalks per plant were obtained in the open-pollinated population. The considerable variability within the lines shows that environment has a large effect on this character.

The variability of seed stalk height is shown in Table IV. Clonal line 11673 has the shortest seed stalks (mean of 79.4 cm), and line 11745 has the tallest seed stalks (mean of 132.3 cm). The means show that there is much variation among the clonal lines. The open-pollinated population has seed stalks ranging in height from 75 to 166 cm, which indicates much variability. The individual clonal lines are also quite variable demonstrating the significant environmental influence.

Plant spread (Table V) does not show much variability among the means of the clonal lines. Clonal lines 11673 and 11669 have the least spread with both lines having a mean of 6.1. Clonal line 11670 has the greatest spread (mean of 6.8). Some individual clonal lines show considerable variability while others do not. The open-pollinated population exhibits quite a range (4.0 to 8.0), but it is not much more variable than the plants in the most variable clonal line. This indicates that variability of plant spread is probably due, in large part, to environment. In addition, different genotypes appear to behave differently in the same environment.

Table VI indicates that the clonal lines exhibit considerable variability for leafiness. Clonal line 11746 has the poorest leafiness rating (mean of 5.6), and clonal line 11673 has the best (mean of 7.3). The open-pollinated population exhibits a wide range in leafiness (2.0

TABLE III

VARIABILITY OF NUMBER OF SEED STALKS PER PLANT IN NINE CLONAL LINES AND AN OPEN-POLLINATED POPULATION OF EASTERN GAMAGRASS

Line	No. of Plants	Mean	Range	Std. Dev.
11673	10	19.1	7 - 36	8.62
11672	10	55.1	36 - 79	14.96
11671	10	29.7	10 - 69	15.56
11670	10	28.6	13 - 49	13.38
11669	10	36.1	17 - 49	10.56
11668	10	28.6	10 - 46	10.82
11745	10	16.6	8 - 26	5.76
11746	10	34.4	18 - 53	11.00
11820	10	20.4	5 - 32	10.24
O. P. Pop.	60	42.0	11 - 86	15.89

$LSD_{.05} = 10.3$

TABLE IV

VARIABILITY OF SEED STALK HEIGHT IN NINE CLONAL LINES AND AND OPEN-POLLINATED POPULATION OF EASTERN GAMAGRASS

Line	No. of Plants	Mean (cm)	Range (cm)	Std. Dev. (cm)
11673	10	79.4	63 - 89	10.02
11672	10	108.8	100 - 121	6.99
11671	10	118.1	91 - 133	11.42
11670	10	86.3	76 - 101	8.22
11669	10	104.7	88 - 121	11.76
11668	10	101.0	80 - 124	14.41
11745	10	132.3	115 - 148	10.36
11746	10	125.4	112 - 141	10.25
11820	10	130.7	113 - 157	13.61
O. P. Pop.	60	113.9	75 - 166	18.73

$LSD_{.05} = 9.8$

TABLE V

VARIABILITY OF PLANT SPREAD IN NINE CLONAL LINES AND AN OPEN-POLLINATED POPULATION OF EASTERN GAMAGRASS

Line	No. of Plants	Mean*	Range*	Std. Dev.*
11673	10	6.1	5.0 - 7.0	0.74
11672	10	6.3	6.0 - 7.0	0.48
11671	10	6.6	5.0 - 8.0	0.97
11670	10	6.8	6.0 - 8.0	0.63
11669	10	6.1	5.0 - 7.0	0.56
11668	10	6.5	6.0 - 7.0	0.52
11745	10	6.4	5.0 - 7.0	0.70
11746	10	6.6	6.0 - 7.0	0.52
11820	10	6.5	6.0 - 7.0	0.52
O. P. Pop.	60	6.4	4.0 - 8.0	1.12

*Subjective rating scale was used. 1 = upright growth habit. 9 = more prostrate growth.

$LSD_{.05} = 0.6$

TABLE VI

VARIABILITY OF LEAFINESS IN NINE CLONAL LINES AND AN OPEN-POLLINATED POPULATION OF EASTERN GAMAGRASS

Line	No. of Plants	Mean*	Range*	Std. Dev.*
11673	10	7.3	6.0 - 9.0	0.94
11672	10	6.7	5.0 - 8.0	0.82
11671	10	5.8	4.0 - 7.0	1.03
11670	10	6.5	5.0 - 8.0	0.85
11669	10	6.3	4.0 - 7.0	0.95
11668	10	6.5	5.0 - 8.0	0.85
11745	10	5.8	5.0 - 7.0	0.92
11746	10	5.6	5.0 - 7.0	0.70
11820	10	6.0	3.0 - 7.0	1.25
O. P. Pop.	60	5.8	2.0 - 8.0	1.30

*Subjective rating scale was used. 1 = very few leaves. 9 = abundant foliage.

$LSD_{.05} = 0.8$

to 8.0), but the standard deviation is not much different than that for the most variable clonal line, which shows that environment has a significant effect on this character.

Variability of the number of racemes per head (Table VII) is great both among the clonal lines and in the open-pollinated population. The means for the clonal lines range from 1.3 for 11673 to 2.2 for 11670 and 11671. In the open-pollinated population, there is a range from 1.0 to 3.4 racemes per head. The individual clones exhibit considerable variability, which indicates that environment is an important factor influencing this particular character.

The number of seeds per raceme (Table VIII) ranges from a mean of 3.5 in 11670 to 7.7 in 11746, which indicates much genetic variability for this character. Although the standard deviation for the open-pollinated population is not much larger than that for the most variable clonal line, the range is much greater than the ranges for the individual clonal lines. In the open-pollinated population, the number of seeds per raceme ranges from 1.1 to 9.6.

Green weight (Table IX) ranges from a mean of 0.90 kg in 11670 to 1.92 kg in 11820 showing that there is considerable variation between the clonal lines. On the other hand, the individual clonal lines also are quite variable showing that environment plays a major role in influencing this character. Of course, sampling error could also make these clonal lines seem to be more variable. The open-pollinated population shows a large range and standard deviation, but the range and standard deviation of the most variable clonal line, 11671, are greater.

From the correlation analysis conducted in the open-pollinated population (Table X), several significant correlations were obtained.

TABLE VII

VARIABILITY OF NUMBER OF RACEMES PER HEAD IN NINE CLONAL LINES AND AN OPEN-POLLINATED POPULATION OF EASTERN GAMAGRASS

Line	No. of Plants	Mean	Range	Std. Dev.
11673	10	1.3	1.0 - 1.8	.3
11672	10	2.0	1.6 - 2.6	.3
11671	10	2.2	1.6 - 2.8	.4
11670	10	2.2	1.6 - 3.0	.5
11669	10	1.9	1.0 - 2.6	.5
11668	10	1.9	1.4 - 2.6	.3
11745	10	2.0	1.6 - 2.8	.4
11746	10	2.0	1.4 - 2.4	.3
11820	10	1.7	1.2 - 2.6	.5
O. P. Pop.	60	2.0	1.0 - 3.4	.6

$LSD_{.05} = 0.36$

TABLE VIII

VARIABILITY OF NUMBER OF SEEDS PER RACEME IN NINE CLONAL LINES AND AN OPEN-POLLINATED POPULATION OF EASTERN GAMAGRASS

Line	No. of Plants	Mean	Range	Std. Dev.
11673	10	6.3	5.1 - 7.2	.8
11672	10	4.5	3.6 - 5.2	.6
11671	10	4.0	3.0 - 5.5	.8
11670	10	3.5	1.8 - 6.0	1.5
11669	10	3.6	2.0 - 6.8	1.6
11668	10	5.7	4.8 - 6.7	0.6
11745	10	4.3	2.6 - 5.0	0.9
11746	10	7.7	6.1 -10.1	1.3
11820	10	5.1	3.3 - 7.2	1.4
O. P. Pop.	60	6.2	1.1 - 9.6	1.7

$LSD_{.05} = 0.98$

TABLE IX

VARIABILITY OF GREEN WEIGHT IN NINE CLONAL LINES AND AN OPEN-POLLINATED POPULATION OF EASTERN GAMAGRASS

Line	No. of Plants	Mean (kg)	Range (kg)	Std. Dev. (kg)
11673	10	1.02	0.55 - 1.50	0.36
11672	10	1.87	1.36 - 2.91	0.54
11671	10	1.25	0.32 - 3.23	0.86
11670	10	0.90	0.45 - 1.27	0.31
11669	10	1.32	0.82 - 2.23	0.43
11668	10	1.58	0.86 - 2.27	0.46
11745	10	1.40	0.64 - 2.32	0.48
11746	10	1.50	1.00 - 2.64	0.47
11820	10	1.92	0.77 - 3.14	0.70
O. P. Pop.	60	1.74	0.27 - 3.18	0.68

$LSD_{.05} = 0.48$

TABLE X

CORRELATIONS OF IMPORTANT CHARACTERS FROM AN OPEN-POLLINATED POPULATION OF EASTERN GAMAGRASS†

Character/	Plant Height	Leaf Width	Seed Stalk No.	Seed Stalk Ht.	Plant Spread	Leafiness	Racemes Per Head	Seeds Per Raceme
Leaf Width	0.042							
Seed Stalk No.	0.054	-0.062						
Seed Stalk Ht.	0.393**	0.425**	0.212					
Plant Spread	0.011	0.258*	0.626**	0.280*				
Leafiness	0.343**	-0.069	-0.223	-0.158	-0.113			
Racemes/Head	0.131	-0.118	-0.020	-0.029	0.047	0.313*		
Seeds/Raceme	-0.074	0.127	0.158	0.044	0.156	-0.186	-0.591**	
Green Weight	0.595**	0.179	0.530**	0.377**	0.473**	0.242	0.144	0.037

†Based on 58 degrees of freedom.

*Significant at 0.05 level.

**Significant at 0.01 level.

Positive correlations significant at the 1 per cent probability level include plant height with height of seed stalks, leafiness, and green weight; leaf width with height of seed stalks; number of seed stalks with plant spread and green weight; height of seed stalks with green weight; and plant spread with green weight. Positive correlations significant at the 5 per cent probability level consist of leaf width with plant spread, height of seed stalks with plant spread, and leafiness with the number of racemes per head. The only significant negative correlation is racemes per head with seeds per raceme which is significant at the 1 per cent probability level.

The analysis of variance and covariance of the nine clonal lines (Table XI) indicate that the genetic correlations, in most instances, are of greater magnitude than the phenotypic correlations, which agrees with earlier results (10). Exceptions include the correlations of the number of seed stalks per plant with leafiness and green weight; and seed stalk height with seeds per raceme. The estimates of genetic correlations were not judged to be highly reliable. The variances of the estimates of the correlations were greater than 1.0 in all cases when the formula presented by Tallis (36) was used.

It can be concluded that considerable variation occurs among the nine clonal lines and in the open-pollinated population. This would probably warrant a selection program. A replicated test should be conducted, however, to get better estimates of variability.

In general, if any of the nine characters were selected for, there would be no detrimental effect (Table X). A possible exception would be selection for greater number of seed stalks and forage green weight. These characters are positively correlated with each other. However,

TABLE XI

GENETIC AND PHENOTYPIC CORRELATIONS FROM ANALYSIS OF VARIANCE AND COVARIANCE OF NINE CLONAL LINES OF EASTERN GAMAGRASS

Character/	Plant Height	Leaf Width	Seed Stalk No.	Seed Stalk Ht.	Plant Spread	Leafiness	Racemes Per Head	Seeds Per Raceme
Leaf Width	0.371 0.470							
Seed Stalk No.	0.396 0.406	0.000 -0.362						
Seed Stalk Ht.	0.631 0.826	0.514 0.640	0.079 -0.084					
Plant Spread	0.000 -0.214	0.000 -0.266	0.206 -0.351	0.225 0.315				
Leafiness	0.167 -0.795	0.025 -0.330	0.205 0.069	0.000 -1.000	0.004 -1.000			
Racemes/Head	0.111 0.213	0.005 -0.266	0.190 0.337	0.169 0.381	0.260 1.000	0.002 -0.848		
Seeds/Raceme	0.086 0.131	0.019 -0.119	0.000 -0.088	0.124 0.084	0.000 -0.060	0.000 -0.015	0.000 -0.356	
Green Weight	0.493 0.904	0.305 0.672	0.479 0.319	0.385 0.718	0.251 -0.488	0.150 -0.444	0.000 -0.095	0.090 0.240

The top number in each pair is the phenotypic correlation and the bottom one is the genetic correlation.

the seed stalks probably do not have good forage quality, and as a result, forage quality may decrease if either seed stalk number or forage green weight would be selected for by itself. Because of the significant negative correlation between racemes per head and seeds per raceme (Table X), if one of these traits were selected for by itself, the other one would be selected against. Thus, both factors should be selected for at the same time.

It was hoped that the genetic correlations from Table XI could be directly extrapolated to the open-pollinated population. However, there is not good agreement between the phenotypic correlations from the analysis of variance and covariance of the nine clonal lines and the phenotypic correlations from the correlation analysis of the open-pollinated population. Because of this, it is difficult to directly extrapolate the genetic correlations calculated to the open-pollinated population. As mentioned previously, the genetic correlations have large variances, which indicate they may not be reliable estimates of the true correlations. A remedy for this situation would be replicated progeny tests to obtain better estimates of the genetic correlations.

CHAPTER V

SUMMARY AND CONCLUSIONS

The objective of this study was to obtain basic information on the reproductive behavior and inherent variability of eastern gamagrass (Tripsacum dactyloides).

Studies of megasporogenesis, embryo sac development, and early embryogenesis were conducted to obtain information concerning reproductive behavior.

Megasporogenesis in clonal lines 11757, 11672, 11668, 11670, and 11669 appears to be normal. Embryo sac development is normal with the primary megaspore undergoing three successive mitotic divisions to produce a typical 8-nucleate sac. Early embryo and endosperm development also seem to be normal in these lines.

From the female spikes that were bagged, only those of 11744 had embryo sacs containing proembryos, which is suggestive of apomixis in this clonal line. Although proembryos were found in non-pollinated embryo sacs, no endosperm was observable. Thus, pollination must be necessary for endosperm development.

Extra nuclei were found in some embryo sacs in all six of the clonal lines, but clonal lines 11757 and 11744 have significantly more embryo sacs with extra nuclei than the other four lines.

In some of the florets, two ovaries are present instead of the normal single ovary. This is especially noticeable in clonal line 11757.

From the study of phenotypic variability, it can be postulated that there is probably sufficient variability in the available plant material to warrant a selection program. Considerable variability among the clonal lines and in the open-pollinated population is exhibited.

The phenotypic correlations in the open-pollinated population show that, in general, there would be no detrimental effect, if any of the nine characters were selected for. However, if one selected for the number of seed stalks or forage green weight by itself, there could be a reduction in forage quality. In addition, racemes per head and seeds per raceme should be selected for in conjunction with each other because they are negatively correlated.

The analysis of variance and covariance of the nine clonal lines gave estimates of phenotypic and genotypic correlations which cannot be extrapolated to the open-pollinated population accurately. The phenotypic correlations are much different in the analysis of variance and covariance of the clonal lines than in the analysis of the open-pollinated population. In addition, very large variances were calculated for the estimated genetic correlations.

Replicated field trials with appropriate genetic populations are needed to determine whether there is enough variability to warrant selection and to get a better estimate of the genetic correlations among the selected agronomic characters.

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