THE EFFECT OF SELFING ON WILD SUGARCANE (SACCHARUM SPONTANEUM) CLONES

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CHAPTER I

INTRODUCTION

The genus Saccharum belongs to the family Gramineae and the tribe Andropogoneae. This genus exhibits a wide morphological and cytological range. The range of diploid numbers in the Saccharum genus extends from 40 to 128. Five species, S. officinarum (2n=80), S. spontaneum (2n=40 to 128), S. robustum (2n=60 and 80), S. barberi (2n=82 to 124), and S. sinense (2n=106 to 121) have been traditionally recognized. The sixth species, S. edule, is generally not regarded as a separate species because of its morphological similarity to S. robustum (Stevenson, 1965).

More recently Grassl (1974) suggested a reclassification of species within the Saccharum genus. He would recognize S. officinarum, S. spontaneum, S. robustum, and S. sanguineum as distinct species, but would reclassify S. barberi and S. sinense as horticultural groups because of evidence that the latter two resulted from intergeneric hybridization. Grassl's suggestions, though apparently taxonomically sound, have not received any official recognition.

S. spontaneum genotypes are characterized by thin and hard tillers, normally pithy and fibrous, having long internodes, good rooting and tillering habit, but having low sugar content. The known genotypes within the S. spontaneum species form a highly complex group, undoubtedly the most complex within the Saccharum genus. This species extends through the tropics and subtropics from the Pacific islands through Asia to the Mediterranean region. Climatical extremes range from the slopes of Himalayas to the Indonesian tropics, thus partially explaining the extreme genetic diversity found within the species. Individual genotypes have widely varying levels of resistance to insects and diseases and tolerance to frost, drought, salinity, etc. (Raghavan, 1953). In fact, S. spontaneum clones have varying heights from 18 inches to 30 feet. There is a considerable range in the

chromosome numbers in this species with 2n=64, 80, 96, and 112 being the most typical across the major ecological areas.

Chromosome number 2n=40 is the lowest recorded so far in this species (Rao and Babu, 1955). In India, where S. spontaneum probably originated, forms with lower chromosome numbers are characteristic of the northwestern region. The 64-chromosome type has the widest distribution in India, but is concentrated mostly in the lower part of the peninsula. Types with chromosome numbers higher than 64 are restricted to northern and mid-western regions (Panje and Babu, 1960).

It seems evident that the origin of several of the different chromosomal types must have been by natural hybridization within this species (Grassl, 1977). The value of S. spontaneum in sugarcane breeding is widely recognized. Nearly all major commercial sugarcane varieties grown throughout the world contain S. spontaneum germplasm, based on their pedigrees. Clones of S. spontaneum are characteristically male fertile and have generally been used as male parents in hybridization programs. Continued efforts have been made to collect and maintain S. spontaneum genotypes due to their recognized potential in breeding programs (Heinz, 1965).

The potential and complexity of S. spontaneum is already recognized, but relatively little work has been done on breeding behavior and chromosomal stability within the species. The overall objective of this study is therefore to understand and appreciate the characteristics of S. spontaneum with particular regard to selfing. As S. spontaneum is believed to have evolved from a complex and diverse genetic background, we would expect interesting morphological and possibly chromosomal variations among the progenies after one generation of selfing. Specifically, the objectives of this study are:

1. To study the chromosomal variability in S_1 (first generation selfing) progenies of several S. spontaneum clones.

2. To search for evidence of chromosomal mosaicism at mitosis in S_1 progenies.

3. To study morphological variability in S_1 progenies.

CHAPTER II

LITERATURE REVIEW

A. Evolution in Sugarcane

The sugarcane species are large tropical grass polyploids in nature belonging to the tribe Andropogoneae. Darlington and Janaki Ammal (1945) suggested that the basic chromosome numbers of Andropogoneae were x = 5, 9, 10, and 12, of which x = 5 was the most common among the genus. The basic chromosome number of Saccharum is subject to a lot of uncertainties, assumptions and discussions. Bremer (1925, 1929), judging from their results from counting a number of Saccharum clones, considered x = 10 as the basic number. The basic number x = 5 might be a possibility judging from the fact that x = 5 is the most common in the tribe Andropogoneae. Janaki Ammal (1936) agreed with Bremer, but she went on to assume that x = 6 or 12 may be another possibility. Her later work with Darlington (1945) and Subba Rao (1947) strongly suggested that x = 10 and 12 were the most probable basic numbers of Saccharum.

However, not all species and varieties of Saccharum have chromosome number that are multiples of 5 or 10 or their aneuploids. Among S. spontaneum species only, numbers like 48, 56, 64, 72, 96, and 112 are more common. In fact, S. officinarum, a more stable species, has chromosome number 2n=80 indicating the basic number could be 8 or 10. Price (1957) considered x = 8 as the most probable basic number of S. spontaneum complex. Dutt and Rao (1933) counted chromosome numbers in S. spontaneum of India and found that the polyploidy developed largely as multiples of 8.

Further attempts were made to involve other genera in determining the basic chromosome number of Saccharum species when hybrid of S. officinarum x Sclerostachya fusca was backcrossed to the male parent, 10 S. officinarum chromosomes, usually assumed to be one chromosome set, were further divided into

two sets, each possessing five chromosomes (Parthasarathy, 1948). One of these two sets was homologous to one of the three genomes of Sclerostachya fusca (n=15). Parthasarathy (1948) therefore concluded that the basic chromosome number of Saccharum was 5. A similar work was done by Raghavan (1951). In this study he observed that the two sets of five basic chromosomes of S. officinarum were homologous with two sets of Narenga (n=15), thus making him agree with the conclusion made by Parthasarathy (1948).

The analysis of genomes in Saccharum had been made by Nishiyama (1956). He studied the meiotic configurations of Saccharum species and their interspecific hybrids. Based on homologies of chromosomes in all these hybrids, he concluded that there were two basic numbers, 8 and 10, since polypoloidy in Saccharum consisted of multiples and combinations of these two basic numbers.

In the middle of the 19th century, as many as 62 species were recorded under the genus Saccharum (Steudel, 1855). About a century later, they were reduced to five, Erianthus, Sclerostachya, and Narenga being transferred to three different genera. Mukherjee (1957) had demonstrated that the latter three genera were interrelated and interbreeding and he termed them as "Saccharum complex."

Saccharum extends through the tropics and subtropics from the Pacific islands through Asia to the Mediterranean and Africa (Panje and Babu, 1960). Erianthus is widely present in the tropics and subtropics region (Mukherjee, 1957); Sclerostachya and Narenga are mainly confined to the humid tropics of South East Asia. Thus the maximum number of this complex was centered in the Indo-Burma-China border region.

Mukherjee (1957) considered Saccharum as the most advanced genus in this complex for vegetative propagation as it had well developed buds and having few rows of root eyes in the nodal region compared to the other genera.

Mukherjee (1957) hypothesized that Saccharum genus might have evolved from Ripidium (formerly Erianthus) either directly or via Sclerostachya. (Under new chemotaxonomy, this includes Narenga genera.) Panje and Babu (1960) reportedly had found S. spontaneum species of 2n=40 type, the lowest within this genera, in North West India, where both the above genera were present. The 2n=80 type happened to coexist in the same area where 2n=40 type predominated but they did not differ significantly from each other (Daniels et al., 1975).

Mukherjee (1957) believed that S. spontaneum 2n=40 type might have arisen from a 2n+n transmission of Sclerostachya (2n=30) x Ripidium (2n=20). Since S. spontaneum 2n=80 type appeared very similar to 2n=40 type, Grassl (1977) considered that the 2n=80 type might have originated from autopolyploidization of 2n=40 type, and the hybridization between these two types produced the 60 chromosomes type. Further hybridization of all these types might have given rise to 50 and 70 chromosomes types, and other intermediates like 45, 55, 65, and 75 chromosomes types.

For S. robustum to be considered as a member of the genus Saccharum, it must have chromosomes in common with that of S. spontaneum. There is an increase in size, complexity, and polyploidy of S. robustum as one moves away from the center of origin of S. spontaneum indicating that S. spontaneum may be one of the ancestors of S. robustum.

Parthasarathy (1948) studied the 40 chromosome pairs of S. officinarum and he found that five pairs of chromosomes appeared in common with Sclerostachya. This had led him to believe that Sclerostachya must be one of the ancestors of S. robustum. Similarly, Li et al. (1948) had found a few chromosomes which were common with that of Miscanthus floridulus, thus suggesting that M. floridulus might be another of its ancestors.

Grassl (1977) found it very easy to hybridize Miscanthus and a few species of Ripidium, therefore making him conclude that Ripidium could replace Miscanthus in furnishing some chromosomes involved in the origin of S. robustum. The Sclerostachya group furnished genes for self incompatibility and size.

S. officinarum was generally believed to have originated from interhybridization of different S. robustum types. S. robustum, which was commonly found in mouths of large rivers, was highly polyploid in nature and was naturally cross-pollinated. This species, due to its robustness, was being used for shelters and fences by the natives of New Guinea. Two or more types might have come together due to selection done by the natives. These types might have interbred with each other freely. The resulting F_1 progenies were found to be similar to both parents. Not until the F_3 generation were linkage blocks sufficiently broken to result in new recombinations which marked the origin of a new species, S. officinarum (Grassl, 1974).

B. Inbreeding and Heterosis

Inbreeding generally refers to the closer kinds of matings such as brother and sister and parent and offspring. Many wild as well as cultivated species are naturally self pollinated and often have special mechanisms to promote selfing. Inbreeding seems not to have any injurious effect in these species in terms of vigor, productivity, and ability to survive. However, most higher plants are naturally cross pollinated and inbreeding depression is often seen in their inbreds.

The inbreeding process is of value in plant improvement to plant breeders in view that it can eliminate abnormal, inferior recessive types. Inbreeding followed by selection may eventually result in complete removal of deleterious genes except if these genes are linked with some good character. The development of distinct inbred lines has become the basis of many methods of plant breeding in cross-pollinated crops.

The most widely used of inbred materials is the development of high-yielding hybrids. The use of inbreeding (selfing and sibbing) in plant breeding has been followed by useful advances in yield and quality of many plant species. A hybrid cross between inbred lines results in progeny of great vigor, size, and productiveness and generally superior to that of the original open pollinated stock. The yield and quality of the crop produced are functions of the particular combination of self-fertilized parental type, and these qualities remain the same whenever the cross is repeated (Shull, 1910).

Selfing represents one of the two extremes of breeding philosophy in sugarcane (Stevenson, 1953). Selfing Saccharum clones may result in broadening of genetic base and derivation of superior individuals. In fact, many selfed progenies of commercial clones performed better than the parent. Many showed normal vigorous growth and significant improvement in sugar content (Ethirajan et al., 1977). Repeated cycles of inbreeding of these commercial clones have resulted in dramatic improvement in sucrose percent juice.

Loss of vigor may occur in the early stages of the selfed line. Stevenson (1959) selfed interspecific hybrid of sugarcane, and he noticed the appearance of defective types in the selfed population. These types might have abnormal cane growth, recumbent habit, precocious growth of buds, freckling or blotching of leaves, and weakness of top growth.

According to Raghavan (1952), there were no S_1 seedlings produced due to the presence of a large accumulation of lethal genes when commercial clones were selfed. However, Stevenson (1959) reported that vigorous selfed seedlings had been obtained from selfing interspecific hybrid varieties. Even after several generations of selfing, satisfactory vigor was maintained for some hybrids. Contradicting Raghavan (1952), Stevenson (1959) believed that self sterility was exceptional; low

fertility was infrequent and moderate, and good fertility was common when these hybrids were selfed.

As a result of progressive selfings, the inbreds approached S. spontaneum characteristics such as in growth, production of thin stalks, narrow leaves, and aggressive rhizomatous tillering habit indicating a reversion to wild characters.

Male sterility was not proven to be a limiting factor to line development, sibbing, and backcrossing. Stevenson (1959) observed that male fertility was still restored in these inbreds. He even reported in some cases male fertility approached 100%.

In selfs of commercial clones, chromosome losses seem to be the rule rather than an exception (Li and Ma, 1951). It is very difficult, though, to determine which chromosomes were lost (Stevenson, 1959). The greatest loss of chromosome is in spontaneum-like clones, having high vigor, narrow leaves, and rhizomatous tillering habit. With successive selfing, there will be progressive thinning of tillers but no reduction in growth vigor. Selfed hybrids having S. officinarum, S. sinense, and S. spontaneum germplasm suffered a large chromosome loss in first generation but tend to be stable after progressive selfing. These losses were a result of meiotic irregularities in the parent. Univalents appear at meiosis which later resulted in chromosome lagging at anaphase and eventually may or may not be included in the gametes.

Selfed clones of S. spontaneum generally resemble their parents in gross morphology. Minor variations were observed but these were not associated with the loss of vigor (Panje and Ethirajan, 1959). Minor differences in features like growth habit, stooling habit, stalk color, tillering capacity, plant height, and leaf characteristics were observed among these selfed progenies. A great percentage of these progenies had higher sucrose percent juice but lower dry matter yields as compared to the parents.

A similar study was later made by Sankaranarayana (1977). The same phenomenon was observed, though a generalization cannot be made because different clones acted differently. He observed that for sucrose percent juice, only 2% of genotypes of SES 108 have values above the parental mean, in contrast to 80% of SES 14 genotypes.

In contrast to S. spontaneum and commercial hybrids, there was an occurrence of inbreeding depression in selfed clones of S. officinarum. Selfed clones of S. officinarum usually have 80 chromosomes. Meiosis was regular as was to be expected in any polyploid species where duplicated chromosome sets were present. Selfing resulted in loss of vigor and fertility in the progenies. Selfed progenies of S. officinarum might not be able to survive well possibly due to the presence of a large reserve of lethal recessive genes in the genetic constitution (Raghavan, 1951).

C. Utilization of Saccharum spontaneum in Sugarcane Breeding

Crossing of S. spontaneum with commercial hybrids and S. officinarum has been practiced in several countries with the objective of transferring specific characters from S. spontaneum into them. The first nobilization was done to transfer disease resistance characters into the S. officinarum. As a result of this, it was discovered that this F_1 hybrid had also certain agricultural stability and superior yielding powers (Panje, 1971).

Many S. spontaneum varieties were collected during The Spontaneum Expedition Scheme, launched in India, in 1947. Since then observations on morphological characters, chromosome numbers, flowering behavior, etc., were made on these collections.

S. spontaneum varieties are known to be able to survive under poor cultural conditions. Although they take a little time to establish themselves, they can keep other vegetation out by physical occupation of space and soil. Generally they have

underground stems or stubble systems that are repeatedly regenerating themselves (this contributes to the ratooning ability of sugarcane).

Generally S. spontaneum varieties can be regrouped into 4-5 ecotypes (Panje, 1971):

a. Tall, foliaceous, thick stemmed, long season, and hygrophilous plant. This type normally exists and occupies most and well aerated sandy soil especially along the river banks and fresh water streams.

b. Mesophilous, medium stature, and foliage. This ecotype is morphologically variable. In some cases tillers tend to grow outwards a small distance underground before emerging. Some exist in small compact clumps. This ecotype occupies arable farming area.

c. Tall or short, highly tufted plants with narrow blades occasionally reduced to the midrib. This ecotype generally is found in open, highly isolated sand banks of perennial rivers.

d. The weed ecotype, a dwarf plant having rod-like tuberous underground stems for water storage. They have scattered stems bearing wing leaf blades.

S. spontaneum varieties are generally not too tolerant to waterlogging but they are more tolerant than S. officinarum. However, they are able to control transpiration rate better than other species (Mallik, 1950) and the root cap cells are only plasmolysed at high concentration of KNO_3 (Panje, 1971).

Tillering ability in S. spontaneum is associated with the increase in the number of stalks per hectare or per germinated bud. It is "the capacity of the cane plant to respond, through tillering to environmental conditions whereby the plant is able to utilize solar energy which otherwise could not be utilized," (Panje, 1971). This character is highly influenced by certain environmental conditions such as latitude displacement (Brandes, 1950) and intensity or duration of daylight.

S. spontaneum varieties show the ability to root even at low temperature, ability to tiller under long days, intense light and/or high temperatures. They have rapid elongation of stalks under conditions of the monsoon, flower early under certain daylength, and have early senescence.

S. spontaneum varieties are frost escaping plants due to brevity of life cycle. They remain inactive during cold season and survive later. They are able to grow well under medium and low temperature.

In the production of any commercial cultivars and in any nobilization program, S. spontaneum has generally been employed as the pollen parent. It is very seldom being used as the seed parent possibly because it is a profuse producer of pollen and is known to be self fertile. Emasculation of individual flowers is almost impossible. There are a few protogynous clones, but the use of these clones has not so far been extensively utilized, for one reason or another (Dutt and Krishnaswamy, 1943).

The importance of S. spontaneum in sugarcane breeding is demonstrated by the fact that almost all commercial clones of today are descendants of S. spontaneums. The benefits derived from interspecific hybridization include increase in yield and disease resistance, improving ratooning ability, and adaptability for growth under stress. S. spontaneum has been the most important donor of these attributes. S. spontaneum clones have been found to be able to survive even if they are exposed to temperature as low as -°4C (Irvine, 1977).

Selection within S. spontaneum for specific characters seems to be most effective in any hybrid improvement program ("nobilization"). Of these attributes selection for percentage sucrose will be most effective in increasing sugar content of the subsequent F_1 hybrids (Roach, 1977). This is supported by the fact that h^2 for percent sucrose in S. spontaneum is equal to 99% (Roach, 1977). In fact, it was found that the predicted and actual responses were in good agreement and a

selection rate of about 30% for percent sucrose would increase percent sucrose of F_1 progenies by about 1.2 units. The second character that has a high heritability value is stalk diameter.

Most of the commercial hybrids of today are derived from the cross between S. officinarum and S. spontaneum. The reduction in the proportion of S. spontaneum chromosomes in order to lessen the undesirable characters like impurities in the juice, imparted by the S. spontaneum parent along with desirable characters such as vigor and disease resistance, is done through backcrossing to the female parent; this process is called "nobilization."

Many studies have been made on the nature of inheritance in S. spontaneum. The capacity of sugarcane to produce the reduced and unreduced type of egg gametes was well demonstrated in earlier work (Kandasami, 1961; Price, 1963; Roach, 1977). The contribution of diploid genome by the pistillate parent was first noticed by Bremer (1923) in natural hybrids of S. officinarum var. Cheribon (n=40) and S. spontaneum var. Glagah (n=56). A similar observation was also made by Dutt and Subba Rao (1933) in interspecific hybrids of S. officinarum var. Vellai (n=40) and S. spontaneum var. Coimbatore (n=32), and by Kandasami (1977).

The contribution of diploid egg gametes by S. officinarum is not confined to the first nobilization cross, but can also occur in second and third nobilization crosses (Bremer, 1929). When S. officinarum is used as a female, chromosome number in the hybrids is the sum of the haploid numbers of parents (n+n).

Not all hybrids of interspecific crosses involving S. spontaneum have a 2n+n chromosome transmission (Roach, 1977). Some n+n hybrids were also found in the progenies of Tabonggo (S. spontaneum, 2n=80) and that of NG 51-2 (S. spontaneum, 2n=80). Kandasami (1977) observed n+n chromosome transmission in hybrids of (S. officinarum x S. spontaneum) x S. spontaneum and also hybrids of second nobilization cane variety x S. spontaneum.

Bremer (1923) suggested that the doubling in pistillate parent occurred in the egg cell during fertilization. Narayanaswamy (1940), while working on megasporogenesis of S. officinarum and S. spontaneum, found a fusion of the two inner megaspore nuclei to give rise to diploid megaspores. The condition happened more often in S. officinarum. Unlike others this condition normally arises due to absence of pairing, double division of univalent and suppression of first division, this condition of post meiosis is unique for sugarcane.

Raghavan (1951), when reviewing work done in India, suggested the 2n+n nature of inheritance will hold true only if the proportion of S. spontaneum chromosomes is about 15-20%. When the proportion of S. spontaneum chromosomes falls below this threshold the reduced gametes on the S. officinarum side are seen to function.

The haploid transmission in S. spontaneum in all its progenies is beyond any doubt. In crosses involving other species (e. g. S. robustum) the same nature of inheritance is also observed. When S. spontaneum is hybridized with S. robustum, where S. spontaneum is the male parent, both 2n+n and n+n transmission are observed (Price, 1968).

D. Meiosis in S. spontaneum

Meiotic irregularities normally occurred in pollen mother cells. Cells with different chromosome numbers coexist within the same anther. Meiotic irregularity is not a new phenomenon. It was reported to have occurred in the pollen mother cells of many crops such as in Datura (Bhaduri and Sharma, 1946), Lolium species (Emsweller, 1949), Gossypium species (Menzel, 1952), Triticinae species (Sachs, 1952), Secale (Muntzing, 1951; Rees and Thompson, 1955), Festuca species (Malik and Thomas, 1966), Narenga species (Jegathesan and Sreenivasan, 1967), and intergeneric hybrid of Triticum and Hordeum (Nakamura et al., 1981).

Aneuploidy and chimerism in interspecific and intergeneric crosses of Triticum and Hordeum (Nakamura et al., 1981) were frequently detected in their pollen mother cells. The complete sterility reported in their pollens may be due to meiotic breakdown caused by chromosome instability. Brown (1950), in his study using gamma radiated cotton plants, found that there were fragmentation, loss of whole chromosome, translocation of chromosome parts, and possible intrachromosomal changes in the pollen mother cells.

Meiotic irregularities in naturally grown S. spontaneum were reported by Nair (1972b). He studied meiosis of twelve varieties of S. spontaneum of varying chromosome number. In almost all these varieties, he observed the occurrence of univalents at diakinesis and metaphase I. There were occurrences of bridges and laggards (which may result in losing of these chromosomes) and unequal distribution of chromosomes. This abnormality was also found in Narenga species (Jegathesan and Sreenivasan, 1967). There were also persisting bridges and spindle abnormalities as found in Triticinae species (Sachs, 1952) in the second meiotic division.

Malik and Thomas (1966) observed a high occurrence of univalents and multivalents in Festuca species. So far multivalents were not being reported in S. spontaneum yet. Shahre and Shastry (1963) proposed that this genetic instability might be due to unequal numerical disjunction of multivalents. Emsweller (1949) observed a loss of one chromosome following doubling in Lolium species.

Most of the above meiotic abnormalities occurred in different frequency in different varieties of S. spontaneum. All these may have resulted in pollen mother cells of varying chromosome numbers within one flower. For example, in the case of variety Imp 212 (2n=61), $30_{II} + 1_{I}$ and $29_{II} + 3_{I}$ cell types were found (Nair, 1972a). The univalents would move at random in most of anaphase I cells resulting in 29/32 and 30/31 separation. This later would give rise to pollen mother cells having slightly different chromosome numbers.

Mehta and Sood (1974) suggested that variability in S. spontaneum might be obtained due to the presence of floating chromosomes within this population. While studying meiosis in pollen mother cells of six cytotypes of S. spontaneum, they found that some of the bivalents did not congress in the equatorial plate. In one case (n=36 cytotype), some of these bivalents desynapsed in up to 30% of the cells. In both these cases, the ultimate result would be chromosomes lagging behind as univalent or bivalents during anaphase. Multipolar spindles were also reported in n=21 cytotypes. They then concluded that in the later canes hyper- and hypo-aneuploid gametes could be produced and, if functional, would produce seedlings with higher or lower chromosome numbers than the parent.

Chromosome elimination 'en block' in Saccharum species during meiosis of the parents may result in great chromosome losses. Subba Rao et al. (1960) noticed a loss of 11 bivalents in the two types of seedlings from Co 602 (2n=96 from 2n=118). When a study was made on the mega and microspore mother cells of the parent clone by Alexander (1965), there was an occurrence of accessory spindle and double-plate metaphase in 10% of both types of cells. He noticed that the main group underwent normal first and second meiotic division but not the accessory group which moved to one pole without undergoing any separation and slowly was degenerated.

Raghavan (1954) seems to agree with Bremer (1951) that this process of elimination was accompanied by a doubling of chromosomes through endomitosis of surviving group, thus resulting in the functioning of an egg having chromosome number neither haploid nor diploid. Pathenogenetic seedlings might have resulted from these eggs. Such eggs were also shown to take part in fertilization thus giving rise to seedlings having significantly higher chromosome numbers.

Meiosis in S. spontaneum varieties is therefore very complex and irregular. As a result of that, we could expect a lot of segregation and irregularities in the chromosome number of their offspring.

E. Somatic Mosaicism in Saccharum

Frankhauser (1945) defined chromosome mosaicism as the coexistence of cells with different chromosome numbers within the same tissue.

Intraplant variation in chromosome number within somatic tissues such as root tips and apical meristems was reported in many vegetatively propagated plants (Sarma, 1956), in dicots (Partanen, 1963) including in Nicotiana species, (Cooper et. al, 1964; Nishiyama and Taira, 1966), Trillium species (Huskins and Cheng, 1949), Allium cepa (Huskins and Cheng, 1950), Hymenocallis calathinum (Snoad, 1955), Rubus species (Britton and Hull, 1957), Oryza sativa (Thakur, 1978), and Saccharum species (Heinz and Mee, 1971; Nair, 1972a, b, and 1973; and Fernandez et. al, 1978).

In most of these cases, these variations were due to chemical treatment, exposure to cold temperature, and chemical mutagens.

Work on Trillium species by Huskins and Cheng (1949) indicated that there was an occurrence of reductional division in mitosis although this occurred in low frequency (3.9%-5.1% of the total cells counted). They studied squashed root tips treated with 4% sodium nucleate for 3-6 days at 2°C. Segregation of individual chromosome pairs into different ratios was observed. They proposed that this somatic meiosis may be due to:

1. separation of chromosomes into two numerically equal groups and,
2. separation of homologues to the same side or being segregated with greater than random frequency.

Cytological behavior of mitosis in onion was also studied by them later (1950). They treated onion bulbs for several days at 5° to 6°C and root tips that

grew from them were squashed and studied. They found that there were groupings of chromosomes at prophase and metaphase. At anaphase and telophase, there were four separate nuclei formed, arranged either quadrilaterally or linearly. In some cases there were two separate nuclei at one end of the cell and a single fused nuclei at the other end possibly giving rise to two haploid nuclei and one diploid nucleus. This may have confirmed their earlier proposal that treatment with sodium nucleate or cold temperature can result in reductional grouping similar to that of meiosis.

Wilson et al. (1949) suggested that disorganized mitosis which lead to mitotic segregation and reduction occurred in some material. Special treatments such as chemicals and low temperature only enhance the frequency slightly. They observed that, when there were any reductional groupings, they always occurred at right angle to the spindle. This sort of mitotic aberration could easily account for the loss of chromosomes in the tissues resulting in somatic mosaicism.

Menzel and Brown (1952) and Shung-Jun Yang (1965) associated mosaic formation in somatic cells to different ploidy levels. The earlier writers observed that somatic mosaicism was exhibited in cells of tetraploid through octoploid plants. There was no occurrence of somatic mosaicism in diploid and triploid plants. They proposed that mosaicism was more likely to be due to spontaneous changes in the chromosome complement in the affected areas than to changes in the genes themselves. However, they concluded that this phenomenon was due to aberrant segregational grouping at mitosis, and more rarely resulted in atypical segregational mitotic division.

The occurrence of irregular groupings of the chromosomes during mitosis was also reported by Snoad (1955). Chromosome number of squashed root tips of Hymenocallis calathinum were counted after colchicine treatment. Some cells were reported to have two distinctly separate spindles and others were a result of

fusion of these irregularly distributed daughter groups. Two or more groups became enclosed within one cell wall thus resulting in the occurrence of cells having proportionately very high chromosome numbers. He proposed that mitotic abnormalities may not be the result of colchicine because peculiarities appeared unusually evident even if the spindle inhibiting effect of colchicine was taken into account.

Britton and Hull (1957) observed mitotic mosaicism in squashed root tips of hexaploid progeny (2n=42) that resulted from selfing of a tetraploid plant of Eldorado of Rubus. They found that, though many cells had 42 chromosomes, a significant proportion of these cells had chromosome numbers that ranged from 2n=24 to 42. Mosaicism was also morphologically expressed in the leaves.

They suggested that the decrease in chromosome number in these cells was associated with abnormalities during mitotic division. They proposed that this decrease occurred in the following sequence:

1. At prophase, there was evidence of irregular groupings of chromosomes.
2. At metaphase, there were two distinct plates of chromosomes, oriented in various ways, each having its own spindle. The total chromosomes in these plates were equal to the number in some of the neighboring cells.
3. At anaphase, there were four groups of chromatids with two (or three) spindles. The number of chromatids going to each pole was dependent on the chromosome number of each grouped metaphase.
4. At telophase, phragmoplasts were seen between the groups of chromatids in the region of the spindles and when there was no spindle, no phragmoplast was formed. In late telophase, three cells were formed, two cells having smaller chromosome number and one binucleated cell. In some cases, when two groups of chromosomes were in close proximity, they may be enclosed by

a single nuclear membrane resulting in a partially binucleated or lobed nucleus.

Fragmentation of chromosomes can also bring about an increase in chromosome number (Sharma and Sharma, 1959). This theory was first not recognized, because fragmentation in a normal chromosome with a localized centromere may result in fragments lacking centromeres. Later it was shown that a chromosome fragment can have its own centromere. The centromeric function or the property of spindle attachment was diffused throughout the chromosome. During metaphase and anaphase the two sister chromatids were arranged on the spindle parallel to each other and regular mitosis will then take place.

Colchicine-treated commercial canes also show somatic mosaicism. Heinz and Mee (1971) obtained somatic variation of 2n=77-117 in one of the colchicine-derived clones of H50-7209 and a range of 2n=25-118 in another. They further reported that the presence of multinucleated cells showed a strong indication of the presence of asynchronous mitotic division, one way by which cells with varying chromosome number can be derived. They suggested that this mitotic instability may have been due to genetic complexity of H50-7209 because it has germplasm from at least three to five species of Saccharum due to the fact that one of its parents originated from a polycross.

Similar study was done by Fernandez et al. (1978), using naturally grown commercial clones cultivated in North West Argentina. They found that all the commercial varieties under study showed a high degree mosaicism and aneuploidy.

Intraplant variation in chromosome numbers of mitotic tissues of naturally grown S. officinarum clones was reported by Nair (1972a). Eight out of eleven clones with 2n=80 and a typical form of S. officinarum showed chromosome mosaicism in root tip cells. Aneuploid chromosome numbers ranging from 73 to 85 was observed with varying percentages in all the eight varieties.

Somatic instability was observed in chromosome counts of Triticinae (Sachs, 1952), and a derivative from Agroclymus turneri (Nielsen and Nath, 1961). Since these progenies were from wide crosses it was postulated that this somatic instability was due to the intergeneric nature of the crosses.

The origin, importance, and genetic complexity of S. spontaneum was well understood. The idea of somatic mosaicism was new in Saccharum, and with this discovery, one would appreciate more of its nature and instability in view of its use in sugarcane breeding.

CHAPTER III

MATERIALS AND METHODS

A. General

This study was conducted at the Hawaiian Sugar Planters' Association Breeding Station in the Maunawili Valley, Island of Oahu, Hawaii. The breeding station is located at a latitude of 21° 30' N and longitude of 157° 40' W. It is situated beneath the Koolau Mountain Range at an altitude of 150 m, where frequent cloud cover and a mean annual rainfall of 2300 mm assure favorable flowering conditions.

At this station, the flowering season normally extends from October through January and peaks in early December. Most of the 67 Saccharum spontaneum clones presently maintained at the station flower early in the season.

Selfing was attempted on 17 pollen-bearing S. spontaneum clones. A minimum of 10 flowering stalks of each clone were collected, cut from the field nursery just prior to anthesis, and isolated in 3.0×0.7^2 m, closely woven cloth lanterns. Lanterns covered most of the length of individual stalks so that pollen contamination was assumed to be negligible. The cut ends of the stalks were kept in a tassel solution (see III, B.) which normally assures tassel survival from anthesis to seed maturity.

Ten of the 17 S. spontaneum clones produced viable seed. Seeds of two of the clones (SES113A and Tabongo) were not transplanted because they had fewer than 10 seedlings. Data on two other progenies (SES 192 and SES 197A) were discontinued because a sample size of ≤ 10 was thought to be too small to justify further evaluation. Six of the clones, four originating from India, one from Egypt, and one from Indonesia, were used in this study because they produced more than 20 viable seedlings each.

B. Description of the Parental S. spontaneum Clones

Information on the six clones used for this study was published by Panje and Babu (1960) and Hawaiian Sugar Planters' Manual (unpublished).

a. SES 84A

Panje and Babu (1960) reported that this clone has a somatic chromosome number of 64. A confirmation of this count was made on ten mitotic cells from its apical tissue. The clone is drought tolerant, resistant to red rot disease (Physalospora tucumanensis) but susceptible to smut (Ustilago scitaminea Syd.). At HSPA, this variety is rated as strongly male fertile and heavy tasseling. It is described as being able to impart its vigor to its progenies. This variety originates from Madras state in India.

b. SES 106B

The somatic chromosome of this clone was confirmed to be 2n=48, by counting 10 cells from its apical tissue. It is resistant to red rot disease and smut. At HSPA, this clone is rated as heavy tasseling. SES 106B originates from Andra Pradesh state in India.

c. SES 231

Somatic chromosome count was done on this clone to confirm a count of 2n=80. It is resistant to red rot disease and smut. There is no incidence of mosaic disease from inoculation or exposure. It has a very heavy stooling habit with short internodes. The exposed stalks are brown in color but the unexposed internodes are green or grey in color. It is found to be tolerant to saline condition. At HSPA it is rated as very sparsely flowering. This variety originates from north Bihar in India.

d. SES 239/43

Somatic chromosome number of this clone was confirmed to be 2n=72. It is resistant to mosaic and tolerant to water logging. It is believed to be a S. spontaneum hybrid. It originates from North Bihar in India having a latitude of 27° 25' N.

e. Aegyptiacum

This clone has a somatic count of 64 chromosomes which was confirmed by mitotic counts on its apical tissue. It is generally non-vigorous but carried drought resistance genes. At HSPA, it is rated as a weak male; the tassels may be male-sterile or fertile depending on environment. It tassels heavily but has poor seed germination ability. It is common on stream and canal banks and was collected in Egypt.

f. Djatirato

A confirmation of this somatic chromosome count of this clone was made and found to be 2n=112. At HSPA, it is rated as highly fertile, very heavy tasselling, and small in stalk size. This clone originates from Java.

C. Tassel and Seed Preservation

The flowering stalks of S. spontaneum clones were collected from the field beginning in early October and concluding in late November 1981. Stalks were cut 2-3 times per week to assure an adequate seed sample for each clone. Stalks were contained in 18-liter pails, filled with a tassel solution consisting of 100 ppm SO_2, 50 ppm H_3PO_4, 25 ppm H_2SO_4, and 25 ppm HNO_3. Stock solution was added to water which was naturally low in ionic potential. Due to volatilization of SO_2, concentrated SO_2 (3.5%) was added to the buckets weekly (every Monday), and whole solution was replaced weekly (every Thursday).

The stalks were kept in the solution until flower ripening but not beyond 30 days. Tassels were cut, put in separate paper bags and stored in a drying chamber until germination. The drying chamber was maintained at 27-35°C at near-zero humidity. Following drying, the fuzz (fluffy appearing mat of seed) was stripped off the raches in preparation for germination.

D. Germination

Germination was done at the Breeding Station in March 1982. The fuzz was spread uniformly on the surface of 0.32 x 0.55 m flats, using vermiculite as a soil medium.

The fuzz was then sprayed with water to establish a close contact between the true seed and the vermiculite. Germination was done in the greenhouse, with temperatures that ranged between 38°C - 43°C and humidity between 50-100%. After 5 days, these flats were moved outdoors to harden under normal temperature and sunlight conditions.

E. Fertilization

A stock solution containing the following was prepared:

		pounds/gallon
1.	Diammonium phosphate	18.5
2.	Urea (46-0-0)	33.0
3.	Muriate of potash (0-0-62)	5.0
4.	Fe (Geigy Sequestrene 10%)	10.0
5.	Zn (" " 14.2%)	5.0
6.	Mn (" " 12.0%)	5.0
7.	Cu (" " 13.0%)	2.5

130 ml of the above stock solution was added to a gallon of water. This gave an N-P-K ratio of 6-3-1, 0.41% Fe, 0.29% Zn, 0.24% Mn, and 0.13% Cu for direct fertilization. At sowing, a single application of 200 ml of diluted stock solution

was added to each flat. Dexon and Panogen (fungicides) were also added to control seedling diseases. After germination, 200 ml of the diluted stock solution and Panogen were added to each germinating flat weekly. In case the seedlings turned yellow, extra Fe was added.

F. Transplanting

Seedlings were transplanted to the field in mid-May 1982 when they were about 2 months old. Clones with a transplant seedling number of at least 20 were selected for this study. SES 84A, SES 106B, SES 231, SES 239/43, Aegyptiacum, and Djatiroto had 46, 47, 38, 23, 42, and 43 selfed seedlings, respectively. The progenies were planted in rows, and the rows were randomized (see Appendix A). Rows were spaced 1.6 m apart with 0.3 m spacing between plants. A fertilizer application consisting of a mixture of N-P-K (1-1-1) was given at transplanting.

G. Growth Parameters

The seedlings were allowed to grow with regular maintenance (as that practiced by HSPA) during the period of data collection. In August (5 months of age) data on plant height and tiller number were taken. Data on a stalk diameter were taken when the plants were 9 months old. During February 1983 (11 months of age), other data were taken which included leaf width, midrib width, leaf angle, stalk diameter, and percent refractometer solids. The survival rate was calculated based on the number of available data for each progeny under study at different periods, i. e. at 5 months, 9 months, and 11 months of age.

a. Plant height - Plant height was measured from the base of the stalks to the top visible dewlap (the triangle feature separating the leaf sheath and leaf blade) of the primary tiller.

b. Tiller number - Every live, visible tiller for each seedling was counted.

c. Tiller size - The diameter of the primary tiller was taken at the fourth internode above ground level.

d. Leaf characteristics - Leaf parameters were taken from the fourth fully visible leaf from the canopy top. Two tillers per seedling were measured. Leaf and midrib width were taken at exactly one-third way from the tip of the leaf. A leaf:midrib width ratio was calculated using these data. The leaf angle was taken as the angle made by the fourth leaf while holding the stalk vertical, and was measured at the base of the leaf.

e. Refractometer solids % expressed juice - Procedures for juice extraction were outlined by Tanimoto (1964). The most mature cane stalks of each stool were cut into small pieces and preserved in a refrigerator. The stalks were then chopped into small aggregates in a Buffalo chopper and a pressure of up to 105 kg/cm^2 was applied to obtain cane juice. Individual juice samples were collected in test tubes. A few mg of mercuric chloride crystals were added to each sample to help preserve it. Samples were frozen if analysis was to be delayed. Since the juice obtained was badly contaminated with colloidal substances, the samples were centrifuged prior to analysis. Percent refractometer solids was read on each sample immediately following centrifugation.

H. Mitosis

Apical meristems were collected from ten randomly chosen selfed progeny per parent clone under study. Mitotic chromosome analysis continued from August 1982 through February 1983.

Pretreatment procedures and chemicals used for mitotic preparation were outlined by Ahloowalia (1965). The growing and actively dividing apical meristem was pretreated with alpha-bromonaphthalene (saturated in water) for 2 hours 45 minutes. The shoot was fixed overnight in Carnoy solution containing 1 part of

ethanol, 3 parts of acetic acid, and 1 part of chloroform. Five percent ferric chloride was added to the above mixture in the amount of 1 ml per 100 ml.

Hydrolysis was done in HCl at 60°C for 25 minutes. The shoot was then rinsed with 70% ethanol for 2 minutes. Staining was done in aceto-orcein set at 38°C for 45 minutes.

The shoot meristem was cut into thin pieces (about 1 mm thick), squashed in 45% acetic acid to a thickness of one cell layer, and spread using thumb pressure. Slight heating was necessary prior to sealing of the coverslip.

The chromosomes were counted under (100X) magnification. Chromosome counts were made on at least 10 cells from each plant examined. For progenies of SES 239/43, in which mosaicism was observed, the frequency of each cell type was calculated by counting the chromosomes of 25-50 cells for each plant examined.

I. Meiosis

Meiotic studies, outlined by Ahloowalia (1982), were attempted during the flowering season (October to January, 1982). However, due to failure of almost all progenies (except progeny of SES 84A) to flower during the season, this study was not continued.

CHAPTER IV

RESULTS

A. Survival Rate

Selfing was done on 17 S. spontaneum clones. The seed set was generally very poor. Seven of the 17 clones did not set viable seed at all (Table 1). Overall 281 inbreds were initially planted. Studies on 20 other inbreds (from progenies of SES 192 and SES 197A) were discontinued. Only 259 inbreds were evaluated at 5 months, 230 inbreds were evaluated at 9 months, and only 220 inbreds could be evaluated at 11 months of age.

B. Chromosome Variability

Initially, all the chromosome numbers of the parental clones were counted and confirmed. Counts from at least 10 cells per inbred seedling are shown in Appendix B. The values given in Table 2 are considered to be the probable diploid number of each inbred seedling. The exact chromosome number was hard to determine due to the overlapping of chromosomes, possibility of double counting, and sometimes due to the sticky nature of chromosomes. Thus assigning a most probable number for each inbred seedling was considered to be reasonable.

The chromosome range in the inbreds of SES 84A was 2n=64 to 78 and the mean was 2n=71. One inbred had a chromosome number equal to that of the parent and the remaining nine had chromosome numbers more than the parent. The chromosome range and mean within the inbreds of SES 106B were found to be 2n=48 to 64 and 2n=58, respectively. Similarly, one inbred had a chromosome number equal to that of the parent and the remaining nine had chromosome numbers greater than the parent.

In contrast to the previous two populations, inbreds of SES 231, Aegyptiacum, and Djatiroto showed both an increase and a decrease in their 2n numbers from that of the parent. One inbred seedling of SES 231 had a 2n number greater than

Table 1

INITIAL NUMBER AND SURVIVAL RATE

OF INBREDS OF 17 S. SPONTANEUM CLONES

Varieties selfed	No. of inbreds planted in field	No. (Percentage) of inbreds surviving after*		
		5 months	9 months	11 months
1. Aegyptiacum	45	42(93)	42(93)	42(93)
2. Djatiroto	50	43(86)	37(74)	37(74)
3. Glagah Kloet.	0	—	—	—
4. Mol. 5801	0	—	—	—
5. Mol. 4009	0	—	—	—
6. SES 84A	48	46(96)	46(96)	45(94)
7. SES 106B	48	47(98)	44(92)	37(77)
8. SES 113A	0	—	—	—
9. SES 182**	10	10(100)	—	—
10. SES 186	0	—	—	—
11. SES 197A**	10	10(100	—	—
12. SES 205A	0	—	—	—
13. SES 231	40	38(95)	37(93)	37(93)
14. SES 239/43	30	23(92)	23(92)	22(88)
15. Tabongo	0	—	—	—
16. US 56-8-2	0	—	—	—
17. US 56-10-3	0	—	—	—
Total No. of Seedlings	281	259	230	220

*Percentage calculated based on the number at planting.
**Not studied

Table 2

CHROMOSOME NUMBERS (2n NUMBER) OF 10 INBREDS*

SELECTED AT RANDOM OF EACH PARENT

		INBRED NUMBER											
Parents	Parent	1	2	3	4	5	6	7	8	9	10	M[a]	S[b]
SES84A	64	64	66	66	70	70	72	72	74	76	78	71	4.54
SES106B	48	48	50	56	58	60	62	62	62	62	64	58	5.48
SES231	80	64	72	72	74	76	78	80	80	80	82	76	5.45
Aegyptiacum	90	60	62	62	64	66	74	74	86	92	96	74	13.33
Djatiroto	112	78	80	86	86	90	90	94	98	100	114	92	10.57

* Most probable diploid number from the counts of at least 10 cells for each inbred.

[a] Mean.

[b] Standard deviation.

the parent, three inbreds were equal and six of them had fewer chromosomes than the parent. The range of 2n number within this population was found to be 2n=64 to 82, and the mean was 2n=76.

Two of the inbreds of Aegyptiacum had 2n numbers greater than the parent and the remaining eight, fewer. The range and mean in 2n number within this family were found to be 2n=60 to 96 and 74, respectively. For Djatiroto, only one inbred seedling had a chromosome number more than the parent; the rest had fewer. The range in 2n number within this population was found to be 2n=78 to 114 and the mean was 2n=92.

C. Somatic Mosaicism

The somatic chromosome count of S. spontaneum var. SES 239/43 was found to be 2n=72 (Panje and Babu, 1960). To confirm this, ten cells were counted from the apical tissue of this variety and its somatic chromosome number was found to be consistent with the reported number. No somatic mosaicism was observed in the parental clone. However, all six inbreds that were studied exhibited somatic mosaicism, i.e. cells having different chromosome within one apical tissue, in varying proportions, (see Fig. 1A-F).

Inbred 1 from variety SES239/43 showed a regular distribution of somatic chromosome numbers within one slide (Fig. 1A). Most of the cells in the slide were single nucleated although occasionally multinucleated cells were found (Fig. 2A, 2B). Almost all countable cells were counted, and they exhibited irregular chromosome counts (Fig. 3). The chromosome numbers ranged between 2n=25-84, the highest frequency being 2n=70. The mean chromosome number was found to be 60 and the coefficient of variance was 24.6% (Fig. 1A).

Inbred 2 exhibited a pattern similar to plant 1 (Fig. 1B). The chromosome numbers ranged betwen 2n=37 to 90 with the highest frequency of occurrence being 2n=44, the mean was 2n=55, and the coefficient of variance was 20.8%.

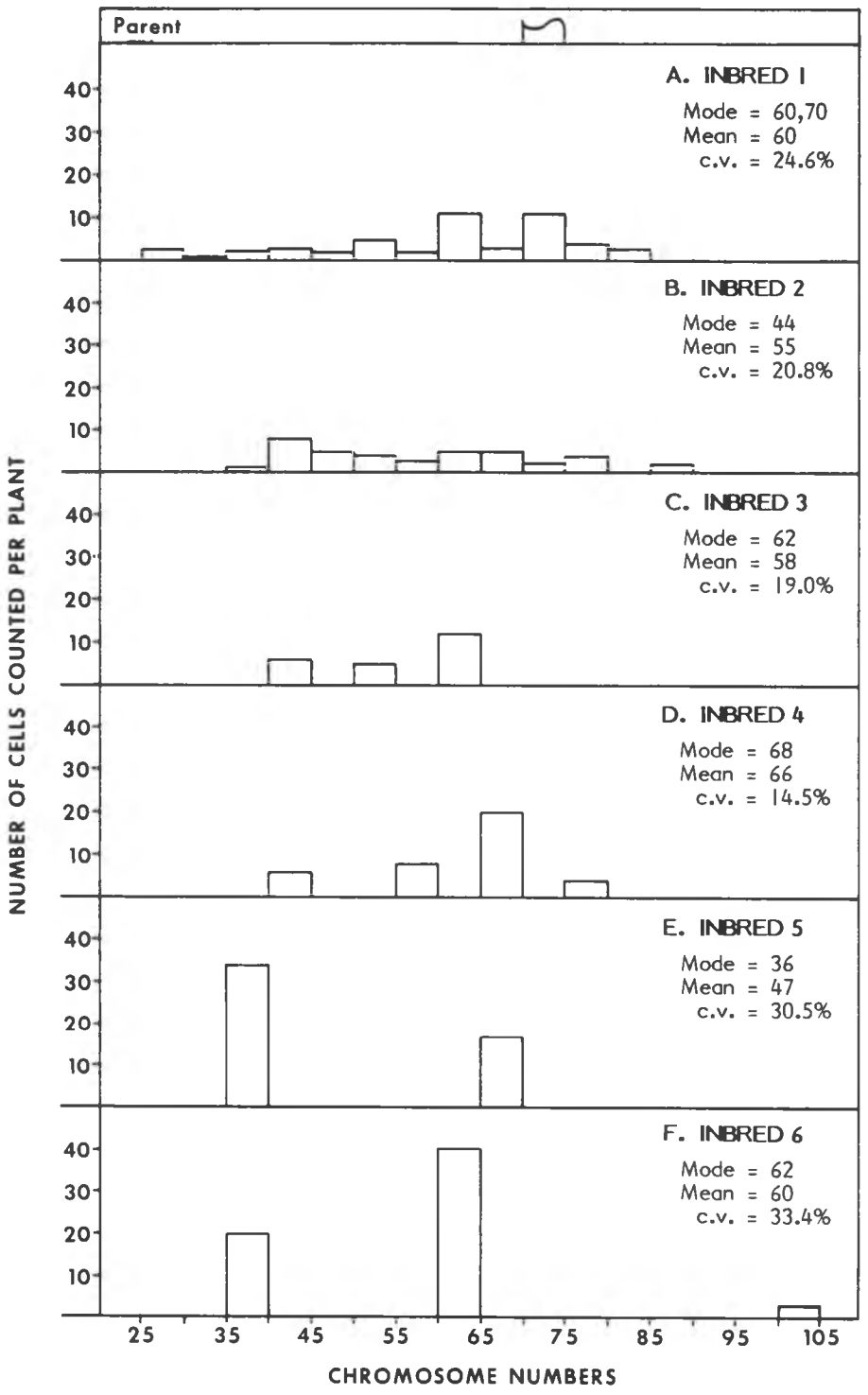

Fig. 1. Distribution of chromosome numbers in six S_1 inbreds of S. spontaneum var. SES 239/43.

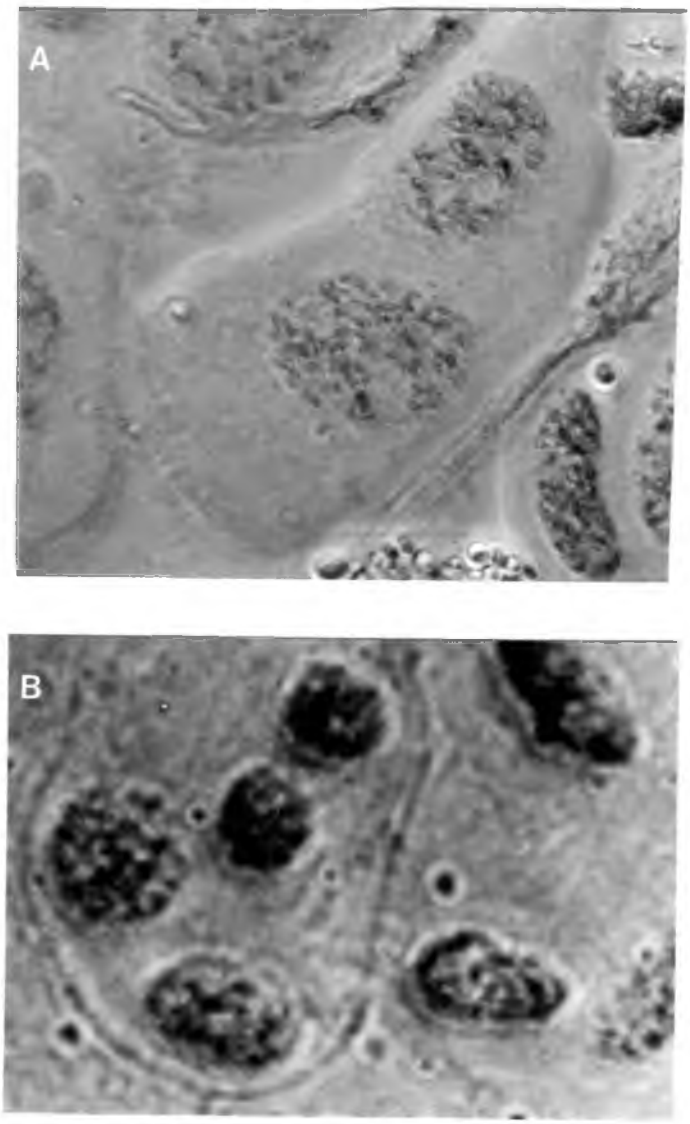

Fig. 2. Multinucleated cells of sugarcane plants from somatic tissues of inbred 1 of SES 239/43. Fig. 2A. = binucleated cell. Fig. 2B. = quadruple nucleated cell.

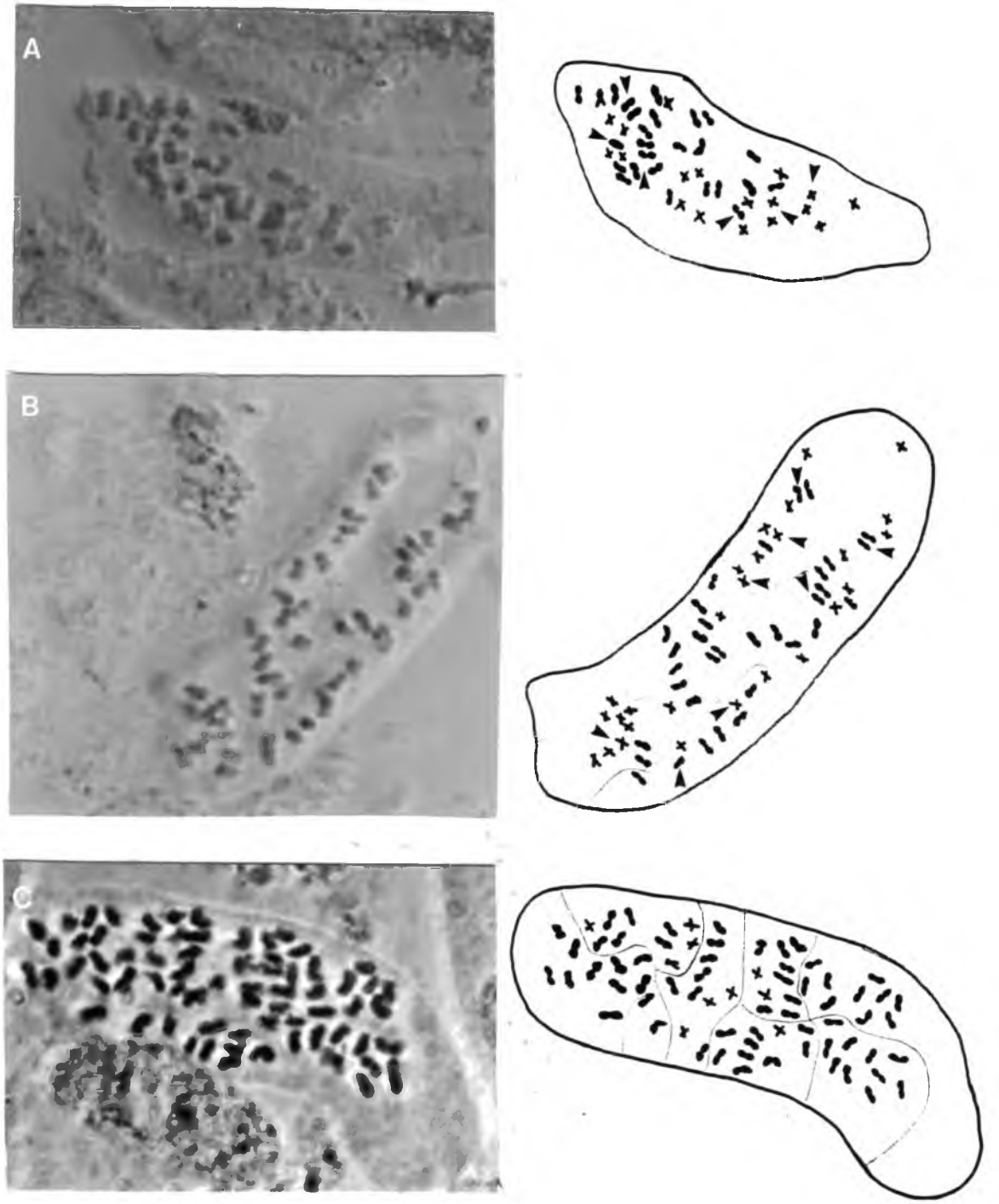

Fig. 3. Mitotic metaphase in apical tissue of inbred 1 of SES 239/43. *(Left is the photograph of cell and right is its idiogram. The arrows on the idiograms indicate chromosomes not clearly shown in the photograph.)* Fig. 3A.=2n=ca 44. Fig. 3B.=2n=ca 60. Fig. 3C.=2n=ca 72.

Inbreds 3 and 4 exhibited another pattern of chromosome distribution, i.e. into different cell types. Inbred 3 showed three cell types, i.e. (1) 2n=40-45, (2) 2n=50-55, (3) 2n=60-65 (Fig. 1C), all in one slide. Chromosome number 2n=62 was the most frequent diploid number of this inbred. Its mean value was 2n=58 and the coefficient of variance was 19.0%. Inbred 4 also showed a similar pattern. The cell types were (1) 2n=40-45, (2) 2n=55-60, (3) 2n=65-70, (4) 2n=75-80 (Fig. 1D and 4). The mean value was 2n=66 and the most frequent diploid number was 2n=68. The coefficient of variance was found to be 14/5%.

Another mosaicism pattern was found to be that of mixtures of haploid/diploid/polyploid type of cells. Out of the 50 cells counted from one slide of inbred 5, 17 cells had somatic count of 2n=65-70 and 34 cells had somatic count of 2n=35-40 thus approximating a 2:1 ratio (Fig. 1E). Most of the cells in the slide were binucleated rather than mononucleated although occasionally there were 3- or 4-nucleated cells. The mean value of this distribution was 2n=47 with a coefficient of variation of 3.5% and mode of 2n=47.

Inbred 6 showed a similar pattern. Twenty of the cells counted have a somatic count of 2n=35-40 and 40 cells had counts between 2n=60-65, again approximating 1:2 ratio (Fig. 1D). When a thorough search was made under the microscope three cells were found to have at least 104 chromosomes (a confirmation on the number was difficult because the chromosomes were not too well spread) (Fig. 5). The mean value for this distribution was 2n=60, with coefficient of variation of 33.4% and mode of 2n=62.

D. Morphological Variations

The inbred derivatives of the S. spontaneum clones under study generally resembled their respective parents and each other, so that there was no basis to suspect that outcrossing may have accidently occurred. However, when detailed observations were made on these inbreds, there was a strong indication that

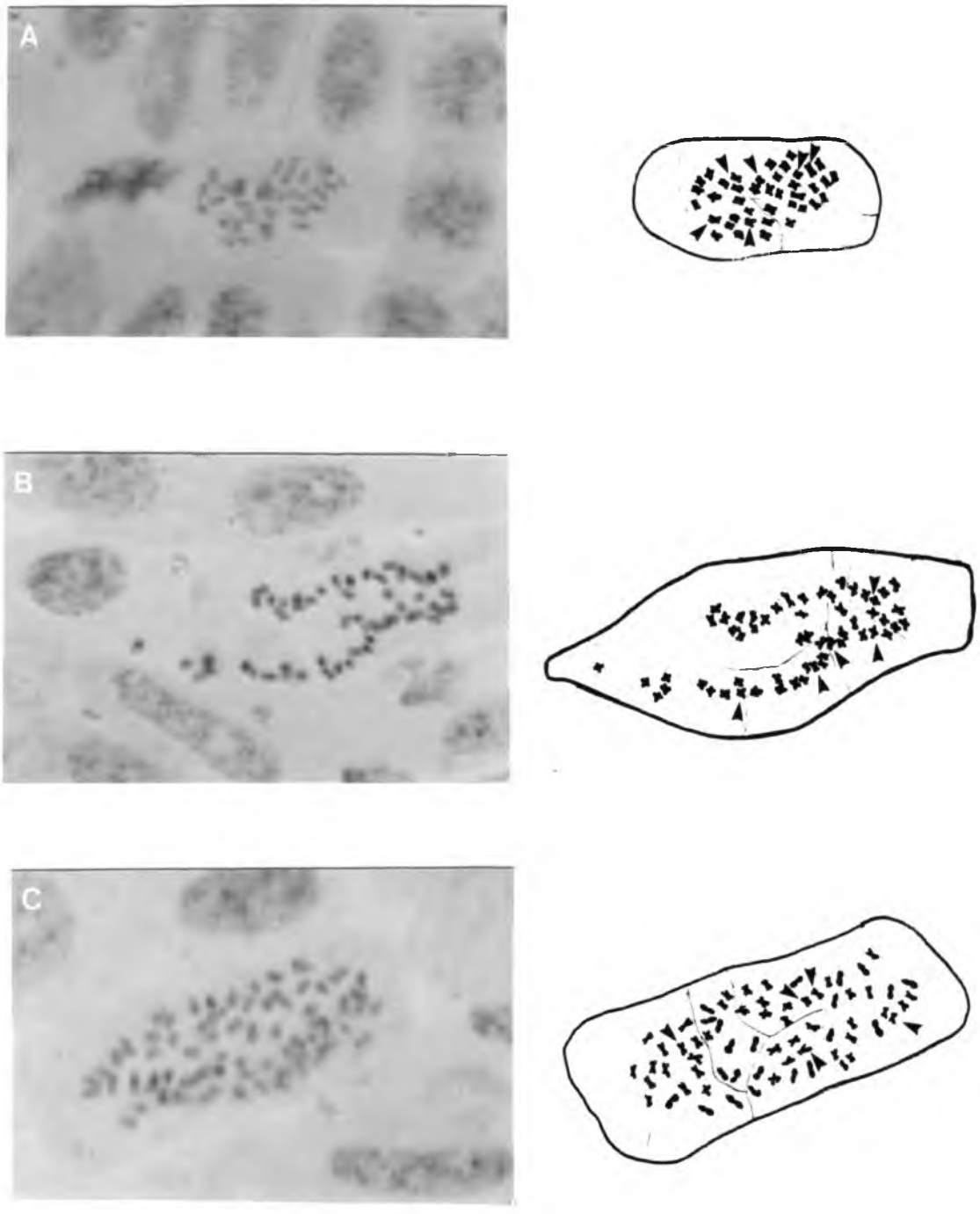

Fig. 4. Mitotic metaphase in apical tissue of inbred 4 of SES 239/43. *(Left is the photograph of the cell and right is its idiogram. Arrow indicates chromosomes not clearly shown in the photograph.)* Fig. 4A. = 2n=ca 40, Fig. 4B. = 2n=ca 56, Fig. 4C. = 2n=ca 67.

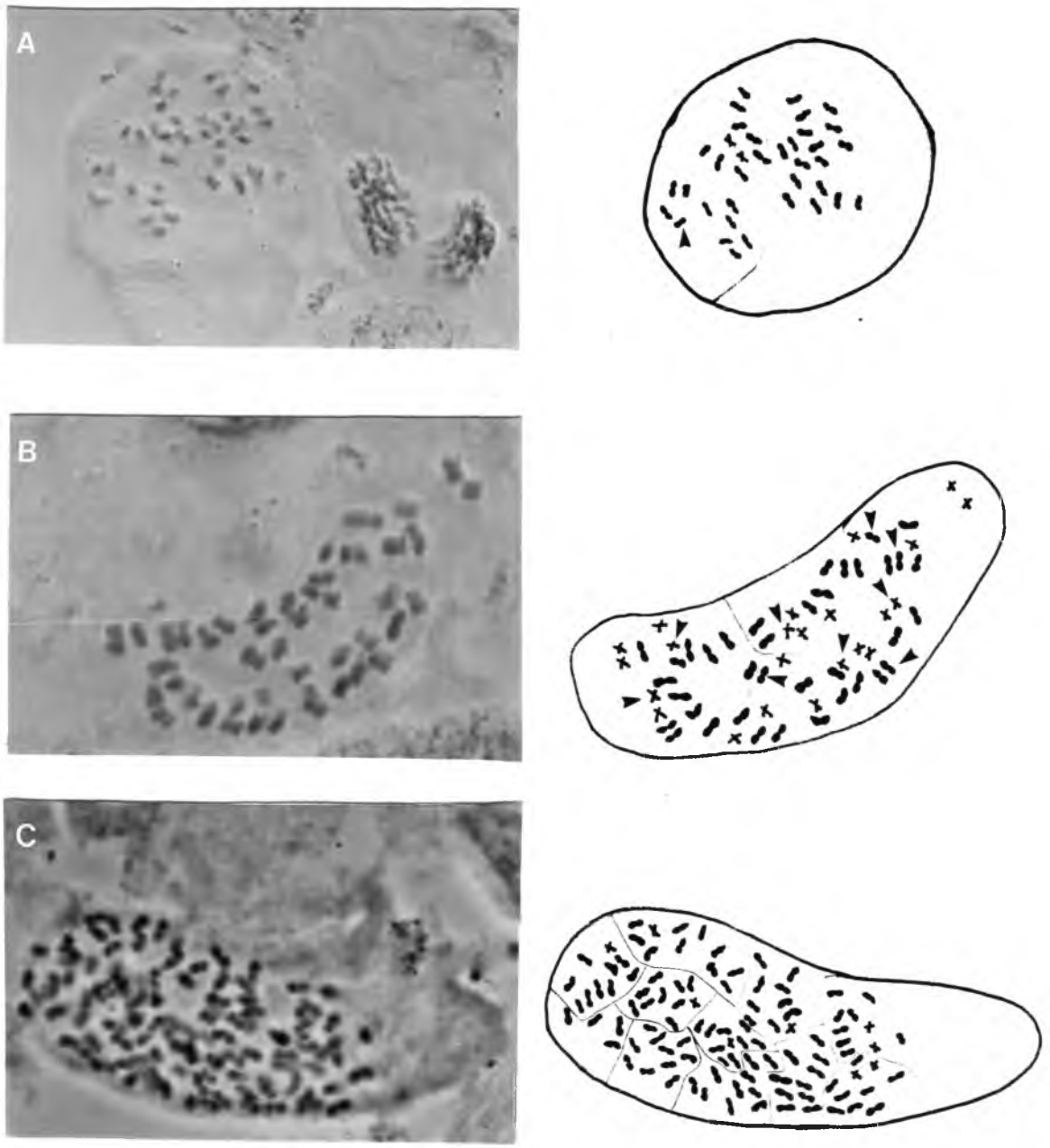

Fig. 5. Mitotic metaphase in apical tissue of inbred 6 of SES 239/43. *(Left is the photograph and right is its idiogram. Arrow on the idiograms indicate chromosomes not clearly shown in the photograph.)* Fig. 5A. = 2n=ca 37. Fig. 5B. = 2n=ca 60. Fig. 5C. = 2n=ca 104.

considerable segregation was occurring for all traits measured in this study. Also, differences in bud characteristics, ligule, dewlaps, hair group, auricle and leaf sheath were apparent within each family. These characters are under genetic control with little affect by environment and are frequently used for identification purposes.

a. Tiller Number

These data were taken when the plants were 5 months old. The distribution pattern of this character is presented in Fig. 6. Inbred derivatives of SES 239/43 showed the highest variation in tiller number. However, the distribution indicates skewness in the pattern. This population shows the highest degree of segregation and also has the highest range of values. Variations within the inbred population of Djatiroto were next highest followed by Aegyptiacum, SES 106B, and SES 84A. Progeny of SES 231 had the least variation. Among all inbreds, the mean values of Aegyptiacum progeny were the highest.

b. Plant Height

The inbred progeny of SES 106B showed the greatest range of plant height values at 5 months age. However, the selfed population of Aegyptiacum had the highest coefficient of variation, followed by SES 239/43, SES 106B, Djatiroto and SES 84A. The inbred population of SES 231 had the least variation (Fig. 7). Progeny of SES 84A had the highest mean.

c. Stalk Diameter

The mean stalk diameter values of SES 231 were the largest among all the inbreds at 9 months age (Fig. 8). The inbreds of Djatiroto had the highest coefficient of variation indicating that this family had the greatest segregation for stalk diameter. The inbreds of SES 231 had

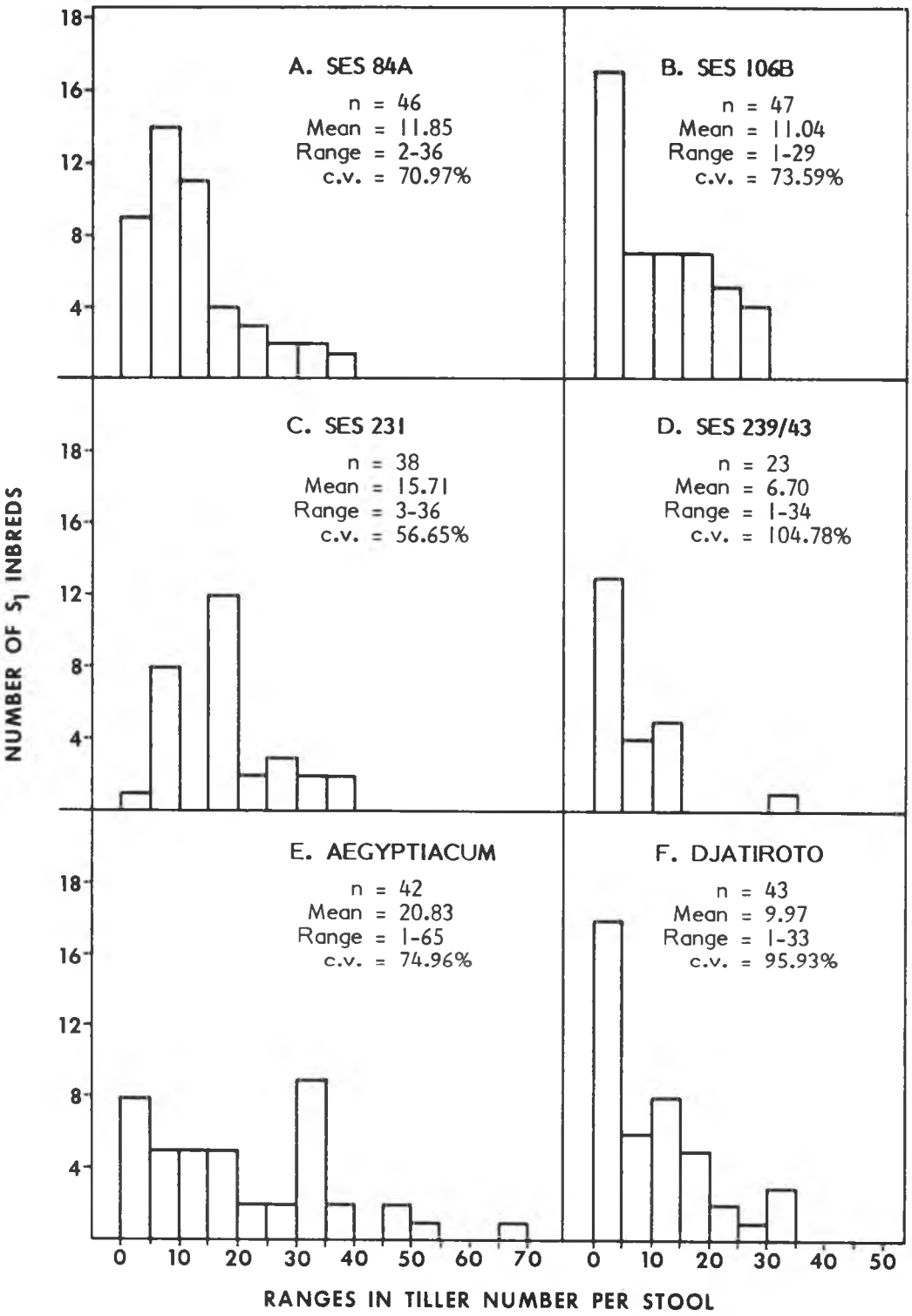

Fig. 6. Distribution pattern for tiller number of all S_1 inbreds.

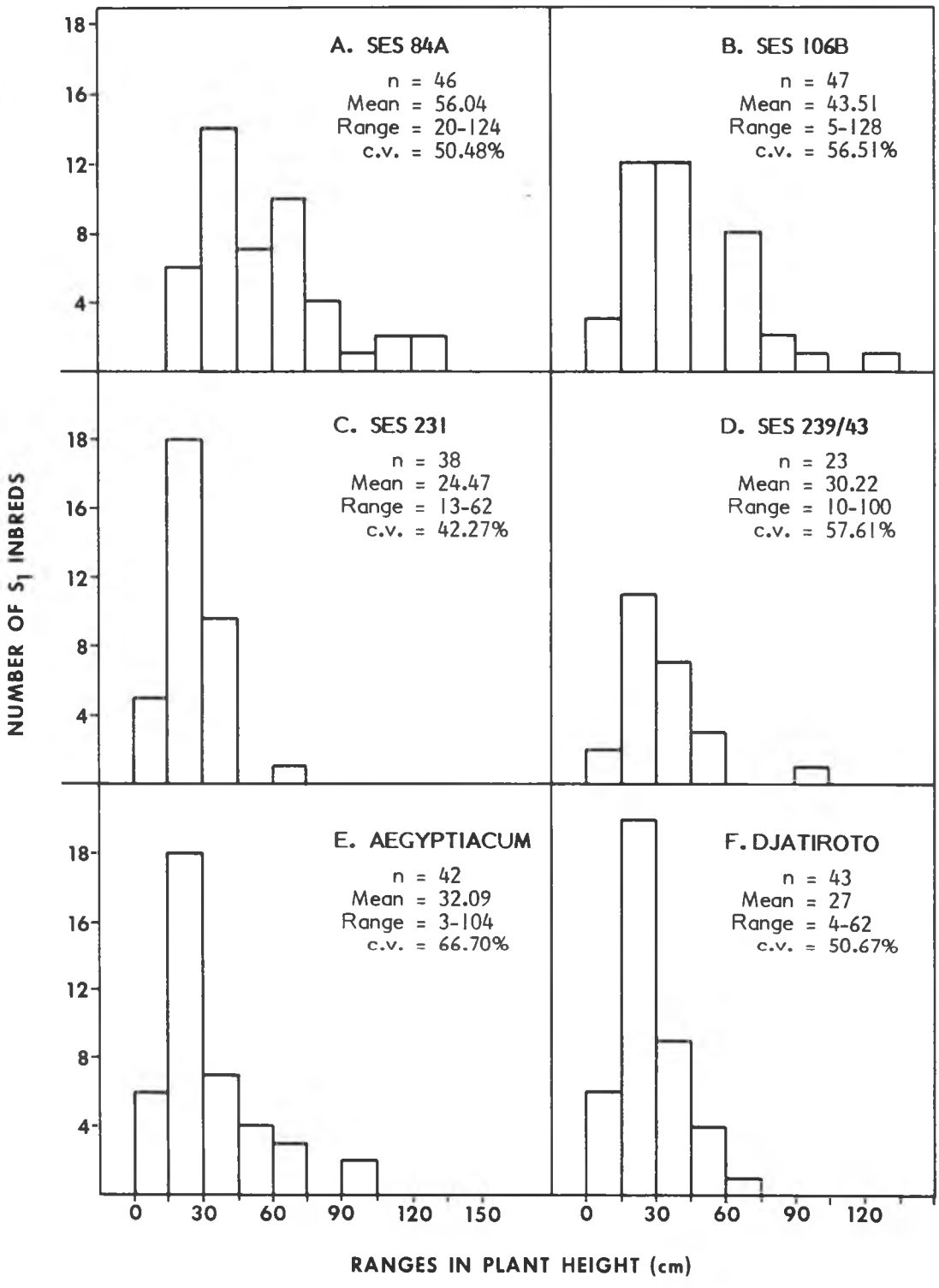

Fig. 7. Distribution pattern for plant height of all S_1 inbreds.

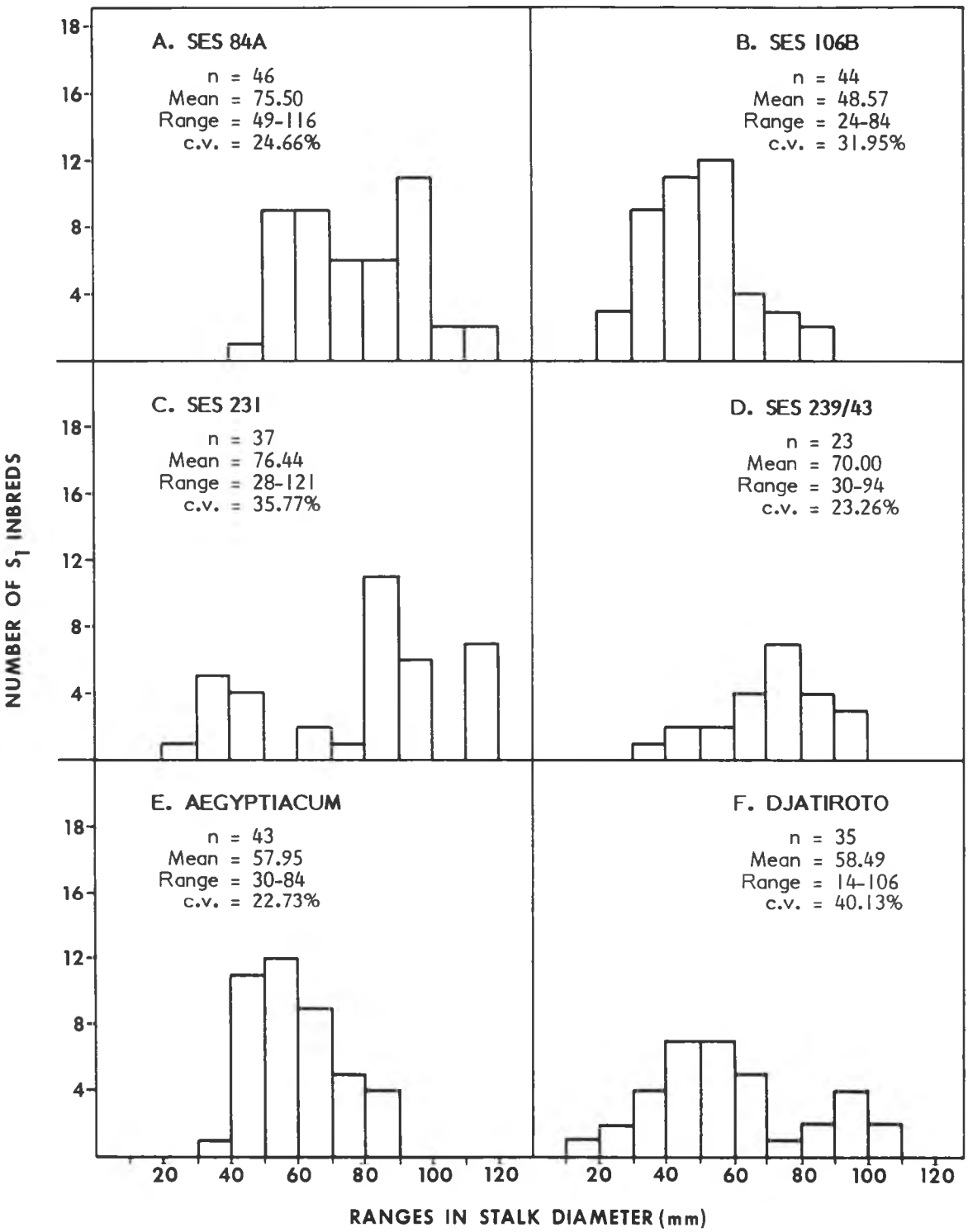

Fig. 8. Distribution pattern for stalk diameter of all S_1 inbreds.

next highest coefficient of variations followed by SES 106B, SES 84A, SES 239/43 and Aegyptiacum. In almost all except SES 231, the pattern generally followed a normal distribution. The mean values of SES 231 were the highest.

d. Refractometer Solids

These data were taken when the plants were 11 months old. The inbreds population of SES 231 had the largest variability in this character followed closely by inbreds of SES 239/43 (Fig. 9). Inbreds of SES 231 had the highest coefficient of variation followed by SES 239/43, Aegyptiacum, SES 84A, and SES 106B. The distribution pattern almost followed a normal distribution. The family that had the least variability was that from Djatiroto although its mean was high. Inbreds of Aegyptiacum had highest mean values.

e. Leaf Angle

The distribution pattern for leaf angle (taken at 11 months age) indicates some skewness within the families of SES 84A, SES 231, SES 239/43, and Aegyptiacum (Fig. 10). The inbred progeny of Djatiroto almost follow a normal distribution pattern, and have the least variability. The mean values of the inbreds of SES 84A compared to SES 84A did not differ significantly (9.1° versus 10.0°) (Table 3). The inbred progeny of SES 106B differed significantly from their parent (37.4° versus 6.0°). Thus, segregation was in the direction of a drooping character rather than an erect leaf. Similarly, the inbreds of SES 239/43 and Djatiroto showed a more drooping characteristic (19.8° versus 10.0°; 7.7° versus 5.0°) than the parent showed. In contrast to the above, the inbreds of SES 231 were generally more erect than the parent (5.08° as compared to 10° of the parent).

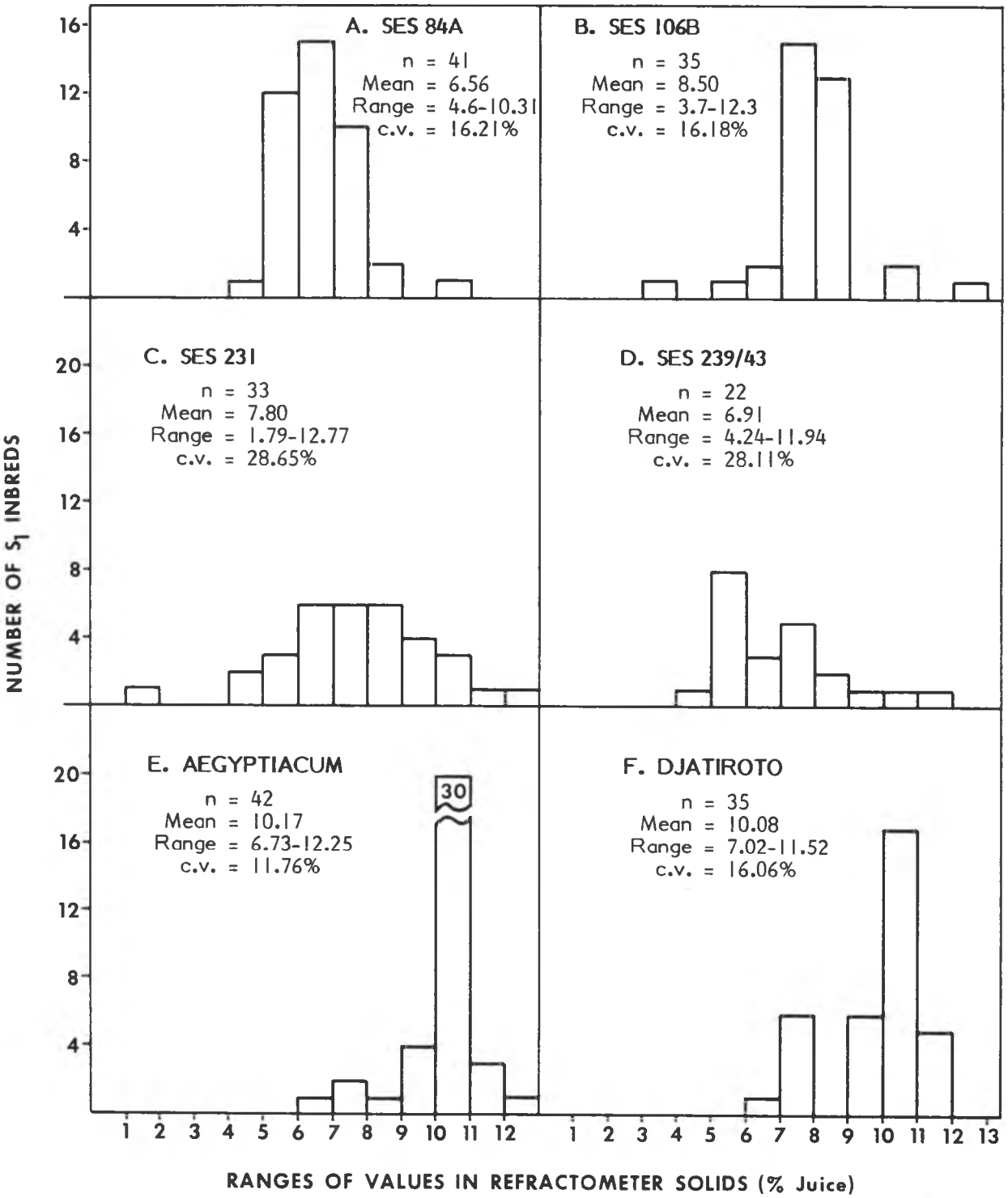

Fig. 9. Distribution pattern for refractometer solids (% juice) of all S_1 inbreds.

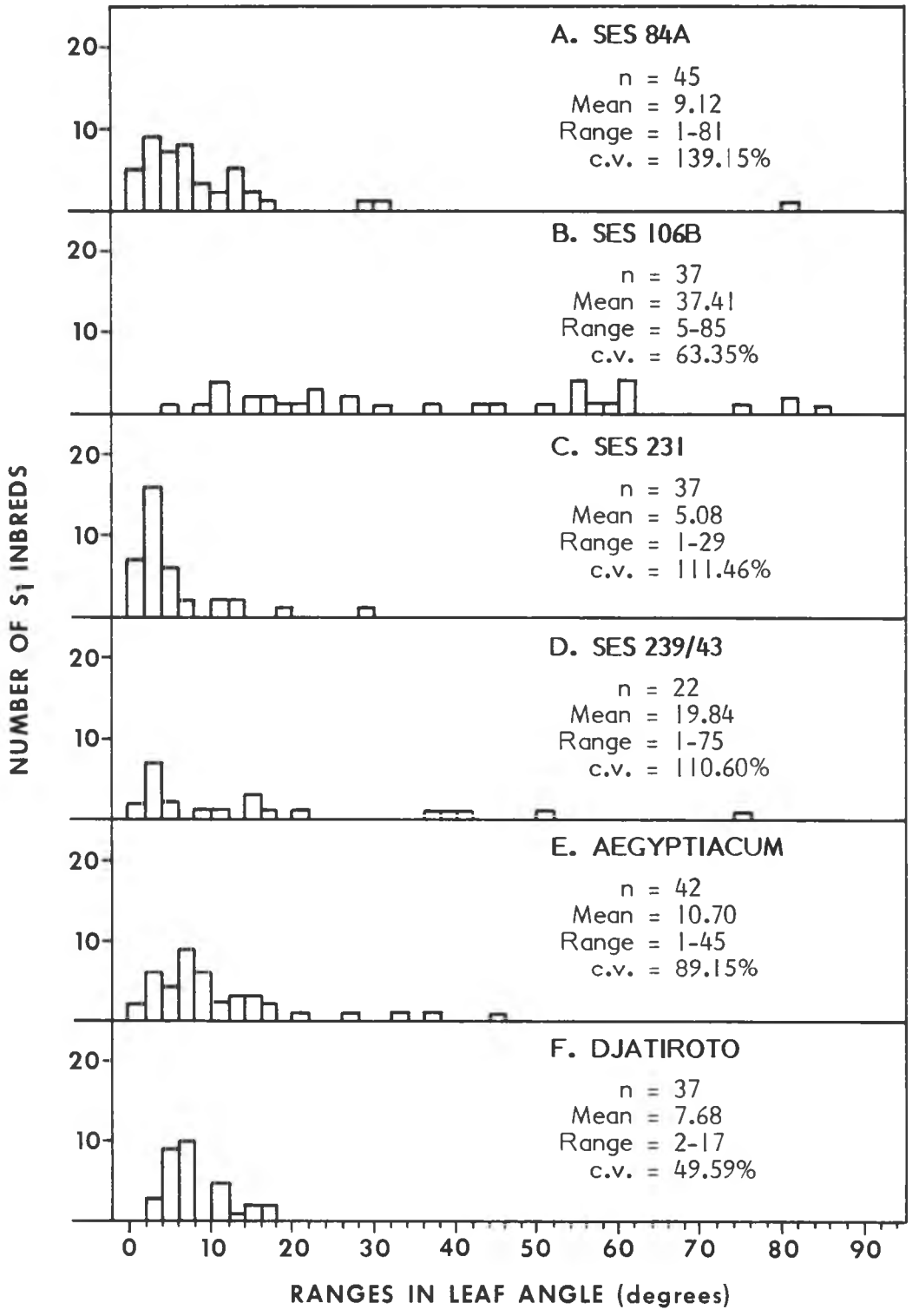

Fig. 10. Distribution pattern for leaf angle of all S_1 inbreds.

Table 3

COMPARISON OF PARENTAL MEAN AND S_1 PROGENY MEAN FOR LEAF ANGLE (DEGREES) AND LAMINA MIDRIB RATIO

	Family	Characters	Parental mean	S_1 Progeny mean
1.	SES 84A	Leaf angle	10.0	9.1
		Lamina midrib ratio	5.5	4.4
2.	SES 106B	Leaf angle	6.0	37.4
		Lamina midrib ratio	11	11
3.	SES 231	Leaf angle	10.0	5.1
		Lamina midrib ratio	4.4	3.8
4.	SES 239/43	Leaf angle	10.0	19.8
		Lamina midrib ratio	5.0	4.1
5.	Aegyptiacum	Leaf angle	10.0	12.5
		Lamina midrib ratio	6.5	5.0
6.	Djatiroto	Leaf angle	5.0	7.7
		Lamina midrib ratio	10.2	5.0

f. Lamina Midrib Ratio

Lamina midrib ratio is the ratio obtained after dividing leaf width by midrib width taken at 11 months age. This ratio can be used for identification purposes to distinguish between varieties in sugarcane. Inbreds of clone SES 106B showed the most variation (Fig. 11). Thus this population showed the highest degree of segregation. Following SES 106B were inbreds of SES 239/43, SES 84A, SES 231, Aegyptiacum, and Djatiroto.

Among all inbreds, population of SES 106B had the highest mean value (Table 3) and did not offer significantly from the parent. The population of Djatiroto had a lower ratio compared to the parent (5.0 compared to 10.2 of the parent). The inbreds of SES 231 have a lower ratio compared to the parent (3.8 compared to 4.4). Similarly this is also observed in the inbreds of SES 239/43 (4.1 compared to 5), Aegyptiacum (5.0 compared to 6.5) and SES 84A (4.4 compared to 5.5).

g. Stooling Habit

The inbreds obtained in this study were grouped under two broad classes for this character, i.e. erect and drooping. Seedlings of the erect type tended to be tall and erect. The drooping type tended to be bushy (Appendix C).

The results for this classification are presented in Table 4. Most of the inbreds of SES 84A, SES 231, and Djatiroto had erect stools. Inbreds of SES 106B and Aegyptiacum were generally drooping. Inbreds of SES 239 were generally widespread, short and having drooping stooling habit.

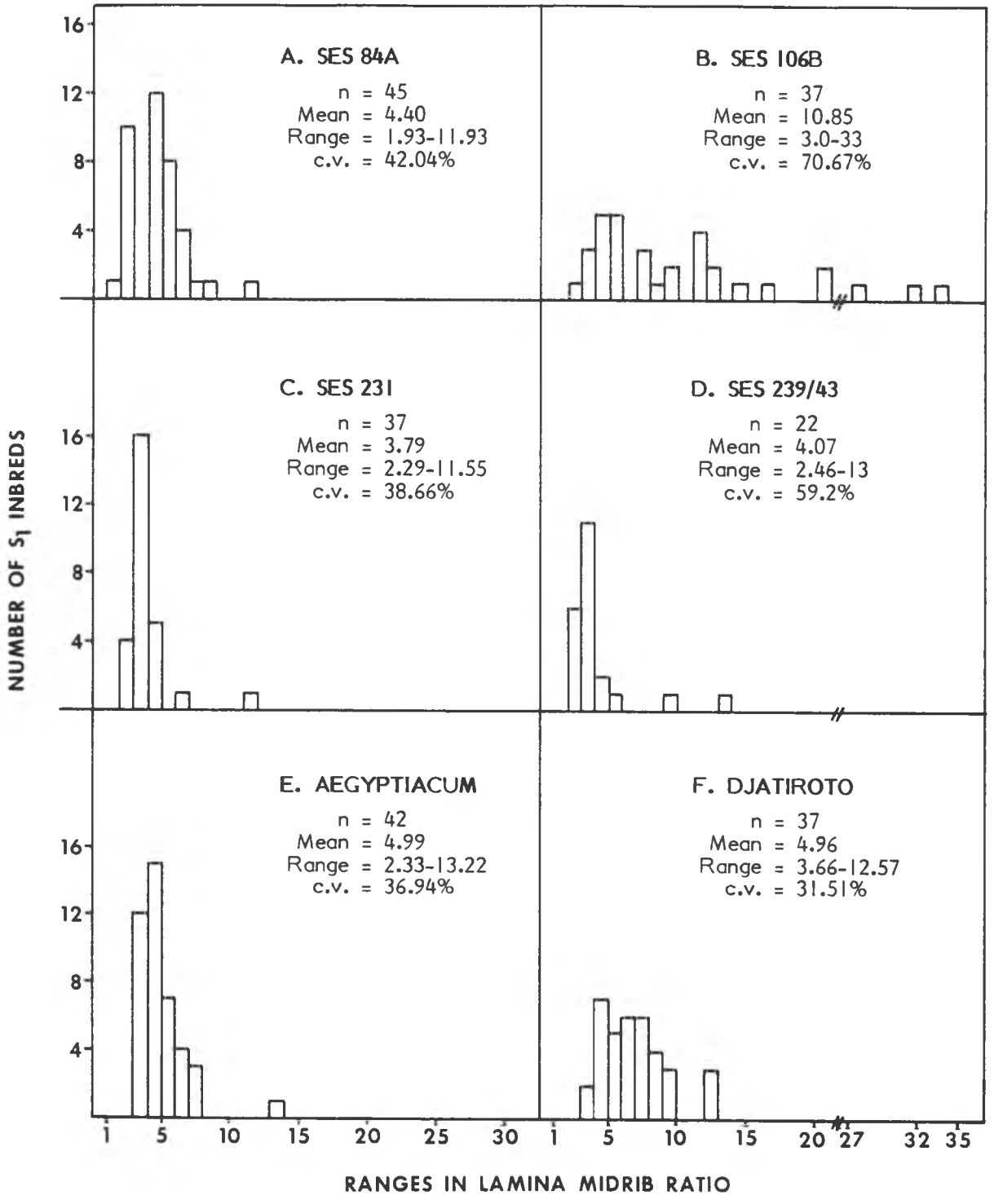

Fig. 11. Distribution pattern for lamina midrib ratio of all S_1 inbreds.

Table 4

STOOLING HABIT OF INBREDS OF

S. SPONTANEUM CLONES

	Parent	Stooling habit			
		Erect		Drooping	
		No.	%	No.	%
1.	SES 84A	45	100		
2.	SES 106B	1	5	21	95
3.	SES 231	36	100		
4.	SES 239/43	1	5	21	95
5.	Aegyptiacum			43	100
6.	Djatiroto	36	97	1	3

CHAPTER V

DISCUSSION

A. Survival Rate

In this study, at least 10 stalks of each variety were selfed, and the number of inbreds obtained from each variety selfed was relatively very small (Table 1). This is probably due to the high production of non-viable seeds and possible existence of genetic self incompatibility. Perhaps the most predominant reason is the inability of the stalks to remain alive in the solution media. S. spontaneum stalks are generally very small, low in sucrose, and therefore it is difficult to keep them alive to seed maturity.

The production of viable gametes is dependent on meiosis of the parent. Review of literature had indicated that meiosis in most S. spontaneum varieties is highly irregular (Nair, 1972a, 1972b; Mehta and Sood, 1974; Alexander, 1965). There are a lot of losses and gains of chromosomes during the anaphase separation. Probably this elimination may have caused the loss of some essential genes that determine the survival of both male and female gametes. As a result, only a low proportion of all gametes may have been capable of producing viable seeds.

Not all of the inbreds planted managed to survive till the end of the experiment. As time progressed, more inbreds were lost. In most of these cases, the inbreds had lower rate of growth and lower rate of tiller production. This is probably due to the cumulative effect of some deleterious genes or the absence of some genes essential for continued growth. As a result of this, these inbreds did not manage to compete during growth. As these inbreds were planted one foot apart, the competition for sunlight, nutrition, and water became more severe with time.

B. Chromosome Variability

Variability in the chromosome numbers among selfs of each variety may be due to some irregularities during meiosis of the parents. The high occurrence of univalents in flowers of almost all S. spontaneum clones is beyond any doubt (Nair, 1972a, 1972b; Mehta and Sood, 1974; Alexander, 1965). In all these cases, one would expect laggards during anaphase thus resulting in chromosome losses in pollen mother cells. If this pollen fertilizes a normal haploid egg, and if it survived, the seedling will have a lower chromosome number. A loss of a few chromosomes is not abnormal in all these cases as seen in progenies of SES 231 (2n=72 resulted from the loss of 8 chromosomes), and Aegyptiacum (2n=86 resulted from the loss of 4 chromosomes).

Selfed progenies of SES 84A and SES 106B did not seem to lose any chromosomes at all. In all these progenies, a gain of 2 to 18 chromosomes was observed indicating unequal distribution of chromosomes during meiotic process in the parent. At anaphase separation, one gamete might have gained chromosomes and the other had lost some chromosomes. Gametes having lower chromosome numbers might not be able to survive to fertilize normal haploid eggs.

Chromosome elimination 'en block' in Saccharum species (Alexander, 1965) during meiosis of the parents may be an acceptable explanation of such great losses observed in selfed seedlings of Djatiroto (2n=78, 80, 86, 90, 94 from 2n=112), Aegyptiacum (2n=60, 62, 64, 66 from 2n=90) and SES 231 (2n=64 from 2n=80).

A probable occurrence of accessory spindle and double plate metaphase might have taken place during meiotic division of the parental types. The main group probably underwent normal first and second meiotic division but not the accessory group. The accessory group moved to one pole without undergoing any separation and degenerated slowly. As a result of this, two gametes having fewer chromosomes will be produced. Since these eliminations were reported to have occurred

both in mega and microspore mother cells, one would expect large losses in their inbreds.

C. Somatic Mosaicism

S. spontaneum var. SES 239/43 was believed to be a S. spontaneum hybrid (Panje and Babu, 1960). Both parents were unknown, but since it originated from North Bihar in India, we would expect that it is a hybrid of S. spontaneum parents having chromosome numbers of 2n=60 to 64 and 2n=80.

From the results, there is a possibility that this instability may be due to complexity in its origin. The cells tend to segregate into cells having various chromosome numbers, possibly those of their earlier components.

This process of elimination might be accompanied by a doubling of chromosomes through endomitosis of the surviving group, thus resulting in the functioning of an egg, having a chromosome number which is neither haploid nor diploid. Pathenogenetic seedlings might have resulted from these eggs. Sub eggs were also shown to take part in fertilization thus giving rise to seedlings having significantly higher chromosome numbers. Perhaps this phenomenon could explain the significant gain in chromosomes of selfed seedlings of SES 84A (2n=78 from 2n=64) and SES 106B (2n=64 from 2n=48). Similarly, Nair (1972b) also found pollen mother cells of S. spontaneum var. SES 128A have a maximum of almost diploid chromosome number.

Thus, if the pollen mother cells happen to fertilize a normal haploid egg, seedlings with almost the triploid number of chromosomes will be obtained. It is hard, however, to determine the mechanisms involved but a detailed analysis of meiotic division of the parental type is necessary to understand this behavior.

There is evidence that 2n=62-72 is the diploid number of most of the cells. This observation of heteroploidy in these selfed clones indicates the variation and diversity in the origin of the hybrid parent.

The occurrence of irregular groupings of chromosomes at prophase as proposed by Britton and Hull (1957) may be the most acceptable explanation for the variation observed in inbreds 1 to 4. As a result of this, multiple spindles are formed leading to formation of cells and cells with variable chromosome number. The occurrence of multi-nucleated cells and cells with low, medium, and high chromosome numbers as seen in inbred 6, lead us to believe that asynchronous mitotic division as suggested by Heinz and Mee (1971), might have taken place.

An approximate 2:1 ratio of the different cell types was seen in inbreds 5 and 6. The chromosome numbers exhibited in cells of this inbred estimated to haploid/diploid relationship as suggested by Huskins and Cheng (1949). This reductional group and the presence of single, double, triple, and quadruple nucleated cells (Fig. 2) suggested the presence of reductional grouping at prophase and possible fusion of two haploids during the formation of nucleolar wall.

Another possible mechanism that helped to explain this phenomenon is the stemline theory proposed by Makino (1957). He postulated the possible existence of several cell populations, each with a different chromosome number naturally coexist within a particular tissue. The frequency of these sub-populations would depend on the environment in which they are grown. In this study somatic mosaicism was observed in seedling canes. There is a possibility that this phenomenon will only be observed in seedling canes and not canes grown from cane setts. A further study is suggested to confirm this.

Krishnamurthi and Tlaskal (1974) suggested the possibility of an irregular distribution of supernumerary chromosomes during mitosis. In this study, a regular distribution was observed in inbreds 1 and 2. This theory may help to explain this phenomenon but is not solely responsible for the observed differences as evidence from the fact that the range of chromosome numbers in all these inbreds was very wide.

In conclusion, there are few mechanisms that were suggested to have caused mosaicism in somatic tissues. Chromosome breakage and fusion, chromosome grouping, split spindles and non-disjunction of chromosomes during the mitotic process, all or any one of them may have contributed to the observed mosaicisms in this variety.

It is hard, however, to determine the exact mechanism that is involved in this unique pattern of division within this family. The relative importance of each theory suggested is unknown. Further detailed work is suggested that requires more time.

D. Morphological Variations

The resemblance of the first generation inbreds to their respective parents indicated that there were no losses or gains of any major genes that were to upset the general chracteristics of a variety and its progenies. When detail studies were made on these S_1 inbreds it was obvious that segregation had taken place. Though most of these segregations approximating a normal distribution pattern but there was skewness in some characteristics. The skewness that was exhibited in characters such as tiller number per stool and leaf angle probably was due to lack of data. In cases like stalk diameter and refractometer solids percent, the coefficients of variation were generally low and the distribution pattern almost showed a normal curve. These particular characters had high heritability values (Roach, 1977). The segregation that was exhibited in all these characters can be deduced as a typical F_2 segregation pattern. The distribution pattern exhibited in characters such as plant height and lamina midrib ratio indicated a certain amount of skewness but generally followed a normal distribution curve.

The generally low and non-significant correlation values between chromosome numbers and morphological characters (Appendix D) suggests the inconsistent nature of the chromosomes lost or gained. Variability in chromosome number

likely affects the growth of these inbreds, but the absence, presence, or duplication of chromosomes directly affecting specific characters is probably more important than total chromosome number, per se. The significant correlation between chromosome numbers and tiller number (SES 106B), stalk diameter (Djatiroto) and leaf angle (Djatiroto) could be a chance event. A similar trend was not observed in other families.

E. General Discussion

Genetic changes may be one of the possible explanations for the observed differences in agronomic performances between the inbreds and their parental clone. The resulting inbreds could be of different genetic combination from the original clone.

In sugarcane, small gain or loss in chromosome numbers may not always be sufficient to evoke a large change in morphology. Perhaps, due to high ploidy level, the loss or gain of a few chromosomes or block of chromosomes does not alter the basic genetic composition of a clone. The duplicated genomes may be the ones that are lost. It is even possible that changes may have occurred without being visibly expressed. To further verify this fact, Babu (1967) selfed a commercial variety Co 421, and found that the inbreds were not so morphologically distinct and the variation was not so marked in spite of the fact that they exhibited a range in chromosome numbers. Similarly, Liu et al. (1977) also observed that increasing chromosome numbers in callus derived plants had not altered the morphology drastically.

In this study, it was found that the inbreds were generally similar to the parental clones and to each other. Selfing of Saccharum spontaneum, therefore, has no effect on the basic genome. The loss or gain of chromosomes are from the duplicated genomes and their effects are relatively small.

Figures 6, 7, 8, 9, 10, and 11 indicate that segregation is taking place within the populations of Saccharum spontaneum clones under study. The segregation is similar to that of any F_2 population. One can safely conclude that these clones are actually hybrids as suggested by Grassl (1977). Selfing of these hybrids had given rise to segregated F_2 populations.

The stooling habit of a genus is highly genetic but also can be affected by environment. Sugarcane tillers may grow erect or they may be sprawling in their early stages of development and bend upwards later. Brandes (1948) found that the Turkestan form of S. spontaneum planted near the equator tillered profusely but looked like a bunch of grass which he considered due to a 'daylength' effect. Hole (1911) suggested that the quality and stability of the soil, direction of wind and water, and quantity of available moisture in the habitat might affect the stooling habit of a genus.

In this study, changes in the phenotypic expression of characters are perhaps due to the fact that they are present in the heterozygous condition. Selfing results in segregation of this heterozygosity into plants having different stooling habits.

The high chromosome number of a S. spontaneum clone is rather unique. One of the S. spontaneum clones under study has 2n=112. This clone, Djatiroto, is believed to be a natural hybrid of S. officinarum x S. spontaneum. Eighty of its chromosomes come from S. officinarum parent and 32 from S. spontaneum parent. Price (1963) generated F_2 seedlings from a S. officinarum x S. spontaneum hybrid (2n=112), and he obtained a set of inbreds having variable chromosome number. The chromosome numbers of F_2 plants occasionally exceeded but often fell short of the F_1 somatic number. Although there was approximate n+n chromosome transmission, the somatic numbers of F_2 plants reflected gains or losses of a few chromosomes during gametogenesis of the F_1 parent as discussed earlier.

The chromosome segregation in Djatiroto is almost similar to that of an F_2. Nine of ten inbreds had chromosome numbers less than the parent. It is a well known fact that the losses in chromosomes usually involved S. spontaneum chromosomes and only rarely those of S. officinarum. The similarities observed in this study with that of Price (1963), strongly indicate the hybrid nature of Djatiroto. It probably arose from S. officinarum x S. spontaneum.

In sugarcane breeding, parents are chosen for further crossing on the basis of high sample mean and/or high sample variance in the progeny for the traits under consideration (Wu et al., 1980). Crosses with low mean but high variance are repeated. If sample variance is high, progeny size should be large because the sample variance fluctuates greatly among small samples (Wu et al., 1978).

The comparison for means and variances of all the inbreds under study is presented in Table 5. Inbreds of Aegyptiacum show the highest mean and variance in tiller number thus indicating that this is the best progeny to be chosen for crosses for this trait. The inbred population of SES 84A is the most appropriate population if one is interested in plant height, since it has the highest values for both means and variances for this trait. The inbred population of SES 231 should be chosen if one is interested in incorporating the trait of large tiller size in sugarcane crosses. They have the best values for this trait. Inbreds of SES 84A have the most erect leaves.

In contrast to other traits, leaf lamina to midrib ratio trait will totally depend on the interest of the breeders. Some breeders are interested in wide leaf lamina for efficiency in photosynthesis but some are interested in narrower leaves for resistance to frost damage. If wide leaves are the main interest, the progeny of SES 106B is the most appropriate. In contrast, if one is interested in narrower leaves, progeny of SES 231 would be the best.

The last trait under study is refractometer solids percent which is indirectly also measuring the proportion of sucrose in the sample. For this trait, progeny of SES 231 shows the highest mean.

CHAPTER VI

SUMMARY AND CONCLUSION

A. Summary

a. Selfing of S. spontaneum clones under study resulted in mortality of some inbreds.

b. Chromosome variability in all these inbreds showed an increase in inbreds of SES 84A and 106B, both a decrease and increase in inbreds of SES 231, Aegyptiacum and Djatiroto.

c. Chromosome mosaicism was observed in six inbreds of S. spontaneum var. SES 239/43.

d. Morphological variations and distribution patterns of some characters indicated that there was a strong genetic influence in percent refractometer solids and stalk diameter. There were both genetic and environmental influence on other characters under study.

B. Conclusion

a. The death of some of these inbreds may be due to failure to withstand competition and may be due to the presence of deleterious genes.

b. Chromosome segregation exhibited in all the inbreds indicates the meiotic instability in the parents.

c. Somatic mosaicism may have resulted due to genetic complexity of the parents.

d. The nature of morphological variations resembles that of any F_2 population indicating the hybrid nature of all the S. spontaneum varieties under study.

APPENDIX A

FIELD LAYOUT AT TRANSPLANTING

Block 1.

Parent plant	Inbred plants									
SES 84A	P_1	P_2	P_3	P_4	P_5	P_6	P_7*	P_8	P_9	P_{10}
SES 239/43	P_1	P_2*	P_3	P_4	P_5*	P_6	P_7*	P_8	P_9	P_{10}
SES 106B	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
SES 231	P_1	P_2	P_3	P_4	P_5	P_6*	P_7	P_8	P_9	P_{10}
SES 197A**	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
SES 182**	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
Aegyptiacum	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9*	P_{10}
Djatiroto	P_1	P_2*	P_3*	P_4	P_5	P_6	P_7	P_8	P_9*	P_{10}

Block 2.

Parent plant	Inbred plants									
SES 239/43	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
SES 84A	P_1	P_2	P_3	P_4	P_5	P_6	P_7*	P_8	P_9	P_{10}
Aegyptiacum	P_1*	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
SES 231	P_1	P_2*	P_3	P_4	P_5*	P_6	P_7	P_8	P_9	P_{10}
SES 106B	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9*	P_{10}
Djatiroto	P_1	P_2	P_3*	P_4	P_5*	P_6*	P_7	P_8	P_9	P_{10}

Block 3.

Parent plant	Inbred plants									
Djatiroto	P_1	P_2	P_3	P_4*	P_5*	P_6	P_7	P_8	P_9	P_{10}*
SES 106B	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9*	P_{10}
SES 84A	P_1	P_2	P_3	P_4	P_5	P_6	P_7*	P_8	P_9	P_{10}
SES 239/43	P_1	P_2	P_3*	P_4*	P_5*	P_6	P_7*	P_8*	P_9	P_{10}
Aegyptiacum	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
SES 231	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}

Block 4.

Parent plant	Inbred plants									
Aegyptiacum	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
Djatiroto	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
SES 231	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8*	P_9*	P_{10}*
SES 106B	P_1	P_2	P_3	P_4	P_5*	P_6	P_7	P_8	P_9	P_{10}
SES 84A	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}

Block 5.

Parent plant	Inbred plants									
SES 106B	P_1	P_2*	P_3	P_4	P_5*	P_6*	P_7*	P_8*		
Aegyptiacum	P_1*	P_2	P_3	P_4	P_5					
Djatiroto	P_1	P_2*	P_3	P_4*	P_5*	P_6	P_7	P_8	P_9*	P_{10}
SES 84A	P_1	P_2	P_3	P_4*	P_5	P_6	P_7*			

* = Plants that remained alive until the end of the experiment.
** = Studies were discontinued.

APPENDIX B

Table 5

CHROMOSOME COUNTS OF 10 CELLS/APICAL TISSUE OF 10 S_1 FOR EACH PROGENY

a. SES 84A

Plant No.	Cell Number 1	2	3	4	5	6	7	8	9	10	Most probable diploid no.	Mean	c.v. (%)
R_2P_3	56	57	59	59	64	65	66	66	66	71	66	63	7.7
R_3P_1	58	60	65	68	69	69	70	70	70	70	70	67	6.7
R_3P_2	70	70	71	71	72	72	72	72	72	72	72	71	1.2
R_3P_3	65	65	72	72	72	72	72	76	78	80	72	72	6.7
R_3P_4	56	58	72	72	72	77	77	77	78	78	78	72	11.4
R_3P_5	61	61	62	63	64	64	64	64	65	67	64	64	2.9
R_3P_6	65	65	65	66	66	66	66	67	67	67	66	66	1.2
R_3P_{10}	48	53	70	70	73	73	73	74	74	74	74	71	12.5
R_4P_{10}	55	56	74	74	74	75	75	76	76	76	76	71	11.6
R_5P_5	68	68	69	69	70	70	70	70	70	78	70	70	4.0

b. SES 106B

Plant No.	Cell Number 1	2	3	4	5	6	7	8	9	10	Most probable diploid no.	Mean	c.v. (%)
R_1P_1	53	55	56	58	58	58	58	58	59	60	58	57	3.6
R_1P_2	46	47	48	48	48	48	48	48	48	49	48	48	1.7
R_1P_3	60	61	61	62	62	62	62	63	63	63	62	62	1.6
R_1P_4	48	48	49	49	49	50	50	50	50	51	50	49	2.0
R_1P_5	40	46	54	55	55	56	56	56	56	57	56	53	10.5
R_1P_9	60	60	60	61	61	61	62	62	62	62	62	61	1.4
R_3P_2	58	58	59	61	61	61	62	62	62	62	62	61	2.7
R_3P_6	58	59	59	61	61	61	61	62	62	62	62	61	2.4
R_3P_9	57	58	60	62	62	63	64	64	64	64	64	61	4.2
*$R_3P?$	58	58	58	59	59	60	60	60	60	61	60	59	1.7

c. SES 231

Plant No.	Cell Number 1	2	3	4	5	6	7	8	9	10	Most probable diploid no.	Mean	c.v. (%)
R_1P_2	72	77	77	80	80	80	80	80	80	86	80	80	7.6
R_1P_3	77	77	79	79	79	80	80	80	80	84	80	80	2.5
R_1P_6	60	63	63	63	64	64	64	64	65	65	64	64	2.3
R_1P_7	60	72	72	72	72	72	72	72	72	87	72	72	8.8
R_1P_8	73	73	80	80	80	80	80	80	84	90	80	80	6.1
R_1P_9	70	71	71	72	72	72	72	72	73	76	72	72	2.2
R_4P_3	68	73	75	76	76	77	78	78	78	78	78	76	4.2
R_4P_7	68	69	70	74	74	74	74	74	76	78	74	73	4.3
R_4P_8	68	70	74	75	75	75	75	76	76	76	76	74	3.7
R_4P_9	80	80	80	82	82	82	82	84	84	86	82	82	2.4

d. Aegyptiacum

Plant No.	Cell Number 1	2	3	4	5	6	7	8	9	10	Most probable diploid no.	Mean	c.v. (%)
R_1P_1	50	58	58	60	62	62	62	62	62	63	62	60	6.5
R_1P_2	58	59	60	61	61	62	62	62	62	62	62	61	2.4
R_1P_5	80	80	84	84	84	86	86	86	86	86	86	84	2.8
R_1P_6	58	60	63	64	64	64	64	64	64	68	64	63	4.2
R_1P_9	70	73	73	73	73	73	74	74	74	74	74	73	1.6
R_2P_5	56	56	58	58	60	60	60	60	61	61	60	59	3.2
R_2P_7	60	68	70	73	73	73	74	74	74	74	74	71	6.2
R_3P_2	80	88	90	90	92	92	92	92	96	98	92	91	5.3
*R_3P?	64	64	65	65	66	66	66	66	68	69	66	66	2.4
*R_3P?	90	95	95	95	95	96	96	96	96	98	96	95	2.1

e. Djatiroto

Plant No.	Cell Number 1	2	3	4	5	6	7	8	9	10	Most probable diploid no.	Mean	c.v. (%)
R_1P_1	55	75	77	77	77	77	78	78	78	78	78	75	9.4
R_1P_2	80	84	88	89	91	93	94	94	94	94	94	90	5.4
R_1P_4	78	88	90	90	90	90	90	92	93	95	90	90	5.1
R_1P_6	106	108	112	112	114	114	114	114	120	123	114	114	4.4
R_3P_3	80	86	90	98	98	99	99	100	100	100	100	95	7.5
R_3P_5	75	80	90	90	98	98	98	98	98	100	98	93	9.4
R_4P_3	80	80	84	86	88	89	89	90	90	90	90	87	4.6
R_4P_7	74	74	75	76	80	82	86	86	86	86	86	81	6.7
R_4P_8	73	75	76	80	80	86	80	86	867	86	86	81	6.5
R_4P_9	78	78	79	79	80	80	80	80	82	82	80	80	1.8
R_5P_2**	43	46	51	54	56	58	60	60	60	60			
	60	60	60	60	61	62	64	64	64	65	Mean = 73		
	67	67	68	72	73	76	75	80	80	80	c.v. = 24.8%		
	80	80	82	84	86	87	88	90	91	95	Mode = 60		
	95	125	130										

*Plant number was unknown.
**Mosaicism was observed.

APPENDIX C

STOOLING HABIT OF S. SPONTANEUM INBREDS

A. Drooping habit

B. Erect habit

APPENDIX D

Table 6

RELATIONSHIP BETWEEN CHROMOSOME NUMBERS AND MORPHOLOGICAL CHARACTERS OF ALL S_1 POPULATIONS

a. SES 84A

Plant No.	Chromosome No. 2n	Plant height (cm)	Tiller No.	Stalk diameter (mm)	Refractometer solids (%)	Leaf angle (degrees)	Lamina midrib ratio
R_2P_3	66	68	21	94	7.0	14	5.47
R_3P_1	70	68	14	90	7.6	4	2.64
R_3P_2	72	60	9	90	5.7	18	5.59
R_3P_3	72	48	10	84	8.6	3	4.98
R_3P_4	78	83	11	72	5.8	8	5.44
R_3P_5	64	86	11	80	6.2	4	2.80
R_3P_6	66	65	5	64	6.7	4	3.75
R_3P_{10}	74	119	18	76	7.3	5	4.98
R_4P_{10}	76	54	20	49	6.2	7	5.30
R_5P_5	70	65	36	70	6.7	3	4.07
Mean	71	71.6	15.5	76.9	6.8	7.0	4.5
Std. dev.	4.5	20.3	8.8	13.8	0.9	5.1	1.1
r values between chromosome number and morphological characters.		0.07	0.07	-0.36	-0.13	0.08	0.58

b. SES 106B

Plant No.	Chromosome No. 2n	Plant height (cm)	Tiller No.	Stalk diameter (mm)	Refractometer solids (%)	Leaf angle (degrees)	Lamina midrib ratio
R_1P_1	58	5	11	55	12.3	54	5.78
R_1P_2	48	36	4	33	8.6	22	11.42
R_1P_3	62	72	8	40	5.6	17	13.01
R_1P_4	50	30	5	30	8.6	50	7.84
R_1P_5	56	46	3	50	7.3	10	11.08
R_1P_9	62	64	11	45	8.3	9	3.12
R_3P_2	62	49	22	54	8.4	10	4.80
R_3P_6	62	70	16	46	8.5	14	3.42
R_3P_9	64	18	27	44	***	58	4.66
Mean	58.2	43.3	11.9	44.1	8.5	27.1	7.2
Std. dev.	5.8	23.3	8.3	8.6	1.9	20.7	3.7
r values between chromosome number and morphological characters.		0.32	0.73*	0.62	-0.17	-0.15	-0.51

c. SES 231

Plant No.	Chromosome No. 2n	Plant height (cm)	Tiller No.	Stalk diameter (mm)	Refractometer solids (%)	Leaf angle (degrees)	Lamina midrib ratio
R_1P_2	80	15	20	11	7.0	13	4.48
R_1P_3	80	33	12	84	12.8	3	3.65
R_1P_6	64	21	17	111	9.8	6	3.51
R_1P_7	72	26	7	105	9.7	1	3.33
R_1P_8	80	10	10	99	11.7	11	2.29
R_1P_9	72	23	18	85	7.4	4	2.96
R_4P_3	78	14	14	36	***	6	3.62
R_4P_7	74	14	14	44	6.9	2	2.90
R_4P_8	76	35	8	28	***	1	3.60
R_4P_9	82	14	9	30	8.6	1	3.30
Mean	76	20.5	12.9	63.3	9.2	4.8	3.4
Std. dev.	5.5	8.6	4.5	37.1	2.2	4.3	0.6
r values between chromosome numbers and morphological characters.		-0.28	-0.26	-0.56	0.16	0.19	0.08

d. Aegyptiacum

Plant No.	Chromosome No. 2n	Plant height (cm)	Tiller No.	Stalk diameter (mm)	Refractometer solids (%)	Leaf angle (degrees)	Lamina midrib ratio
R_1P_1	62	21	14	74	10.6	6	3.99
R_1P_2	62	13	16	70	11.2	5	4.64
R_1P_5	86	70	28	49	10.6	8	3.30
R_1P_6	64	18	21	48	7.3	21	4.92
R_1P_9	74	24	3	60	10.9	14	6.09
R_2P_5	60	39	65	68	10.6	6	4.63
R_2P_7	74	22	31	50	10.8	15	7.00
R_3P_2	92	20	31	60	10.2	7	4.04
Mean	72	28.4	26.1	59.9	10.3	10.3	4.8
Std. dev.	12.0	18.4	18.4	10.2	1.2	5.8	1.2
r values between chromosome numbers and morphological characters.		0.37	-0.07	-0.48	0.12	-0.04	0.18

e. Djatiroto

Plant No.	Chromosome No. 2n	Plant height (cm)	Tiller No.	Stalk diameter (mm)	Refractometer solids (%)	Leaf angle (degrees)	Lamina midrib ratio
R_1P_1	78	42	3	89	9.3	4	4.75
R_1P_2	94	19	1	**	**	**	**
R_1P_4	90	62	7	87	11.13	4	4.17
R_1P_6	114	24	2	44	11.23	15	4.75
R_3P_3	100	39	3	30	***	12	7.00
R_3P_5	98	27	4	**	**	**	**
R_4P_3	90	28	8	65	10.64	6	8.91
R_4P_7	86	15	10	100	10.80	10	6.50
R_4P_8	86	17	7	96	10.65	9	9.22
R_4P_9	80	27	13	76	10.61	4	7.02
Mean	92	30	5.8	73.4	10.6	8.0	6.5
Std. dev.	10.6	14.2	3.9	25.3	0.6	4.2	1.9
r values between chromosome numbers and morphological characters.		0.09	0.58	0.75*	0.65	0.83*	0.21

* Significant at 0.05 level.
** Died.
*** Data unable to be processed due to insufficient number of tillers.

LITERATURE CITED

Ahloowalia, B. S. 1965. A root tip squash technique for screening chromosome number in Lolium. Euphytica 14:170-172.

Ahloowalia, B. S. 1982. Personal communication.

Alexander, M. P. 1965. Chromosome elimination en block in Saccharum. Proc. ISSCT XII:871-874.

Bhaduri, P. N. and Sharma, A. K. 1946. Cytogenetics of Datura fastuosa L. Bull. Torry Bot. Club B. 73:438-458.

Brandes, E. W. 1950. Changes in seasonal growth gradients in geographically displaced sugarcane. Proc. ISSCT VII:1-32.

Bremer, G. 1923. A cytological investigation of some species and species-hybrids within the genus Saccharum. Genetica 5:97-148.

Bremer, G. 1925. The cytology of the sugarcane. III. The chromosome numbers of primitive forms of the genus Saccharum. Genetica 7:293-322.

Bremer, G. 1929. Short remarks on cytology of Saccharum. Proc. ISSCT III:403-408.

Bremer, G. 1951. Problem in breeding and cytology of sugarcane. Euphytica 10:59-78.

Britton, D. M. and Hull, J. W. 1957. Mitotic instability in Rubus. J. Heredity 48:11-20.

Brown, M. S. 1950. Cotton from Bikini. J. Heredity 41:115-121.

Cooper, L. S., Cooper, D. C., Hildebrandt, A. C., and Riker, A. J. 1964. Chromosome numbers in single cell clones of tobacco tissue. Amer. J. Bot. 31:284-290.

Daniels, J., Smith, P., Paton, N., and Roach, B. T. 1975. 2n=80 S. spontaneum and the origin of sugarcane. ISSCT Sugarcane Breeders' Newsletter 35:19-20.

Darlington, C. D. and Janaki Ammal, E. K. 1945. Chromosome Atlas of Cultivated Plants. George Allen and Unwin Ltd. London. 397 pp.

Dillewijn, C. 1952. Botany of sugarcane. The Chronica Botanica Co., Book Dept. Waltham, Mass, U.S.A.

Dutt, N. L. and Krishnaswamy, M. K. 1943. Protogyny in Uganda spontaneum [Saccharum spontaneum]. Curr. Sci. 12:24-26.

Dutt, N. L. and Subba Rao, K. S. 1933. Observations on the cytology of sugarcane. Ind. J. Agric. Sci. 3:37-56.

Emsweller, S. 1949. Polyploidy in Lolium longiflorum. Amer. J. Bot. 36:135-144.

Ethirajan, A. S., Santhakumariamma, P. N., and Velayutham, N. 1977. Studies on successive inbred generations of selected sugarcane cultivars. Proc. ISSCT XVI:269-274.

Fernandez, A. M. F., Cristobal de Hinozo, M. E., and Lozzia de Canelada, M. E. 1978. Citogenetica De La Cana De Azucar. Lilloa XXXI(1) 23-30.

Frankhauser, G. 1945. The effect of changes in chromosome numbers on amphibian development. Quart. Rev. Biol. 2:20-78.

Grassl, C. O. 1974. The origin of sugarcane. ISSCT Sugarcane Breeders' Newsletter 34:10-18.

Grassl, C. O. 1977. The origin of the sugar producing cultivars of Saccharum. ISSCT Sugarcane Breeders' Newsletter 39:8-33.

Heinz, D. J. 1965. Wild Saccharum species for breeding in Hawaii. Proc. ISSCT XII:1021-1026.

Heinz, D. J and Mee, G. W. P. 1971. Morphologic, cytogenetic and enzymatic variation in Saccharum species hybrid clones derived from callus tissue. Amer. J. Bot. 58(3):257-262.

Hole, R. S. 1911. On some Indian forest grasses and their ecology. Ind. For. Mem. Forest Bot. Series, I, Part 1.

Huskins, C. L. and Cheng, K. C. 1949. Spontaneous and induced variation and mitosis. J. Heredity 40:3-6.

Huskins, C. L. and Cheng, K. C. 1950. Segregation and reduction in somatic tissue. IV. Reductional groupings induced in Allium cepa by low temperature. J. Heredity 41:13-18.

Irvine, J. E. 1977. Identification of cold tolerance in Saccharum and related genera through refrigerated freeze screening. Proc. ISSCT XVI:147-156.

Janaki Ammal, E. K. 1936. Cytogenetic analysis of Saccharum spontaneum L. I. Chromosome studies in some Indian forms. Ind. J. Agric. Sci. 6:1-8.

Jegathesan, D. and Sreenivasan, T. V. 1967. Cytogenetical studies in Narenga porphyrocoma. I. Study of karyotype and abnormalities in meiosis. Cytologia 32:11-18.

Kandasami, P. A. 1961. Interspecific and intergeneric hybrids of Saccharum spontaneum L. I. Functioning of gametes. Cytologia 26:117-123.

Kandasami, P. A. 1977. Cytogenetical studies of interspecific and intraspecific hybrids of Saccharum. Proc. ISSCT XVI:251-267.

Krishnamurthi, M. and Tlaskal, J. 1974. Fiji disease resistant Saccharum officinarum var. Pindar sub-clones from tissue cultures. Proc. ISSCT XV:130-137.

Li, H. W., Loh, C. S., and Lee, C. L. 1948. Cytological studies on sugarcane and its relatives. I. Hybrids between Saccharum officinarum, Miscanthus japonicus, and Saccharum spontaneum. Acad. Sinica. Bot. Bull. 2:147-160.

Li, H. W. and Ma, T. H. 1951. List of the chromosomal number of sugarcane varieties and sugarcane relative plants. Rept. Taiwan. Sug. Expt. Stn. No. 7.

Makino, S. 1957. The chromosome cytology of the ascites tumors of rats, with special reference to the concept of the stemline cell. Int. Rev. Cytol. 6:25-84.

Malik, C. P. and Thomas, P. T. 1966. Chromosomal polymorphism in Festuca arundinacea. Chromosoma (Berl.) 18:1-18.

Mallik, A. K. 1950. A preliminary study of drought resistance in sugarcane. Ind. J. Agric. Sci. 29:143-146.

Mehta, P. N. and Sood, O. P. 1974. Floating Chromosomal Populations in Saccharum spontaneum L. Cytologia 39:681-696.

Menzel, M. Y. 1952. Spontaneous somatic reduction in chromosome number in polygenomic Gossypium hybrid. Tenn. Acad. Sci. J. 27:212-213.

Menzel, M. Y. and Brown, M. S. 1952. Polygenomic hybrids in Gossypium. II. Mosaic formation and somatic reduction. Amer. J. Bot. 39:59-69.

Mukherjee, S. K. 1957. Origin and distribution of Saccharum. Bot. Gaz. 119:55-61.

Muntzing, A. 1951. Cytogenetic properties and practical value of tetraploid rye. Hereditas 37:17-84.

Nair, M. K. 1972a. Cytogenetics of Saccharum officinarum L., Saccharum spontaneum L. and S. officinarum x S. spontaneum Hybrids. I. Chromosome mosaics. Cytologia 37:565-575.

Nair, M. K. 1972b. Cytogenetics of Saccharum. III. Karyotype analysis and meiosis in S. spontaneum. The Nucleus 15:107-117.

Nair, M. K. 1973. Cytogenetics of Saccharum officinarum L., Saccharum spontaneum L. and S. officinarum x S. spontaneum Hybrids. I. The probable origin of 'atypical noble canes.' Cytologia 38:25-43.

Nakamura, C., Keller, W. A., and Fedak, G. 1981. In vitro propagation and chromosome doubling of a Triticum crassum x Hordeum vulgare intergeneric hybrid. Theor. Appl. Genet. 60:89-96.

Naranaswamy, S. 1940. Megasporogenesis and origin of triploids in Saccharum. Ind. J. Agric. Sci. 10:534-551.

Nielsen, E. L. and Nath, J. 1961. Somatic instability in derivatives from Agroelymus turneri resembling Agropyron repeus. Amer. J. Bot. 48(4):345-349.

Nishiyama, I. 1956. Basic numbers in the polyploidy of Saccharum. J. Heredity 47:91-99.

Nishiyama, I. and Taira, T. 1966. The effects of kinetin and indoleacetic acid on callus growth and organ formation in two species of Nicotiana. Jap. J. Genet. 41:357-365.

Panje, R. R. and Ethirajan, A. S. 1959. Studiens in Saccharum spontaneum. Preliminary studies in inbreeding. Proc. ISSCT X:751-754.

Panje, R. R. and Babu, C. N. 1960. Studies in Saccharum spontaneum. Distribution and geographical association of chromosome numbers. Cytologia 25(2):152-172.

Panje, R. R. 1971. The role of Saccharum spontaneum in sugarcane breeding. Proc. ISSCT XIV:217-223.

Partanen, C. R. 1963. Plant tissue culture in relation to developmental cytology. Int. Rev. Cytol. 15:215-243.

Parthasarathy, N. 1948. Origin of noble canes (Saccharum officinarum L.). Nature 161:608.

Price, S. 1957. Cytological studies in Saccharum and allied genera. III. Chromosome numbers in interspecific hybrids. Bot. Gaz. 118:146-159.

Price, S. 1963. Cytogenetics of modern sugarcanes. Eco. Bot. 17:97-106.

Price, S. 1968. Chromosome numbers in miscellaneous clones of Saccharum and allied genera. Proc. ISSCT XIII:921-926.

Raghavan, T. S. 1951. The sugarcanes of India. Some cytogenetic considerations. J. Heredity 42:199-206.

Raghavan, T. S. 1952. Ann. Rept. of the Second Cane Breeding Officer. Sugarcane Breeding Institute. Coimbatore.

Raghavan, T. S. 1953. Cytogenetics of sugarcane. Ind. J. Agric. Sci. 22:93-104.

Raghavan, T. S. 1954. Cyto-genetics in relation to sugarcane breeding. Cytologia 19:133-143.

Rao, J. T. and Babu, C. N. 1955. Chromosome numbers in Saccharum spontaneum. Curr. Sci. 24:53-54.

Roach, B. T. 1977. Utilization of Saccharum spontaneum in sugarcane breeding. Proc. ISSCT XVI:43-58.

Rees, H. and Thompson, B. 1955. Localization of chromosome breakage at meiosis. Heredity 9:359-407.

Sachs, L. 1952. Chromosome mosaics in experimental amphiploids in the Triticinae. Heredity 6:157-170.

Sankanarayanan, P. 1977. Utilization of wild germplasm Saccharum spontaneum in breeding. Proc. ISSCT XVI:233-238.

Shahre, M. L. and Shastry, S. V. S. 1963. Meiosis in garden roses. Chromosoma (Berl.)13:702-770.

Sharma, A. K. 1956. A new concept of a means of specialism in plants. Caryologia 9:93-130.

Sharma, A. K. and Sharma, A. 1959. Chromosomal alternations in relation to speciation. Bot. Rev. 25:514-544.

Shull, G. H. 1910. Hybridization methods in corn breeding. Am. Breeders Magazine 1:98-107.

Shung, J. Y. 1965. Numerical chromosome instability in Nicotiana hybrids. II. Intraplant variation. Canadian J. Genet. Cytol. 7:112-119.

Stevenson, G. L. 1953. The use of selfing and inbreeding with sugarcane. Proc. ISSCT VIII:509-519.

Stevenson, G. L. 1959. Inbreeding with sugarcane in Barbados. Proc. ISSCT X:670-681.

Stevenson, G. L. 1965. Genetics and Breeding of sugarcane. Longmans, Green and Co., Ltd., London. 248 pp.

Steudel, E. G. 1855. Synopsis plantarum glumacearum 1:405-409.

Snoad, B. 1955. Somatic instability of chromosome numbers in Hymenocallis calathinum. Heredity 9:129-134.

Subba Rao, K. S. 1947. Studies in sugarcane cytogenetics and floral characteristics. (Msc. Thesis. Assoc, IARI).

Subba Rao, K. S., Batcha, M. B. G., and Sankanarayanan, P. 1960. Proc. 4th All Ind. Conf. Sug. Res. and Dev. Workers.

Tanimoto, T. 1964. The press method of cane analysis. The Hawaiian Planters' Record 57:133-150.

Thakur, R. 1978. Studies in a large clonal population of haploid rice. Riso 27(2):121-126.

Wilson, G. B. and Cheng, K. C. 1949. Spontaneum and induced variation in mitosis. J. Heredity 40:3-6.

Wu, K. K., Heinz, D. J, Meyer, H. K., and Ladd, S. L. 1977. Selection studies in sugarcane (Saccharum sp. hybrids). III. A method to determine sample size for the estimation of population variance. Theor. Appl. Genet. 51:29-33.

Wu, K. K., Heinz, D. J, Meyer, H. K., and Ladd, S. L. 1978. Minimum sample size for estimating progeny mean and variance. Crop Science 18:57-62.