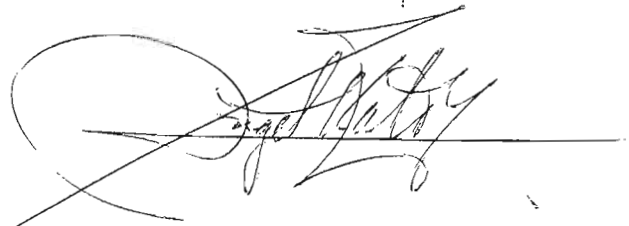


Para el Dr. E. J. Wellhausen
con todo el respeto y la
estimación del autor.



México, D.F. Octubre 8, 1975.

CYTOLOGICAL STUDIES OF MAIZE [ZEA MAYS L.] AND TEOSINTE
[ZEA MEXICANA (SCHRÄDER) KUNTZE] IN RELATION TO THEIR
ORIGIN AND EVOLUTION.

A Dissertation Presented

By

TAKEO ANGEL KATO YAMAKAKE

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

DOCTOR OF PHILOSOPHY

May 1975

Plant and Soil Sciences

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Approved as to style and content by:

Walton C. Galinat

Dr. Walton C. Galinat, Chairman of Committee

William H. Lachman

Dr. William H. Lachman, Member

Carl P. Swanson

Dr. Carl P. Swanson, Member

Franklin W. Southwick

Dr. Franklin W. Southwick, Head
Department of Plant and Soil Sciences

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Cytological Studies of Maize [Zea mays L.] and Teosinte
[Zea Mexicana (Schrader) Kuntze] in Relation to their
Origin and Evolution. [May 1975]

Takeo Angel Kato Yamakake, B.S., Escuela Nacional de Agricultura, Mexico.

M.S., North Carolina State University

Directed by: Dr. Walton C. Galinat

A B S T R A C T

Previous extensive analysis of chromosome knob distributions in maize suggested that similar studies in both teosinte and maize might provide further insights into their origin and evolution.

A comparative study of teosinte and maize pachytene chromosome morphology was undertaken from three different aspects: 1] the basic characteristics of length, arm ratio, chromomere distribution and knob position and size; 2] the knob frequency distribution in teosinte collections from its whole distribution range in Mexico and Guatemala, and maize collections from central Mexico; and 3] the presence of small inversions in teosinte chromosomes from different regions.

The length of corresponding teosinte and maize chromosomes is variable, but no differences exist at the species level. However, length differences may exist among races. The arm ratio and chromomere distribution are similar in both genomes. Various knob types may or may not be found at each of many fixed positions on the chromosomes. More knobbed positions were found in teosinte than in maize chromosomes. Mexican teosinte having mostly intercalary knobs is more similar to maize than Guatemalan teosinte, possessing only terminal knobs. These results confirm and substantiate

other studies that both species possess the same basic genome. Consequently, all existing teosintes are derivatives of a common ancestor and maize must have originated from a Mexican type of teosinte population complex.

Knobs at different positions on the chromosomes of teosinte and maize populations are not distributed at random, but populations having various unique knob combinations are found at the regional or racial level. In many cases, these knob combinations are sharply distinct in the races from adjacent regions. In some chromosomes, or chromosome arms possessing more than one knob position, their knobs tend to appear in different regions or races. These results indicate that: 1] the several knob types at different positions possess various adaptive values, so that different selection pressures can act upon them bringing about the non-random distribution observed; 2] knobs at different positions on the same chromosome have either or both compensatory or complementary effects; and 3] no evidence exists of a knob shifting mechanism in teosinte and maize chromosomes, so the existing populations are derivatives from a common ancestral population complex which had all of the knobs known at present.

Many knobs that exist in high frequencies in teosinte are absent in sympatric and hybridizing maize populations. Some of these knobs, however, are present in high frequencies in maize from regions where no teosinte exists at present. These facts indicate that at least the segments carrying these knobs do not introgress from teosinte into maize, strengthening the idea that knobs have different adaptive values.

Several inversions already reported in the literature and two new ones, causing a low frequency of pollen abortion, were found in teosinte

plants. The same inversions are present in widely separated populations without any geographical connection between them, a fact that supports further the idea that all teosintes were derived from a common population complex.

The known naturally occurring inversions in teosinte and maize populations are knobless or small knobbed, and usually located at or near known knob positions. Therefore, they might have different adaptive values and be knob substitutes for accomplishing the same role in evolution.

Based on the fact that knobs reduce the recombination rate in the segments carrying them, the possibility is proposed and discussed that knobs favor the development of supergenes, acquiring in this way various adaptive values.

That the process of genetic drift is not adequate for explaining all the results obtained in the present studies is also discussed.

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CHAPTER I

INTRODUCTION

General Antecedents

At present the origin of both maize and teosinte is still controversial. There are now two main currents of thinking prevailing in this respect. One theory considers that modern maize is a domesticated descendant of a now extinct wild maize which was also a progenitor of teosinte [Mangelsdorf, 1974]. The second theory, on the contrary, postulates that teosinte is the wild ancestor from which modern maize originated during domestication [Galinat, 1971, 1974a; Iltis, 1961; deWet and Harlan, 1972; Beadle, 1972]. At any rate both theories accept that among the members of the Maydeae teosinte is the closest relative of maize. Although many cytological considerations have been given for reaching the conclusion on the close relationship between maize and teosinte, the main foundations of both theories on the origin of these plant species are largely based on morphological grounds [Galinat, 1971, 1974a; Beadle, 1972; Mangelsdorf, 1974].

Chromosome knobs are heterochromatic structures of variable size found at fixed positions on the chromosomes of Zea, Tripsacum, and other members of the Gramineae. These structures of maize have been used in diverse ways, as chromosomal markers in cytogenetic studies of chromosomal aberrations, in investigations regarding the association between knobs and morphological and physiological characters, in the form of average numbers have been used in studies of racial characterization and relationships, etc. However, these studies have not contributed much to-

ward the clarification of the problem concerning the origin and evolutionary relationships of maize and teosinte.

The preliminary studies of McClintock [1959, 1960] on chromosome knob constitutions and their geographical distribution in several selected races of maize in Latin America suggested that the knobs did not occur at random but they followed specific distribution patterns and she recommended an extensive study of the American races of maize. This task was first undertaken by Longley and Kato [1965] but unfortunately the data obtained were analyzed in terms of average knob numbers instead of knob positions. Recently, more extensive studies on chromosome morphology of maize populations from all over the Americas have resulted in the main conclusion that present maize populations are the result of admixtures of different original maize germplasms. These germplasms came from several localities by independent domestication from the wild ancestor which in turn was probably already differentiated into several distinct racial types [Kato and Blumenschein, 1967; McClintock, Blumenschein and Kato, unpublished].

So far, the chromosome morphology studies on teosinte are still limited and incomplete [Longley, 1937; Ting, 1958a, 1964]. This has been especially due to the fact that extensive collections from populations of different geographical regions have not been made until recently [Wilkes, 1967, 1972b].

With these antecedents in mind, it has been thought that the collection of chromosome knob data from teosinte populations on a larger scale eventually could lead into more insights into the problem of the origin and further evolution of maize and teosinte. Therefore an investigation

has been undertaken of the chromosome morphology of maize and teosinte with the following objectives.

Objectives

1. To obtain further knowledge on the chromosome morphology of maize populations from regions where teosinte is also growing.
2. To obtain information on the chromosome morphology of teosinte populations from all the regions where it grows in the wild.
3. To make a comparative study of teosinte and maize with the information gathered in points 1 and 2. As a result, new insights may be gained on the problem of the origin and further evolutionary relationships between maize and teosinte populations.

Significance

The main purpose of this research is to shed light on the problem of the origin and evolution of maize and teosinte, the closest relatives among the members of the Maydeae. The importance of clarifying the origin of these plants resides not only in the academic value, but also in the fact that this is basic in understanding the genetic mechanisms that were responsible in transforming one species into the other. The more that is understood about the genetic mechanisms, the greater the opportunity to manipulate intelligently these plant species for man's benefit.

It is well known that maize is one of the world's most important food plants. Although genetic variability in maize is still available in many countries and this is being used in breeding new superior varieties of maize, the breeder eventually will have to look to its relatives for new kinds of genetic variability to solve specific problems or for trying new

ways of improving maize varieties. Logically, he will find the use of the closest relative, teosinte, most promising. Here is where the knowledge gained on the genetic mechanisms that operated during the divergence and continued separation of the two species will find practical application. The more knowledge that is available when it is needed, the faster man can solve his problems.

CHAPTER I I

REVIEW OF LITERATURE

Morphology and Taxonomy

General morphology. The general descriptive morphology of the maize and teosinte is now universally accepted without any objections. However, there have been differences in opinion about the morphological and taxonomic relationships in maize and its relatives.

The general morphology of maize has been described in much detail by Arber [1934], Anderson [1944a], Anderson and Brown [1948], Anderson and Cutler [1942], Bonnett [1948, 1953, 1954], Cutler and Cutler [1948], Kieselbach [1949], Sass [1955], Weatherwax [1916, 1917, 1918, 1935 and 1955], and others.

The stalk consists of a series of nodes separated by internodes of varying lengths. Each node bears a leaf, which consists of a sheath and a blade. The leaves at successive nodes usually have an alternate and distichous arrangement. Each leaf subtends an axillary bud enclosed in a prophyll. The axillary buds located at the lower end of the main stem may develop into tillers or lateral vegetative branches with a morphological structure similar to that of the main axis. On the other hand, the buds borne at upper nodes have short internodes and modified sheaths, the husks, that enclose a terminal female or pistillate inflorescence [the ear]. The main axis is terminated by the male or staminate inflorescence [the tassel].

The male inflorescence usually is formed by a central axis and several spreading lateral branches, which may have secondary and tertiary

branches. On this basic framework the inflorescence is organized into spikelets which are arranged in pairs, one member being sessile and the other pedicellate. The upper portion of the central axis, the central spike, shows a polystichous arrangement of the pairs of spikelets, while the lateral branches usually have a distichous arrangement. Each spikelet consists of two functional male florets all enclosed in two empty or sterile glumes. Each of the two florets of a spikelet is enclosed by two floral bracts, the lemma and the palea, enclosing three stamens, two lodicules and a rudiment of a pistil.

The female inflorescence, commonly called the ear, consists of pistillate spikelets borne in pairs forming many longitudinal rows on a thick axis, commonly called the cob of the ear. Each row of paired spikelets usually forms only two rows of kernels [caryopses] in the mature ear. This is so because only the upper flower of each spikelet is usually functional, the other remaining suppressed. The rachilla elongates to elevate the kernel above the empty glumes. The kernel enlarges and, thereby, emerges from its lemma and palea. Both pistillate spikelets may appear to be sessile because the pedicels may be short and fused into the cupule floor. The pistil of the female flower consists of a fused tricarpelar ovary, two of the carpels giving rise to two long styles or 'silks' which fuse except at the tip. The ovary contains a single campilotropous ovule with two integuments. Rudimentary stamens are present.

The vegetative morphology of the teosinte plant has been found to be basically similar to that described for the maize plant [Mangelsdorf, 1974; Mangelsdorf and Reeves, 1939; Weatherwax 1935, 1955; Wilkes, 1967.] The main differences between teosinte and maize are found in the inflor-

escences, especially the female inflorescences. The male inflorescence of teosinte has the same basic structure as that of maize with the exception that the former plant does not have a polystichously arranged central spike. While it is claimed by Wilkes [1967] that the Guatemalan teosintes do not possess a central spike in their tassels, one appears to be present in this teosinte when grown in our cultures at Waltham, Mass. The Mexican teosintes show conspicuous central spikes, and contrary to the maize central spike, always have a distichous arrangement of their spikelet pairs. The female inflorescences consist of two rows of single pistillate spikelets borne in alternately arranged cupulate rachis segments. Each female spike is enclosed in a husk. In each cupule only a single spikelet develops to maturity, the other spikelet of the pair remains as a "microscopic rudiment" on one side of the fruit case [Weatherwax, 1918, 1935, 1955].

A quantitative variation of different morphological traits in maize has been given in a series of publications describing the hundreds of races of maize found in Latin America [Wellhausen et al. 1952, 1958; Roberts et al. 1957; Hatheway, 1957; Brieger et al. 1958; Brown, 1960, Ramirez et al. 1960; Grobman et al. 1961; Timothy et al. 1961, 1963; and Grant et al. 1963]. Similar information for the races of teosinte has been given by Wilkes [1967].

Homology and origin of the inflorescences. The central spike of the tassel and ear of maize, having a polystichous arrangement of the spikelet pairs, uniquely deviate from the basic distichous structural pattern of the Gramineae. This situation was realized by Wigand as early as 1854 and since that time the finding of an explanation to the problem of the

origin of the maize inflorescences, but especially the female inflorescence or the ear, has been the central subject of the more general theme about the origin of the maize plant [Galinat, 1956, 1959, 1963, 1967, 1970, 1971, 1974a; Mangelsdorf, 1945, 1959, 1965, 1974; Mangelsdorf and Reeves, 1939; Weatherwax, 1918, 1935, 1955].

Two aspects have been of importance in the study of the tassel and ear of maize: the homology between the two types of inflorescences and the phylogenetic mechanism for their origin.

That both structures of the inflorescences of maize are homologous to each other has been clearly pointed out in early investigations [Weatherwax, 1935] or in other words as Mangelsdorf [1945] stated "since the appearance of Montgomery's paper [in 1906] the homology of the ear with the central spike of the tassel has not been seriously challenged except, perhaps, by indirection".

Further investigations have provided definitive evidence about the tassel-ear homology and also that this homology is valid between maize and its closest relatives, teosinte and Tripsacum.

That distichy and polystichy are expressed in segregates of maize-teosinte hybrids showing a high correlation between the central spike of the tassel and the ear has been found by Langham [1940].

Clear evidences showing correlations between tassel internode condensation and increase in row number in the ear, tassel branch length and ear length, tassel branch pattern and ear shape, and tertiary branches and irregular rowing in the ear, have been found by Anderson [1944a]. Further studies of Anderson and Brown [1948] on the row numbers in the tassel as well as in the ear, have shown that the homology between the

two inflorescences, with respect to this character, is basically very simple [the row numbers in the ear are correlated with the number of spikelet pairs per whorl in the tassel] and that in those cases where this correspondence is obscure, the cause is found in the action of the phenomenon of condensation or telescoping of successive internodes.

In the progeny of the cross between Guarani maize from Paraguay and a pod corn, Mangelsdorf [1945] has found plants bearing long and stretched ears, some of them with basal branches resembling the tassels. The main difference was that instead of having staminate spikelets, they had pistillate spikelets. He concluded that "there is now no doubt, if there was doubt before, that the ear is the homologue of the central spike of the tassel".

By studying the vascular organization of the central spike of the tassel and the cob of the ear of maize, Laubengayer [1949] and Reeves [1950, 1953] suggested the now obsolete idea that both organs possess two separate vascular systems, one inside the other, and both forming a cylindrical "hollowed" network. Galinat [1959] has shown that the two apparent vascular systems actually are connected in the glume cushions. The innermost vascular bundles are large and supply mainly the spikelets. The outer bundles are small and supply chiefly the rind of the rachis and the outer glume of the spikelets. One of the differences between the vascular system in the central spike of the tassel and that in the cob resides in the amount of central pith tissue surrounded by the larger bundles.

Another approach toward the demonstration of the homology between the tassel and the ear of maize and its relatives has been those studies regarding the internal anatomy and development of the prophyll and the cu-

pule, and applying the concept of the phytomer, the basic structural unit of the plants. Galinat [1956, 1959, 1970] concluded that, to the traditional definition of the phytomer as composed by an internode, a leaf and an axillary bud, should be added a fourth component, the prophyll, and that this component is developed as the cupule or its lining tissue in the ear, as the pulvinus in the axils of the tassel branches, the palea in the staminate and pistillate flowers and the prophylls proper in the axils of lateral branches in the tillers and the lateral ear bearing shoots [shanks].

Through detailed developmental investigations of the vegetative and floral primordia of maize, Bonnett [1948, 1953, 1954] has also established the tassel-ear homology, since he has found that the early stages of the development of the two types of inflorescences essentially do not differ in the component elements formed and the origin and further growth of the primordia of these components. Furthermore, he concluded that any dissimilarity in the morphology of the mature tassel and ear is the product of the differences in action patterns of the later stages of the developmental controlling systems. This conclusion seems to be further supported by the common observation that in the tillers of normal maize, and under certain genetic or environmental conditions, portions or the whole tassel of the main stalk can develop pistillate spikelets and the ear can develop staminate segments or anthers intermixed with pistillate spikelets [Bonnett, 1948; Cutler and Cutler, 1948; Mangelsdorf, 1945, 1974; Mangelsdorf and Reeves, 1939; Reeves, 1950, 1953; Weatherwax, 1918, 1935, 1955].

Although the above mentioned studies have definitely established the homology between the tassel and the ear, this homology does not give any explanation about the origin of the inflorescences of maize as such.

Therefore, parallel to the studies regarding tassel-ear homology, several hypotheses on the probable origin of these inflorescences, especially of the ear, have been proposed [Galinat, 1963, 1967, 1970, 1974a; Mangelsdorf, 1945, 1948, 1974; Mangelsdorf and Reeves, 1939; Weatherwax, 1918, 1935, 1955].

The early hypotheses have been reviewed by Weatherwax [1918, 1935], Mangelsdorf [1945] and Mangelsdorf and Reeves [1939]. These are three: [1] the fusion hypothesis; [2] hypothesis of the twisting of a two-ranked spike, and [3] the hypothesis of the contraction or reduction of branches.

1. The fusion hypothesis was first proposed by Hackel [1890] and regards the ear of maize as formed by double rows of kernels, each row corresponding to a single spike of teosinte.

The appearance of branched ears with some frequency in populations of maize has been one of the major evidences supporting this hypothesis. However, it is impossible to obtain ears with ten, fourteen, or eighteen rows if these are the result of the fusion of distichous spikes, since each of the latter would contribute four rows. Therefore, the fusion theory presents "mathematical inconsistencies" as concluded by Weatherwax [1918, 1935].

When a variety of maize from the Guarani Indians of Paraguay is crossed to pod corn and backcrossed to Guarani maize, the progeny plants produced very elongated and stretched ears with the terminal portions resembling the central spike of the tassel. In some cases these ears possessed basal branches. Mangelsdorf [1945] studying these ears interpreted them as ears corresponding to an association between a central spike and upper lateral branches of the tassel. He concluded that this evidence

completely invalidates the fusion hypothesis.

Frequently central spikes of tassels bear segments with an ear-like structure. Since the staminate and the pistillate portions are on different parts of the same axis, there would be difficulty to explain this fact by accepting the fusion theory [Reeves, 1950, 1953]. Also Reeves [1953] by studying branched ears and stems and assuming that "the maize plant with tillers has the same relation to the plant without tillers as the ear with monopodial branches has to the unbranched ear", concluded that the branched ears "are no better evidence for fusion in the ear than branched culms are for fusion in the culm".

2. The second hypothesis was advanced by Collins [1919] after his studies of pistillate spikes of an F_2 progeny of a maize x teosinte hybrid. In these spikes he found a series of transitional forms between the teosinte spike with the typical ear of maize. The steps are: a] the suppressed pistillate spikelet becomes functional; b] the axis of the rachis segments shorten and become more numerous and crowded; c] adjacent rachis segments are positioned at the same plane and unite or become "yoked" in pairs; and d] the axis of the spike twists so that the "yoked" rachis segments become perpendicular from each other forming a four-rowed ear and further twisting of the axis will form ears with higher ranking. His main evidence of "yoking" in the ear of maize was that the dropping of rows always occurred on opposite sides of the ear and not from two adjacent rows.

This hypothesis has been objected mainly by Weatherwax [1935] who found no evidence of "yoking" of spikelet pairs on opposite sides of the rachis in the ears of maize. He showed that the dropping of rows is al-

ways by pairs of adjacent rows and not by two rows from opposite sides of the rachis as stated by Collins [1919]. Also Mangelsdorf [1945] stated that probably the yoking has not occurred in the origin of the maize ear simply because the spikelets on opposite sides of the rachis behave differently from each other.

3. The hypothesis of the reduction of branches was first advanced by Collins [1912] who proposed that the ear of maize originally was a panicle, the branches of which were shortened until each of them were represented only by a pair of spikelets. Descriptions of the ramosa mutant in maize by Weatherwax [1918] has given further support to this hypothesis. However, Weatherwax [1935] indicated that this hypothesis explains satisfactorily the origin of spikelet pairs, but still fails in explaining the origin of polystichy of the ear of maize. He proposes, then, an alternate interpretation based on the theory of spiral phyllotaxy. However, Mangelsdorf [1945] objects to this interpretation because it is only descriptive and not explanatory of the distichous and polystichous conditions. Anderson [1944] further modified the concept of branch reduction of Collins by suggesting that a whorl of spikelets resulted from the condensation of the secondary branches down onto the primary axis.

In his studies of the South American Guarani maize crossed with pod corn as mentioned above, Mangelsdorf [1945] concluded that in all cases the ears of maize represent compact inflorescences, indicating that in the origin of the ear this characteristic, compaction or shortening of internodes, has been very important.

Reeves [1950, 1953] by showing a series of tassels with different

degrees of branch reduction, interpreted them as representing the probable steps of the ear evolution from a paniculate type of inflorescence. Based on these observations he supported the idea that the "unbranched ear of maize is the immediate result of the condensation of a panicle with many polystichously arranged branches". He also concluded that the origin of the polystichy had in its first stages a decrease in length and later elimination of internodes.

The condensation index or the average number of spikelet pairs per apparent node was devised by Anderson [1944a]. By using this index in correlation studies between tassel condensation and row number of the ear, Anderson and Brown [1948] concluded that the tassel is "fundamentally distichous throughout" with the upper part or central spike having branches reduced to whorls of paired spikelets and with an intermediate portion of the tassel with partially reduced branches. This tassel organization has been interpreted by them as the result of a gradual action, from base to tip of the tassel, of the phenomenon of condensation or telescoping of successive internodes. This process also operates in the same way in the ear and those varieties of maize with very compact ears have accumulated several modifier genes during domestication which permitted condensation of the ear without fasciation.

Once the importance of the concept of compaction, condensation or telescoping of internodes in the evolutionary development of the inflorescences of maize was realized and well established, the hypothesis of the reduction of branches became modified in its basic proposition of a lateral reduction of branches by a concept of vertical reduction of the axis and the way was opened toward a better understanding of the evolu-

tionary steps for the development of the tassel and the ear of present maize. Also a new concept is introduced into the hypothesis, the differential action of several sets of genetic factors on similar primordia for developing morphologically different but homologous organs.

In a series of studies regarding the comparative developmental morphology of floral parts of maize and its relatives and using the phytomer concept, Galinat [1956, 1957, 1959, 1963, 1969, 1970, 1971, and 1974a] has been able to develop an explanation of the most probable evolutionary steps by which the ear of modern maize was originated under domestication from its wild ancestor, teosinte; the basic sequence of his developments are as follows:

1. The cupulate rachis segment has evolved in several ways in different genera and species, in some cases as a structural device for protecting the caryopses and in other cases only as a structural support for the caryopses. This evolution of the cupule is seen "in a graded series of species starting with the *Andropogoneae* and terminating with the *American Maydeae*" [Galinat, 1956].

2. The morphological organization of maize and its relatives is better understood as a structure composed of a fundamental set of organs, the phytomer, which can be variously developed through the action of different genetic factors in the different species and also in space and time within a given species, but always maintaining their respective homology [Galinat, 1957, 1963, 1969].

3. In the domestication of maize from its wild ancestor, it could have been selected during the accumulation of genes affecting condensation and differentiation of the staminate and pistillate inflorescences. In

this way a plant type with a very compact, unbranched, many rowed ear covered by husks and a less compact, flexible and branched tassel, could have originated [Galinat, 1969, 1970, 1971, 1974a].

4. The ears as found in modern maize have been evolved from an ancestral spike like that of present teosinte. This conclusion has been reached by a comparative study of the cupule morphology of modern teosinte, maize and their hybrid progeny and also of archaeological maize remains. It was shown that in this material a series of cupule shapes are found intergrading between two extremes, teosinte and modern maize, which represent a part of the postulated evolutionary sequence [Galinat, 1970, 1971, 1974a].

Taxonomy. Maize, Zea mays L., and teosinte Zea mexicana [Schröder] Kuntze and Zea perennis [Hitchcock] Reeves and Mangelsdorf, together with the genus Tripsacum, form the American Maydeae of the family Gramineae. The other members of this tribe, Coix, Polytoxa, Chionacne, Trilobachne, and Schlerachne, are all Asiatic [Hackel, 1890; Hitchcock, 1922, 1930, 1951; Reeves and Mangelsdorf, 1942; Wilkes, 1967; Mangelsdorf, 1974].

The name of maize, Zea mays L., has been maintained unchanged since Linneus described and named it in 1753. Teosinte, which was described by Schröder in 1832 for the first time under the name of Euchlaena mexicana, contrary to maize has had several names since different authors, by describing different specimens introduced into Europe from Mexico and Guatemala, have given different botanical names [e.g. Reana giovanninii Brignolia; Reana luxurians Durien; Reana angustifolia Durien; Euchlaena luxurians Durien et Ascherson; Euchlaena bourgaei Fournier]. Furthermore, during the botanical history of maize and teosinte, both were placed under different tribes of the grass family [e.g. Zeineae, Paniceae, Phalarideae,