

Genetic Diversity, Cytology, and Systematic and Phylogenetic Studies in Zingiberaceae

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ABSTRACT

Members of the Zingiberaceae, one of the largest families of the plant kingdom, are major contributors to the undergrowth of the tropical rain and monsoon forests, mostly in Asia. They are also the most commonly used gingers, of which the genera *Alpinia*, *Amomum*, *Curcuma*, and *Zingiber*, followed by *Boesenbergia*, *Kaempferia*, *Elettaria*, *Elettariopsis*, *Etingera*, and *Hedychium* are the most important. Most species are rhizomatous, and their propagation often occurs through rhizomes. The advent of molecular systematics has aided and accelerated phylogenetic studies in Zingiberaceae, which in turn have led to the proposal of a new classification for this family. The floral and reproductive biology of several species remain poorly understood, and only a few studies have examined the breeding systems and pollination mechanisms. Polyploidy, aneuploidy, and structural changes in chromosomes have played an important role in the evolution of the Zingiberaceae. However, information such as the basic, gametic, and diploid chromosome number is known for only a few species. In this review we highlighted the need to undertake concentrated efforts to address poorly known areas and perform phylogenetic studies to achieve a consensus on the number of genera and species in the family; investigate the pollination biology and breeding system in members of this family; conduct cytological studies to acquire reliable information regarding basic, gametic, and somatic chromosome numbers and ploidy levels in different species; and perform genetic diversity studies to determine the genetic base of the existing genetic resources in the Zingiberaceae.

Keywords: *Alpinia*, *Curcuma*, pollination-biology, polyploidy, taxonomy, *Zingiber*

Abbreviations: cpDNA, chloroplast DNA; ITS, internal transcribed spacer; nrDNA, nuclear ribosomal DNA; RAPD, random amplified polymorphic DNA; Rubisco, ribulose biphosphate carboxylase

CONTENTS

INTRODUCTION.....	56
General introduction.....	56
Distribution and habitat specialization.....	57
Mode of propagation.....	57
TAXONOMIC DESCRIPTION.....	57
Controversies regarding the number of genera and species.....	57
General taxonomic features.....	58
SYSTEMATICS AND PHYLOGENETIC STUDIES IN THE ZINGIBERACEAE.....	58
The Evolutionary relationships based on molecular data.....	58
Phylogenetic relationships based on seed characters and isozymes.....	59
FLORAL BIOLOGY AND BREEDING SYSTEMS IN THE ZINGIBERACEAE.....	59
Floral and reproductive biology.....	59
Pollination biology.....	59
CYTOLOGICAL OBSERVATIONS.....	60
Chromosome number.....	60
Polyploidy.....	60
Cytoplasmic inheritance.....	60
GENETIC DIVERSITY.....	60
Molecular markers.....	60
Comparative anatomical features.....	61
CONCLUSIONS AND RECOMMENDATIONS.....	61
ACKNOWLEDGEMENTS.....	61
REFERENCES.....	61

INTRODUCTION

General introduction

Members of the Zingiberaceae, one of the largest families of the plant kingdom, are distributed mainly in tropical Asia.

They not only comprise a prominent fraction of the undergrowth of tropical rain and monsoon forests but are also found, albeit infrequently, in secondary forests (Chen 1989). Members of the Zingiberaceae are usually aromatic in all or at least one of their plant parts. Thus, species belonging to this family are important natural sources of spices, herbal

medicines, natural dyes, perfumes, and multipurpose aesthetic compounds (Siriruga 1999). Some members of the Zingiberaceae yield dyes, spices, perfumes, and medicines, while others are cultivated for their showy flowers (Te-lin and Larsen 2000). The most often used gingers come from the genera *Alpinia*, *Amomum*, *Curcuma*, and *Zingiber*, and to a lesser extent, *Boesenbergia*, *Kaempferia*, *Elettaria*, *Elettariopsis*, *Etilingera*, and *Hedychium*. Among these, three species, *Zingiber officinale*, *Curcuma longa* (= *Curcuma domestica*), and *Elettaria cardamom*, are the most important commercially. Zingiberaceous plants vary in height and size, from gigantic erect leafy shoots, which in some species achieve heights of more than 8 m (e.g., *Etilingera elatior*), to plants as small as 10 cm or at nearly ground level, such as *Kaempferia galanga* (Larsen *et al.* 1999).

Distribution and habitat specialization

Zingiberaceae are scattered over a wide belt of tropical and subtropical regions, primarily in tropical Asia, where the region's long history of a stable, damp, and hot climate together with its wide array of habitats probably favored the development and differentiation of these plants (Chen 1989). Within tropical Asia, the diversity-rich Malesian region, which includes Malaysia, Indonesia, Brunei, Singapore, the Philippines, and Papua New Guinea, contains 24 genera and about 600 species (Larsen *et al.* 1999). The members of this family are distributed pantropically, with one genus (*Renealmia*) in the Neotropics, four (*Aframomum*, *Aulotandra*, *Siphonochilus*, and *Renealmia*) in Africa, and the rest distributed in East Asia and the Pacific islands (Kress *et al.* 2002).

The distribution of the Asian genera into different zones in the tropics is distinguished by their various characteristics, which most likely are the result of evolutionary changes and their adaptation to several climatic, geographic, and topographic conditions. For example, the genus *Roscoea* is found in the Himalayan range and is therefore adapted to the harsh conditions and high elevations of those mountains. Thus, species of *Roscoea* have closed leaf sheaths, a reduced number of rhizomes, and long roots reaching deeply into the soil since the fleshy rhizomes cannot be accommodated on the surface of the rocky soil (Chen 1989). Similarly, *Aframomum* is the largest genus of the African Zingiberaceae (c. 80 species), as well as the largest among

genera representing the understory herbs of the African rain forest. Its range extends from Senegal to Ethiopia in the north and Angola to Madagascar in the south; it is also found on the Gulf of Guinea islands of São Tomé and Príncipe (Harris *et al.* 2000).

Mode of propagation

The biology of the Zingiberaceae is poorly known. With the exception of a few species, the predominant mode of reproduction in the Zingiberaceae is asexual and propagation is often through underground rhizomes. The plants have prostrate or tubular rhizomes that are sympodial and promote the formation and development of axillary buds. Sexual reproduction is largely diminished or even lost with increasing ploidy levels. Moreover, in some species, such as those of the genus *Globba*, the flowers are often replaced by bulbils (Chen 1989). Due to their vegetative mode of reproduction, crop improvement in zingiberaceous plants is confined to clonal selection, mutation breeding, and the induction of polyploidy (Ravinderan *et al.* 2005).

TAXONOMIC DESCRIPTION

Controversies regarding the number of genera and species

Discrepancies pertaining to the number of genera and species in the family Zingiberaceae have been reported. According to different reports Zingiberaceae comprises about 45 genera and 1000 species (Ghazanfar and Smith 1982), 52 genera and 1500 species (Chen 1989), 53 genera and over 1200 species (Kress 1990), and 50 genera and 1300 species (Te-lin and Larsen 2000). This ambiguity occurs because the Zingiberaceae are still in an active stage of evolution (Larsen *et al.* 1999), and the relationships between several newly described species and genera are not yet fully understood (Te-lin and Larsen 2000). Furthermore, nearly one-quarter of the genera in the Zingiberaceae are monospecific (morphologically very distinctive), but criteria that distinguish these species at the generic level have yet to be established (Kress *et al.* 2002). Geographically large areas, such as Sumatra and Borneo on the Malayan Peninsula, are still insufficiently explored, especially with respect to their ginger flora. Thus, many new species will undoubtedly be discovered (Larsen *et al.* 1999). The confusion and contro-

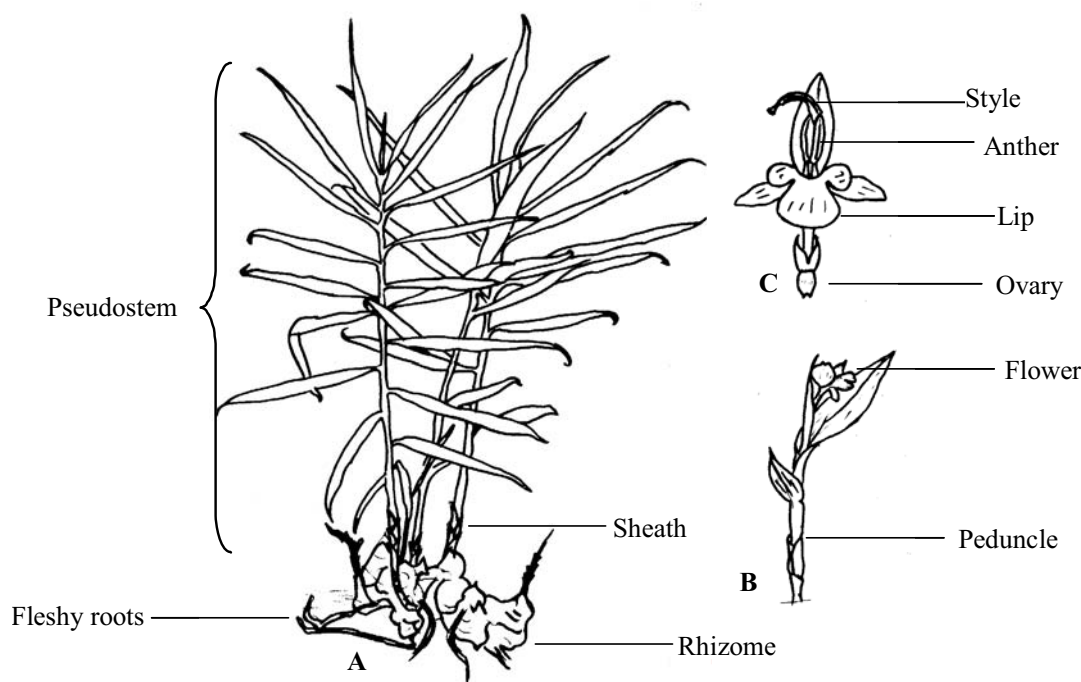


Fig. 1 Typical ginger (*Z. officinale*) plant showing different parts. (A) Complete plant; (B) Inflorescence; (C) Individual flower.

ersies over the number of genera and species mandate the need for a comprehensive and integrated approach to resolve these issues gradually and systematically. This approach should also include integrating the information documented in the different countries where the Zingiberaceae grow.

General taxonomic features

A brief taxonomic description of Zingiberaceae with relevant explanation of different plant parts (Fig. 1) is given as under.

Plants are perennial herbs, terrestrial, rarely epiphytic, aromatic, with fleshy, tuberous or non-tuberous rhizomes, and often with tuber-bearing roots. Stems are usually short and replaced by pseudostems formed by leaf sheaths.

Leaves are simple, with few to many distichously arranged blades; those toward the base of the plant are usually bladeless and reduced to sheaths. The leaf sheath is usually open on the side opposite the lamina. The leaf blade is usually suborbicular or lanceolate to narrowly strap-shaped, rolled longitudinally in the bud, glabrous or hairy, with a prominent midvein and complete margins.

Ligules are usually present in nearly all species, whereas a petiole, usually located between the leaf blade and sheath, is present in some species. Its form differs in different species; for example, it is cushion-like in Zingiber.

Inflorescence is terminal on pseudostems or borne on separate leafless shoots that are sheath-covered and arise from rhizomes. Inflorescence is either cylindrical or fusiform, but sometimes globose, and lax to dense with few to many flowers.

Flowers are generally bisexual, epigynous, and zygomorphic. They are solitary in the axils of bracts or in cincinni, with or without bracteoles (secondary bracts). The calyx is tubular; the usually slender corolla tube is divided into three subequal-sized petals.

Ovaries are inferior and initially 3-loculed but may change to 1- or 3-loculed when mature.

Fruits are capsular, fleshy or dry, dehiscent or indehiscent, sometimes berrylike. Seeds may be few or many, arilate; aril, often lobed or lacerate (Baker 1892; Ghazanfar and Smith 1982; Te-lin and Larsen 2000).

SYSTEMATICS AND PHYLOGENETIC STUDIES IN THE ZINGIBERACEAE

The Zingiberaceae comprise the largest family in the order

Zingiberales. The first classification of this family was proposed in 1889 and later refined. The current classification of the Zingiberaceae which is based on both vegetative and floral characteristics includes four tribes; Hedychieae, Alpinieae, Zingiberaceae and Globbeae (Larsen 1998; Kress *et al.* 2002). Larsen (1980) proposed a classifying scheme (key) and five genera, i.e., *Caulokaempferia*, *Boesenbergia*, *Kaempferia*, *Scaphochlamys*, and *Hedychium*.

The Evolutionary relationships based on molecular data

Over the past decade, several thousand phylogenetic analyses with particular emphasis on sequencing *rbcL*, the plastid gene encoding the large subunit of Rubisco (ribulose biphosphate carboxylase), have been used to reclassify all families of flowering plants (Savolainen and Chase 2003). The study of the Zingiberaceae has proceeded slowly and many genera of this family remain to be studied (Siriruga 1999). Nonetheless, the use of molecular systematics such those by Kress *et al.* (2002), have led to the proposal for a new classification of the Zingiberaceae based on DNA sequences of the ITS and plastid *matK* regions (Fig. 2). According to those authors, the Zingiberaceae can be divided into four subfamilies and four tribes: Siphonochiloideae (Siphonochileae), Tamijioideae (Tamijieae), Alpinioideae (Alpinieae, Riedelieae), and Zingiberoideae (Zingiberaceae, Globbeae). Several individual studies involving molecular systematics have refined current understanding of the phylogenetic relationships among species of different genera in the Zingiberaceae. Newly designed primers for the amplification of cpDNA intergenic *petA-psbJ* sequences were used to study the phylogeny of 14 *Boesenbergia* species from Thailand, which resulted in greater insight into the species relationships among members of this genus (Ngamriabsakul and Techaprasan 2006). Parsimony analyses based on the plastid *matK* region and the nuclear internal transcribed spacer (ITS) loci for different species of the subfamilies Alpinioideae, Zingiberoideae, and Tamijioideae, and the out-group genus *Siphonochilus* (Siphonochiloideae) identified six polyphyletic clades containing species of *Alpinia* distributed across the tribe Alpinieae. These results were generally supported by a Bayesian analysis of the combined data (Kress *et al.* 2005). DNA sequence data of the plastid group II intron *rps16* and the ITS were used to examine the relationship of the pantropical subfamily Alpinioideae. A strict consensus tree was obtained that supported the new classification of the Zingiberaceae into two subfamilies,

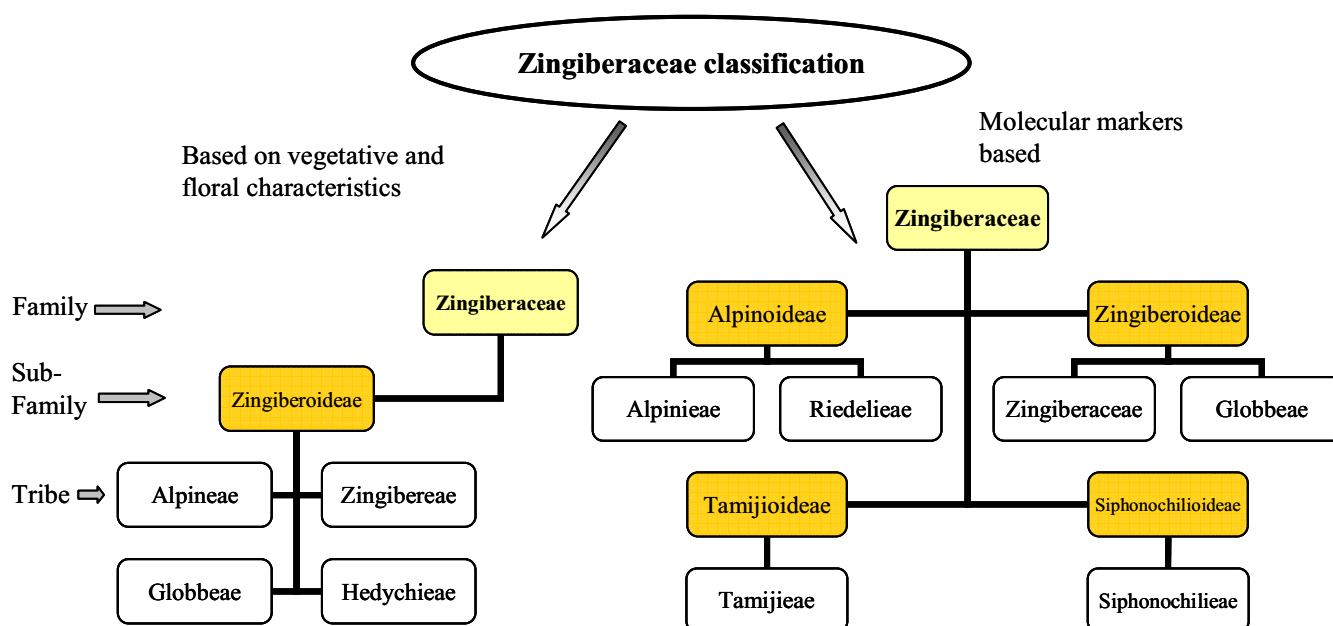


Fig. 2 Classification of Zingiberaceae family based on morphological characters (Larsen *et al.* 1998) and molecular markers (Kress *et al.* 2002).

Alpinoideae and Zingiberoidae, as proposed by Kress *et al.* (2002). With few exceptions, the subfamily Alpinoideae appeared as a monophyletic group (Pederson 2004).

A new infrageneric classification system for *Globba* recognized three subgenera and seven sections following parsimony and Bayesian analyses of nuclear ITS and plastid *trnK-matK* data from a broad sampling of *Globba* and related genera (Williams 2004). The evolutionary relationships among 13 genera of the Zingiberaceae based on comparative sequencing of the nuclear ribosomal ITS and the chloroplast *matK* regions showed that *Amomum*, which had been defined as polyphyletic with three major groups of species, did not correspond with any previously recognized sectional classification of the genus. Moreover, *Paramomum* was identified as a sister to *Elettariopsis*, and both were embedded within one group of *Amomum* (Xia *et al.* 2004). The unusually low level of sequence variation observed in 28 species of *Aframomum* using ITS nuclear ribosomal (nrDNA) suggested a recent radiation of this genus in Africa. However, its high variability in vegetative, floral, and fruit characteristics was not reflected in the ITS sequence data, which may be attributable to rapid radiation under the conditions of the Pleistocene climate change and to the effective dispersal of seeds by primates (Harris *et al.* 2000). With the considerable growing interest in the taxonomy and classification of the Zingiberaceae, it is hoped that new and more detailed insights together with a better understanding of species relationships within the family will emerge.

Phylogenetic relationships based on seed characters and isozymes

For the first time, phylogenetic relationships in the different tribes of the Zingiberaceae were studied on the basis of seed characters, which suggested that Hedychieae, Zingiberaceae, and Globbeae are closely related, although Hedychieae and Zingiberaceae differ from Globbeae, which has a multilayered exotestal epidermis. Moreover, the Zingiberaceae and Costaceae are connected through the tribe Alpinaceae, with a sclerenchymatous type of endotesta (Liao and Qi-Gen *et al.* 2000). Based on isozyme analysis, the relationships among 11 taxa of *Boesenbergia*, six taxa of *Kaempferia*, and two taxa of *Scaphochlamys* from Southern Thailand were studied. The results showed a higher degree of relationship between *Boesenbergia* and *Scaphochlamys* than between *Boesenbergia* and *Kaempferia* (Vanijajiva *et al.* 2003).

Distribution of the Zingiberaceae is vast, and various species are scattered over a large area. During their evolution, Zingiberaceae species adapted to the different geographical conditions within the Asian tropics, which ultimately resulted in a nonuniform distribution of species. This is also one of the reasons for the lack of a comprehensive effort to address the phylogeny of the Zingiberaceae. The individual reports mentioned above have yielded a good deal of information, which has been used to establish the current classification of gingers among various genera; however, confusion remains. The availability of specimens representing species from all of the Asian countries seems to be the main hurdle. The best way to address this issue is to initiate a collaborative effort, such as that made by several organizations under the umbrella of the FAO, (e.g., ICARDA, IPGRI). Such efforts should not focus solely on phylogenetic relationships, but also on the sustainable use and conservation of the resources presented by Zingiberaceae species.

FLORAL BIOLOGY AND BREEDING SYSTEMS IN THE ZINGIBERACEAE

Floral and reproductive biology

The ginger family is endowed with many types of breeding systems such as gynodioecy, andromonoecy, flexistylly,

protandry, and self-incompatibility. The major pollinators, that is, bees, hawkmoths, butterflies, and birds, correspond to the different morphological characteristics of the flowers (Gao *et al.* 2005) and are active at different times and at different stages of flowering (Gao *et al.* 2004).

Only a few studies have examined the breeding systems and pollination mechanisms of different members of the Zingiberaceae. These led to the identification of a new floral mechanism, studied in *Alpinia kwangsiensis* and *A. blepharocalyx*, referred to as “flexistylly,” which avoids self-compatibility and promotes outcrossing in these plants (Li *et al.* 2002; Zhang *et al.* 2003a). The two floral phenotypes, *cataflexistyle* (individuals in which the stigma is held erect above the dehiscent anther when anthesis begins in the morning and moves downward to under the anther at noon) and *anaflexistyle* (individuals in which the receptive stigma is initially downward-facing under the indehiscent anther and moves into a reflexed superior position above the anther as it begins to shed pollen at midday), are manifested as synchronous stigmatic movements that are correlated with the foraging behavior of floral visitors (Li *et al.* 2002; Zhang *et al.* 2003a). In addition, a new pollination mechanism discovered in *Caulokaempferia coenobialis* was reported in which a film of pollen is transported from the anther by an oily emulsion that slides sideways along the flower’s style and into the individual’s own stigma, thus facilitating self-pollination (Gao *et al.* 2005). The unique floral mechanisms discovered in different genera have provided further evidence regarding the diversity of pollination and breeding systems of gingers. Similarly, recent comparative morphological and ontogenetic data on the flowers and bulbils of *Globba* have provided detailed information that has aided in clarifying their homologies among zingiberaceous plants (Box and Rudall 2006). The insights gained from these studies on the floral biology and breeding systems of the Zingiberaceae also indicate that much more remains to be discovered about the floral and reproductive biology of its member species. This information can be used to devise better propagation strategies, as the lack of sexual reproduction is one of the constraints in the Zingiberaceae.

Pollination biology

The Zingiberaceae comprise a large family of animal-pollinated tropical monocotyledons, and its members display a broad range of pollination and breeding systems (Sakai *et al.* 1999). Pollen grains of the Zingiberaceae are spherical, subspherical, ovoid, or prolate and range in size from 36 to 225 μm . These are aperturate or non-aperturate, less resistant to acetolysis, and their walls are composed of a very thin exine and a thick intine (Liang 1988). Pollen histochemistry and the pollen:ovule ratio (P/O) are used to study the relationship between pollination mechanism, breeding systems, and pollen nutrition types. However, pollen nutrition types do not seem to be related to mating systems, and P/O ratios in members of the Zingiberaceae with the same breeding system are variable (Wang *et al.* 2004). The lower P/O ratios in this family are presumed to be related to its highly efficient pollination systems, which are mediated by pollen that can be quite glutinous, and the relatively large area of its stigmas (Wang *et al.* 2004). Pollinator activity in terms of the frequency and behavior of the visitors, quantity and quality of the pollination services, and the effect of flowering behavior influence the pollination ecology of *C. longiflora* and other zingiberaceous plants (Gao *et al.* 2004). A newly discovered protandrous mechanism in *C. longiflora* consists of flowering over 2 days to avoid autogamy. Under field conditions, all individuals of this species usually produce only one flower every other day to minimize geitonogamy (Gao *et al.* 2004).

Table 1 Basic, gametic and diploid/somatic chromosome numbers of different species in different genera of the Zingiberaceae.

Genus	Species studied	Basic No. (x)	Gametic No. (n)	Diploid/somatic No. (2n)
<i>Alpinia</i>	12	12		48
<i>Amomum (Phaeomeria)</i>	1	12	--	48, 52
<i>Boesenbergia</i>	9	10	--	20, 24, 36
<i>Brachychilus</i>	1	16	--	32
<i>Cautleya</i>	1	--	13	34
<i>Curcuma</i>	9	--	--	32, 42, 61, 62, 63, 64, 86
<i>Eletteria</i>	1	12	--	48, 52
<i>Etilingera</i>	1	12	--	24
<i>Globba</i>	32	16, 12	16, 12	24, 32, 48, 64
<i>Hedychium</i>	9	9, 17, 26	--	34, 36, 52, 54, 66
<i>Kaempferia</i>	7	11	--	22, 24, 36, 54
<i>Phaeomeria (Nicolaja)</i>	1	12	--	48
<i>Roscoea</i>	2	--	13	42, 24
<i>Scaphochlamys</i>	2	--	14	13
<i>Zingiber</i>	6	11, 12	--	22, 55

CYTOLOGICAL OBSERVATIONS

Chromosome number

Information regarding basic, gametic, and diploid/somatic chromosome numbers reported in 36 independent studies of 94 species belonging to 15 genera of Zingiberaceae is summarized in **Table 1**. The basic chromosome number observed in different species varies from 9 to 26, with 12 as the most frequent chromosome number. Gametic numbers were reported for only a few of the genera, whereas extensive information exists concerning diploid/somatic chromosome numbers. Diploid chromosome numbers vary widely in different species, ranging from 13 to 86. Widely varying chromosome numbers were found in *Curcuma*, followed by *Hedychium*, *Globba* and *Kaempferia*, and *Boesenbergia*. Ramachandran (1961) investigated the cytology of six *Curcuma* species and found that their chromosomes were small (0.6 μm and 1.7 μm in length) and that the basic chromosome number of the genus was 21. Eksomtramage *et al.* (2002) studied chromosome numbers in 22 species belonging to 10 genera of the Zingiberaceae. Somatic numbers ranged from 20 to 48 and showed diploidy and polyploidy. According to the cytology data, Zingiberaceae is a natural group that can be divided into two subfamilies, Zingiberoideae and Costoideae, and four tribes, Globbeae, Zingibereae, Alpinieae, Hedychieae (Chen 1989). However, disagreement exists among the various reports pertaining to chromosome numbers in some zingiberaceous species, which has led to further confusion. Moreover, many of these reports are older and conducted before sophisticated techniques were available for in-depth studies. Consequently, new studies are needed to resolve existing controversies over chromosome counts in the Zingiberaceae.

Polyploidy

Polyploidy has been a pervasive force in plant evolution and is extremely common in all major groups of plants. However, the extent of polyploidy depends on the base number, as the product of the two determines chromosome number (Stace 2000). Polyploidy, aneuploidy, and structural changes in chromosomes have played a major role in the evolution of the Zingiberaceae (Sharma and Bhattacharya 1959). *Curcuma* is an important member of the Zingiberaceae, and the herbaceous perennial habit of these plants, their vegetative mode of propagation, and the small size of their chromosomes favor the perpetuation of polyploidy types in this genus. The basic chromosome number of *Curcuma* (21) is too high to be the primary one and may instead be a secondary balance resulting from amphidiploidy derived from a combination of the 9 and 12 chromosomes found in many Zingiberaceae genera (Ramachan-

dran 1961). Allotetraploids and heteroploids are found in Malayan and Thai *Globba* species (Larsen 1972; Lim 1972) and multiple triploid formations may be one of the most important forces in *Globba* speciation (Takano and Okada 2004). The tribe Hedychieae has a wide range of basic chromosome numbers and is at an active stage of evolution, whereas the basic chromosome number in the Zingiberaceae is thought to be 12 (Chen 1989). The increase or decrease in chromosome numbers from the original basic number of 11, the basic number found in the Zingiberales, has not occurred through simple evolutionary loss or gain of chromosomes, but through complex structural changes in the chromosomes themselves (Mahanty 1970).

Cytoplasmic inheritance

Cytoplasmic DNA of male reproductive cells was examined under epifluorescence microscopy to determine the potential for cytoplasmic inheritance in different families, including the Zingiberaceae. In *Costus lacerus*, *Curcuma domestica*, *Etilingera erythrophylla*, and *Hedychium spicatum*, the cytoplasmic DNA of generative cells or sperm did not fluoresce, which suggests maternal cytoplasmic inheritance in these species. In contrast, the cytoplasmic DNA of *Globba barthri*, *Globba racemosa*, *Zingiber purpureum*, and *Zingiber zerumbet* male reproductive cells displayed fluorescence, indicating the potential for biparental cytoplasmic inheritance in these species (Zhang *et al.* 2003b).

GENETIC DIVERSITY

Despite its large size, only a few of the genera belonging to the Zingiberaceae have been the focus of attention, specifically, *Zingiber*, *Curcuma*, *Alpinia*, *Kaempferia*, *Boesenbergia*, *Globba*, and *Amomum*. Zingiberaceae species display diversity in habitat, morphology, and ethnobotanical use. However, little is known about the genetic relationships among taxa and genetic diversity has been investigated in only a few genera, primarily due to the lack of suitable molecular markers (Jatoi *et al.* 2006). To our knowledge, microsatellite markers, which provide reliable molecular detection of diversity, have not yet been developed for zingiberaceous plants. Of the Zingiberaceae species examined in diversity studies, the genera *Boesenbergia* and *Alpinia* have received considerable attention, mainly in Thailand, and *Z. officinale* and *Curcuma*, have been studied mainly in India, China, and to some extent in neighboring countries (e.g., Bangladesh, Myanmar). The results of these diversity studies are briefly described in the following section.

Molecular markers

Since their discovery, molecular markers have played a tre-

mendous role in delineating the diversity of different crop plants, including members of the Zingiberaceae. A few of these studies used molecular markers, mainly random amplified polymorphic DNA (RAPD), to analyze the genetic diversity of this family. A RAPD-based assay revealed that the genetic diversity of *Curcuma zedoaria* was maintained to a greater extent in hilly populations than in plains and plateau populations (Islam *et al.* 2005). Analysis of the genetic diversity of *Zingiber*, *Curcuma*, and *Alpinia* using rice microsatellites as RAPD markers also demonstrated high polymorphism and confirmed the usefulness of these markers in genetic diversity studies of the Zingiberaceae (Jatoi *et al.* 2006). Significant variation among 16 elite cultivars of *Zingiber officinale* (Nayak *et al.* 2005) and 17 of *C. longa* (Nayak *et al.* 2006) was determined using cytological and RAPD markers. Metabolic profiling was used as in the diversity assessment of plants belonging to different species in the genus *Zingiber*, in which chemotaxonomic investigations were shown to best reveal both diversity and relationships among species (Jiang *et al.* 2006). Eleven species of *Boesenbergia*, six species of *Kaempferia*, and two species of *Scaphochlamys* from Southern Thailand were analyzed for diversity and species relationship based on RAPD markers. The results clarified doubts that had arisen based on their morphologic classification (Vanijajiva *et al.* 2005). Within a natural population of wild and cultivated individuals of *Siphonochilus aethiopicus*, estimation of genetic diversity using horizontal starch gel electrophoresis indicated exceptionally high allelic heterogeneity due to the synergistic effects of vegetative and sexual reproduction (Makhuvha *et al.* 1997). Diversity studies were confined mainly to the species valued as spices, such as *Z. officinale* and *C. longa*. There may be some unpublished information on the generation of SSR markers specific to *Zingiber* (pers. comm., Prof. Halijah Ibrahim, University of Malaya).

Comparative anatomical features

Variation in leaf anatomical structures was used as tool to study the interspecific variability of 20 *Alpinia* species. Interspecific variation in the structure of the leaf midrib and petiole was shown (Hussin *et al.* 2000). Species-specific anatomical variations in the leaves, flowers, and rhizomes of ginger (*Z. officinale*) and a subsequent comparison with other species of the genus *Zingiber* yielded useful information that could be applied to identify different species (for details see the review by Ravinderan *et al.* 2005). Diversity assessment and species identification in *Curcuma* have also been carried out using isozymes to clarify taxonomic confusion. The polymorphism displayed by these allozyme markers allowed the various taxa analyzed to be distinguished (Apavatjirut *et al.* 1999).

CONCLUSIONS AND RECOMMENDATIONS

The Zingiberaceae is of paramount importance in the plant kingdom as members of this family have been used by humans throughout their history. The plants are used fresh or in processed form for medicinal, aromatic, perfume, ornamental, and aesthetic purposes. Cultivation of zingiberaceous plants ranges from subsistence farming to backyard plantation.

Since the many species ascribed to this family are at an active stage of evolution, much uncertainty exists about their taxonomic classification, and several genera are under revision. Recently, the advent of molecular systematics has assisted in the taxonomic classification of the Zingiberaceae and has accelerated phylogenetic studies of this family. Several individual efforts in this regard have yielded valuable information that has helped to elucidate the evolutionary relationships among the many genera and species. However, a comprehensive and concentrated effort is still needed to obtain a comprehensive classification of the family and thereby resolve discrepancies concerning the

number of genera and species in the Zingiberaceae. With increased sampling, a more complete phylogenetic classification of the family, and perhaps new generic circumscriptions, will become feasible.

One area yet to be explored in depth is the complex breeding systems found in the Zingiberaceae. Many species have undergone complex evolutionary and adaptive modifications. Additional knowledge of pollination biology and breeding systems will help researchers to devise breeding strategies in many of the zingiberaceous species that are prone to sexual reproduction. We therefore recommend that further studies to be carried out on the pollination and breeding systems of a wider range of gingers, while phylogenetic research should focus on identifying the evolution of pollination and breeding systems in the Zingiberaceae.

Most of the species from this family propagate clonally, and thus are likely to contain a narrow genetic base. Knowledge of genetic diversity, such as that obtained through molecular marker studies of a large number of species together with morphological evaluation, is of prime importance to devise conservation and crop-improvement strategies.

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