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CYTOGENETICS OF TROPICAL BULBOUS ORNAMENTALS. IX. BREEDING SYSTEMS IN *ZEPHYRANTHES*

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SUMMARY

Zephyranthes is a rather versatile genus and contains self-incompatible and self-compatible taxa coupled with positional barrier between stigma and anthers. The two may augment or run counter to each other. Furthermore, the genus also contains sexual and agamospermous species. The latter are often self-pollinated and pseudogamous. *Z. sulphurea* (2n = 48) when pollinated with *Z. candida* (2n = 40 and 41) has consistently given rise to seedlings with maternal chromosome number and morphology. *Z. lancasteri* and cv. 20 also behave similarly. This is a strong pointer for their being agamospermous, although a final proof will come from an embryological study. The intraspecific polymorphism within agamospermous taxa in the genus may be the result of autosegregation. On the other hand, the crosses involving sexual species like *Z. candida* (2n = 41) as the female parent have generated a large heterogeneous progeny ranging in chromosome number from 2n = 33 to 48 depending upon the number in the male parent. Such versatility of the breeding system together with chromosomal repatterning, hybridization, polyploidy and vegetative multiplication/apomixis explains the origin and preservation of an astonishing range in chromosome numbers from 2n = 18 to 96.

INTRODUCTION

For a proper understanding of the dynamics and statics of any group of plants, knowledge of its breeding system is essential. More often it not only clarifies the nature of variation in the group but also helps in chalking out effective plant breeding procedures. Among species and cultivars of the genus *Zephyranthes* (Amaryllidaceae) some taxa seed very heavily, leaving hardly any undeveloped ovule in an ovary, while others produce very few or no seed at all. Coupled with this, there are some well-known cases of apoximis already reported in the genus (PACE, 1913; BROWN, 1951; COE, 1953, 1954). This leads to the suspicion that in this complex there may be different modes of reproduction and/or mating systems. Accordingly, a number of experiments were made to ascertain the type of pollination and reproduction.

OBSERVATIONS

Pollination

In most taxa, the stigma becomes receptive by the time anthesis takes place which on

Table 1. Relationship of style length and breeding system number of taxa.

Style	Self-compatible	Self-incompatible	Total
Long (5–15 mm)[1]	–	7	7
Equal (0–1 mm)[1]	16	20	36
Short (3–7 mm)[1]	14	3	17
Total	30	30	60

[1] Difference in the level of stigma and anther

cool days is between 10 h 00 and 11 h 00 but on warm days between 08 h 00 and 09 h 00. Bees usually visit flowers in search of nectar at the base of the corollary tube. These help both in self and cross pollination.

The difference in height of anthers and stigma is superimposed on this situation. Data on 60 taxa have been summarized in Table 1. It is apparent that although intergrading types occur, there are broadly speaking three categories (Fig. 1). The more common condition is seen in 36 taxa in which the two organs are nearly at the same level, or the difference either way is negligible and they may be self-compatible or self-incompatible. In cv. 56 ($2n = 43$) of this group, with stigma at about the same level as the anthers, about 80% flowers are self-pollinated in nature. However, there is no seed set after self-pollination either in nature or by hand. In this case the pollen tube growth through the style was perfectly normal and the ovary continues to swell for 4–5 days and thereafter dries. Perhaps fertilization occurs.

One of the extremes is where a stigma is 5–15 mm higher than the level of anthers and thus self-pollination is not possible. Such taxa have been referred as long-styled and are self-incompatible. Seed set is possible only through cross-pollination. Most important species and cultivars of this group are *Z. grandiflora*, *Z. insularum*, *Z. rosea* *Z. puertoricensis* and 3 cultivars. Self-pollination by hand in *Z. grandiflora* where the stigma is about 13 mm above the anthers, proved to be a total failure. The other extreme is when stigma is 3–7 mm shorter than the height of the anthers and most im-

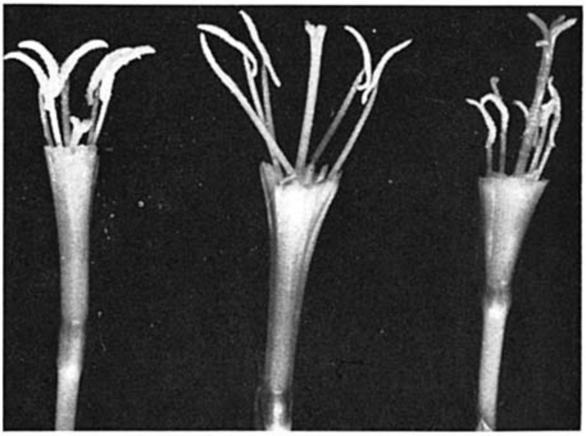

Fig. 1. Three broad classes in positional difference between the level of stigma and anthers.

portant taxa showing this condition are *Z. lancasteri* and 16 cultivars. Fourteen of these are self compatible and 3 are self-incompatible.

From an analysis of 60 taxa, 50 % each were self-compatible and self-incompatible (Table 1). An important feature is that in the self compatible category no taxon was long-styled, but 23.3 % have a short style and in the remaining 26.7 % the stigma is at the same level as the stamens. The corresponding figures in the self-incompatible category are 5 and 33.3 %, respectively. The remaining 11.7 % taxa are long-styled.

Mode of reproduction

This was investigated after making a number of self and cross pollinations involving 14 species and cultivars (*Z. candida*, *Z. grandiflora*, *Z. insularum*, *Z. lancasteri*, *Z. puertoricensis*, *Z. rosea*, *Z. sulphurea* and cultivars 9, 10, 12, 14, 20, 56 and 59) at homoploid and heteroploid levels ($2n = 24, 25, 28, 40, 41, 43, 45–48$) in different combinations. These taxa were selected both on account of their horticultural value and chromosome number and the experiments have been made with the dual purpose of raising new variants and also unravelling the nature and types of reproduction. So far about 1,200 crosses have been made but seeds were obtained in only 23 combinations involving 575 crosses. However, only 8 combinations have been investigated to date and the data are summarized in Table 2. Mature plants from seedling progenies from these combinations could not be compared phenotypically with their parents in all cases, because nearly 3 years are required for seedlings to reach maturity. Therefore, in most cases seedlings were only scored for chromosome number in relation to their parents and inferences drawn wherever possible.

It is apparent that *Z. candida* ($2n = 41$) is a good seed parent because it gave 50 to 80 % success, yielding sizable seedling progenies irrespective of the chromosome number of the male parent. Heteroploid progeny results after both selfing and crossing. In absence of the phenotypic characters of the progeny it is difficult to decide whether *Z. candida* is sexual or an apomict with autosegregation (Gustafsson, 1947). It has not been possible to compare the karyotype of seedlings with $2n = 41$ with the maternal parent ($2n = 41$). In this connection it may be pointed out that Yokouchi (1965) obtained seedlings with $2n = 38$ from the cytotype with the same number of this species and the karyotype tallied in all cases. Similarity cv. 20 ($2n = 48$) and cv. 59 ($2n = 46$) are also very good seed parents and set seeds irrespective of the chromosome number of the male parent.

In contrast to this situation, selfing in *Z. grandiflora* did not succeed indicating its self-incompatible nature. The failure of crosses between *Z. grandiflora*, and *Z. puertoricensis* and *Z. lancasteri* may indicate either female sterility in the former or all the three have the same incompatibility alleles. In general the same is true of *Z. puertoricensis* except that only one seedling was obtained after selfing. It was very weak and had $2n = 23$, which is lower than the number in *Z. puertoricensis* ($2n = 25$) itself. Evidently both *Z. grandiflora* and *Z. puertoricensis* are bad seed parents and may be avoided in any breeding programme.

Z. sulphurea ($2n = 48$) is distinctive in that it shows prolific seed production after selfing and crossing with two cytotypes of *Z. candida* ($2n = 40, 41$). In all cases the chromosome number was the same as in *Z. sulphurea* itself. Preliminary investigation of the karyotype of the seedling showed that it matches well with the female parent.

Table 2. Crossability and chromosome number in seedling progenies in some *Zephyranthes* hybrids.

Cross	Chromosome number (2n); female × male	Percentage success	Chromosome number (2n) in progeny (number of seedlings)
Z. candida (esmasculated)	41	–	–
Z. candida × *Z. candida*	41 × 41	80	41(3), 43(2), 44(2) and 48(1)
Z. candida × cv. 9	41 × 47	50	40(2), 41(1), 40(2) and 44(3).
Z. candida × cv. 14	41 × 45	80	33(1), 38(3), 40(2) and 41–44 (1 each).
Z. candida × *Z. sulphurea*	41 × 48	50	45(1), 46(1)
Z. grandiflora × *Z. grandiflora*	48 × 48	–	–
Z. carinata × *Z. lancasteri*	48 × 48	–	–
Z. grandiflora × *Z. puertoricensis*	48 × 25	–	–
Z. lancasteri × *Z. lancasteri*	48 × 48	100	not investigated
Z. lancasteri × *Z. puertoricensis*	48 × 25	–	–
Z. puertoricensis × *Z. puertoricensis* × *Z. puertoricensis*	25 × 25	0.5	23(1)
Z, puertoricensis *Z. candida*	25 × 41	–	–
Z. puertoricensis × *Z. grandiflora*	25 × 48	–	–
Z. puertoricensis × *Z. lancasteri*	25 × 48	–	
Z. sulphurea (emasculated)	48	–	–
Z. sulphurea × *Z. sulphurea*	48 × 48	100	48(4)
Z. sulphurea × *Z. candida*	48 × 40	100	48(2)
Z. sulphurea × *Z. candida*	48 × 41	100	48(3)

This species is one of the few in our collection in which seed production and seed germination are very high. The progeny in this case is matroclinous. An obligate apomictic reproduction is very strongly suspected which can be confirmed only after embryological studies are complete.

One of the important points in this direction is that when *Z. candida* (2n = 41) is selfed it yields heteroploid progeny indicating selection of different chromosome numbers in gametes. However, when it is used as male parent against *Z. sulphurea* none of the male gametes with different chromosome numbers succeed, because all seedlings contain the maternal number (2n = 48). Furthermore, the pollen of *Z. sulphurea* was successfully involved in crossing with *Z. candida* (2n = 41). The two seedlings tested so far have 2n = 45 and 46, respectively. An investigation of pollen mitosis showed n = 24 in *Z. sulphurea*. Assuming that pollen with this number was selected, the constitution of eggs selected from *Z. candida* (2n = 41) is n = 21 and 22, which is close to the expected.

Chromosomal polymorphism in progeny

One of the interesting aspects of this study is that by simple selfing of *Z. candida* (2n = 41), only 3 seedlings out of 8 had the parental number. Whether they had the parental karyotype has not been investigated. The other numbers are 2n = 43, 44 and 48. Similarly, from *Z. candida* (2n = 41) × cv. 9 (2n = 47) only one seed had the maternal number, while the remaining had 2n = 40, 42 and 44 all of which are new. Only one seedling had 2n = 41 in the progeny of *Z. candida* (2n = 41) × cv. 14 (2n = 45) in which case an interesting series of numbers 2n = 33, 38, 40, 42, 43 and 44 was obtained. The first three and in particular 2n = 33 is much lower than the parental number. These data are a strong pointer to the manner by which new cytotypes may arise or have arisen in nature and under garden conditions in this genus.

DISCUSSION

The characteristics of the breeding system in a group of plants exercise strong influence on the genetical architecture of its taxa which in turn affect their evolutionary potentialities. Judged from this angle, *Zephyranthes* is a very versatile genus. It contains self-compatible and self-incompatible taxa. These characteristics may be coupled with the presence of a positional barrier between stigma and anthers. Sometimes the two augment each other in-as-much-as self-incompatible species like *Z. grandiflora* has the stigma well above widely spaced anthers making self-pollination most unlikely (Brown, 1915; present authors). Other well-known self-incompatible taxa are *Z. caudata* and *Z. atamasco* (see Fryxell, 1957). Taxa with long styles are self-incompatible and those with short styles are mostly self-compatible. However, where style and stamens are of equal length, the taxa may be self-compatible or incompatible. Where self-compatibility is associated with anthers and stigma being at the same level, selfing can not be avoided. This is seen in *Z. texana* (now *Habranthus texanus*; 2n = 24, Pace, 1913; Brown, 1951) and 16 cultivars (present authors).

The genus contains sexual and apomictic species. To the later belong the well-known cases of *Z. texana* (*Habranthus texanus*), *Z. drummondii*, *Z. brazosensis*, and *Z. pedunculata* (all 2n = 48; Coe, 1953, 1954). These show agamospermy with pseudogamous diplosporous gametophytic apomixis. Further, apomictic species like *Z. texana* are self-pollinated, the related sexual species are self-incompatible, a condition also seen by Gustafsson (1947).

In the present work direct evidence for apomixis has been obtained in *Z. sulphurea* (2n = 48), a very common garden cultivar. The chromosome number in selfed and crossed progeny of this species with *Z. candida* (2n = 40 and 41) has given consist ently plants with maternal number of 2n = 48. While the final proof of apomixis will come from embryological examination, the seedling progeny in this case has been found to be phenotypically identical with the maternal parents. Other taxa where apomixis, is suspected are *Z. lancasteri* and cv. 20.

Coe (1954) has reported considerable plant to plant variation in karyotype of apomictic *Z. drummondii* (2n = 48) although there is consistency within the same plant. Furthermore, in another apomictic species *Z. brazosensis* (2n = 48), he has noted plants with 2n = 55 and 56, and 58 and 59. How such intraspecific aneuploidy coupled with karyotypic difference arises is not precisely known, but according to him it probably resulted from abnormalities in nuclear divisions either in some somatic and/or reproductive tissues. However, the point of interest is that such abnormalities are sufficient to cause sterility in sexual species but evidently in these apomicts a wide range of cytological variation is tolerated.

An apparently similar situation to that in apomictic *Z. brazosensis* has been obtained in the present study (Table 2) in crosses with *Z. candida* (2n = 41) as the female parent. How far variation in chromosome number generated from these crosses is due to sexual and/or apomictic reproduction with autosegregation, is difficult to answer at present. In this connection it may be of interest to note that YOKOUCHI (1965) found same chromosome number and morphology in the seedlings from *Z. candida* (2n = 38). The origin of seedlings with numbers lower than the female parent, like 2n = 33, 38 and 40 in *Z. candida* (2n = 41) × cv. 14 (2n = 45) and cv. 9 (2n = 47) and in selfed progeny of *Z. puertoricensis* (2n = 25) is interesting. Similar variation in chromosome number has also been observed after natural, controlled interspecific hybridization and backcrossing in *Zephyranthes* and related genera (FLORY, 1954, 1964; FLORY and FLAGG, 1961; FLORY et al., 1968). This is due to the fact that the parents involved are all polyploids with the result that there is enough duplicated genic material and they can withstand losses of chromosome parts and whole chromosome or even chromosomes (RAINA and KHOSHOO, 1971b). The classical example is *Triticum aestivum* in which 21 different nullisomics have been obtained.

Such aneuploid progeny can arise both in sexual as well as apomictic taxa. Well-known cases of intraspecific aneuploid-polyploid pattern in sexual taxa are found in garden cultivars of *Hyacinthus orientalis* (2n = 2x = 16, 17, 19–23, 25–31; DARLINGTON et al., 1951), *Narcissus bulbocodium* (2x-3x, 4x, 5x, 6x; DARLINGTON, 1963), etc. There are many wild taxa showing such a pattern, like *Erophila verna* (2n = 14, 24, 30, 32, 34, 36, 40, 52, 54, 58, 64; WINGE, 1940), *Claytonia virginica* (2n = 14–36, 38, 42, 44, LEWIS, 1962, 1970), etc. (see DARLINGTON and WYLIE, 1955; CAVE et al., 1956–64; ORNDUFF, 1965–66). In the last taxon there is a mixture of aneuploid-polyploid cytotypes superimposed on which is aneusomaty in pollen mother cells. Among the apomicts with frequent occurrence of aneuploidy mention may be made of *Poa* (2n = 48–124, 82–147, 39–92, 31–57; GUSTAFSSON, 1947), *Potentilla, Calamagrostis,* etc. (STEBBINS, 1950). In all such cases there are abundant meiotic irregularities in embryo-sac mother cells (GUSTAFSSON, 1947). Direct result of all such aberrations is the wide range of chromosome numbers found in sex cells which has been shown to occur in the male meiosis of *Z. puertoricensis* with 2n = 25 (RAINA and KHOSHOO, 1971c). It is, however, not known if this species is sexual or apomictic. In any case, the total disregard for chromosome balance is apparent.

In conclusion it may be pointed out that the studies of the present authors (RAINA and KHOSHOO, 1971a, 1971b, 1971c) have shown that the genus contains and maintains efficiently an astonishing array of chromosome numbers (2n = 18, 22, 24, 25, 28, 38, 40–50, 54, 58–61, 66, 69, 72, 73, 96). Such a cytogenetic pattern in the genus perhaps began with x = 6 and owes its origin to chromosomal repatterning due to periand paracentric inversions, interchange heterozygosity and misdivision. This is coupled with self-compatibility/incompatibility and sexual/apomictic reproduction together with hybridization and polyploidy. The interchange polyploids because of their abnormal meiosis produced sex cells with a wide range of numbers, which are well tolerated because of the inherent polyploid constitution. This in turn leads to a cycle of aneuploid variability. All together release tremendous variability and the genetic system in the genus has potentialities to conserve and preserve all heterozygosity through agamospermy and in particular through vegetative multiplication. With such a pattern morphological diversity and taxonomical complex nature of the genus is quite natural.

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