# Interspecific Hybridization in *Cucumis*—Progress, Problems, and Perspectives

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Interspecific hybridization is used to improve crops by transferring specific traits, such as pest and stress resistance, to crops from their wild relatives (Bowley and Taylor, 1987). When applicable, this approach is a very effective method of gene transfer. In nature, ≈30% to 35% of flowering plant species were created by interspecific hybridization, followed by chromosome doubling (Stebbins, 1971). Starting with interspecific hybridization, allopolyploids, such as allotetraploids, can be developed by doubling the chromosome number of the $F_1$ hybrid. Successful construction of an allopolyploid results in the creation of a new combination of genomes, or the production of a species that did not exist previously.

However, great effort may be required to hybridize cultivated and wild species. The first man-made interspecific hybrid was synthesized in 1717 between carnation (*Dianthus caryophyllus* L.) and sweet william (*Dianthus barbatus* L.) (Stalker, 1980). Since then, thousands of interspecific crosses have been attempted, but success has been rather limited. Chromosomal, genetic, cytoplasmic, or mechanical isolation barriers can handicap successful hybridization and utilization. It took plant breeders about 100 years to produce triticale—a new crop species created from the cross of wheat (*Triticum aestivus* L.) and rye (*Secale cereale* L.) (Zillinsky, 1985). Significant benefits and difficulties make interspecific hybridization an important objective for geneticists and plant breeders.

Interspecific hybrids in the Cucurbitaceae have been produced in several genera, including *Cucumis* (Deakin et al., 1971), *Citrullus* (Valvilov, 1925), *Luffa* (Singh, 1991), and *Cucurbita* (Weeden and Robinson, 1986). In the genus *Cucumis*, an amphidiploid was reported from the cross of *C. anguria* L. and *C. dipsaceus* E. ex S. (Yadava et al., 1986). However, in the *Cucurbitaceae* only in *Cucurbita* has interspecific hybridization been successfully utilized for crop improvement (Robinson and Decker-Walters, 1997).

*Cucumis* contains two species of economic importance, melon (*C. melo* L., 2n = 24) and cucumber (*C. sativus* L., 2n = 14). The importance of wild *Cucumis* species has long been recognized because they possess resistance to pathogens, such as powdery mildew [caused by *Sphaerotheca fuliginea* (Schlechtend.: Fr) Pollacci], downy mildew [caused by *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev], anthracnose [caused by *Colletotrichum orbiculare* (Berk. & Mont) Arx], and fusarium wilt (caused by *Fusarium oxysporum* Schlechtend.: Fr.) (Kirkbride, 1993; Leppick, 1966; Lower and Edwards, 1986). Genetic variation is relatively limited in cucumber (Staub et al., 1987); thus, efforts to create interspecific hybrids become more critical and meaningful. In 1859, Naudin first tried to cross melon with cucumber and other species (Naudin, 1859). Historically, various approaches (traditional and biotechnological) for interspecific hybridization have been used in *Cucumis* to overcome the fertilization barriers between cucumber, melon, and wild species, but with only limited success.

The recent cross between cucumber and *C. hystrix* Chakr. (2n = 24) was the first repeatable cross between a cultivated *Cucumis* species and a wild relative (Chen et al., 1997b), and represented a breakthrough in interspecific hybridization in *Cucumis*. The success of this cross was even more surprising because the parental species have different chromosome numbers. The original $F_1$ hybrid (2n = 19), obtained by embryo rescue following pollination of *C. sativus* by *C. hystrix* (Fig. 1A), has 7 chromosomes from *C. sativus* and 12 from *C. hystrix*, and was both male- and female-sterile. To restore fertility, reciprocal crosses were made and the chromosome numbers of the progeny were successfully doubled (Fig. 1B) (Chen et al., 1998). Pollen grains were produced by these progeny when *C. hystrix* was used as the seed parent; the plants produce fertile flowers (Fig. 1C) and set fruit (Fig. 1D) with viable seeds (Fig. 2), indicating that fertility was restored. This restoration of fertility marked the creation of a new synthetic species, which has close phylogenetic relationships with its parental species, but is distinctively different from each. It has the genome HHCC and chromosome number 2n = 4x = 38. This synthetic species might be useful as a new *Cucumis* crop. In addition, as a *C. hystrix* x *C. sativus* hybrid, it might be useful as a bridging species for transfer of useful traits to cucumber.

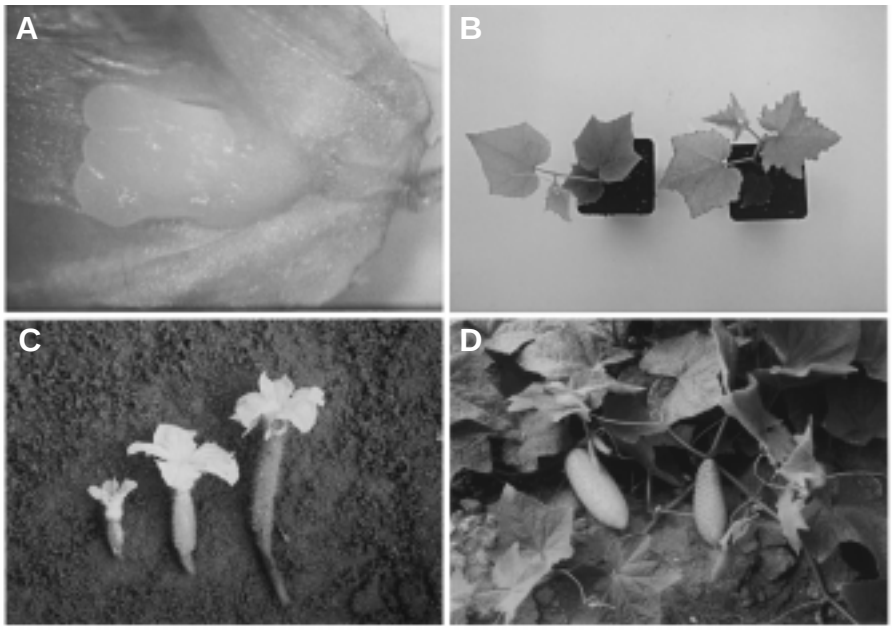


Fig. 1. (**A**) Embryo obtained from the interspecific hybrid between *Cucumis sativus* and *C. hystrix*. (**B**) The $F_1$ diploid, sterile, hybrid plant form embryo rescue (**left**) and its chromosome-doubled tetraploid, fertile plant (**right**). (**C**) Female flowers of *C. hystrix* (**left**), *C. sativus* (**right**), and the $F_1$ hybrid (**middle**).(**D**) Fruits set on the amphidiploid.

Received for publication 18 Mar. 1999. Accepted for publication 12 July 1999. Journal paper no. 4482 of South Carolina Agricultural Experiment Station, Clemson, S.C. The authors sincerely thank Dr. Yosuke Tashiro, Dr. Shiro Isshiki, and Dr. Sadami Miyazaki, Faculty of Agriculture, Saga Univ. of Japan, Saga, for the isozyme data presented in this paper, and Dr. Bill Rhodes and Dr. Vance Baird, Dept. of Horticulture of Clemson Univ., and Dr. Jack Staub, USDA/ARS, the Univ. of Wisconsin–Madison, for their review of this paper. This research was supported by the National Education Committee of the Peoples Republic of China, and by Research Grant Award No. US-2809-96R from the United States–Israel Binational Agricultural Research and Development Fund (BARD). The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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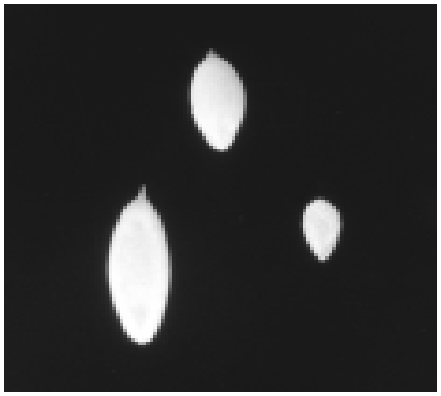

Fig. 2. Seeds harvested from the amphidiploid (**below**) and its diploid progenitors (*Cucumis hystrix*, **upper left**; *C. sativus*, **upper right**).

## PROGRESS: SYSTEMATIC STUDIES AND INTERSPECIFIC CROSSES IN *CUCUMIS*

The genus *Cucumis* includes two distinct groups or subgenera, different in their origins and basic chromosome numbers (Jeffrey, 1980). Melon, and most other species in this genus with the basic chromosome number n = 12, are referred to as the African group. *Cucumis sativus* var. *sativus* and *C. sativus* var. *hardwickii* (Royle) Alefeld, with the basic chromosome number n = 7, are referred to as the Asian group. Under the current systematic system (Fig. 3), 30 species are grouped into six series in the subgenus *melo* (Kirkbride, 1993), instead of four groups (Jeffrey, 1980). *Angurioidei* is the largest of the six series in the subgenus *melo*, and includes 19 species that are cross-compatible and can stimulate fruit set in members of the series *melo*. The species *C. sativus* and *C. hystrix* are included in the subgenus *Cucumis* (Kirkbride, 1993).

Successful utilization of wild species to improve a crop species largely depends on species relationships. To understand the phylogenetic affinities among species, studies on comparative morphology, crossability, chromosome pairing, isozyme variability, and DNA variation in *Cucumis* have been carried out (Table 1). Although the number of groups varied with each study, the basic phylogenetic trees developed from the different experiments were similar. For instance, most of the African *Cucumis* species form a close group (*Anguria*), which is distant from both melon (*C. melo*), and the other isolated species, such as *C. metuliferus* E. Meyer ex Naudin, *C. sagittatus* P., and *C. humifructus* Stent, which are all far from each other. Cucumber (*C. sativus*) is the most distant species within the genus (Perl-Treves and Galun, 1985; Perl-Treves et al., 1985).

In 1989, *Cucumis hystris* Chakr., a wild *Cucumis* species, was rediscovered and identified by Jinfeng Chen et al. in Yunnan Province of China (Chen et al., 1994). This unique species may improve our understanding of phylogenetics in *Cucumis*. *Cucumis hystrix* is the only 2n = 24 *Cucumis* species native to Asia (Figs. 4 and 5). This finding challenges the basic chromosome number theory that

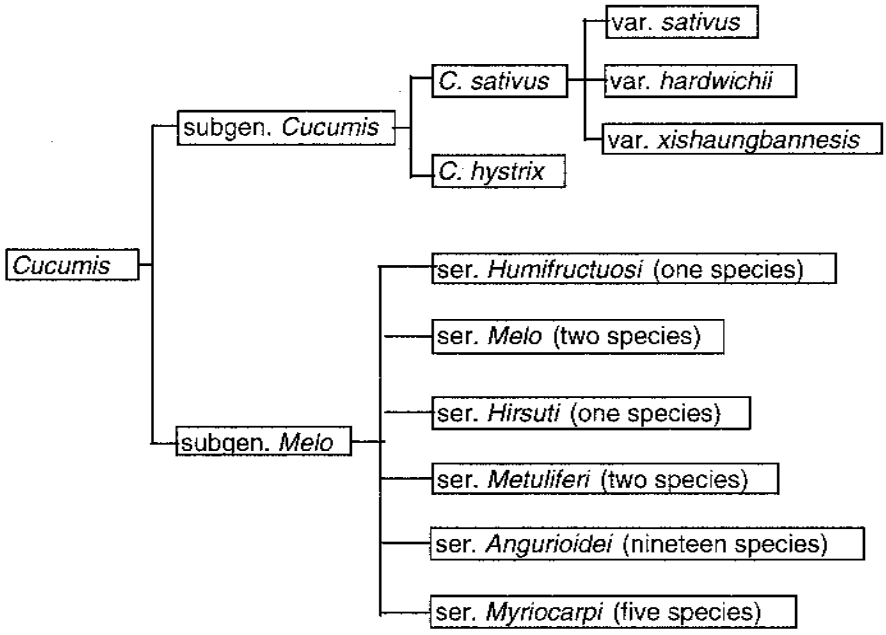


Fig. 3. The current *Cucumis* systematic system proposed by Kirkbride (1993).

Table 1. Grouping of *Cucumis* species by the studies on phylogenetic affinity.

| Methods | No. groups | No. species used | Source |
|---|---|---|---|
| Crossability | 4 | 14 | Deakin et al., 1971 |
| Morphology and chromosome pairing | 5 | 13 (2n = 24) | Singh and Yadava, 1984a |
| Crossability, chromosome pairing, and pollen fertility | 3 | 8 | Singh and Yadava, 1984b |
| ChlDNA variation | 6 | 21 | Perl-Treves and Galun, 1985 |
| Isozyme variability | 6 | 21 | Perl-Treves et al., 1985 |
| Isozyme pattern | 6 | 24 | Puchalski and Robinson, 1990 |

African *Cucumis* have n = 12, and that Asian *Cucumis* have n = 7, which has governed the understanding of systematics and phylogenetics in *Cucumis* for decades. The taxonomic position of *C. hystrix* is of special interest because it bears a morphological resemblance and biochemical affinity to *C. sativus* while its chromosome number is the same as *C. melo* (Chen et al., 1995). Isozyme variability suggested a phylogenetic relationship between *C. hystrix* and both *C. sativus* and *C. melo* (Chen et al., 1997a). For instance, *C. hystrix* has four bands in the pattern of malate dehydrogenase (MDH) (Fig. 6). The first band is shared by all three species, indicating the common property of this genus. The second and third bands are identical with those in *C. melo* and *C. sativus*, respectively, indicating a connection to each species. The fourth band is a unique or specific band that distinguishes *C. hystrix* from both *C. melo* and *C. sativus*.

The first comprehensive crossability analysis of the genus was published by Deakin et al. (1971), who observed that crosses among wild species are frequently possible, but that all attempts to cross any of these with the two cultivated species, *C. sativus* and *C. melo*, failed. Other more successful interspecific hybridization studies between cultivated *Cucumis* crops and the wild relatives are presented in Table 2. However, in practice, most of these results were not repeatable and did not result in fertile hybrids. Our current understanding of the cross relationship based on the previous experiments is presented in Fig. 7. More work is needed for a precise placement of *C. hystrix* in the genus *Cucumis* and a better understanding of its specific relationship. Knowledge of species relationships are the key to success.

## MAJOR PROBLEMS IN INTERSPECIFIC HYBRIDIZATION AND THEIR SOLUTIONS

### Hybridization barriers

Many experiments have indicated the presence of a strong barrier to interspecific hybridization in *Cucumis*. The nature of cross-incompatibility between cultivated *Cucumis* species and their wild relatives is not well understood. Incompatibility is characterized by delayed growth of pollen, or arrested pollen tube growth in the stigma, or inability of pollen tubes to reach the ovules (Kishi and Fukishita, 1969), as well as lack of cell division of the zygote, and abortion of the endosperm (Kishi and Fukishita, 1970).

Several traditional approaches in interspecific hybridization have been used to overcome the hybridization barriers in *Cucumis*.

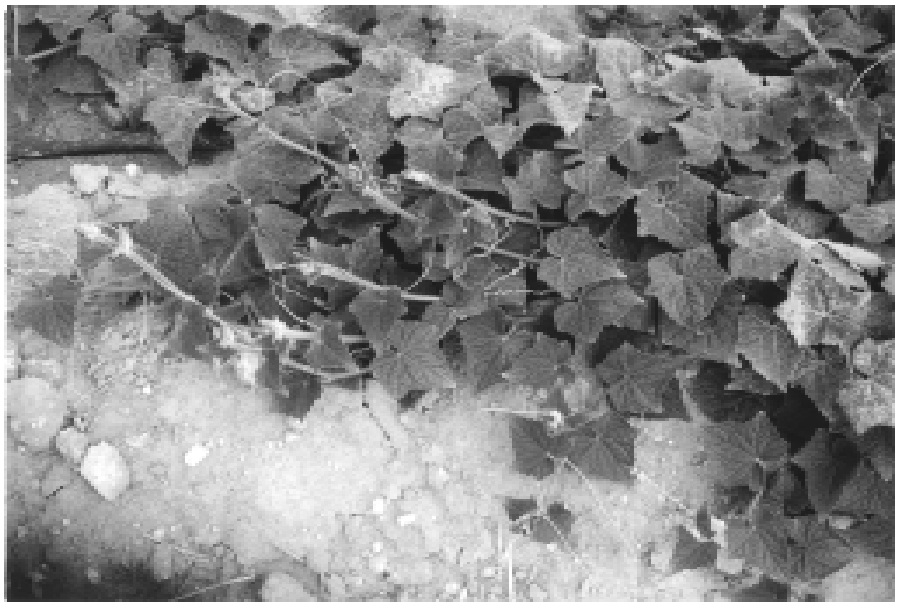

Fig. 4. *Cucumis hystrix* plant in the field.

Fig. 5. Fruits on *Cucumis hystrix* plant.

These include growth regulator application (Custers and Den Nijs, 1986), pollen irradiation (Beharav and Cohen, 1994), use of mentor pollen (Kho et al., 1980), and bud pollination (Chatterjee and More, 1991). Biotechnological techniques such as somatic hybridization have also been suggested as possible tools for overcoming these barriers in *Cucumis* (Chartterjee and More, 1991; Tang and Punja, 1989). Likewise, fusion of *C. sativus* and *C. melo* protoplasts has been attempted, but the results indicated that successful hybridization is still unpredictable (Fellner et al., 1996).

The interspecific hybrid between *C. sativus* and *C. hystrix* (Chen and Staub, 1997) represents an important step in interspecific hybridization in *Cucumis*. If *C. hystrix* and *C. melo* are cross-compatible and if the $F_1$ derived from either interspecific hybridization can be made fertile through crossing and/or chromosome doubling, then *C. hystrix* could act as bridge species between *C. melo* and *C. sativus*.

### Postfertilization abortion and embryo rescue

In higher plants, postzygotic failure of hybrid embryos is often due, not to incompatibility between the parental chromosomes, but incompatibility problems in the endosperm. In such cases, embryos from interspecific hybridization have to be rescued; otherwise, they will fail due to embryo abortion and/or endosperm degeneration. Successful embryo rescue in tissue culture allows further advances in interspecific hybridization.

Embryos can sometimes be rescued, even if they are immature or lack endosperm (Laibach, 1925). In *Cucumis*, fruits with inviable seeds were obtained in the cross between *C. prophetarum* L. and *C. melo* (Singh and Yadava, 1984b). The authors believed that the barriers between these two species were postzygotic. If the embryo rescue technique had been employed, the experiment might have been successful. Interspecific hybrid embryos from reciprocal hybridizations in our studies were rescued successfully (Fig. 1A). Rescued embryos started growing within 3 d, turned green in 5 d, and rooted in 8 d on MS medium (Murashige and Skoog, 1962). About 40% of the embryos developed into whole plants (Chen et al., 1997b).

### Sterility in $F_1$ hybrids

In our review of the literature on utilizing germplasm of wild species for crop improvement, a common problem was sterility in $F_1$ hybrids. In many cases, this sterility was associated with meiotic abnormalities, and was a large obstacle that followed hybridization and hindered utilization.

The ability to cross *C. sativus* and *C. hystrix* offered the promise of moving desirable characters from *C. hystrix* to *C. sativus*. However, self-pollination and backcrossing of the $F_1$ plants to either parent was unsuccessful because the original hybrid was both male- and female-sterile, probably because of the nonfunctional gametes containing odd chromosome numbers. When chromosomes were doubled, each chromosome had a homologous partner for pairing during meiosis; if there were no cytoplasmic incompatibility, the chromosome-doubled $F_1$ hybrid might have produced viable gametes, and fertility restoration was anticipated.

External application of chemical agents is the usual way to double chromosome number. Among various agents, colchicine was one of the antimitotic substances most frequently used for this purpose (Chen and Staub, 1997). Colchicine in an aqueous solution of ≈0.05% to 0.5% (w/v) is believed to be the most effective dosage for many plant species. Since colchicine is poisonous to plants, germinating seeds or young seedlings are often preferred for treatment because they grow rapidly and recover more readily than more mature plants do.

When the experimental material does not respond well to chemical treatment, in vitro chromosome doubling (spontaneous polyploidy as a consequence of tissue culture) could be an alternative (D'Amato, 1977). When and how the polyploidization happened in tissue culture was not entirely clear, but it occurred at a low rate during plant formation from axillary buds (Adelberg et al., 1994), callus (Osifo et al., 1989), and culture of protoplasts (Tabei et al., 1992). Polyploidization can be generalized as a universal phenomenon in melon tissue culture (Ezura et al., 1992), although genotype is an important factor in determining the rate of chromosome doubling (Adelberg and Chen, 1998). In our work with interspecific embryos, ≈7% of the regenerates were chromosome-doubled $F_1$ hybrids after organogenesis (Chen et al., 1998). More importantly, the polyploid regenerates obtained

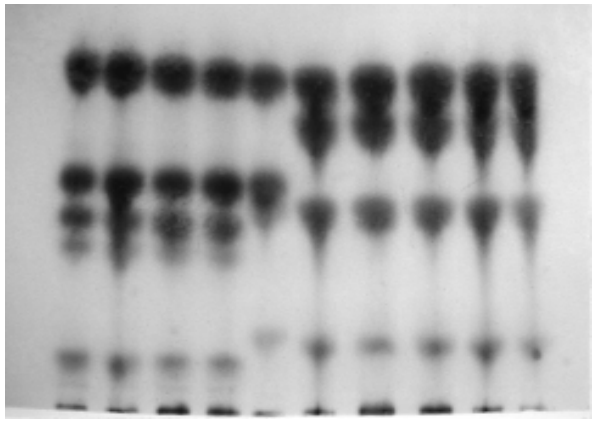

Fig. 6. Zymogram of malate dehydrogenase. Melon group (lines 1–4) has five bands, *Cucumis hystrix* (line 5) and cucumber group (line 6–10) have four bands. The first band in *C. hystrix* is the common band shared by all samples; the second is the same as in the melons while the third is the same as in cucumbers. The fourth band is a unique, and distinguishes *C. hystrix* from both melon and cucumber.

Table 2. Wide-cross attempts between cultivated and wild *Cucumis* species.

| Cross | Result | Source |
|---|---|---|
| *C. sagittatus* x *C. melo* | Embryos only | Deakin et al., 1971 |
| *C. metuliferus* x *C. melo* | Embryos only | Fassuliotis, 1977 |
| *C. sativus* x *C. melo* | Globular stage embryos only | Niemirowicz-Szczytt and Kubicki, 1979 |
| *C. metuliferus* x *C.melo* | Fertile $F_1$ | Norton and Granberry, 1980 |
| *C. prophetarum* x *C. melo* | Fruit with inviable seeds | Singh and Yadava, 1984b |
| *C. zeyheri* x *C. sativus* | Fruit with inviable seeds | Custers and Den Nijs, 1986 |
| *C. sativus* x *C. metuliferus* | Embryos only | Franken et al., 1988 |
| *C. melo* x *C. metuliferus* | Embryos only | Soria et al., 1990 |
| *C. sativus* x *C. hystrix* | Sterile plants (2n and 4n) | Chen et al., 1997b |
| *C. hystrix* x *C. sativus* | Fertile plants (4n) | Chen et al., 1998 |

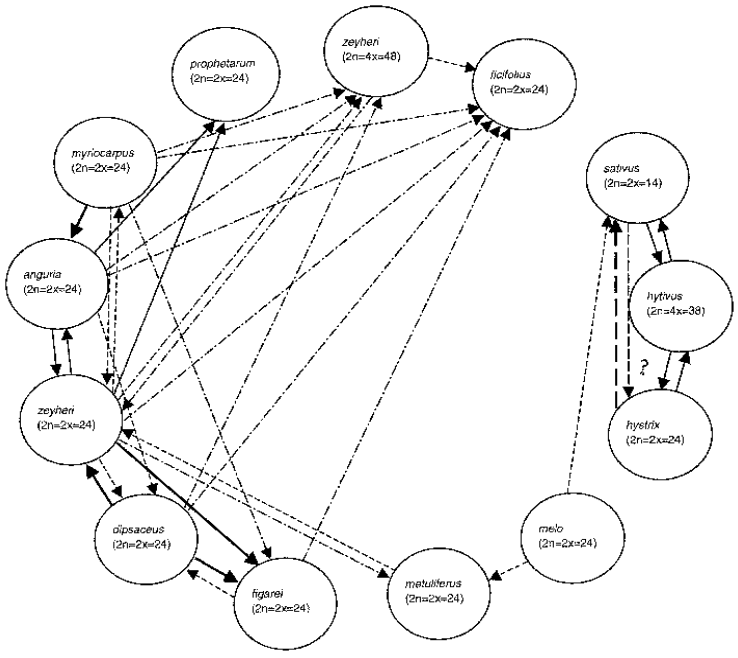


Fig. 7. Polygon of crossability in *Cucumis* species (modified from Nijs and Visser, 1985). Arrows point to the female parent. Moderately to strongly self-fertile and cross-fertile hybrids (**thick solid line**); sparingly self-fertile and moderately cross-fertile hybrids (**thin solid line**); self-fertile, usually not cross-fertile hybrids (**dashed and dotted line**); inviable seeds or seedlings (**dashed line**); self-sterile and cross-sterile hybrids (**thick dashed line**); self-sterile and cross-fertile hybrids (**long dashed line**). Absence of a line indicates that seeded fruits were not obtained; question mark means that the information needs to be confirmed.

through somaclonal variation were non-chimeral and vigorous.

## PERSPECTIVE: POTENTIAL OF UTILIZING OTHER WILD SPECIES

An important long-term objective for *Cucumis* breeders is the introduction of genes from wild relatives. Some wild relatives, such as *C. metuliferus* E. Meyer ex Naudin (nematode resistance) and *C. figarei* Naudin (virus resistance), have long been attractive to scientists. However, progress through conventional crossing has been limited for lack of techniques and knowledge of species relationships. *Cucumis hystrix* is an important species for the investigation of phylogenetic relationships, especially between species with basic chromosome numbers of n = 7 and n = 12. New knowledge gained by investigation of these relationships might eventually enable us to successfully accomplish crosses between cultivated *Cucumis* and the other wild species.

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