Euphytica **128:** 371–374, 2002.

Incompatibility in diploid and tetraploid crosses of *Cucumis sativus* and *Cucumis metuliferus*

S. Alan Walters[1] & Todd C. Wehner[2]
[1]*Department of Plant, Soil, and General Agriculture, Southern Illinois University, Carbondale, IL 62901-4415, U.S.A.;* [2]*Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609, U.S.A.*

Received 3 January 2001; accepted 26 April 2002

Key words: African horned cucumber, cucumber, *Cucumis sativus, C. metuliferus*, interspecific hybridization, polyploidy, vegetable breeding

Summary

The African horned cucumber (*Cucumis metuliferus* Naud.; 2x = 2n = 24) contains genes that can confer resistance to many important cucumber (*C. sativus* L.; 2x = 2n = 14) pests [e.g., root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood]. Cucumber is highly susceptible to this root-knot nematode species, and a recent screening of *C. sativus* accessions in the U.S. National Plant Germplasm collection did not identify sources of resistance. Thus, autotetraploids of *Cucumis sativus* and *C. metuliferus* were created to recover fertile resistant interspecific progeny. Autotetraploids were obtained at the highest rate when seeds were immersed in 0.5% colchicine for a period of 6 to 8 hrs. Treatment durations less than 6 hrs produced few tetraploids, and durations of 10 hrs or more were lethal to seeds or developing seedlings. Crosses between *C. sativus* and *C. metuliferus* were made using diploid and tetraploid lines in all possible combinations, including reciprocals. Fruit development occurred in crosses when diploid and tetraploid *C. sativus* were used as the female parent. However, seeds developed only in fruit of *C. sativus* (4n) × *C. metuliferus* (2n) crossings. Seeds from these crosses, however, were flat and not viable. No fruit development occurred in crosses where *C. metuliferus* was used as the female parent.

Introduction

Significant progress has been made in breeding for root-knot resistance in many important horticultural crops such as common bean (*Phaseolus vulgaris* L.), southern pea (*Vigna unguiculata* L.), tomato (*Lycopersicon esculentum* Mill.), and sweetpotato [*Ipomoea batatas* L. (Lam.)] (Sasser & Kirby, 1979). However, no such progress has been made in cucumber (*Cucumis sativus* L.). Attempts to produce viable interspecific hybrids between cucumber and several related root-knot nematode resistant wild *Cucumis* species (e.g., African horned cucumber, *C. metuliferus* Naud.) have failed (Deakin et al., 1971; Fassuliotis, 1977; Fassuliotis, 1979). Interspecific hybrids in *Cucumis* have been obtained (Chen & Adelberg, 2000; Dane, 1991; Deakin et al., 1971; Fassuliotis, 1977; Fassuliotis & Nelson, 1988). But interspecific hybrids have not been obtained between *C. sativus* and *C. metuliferus*. Franken et al. (1988) found that hybrid plant development between these two species is restricted by strong barriers to crossing including pollen tube growth arrestment in the stylar region of the pistil or abortion of the hybrid embryo at the globular stage. Previous research (Norton & Granberry, 1980; Walters et al., 1993; Wehner et al., 1991) indicates that the African horned cucumber is resistant to several root-knot nematode species. The incorporation of this resistance into cultivated cucumber to control root-knot nematodes would be beneficial to growers, and genetic resistance would contribute to reduced nematicide use.

Successful exchange of genes between *C. sativus* and related wild species is difficult using conventional hybridization techniques, since the chromosome number of *C. sativus* is different (n = 7) from most other species of *Cucumis* (n = 12) (Chen & Adelberg, 2000; Dane, 1991; Deakin et al., 1971; Robinson & Decker-

Walters, 1997). Several attempts have been made to introduce economically important characteristics from wild into cultivated *Cucumis* species (*C. sativus* and *C. melo* L.) with little success (Deakin et al., 1971; Dane, 1991). Only recently has interspecific hybridization been achieved between *Cucumis sativus* and a wild relative (*C. hystrix* Chakr., 2n = 24) (Chen et al., 1998). Interspecific hybridization in this cross was obtained by first rescuing the F_1 hybrid following the pollination of *C. sativus* by *C. hystrix*; however, the resulting F_1 hybrid was both male- and female-sterile. Fertility was restored by making reciprocal crosses which successfully doubled the number of chromosomes in the progeny. Successful fruit set with viable seeds occurred when pollen grains from the progeny were placed on female flowers of *C. hystrix* (Chen et al., 1998). However, embryo rescue has not been successful following pollinations of *C. sativus* by *C. metuliferus* (den Nijs & Custers, 1990).

Induced polyploidy has facilitated gene transfer between some related species when crossed at different ploidy levels (Hadley & Openshaw, 1980; Stoskopf et al., 1993). The objectives of this research were to create fertile autotetraploids of *C. sativus* and *C. metuliferus*, and to use these progeny in interspecific hybridization using all cross combinations of parental lines and derived autotetraploids.

Materials and methods

Development of polyploids. Smith & Lower (1973) reported that diploid cucumber seeds (2n = 14) soaked in 0.5% colchicine for 6 to 24 hrs at 21 °C produced tetraploids (4n = 28). Seeds of *C. sativus* 'Sumter' and *C. metuliferus* (PI 482454) were immersed in 0.5% colchicine for 0 to 20 hrs to determine the optimum immersion time for each species to induce tetraploidy. *Cucumis metuliferus* (PI 482454) was utilized as this accession has resistance to several root-knot nematode species (Walters et al., 1993). The experiment consisted of three replications of ten seeds of each species (*C. sativus* and *C. metuliferus*) soaked in 0.5% colchicine for one of 11 durations (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20 hrs). Before treatment, seed were pre-germinated by soaking in distilled water for 24 hrs (*C. sativus*) or 72 hrs (*C. metuliferus*) at 26 °C. After colchicine treatment, seeds were rinsed twice in deionized water and planted in 50-mm diameter clay pots containing a peat-lite mix (Scotts-Sierra Horticultural Products Company, Marysville, Ohio). Greenhouse temperatures averaged 24 to 32 °C (day) and 20 to 24 °C (night). Pots were irrigated twice daily and fertilized once a week using Peter's® 20-20-20 (N-P-K) (W.R. Grace & Co., Fogelsville, PA).

Ploidy evaluation was conducted on the seedlings that survived four weeks after planting. The acetocarmine technique (Sass, 1958; Smith, 1947) was used on root-tip smears to determine the ploidy level of each plant. Root tips, about 1 cm in length, were placed into Farmer's fixative (3 parts 95% ethanol: 1 part glacial acetic acid) for 24 hrs at room temperature, and then rinsed with distilled water. Root tips were then hydrolyzed in 5N HCl for 1.5 hrs, and rinsed twice with distilled water. The 1-mm end of each root tip was cut and placed onto a microscope slide. Several drops of a 1% acetocarmine stain (1g carmine in 100 ml of boiling 45% acetic acid) were then added to the slide. The slide was heated gently, and the root tissue was teased apart using a rusty dissecting needle (to provide iron to the solution). A cover slip was placed over the root tissue on the slide, and the cells were dispersed by tapping the eraser end of a pencil on the cover slip. The slide was gently reheated, placed between the folds of a paper towel, and cells on the slide were flattened by applying slight pressure to the surface of the cover slip. Finally, root-tip cells were examined under a microscope to determine the ploidy level of the plant.

The goal of this experiment was to determine the optimal immersion time for the creation of *C. sativus* and *C. metuliferus* tetraploids. This information would then be used to produce a sufficient number of *C. sativus* and *C. metuliferus* tetraploids for attempts at interspecific hybridization between these two species.

Interspecific hybridization. Initially, crosses were made using conventional pollination techniques for cucurbits (Robinson & Decker-Walters, 1997; Whitaker & Davis, 1962) to develop diverse *C. sativus* and *C. metuliferus* materials. For *C. sativus*, a double cross hybrid was made between Gy 14 × PI 183967 [*C. sativus* var. *hardwickii* (R.) Alef.] and 'Slice' × 'Wis. SMR-18'. *Cucumis sativus* var. *hardwickii* is genetically and morphologically distinct from *C. sativus* var. *sativus*, but is cross compatible with *C. sativus* var. *sativus* (Robinson & Decker-Walters, 1997). *Cucumis sativus* var. *hardwickii* was used to increase the diversity of the germplasm base for *C. sativus*. For *C. metuliferus*, a double cross hybrid was formed from PI 482454 × PI 482449, and PI 482450 × PI 482442. Interspecific crosses were made using this di-

verse material. Walters et al. (1993) provides specific characteristics about the cultigens used to develop the diverse *C. sativus* and *C. metuliferus* germplasm.

This experiment was conducted with the goal of obtaining fertile progeny by crossing diploids and tetraploids of the diverse *Cucumis sativus* with diploids and tetraploids of the diverse *C. metuliferus*. Initially, 50 seeds of each diverse *C. sativus* and *C. metuliferus* hybrid were pregerminated as described previously, and then immersed in 0.5% colchicine for 8 hrs. This immersion time period was determined in the previous experiment as the time to kill approximately half of the seeds and to produce a sufficient number of tetraploids. After soaking, seeds were rinsed twice in deionized water and planted in 50-mm-diameter (volume) clay pots containing the same peat-lite mix as described previously. The ploidy level of surviving plants was determined, and tetraploid plants were then transplanted into 150-mm-diameter (1750-cm^3 volume) plastic pots containing the peat-lite mix in a greenhouse. At the same time, diploid hybrids of diverse *C. sativus* and *C. metuliferus* were also planted into the same size pots containing the identical soil mix as described for the tetraploids.

The greenhouse temperature was approximately 35 °C day and 24 °C night. Plants were irrigated twice daily using drip irrigation with fertilizer injection. With the advent of flowering, crosses (including reciprocals) were made in all combinations using diverse diploid and tetraploid hybrids of *C. sativus* with diverse diploid and tetraploid hybrids of *C. metuliferus*. Crosses (and reciprocals) were made using diploid *C. sativus* by diploid *C. metuliferus*, diploid *C. sativus* by tetraploid *C. metuliferus*, tetraploid *C. sativus* by diploid *C. metuliferus*, and tetraploid *C. sativus* by tetraploid *C. metuliferus*.

Results and discussion

Development of polyploids. Seed immersion in 0.5% colchicine for 10 hrs or longer resulted in seedlings that did not develop normally (i.e., hypocotyls and radicals were abnormal). Roots of plants treated with colchicine for 10 hrs or more were thick and grew poorly, and often resulted in the death of seedlings over several weeks (data not presented). Best results for both *C. sativus* and *C. metuliferus* were obtained when seeds were immersed in 0.5% colchicine for 6 to 8 hours (data not presented). The greatest percentage of tetraploids was produced at 6 and 8 hours for *C. sativus* (20%) and 8 hours for *C. metuliferus* (40%). Less than a 6-hour duration produced 20% and 33% or less tetraploids for *C. sativus* and *C. metuliferus*, respectvely. Kubicki (1962) found the optimum duration for the production of tetraploids using cucumber seeds soaked in a 1% and 0.4 to 0.7% solution of colchicine was 6 to 24 hours and 12 to 24 hours, respectively. Our data recapitulate their results. Therefore, an 8-hour soak in a 0.5% colchicine solution allowed for the sufficient production of tetraploids of *C. sativus* and *C. metuliferus* for our purposes.

Interspecific hybridization. Regardless of the ploidy levels of the parents, when female flowers of *C. metuliferus* was pollinated with *C. sativus* no fruit development occurred (data not presented). When *C. sativus* was used as the female parent in crosses with *C. metuliferus*, a small percentage (19%) of the plants produced fruit. However, in most cases, seed did not develop in the fruit, and those that did develop in *C. sativus* (female parent-4n) × *C. metuliferus* (male parent-2n) were flat and not viable. In the *C. sativus* (4n) × *C. metuliferus* (2n), seed set was obtained in 5 of the 31 fruit that had developed to be at least 5 cm in diameter (data not presented). Franken et al. (1988) obtained similar results with no seed development occurring out of 78 pollinations made between *C. metuliferus* (female parent) × *C. sativus* (male parent); however, of the 78 pollinations made when *C. sativus* (female parent) was crossed with *C. metuliferus* (male parent), two fruit developed non-viable seeds (or 3% of pollinations).

Fruit development in some instances may have been due to parthenocarpic fruit enlargement. Parthenocarpy is a common occurrence in cucumber (Tatioglu, 1992), especially plants grown in the greenhouse as they reach the end of their life cycle. It is likely that the *C. sativus* hybrid had parthenocarpic tendencies, since unpollinated fruit in that group developed at about the same frequency (data not presented). One of the parents utilized in the development of the diverse *Cucumis sativus*, Gy 14, often sets parthenocarpic fruit under greenhouse conditions.

Cucumis sativus is not closely related to any of the spiny-fruited, 12-chromosome species from Africa, such as *C. metuliferus* (Chen & Adelberg, 2000; Deakin et al., 1971; den Nijs & Custers, 1990). However, commercial *Cucumis sativus* germplasm would benefit from the incorporation of genes for multiple disease and insect resistances present in *C. metuliferus* (den Nijs & Custers, 1990). Within *Cucumis*, *C. melo*,

C. sativus, and *C. metuliferus* appear to be isolated species (den Nijs & Custers, 1990). The chromosome number of *C. sativus* (n = 7), along with their fruit morphology of scattered short, sharp, stiff spines, and natural distribution in India, indicate that *C. sativus* is not closely related to *C. melo*, *C. metuliferus*, or other *Cucumis* species from Africa (Deakin et al., 1971). Utilizing cluster analysis based upon horizontal starch gel protein electrophoresis to evaluate variation, Staub et al. (1992) found that the clustering procedure separated *C. sativus* from seven African *Cucumis* species that were studied. None of the crosses (or reciprocal crosses) of diploid or tetraploid *C. sativus* with diploid or tetraploid *C. metuliferus* were successful in producing viable seeds in our study. It appears that the two species, although somewhat related, are reproductively isolated. *Cucumis sativus* probably originated in India, while *C. metuliferus* probably originated in southern Africa (Dane, 1991). Evidence of divergence in these two species is indicated by the different number of chromosomes in *C. sativus* compared to *C. metuliferus*, and lack of interspecific hybridization.

Acknowledgements

The use of trade names in this publication does not imply endorsement by the NCARS of the products named, nor criticism of similar ones not mentioned. This paper is based on a portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree.

References

Chen, J.F., J.W. Adelberg, J.E. Staub, H.T. Skorupska & B.B. Rhodes, 1998. A new synthetic amphidiploid in *Cucumis* from a *C. sativus* × *C. hystrix* F_1 interspecific hybrid. In: J. McCreight (Ed.), Cucurbitaceae '98 – Evaluation and Enhancement of Cucurbit Germplasm. ASHS Press, Alexandria, Va.

Chen, J.F. & J. Adelberg, 2000. Interspecific hybridization in *Cucumis*-progress, problems, and perspectives. HortScience 35: 11–15.

Dane, F., 1991. Cytogenetics of the genus *Cucumis*. In: T. Tsuchiya & P.K. Gupta (Eds.), Chromosome Engineering in Plants: Genetics, Breeding, and Evolution, Part B. Elsevier Science Publishing Company, Inc., New York.

Deakin, J.R., G.W. Bohn & T.W. Whitaker, 1971. Interspecific hybridization in *Cucumis*. Econ Bot 25: 195–211.

den Nijs, A.P.M. & J.B.M. Custers, 1990. Introducing resistances into cucumbers by interspecific hybridization. In: D.M. Bates, R.W. Robinson & C. Jeffrey (Eds.), Biology and Utilization of the Cucurbitaceae. Cornell Univ. Press, Ithaca, NY.

Fassuliotis, G., 1977. Self-fertilization of *Cucumis metuliferus* Naud. and its cross-compatibility with *C. melo* L. J Amer Soc Hort Sci 102: 336–339.

Fassuliotis, G., 1979. Plant breeding for root-knot nematode resistance. In: J.D. Sasser & C.C. Carter (Eds.), Root-Knot Nematodes (*Meloidogyne* species): Systematics, Biology and Control. Academic press, New York.

Fassuliotis, G. & B.V. Nelson, 1988. Interspecific hybrids of *Cucumis metuliferus* × *C. anguria* obtained through embryo culture and somatic embryogenesis. Euphytica 37: 53–60.

Franken, J., J.B.M. Custers & R.J. Bino, 1988. Effects of temperature on pollen tube growth and fruit set in reciprocal crosses between *Cucumis sativus* and *C. metuliferus*. Plant Breed 100: 150–153.

Hadley, H.H. & S.J. Openshaw, 1980. Interspecific and intergeneric hybridization. In: W.R. Fehr & H.H. Hadley (Eds.), Hybridization of crop plants. American Society of Agronomy, Madison, Wis.

Kubicki, B., 1962. Polyploidy in muskmelons (*Cucumis melo* L.) and cucumbers (*Cucumis sativus* L.). Genetica Polonica 3: 161–179.

Norton, J.D. & D.M. Granberry, 1980. Characteristics of progeny from an interspecific cross of *Cucumis melo* with *Cucumis metuliferus.* J Amer Soc Hort Sci 105: 174–180.

Robinson, R.W. & D.S. Decker-Walters, 1997. Cucurbits. CAB International, Wallingford, UK.

Sass, J.E., 1958. Botanical Microtechnique. Iowa State College Press, Ames.

Sasser, J.N. & M.F. Kirby, 1979. Crop cultivars resistant to root-knot nematodes, *Meloidogyne* species. Cooperative Publication of North Carolina State University Department of Plant Pathology and United States Agency for International Development, North Carolina State University Graphics, Raleigh, N.C.

Smith, L., 1947. The acetocarmine smear technique. Stain Technol 22: 17–31.

Smith, O.S. & R.L. Lower, 1973. Effects of induced polyploidy in cucumbers. J Amer Soc Hort Sci 98: 118–120.

Staub, J.E., L.D. Knerr & D.J. Holder, 1992. Phylogenetic relationships among several African *Cucumis* species. Can J Bot 70: 509–517.

Stoskopf, N.C., D.T. Tomes & B.R. Christie, 1993. Plant Breeding: Theory and Practice. Westview Press, Inc., Oxford, UK.

Tatioglu, T., 1992, Cucumber *Cucumis sativus* L. In: G. Kalloo & B.O. Bergh (Eds.), Genetic Improvement of Vegetable Crops. Pergamon Press, Oxford, U.K.

Walters, S.A., T.C. Wehner & K.R. Barker, 1993. Root-knot nematode resistance in cucumber and horned cucumber. HortScience 28: 151–154.

Wehner, T.C., S.A. Walters & K.R. Barker, 1991. Resistance to root-knot nematode in cucumber and horned cucumber. J Nematol 23: 611–614.

Whitaker, T.W. & G.N. Davis, 1962. Cucurbits. Leonard Hill, London, 250 pp.