Morpho-agronomic characteristics of the interspecific hybrid *Cucurbita ficifolia* × *C. maxima*

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The aim of the investigation was to describe some of the main morpho-agronomic characteristics of the interspecific hybrid *Cucurbita ficifolia* × *C. maxima*, and to define the traits which can be used for distinguishing this hybrid from the parental species. The hybrid was obtained using emryo-rescue technique. The plants were extremely vigorous and were growing and flowering until the first frost. The ratio male versus female flowers was ranging between 1 : 40 and 1 : 80. Most of the male and female flowers were sterile or partly sterile. The majority of the analysed traits were intermediate regarding the parental species. The most appropriate traits for the determination of the interspecific nature were the leaf margin, the shape and colour of ripe fruits and the shape of fruit stalks. The fruit exterior was extremely hard (similarly to the one of *C. ficifolia*) and cracked in several places.

Keywords: interspecific hybridisation, *Cucurbita ficifolia*, *Cucurbita maxima*, flowering, morphology

INTRODUCTION

The genus *Cucurbita* L. originated in America (Purseglove 1977). Most of the species are monoecious annual or perennial climbing herbs. The stems can be long-running, intermediate or short. Plants with short stems have a bushy appearance. Leaves are simple (not divided into leaflets), alternate, shallow to deeply lobed.

Flowers are relatively large and solitary. Their colour varies from light to dark yellow. Calyx and corolla are usually five-lobed. The staminate flowers appear in most instances before the female ones. Among the exceptions are some of the interspecific hybrids. Flowers have long peduncles and the male reproductive organ consists of three stamens which are fussed together. Pollen is sticky. The pistillate flowers are short-pedunculate and consist of 3-5 carpels. The ovary is inferior, oblong or discoid, with numerous ovules. The style is thick and the stigma is usually divided in three parts, each of them having two lobes. Fruits are fleshy, indehiscent pepos which vary in size, shape and colour, depending on species and genotype. Most of the fruits have numerous flat seeds.

The genus *Cucurbita* includes wild and cultivated species. The cultivated species are: *C. argyrosperma* Huber, *C. ficifolia* (Bouché) Britton, *C. maxima* Duch. ex Lam., *C. moschata* (Duch. ex Lam.) Duch. ex Poir. and *C. pepo* L. The main problems associated with their production are susceptibility to pests and diseases (viral diseases are becoming a very serious problem), and susceptibility to drought, excess humidity and low temperatures. The genetic variability within one species is too narrow to solve these problems. One of the solutions is to create additional variation by interspecific hybridisation.

Interspecific hybrids between some of the *Cucurbita* species appear spontaneously in nature (Decker-Walters et al. 1990; Wilson 1990; Wilson et al. 1994; Wessel-Beaver et al. 2004). Among the most 'flexible' species is *C. argrosperma*. It has been used as one of the parents in several artificially conducted interspecific combinations such as *C. lundelliana* L. H. Bailey, *C. moschata*, *C. okeechobeensis* (Small) L. H. Bailey and *C. pepo* (Robinson and Decker-Walters 1997).

C. ficifolia and *C. maxima* are morphologically very different species. There are also significant genetic and phylogenetic differences what was demonstrated by Sanjur et al. (2002), and differences in genome size (Šiško et al. 2003). Regarding the genome size (measured by flow cytometry), higher values were obtained for *C. ficifolia*. This is an old species which has been cultivated since ancient times in the highlands of Mexico, Central and South America. The cultivation of *C. maxima* started in South America (Purseglove 1979) and from there it spread to other parts of the world. Both species have 2n=40 chromosomes (Robinson and Decker-Walters 1997). According to data presented in the publication of Fedorov (1969), the chromosome number of some genotypes can be different, due to aneuploidy.

Hybrids between *C. ficifolia* and *C. maxima* are new and no systematic data about their morphology can be found

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in the literature. The main reason for including *C. ficifolia* was its adaptation to a cool climate and high elevations. The aim of this paper is to describe the main morpho-agronomic traits and define the traits which can be used for distinguishing this interspecific hybrid from the parental species.

MATERIAL AND METHODS

The investigation was associated with the breeding programme aimed at creating genotypes which would be highly productive, with good quality and suitable for organic production. The program was largely based on interspecific hybridisation, and involved all cultivated *Cucurbita* species. The final aim of the hybridisation was to create a series of poly-species hybrids which would be used in the recurrent selection programme.

The first crosses between *C. ficifolia* and *C. maxima* were conducted in 1999, and failed. The female component was *C. ficifolia* (the accession Grif 9447) whereas the male was *C. maxima* (a local population characterised by relatively small green, red-orange-green, grey-green and red-orange fruits). A year later, these crosses were repeated (using the same parents) and combined with the embryo-rescue technique. The whole procedure was described by (Šiško et al. 2003). The embryos developed shoots and roots within 2-3 weeks. The developing plantlets were maintained by subculturing until February 2004 when they were transferred into pots (placed in a screenhouse), and three months later to the field (a fertile plot in near Brežice, in the south-eastern part of Slovenia). The plants were weak and very sensitive to the changing weather conditions (at the beginning of May). The total number of the survived hybrid plants was 17 (12 plants died). In the same field, there were also the parental plants (30 individuals of each parent).

The morpho-agronomic evaluation took place from August 12 to October 16 (2004). The evaluated qualitative traits were: the length of the vegetation period, growth vigour, shape of leaves, characteristics of the leaf edge, shape of (male and female) flowers, shape and colour of fruits, shape and colour of seeds, and shape of fruit stalk. The analysed quantitative traits were (Fig. 1): (a) length of the leaf petiole, (b) length of the main vain (connecting the central vein junction with the tip of leaf lamina), (c) length of the second largest (lateral) vein, (d) lamina width, (e) length of the secondary vein running along lamina edge, (f) width of the central lobe, (g) distance between the attachment of the lamina with the petiole and the base of the first incision next to the main vein, and (h) the ratio b/d. The measurements were taken from 26 to 28 August (2004). The statistical analysis was based on a random sample of 50-61 mature leaves and included minimum, maximum, mean value, standard deviation, standard error of mean, C.V.(%) and correlation coefficients.

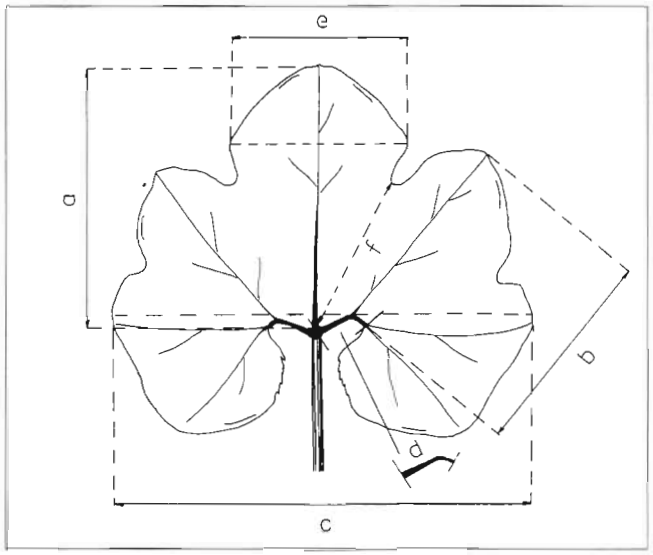

Fig. 1. Investigated leaf lamina parameters: a - length of the main vain vein (connecting the central vein junction with the lamina tip); b - length of the second largest (lateral) vein; c - lamina width; d – length of the secondary vein running along lamina margin; e - width of the central lobe; f - distance between the attachment of the lamina with the petiole and the base of the first incision next to the main vein.

RESULTS AND DISCUSSION

Length of the vegetation period and growth vigour

The exact determination of the length of the vegetation period in the temperate climate is not possible because of frost. One of the indicators of this trait can be the length of the flowering period. The appearance of new flowers can be considered as an indication that the plant continues to grow. The male parent (*Cucurbita maxima*) stopped flowering in mid September (the last flower was observed on September 17). However, there were some materials (hybrid populations) of the same species which continued to produce flowers until October 17. The last flower of the female parent (*C. ficifolia*) was observed on November 6. The interspecific hybrids (*C. ficifolia* × *C. maxima*) were flowering until the first frost (on November 16). The hybrid plants were much more vigorous than the parents. Their root system was much more developed than the one of the parental species. The plants remained perfectly healthy and there were no signs of diseases and/or other damages until the first frost.

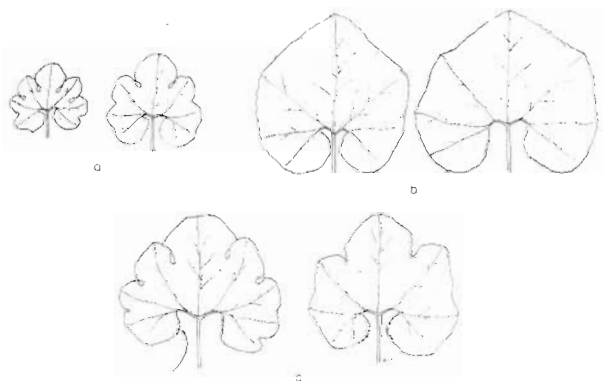

Fig. 2. Leaf lamina shape: a – *Cucurbita ficifolia*, b – *C. maxima*, c – F_1 hybrid (*C. ficifolia* × *C. maxima*). The arrow indicates the part of the lamina margin which can be used as an indicator of the interspecies hybrid origin.

Leaves

Qualitative traits

The hybrid plants were characterised by lobed laminas, similarly to *C. ficifolia*, although not so deeply (Fig. 2). If there were no lobes, the outline of leaf laminas would be almost identical to *C. maxima*. The leaf margin was serrate. The teeth were longer than those of the parents (Fig. 3). The largest teeth were in the region where the margin approached the junction of the petiole with the lamina (Fig. 2).

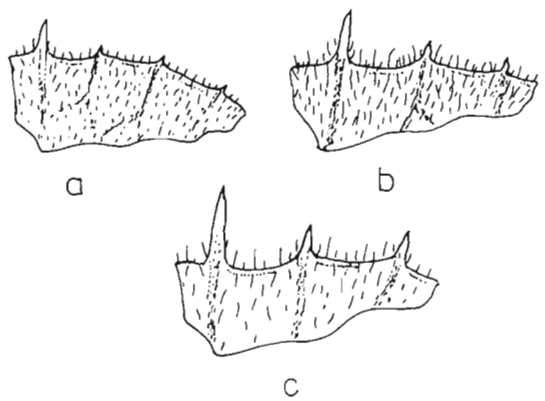

Fig. 3. Detail of lamina edge: a – *C. ficifolia*, b – *C. maxima*, c – F_1 hybrid (*C. ficifolia* × *C. maxima*).

Quantitative traits

The average values of the studied traits of hybrids were in most instances between the two parental species, but much closer to those of *C. maxima* (Table 1). The lowest average values were obtained for *C. ficifolia*. The exception, however, was the ratio between the length of the main vein (connecting the central vein junction with the lamina), and the lamina width. Most of the highest average values belonged to *C. maxima*. The exceptions were the length of the second largest (lateral) vein, the length of the secondary vein running along lamina margin (Fig. 1) and the ratio mentioned before. This ratio expressed the lowest variation (in all three materials). The highest variation was established in *C. ficifolia* for the length of the secondary vein running along the lamina margin (C.V. = 41.936).

Table 1. Mean values and variation of the main leaf traits of *Cucurbita ficifolia*, *C. maxima* and F_1 hybrid (*C. ficifolia* × *C. maxima*).

Female parent (*Cucurbita ficifolia*)							
Trait	N	Min.	Max.	Mean	SEM	St.Dev.	C.V.(%)
L. Pet. L.	51	14.5	29.1	20.369	0.447	3.190	15.661
M. V.	51	9.6	18.8	13.324	0.234	1.669	12.524
L. V.	51	7.2	12.4	9.853	0.165	1.175	11.928
Lam. W.	51	15.1	23.2	19.429	0.300	2.146	11.046
S. V. E.	51	1.6	9.9	2.639	0.155	1.107	41.936
C. L. W.	51	6.7	11.5	9.051	0.145	1.037	11.453
Incis. B.	51	3.7	9.0	6.820	0.136	0.970	14.218
M.V./Lm.W.	51	0.61	0.98	0.687	0.008	0.061	8.880
Male parent (*Cucurbita maxima*)							
Trait	N	Min.	Max.	Mean	SEM	St.Dev.	C.V.(%)
L. Pet. L.	52	16.5	50.5	34.810	1.363	9.827	28.230
M. V.	52	13.1	27.8	19.212	0.404	2.912	15.159
L. V.	52	9.5	17.5	14.117	0.259	1.866	13.215
Lam. W.	52	19.8	35.5	28.806	0.528	3.811	13.229
S. V. E.	52	2.3	5.4	3.815	0.108	0.778	20.406
Incis. B.	50	10.9	18.5	15.508	0.253	1.790	11.542
M.V./Lm.W.	52	0.59	0.98	0.668	0.009	0.064	9.545
Hybrid progeny (*C. ficifolia* × *C. maxima*)							
Trait	N	Min.	Max.	Mean	SEM	St.Dev.	C.V.(%)
L. Pet. L.	61	17.8	38.2	29.144	0.645	5.037	17.284
M. V.	61	14.1	27.1	19.190	0.362	2.829	14.745
L. V.	61	11.5	20.2	14.897	0.272	2.122	14.244
Lm. W.	61	21.4	36.5	28.072	0.474	3.704	13.195
S. V. E.	61	2.5	6.1	4.292	0.085	0.661	15.403
C. L. W.	61	8.5	17.7	12.603	0.237	1.855	14.719
Incis. B.	51	7.3	14.2	10.788	0.251	1.791	16.604
M.V./Lm.W.	61	0.45	0.82	0.685	0.008	0.061	8.984

SEM – standard error of mean; L. Pet. L. – leaf petiole length; M. V. - length of the main vain (connecting the central vein junction with the lamina tip); L. V. - length of the second largest (lateral) vein; Lm. W. - lamina width; S. V. E. - length of the secondary vein running along lamina margin; C. L. W. - width of the central lobe; Incis. Base - distance between the attachment of the lamina with the petiole and the base of the first incision next to the main vein; M. V./ Lm. W. represents the ratio.

Relationships among studied quantitative traits

Most of the correlation coefficients were similar in all three materials (i.e. parental species and the hybrid progeny - Table 2). The exceptions were the correlation coefficients between the length of the secondary vein running along the lamina margin and the rest of the traits estimated for *C. ficifolia* (their values were lower). When taking into consideration all correlation coefficients, the hybrid progeny appeared to be more similar to *C. maxima*.

Flowers

Female flowers

The number of female flowers per plant varied from 25 to more than 40. Most of the qualitative traits of the female flowers of the hybrid plants was intermediate (Fig. 4). The most obvious intermediate trait was the shape of the corolla. The base of the corolla of *C. maxima* was relatively broad, while *C. ficifolia* had a shape of a funnel. Another intermediate trait which could be very useful for differentiating the interspecific hybrids (*C. ficifolia* × *C. maxima*) was the region between the ovary and the base of the calyx (it was relatively thick in *C. ficifolia* and very thin in *C. maxima*).

Male flowers

The corolla shape of the male flowers of hybrid plants was intermediate and very similar to the one of the female flowers (Figs. 4, 5). The male flowers were relatively rare and most of them were sterile or partly sterile. Ratio male : female flowers was ranging between 1 : 40 and 1 : 80.

Fruits

The hybrid plants started to develop fruits relatively late. The first fruits appeared in the last decade of July, approximately two months after the appearance of the first female flowers. On November 6 (2004), the total number of fruits (of 17 plants) having the diameter at least 10 cm was 154. This means that the average number of fruits per plant was 9.06. The largest fruit was 14 cm long and its diameter was 21.5 cm.

Table 2. Phenotypic correlation coefficients among studied leaf traits of *Cucurbita ficifolia*, *C. maxima* and F_1 hybrid (*C. ficifolia* × *C. maxima*).

Female parent (*Cucurbita ficifolia*), N = 51

	A	B	C	D	E	F	G
B	0.466**						
C	0.599**	0.764**					
D	0.592**	0.723**	0.841**				
E	0.140	0.043	0.140	0.099			
F	0.276	0.693**	0.631**	0.742**	-0.083		
G	0.448**	0.434**	0.511**	0.636**	0.280*	0.345*	
H	-0.082	0.514**	0.037	-0.218	-0.065	0.056	-0.165

Male parent (*Cucurbita maxima*), N = 52

	A	B	C	D	E	F	G
B	0.508**						
C	0.540**	0.775**					
D	0.585**	0.820**	0.794**				
E	0.352*	0.611**	0.529**	0.741**			
F	-	-	-	-	-		
G	0.640*	0.762**	0.799**	0.807**	0.565**	-	
H	-0.029	0.421**	0.138	-0.159	-0.097	-	0.094

Hybrid progeny (*C. ficifolia* × *C. maxima*), N = 61

	A	B	C	D	E	F	G
B	0.280*						
C	0.371**	0.743**					
D	0.381*	0.779**	0.877**				
E	0.588**	0.465**	0.635**	0.701**			
F	0.238	0.683**	0.523**	0.685**	0.542**		
G	0.327*	0.510**	0.665**	0.690**	0.551**	0.517**	
H	-0.092	0.448**	-0.075	-0.206	-0.265*	0.090	-0.097

* $P < 0.05$, ** $P < 0.01$

A - leaf petiole length; B - length of the main vain (connecting the central vein junction with the lamina tip); C - length of the second largest (lateral) vein; D - lamina width; E - length of the secondary vein running along lamina margin; F - width of the central lobe; G - distance between the attachment of the lamina with the petiole and the base of the first incision next to the main vein; H - M. V./ Lm. W.

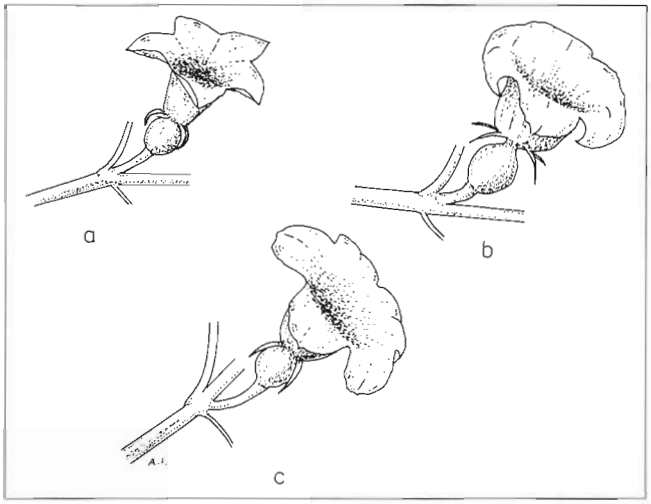

Fig. 4. Female flowers: a – *C. ficifolia*, b – *C. maxima*, c – F_1 hybrid (*C. ficifolia* × *C. maxima*).

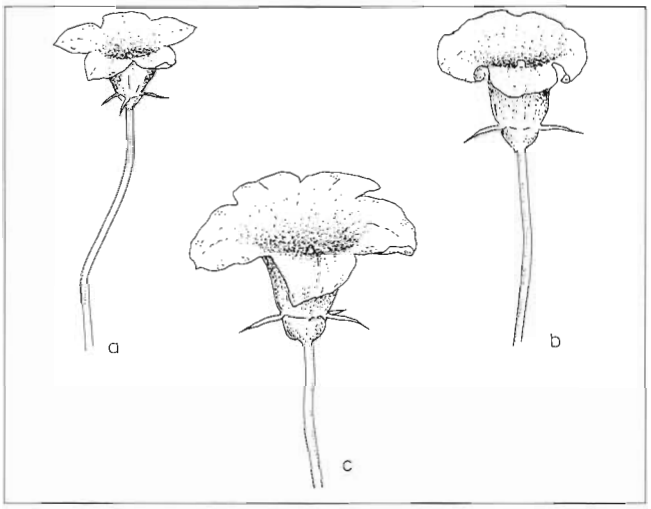

Fig. 5. Male flowers: a – *C. ficifolia*, b – *C. maxima*, c – F_1 hybrid (*C. ficifolia* × *C. maxima*).

The ripe fruits of *C. ficifolia* were a bit elongated. The fruit stalk was relatively thin but the part connected to the fruit was broaden. The fruits of *C. maxima* were round or a bit flat, having a thick fruit stalk which was a bit thinner in the region attached to the fruit. The shape of the fruit stalks of the hybrid progeny was intermediate (Fig. 6). The shape of fruits, however, was more similar to *C. maxima*. The colour of the fruit exterior was intermediate. The tissues inside the hybrid fruits were light yellow to yellow-green (*C. ficifolia* was characterised by light green or white (transparent) pigmentation, whereas the tissues of *C. maxima* were dark yellow). The shape and colour of the seed coat was inherited from the male parent (*C. maxima*). Empty seeds, however, were dark brown (but much lighter than the seeds of *C. ficifolia*). Most of the seeds were empty (without embryo).

The fruit exterior was extremely hard and was inherited from the female parent (*C. ficifolia*). Its growth and the expansion of the tissues below was not balanced, and the consequence were numerous cracks. These cracks healed up very fast due vigorous growth and therefore did not cause rotting. The cracks became less frequent at the end of October, when the weather became cooler and more humid.

The most useful traits of the interspecific hybrid *C. ficifolia* × *C. maxima* are probably higher tolerance to low temperatures and vigorous root system. The main problem, which needs to be solved, is sterility. Another problem, which is at least partly associated with sterility, can be the unstable 'composed' genome. The progeny may start 'approaching' one of the parental species.

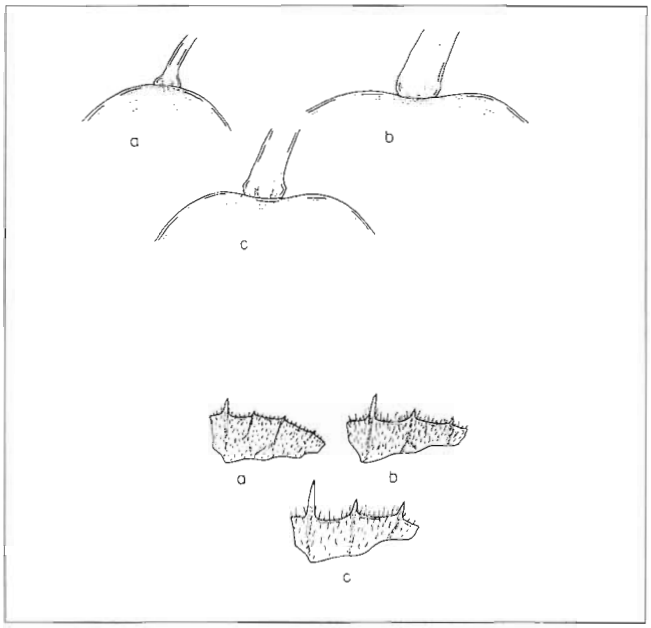

Fig. 6. Base of the fruit stalk: a – *C. ficifolia*, b – *C. maxima*, c – F_1 hybrid (*C. ficifolia* × *C. maxima*).

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