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Interspecific crosses of onion with distant *Allium* species and characterization of the presumed hybrids by means of flow cytometry, karyotype analysis and genomic in situ hybridization

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Abstract Interspecific crosses were made by hand-pollination of *Allium cepa* with pollen of 19 species belonging to nine sections of two subgenera of the genus *Allium*. In all cases viable plantlets were obtained from ovary culture. The efficiency depended on the relationship of the pollen donor to *A. cepa*. The hybrid character of the regenerants was checked by morphological comparisons with the parents and/or by one or more cytological methods such as flow cytometric DNA measurement, karyotype analysis, and genomic in situ hybridization (GISH). Hybrids were confirmed for 18 new species combinations. The viable hybrid of the most distant cross resulted from crossing *A. cepa* with *A. sphaerocephalon*. The relevance of the verification methods and the potential use of the hybrids for breeding purposes are demonstrated.

Key words *Allium cepa* · DNA content · Ovary culture · Verification of hybrids · Wide crosses

Introduction

Since the earliest breeding experiments, crosses between species have been made in order to achieve new forms and to transfer desirable features from one species into another, e.g. via introgression from wild into related crop species.

Within the genus *Allium*, a few crosses between onion (*A. cepa* L.) and its closest relatives of the section *Cepa* have proved successful (for a review see Van Raamsdonk et al. 1992). Most of the primary hybrids from crosses of *A. cepa* with *A. fistulosum* L. (Emsweller and Jones 1935 a,b; Levan 1936; Maeda 1937; Van der Meer and van Benekom 1978; Doležel et al. 1980), *A. galanthum* Kar. et Kir., *A. oschaninii* O. Fedt., and *A. pskemense* B. Fedt. (Saini and Davis 1967; McCollum 1971,1974) were sterile. Using GISH, the sterile Japanese form *A. wakegi* Araki was also found to be a natural hybrid of *A. cepa* with *A. fistulosum* (Hizume 1994). Only some cases gave further progeny generations. A natural fertile amphidiploid hybrid of *A. cepa* with *A. fistulosum* has been reported by Jones and Clarke (1942). The variety 'Beltsville Bunching' is an amphidiploid hybrid between *A. cepa* and *A. fistulosum* (Dowker 1990). Fertile amphidiploids were obtained by fertilization of unreduced gametes of a hybrid between *A. cepa* and *A. fistulosum* (Levan 1941) and by colchicine treatment of hybrids of *A. cepa* with *A. galanthum* (McCollum 1980) and *A. pskemense* (Havránek, personal communication). Backcross analysis of the *A. cepa* × *A. fistulosum* hybrids with *A. cepa* is in progress (Cryder et al. 1991; Bark et al. 1994).

By crossing with *A. roylei* Stearn it was possible to introduce resistance against *Peronospora destructor* [Berk.] Casp. into onion (Kofoet et al. 1990; Van der Meer and de Vries 1990). Similarly, backcrosses of a hybrid of *A. cepa* (susceptible) with *A. nutans* (resistant) led to an F_2 plant resistant to *Peronospora* (Pavlenko 1993 a,b).

Crosses of onion with species of other sections or subgenera were mostly unsuccessful; this includes crosses with *A. angulosum* L., *A. schoenoprasum* L., *A. senescens* L. (Gonzalez and Ford-Lloyd 1987), and *A. sphaerocephalon* L. (Bino et al. 1989). The widest crosses so far reported to be successful with onion were those with *A. nutans* L. belonging to the section *Rhizirideum* (Yurieva and Titova 1984), with *A. chinense* G. Don., section *Sacculiferum*, (Nomura et al. 1994), and with a unique fertile garlic clone (Ohsumi et al. 1992). Garlic belongs to the subgenus *Allium*; therefore, this cross is especially remarkable.

Embryo rescue has been employed as a method to facilitate distant crosses in several species belonging to the genus *Allium* (Doležel et al. 1980; Yurieva and Titova 1984; Nomura and Oosawa 1990). Instead of isolating the embryos proper we used the less-time and labour consuming culture of entire ovaries (Gonzalez and Ford-Lloyd 1987; Nomura et al. 1994) as a method for in vitro embryo rescue (Keller 1990 a,b; 1992).

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Several methods are available for the verification of interspecific hybrids. Morphological evaluation is possible if paternal or intermediate features of flowers, leaves, or epidermal structures (Pavlenko 1993 a,b) etc. are recognizable. However, these are frequently not evident at early stages of plantlet development.

If the parents clearly differ in genome size, flow cytometric measurement of DNA content may detect diploid hybrids at early developmental stages. However, since differences in DNA content may be due to reasons other than hybrid status, additional methods confirming the hybrid nature of the investigated material need to be applied. As soon as root tips are developed, karyotype analysis of colchicine-arrested and Feulgen-stained meristematic metaphase cells can be performed. If the chromosomes of the parental complements differ in size, number and/or morphology then simple karyotyping is most conclusive.

If the parental karyotypes are not clearly distinguishable, molecular methods such as genomic in situ hybridization (GISH – Schwarzacher et al. 1989), isozyme or DNA techniques may be applied.

Here we summarize our attempts to produce hybrids by crossing *A. cepa* with several species of different sections of the subgenera *Rhizirideum* and *Allium*, according to the subdivision of the genus given by Hanelt et al. (1992), and to confirm their hybrid status by means of cytological methods.

Materials and methods

Plant material

For methodical reasons *A. cepa* was used as the seed parent in all crosses. The pollen was obtained from accessions of the *Allium* collection in the Taxonomy Department of the IPK Gatersleben (see Table 1). The accessions were selected according to pollen availability at the flowering time of the seed parent.

Crosses within the subgenus *Rhizirideum*

Tetraploid accessions

Previously, the name *A. ledebourianum* Roem. et Schult. instead of *A. altyncolicum* has been used for this pollen donor (Keller 1992; Keller et al. 1994). A recent re-investigation revealed this accession to be a natural occurring amphidiploid hybrid between *A. ledebourianum* and *A. schoenoprasum*, characterized as *A. altyncolicum* Friesen (Friesen 1987, 1996). *A. senescens* is a predominantly tetraploid species closely related to *A. nutans* which has already yielded hybrids with *A. cepa* (Yurieva and Titova 1984). Hybrids of *A. cepa* with *A. altyncolicum* and with *A. senescens*, respectively, are triploid.

In our collection *A. globosum* is represented by both diploid and tetraploid accessions. The accessions may be heterogeneous within themselves. Therefore, diploid and triploid hybrids may be expected and a ploidy check is necessary for each hybrid.

Diploid accessions

In the following cases, only diploid accessions of a species have been selected for crosses: *A. albidum* Fisch. ex Bieb., *A. angulosum*, *A. carolinianum* DC., *A. chevsuricum* Tscholok., *A. flavellum* Vved., *A. hymenorrhizum* Ldb., *A. jodanthum* Vved., *A. karelinii* Poljak., *A. lineare* L. s. l., *A. obliquum* L., *A. rubens* Schrad. ex Willd., *A. saxatile* M. Bieb. and *A. victorialis* L..

Crosses with species of the subgenus *Allium*

Crosses with the subgenus *Allium* are interesting both because of the large phylogenetic distance between the parental species and because the subgenus *Allium* comprises several important crop species such as garlic (*A. sativum* L.) and leek (*A. porrum* L.). The cross between *A. cepa* and *A. sphaerocephalon* is, therefore, an important precedent for such a wide cross.

Pollination and in vitro culture

Onions were grown in growth chambers for optimal flowering, synchronization, and avoidance of cross-pollination. Flowers were daily emasculated as soon as the buds opened. All flowers were hand pollinated using fresh pollen directly from the anthers of the pollinators (Fig. 1). The pollination was performed when the styles were ripe, and the flowers were excised as soon as the styles wilted, usually 3–5 days after pollination. Flowers were sterilized in 3% sodium hypochlorite solution and washed three times in de-ionized water. Subsequently, the ovaries were separated from the remaining part of the flowers and placed in an upright position on the medium.

In vitro culture has been performed according to the method described for haploid production (Keller 1990 a,b). The ovaries have been cultured on BDS medium (Dunstan and Short 1977), containing 2 mg/l indole butyric acid (IBA), 1.2 mg/l benzylamino purine (BAP), 500 mg/l glutamine, and 100 g/l sucrose, in the dark at 27°C. One month later, the ovaries were transferred to light (16 h/day at 25°C) and, another week later, to BDS medium +2 mg/l IBA and 0.12 mg/l BAP. Plantlets breaking through the ovary wall were transferred to BDS medium without hormones. After some months of subculture on this medium (Fig. 1) they were either planted into soil or included in the maintenance cycle of the in vitro genebank (Keller 1991).

Hybrid verification

Flow cytometry

Suspensions of nuclei stained with the DNA-specific fluorescent dye DAPI (4′,6-diamidino-2-phenylindole) were prepared by chopping leaves with a razor-blade in DAPI staining solution (PARTEC GmbH). Nuclear suspensions were filtered through a nylon membrane with a mesh width of 100 µm and analyzed with a FACStar^{Plus} flow cytometer (Becton and Dickinson). Fluorescence was excited by an argon-ion laser INNOVA 90-5 (Coherent) using multiline optics in the UV range (351.1–363.8 nm) with 100 mW output power. The results were analysed by means of the program LYSIS II (Becton and Dickinson) using the fluorescence pulse area histogram for evaluation. The G_1 peak of a diploid onion standard as reference sample was set to channel 400 in the 1024 channel histogram by calibration of the fluorescence photomultiplier sensitivity via the photomultiplier high voltage. For determination of DNA content, proportionality to fluorescence intensity was assumed.

Karyotype analysis

Metaphases were prepared from root-tip meristems of plantlets after treatment in 0.05% colchicine for 2 h, fixation in ethanol:acetic acid (3:1), digestion in 1% pectinase (Sigma) and 1% cellulase (Onozuka R10) at 37°C for 30 min, and squashing in 45% acetic acid. For microscopic inspection of the karyotype, the root tips were Feulgen-stained before squashing.

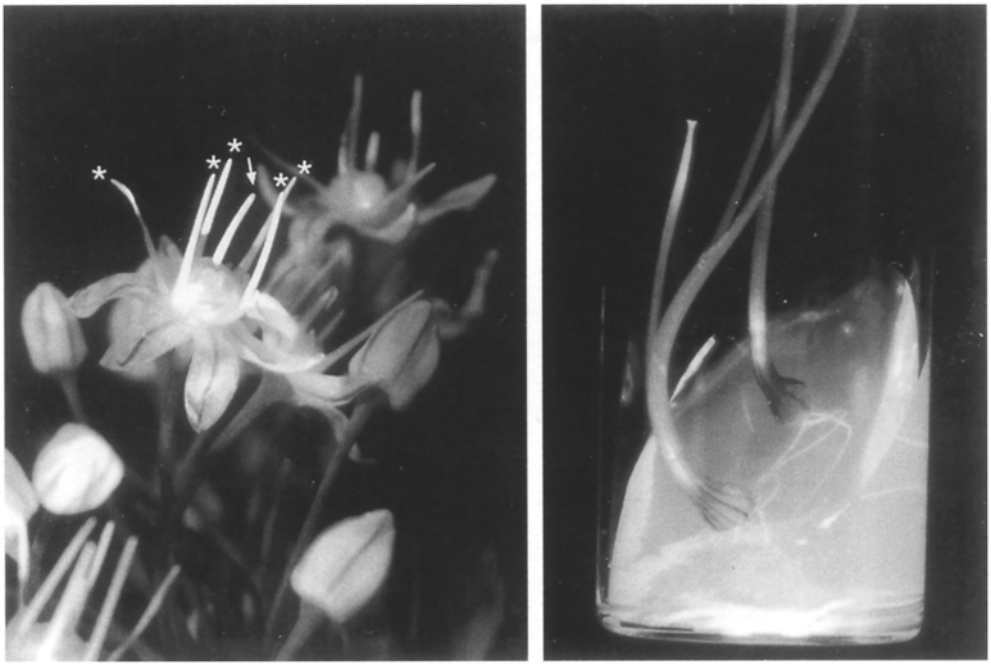


Fig. 1 Left: emasculated onion flowers ready for pollination, stigma marked by an *arrow*, the ends of emasculated filaments shown by *asterisks*; right: hybrid plantlet of *A. cepa* × *A. globosum* produced by in vitro culture

Table 1 Regeneration via ovary culture and survival of plantlets resulting from pollination of onion by the pollen of distant *Allium* species. Abbreviations: PA – number of pollinator accessions used for crossing, OC – number of cultivated ovaries; figures in the column of relative regeneration frequencies, bearing the same letter, belong to the same significance group in the χ^2 test

Section	Species	PA	OC	Regeneration		Plants	
				abs.	rel. %	abs.	rel. %
Allium	*sphaerocephalon*	1	304	6	2.0cd	6	2.0
Anguinum	*victorialis*	3	304	3	1.0d	2	0.6
Campanulata	*barsczewskii*	1	224	2	0.9d	2	0.9
	flavellum	2	330	3	0.9d	2	0.6
	jodanthum	1	517	9	1.7d	2	0.4
Cepa	*altaicum*	1	507	32	6.3c	14	2.8
Oreiprason	*carolinianum*	1	320	20	6.2c	11	3.4
	chevsuricum	1	78	20	25.6a	11	14.1
	globosum	3	933	38	4.1cd	32	3.4
	hymenorrhizum	3	553	29	5.2c	22	4.0
	saxatile	2	681	83	12.2b	53	7.8
Petroprason	*obliquum*	1	508	8	1.6d	6	1.2
Reticulato-bulbosa	*lineare s.l.*	1	189	14	7.4c	13	6.9
Rhizirideum	*albidum*	1	117	7	6.0c	5	4.3
	angulosum	2	206	9	4.4cd	9	4.4
	rubens	1	179	8	4.5cd	2	1.1
	senescens	4	1917	22	1.1d	3	0.2
Schoenoprasum	*altyncolicum*	1	404	8	2.0d	4	1.0
	karelinii	1	896	15	1.7d	1	0.1
Total		31	9167	336	3.7	199	2.2

Genomic in situ hybridization

Genomic DNA of *A. cepa* was prepared from leaf tissue (1 g) of young plants according to Pich and Schubert (1993). The DNA was labelled via nick translation (Langer et al. 1981) with Bio-16-dUTP (Boehringer Mannheim) and used for fluorescent in situ hybridization (Fuchs and Schubert 1995).

Slides with metaphase spreads were pre-treated with DNase-free RNase (50 µg/ml), proteinase K (1 µg/ml) and paraformaldehyde (4%). Amounts of 20–40 ng of labelled DNA from *A. cepa* and 10 µg of tRNA from *E. coli* were used per slide for hybridization overnight at 37°C in 50% formamide, 10% dextran sulphate and 2 × SSC. Post-hybridization washing steps were performed in 50% formamide at 42°C, 1 × SSC and 0.1 × SSC at 60°C. Hybridization signals of the

biotinylated probes were detected with streptavidin FITC (CAMON) and, if necessary, amplified via biotinylated antistreptavidin (CAMON) and a further layer of streptavidin FITC. After counterstaining with propidium iodide (Sigma), slides were inspected using a Zeiss Axioskop. Photographs were taken on Kodak Ektachrome 400 slide films.

Results

In total, 11 *A. cepa* plants were pollinated with the pollen of 19 donor species. From 38 different single-plant combinations 336 regenerants developed in ovary culture. Of these, 199 plants survived (Table 1). Out of 135 regenerants investigated, 108 (i.e. 80%) were confirmed to be hybrids by morphological comparison, flow cytometry and karyotype analysis including GISH (Table 2). Of 19 species combinations 18 indeed yielded hybrids. In one combination (*A. cepa*×*A. barsczewskii*) only two regenerants with a haploid *A. cepa* chromosome complement have developed. In *A. cepa*×*A. flavellum*, in addition to one true hybrid individual, a plant with a diploid *A. cepa* chromosome complement was obtained.

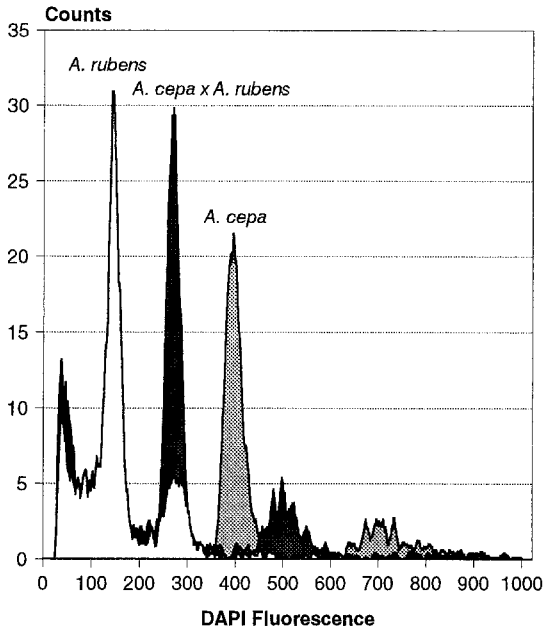


Fig. 2 Example of a clear difference between the parental histograms compared with that of the hybrid *A. cepa*×*A. rubens*

Table 2 Results of hybrid verification after crosses of *A. cepa* with wild species. Abbreviations: DNA cs: DNA content similar, F: flow cytometry, K: karyotype analysis, G: GISH, V: verified by..., * no further investigations because of clear morphological evidence and the verification by flow cytometry

Species	Regenerants investigated				V	Remarks
	Total	F	K	G		
albidum	5	5	1	1	1	DNAcs,V:G
altaicum	6	6	0	0	6	V:F*
altyncolicum	4	4	4	0	4	DNAcs,V:K 3 × triploid, 1 × tetraploid
angulosum	3	2	1	1	3	V:F,K,G
barsczewskii	2	0	2	0	0	2 × haploid
carolinianum	11	11	0	0	8	V:F
chevsuricum	7	6	1	1	1	DNAcs,V:G;
flavellum	2	2	2	1	1	V:F,K,G 1 × like *cepa*
globosum	24	23	3	1	23	V:F,K,G 2 × triploid
hymenorrhizum	15	15	1	1	12	V:F,K,G;
jodanthum	2	2	1	0	2	V:F,K
karelinii	1	1	1	0	1	V:F,K
lineare s.l.	8	8	1	1	1	DNAcs,V:G 1 × polyploid
obliquum	6	6	0	0	6	V:F*
rubens	1	1	1	0	1	V:F,K
saxatile	32	32	1	1	32	V:F,K,G
senescens	3	1	2	0	3	V:K 3 × polyploid
sphaerocephalon	1	0	1	1	1	V:G
victorialis	2	1	1	1	2	V:G 1 × polyploid
Totals	135	126	24	10	108	

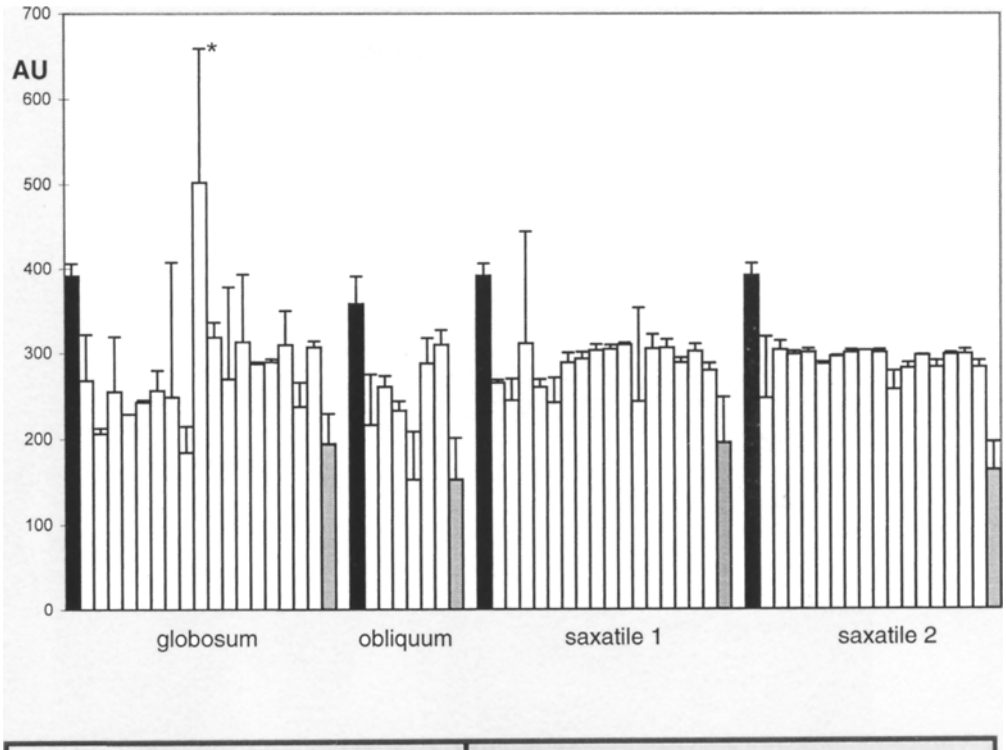


Fig. 3 Examples of flow cytometric DNA measurements. Each value is the average from two independent leaf segments (standard deviation given by *bars*). *Black columns*: mother plants (*A. cepa*); *grey columns*: father plants (mentioned in the legend). *AU* – flow cytometric arbitrary units, * polyploid regenerant

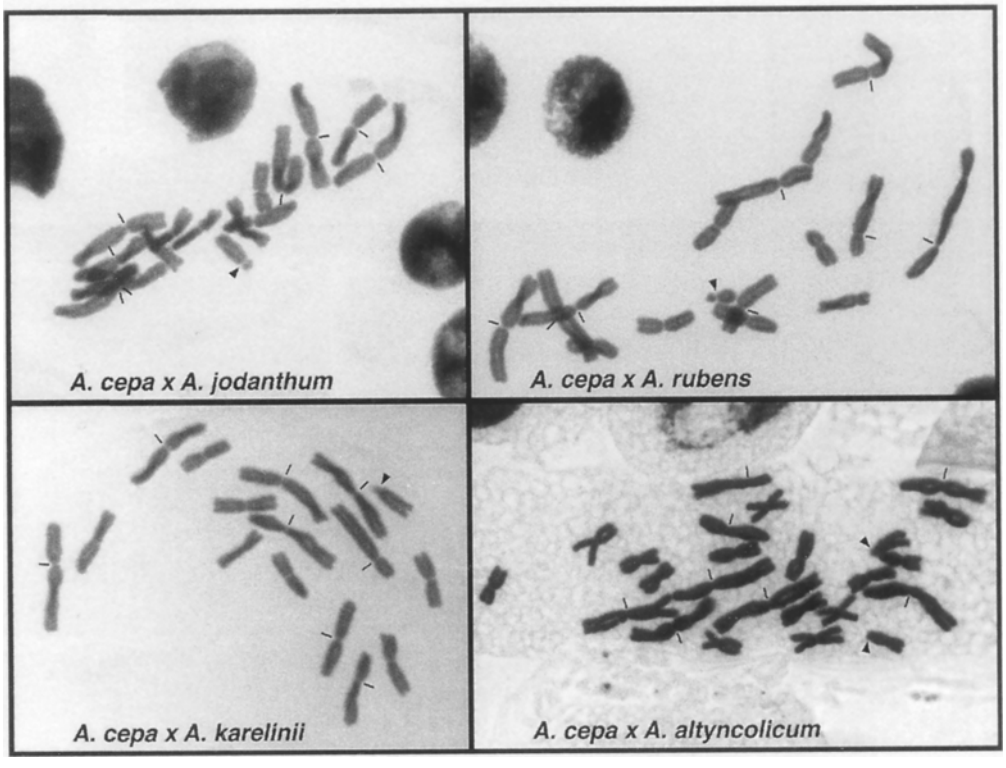
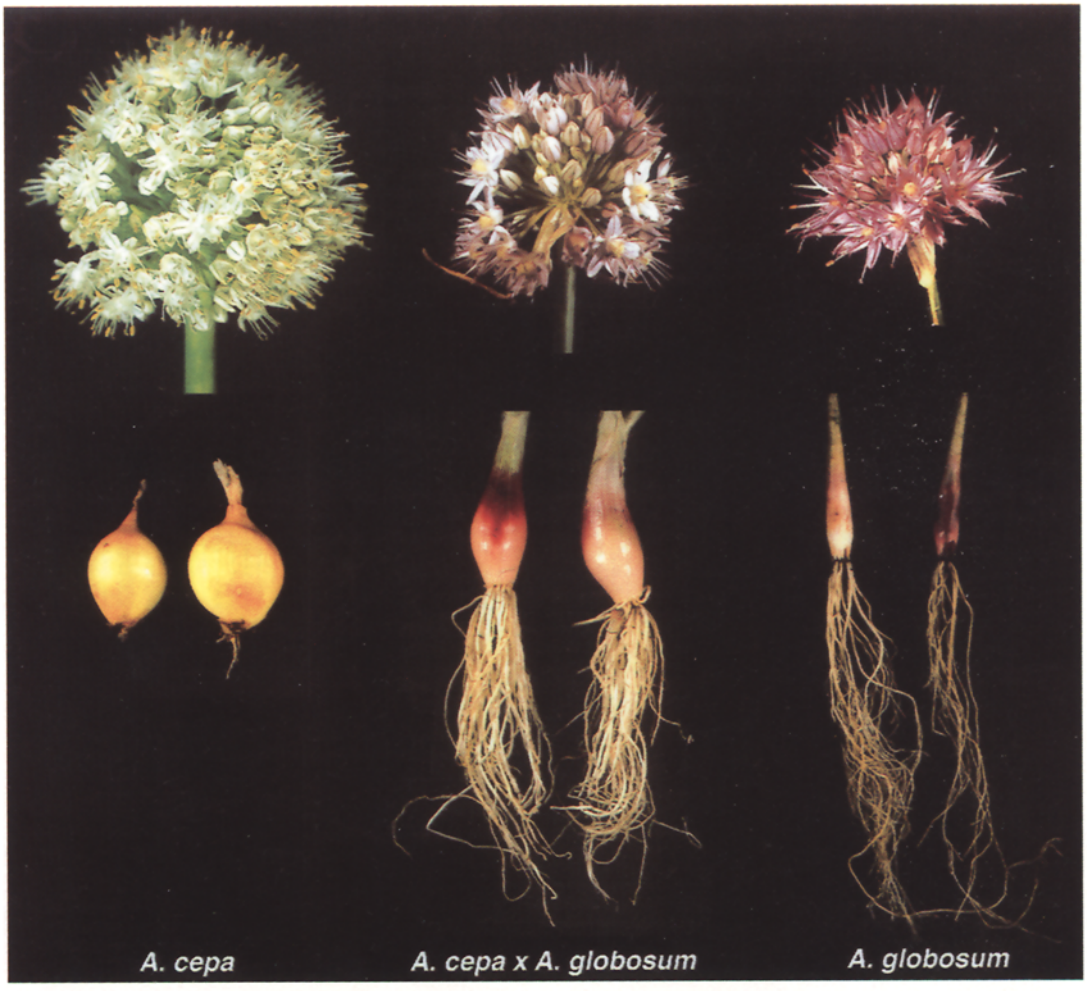


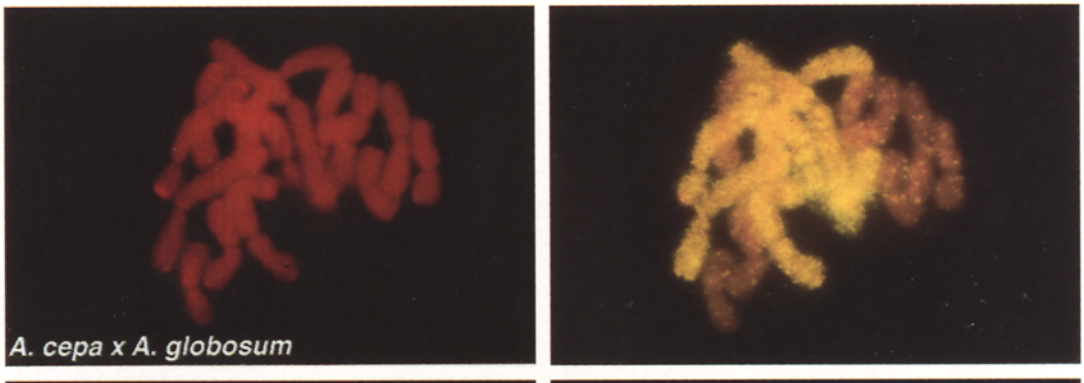
Fig. 4 Complete Feulgen-stained metaphases from hybrids of *A. cepa* with *A. jodanthum* (2n=16), *A. rubens* (2n=17), *A. karelinii* (2n=16), and *A. altyncolicum* (3n=24), respectively. The eight large *A. cepa* chromosomes are marked by a *bar* at the centromere in each metaphase. Marker chromosomes of the paternal chromsome complements are labelled by *arrowheads*

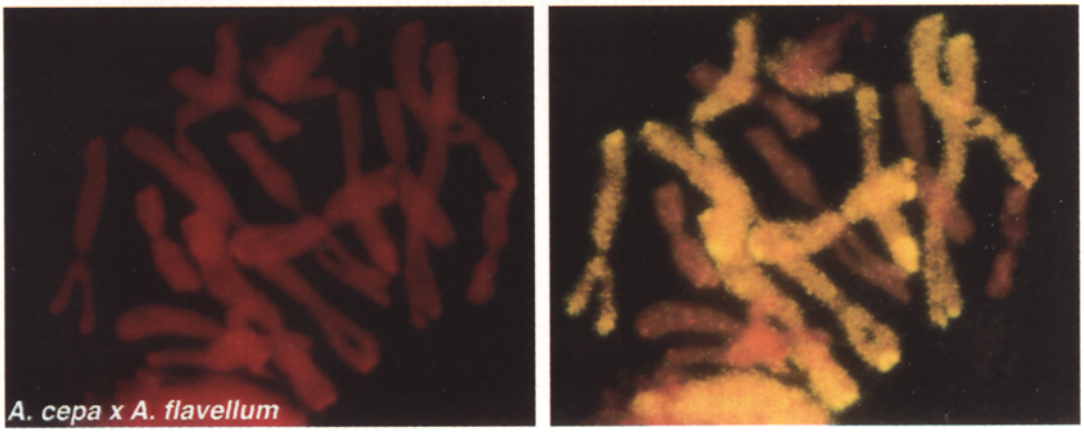
Regeneration frequency and plant survival

Regeneration frequency and subsequent survival of plants were found to be dependent on the combination of parents. The highest frequencies were observed in crosses with species of the subgenera *Oreiprason* [*A. chevsuricum* (25.6%) and *A. saxatile* (12.2%)], *Reticulato-bulbosa* and *Cepa*. Lower frequencies resulted from crosses with species of the subgenera *Campanulata*, *Schoenoprasum*, *Petroprason* and *Allium*.

The survival rates were as high as 14.1% (cross with *A. chevsuricum*) and 7.8% (cross with *A. saxatile*). Even in the widest cross (*A. cepa* × *A. sphaerocephalon*) 2% of cultured ovaries developed into hybrid plantlets (Table 1).


A. cepa
A. cepa x A. globosum
A. globosum


A. cepa x A. globosum


A. cepa x A. flavellum

Flow cytometry

Flow cytometrical analyses have been performed for all sufficiently large plants except for those of the cross with *A. barsczewskii*. If the DNA contents of the parents differ significantly, the hybrids show an intermediate DNA content as in the cross *A. cepa*×*A. rubens* (Fig. 2). Clear evidence for the hybrid nature was also obtained by this method for crosses with *A. altaicum* (6 plants), *A. angulosum* (2), *A. karelinii* (1), *A. obliquum* (6), and *A. saxatile* (32) and partly for the crosses of *A. cepa* with *A. carolinianum* (8 of 11), *A. flavellum* (1 of 2), *A. globosum* (21 of 23), *A. hymenorrhizum* (12 of 15), and *A. jodanthum* (1 of 2). Figure 3 shows, as an example, flow cytometric data for the crosses of *A. cepa* with *A. globosum*, *A. obliquum*, and *A. saxatile*. Because of similar nuclear DNA contents, hybrid verification by flow cytometric DNA measurement is not possible for hybrids of *A. cepa* with *A. albidum*, *A. altyncolicum*, *A. chevsuricum* and *A. lineare*. Obvious polyploid progeny occurred among crosses of *A. cepa* with *A. altyncolicum*, *A. globosum*, *A. lineare*, *A. senescens*, and *A. victorialis*. Polyploid plants need further investigation.

Karyotype analysis

Analysis of complete Feulgen-stained metaphase plates has been performed on regenerants from ovaries derived from crosses of *A. cepa* with *A. albidum, A. altyncolicum, A. angulosum, A. barsczewskii, A. chevsuricum, A. flavellum, A. globosum, A. hymenorrhizum, A. jodanthum, A. karelinii, A. lineare, A. rubens, A. saxatile, A. senescens,* and *A. sphaerocephalon*. The hybrid character is directly visible when the chromosomes of the parental complements differ in size and/or morphology (Fig. 4). This was the case in the investigated crosses with *A. altyncolicum, A. angulosum, A. flavellum, A. globosum, A. hymenorrhizum, A. jodanthum, A. karelinii, A. rubens, A. saxatile*, and *A. senescens*. In a triploid hybrid from the cross *A. cepa*×*A. altyncolicum* two acrocentric chromosomes clearly belong to the two *altyncolicum* genomes whereas the eight larger chromosomes represent the single *cepa* complement (Fig. 4).

Genomic in situ hybridization

GISH was used successfully to verify hybrids from crosses of *A. cepa* with *A. albidum* (Fig. 5), *A. angulosum, A. chevsuricum, A. globosum, A. flavellum, A. hymenorrhizum, A. lineare, A. saxatile, A. sphaerocephalon* and *A. victorialis*. In five of these combinations (*A. albidum, A. chevsuricum, A. lineare, A. sphaerocephalon* and *A. victorialis*) no conclusive results from flow cytometry and/or karyological analysis were available due to the similar parental DNA content and chromosome morphology or the lack of sufficiently developed plant material (*A. cepa*×*A. victorialis*).

Fig. 5 Morphological comparison of flower and bulbs of hybrid plants from a cross of *A. cepa*×*A. globosum* with those of the parental species (above); genomic in situ hybridization (GISH) of total labelled *A. cepa* DNA to the chromosomes of a hybrid between *A. cepa* and *A. globosum* (middle); and to the chromosomes of a hybrid between *A. cepa* and *A. flavellum* (below). On the left the same metaphases after propidium iodide staining

Discussion

Crosses with wild *Allium* species might be useful for the introduction of genes for resistance or for the production of interesting metabolites (flavour, carbohydrates) into onion.

The suitability of the different interspecific hybrids for breeding purposes depends on the mode of crop propagation. In the case of vegetative propagation (as in garlic, shallots and rakkyo), the primary hybrids could be used directly if they possess any interesting features such as cold tolerance, disease resistance, special flavour characters or even higher yield potential. If seed production is required, the sterility of the hybrids has to be overcome. This requires several steps. First amphidiploids may be generated by colchicine treatment. These can either be propagated by selfing, if no inbreeding depression occurs, crossed to autotetraploid *A. cepa* (D'Amato 1948) and/or diploidized by ovary culture to produce substitution lines.

Crosses of amphidiploid hybrids with either tetraploid *A. cepa* or tetraploid leek could be of interest to breeders.

Fertile tetraploid onions have been produced successfully by Virnich (1967). Cross breeding of such tetraploids with tetraploid *A. fistulosum* has been discussed by Van der Meer (1984), although the latter material was reported to be of low fertility (Toole and Clarke 1944; Davis 1955; Chattacharya 1976). This strategy offers an oppurtunity for breeding research when in future amphidiploids are produced from the primary hybrids presented here.

Several hybrids, possessing clearly different morphological characters (Fig. 5), have already reached the flowering stage. So far they are seed sterile. Colchicine treatment is envisaged as a means of producing amphidiploid fertile plants.

Especially for young plantlets and for crosses of morphologically similar plant species, cytological checking of regenerants from ovary culture after cross pollination is indispensable to prove the hybrid status and to exclude haploid regenerants and those descending from unreduced egg cells or sporophytic cells of the nucellus.

Concerning confirmation of the hybrid status, the flow cytometric test proved to be very efficient. It can easily be used for large scale screening. However, it provides no more details than does DNA content about the tested plant and may fail in the case of polyploidy, aneuploidy, or a similar DNA content of the parents. Simple karyotype analysis can be conclusive even when the parental genomes are of similar size provided that the chromosome morphology is different. If both methods fail, GISH usually provides a reliable tool.

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References

Bark OH, Havey MJ, Corgan JN (1994) Restriction fragment length polymorphism (RFLP) analysis of progeny from an *Allium fistulosum*×*A. cepa* hybrid. J Am Soc Hortic Sci 119:1046–1049

Bino RJ, Janssen MG, Franken J, de Vries JN (1989) Enhanced seed development in the interspecific cross *Allium cepa*×*A. sphaerocephalon* through ovary culture. Plant Cell Tissue Org Cult 16:135–142

Chattacharyya R (1976) A tetraploid *Allium cepa* from Bangladesh. Cytologia 41:513–521

Cryder CM, Corgan JN, Urquhart NS, Clason D (1991) Isozyme analysis of progeny derived from (*Allium fistulosum*×*Allium cepa*)×*Allium cepa*. Theor Appl Genet 82:337–345

D'Amato F (1948) Autotetraploidia spontanea in *Allium cepa* L. Caryologia 1:48

Davis EW (1955) Nature of sterility in the amphidiploid *Allium cepa-fistulosum*. Am J Bot 42:41–48

Doležel J, Novák FJ, Lužný J (1980) Embryo development and in vitro culture of *Allium cepa* and its interspecific hybrids. Z Pflanzenzücht 85:177–184

Dowker BD (1990) Onion breeding. In: Rabinowitch HD, Brewster JL (eds) Onions and allied crops. Vol. I. CRC Press, Boca Raton, pp 215–232

Dunstan DL, Short KC (1977) Improved growth of tissue cultures of the onion, *Allium cepa*. Physiol Plant 41:70–72

Emsweller SL, Jones HA (1935a) An interspecific hybrid in *Allium*. Hilgardia 9:265–273

Emsweller SL, Jones HA (1935b) Meiosis in *Allium fistulosum, Allium cepa* and their hybrid. Hilgardia 9:277–288

Friesen N (1987) A new species of *Allium* (*Alliaceae*) from the Altai mountains (in Russian). Bot Žurn 72:815–820

Friesen N (1996) A taxonomical and chorological revision of the section *Schoenoprasum* Dumort. (Genus *Allium* L.). Candollea (in press)

Fuchs J, Schubert I (1995) Localization of seed protein genes on metaphase chromosomes of *Vicia faba* via fluorescence in situ hybridization. Chromosome Res 3:94–100

Gonzalez LG, Ford-Lloyd BV (1987) Facilitation of wide-crossing through embryo rescue and pollen storage in interspecific hybridization of cultivated *Allium* species. Plant Breed 98:318–322

Hanelt P, Schultze-Motel J, Fritsch R, Kruse J, Maaß H, Ohle H, Pistrick K (1992) Infrageneric grouping of *Allium* – the Gatersleben approach. In: Hanelt P, Hammer K, Knüpffer H (eds) The genus *Allium* – taxonomic problems and genetic resources. Proc Int Symp Gatersleben, June 11–13, 1991. Inst Genet Crop Plant Res, Gatersleben, pp 107–123

Hizume M (1994) Allodiploid nature of *Allium wakegi* Araki revealed by genomic in situ hybridization and localization of 5s and 18s rDNAs. Jpn J Genet 69:407–415

Jones HA, Clarke AE (1942) A natural amphidiploid from an onion species hybrid *Allium cepa* L.×*Allium fistulosum* L. Heredity 33:25–32

Keller J (1990a) Culture of unpollinated ovules, ovaries, and flower buds in some species of the genus *Allium* and haploid induction via gynogenesis in onion (*Allium cepa* L.). Euphytica 47: 241–247

Keller J (1990b) Results of anther and ovule culture in some species and hybrids in the genus *Allium* L. Arch Züchtungsforsch 20: 189–197

Keller J (1991) In vitro conservation of haploid and diploid germplasm in *Allium cepa* L. Acta Hortic 289:231–232

Keller J (1992) In vitro cultivation of *Allium* species – a method for application in plant breeding and germplasm conservation. In: Hanelt P, Hammer K, Knüpffer H (eds) The genus *Allium* – taxonomic problems and genetic resources. Proc Int Symp Gatersleben, June 11–13, 1991. Inst Genet Crop Plant Res Gatersleben, pp 137–152

Keller ERJ, Eickmeyer F, Lux H, Maaß HI, Schubert I (1994) Ergebnisse zur entfernten Bastardierung bei *Allium*, Untergattung *Rhizirideum*. Vortr Pflanzenzücht 28:309–311

Kofoet A, Kik C, Wietsma WA, de Vries JN (1990) Inheritance of resistance to downy mildew (*Peronospora destructor* [Berk.] Casp.) from *Allium roylei* Stearn in the backcross *Allium cepa* L.×(*A. roylei*×*A. cepa*). Plant Breed 105:144–149

Langer PR, Waldrop AA, Ward DC (1981) Enzymatic synthesis of biotin-labeled polynucleotides: novel nucleic acid affinity probes. Proc Natl Acad Sci USA 78:6633–6637

Levan A (1936) Die Zytologie von *Allium cepa*×*A. fistulosum*. Hereditas 21:195–214

Levan A (1941) The cytology of the species hybrid *Allium cepa*× *fistulosum* and its polyploid derivatives. Hereditas 27:253–272

Maeda T (1937) Chiasma studies in *Allium fistulosum, Allium cepa* and their F_1, F_2, and backcross hybrids. Jpn J Genet 13:146–159

McCollum GD (1971) Sterility of some interspecific *Allium* hybrids. J Am Soc Hort Sci 96:359–362

McCollum GD (1974) Chromosome behavior and sterility of hybrids between the common onion, *Allium cepa*, and the related wild *A. oschaninii*. Euphytica 23:699–709

McCollum GD (1980) Development of the amphidiploid of *Allium galanthum*×*A. cepa*. J Hered 71:445–447

Nomura Y, Oosawa K (1990) Production of interspecific hybrids between *Allium chinense* and *A. thunbergii* by *in ovulo* embryo culture. Jpn J Breed 40:531–535

Nomura Y, Maeda M, Tsuchiya T, Makara K (1994) Efficient production of interspecific hybrids between *Allium chinense* and edible *Allium* spp. through ovary culture and pollen storage. Breed Sci 44:151–155

Ohsumi C, Kojima A, Hinata K, Etoh T, Hayashi T (1992) Interspecific hybrids between onion and garlic. In: Hanelt P, Hammer K, Knüpffer H (eds) The genus *Allium* – taxonomic problems and genetic resources. Proc Int Symp Gatersleben, June 11–13, 1991. Inst Genet Crop Plant Res Gatersleben, pp 233–239

Pavlenko EP (1993a) Gistometricheskij analiz elementov pokrovnoj tkani lista meshvidovykh gibridov luka v svjazi s ustojchivost'ju k peronosporozy. Bjul Glavn Bot Sada 167:88–95

Pavlenko EP (1993b) Osobennosti pokrovnoj tkani lista meshvidovykh gibridov luka v svjazi s ustojchivost'ju k peronosporozu. Bjul Glavn Bot Sada 168:65–71

Pich U, Schubert I (1993) Midiprep method for isolation of DNA from plants with a high content of polyphenolics. Nucleic Acids Res 21:3328

Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1989) In situ localization of parental genomes in a wide hybrid. Ann Bot 64:315–324

Saini SS, Davis GN (1967) Compatibility in some *Allium* species. Proc Am Soc Hort Sci 91:401–409

Toole MG, Clarke AE (1944) Chromosome behaviour and fertility of colchicine-induced tetraploids in *Allium cepa* and *Allium fistulosum*. Herbertia 11:295–303

Van der Meer QP (1984) Exchange of genes between *Allium cepa* L. and *Allium fistulosum* L. Proc 3rd. Eucarpia *Allium* Symp Wageningen, pp 65–69

Van der Meer QP, de Vries JN (1990) An interspecific cross between *Allium roylei* Stearn and *Allium cepa* L., and its backcross to *A. cepa*. Euphytica 47:29–31

Van der Meer QP, van Benekom JL (1978) Improving the onion crop (*Allium cepa* L.) by transfer of characters from *Allium fistulosum* L. Biul Warzywniczy 22:87–91

Van Raamsdonk LWD, Wietsma WA, de Vries JN (1992) Crossing experiments in *Allium* L. section *Cepa*. Bot J Linn Soc 109:293–303

Virnich H (1967) Untersuchungen über das Verhalten der männlichen Sterilität und anderer Eigenschaften bei polyploiden Zwiebeln (*Allium cepa* L.) als Grundlage für eine Nutzung in der Hybridzüchtung. Z Pflanzenzücht 58:205–244

Yurieva NA, Titova IV (1984) Embryo culture in vitro of interspecific hybrids of *Allium* L. genus. In: Novák FJ, Havel L, Doležel J (eds) Proc Int Symp Plant Tissue and Cell Culture Application to Crop Improvement. Olomouc, Czechoslovakia 24.–29.9.1984, Prague, pp 463–464