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Bulb-type onion introgressants posessing *Allium fistulosum* L. genes recovered from interspecific hybrid backcrosses between *A. cepa* L. and *A. fistulosum* L.

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Abstract *Allium fistulosum* possesses a number of traits which would be desirable in *A. cepa*. Thus far, no commercial *A. cepa* cultivars have been released which harbor *Allium fistulosum* traits. F_1BC_3 populations were generated for this study by backcrossing *A. cepa* to *A. cepa*×*A. fistulosum* hybrids. The F_1BC_3 plants were evaluated for plant morphology, floral characters, male-sterile cytoplasm, soluble solids and pungency, and isozymes. Overall growth habit and floral characters of the F_1BC_3 plants were much like *A. cepa.* We report here the recovery of recombinant, bulbing, and fertile *A. cepa*-type onions that exhibit *A. fistulosum* isozyme alleles and morphological markers. Recombination between *A. cepa* and *A. fistulosum* genomes was achieved using the introgression strategy of backcrossing *A. fistulosum* into *A. cepa*, thereby ameliorating the nuclear cytoplasmic barriers that occurred in previous less successful introgression attempts when plants were not in *A. cepa* cytoplasm. We believe this report to be the first demonstration of onion introgressants that are like *A. cepa* in appearance, are male- and female-fertile, and possess *A. fistulosum* genes.

Key words Introgression · Interspecific hybridization · Isozymes · Japanese bunching onion · Male sterility

Introduction

Bulb onion (*Allium cepa* L. 2n=2x=16) and Japanese bunching onion (*A. fistulosum* L. 2n=2x=16) are taxonomically closely related (Hanelt 1990). *A. cepa* and *A. fistulosum* differ botanically in that *A. cepa* produces bulbs as the consumed food, while *A. fistulosum* produces no or only a slight bulb. The species can be dinstinguished by leaf and floral morphology and by polymorphic isozyme alleles of several isozyme loci (Peffley et al. 1985). Japanese bunching onion possesses traits such as resistance to pink root (*Phoma terrestris* E.M. Hans.) (Porter and Jones 1933), smut (*Urocystis cepulae* Frost) (Jones and Mann 1963), leaf rot (*Botrytis squamosa* Walker) and onion fly (*Hylemyia antiqua* Bouche) as well as high soluble solids and cold hardiness (Van Der Meer and Van Benekom 1978). Incorporation of these traits into *A. cepa* could be valuable for the genetic improvement of bulb onion cultivars.

The first breeding effort to introduce *A. fistulosum* traits into *A. cepa* was initiated more than 60 years ago by Emsweller and Jones (1935). Since then numerous efforts using interspecific hybridization have been made to introgress *A. fistulosum* into *A. cepa.* Interspecific hybrids between *A. cepa* and *A. fistulosum* have been readily obtained with *A. cepa* as either the seed parent (Currah et al. 1984; Emsweller and Jones 1935; Van Der Meer and Van Benekom 1978) or the pollen parent (Corgan and Peffley 1986). The F_1 hybrids are intermediate in morphology: bulbing slightly, predominated slightly by *A. fistulosum* leaf characters, cup-shaped florets, intermediate flower length, angle, and inflorescence type, and flowering midway in time between the two parents (Currah and Ockendon 1988; Emsweller and Jones 1935; Van Der Meer and Van Benekom 1978). In all cases, the F_1s are highly sterile. Studies of progeny with *A. fistulosum* cytoplasm showed that fertility was not much improved in BC_1 and BC_2 plants, suggesting nuclear-cytoplasmic incompatibility interactions between *A. fistulosum* and *A. cepa* when *A. fistulosum* was used as the female parent (Ulloa-G et al. 1994, 1995). A bridge cross involving *A. roylei* has been proposed as a means to increase the possibility of genetic introgression from *A. fistulosum* into *A. cepa* (Khrustaleva and Kik 1998). Yet despite various efforts for numerous decades, onion cultivars possessing *A. fistulosum* genes have not been released.

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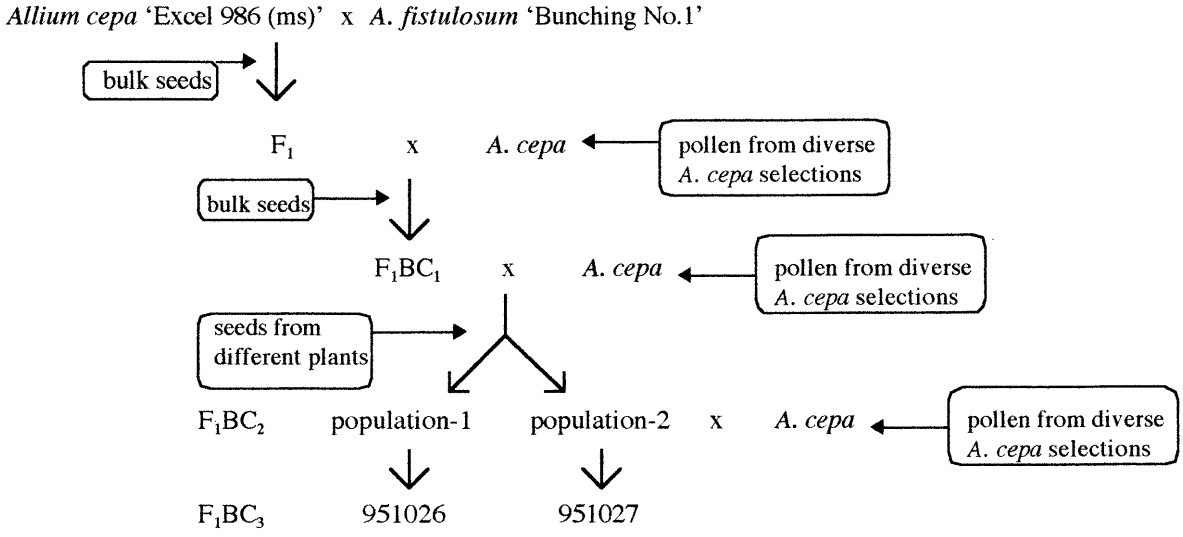

Fig. 1 Schematic representation of derivation of advanced backcross populations F_1BC_3 951026 and 951027

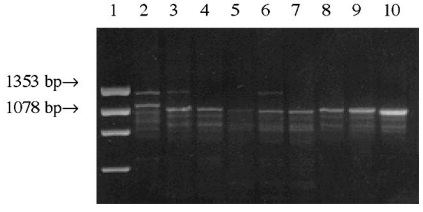

Fig. 2 Agarose gel electrophoresis of PCR products amplified with primers A (5′ATTACAAATGCGATGCTCT-3′) and B (5′-TCTACCGATTTCGCCATATC-3′). *Lane 1* ϕ x174 DNA digested with *Hae*III, *lane 2* N-cytoplasmic onion like with 1.1-kb band, *lane 3*, *A. cepa* 'Excel' with S-cytoplasm with 1.0-kb band, *lanes 4-10*, F_1BC_3 951027 plants 951027-91, 11, 75, 64, 46, 3, and 97

Three advanced backcross populations have been studied for the introgression of *A. fistulosum* into *A. cepa*. The results are reported here. F_1BC_3 backcross populations were derived from the crossing of *A. cepa* to an *A. cepa*×*A. fistulosum* F_1 hybrid. Over 100 F_1BC_3 plants were investigated for various characters – morphology, fertility, cytoplasm, isozymes – and investigated cytogenetically with karyotypes and in situ hybridization. Cytogenetic evidence is reported elsewhere (Hou and Peffley in preparation). Not all characters could be evaluated in all plants due to the bolting or non-bolting of some plants; some individuals survived the duration of the study, while others were lost before a complete set of information was collected. In all, sufficient numbers of backcross individuals were studied to gain an insight into genome recombination between *A. cepa* (bulb onion) and *A. fistulosum* (Japanese bunching onion).

In the study reported here, three F_1BC_3 populations were generated in *A. cepa* cytoplasm. Fertile, recombinant, bulb-type onions possessing *A. fistulosum* isozyme alleles were recovered, demonstrating for the first time that economically useful introgressants can result from *Allium* interspecific hybridization.

Materials and methods

Plant materials

Three F_1BC_3 populations, 951026, 951027, and 951029, each derived from (*Allium cepa* × *A. fistulosum*) × *A. cepa* F_1 hybrids were made available to us by Dr. J.N. Corgan, New Mexico State University. Pedigrees for 951026 and 951027 are diagramed in Fig. 1. In both populations the seed parent was male-sterile *A. cepa* 'Excel 986A', and the pollen parent was *A. fistulosum* 'Bunching No. 1'. Population 951029 was generated following a similar strategy except that the seed parent was the partially male-sterile *A. cepa* New Mexico State University breeding line 93121, and the pollen source was from F_2 plants of an *A. fistulosum* 'Ishikura'×*A. cepa* 'Yelow Grano' hybrid. Crosses that generated the original interspecific F_1 hybrids were made in the field under caged conditions. Subsequent backcrosses were made under open field conditions using pollen from diverse *A. cepa* sources available at the time of the F_1 hybrids' flowering. Given the uncontrolled open pollination, it is possible that some plants in the backcross populations could have been either true backcrosses to *A. cepa*, sib-populations from other F_1 plants, or selfed populations resulting from self-pollination. Seeds of each F_1BC_3 population were sown in the Texas Tech University greenhouse on June 24, 1996, and seedlings were transplanted to the field on September 23, 1996. The plants were grown in the field throughout the winter. In the spring of 1997, 4 of 15 plants of population 951026, 53 of 100 plants of population 951027, and 13 of 27 plants of 951029 had survived. Among these plants, 35 did not bolt but formed bulbs that matured in the early summer; 35 plants bolted and some produced seeds.

Cytoplasm

Polymerase chain reaction (PCR) analysis was performed on genomic DNA of both fertile and sterile F_1BC_3 plants to confirm that S-cytoplasm had been maintained. DNA was extracted following Wettasinghe and Peffley (1998). Primers used for amplification were oligonucleotides A (5′-ATTACAAATGCGATGCTCT-3′) and B (5′-TCTACCGATTTCGCCATATC-3′) (Taberlet et al. 1991). An N-cytoplasmic maintainer line (Rio Colorado Seed Company, Bakersfield, Calif.) was used as the control for the 1.1-kb fragment, and 'Excel 186A' was used as the control for the S-cytoplasmic 1.0-kb fragment. PCR protocols and conditions followed Havey (1995).

Morphological observation

Bulbing plants were evaluated for morphology at maturity. Characters recorded were: leaf shape, fistulose or unifacial; leaf arrangement, distichous spreading or monostichous erect; bulb neck length, measured from the top of the bulb to the first leaf in *A. cepa* types; and the entire length of the pseudostem in *A. fistulosum* types for the relative comparison. Differences between the mean neck length of the F_1BC_3 population and that of *A. cepa* or *A. fistulosum* were tested by an analysis of variance with SAS (SAS Institute, Cary, N.C.) statistical software. Bulbs were scored under field conditions for pink root incidence; 191 plants of *A. cepa* 'TG1015' and 41 plants of *A.fistulosum* 'Ishikura' were scored as controls. Bulbs were scored as 0, no symptoms exhibited; 1, slight pink color on a few roots, up to 25%; 2, 25–50% roots with pink color; 3, 50–75% roots with pink color; and 4, more than 75% pink roots. Incidence percentage was calculated as the ratio of number of infected plants over the total number of plants

Table 1 Characteristics of bulbing F_1 BC_3 plants

Population	Plants observed	Isozymes[a]			Neck length (cm)	Bulb size (cm)		Leaf size (cm)			Bulb[b] color	Foliage[c]	Pink root[d]	SSC[e] (%)	Pyruvate[e] (μ*M*/ml)
		ADH	PGI	EST		Height×width		Length×width×thickness							
951026	# 1				8.0	6.4	4.5	34	0.8	0.5	y	1	0		
	# 8				10.7	6.6	5.1	19	0.9	0.7	y	2	0	5.2	7.02
	#14				6.0	5.1	4.1	31	0.7	0.7	wp	1	0	7.9	8.76
951029	# 1				8.0	5.5	3.8	19	0.5	0.9	w	2	0		
	#12				6.5	Divided bulbs		36	0.5	0.9	y	2	0		
	#14				8.3	5.2	5.5	29	0.8	0.6	y	1	0	6.1	3.72
	#21			f/c	10.5	4.9	5.0	21	0.7	0.7	w	3	0	8.8	6.85
	#22				9.5	6.1	5.6	37	0.7	0.7	y	3	0	7.3	7.09
951027	# 3				6.5	5.3	5.7	40	1.0	0.6	w	2	0	8.0	6.02
	# 4				5.0	4.4	2.9	17	0.6	0.2	w	2	0	6.4	6.15
	# 7				8.0	5.0	6.0	31	1.0	0.4	w	3	1		
	#10				7.0	5.1	7.2	30	0.9	0.8	y	2	0		
	#16				9.5	5.2	6.9	35	1.0	0.5	y	1	0		
	#18				4.0	4.0	3.3	22	0.7	0.3	w	3	0	9.3	8.12
	#21				9.8	5.5	6.9	47	1.6	0.9	y	1	0		
	#23				8.5	4.7	5.4	32	1.0	0.5	yw	2	1	8.3	11.76
	#25				6.5	4.0	4.0	33	1.3	0.7	wyp	2	0	10.2	7.55
	#30	3/1			5.5	Divided bulbs		22	0.5	0.5	w	3	0	11.4	9.76
	#31				7.0	5.1	4.5	30	1.0	0.7	y	3	0	7.1	6.12
	#33				4.0	4.0	4.2	30	0.7	0.5	w	2	0	9.0	10.69
	#42				10.0	4.5	5.2	30	1.0	0.4	wyp	2	1		
	#43	3/1			4.0	2.8	2.6	16	0.6	0.2	y	2	0	12.1	7.05
	#45				6.0	Divided bulbs		23	0.4	0.4	w	2	1	5.1	7.09
	#54		2/1		4.5	3.2	3.1	25	0.8	0.6	y	3	0	9.2	6.62
	#57		2/1		9.5	6.3	6.3	44	1.0	0.9	w	3	0	8.2	5.19
	#62				5.5	4.0	6.2	22	0.7	0.6	y	1	0		
	#71	3/2	2/1		7.0	4.7	4.8	27	0.9	0.2	y	3	0	6.0	6.25
	#74				6.5	4.3	4.5	27	0.8	0.3	y	2	0	6.9	7.39
	#81				9.5	4.8	5.8	27	1.0	0.9	y	2	0		
	#83				6.5	4.2	4.8	34	1.3	0.6	yw	2	0	7.4	8.49
	#84				9.5	4.2	5.6	26	1.0	0.9	y	1	0	7.2	9.59
	#88				6.5	5.9	6.0	29	0.8	0.7	y	1	0	6.3	7.82
	#95				3.5	2.3	1.7	13	0.4	0.4	y	3	0		
	#96			f/c	9.5	4.9	5.5	24	0.7	1.0	yw	1	0	8.1	6.12
	#97	3/1	2/1	f/c	16.5	Non-bulbing		39	1.2	1.3	w	3	0		
	#99					4.5	3.7				y	0		6.8	6.36
Ishikura	# 1	3/3	2/2	f	19.0	Non-bulbing		30	1.0	1.0	w	3		5.7	6.43
	# 2	3/3	2/2	f	19.5	Non-bulbing		50	1.5	1.4	w	3			
	# 3	3/3	2/2	f	20.0	Non-bulbing		46	1.4	1.4	w	3			
	# 4	3/3	2/2	f	19.0	Non-bulbing		43	1.5	1.5	w	3			
	# 5	3/3	2/2	f	18.0	Non-bulbing		50	1.6	1.6	w	3			
	# 6	3/3	2/2	f	20.0	Non-bulbing		49	1.5	1.5	w	3			
New Mexico Sunlite															
	# 1	1 or 2	1/1	c	3.8	4.3	4.9	36	0.9	0.5	y	1		7.9	8.21
	# 2	1 or 2	1/1	c	2.5	4.5	4.8	27	0.7	0.5	y	1			
	# 3	1 or 2	1/1	c	2.7	4.6	4.9	29	0.8	0.6	y	1			
	# 4	1 or 2	1/1	c	2.9	5.5	5.8	26	0.9	0.7	y	1			
	# 5	1 or 2	1/1	c	3.6	4.1	5.0	27	0.7	0.6	y	1			
	# 6	1 or 2	1/1	c	3.1	5.5	5.4	27	0.8	0.5	y	1			

[a] Only plants with *A. fistulosum* isozyme alleles are indicated
[b] y, Yellow; w, white; p, pink; yw, between yellow and white; wp, pink color on white bulb; wyp, mix of white, yellow and pink
[c] 1, *A. cepa* type; 2, intermediate; 3, *A.fistulosum* type
[d] 0, No symptoms; 1, slight pink root; 2, 25–50% pink root; 3, 50–75% pink root; 4, more than 75% pink root
[e] SSC and pyruvate data for 'Ishikura' and 'New Mexico Sunlite' are averages of 7 plants

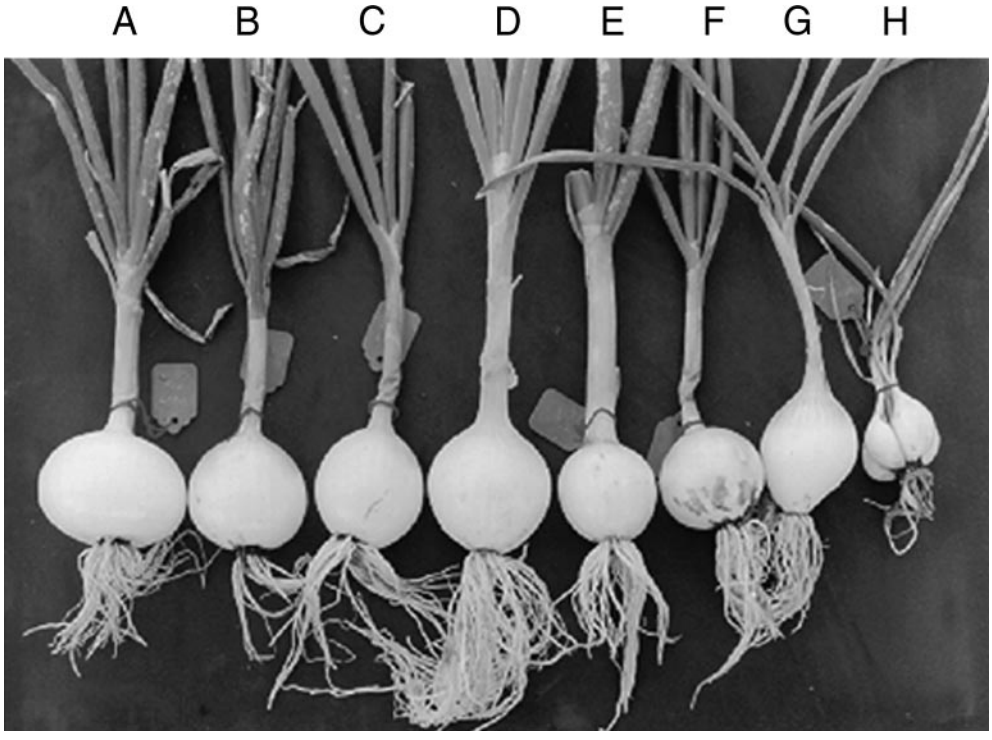

Fig. 3 Photographic representation of F_1BC_3 plants. *A*, *B*, and *G A. cepa*-type bulbing and leaf morphology, *C*, *D*, and *E*, *A. cepa*-type bulbing and *A. fistulosum*-type neck and leaf characters, *F A-cepa*-type bulb and *A. fistulosum* leaf characteristics, *H* small divisions similar to *A. fistulosum. Plant D* possesses the *A.fistulosum* allele, *Adh-1*^{3}

Table 2 Pink root incidence of F_1BC_3 population, *A. cepa* 'TG1015' and *A. fistulosum* 'Ishikura'

Material	Plants tested	Ratings[a]					Incidence percentage	Average Index
		0	1	2	3	4		
F1BC3	37	31	4				11.43	0.03
Ishikura	41	38	2	1			7.32	0.02
TG1015	191	110	28	27	20	6	42.41	0.22

[a] Incidence readings were 0=no symptoms, 1=slight-25% pink roots, 2=25–50% pink roots,3=50–75% pink roots, 4=more than 75% pink roots

investigated. Average incidence index was calculated as ∑ (rating×number of plants at the rate(/(maximum rating number×number of plants tested).

The 35 plants which flowered were evaluated for the following characters: scape type (swollen, intermediate, or straight), cyme opening (random, or center to outside), corolla color (white, intermediate, or green), floret shape (open, intermediate, or closed), and anthesis date. Pollen viability was recorded as stainability of the microspores following Peffley et al. (1985). Seed set of each plant was recorded at seed maturity following open pollination in the field.

Soluble solids and pungency level measurement

Bulbs were stored at 28°C for 3 months; 11 bulbs rotted, and the remaining 24 bulbs were measured for soluble solid content (SSC) and puncency. Three samples of each bulb were taken with a 7-mm diameter corer and smashed together with a garlic press to release a slurry that was used for SSC and pungency tests. The samples of 7 plants of the *A. fistulosum* 'Ishikura' control were collected from the lower 0–3 cm. SSC of the samples was measured with a hand-held refractometer (ATAGO, Japan). Pungency was estimated by the pyruvate concentration as measured with a Beckman 2000 spectrophotometer following Randle and Bussard (1993). Correlations between SSC and pyruvate concentration were analyzed with SAS statistical software.

Isozyme analysis

The isozymes investigated were alcohol dehydrogenase (ADH, E.C.1.1.1.1) and phosphoglucoisomerase (PGI, E.C.5.3.1.9) (following Peffley et al. 1985), and esterase (EST, E.C.3.1.1.) (Hou et al. 1998). Plants were scored for the presence of *A. cepa* and *A. fistulosum* alleles. A plant with *Adh-1*^{1}, *Adh-1*^{2}, or *Pgi-1*^{1} was scored as an *A. cepa* homozygote c/c; *Adh-1*^{3}/*Adh-1*^{3} or *Pgi-1*^{2}/*Pgi-1*^{2} as an *A. fistulosum* homozygote f/f; and if alleles from both *A. cepa* and *A. fistulosum* were present, the individual was scored as a heterozygote c/f. Goodness of fit of the observed plant numbers with ADH and PGI *A. fistulosum* alleles in populations 951026 and 951027 was tested with χ^2. Since the genotype of the F_2 plants which provided pollen to generate 951029 was unknown, the expected ratio for this population was not determined.

Results

Cytoplasm

PCR analysis revealed a 1.0-kb fragment in all of the F_1BC_3 plants that were amplified (Fig. 2), confirming that the F_1BC_3 plants studied had S-cytoplasm as in the *A. cepa* parent.

Table 3 Characteristics of flowering F_1BC_3 plants

Population	Plants observed	Isozymes[a]			Scape shape	Fertility[b]		Cyme[c] opening	Corolla[d] color	Floret[e] shape	Anther[f] color	Anthesis date (month/day)
		ADH	PGI	EST		Pollen (stainability) (%)	Seeds (no.)					
951026	#15				1	92.7	90	1	1	1	y	>6/2
951029	#8				1	1.3	48	1	1	2	g	6/2–6/9
	#15				1	88.1	99	1	1	1	y	>6/2
	#16				1	19.6	35	1	1	1	yg	>6/2
	#17				1	2.6	1	1	1	1	yg	6/2–6/9
	#20				1	87.1	50	1	1	2	yg	>6/2
	#24				1	67.2	188	1				6/2–6/9
	#26				1	93.6	57	1				>6/2
	#27				1	47.7	26	1				<6/9
951027	#5				1	80.7	48	1	1	2	y	6/2–6/9
	#6				1	94.1	19	1				>6/2
	#11				1	75.8	194	1				>6/2
	#15				1	78.5	57	1				<6/9
	#22				1	89.7	71	1	1	1	y	>6/2
	#26				1	80.2	136	1	1	1	y	6/6–6/9
	#36				1	0.8	59	1	1	2	yg	>6/2
	#39	3/1			1	90.1	14	1	1	2	y	>6/2
	#42				1	72.9	1	1				<6/9
	#46				1	19.8	163	1	1	2	yg	>6/2
	#48				1	8.9	3	1				6/2–6/9
	#56				1	88.5	191	1	1	1	y	>6/2
	#61				1	86.6	153	1	1	2	y	<6/9
	#64				1	67.2	56	1				<6/9
	#70				1	0	53	1	1	2	y	>6/2
	#75			f/c	1	92.9	6	1				>6/2
	#76				1	90.7	14	1				>6/2
	#78				1	0.1	7	1				>6/2
	#79				1	96.6	49	1				<6/9
	#82				1	13.2	227	1				>6/2
	#87				1	88.8	115	1				>6/2
	#89				1	3.5	41	1	1	2	y	<6/9
	#90				1	84.3	90	1	1	1	y	>6/2
	#91				1	5.7	0	1			y	>6/2
	#92				1	19.3	88	1	1	2		>6/2
	#93				1	0.1	26	1	1	2	y	>6/2
A. fistulosum Ishikura[z]		3/3	2/2	f	3	94.9	159	3	3	3	yg	>5/22
A. cepa New Mexico Sunlite[z]		1 or 2	1/1	c	1	94.0	202	1	1	1	y	<6/2

[a] Indicated in Materials and methods, only plants with *A. fistulosum* isozyme alleles are indicated
[b] Control data of fertility for 'Ishikura' and 'New Mexico Sunlite' were averaged from 10 plants
[c] 1, random as A. cepa; 3, centered as A. fistulosum
[d] 1, A. cepa type; 3, A. fistulosum transparent type
[e] 1, A. cepa cup-like; 3, A. fistulosum dose type; 2, intermediate
[f] y, yellow; g, green; yg, between yellow and green

Morphological evaluation

The morphology of F_1BC_3 plants that bulbed was similar to that of *A. cepa* (Table 1). *A. fistulosum* characteristics observed included fistulose leaves (Fig. 3, plant D); monostichous erect-grown foliage (Fig. 3, plant D); garlic-like, small divided bulbs (Fig. 3, plant H); and long necks (Fig. 3, plant D and plant E). Plant 951027-97 did not form a bulb but grew like *A. fistulosum* only with leaf characters intermediate between the two species. The mean neck length of the F_1BC_3 plants (7.25 cm) was significantly longer than the mean neck length of 'New Mexico Sunlite' (3.1 cm) (F=16.60, P<0.005) but significantly shorter than 'Ishikura' (19.08 cm) (F=7.97, P<0.03).

Of the 35 F_1BC_3 bulbs scored for pink root, the incidence percentage and average index were lower than 'TG1015' but slightly higher than 'Ishikura' plants (Table 2).

The SSC in the F_1BC_3 plants ranged from 5.1% to 12.1%, with a mean SSC of 7.85%. The mean SSC of the backcross plants was significantly higher than the mean SSC of the control 'TG1015', 5.67% (F=11.54, P<0.01). The SSC of 'TG1015' ranged from 5.1% to 6.2%; pyruvate concentration was 3.72–11.76 μ*M*/ml). The mean value of the F_1BC_3 plants (7.44 μ*M*/ml) was not significantly different from that of the control 'TG1015' (6.43 μ*M*/ml) (F=1.91, P=0.2458). The correlation between SSC content and pungency value was R^2=0.1165 (P<0.025).

Characteristics of the plants that flowered are given in Table 3. Many plants resembled *A. cepa* in bulb and leaf characters, had swollen scapes, a white corolla color, and

Table 4 χ^2 test of F_1BC_3 plants exhibiting *A. fistulosum* isozyme alleles

Isozymes	Observed number	Expected number	χ^2 value	*p* value
ADH	8	14	7.1428	0.05–0.025
PGI	6	14		
ADH×PGI	2	2		

randomly opening cymes as in *A. cepa.* Anther color varied from yellow to dark green with most plants having yellow pollen. Perianths, with few variants, were open as in *A. cepa.* Perianths on some flowers were intermediate in petal transparency yet opened widely as in *A. cepa*; *A. fistulosum* perianths are considerably more transparent than those of *A. cepa*. Pollen stainability varied from 0 to 96.6% (Table 3), falling into two major groups, between 0 and 20% and between 70% and 100%. Seed set of the F_1BC_3 plants was from none to 227 seeds per umbel. In 9 plants, the pollen stainability was high (>80%), as was the seed set (>71 seeds per umbel). Other plants had either low pollen stainability with high seed set, high pollen stainability with low seed set, or low pollen stainability and low seed set. Plant 951027-75, which resembled the interspecific F_1 hybrid in bulbing, was fertile with 92.9% pollen stainability and produced 6 seeds.

Isozymes

In 115 plants of F_1BC_3 populations 951026 and 951027, 8 had *A. fistulosum* ADH alleles and 6 plants had *A. fistulosum* PGI alleles (Tables 1 and 3). Two plants had both ADH and PGI *A. fistulosum* alleles. The number of plants observed with *A. fistulosum* ADH and PGI alleles was significantly lower than the expected number (Table 4). In 51 F_1BC_3 plants of populations 951026, 951027, and 951029, 6 plants had *A. fistulosum* esterase alleles. Of these 6 plants, 1, 951027-97, which was phenotypically like an F_1 hybrid with no bulb, also had *A. fistulosum* ADH and PGI alleles. Of the plants transplanted to the field, 7 formed bulbs similar to *A. cepa*; 2 flowered and had high pollen viability and set seeds. Of the 8 plants that had *A. fistulosum* isozyme alleles and did not bolt, 5 were monostichous, typical of *A. fistulosum*; 2 plants were intermediate; and 1 plant was distichous as in *A. cepa.* The other morphological characters in these plants were apparently not linked to any character in *A. fistulosum.*

Discussion

The goal of introgressive hybridization is increased genetic diversity of a species, thereby altering its adaptive potential (Lewontin and Birch 1966). Agronomically acceptable and superior *A. cepa*-type bulbs with *A. fistulosum* genes conferring traits not found in *A. cepa* have been the goal of plant breeders for decades. We describe in this report the development of stable (fertile) introgressants from these populations and agree with Stebbins' report (1950) that the stabilization of hybrid derivatives can occur which result in new types. In this study we recovered *A. cepa* bulb-type individuals that expressed *A. fistulosum* morphological traits. *Allium fistulosum* isozyme alleles provided unambigous evidence that *A. fistulosum* genes had been introgressed into *A. cepa*-type bulbing onions. Recombinant bulbs were recovered that possessed *A. fistulosum* isozyme alleles, yet were phenotypically like *A. cepa*, grew vigorously, differed in SSC and pungency levels, had high pollen fertility, and produced seeds. Others have reported new genes and new genotypes produced through recombination (Golding and Strobeck 1983). These plants provide unequivocal evidence that introgression occurred between *A. fistulosum* and *A. cepa.*

The general use of wild or related species as gene sources to improve cultivated crops has been successful in other crops (Kalloo 1992). However, the recovery of useful introgressants of *A. cepa*-type bulbs with *A. fistulosum* genes has not yet been reported. The difficulty in recovering introgressants has been due mainly to sterility in F_1 interspecific hybrids and hybrid derivatives. There is evidence to suggest that cytoplasm selection may be a strategic means to circumvent to nuclear-cytoplasmic incompatibility. Ulloa-G et al. (1994, 1995) report high sterility in *A. fistulosum*×*A. cepa* BC_1 and BC_2 plants and suggest that the sterility is due to nuclear-cytoplasmic incompatibility between *A. fistulosum* and *A. cepa* when plants are in *A. fistulosum* cytoplasm. Khrustaleva and Kik (1998) have reported recently the use of a bridge cross involving *A. roylei* for the purpose of increasing the possibility of genetic introgression from *A. fistulosum* into *A. cepa.* This strategy, however, could in fact heighten the difficult of introgression in onion by introducing genes from a third species, *A. roylei.* The success in generating the F_1BC_3 populations and recovering *A. cepa*-type bulbs in this study was likely because the cytoplasm of the seed parent of the initial F_1 hybrids was *A. cepa.*

Recovery of the advanced backcross plants in this study was due in large part to the large scale unrestricted bee-assisted pollination of interspecific F_1 hybrids and backcross progeny. Unrestricted crossing to the pollen donors very likely allowed for the recovery of a large number of seeds. The caveat to this strategy is that the precise pollen donors of these backcrosses cannot be identified. Even though it is regrettable that the exact pedigree is not known, the unrestricted pollinations allowed the possibility for recovery of fertile bulb-type onions with introgressed *A. fistulosum* genes.

Low levels of introgression can be expected in interspecific hybrid backcrosses since the number of markers per backcross individual is halved in each successive generation. Morphology and molecular markers provide two types of estimates of the extent of interspecific gene flow. Most morphological characters of the F_1BC_3 plants were like *A. cepa*, but *A. fistulosum* characters such as long neck and monostichous leaf attachments were found in some plants; some recombinant plants possessed *A. fistulosum* and *A. cepa* isozyme alleles and others only *A.*

cepa alleles, yet still expressed *A. fistulosum* traits. Linkage relationships between each isozyme and morphological characters could not be calculated since there were limited numbers of plants possessing each *A. fistulosum* isozyme. Not all the plants had common *A.fistulosum* isozyme alleles, suggesting that there is no linkage between these leaf characters and ADH, PGI or EST loci. As introgression and selections continue, recombinant bulb-type onions with agronomically acceptable short stems will probably be recovered, as evidenced by the recovery of recombinant plants with *A. fistulosum* isozyme alleles and desirable *A. cepa* characters.

Possible increases in pungency has been a concern when introgressing *A. fistulosum* germplasm into *A. cepa*. Our results show that introducing *A. fistulosum* genes into *A. cepa* does not mean that pungency has to accompany desirable *A. fistulosum* traits. This is not a pressing concern since there was a low correlation between SSC and pyruvate concentration in the F_1BC_3 plants (R^2=0.1165). Our results with interspecific hybrid plants agree with those of Simon (1995): genes controlling SSC and pungency segregate independently. In our study, both bulbs with high SSC and low pungency, and low SSC and high pungency were recovered, providing encouragement in our efforts to recover *A. cepa*-type with high solids but low pungency.

Gene flow from *A. fistulosum* into *A. cepa* in these populations has resulted in novel individuals never before described in previous interspecific hybridization attempts. Stebbins (1950) reported decades ago that the establishment of new types have resulted from introgression. The type of evolution in our backgross populations is likely localized (Heiser 1973) where gene flow is transitory and has only a slight long-term evolutionary significance. It is unlikely that barriers isolating these two *Allium* species have been broken, rather, that the populations were backcrossed into *A. cepa* cytoplasm and recombinant individuals express a combination of genes that allow them to be more fit. Left to their natural, unselected reproduction, the species would probably segregate into the parental types. Regardless, if an introgressant is recovered from a localized pocket of hybridization, introgression by backcrossing of this type may be of great significance (Reiseberg and Brunsfeld 1992). The significance of our study has been to document with morphological and molecular markers the recovery of introgressants resulting from interspecific hybridization, namely, recombinant bulb-type onions with *A. fistulosum* genes that are pollen- and egg-fertile. Agronomically acceptable and superior *A. cepa*-type bulbs harboring *A. fistulosum* genes have been the goal of plant breeders for decades. We have demonstrated that what was once a dream is now becoming a practical possibility.

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