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Electrophoretic analysis of *Allium* alien addition lines

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Summary. Meiotic pairing in an interspecific triploid of *Allium cepa* and *A. fistulosum*, 'Delta Giant', exhibits preferential pairing between the two *A. cepa* genomes, leaving the *A. fistulosum* genome as univalents. Multivalent pairing involving *A. fistulosum* chromosomes occurs at a low level, allowing for recombination between the genomes. Ten trisomics were recovered from the backcross of 'Delta Giant' × *A. cepa* cv., 'Temprana', representing a minimum of four of the eight possible alien addition lines. The alien addition lines possessed different *A. fistulosum* enzyme markers. Those markers, *Adh-1*, *Idh-1* and *Pgm-1* reside on different *A. fistulosum* chromosomes, whereas *Pgi-1* and *Idh-1* may be linked. Diploid, trisomic and hyperploid progeny were recovered that exhibited putative pink root resistance. The use of interspecific plants as a means to introgress *A. fistulosum* genes into *A. cepa* appears to be successful at both the trisomic and the diploid levels. If introgression can be accomplished using an interspecific triploid such as 'Delta Giant' to generate fertile alien addition lines and subsequent fertile diploids, or if introgression can be accomplished directly at the diploid level, this will have accomplished gene flow that has not been possible at the interspecific diploid level.

Key words: Isozymes – Pink root resistance – Plant breeding – Trisomics – Interspecific onion

Introduction

Allium cepa L. is the most widely-grown onion species worldwide. In the United States and Europe it accounts for virtually all of the commercial onion production and is worth more than $ 200 million annually in the United States alone (Ware and McCollum 1980).

A. cepa is susceptible to pink root disease, caused by the imperfect, soil-borne fungus, *Pyrenochaeta terrestris* (Hans.) (Gorenz et al. 1949). *P. terrestris* proliferates rapidly and is persistent in soil over long periods of time, resulting in dwindling land area suitable for onion production.

In *A. cepa* only partial resistance to *Pyrenochaeta* has been reported (Jones and Perry 1956; Nichols et al. 1960; Perry and Jones 1955). Resistant, short-day cultivars, have been developed by U.S. scientists that carry the PRR (pink root resistant) suffix. However, the degree of resistance in these cultivars is not adequate to prevent serious losses. Other species of *Allium*, unlike *A. cepa*, are reported to be highly resistant or immune to pink root (Porter and Jones 1933). These include *A. ampeloprasum* L. subsp. *porrum* and *A. fistulosum* L.

The general use of wild species as a source for disease resistance breeding has been highly successful in other crop plants (Rick 1982). Additional sources of genes for disease and pest resistance are often found in wild relatives of a cultivated species (Saini and Davis 1967). Of the resistant *Allium* species, *A. fistulosum*, because of its comparatively close relationship to *A. cepa*, is a likely source of useful PR resistance. If the gene(s) that confer resistance could be transferred to *A. cepa*, the improvement in onion pink root resistance would be significant. *A. fistulosum* contains other characters such as cold hardiness, resistance to *Botrytis* and smut, and increased solids that would also be valuable if incorporated into *A. cepa* cultivars (van der Meer and van Benekom 1978).

Although hybrids between *A. cepa* and *A. fistulosum* have been obtained (Emsweller and Jones 1935a, b), there are no reports of successful introgression of genes from *A. fistulosum* into *A. cepa* (Van Der Meer 1978). In all cases, the F_1 hybrids were highly sterile. At least part of the sterility is the result of chromosomal rearrangements between the two species (Emsweller and Jones 1935a, b; Levan 1936; Maeda 1937).

In cases where diploid hybrids are sterile, development of alien addition lines has proved to be a useful approach for introgressing genes from wild species. Sears (1956) transferred genes for leaf rust resistance from *Aegilops* into wheat. More recently, this approach was employed in transferring nematode resistance from *Beta procumbens* Chr. Sm. into *B. vulgaris* L. (Savitsky 1978).

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The object of this research has been to generate a series of alien addition lines and evaluate their potential for facilitating introgression between *A. fistulosum* and *A. cepa.* If *A. cepa* lines could be produced with an extra chromosome from *A. fistulosum* carrying the gene(s) for PRR, gene transfer by crossing over with the *A. fistulosum* chromosome might be possible. The availability of a triploid interspecific hybrid between *A. cepa* and *A. fistulosum,* 'Delta Giant', (Perkins et al. 1958) provides an opportunity to develop such alien addition lines.

Materials and methods

Meiotic studies

Meiosis was examined in 'Delta Giant' (2n = 3x = 24) and compared with that of *A. cepa* and *A. fistulosum* accessions (2n = 2x = 16). Inflorescences of field grown plant materials were collected over several months during summer. Floral buds were fixed in 6 : 3 : 1, ethanol : chloroform : glacial acetic acid and stored in 70% ethanol until viewing. Meiocytes were viewed during first prophase, metaphase and anaphase in all plant material. Microspores undergoing the first mitotic division were studied in 'Delta Giant'.

Pollen evaluation

Pollen was removed from five anthers of the parental species and the triploid 'Delta Giant' with a spear point needle and placed in a microfuge tube. One hundred µl of germination media (Tanksley et al. 1981) without agar was added to the pollen and mixed using a mini-vortex. Thirty µl of the dispersed pollen was then micropipetted into three petri dishes containing the artificial germinating media. Germination proceeded for 24 h at 25 °C. This procedure was repeated twice daily, morning and late afternoon, for 3 days. The germination percentage reported is the average over all reps.

Stainability of pollen from the parental species and 'Delta Giant' was recorded. Pollen from three anthers of each genetic line was placed in a microfuge tube. Two drops of acetocarmine were added. Vibrating with a Vortex dispersed the pollen. The solution was micropipetted onto a slide for viewing. A minimum of 100 pollen grains were observed per sample.

Backcross of 'Delta Giant' to A. cepa cv. 'Temprana'

'Delta Giant' was backcrossed to a diploid *A. cepa,* cv. 'Temprana', using *A. cepa* as the pollinator. Plants of each cultivar were planted in 2.5 m rows and enclosed in mosquito netted cages to prevent outcrossing; bees were used to ensure pollination and crossing within cages.

After seed matured, umbels were harvested from the backcross of 'Delta Giant' to 'Temprana'. A total of 224 seeds were collected from approximately 50 umbels representing 10 plants.

Chromosome counts of backcross progeny

Of the 157 seeds that germinated, 142 survived and were planted in a greenhouse. Chromosome counts were obtained from metaphase cells of root tips that had been fixed in 3 : 1, ethanol : glacial acetic acid and stored in 70% ethanol. Roots were stained with acetocarmine, squashed on the slide and viewed under 40X magnifcation. Only after examining several root tips with at least three definitive counts was a plant's chromosome number recorded.

Isozyme analysis

A. cepa, A. fistulosum, 'Delta Giant' and all progeny were assayed electrophoretically for the following enzymes: alcohol dehydrogenase (ADH), (E.C.1.1.1.1); acid phosphatase (APS), (E.C.3.1.3.2); esterase (EST), (E.C.3.1.1.2); glutamate oxaloacetate transaminase (GOT), (E.C.2.6.1.1); glycerate dehydrogenase (GDH), (E.C.1.1.1.29); isocitrate dehydrogenase (IDH), (E.C.1.1.1.42); malate dehydrogenase (MDH), (E.C.1.1.1.37); peroxidase (PRX), (E.C.1.11.1.7); 6-phosphogluconate dehydrogenase (6PGDH), (E.C.1.1.1.44); phosphoglucoisomerase (PGI), (E.C.5.3.1.9); phosphoglucomutase (PGM), (E.C.2.7.5.1); shikimic dehydrogenase (SKDH), (E.C.1.1.1.25), and triose phosphate isomerase (TPI), (E.C.5.3.1.1). Recipes and electrophoretic procedures were according to Vallejos (1983).

Only those enzymes for which reproducible banding patterns were observed were pursued in this study. Putative loci encoding individual isozymes were given gene symbols (Table 1). Allelic differences between the two parents were obtained for five enzyme loci.

Pink root screening

A. cepa, 'Delta Giant' and the backcross progeny were screened in the field for pink root resistance using parental plants for comparison. Only progeny planted in the field screening were inoculated with the pink root organism. The inoculum was obtained from onion roots infested with *P. terrestris* that were removed from the host plants and made into an aqueous, non- buffered slurry using a Waring blender[1]. At

Table 1. List of *Allium* enzyme loci exhibiting resolution

Locus	Plant material	Gel system
Adh-1[a]	seed	TC
	lvs	TC
	rts	TC
Aps-1, 2	lvs	TC
	rts	His
Est-1	lvs	TC
Gdh-1[a]	rts	His
Got-1, 2, 3	rts	TC
	rts	His
Idh-1[a]	rts	His
Mdh-1	lvs	His
Prx-1	lvs	TC
6Pgdh-1, 2	lvs	His
Pgi-1[a]	rts	His
Pgm-1[a]	rts	TC

TC = Tris citrate (Vallejos and Tanksley 1983)
His = Histidine
[a] Loci for which 'Delta Giant' is heterozygous

1 Mention of a company name or trademark is for the readers' benefit and does not constitute endorsement of a particular product by New Mexico State University of Texas Tech University over others that may be commercially available

time of transplanting to the field, 500 ml of the slurry was applied to the root zone of each entry.

A pink root rating of 0 at plant maturity indicated the root mass was totally free of any pink color indicating the presence of *P. terrestis* toxin (Lindsey and Corgan 1976) and a rating of 10, all roots had pink color.

Evaluation of fistulose leafing characteristic

'Delta Giant', 'Temprana', and 'Delta Giant' × 'Temprana' backcross progeny were evaluated for the presence of *A. fistulosum* characters. Fistulose (round) leafing habit is the most reliable differentiating characteristic of *A. fistulosum* and *A. cepa* as *A. fistulosum* possesses circular, fistulose leaves while *A. cepa* leaves are flattened on one side (Jones and Mann 1963).

Results and discussion

Meiotic studies

Analysis of meiosis in *A. fistulosum* and *A. cepa* are in accordance with those of earlier research in that eight bivalents of both parental species were found in diakinesis and metaphase I (Emsweller and Jones 1935b; Levan 1941; Maeda 1937; Cochran 1950). *A. cepa* pairing was observed to be normal with no bridges or fragments; in only a few instances were bridges observed in *A. fistulosum*. When *A. cepa* and *A. fistulosum* genomes are present in the diploid, interspecific state, bivalent pairing does occur with greater or lesser numbers of bivalents depending upon the parents used in the cross (Emsweller and Jones 1945), suggesting homoeology exists between the species.

Meiotic data collected on the triploid, interspecific 'Delta Giant' would indicate that the most common condition of meiotic pairing is bivalents (presumably the two *A. cepa* genomes) and univalents (*A. fistulosum*) (Fig. 1 and Table 2). In only 3% of the cells observed could multivalent associations be detected (Table 2). Because of the difficulty of spreading of the meiotic chromosomes, all 24 chromosomes could not usually be viewed; therefore, only the minimum number of chromosomal associations are reported.

Pairing between *A. cepa* and *A. fistulosum* does occur in diploid hybrids (Emsweller and Jones 1935b; Levan 1941; Maeda 1937; Cochran 1950). Observations of bivalent pairing in the triploid interspecific hybrid, however, suggest preferential pairing is occurring among *A. cepa* homologues, leaving *A. fistulosum* univalents, with only occasional multivalent pairing involv-

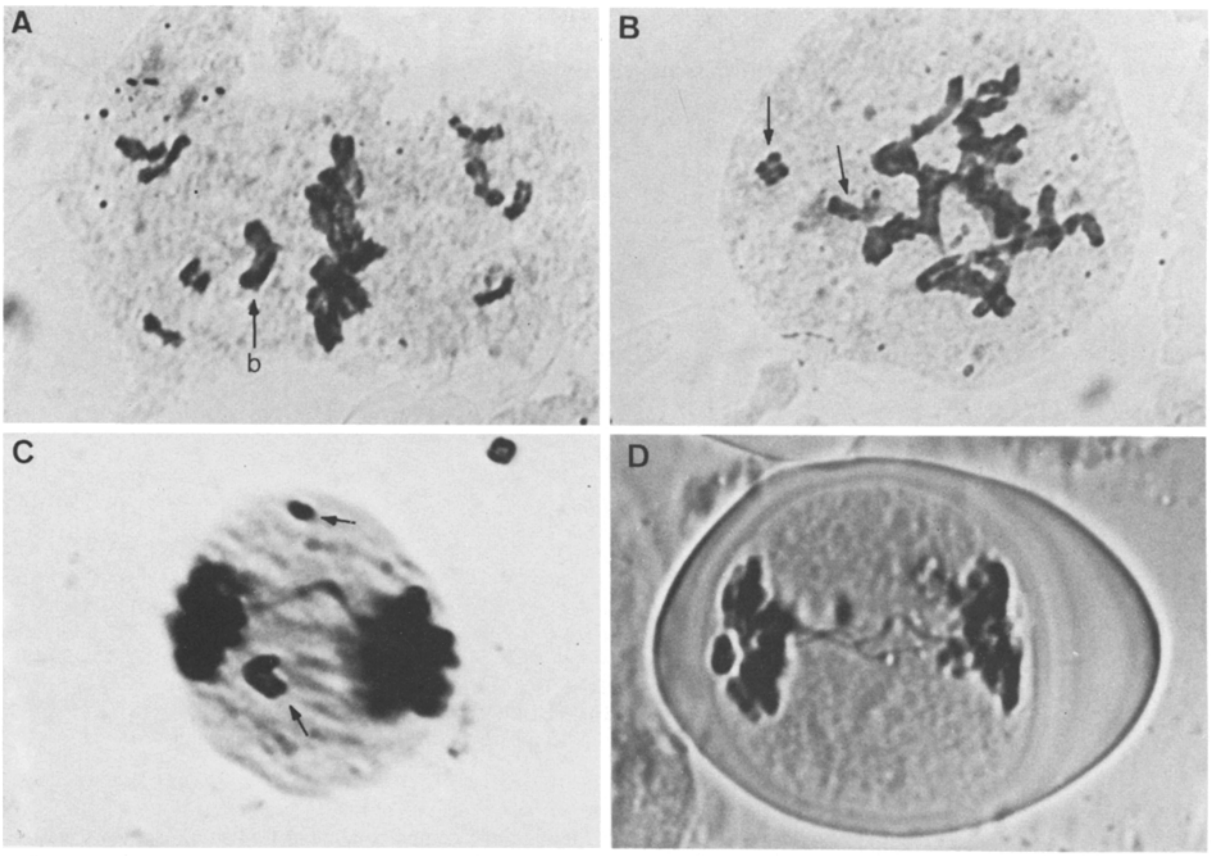

Fig. 1 A–D. Photographs of 'Delta Giant' shallot meiosis. **A** metaphase with 8 univalents, bivalent (b); **B** metaphase with 2 univalents (*arrows*); **C** anaphase with bridge and laggards (*arrows*); **D** double bridge in anaphase

Table 2. Minimum number of chromosomal associations observed in meiocytes of 'Delta Giant' shallot

Minimum no. of associations per cell	No. observed	% of total cells
Univalents		
8	37	26
7	4	3
6	8	6
5	16	11
4	13	9
3	17	12
2	15	10
1	33	23
Bivalents		
8	28	20
7	9	6
6	10	7
5	9	6
4	6	4
Multivalents	5	3

Table 3. Bridges and laggards observed in shallot 'Delta Giant' meiosis

Meiotic condition	No. observed
Without bridge	
no laggard/fragment	47 (23%)
with laggard/fragment	117 (59%)
With bridge	
no laggard/fragment	1 (1%)
with laggard/fragment	30 (15%)
Double bridge	6 (3%)

ing *A. fistulosum* chromosomes. This proposal is supported by data on transmission of enzyme markers (see *"Pollen evaluation"*).

Meiotic data from this study is in contrast to an earlier study reported by Levan (1941). Working with an independent interspecific triploid, Levan observed a preponderance of trivalent formation, with a few univalents. A possible explanation for this discrepancy may be the result of the different combinations of *A. cepa* and *A. fistulosum* parents used in the two studies. Similar variability for pairing in diploid hybrids has been reported (for a review see Emsweller and Jones 1945).

Other abnormalities were present in the first meiotic division of 'Delta Giant'. No attempts were made to differentiate between fragments or laggards. Bridges and fragments and/or laggards were observed in anaphase I and persisting into telophase I (Fig. 1). Because of the high frequency of fragments and/or laggards without the presence of bridges, the possibility exists that some fragments were actually laggards (Table 3).

A. cepa pairing was observed to be normal with no bridges or fragments. Only in a very few instances were bridges observed in *A. fistulosum,* not enough to account for the 15% bridge formation observed in 'Delta Giant'. Assuming the *A. cepa* parent(s) of 'Delta Giant' not to be heterozygous for an inversion, and knowing that pairing between *A. cepa* and *A. fistulosum* does occur (Emsweller and Jones 1935 b, 1945; Levan 1941; Maeda 1937; Cochran 1950), there is evidence for at least one inverted segment differing between *A. cepa* and *A. fistulosum.* The presence of bridges suggests that pairing between the genomes occurred.

Pollen evaluation

A higher percentage of grains absorbed stain than germinated in all entries examined. *A. fistulosum* germinated 32% and stained 76% while the values for *A. cepa* were 35% and 90%, respectively. In both studies the performance of 'Delta Giant' was less when compared to the parental species, 10% germination and 35% stainability. Pollen tubes of 'Delta Giant', while much fewer in number, were also quite short (Fig. 2 a) compared to the apparently vigorous, lengthy, tube growth of the parents (Fig. 2 b). Triploids are known to have reduced fertility (Burnham 1962) and a reduction in pollen germination and stainability which, in this case, may be confounded with the effects of interspecific hybridization.

Pollen mitosis

Pollen mitosis proved to be an excellent tool to evaluate the chromosome numbers of immature 'Delta Giant' microspores. Observing the actual number of chromosomes per gamete provides an understanding of the segregation of 'Delta Giant' chromosomes during meiosis. Chromosome numbers among the microspores examined varied from 2–22, with a normal, bell-shaped distribution curve (Fig. 3). The majority of the spores contained 11 or 12 chromosomes (Fig. 4). At this stage, virtually 100% of the microspores absorbed stain. Not until the microspores matured did the stainability percentage begin to drop to 30%. An apparent lethal effect was expressed as the pollen matured.

Chromosome counts of backcross progeny

Chromosome counts were made on root tip squashes of the progeny derived from 'Delta Giant' × *A. cepa.* Because the chromosome number of the male gametes varied (see *Pollen mitosis*), it would be expected that the progeny derived from the male 'Delta Giant' gametes would also reflect this chromosomal variation.

However, the majority (109) were diploid with 16 chromosomes, 10 were trisomic with 17 chromosomes, and 6 had more than 17 chromosomes. Possibly hyperploid zygotes and/or seedlings experienced low survival rates and, therefore, few progeny with extra, alien chromosomes persist to the seedling stage.

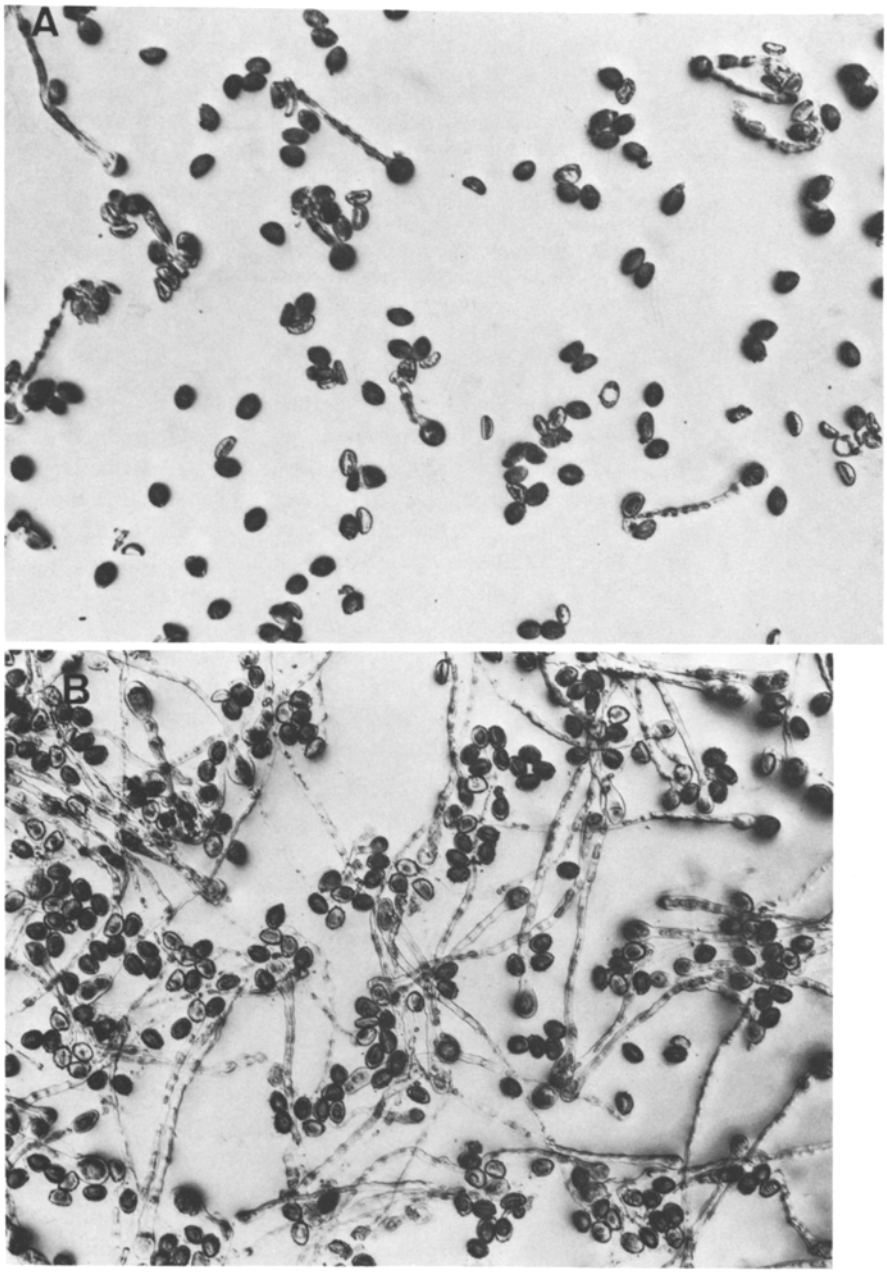

Fig. 2 A, B. Pollen germination. **A** Delta Giant' shallot pollen germination; **B** *A. cepa* pollen germination

Isozyme analysis

Loci encoding individual isozymes that exhibited resolution are listed in Table 1. Segregating isozyme markers could thus be used to detect the presence or absence of *A. fistulosum* chromosomes of chromosome segments. Figure 5 displays photographs of starch electrophoretic gels stained for those isozymes with allelic segregation. Each profile displays *A. cepa* and *A. fistulosum* parental species as well as interspecific material.

Alcohol dehydrogenase (ADH). Three alleles code for ADH activity, two *A. cepa* (alleles 1 and 2) and one *A. fistulosum* (allele 3) (Fig. 5a). The darker banding of allele 3 possibly represents a duplication of the *A. fistulosum* adh locus. The locus corresponding to the genes coding for this isozyme was assigned *Adh-1.*

Glycerate dehydrogenase (GDH). Two zones of NADP-dependent activity were detected for GDH (Fig. 5b). Heterozygous individuals were found to have a band of intermediate mobility, suggesting the enzyme is a dimer. The locus corresponding to the isozyme represented was assigned *Gdh-1.*

Isocitrate dehydrogenase (IDH). An IDH (isocitrate dehydrogenase) NADP-dependent isozyme profile appears in Fig. 5c. Diploid *A. cepa* is homozygous for the

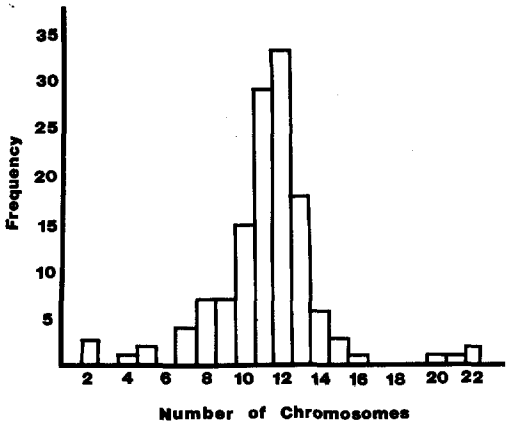

Fig. 3. Chromosome numbers in 'Delta Giant' shallot microspores

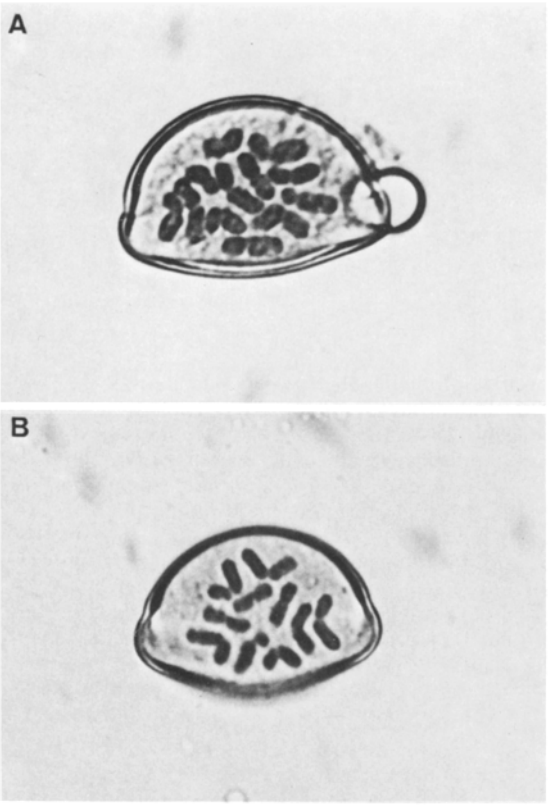

Fig. 4 A, B. 'Delta Giant' shallot microspore mitotic metaphase. **A** with 12 chromosomes; **B** with 10 chromosomes

slow allele. Diploid *A. fistulosum* is homozygous for the fast allele. IDH is presumably a dimer, therefore, two alleles are reflected in this profile. Interspecific heterozygotes between *A. cepa* and *A. fistulosum* display a slow and a fast band with the hybrid molecule of intermediate mobility. *Idh-1* was assigned to the locus responsible for IDH activity.

Phosphoglucoisomerase (PGI). Numerous bands of activity were detected for PGI (Fig. 5 d). These multiple bands have been found to be governed by alleles at a single locus, presumably by posttranscriptional modification (Peffley and Tanksley, unpublished). Heterozygous individuals were found to have continuous banding from the anodal front into the cathodal slice. The locus corresponding to the gene coding for this isozyme was given the symbol *Pgi-1*.

Phosphoglucomutase (PGM). Two zones of PGM activity were detected (Fig. 5 e). Heterozygous individuals possessed two bands. No intermediate band was detected in the hybrid, and this observation is in keeping with monomic nature of the enzyme. *Pgm-1* was assigned to the locus responsible for PGM activity.

Analysis of backcross progeny

The backcross progeny were analyzed electrophoretically using the enzyme markers in Table 1. Among the diploid entries, no *A. fistulosum* alleles were detected.

Of the 10 trisomic plants, three have been identified as alien addition lines containing *A. fistulosum* allozymes in trisomic dosage (Fig. 6). These are entries number 5, a *Pgm-1* addition line, number 59, an *Adh-1* addition line, and number 126, an *Idh-1* addition line that also contained the *Pgi-1 A. fistulosum* allele. *Pgm-1, Adh-1* and *Idh-1* appear to be segregated independently of each other and were associated with different alien additions lines, suggesting that in *A. fistulosum* these loci are on different chromosomes whereas *Idh-1* and *Pgi-1* may reside in the same linkage group. Entry 43 and 126 were host to dormancy and disease problems and could not be further evaluated.

According to these results, enzyme markers have been found for three chromosomes. However, additional trisomics were recovered that possessed no *A. fistulosum* enzyme markers, thus the potential exists that other of the five remaining *A. fistulosum* chromosomes may have been represented in the addition series. The segregation of enzyme markers in the hyperploids was investigated for *Adh-1, Gdh-1, Idh-1, Pgi-1* and *Pgm-1* loci (Fig. 7). As the number of extra chromosomes increased, so did the presence of multiple gene markers from *A. fistulosum,* indicating the presence of more than one *A. fistulosum* chromosome. Not all the progeny exhibited the *A. fistulosum* genetic markers in the trisomic dosages, reflecting probable variation in the transmission of the *A. fistulosum* chromosome(s).

Pink root screening

In all cases, *A. cepa,* cv. 'Temprana' was susceptible (rating average = 7.3) and 'Delta Giant' extremely re-

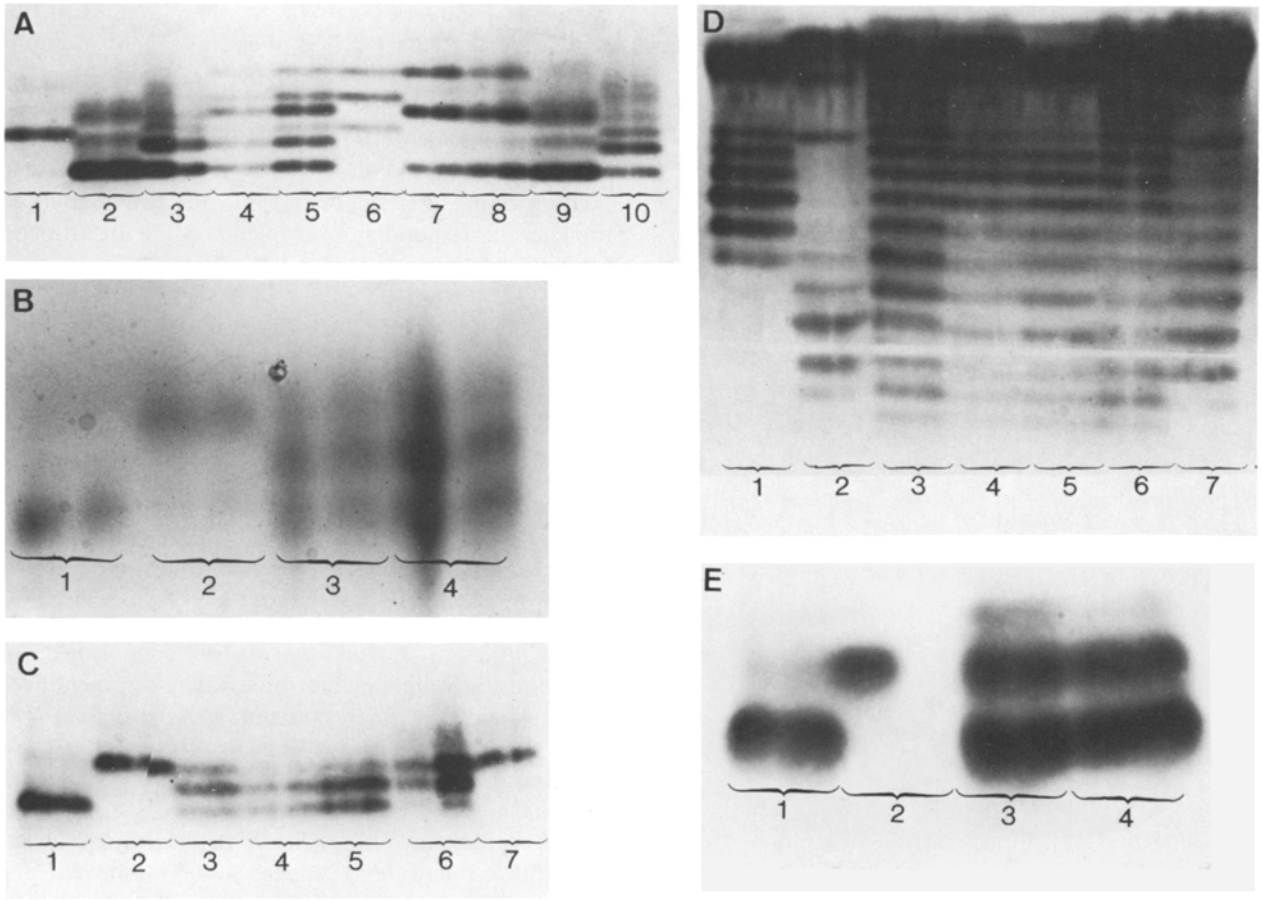

Fig. 5 A–E. Photographs of *Allium* electrophoretic gels. **A** Alcohol dehydrogenase profile; 1: *A. cepa* = *Adh-1*^{1}/*Adh-1*^{1}; 2: *A. fistulosum* = *Adh-1*^{3}/*Adh-1*^{3}; 3: diploid *A. fistulosum* × *A. cepa* = *Adh-1*^{1}/*Adh-1*^{3}; 4: 'Delta Giant' = *Adh-1*^{1}/*Adh-1*^{2}/*Adh-1*^{3}; 5: progeny 59 = trisomic dosage = *Adh-1*^{1}/*Adh-1*^{2}/*Adh-1*^{3}; 6: *A. cepa* heterozygote, progeny 2 = *Adh-1*^{1}/*Adh-1*^{2}; 7: progeny 65 = *Adh-1*^{2}/*Adh-1*^{3}; 8. progeny 82 = *Adh-1*^{2}/*Adh-1*^{3}; 9: progeny 89 = *Adh-1*^{3}/*Adh-1*^{3}; 10: progeny 98 = trisomic dosage = *Adh-1*^{1}/*Adh-1*^{1}/*Adh-1*^{3}. **B** Glycerate dehydrogenase profile; 1: *A. cepa* = *Gdh-1*^{1}/*Gdh-1*^{1}; 2: *A. fistulosum* = *Gdh-1*^{2}/*Gdh-1*^{2}; 3: diploid *A. fistulosum* × *A. cepa* = *Gdh-1*^{1}/*Gdh-1*^{2}; 4: 'Delta Giant' = *Gdh-1*^{1}/*Gdh-1*^{1}/*Gdh-1*^{2}. **C** Isocitrate dehydrogenase profile; 1: *A. cepa* = *Idh-1*^{1}/*Idh-1*^{1}; 2: *A. fistulosum* = *Idh-1*^{2}/*Idh-1*^{2}; 3: diploid *A. fistulosum* × *A. cepa* = *Idh-1*^{1}/*Idh-1*^{1}/*Idh-1*^{2}; 4: 'Delta Giant' = *Idh-1*$_1$/*Idh-1*^{1}/*Idh-1*^{2}; 5: progeny 48 = trisomic dosage = *Idh-1*^{1}/*Idh-1*^{1}/*Idh-1*^{2}; 6: progeny 82 = *Idh-1*^{1}/*Idh-1*^{2}; 7: progeny 89 = *Idh-1*^{2}/*Idh-1*^{2}. **D** Phosphoglucoisomerase profile; 1: *A. cepa* = *Pgi-1*^{1}/*Pgi-1*^{1}; 2: *A. fistulosum* = *Pgi-1*^{2}/*Pgi-1*^{2}; 3: *A. fistulosum* × *A. cepa* = *Pgi-1*^{1}/*Pgi-1*^{2}; 4: 'Delta Giant' = *Pgi-1*^{1}/*Pgi-1*^{1}/*Pgi-1*^{2}; 5: progeny 48 = *Pgi-1*^{1}/*Pgi-1*^{2}; 6: progeny 82 = *Pgi-1*^{1}/*Pgi-1*^{2}; 7: progeny 89 = *Pgi-1*^{1}/*Pgi-1*^{2}. **E** Phosphoglucomutase profile; 1: *A. cepa* = *Pgm-1*^{1}/*Pgm-1*^{1}; 2: *A. fistulosum* = *Pgm-1*^{2}/*Pgm-1*^{2}; 3: diploid *A. fistulosum* × *A. cepa* = *Pgm-1*^{1}/*Pgm-1*^{2}; 4: 'Delta Giant' = *Pgm-1*^{1}/*Pgm-1*^{1}/*Pgm-1*^{2}.

sistant (rating average = 1.0) (Table 4). All groups of the progeny had higher average levels of pink root resistance than 'Temprana'; however, only 'Delta Giant' was significantly more resistant than the other populations.

Highly resistant plants (rating ≤ 3) were found in each of these progeny groups (Table 4), suggesting segregation and possible introgression of *A. fistulosum* pink root resistance genes in the progeny. The possible presence of pink root resistant genes from *A. fistulosum* in these progeny will need to be confirmed by more intensive screening in future generations.

Evaluation of fistulose leafing characteristic

The cross-sectional leaf shape of 'Delta Giant' is intermediate between *A. fistulosum* and *A. cepa* while 'Temprana' has flattened leaves. Leaf shape of the backcross progeny was variable, but conformed more to *A. cepa* than *A. fistulosum*. Five (3%) backcross progeny, three diploids and two hyperploids, had fistulose leaf characteristics. All the progeny that exhibited fistulose leaves were resistant to pink root (rating ≤ 3); however, not all resistant progeny had fistulose leaves. Progeny exhibit-

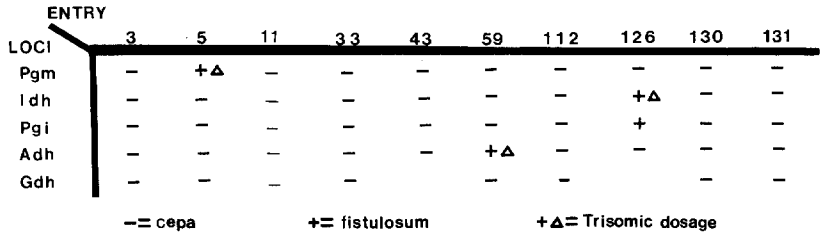

Fig. 6. Segregation of enzyme markers in 'Delta Giant' shallot trisomic progeny (2n = 17)

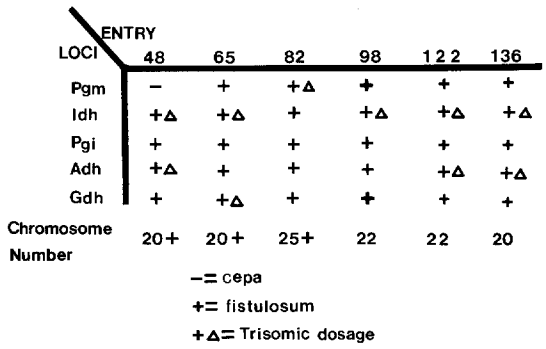

Fig. 7. Segregation of enzyme markers in 'Delta Giant' shallot hyperploid backcross progeny

Table 4. Mean pink root ratings of field pink root screened 'Delta Giant' shallot backcross progeny

Entry	No. of observations	Pink root rating ($\bar{x}$)	SD	Range
'Delta Giant'	3	1.0	0	1
'Temprana'	3	7.3	2.1	5– 9
Diploid	84	5.5	2.3	1–10
Trisomic	10	4.2	2.3	1– 8
Hyperploid	6	4.3	2.7	1– 8

ing both fistulose leaves and pink root resistance may have not been challenged sufficiently in the screenings (discussed above) or the characteristics may have come from *A. fistulosum* and are true recombinants. Segregating generations of resistant plants will be investigated to ascertain if the apparent resistance is genetic.

The frequency of fistulose leaves in the hyperploids was greater than in the diploid population, suggesting that as the number of *A. fistulosum* chromosomes or chromosome segments increase, the frequency of this *A. fistulosum* character increases. There does not appear to be any clear relationship between those entries containing *A. fistulosum* isozymes and those receiving pink root resistance ratings.

Fertility of the progeny

Of 20 diploids that had relatively low pink root resistance ratings (rating ≤ 3), 19 were fully fertile and one was sterile. Of five trisomics that flowered, four were fertile with normal seed set and one, the *Pgm-1* addition line, was only partially fertile. The hyperploids were variable in fertility from partially fertile to highly sterile.

It appears a low level of recombination may be occurring between the species at the diploid level as fertile, apparently resistant plants with the fistulose leafing characteristic (see *"Evaluation of fistulose leafing characteristic"*) have been recovered. One alien addition line was recovered that possessed the *Pgm-1 A. fistulosum* allele and was apparently resistant to pink root.

References

Burnham CR (1962) Discussions in cytogenetics. CR Burnham, University of Minnesota

Cochran FD (1950) A study of the species hybrid, *Allium ascalonicum* and *Allium fistulosum* and its backcrossed progenies. Am Soc Hortic Sci 55:293–296

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

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

Emsweller SL, Jones HA (1945) Further studies on the chiasmata of the *Allium cepa* × *A. fistulosum* hybrid and its derivatives. Am J Bot 32:370–379

Gorenz AM, Larson RH, Walker JC (1949) Factors affecting pathogenicity of pink root fungus of onion. J Agric Res 78:1–18

Jones HA, Mann LK (1963) Onions and their allies. Interscience, New York, p 32

Jones HA, Perry BA (1956) Inheritance of resistance to pink root in the onion. J Hered 47:33–34

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* × *A. fistulosum* and is polyploid derivatives. Heredity 27:253–272

Lindsey DL, Corgan JN (1976) Pink root intensity and root-knot nematode populations associated with onions in the southern New Mexico Rio Grande Valley. NMSU Rsh Rep 326

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

Nichols CG, Larson RH, Gableman WH (1960) Relative pink root resistance of commercial onion hybrids and varieties. Proc Am Soc Hortic Sci 76:468–469

Perkins DY, Kehr AE, Brown RT, Tims EL, Miller JC (1958) 'Delta Giant' – a new long season shallot. LSU Circular No 52

Perry BA, Jones HA (1955) Performance of short-day pink-root-resistant varieties of onions in southern Texas. Proc Am Soc Hortic Sci 66:350–353

Porter DR, Jones HA (1933) Resistance of some of the cultivated species of *Allium* to pink root (*Phoma terrestris*). Phytopathology 23:290–298

Rick CM (1982) The potential of exotic germplasm for tomato improvement. In: Plant improvement and somatic cell genetics. Academic Press, London New York, pp 1–28

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

Savitsky H (1978) Nematode (*Heterodera schachtii*) resistance and meiosis in diploid plants from interspecific *Beta vulgaris* × *B. procumbens* hybrids. Can J Genet Cytol 20:177–186

Sears ER (1956) The transfer of leaf-rust resistance from *Aegilops umbellulata* to wheat. Brookhaven Symp Biol 9:1–22

Tanksley SD, Zamir D, Rick CM (1981) Evidence for extensive overlap of sporophytic and gametophytic gene expression in *Lycopersicon esculentum*. Science 213:453–455

Ware GW, McCollum JP (eds) (1980) Producing vegetable crops. Interstate Printers and Publishers, Danville, Ill, p 359

Vallejos CE, Tanksley SD (1983) Segregation of isozyme markers and cold tolerance in an interspecific backcross of tomato. Theor Appl Genet 66:241–247

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