This is to certify that the

dissertation entitled

Introgression of Allium fistulosum into Allium cepa

through Hybridization

presented by

Uma Shanker Gupta

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Plant Breeding and Genetics

Lowell P. Ewart
Major professor

Date 1/6/93

INTROGRESSION OF *ALLIUM FISTULOSUM* INTO *ALLIUM CEPA* THROUGH HYBRIDIZATION.

by

Uma Shanker Gupta

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Plant Breeding and Genetics Program
Department of Horticulture

1993

ABSTRACT

INTROGRESSION OF *ALLIUM FISTULOSUM* INTO *A. CEPA* THROUGH HYBRIDIZATION.

by

Uma Shanker Gupta

Allium cepa L. (onion) is an important vegetable crop which unfortunately lacks resistance to many insects and diseases. *Allium fistulosum* (Japanese bunching onion), on the other hand, has been widely recognized as the allied onion species with resistance to many of the insects and diseases that attack the bulb onion. Plant breeders all over the world have been trying to transfer the resistance and other economic characters from *A. fistulosum* into *A. cepa*. To date, no published study has shown development of a regularly pairing interspecific hybrid line with *A. cepa* characteristics.

This study describes a classical genetic introgression experiment which used the *A. cepa-fistulosum* allopolyploid, Beltsville Bunching, as the initial *A. fistulosum* genome source. Morphological, cytogenetic, molecular and reproductive evidences were collected and by the 5th generation, they show that the interspecific derivatives externally resemble *A. cepa*, chromosome pairing is stabilizing, a DNA polymorphism found in *A. fistulosum* has been carried over in the interspecific backcrosses, and the reproductive barrier is broken.

The interspecific derivatives were screened for *Fusarium* and pinkroot resistance. Some of the derivatives show strong resistance to both diseases. The data indicate that the resistance in both cases could be a simply inherited dominant. It is possible that the same gene is imparting the resistance / tolerance to both diseases.

DEDICATION

This study is respectfully dedicated to Mrs. Jo Ewart.

ACKNOWLEDGMENTS

I am grateful to many for their help and guidance in this study. I would like to thank Dr. Jim Hancock, Dr. Bernie Zandstra and Dr. Joanne Whallon for their accurate criticisms and suggestions in the preparation of this manuscript. I would also like to thank people from Dr. Hancock's lab, Pete, Jeff and Don, for their help in the RAPD analysis.

This study could not be completed without the teaching, guidance and encouragement of Lowell Ewart, my major professor. I could not have asked for a better professor. He is a true friend, philosopher and guide. I have learned a great deal from his experience as a plant breeder. Thanks Lowell.

Lastly I would like to thank my sons, Sonny and Manish, and wife, Kumud, for their patience during the course of this study.

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INTRODUCTION

Allium cepa L. is a diploid species (2n = 16) which has been cultivated since prehistoric times. Historical references to onion grown for food date back to 3000 BC (Hanelt 1990). Although the genus *Allium* consists of hundreds of species, only a few are commercially important food crops: *A. cepa* (onion), *A. sativum* (garlic), *A. ampeloprasum* (great headed garlic, leek, kurrat), *A. fistulosum* (Japanese bunching onion), *A. schoenoprasum* (chive), *A. chinense* (rakkyo) and *A. tuberosum* (Chinese chive). None of the above species will cross freely with *A. cepa* by conventional means. Experimental hybrids between *A. cepa* and *A. fistulosum*, however, have frequently been reported (Emsweller and Jones 1935, Maeda 1937, Levan 1941). An amphidiploid (2n = 32) hybrid, Beltsville Bunching (*A. cepa* x *A. fistulosum*), and a triploid, Delta-Giant, (*A. ascalonicum* x *A. fistulosum*) have been used as *A. fistulosum* genome sources in the ongoing attempts by breeders over the past 50 years to introduce desirable characters from *A. fistulosum* (Jones et al., 1963). Apart from some genetic studies, most researchers have abandoned such work before the interspecific hybrids could be utilized in a breeding program. The lack of progress is primarily attributed to the meiotic irregularities occurring in *A. cepa* x *A. fistulosum* hybrids and their derivatives which cause sterility and severely hamper transfer of genetic material between the two species (Dowker 1990, Jones 1990).

Allium fistulosum possesses many economically important characters - resistance / tolerance to pinkroot (*Pyrenochaeta terrestris*; Porter and Jones 1933), smut (*Urocystis cepulae* F.; Felix 1933), leaf rot (*Botrytis squamosa*; Maude 1990a), neck rot (*Botrytis allii*; Maude 1990b), and thrips (*Thrips tabaci*; Jones et al., 1934, Soni and Ellis 1990) as well as high soluble solid content and cold hardiness

(Cryder 1988). In short, a successful genetic introgression is very desirable and could alleviate many of the short-comings associated with the cultivated bulb onion.

The current study evaluated an on-going *A. cepa* - *A. fistulosum* backcrossing program. The major hypotheses tested are (a) if there is an actual genetic integration from *A. fistulosum* into *A. cepa*, the recurrent parent. (b) if the interspecifically derived hybrids are cytogenetically stabilized. (c) if the fertility of the interspecific hybrids has significantly improved. (d) if the interspecific hybrid lines carry resistance / tolerance to *Fusarium* and pinkroot, two important diseases of the bulb onion. The purpose of this study was to evaluate the effectiveness of the program / procedure and to assess the potential use of the developed plants in the on-going onion breeding program at Michigan State University.

LITERATURE REVIEW

The botanical classification of *Allium* is still in controversy (Jones et al., 1963; Cronquist 1968; Hanelt 1990). Initially, *Allium* was placed in the family *Liliaceae*, then it was transferred into *Amaryllidaceae* on the basis of inflorescence structure, and later *Amaryllidaceae* was included in *Liliaceae*. Thus, *Allium* was again included in *Liliaceae*. Modern taxonomy, however, now puts *Allium* and its closest relatives in a distinct family *Alliaceae* (Hanelt 1990). The following hierarchy is currently accepted:

Class :	*Monocotyledones*
Superorder :	*Liliifloreare*
Order :	*Aspargales*
Family :	*Alliaceae*
Tribe :	*Allieae*
Genus :	*Allium*

Allium is a large genus of about 600 species - mostly perennial and bulbous (Hanelt, 1986). It is widely distributed over the warm-temperate and temperate zones of the northern hemisphere. It is estimated that the total world onion bulb production is more than 250 million MT and that the value of this production approaches 5 billion dollars annually (FAO, 1988).

In the *Allium* genus as a whole, there are three basic chromosome number groups, x=7, x=8, and x=9. The x=7 species are largely confined to the New World, while the majority of species (about 500) which are in the Old World are mainly x=8. There are only a few species with x=9 (Jones, 1990). All cultivated edible forms have the same basic chromosome number of x=8.

Allium cepa (Bulb Onion)

Allium cepa is the most widely known edible *Allium*. It has been in use for at least 3000 years. The species is an out-breeder and current improvement programs utilize hybrid varieties, as well as open pollinated selections (Dowker et al., 1983). *A. cepa* is a diploid and has a complement of eight pairs of metacentric and submetacentric chromosomes (2n = 2x = 16). With the development of Giemsa c-banding techniques, it is now possible to distinguish and name the eight chromosomes of the basic set individually (Kalkman, 1984, de Vries, 1990). *A. cepa* has a nuclear DNA value of 33.5 pg per 2C nucleus (Jones et al., 1968, Ranjekar et al., 1978). The quality of DNA is such that the G + C base pairs comprise about 32% of the total and up to 50% is in the form of repetitive sequences. There is no satellite DNA (Jones, 1990). Meiosis is regular with eight bivalents. Chiasmata are formed mainly in the distal and interstitial regions of the chromosome arms.

Allium fistulosum (Japanese bunching onion)

Allium fistulosum is a perennial, diploid out-breeder, widely used as an edible green onion in Asia. The species exhibits high levels of resistance to several important pests and diseases. *A. fistulosum* has been observed to possess resistance / tolerance to pink-root (Porter and Jones 1933, Corgan 1985), onion smut (Clarke and Mackay 1946), downy mildew (Henning and Mildenberger 1988, Weitsma and Kik 1989) and thrips (Jones and Mann 1963).

The mitotic chromosome complement resembles that of *A. cepa*, although the homeologues are smaller in both size and DNA amount. The nuclear DNA value is

26.3 pg, only 78.5% of the amount found in *A. cepa*. The difference is unevenly distributed among the eight pairs of chromosomes (Jones et al., 1968, Fiskejo, 1975). The chiasmata are highly proximal (adjacent to the centromere) forming cruciform shaped bivalents (Levan, 1933). It is assumed that as a result of the restricted chiasmata location, there is an absence of crossing-over in the bulk of the chromosome arms and the arms behave as super-genes.

Inter-specific hybridization in *Allium*

It is customary to look for resistance genes in the allied wild relatives of an established crop. Domestic selections of *A. fistulosum*, however, retain many valuable resistance characteristics generally attributed to wild species (Porter and Jones 1933, Corgan 1985, Clarke and Mackay 1946, Henning and Mildenberger 1988, Weitsma and Kik 1989, Jones and Mann 1963).

Other *Allium* species have also been found to possess resistance characteristics but to a lesser extent than *A. fistulosum*. *A. roylei* is resistant to downy mildew (Weitsma and Kik 1989, Meer and de Vries 1990), pinkroot (Saini and Davis 1967) and to thrips (Saini and Davis 1967). *A. galanthum* was found to be tolerant to pinkroot (Saini and Davis 1967) and *A. pskemense* is tolerant to thrips and downy mildew (Saini and Davis 1967).

Artificial inter-specific hybridization in *Allium* species was started in the early thirties. The following hybridization attempts have been reported: *A. fistulosum* x *A. cepa* (Emsweller and Jones 1935, Jones and Clarke 1942, Emsweller and Jones 1945, Clarke and Mackay 1946, Corgan 1985, Peffley 1987, Bino et al., 1988, Lu et al., 1989), *A. fistulosum* x *A. altaicum* (McCollum 1982, Kokoreva et al., 1987), *A.*

cepa x *A. galanthum* (Saini and Davis 1967, McCollum 1971, McCollum 1982, Wietsma and de Vries 1988), *A. cepa* x *A. pskemense* (Saini and Davis 1967, McCollum 1971, Wietsma and de Vries 1988), *A. cepa* x *A. oschaninii* (McCollum 1974, McCollum 1982, Tarasova and Pole 1984, Wietsma and de Vries 1988), *A. roylei* x *A. cepa* (Wietsma and de Vries 1988, Meer and de Vries 1990), *A. cepa* x *A. drobovii* (Saini and Davis 1967), *A. drobovii* x *A. galanthum* (Saini and Davis 1967), *A. drobovii* x *A. pskemense* (Saini and Davis 1967), *A. drobovii* x *A. roylei* (Saini and Davis 1967), *A. fistulosum* x *A. ascalonicum* (Tashiro 1980, Tashiro 1981, Tashiro 1984), *A. cepa* x *A. vavilovii* (Wietsma and de Vries 1988), and *A. fistulosum* x *A. roylei* (Wietsma and Kik 1989). *A. cepa* crosses easily with *A. galanthum*, *A. drobovii* and *A. pskemense* (Saini and Davis 1967, McCollum 1971). Limited success has been reported with *A. cepa* crossed with *A. vavilovii* and *A. roylei* (Wietsma and de Vries 1988, Wietsma and Kik 1989). Few of the other crosses produced any fertile hybrid plants. The success rate of *A. cepa* and *A. fistulosum* crosses is less than 0.5% in a controlled environment (Emsweller and Jones 1935, Jones and Clarke 1942, Emsweller and Jones 1945, Clarke and Mackay 1946, Corgan, 1985).

Artificial inter-specific hybridization between *A. cepa* and *A. fistulosum* started in 1931 at the Agricultural Experiment Station in Davis, California (Emsweller and Jones 1935). The major reason for the introduction of such a breeding procedure was that *A. fistulosum* (var Nebuka), as noted earlier, was resistant to many economically important diseases at the time. *A. fistulosum* as a non-bulbing item, did not have much economic importance in America, and it was natural to try to incorporate the resistance characteristics into the bulbous onion populations. Yellow Globe Danvers (*A. cepa*), an inbred, was used as the female parent and Nebuka (*A. fistulosum*) was used as the pollen parent. Only seven seeds were secured after hundreds of pollinations. Only one seedling survived from these seven

seeds. Many more attempts were made to hybridize *A. cepa* and *A. fistulosum* in the following years. In spite of obtaining some seeds, all the plants from such crosses were found to be mostly self-sterile (Emsweller and Jones 1935).

The following are some of the characteristics of the early developed hybrids (Emsweller and Jones 1935 Emsweller and Jones 1945, Levan 1941):

Bulbing: *A. cepa* is bulbing but *A. fistulosum* is non-bulbing. The hybrid plants showed none or very slight bulbing. Appendix A shows the bulbing in *A. cepa*, *A. fistulosum* and the F_1 hybrid.

Life Cycle: *A. fistulosum* is a perennial, *A. cepa* is a biennial (bulbing one year and setting seed the second year), the hybrids were perennials.

Date of Flowering: *A. fistulosum* flowers early, while *A. cepa* flowers late. The hybrids were intermediate.

Inflorescence: In *A. cepa*, anthesis occurs over the entire inflorescence throughout the flowering period, while in *A. fistulosum*, the terminal flowers open first and anthesis proceeds progressively towards the base. At the tip of the umbel, seed may be practically mature while towards the base, buds are just opening. In the hybrids, the terminal flowers open first, although this process is less clearly defined than in *A. fistulosum*. They mature progressively towards the base with anthesis over the entire inflorescence.

Flower Habit: In *A. cepa*, the flowers are fully expanded at anthesis, while in *A. fistulosum*, the perianth remains erect. The hybrid resembles *A. fistulosum*.

Fertility: Both *A. cepa* and *A. fistulosum* were fertile in both sexes. The hybrids were usually self-sterile, but were found to be partially fertile as a pollen parent. Two hybrids, Beltsville Bunching and Delta Giant, have been released as commercial cultivars of green bunching onion.

Leaf Shape: *A. fistulosum* has circular leaves while *A. cepa* has semi-circular leaves. The hybrids resembled *A. cepa*.

Vigor: The hybrids grow considerably taller than either parent and also exhibited hybrid vigor in many other respects.

Chiasmata formation: *A. fistulosum* forms cruciform chiasmata located closely on each side of the centromere. *A. cepa* forms randomized chiasmata many of which are at the distal position of the arm. In most cases, the hybrids showed a combination of both, though the random form dominated.

Hybrids, when obtained, produced only a few F_2 seeds. Emsweller and Jones (1935) produced three F_2 seeds from a White Persian x Nebuka cross. Maeda (1937) obtained a few F_2 plants from the cross of *A. fistulosum* var Hidanegi x *A. cepa* var Yellow Globe Danvers. Levan (1941) grew several F_2 plants from such a cross and all had more than a diploid complement of sixteen chromosomes, but only one was tetraploid. Emsweller and Jones (1935) obtained more seeds when the F_1 hybrid was used as the pollen parent in the backcross to *A. fistulosum*, than when the F_1 was used as the pollen parent in the backcross to *A. cepa*. Levan (1941) never succeeded in backcrossing his hybrid plants.

Cytological observation of hybrid cells showed variable numbers of bivalents. Most studies reported 8 bivalents in more than 70% of the pollen mother cells (Emsweller

and Jones 1935, Maeda 1937). Univalents in the remaining cells varied in number from two to eight. Chromatin bridges and fragments occurred frequently. In other cases, the pairing was much more irregular, regular pairing being shown in less than two percent of the division figures (Levan 1941).

Pairing between *A. cepa* and *A. fistulosum* chromosomes does occur in the diploid hybrid (Emsweller and Jones 1935, Levan 1941, Maeda 1937, Cochran 1950). Bivalent pairing occurs with a greater or lesser number of bivalents depending upon the parents used in the cross (Emsweller and Jones 1945). Most studies show variations in the frequency of chromosome pairing, number of chromosome bridges and fragments, and heteromorphic chromosomal associations. Emsweller and Jones (1935) found that chromosomes of unequal length paired and sometimes each had long unpaired arm extending beyond the end of the other. Number of bivalents in the adjacent cells was variable and occasionally bridges and fragments were found at the anaphase I stage. Many studies found that the chiasmata in the interspecific hybrid was a mixture of randomized and localized formations (Emsweller and Jones 1945, Cocharan 1950). Levan (1941) reported that complete bivalent pairings were not typical and multivalents were frequently formed.

Several researchers have observed heteromorphic bivalents in the interspecific hybrids (Emsweller and Jones 1935, Maeda 1937). Bridges and fragments in anaphase I are reported and interpreted as a result of inversion (Emsweller and Jones 1935, Emsweller and Jones 1938). Peffley (1986) reported formation of multivalents suggesting occurrences of translocations in the two species chromosomes. In a further study Peffley et al., (1989) presented evidence for three independent reciprocal translocations between *A. cepa* and *A. fistulosum* chromosomes by showing occurrence of tetra- and pentavalent associations. Ring univalents and isochromosomes were also observed.

Sterility of the interspecific hybrids generally blocked progress towards integrating *A. cepa* and *A. fistulosum*. Before efforts in this direction completely stopped, however, nature provided a new hope. In the late thirties, Jones and Clarke (1942) reported a natural amphidiploid between *A. cepa* var 'Australian Brown' and *A. fistulosum* var 'Nebuka'. This amphidiploid occurred spontaneously by natural doubling of the hybrid chromosomes. The amphidiploid was pollen fertile, uniform in appearance, perennial in habit and more vigorous than either of the original parents. The haploid chromosome number was 16 and meiosis was quite regular. Fragments, chromatin-bridges and micro-nuclei were observed but not as frequently as in the diploid interspecific hybrids. The pollen fertility of the amphidiploid gave new hope to onion improvement programs. Polyploidy was considered the possible route to follow. This idea and optimism, however, did not last for long as the amphidiploids were found to be poor seed setters in comparison to either of the parents (Davis 1955). Nevertheless, the amphidiploids form very slight bulbs and are used as edible green onion. In 1950, a highly pinkroot resistant, amphidiploid, green onion cultivar, Beltsville Bunching (*A. cepa* var 'White Portugal' x *A. fistulosum* var 'Nebuka') was released to the seedsmen by the United States Department of Agriculture and California Agriculture Experiment Station (Jones and Mann 1963).

A similar amphidiploid was developed by Davis (1955) by artificially doubling the chromosomes of the cross *A. ascalonicum* (shallot) x *A. fistulosum*. Shallot is very closely related to *A. cepa* and is now considered to be just another form of *A. cepa* (Jones and Mann 1963, Jones 1990). The two cross readily and exhibit normal meiotic behavior. Shallot differs from onion mainly that they are small in stature, have slender awl-like leaves, and have small, ovate-oblong angular bulbs that break up into distinct sets that cohere at the base (Cochran 1950).

In the mid - fifties Delta Giant, a triploid shallot, was released. It was derived from the *A. fistulosum* - shallot amphidiploid backcrossed to shallot. Delta Giant is prolific, tolerates hot weather and can be grown both earlier and later than regular shallot cultivars grown in the South, especially in Louisiana (Jones and Mann 1963).

New Mexico State University (NMSU) has been actively involved in the integration of *A. cepa* and *A. fistulosum* genomes since the late seventies. NMSU has used the triploid Delta Giant as an *A. fistulosum* source in this integration research (Corgan 1985, Peffley 1986) and has succeeded in obtaining trisomic backcross progenies to *A. cepa* (Corgan 1985, Peffley 1986, Cryder et al. 1989). Scientists at NMSU and now at Texas Tech (Peffley et al. 1985, Peffley 1986, Peffley 1987, Peffley et al. 1989) are currently developing isozyme marker maps using the trisomic alien addition lines. They are using cytological and isozymatic information to keep track of the integration of the genetic materials. At the same time molecular markers (isozyme) are identified which are found in the trisomic addition line. These scientists hope to accomplish gene flow between *A. cepa* and *A. fistulosum* at the diploid level using these addition lines (Peffley et al. 1985, Peffley 1986, Peffley 1987, Peffley et al. 1989). The isozyme markers can play a significant role in the screening of the backcrosses from the selfs in an open pollinated crossing program using *A. cepa* as the female parent.

MATERIALS AND METHODS

In 1966, Q. P. van der Meer at the Institute of Horticultural Plant Breeding (IVT), Wageningen, the Netherlands, began making interspecific crosses between *A. cepa* and *A. fistulosum*, using Beltsville Bunching (allopolyploid) as the source for the *A. fistulosum* genome. Parents and backcross (BC) generations up to BC_3 were saved and maintained over the next 20 years. In 1986, L. Ewart of Michigan State University made a comprehensive cytogenetic study of the available *A. cepa-fistulosum* germplasm, furthered the backcross generation to BC_4 and made some other crosses with the available materials at IVT (Ewart 1986). The current study is a continuation of the experimentation started in 1986 with the germplasm brought from IVT. The following studies were planned with the interspecific derivative materials:

i. **Advancement of the backcross generation**: This study was initiated to determine if the barriers of fertilization, which were initially present, were broken by the current generation. Since the material was brought from an extra long day period region, this required application of a flowering synchronization procedure as described later in the hybridization sub-heading.

ii. **Cytogenetic observations**: Such observations were deemed necessary to determine whether initially observed chromosomal aberrations still persisted or were significantly decreased, and to see if the materials were comparable to regular diploid *A. cepa*.

iii. **Genome integration**: RAPD markers were used for this study to verify if the interspecific genome was carrying *A. fistulosum* DNA.

iv. **Disease screening**: this study was initiated to determine if the introgressed lines carried resistance / tolerance to *Fusarium* and pinkroot, two important onion diseases.

Interspecific plants, 1253-1, 1253-2, 1253-3 and 1255, all in the backcross 4 generation were used in the study. The pedigree of the above plants is described in the Appendix B. The following MSU *A. cepa* inbreds were used to make backcrosses with the above interspecific materials - 611, 1731, 1849 and 2399.

Table 1 shows the pedigree of the materials used in 1989. The selfs are designated as ⊕ throughout the manuscript. As is customary, the first pedigree listed in a cross is the female parent. Table 2 shows the new designations given to the old plants when their bulbs were repotted after they were used once in the crossing process. Table 3 shows the crosses and selfs successfully made in 1990.

Table 1: Interspecific *Allium* Germplasm Developed at Michigan State University in 1989.

Cross	Field #
611 x 1253-1	90-1
1253-1 ⊕	90-2
1253-2 ⊕	90-3
1253-3 ⊕	90-4
1253-3 ⊕	90-5
1255 ⊕	90-6

Table 2: Field numbers used after repotting the 1989 *Allium* interspecific derivative plants.

Original Pedigree	New Field #
1253-1	89- 3
1253-2	89-12
1253-3	89-14
1255	89- 4

Table 3: Interspecific *Allium* Germplasm Studied and Developed at Michigan State University in 1990.

Cross	Field #[z]
89-4-2 ⊕	90-11
89-4-3 ⊕	90-12
89-4-4 ⊕	90-13
89-12-4 ⊕	90-14
611 x 89-3-2	90-15
611 x 89-4-2	90-16
611 x 89-4-4	90-17
611 x 89-12-4	90-18
2399 x 89-3-1	90-19
2399 x 89-4-1	90-20
2399 x 89-4-2	90-21
1731 x 89-3-2	90-22
1731 x 89-4-4	90-23
89-4-1 x 1849	90-26

[z] The field # given in table 3 is the pedigree assigned to the seed when it was grown in 1990 and 1991. Some of these plants were used for cytogenetic and RAPD investigations.

Hybridization

A. cepa, as well as interspecific material seedlings were vernalized in a growth chamber and were grown under environmental conditions designed to decrease the seed-to-seed generation from two years to about nine months by bypassing the bulbing stage. The generation reduction procedure was based on the results of the extensive physiological studies done by Brewster (1977a, 1977b, 1983, 1985a, 1985b), which was reported and used by Bacher (1989). Three major factors were controlled: First the nutrition was drastically reduced and the photoperiod was increased to 24 hours. Second, the plants were grown initially at 17^{o}C for the first 100 days to initiate growth and then at 9^{o}C for 40 days to vernalize the plants. During these two temperature periods, the plants were grown under cool white light (200 micromole per sq meter per second). This procedure by-passes the bulbing phase allowing the plants to initiate flowering instead.

Pollination Procedure: The onion inflorescence is an umbel bearing 50 to more than 1000 flowers, though 300 - 500 is a more usual situation. A single umbel may continue to flower over 3 to 4 weeks (Currah and Ockendon 1978). Though the number of flowers opening initially is small, it rapidly increases with a peak between 4 and 10 days in which 50 or more flowers open in a single day (Currah and Ockendon 1978). The opening of the flowers is strongly affected by temperature and day-length (Masuda 1956). Stigmatic exudates, though essential for pollen capture, were not found to play an essential role in pollen germination (Currah and Ockendon 1978). Stigmata have been found to be most receptive between the 3rd and 5th day of opening of the flower (Moll 1954), and stigma receptivity is strongly influenced by temperature (Chang and Strukmeyer 1976a and 1976b).

The umbels that were going to be used as seed parents were bagged as soon as the first flowers started to open in the greenhouse. In most crosses, the interspecific derivatives were used as pollen parents and were allowed to flower freely There was one exception in the second year when 89-4-1, an interspecific derived plant was used as a female and was bagged. *A. cepa* plants were used as females and were emasculated twice daily and rebagged. Because of a limited number of heads, the same heads were used for crossing and selfing in many cases. Crossing was accomplished either by using a camel brush for pollination or by placing male and female heads in a single bag along with flies which did the pollination. Some selfing also was done in the same way when a single flower head could be used.

Disease Screening

Seedlings from selfs of the interspecific derived germplasm were screened for two major onion diseases, basal rot and pinkroot. Basal rot, caused by *Fusarium oxysporum f.* sp *Cepae* and pink root, caused by *Pyrenochaeta terrestris*, are two very devastating soil-born onion diseases. Basal rot has long been recognized as a serious soil-born disease in the midwestern and eastern United States (Abawi and Lorbeer 1971a and 1971b). Pink root has long been a severe yearly problem in the southern and southwestern United States, and it may be severe on midwestern organic soils during periods of relatively high temperature (Gorenz 1949 and Walker 1961). Both basal rot and pink root develop best under controlled conditions at soil temperatures of 26-30^{O}C (Walker and Tims 1924, Gorenz 1949, Kehr et al., 1962).

Inoculum Preparation:

Pink-root: PR-4 and PR-6 strains of *Pyrenochaeta terrestris* were used in this screening study. These inocula were originally developed at the University of Wisconsin by Dr. Clint Peterson, a USDA onion breeder. In this study, they were obtained from Dr. Mel Lacy of the Department Botany and Plant Pathology, Michigan State University, who had obtained some of this material and prepared the inoculum for the screening.

The inoculum is kept as infected muck soil at 4^{o}C. When needed, it is incubated in Czapek's sucrose nitrate solution unshaken for 6-9 day. Then it is diluted to 1/20. One ml of inoculum is used per gram of white silica sand (Dr. Lacy's laboratory protocol).

Basal-rot: F-593 (University of Wisconsin), F110A and F156B (from New York) strains of *Fusarium oxysporum* were used in this study. The inoculum was also obtained from Dr. Mel Lacy of the Department Botany and Plant Pathology, Michigan State University.

Ten days prior to seeding, the inoculum was placed on potato dextrose agar and grown at 26^{o}C. Three days prior to seeding, 7-9 mm discs were cut from the agar and placed in 250 ml flasks containing 50 ml potato dextrose broth, one disk per broth flask. Then the inoculum was increased on a rotary shaker at 24-26^{o}C. After 72 hours of growth, the broth was mixed for two minutes in a blender at low speed. Then it was centrifuged at approximately 3400 RPM for 10 minutes. The supernatant was discarded, and the spores were resuspended in distilled water. The spores were counted with a hematocytometer to determine the concentration so that 20,000 spores per gm of sand could be used.

Growing medium preparation: Though the inocula were developed differently, the rest of the screening procedure was much the same for both of the diseases. The procedure was first developed for pinkroot (Krueger 1986) and currently is used for both *Fusarium* and pinkroot (Gabelman 1988). In both cases, a spore concentration of 20,000 per gram of sand was used. Two 50 x 20 x 11 cm stainless steel pans filled with 20 kg of white sand were used for each of the inocula. The pans were sterilized in an autoclave. A smaller pan was used as a control and as an indicator for normal seed germination without any inoculum for each disease. The other two pans were inoculated with the appropriate amount of spore suspensions prepared by adding the necessary amount to distilled water. About 1 cm deep grooves were made 3 cms apart across the sand, and were seeded with *A. cepa* and the interspecific derivative seeds. Owing to limited supply of seeds from the interspecific derived germplasms, only 10 seeds were planted per row. The pans were covered with aluminum foil and placed in temperature-controlled water baths set at 26^{o}C for pink root and at 24^{o}C for basal rot. The foil was removed after the seedlings had emerged and the seedlings were grown under high intensity discharge lights using a 15-16 hour photoperiod. When the cotyledons reached 1 cm in height, the sand temperature for basal rot was increased to 26-28^{o}C. The optimum temperature of growth for *Fusarium oxysporum* is 26-30^{o}C (Walker and Tims 1924, Davis and Henderson 1937, Abawi and Lorbeer 1972). Plants were watered with distilled water as needed and were fertilized with 20:8.6:16.6 N:P:K at 100 ppm after two weeks. The number of seedlings were counted on the 10th and the 21st day after planting.

Experimental Design: Owing to lack of adequate seeds, two of the interspecific derivative lines were replicated only once in the *Fusarium* (89-4-3 self and 89-4-4 self) and pinkroot (89-4-4 self) screening experiments. Completely randomized design (CRD) does not impose any restriction on the number of replications per

treatment (Steel et al., 1980); and so CRD was chosen for the planning of the experiment. However, the inclusion of singly replicated lines diminishes greatly the resolving power of an analysis and the inequality of replications does not allow for the separation of block effects. All the observations of an experiment were randomized in a single pool for each experiment, as required by the design.

For the pinkroot screening, two replications of 89-4-2 and 89-4-3 selfs and one replication of 89-4-4 self with four replications of each of three *A. cepa* inbred checks, were used. For the *Fusarium* screening experiment, three replications of 89-4-2 self and one replication each of 89-4-3 and 89-4-4 selfs with four replications of each of three *A. cepa* inbred checks, were used. Each experiment had seventeen total experimental units.

Cytology

Flower bud samples were collected in the morning from plants and were fixed in 6:3:1 ethanol:chloroform:glacial acetic acid (Peffley 1986) solution for about 24 hours. They were transferred to 70% ethanol for storage in a refrigerator. The refrigerated samples were used as needed. The meiocytes were stained with aceto-carmine. Metaphase I and anaphase I were the stages examined under a light microscope. At least three slides were prepared for each sample and the whole field of each slide was viewed for appropriate cells.

Random amplified polymorphic DNA (RAPD)

Two types of molecular markers have been found useful for developing detailed genetic maps in plants - restriction fragment length polymorphisms (RFLPs) and

random amplified polymorphic DNAs (RAPDs). A majority of previous studies have used RFLPs, which can detect genetic changes as base substitution and insertions / deletions. RFLPs are often codominant but are useful only in the region of low or single copy sequences (Burr et al., 1983; Tanksley et al., 1989). In 1990, a new technique, RAPD, was developed (Williams et al., 1990). This technique relies on the differential enzymic amplification of small DNA fragments using the polymerase chain reaction (PCR) with arbitrary oligonucleotide primers (usually 10-mers). Polymorphism results either from insertion / deletion in the amplified regions or from base changes that alter primer binding causing the region not to amplify. The procedure is rapid, requires only a small quantity of DNA which does not need to be of high quality; and best of all, it does not require the use of radioactive probes. Since no hybridization is involved, it can be used to detect polymorphism in regions of highly repeated sequences, which is not possible with the RFLP approach. RAPD provides dominant markers, since, polymorphism is detected as the presence or absence of bands.

A RAPD study was done using arbitrary DNA primers to determine if there was a polymorphism in *A. cepa*, *A. fistulosum* and the interspecific derived germplasm. Total DNA was obtained from samples of each of the materials (*A. cepa*, *A. fistulosum* and the interspecific germplasms). Six different lines of *A. cepa*, two different sources of *A. fistulosum* genome, Odessa (*A. fistulosum* cultivar) and Beltsville Bunching (amphidiploid) and three different interspecific hybrid lines were used in the study. Two oligonucleotide primers (10-mers) were used to amplify the DNA obtained from the above sources. Table 4 shows the dNTP sequence of the oligonucleotide primers and Table 5 shows the plant materials used in the study.

Table 4: **Sequences of the primer codes used in the RAPD analysis in the *Allium* Interspecific derivative development study.**

Code	Sequence
LE2	5'-GCACGATTTG-3'
RW2	5'-GGCCACCGTC-3'

Table 5: **Various lines, accessions and crosses used in the RAPD analysis used in the *Allium* interspecific derivative development study.**

A. cepa[z]	*A. fistulosum*	Interspecific Hybrid
420	Odessa	90-26-6
611	Beltsville Bunching[y]	90-12-18
1731		90-11-9
1849		
2399		
8155		

[z] Inbreds used: 420 is a Univ. of Wisconsin line; 611, 1731, 1849, 2399 and 8155 are MSU inbred lines. All these lines were used in this study, for making backcrosses as well as disease screening.

[y] Beltsville Bunching is an amphidiploid containing 2n *A. cepa* and 2n *A. fistulosum* genomes.

DNA Extraction: Samples were taken from newly emerging leaf tissue (5 g fresh weight) of three interspecific germplasm, one *A. fistulosum*, one Beltsville Bunching and six *A. cepa* inbreds. The tissue was immediately frozen in liquid nitrogen and processed the same day.

Total genomic DNA was obtained by grinding tissue to a fine powder with a mortar and pestle in the presence of liquid nitrogen. Approximately 30 ml of extraction buffer (15 ml of 100mM Tris.HCl, pH 8; 200mM Na2EDTA pH 8; 500 mM NaCl; 10 mM B-mercaptoethanol) was added to the cold mortar for each sample. The ground leaf tissue samples were filtered through four layers of cheese cloth into 30 ml oakridge tubes (Du Pont). The samples were incubated at 60^{o}C for an hour. After cooling the tubes to room temperature, the tubes were filled with equal volumes of chloroform:isoamyl alcohol (24:1 v/v). The tubes were centrifuged at 10,000 RPM for 10 minutes. The aqueous layer of each sample was put in another oakridge tube to which 10 ml of chloroform:isoamyl alcohol was added and then centrifuged at 10,000 RPM for 10 minutes at 20^{o}C. The top layer of each sample was pipetted off into a clean corex tube (Corning), and 1/10 volume of 3M Na-acetate and 3/4 vol of ice cold isopropanol was added. DNA was precipitated by gently inverting the tubes. The tubes were kept at -20^{o}C for an hour and then centrifuged at 10,000 RPM for 10 minutes at 4^{o}C to pellet the DNA. The supernatant was poured off and pellets were air dried until no odor of isopropanol was apparent. The pellets were dissolved in 400 μl of 1 TE and the content was transferred to Eppendorf tubes (Perkin Elmer). 20 μg of RNAase were added and the tubes were incubated for an hour at 37^{o}C. 40 μl of 3M Na-acetate and 800 μl of ice cold 100% ethanol was added to the tubes. The tubes were placed on ice for half an hour. The tubes were shaken to mix the layers and to precipitate the DNA. The tubes were centrifuged at 13,000 RPM for 12 minutes at 4^{o}C to pellet the DNA.

The supernatant was poured off and the tubes were filled with 70% ethanol and again centrifuged for 5 minutes at 5,000 RPM. After discarding the supernatant liquid, the pellets were air dried until no odor of ethanol was noted. 400 μl 0.1 TE was added to the tube to resuspend the DNA. The tubes were stored at -20^{o}C.

RAPD Procedure: Amplification reactions were performed in volumes of 25 μl containing 10 mM Tris-Cl, pH 8.0, 50 mM KCl, 2 mM $MgCl_2$, 0.001% gelatin, 100 μM each of dATP, dCTP, dGTP, dTTP, (Pharmacia), 0.2 M primer, 25 ng of genomic DNA and 0.5 units of *taq* DNA polymerase (Perkin Elmer Cetus). Amplification was performed in a Perkin Elmer Cetus DNA Thermocycler programmed for 47 cycles of 1 minute at 94^{o}C, 1 minute at 35^{o}C, 2 minutes at 72^{o}C, using the fastest available transitions between each temperature (Williams et al., 1990). The amplification products were analyzed by electrophoresis in 1.8% agarose gels at 45 volts for 20 hours, and detected by staining with ethidium bromide. Lambda / HindIII and lambda / DraI fragments were used as size markers.

RESULTS AND DISCUSSION

Allium cepa is a biennial crop and normally takes 2 years to complete its life cycle. *A. cepa* and the interspecific derivatives were grown under the cultural procedure outlined in the Materials and Methods section. The seed to seed generation time was effectively reduced to approximately **nine** months in most cases. Thus, the crosses (and selfings) could be made in 1989 and 1990 consecutively.

Morphology

A. fistulosum differs from *A. cepa* in having a bunching (many) stem, round leaves, short stature, oval inflorescence, non-bulbing stem (Figure 1a) and perennial habit; on the other hand, *A. cepa* has a non-bunching (much fewer) stem, flatter or ovate leaves, larger stature, spherical inflorescence, a bulbing stem and a biennial life cycle. The BC_3 plants had slight characteristics of *A. fistulosum* (bunching stem, slightly formed bulbs, and rounder leaves), where as, the BC_4 generation plants had all the observable characteristics matching *A. cepa* (Figure 1b). Moreover, the backcross generation plants had a distinct vigor and were easily distinguishable from either of the parents. Figures 1 to 3 show the various morphological characters of *A. cepa*, *A. fistulosum* and the interspecific germplasms.

The morphological development of the interspecific germplasm has proceeded as expected in a backcross breeding program. Initially, bunching type stems with very slight bulbing were common (up to BC_3, Ewart, 1986). The BC_4 plants were non-bunching (most had a single stem) and visually it was not possible to distinguish between derived bulbs and original *A. cepa* bulbs (Figure 3). These plants have become biennial since BC_4, while up to the BC_3 generations, they had slight but unmistakable properties of a perennial nature (Ewart, personal communication). The leaf type has changed to the morphology of *A. cepa*.

(a)

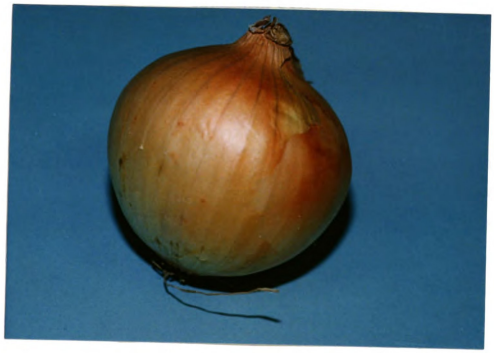
(b)

Figure 1: *A. fistulosum* and interspecific derivative (BC_4) plants. (a) *A. fistulosum* var Odessa. (b) interspecific derivative, 89-4-2 (BC_4).

(a)

(b)

Figure 2: Inflorescence of *A. fistulosum* and interspecific derivative (BC_4) plants. (a) *A. fistulosum* var Odessa. (b) interspecific derivative, 89-4-3. The diameter of the interspecific inflorescence is more than twice that of *A. fistulosum* and similar to *A. cepa*.



(a)

(b)

Figure 3: Bulbs of *A. cepa* and interspecific derivative (BC_4 self) plants. (a) *A. cepa* (b) interspecific derivative, 90-11.

Cytogenetic Observations

A. cepa and *A. fistulosum* have eight pairs of chromosomes each and Beltsville Bunching, the amphidiploid, has sixteen pairs. The major difference between the *A. cepa* and *A. fistulosum* chromosomes are size and chiasmata type. On an average, *A. cepa* chromosomes are larger than *A. fistulosum* chromosomes and *A. cepa* genome contains 33% more DNA than *A. fistulosum* genome. Randomized chiasmata are characterized by the random nature of the crossing-over between the homologous chromosomes. Such chiasmata show pairing closer to the end of the arms forming characteristic oval (semi-circular) shapes. Localized chiasmata, on the other hand, are formed by pairing of homologous chromosomes closer to the centromere. Because of the tension near the centromere, chromosome arms are stretched towards the opposite direction, developing the characteristic cruciform structure. It is not entirely known that localized chiasmata are formed by non-random (localized) crossing-over. If they are, the genetic combination in the chromosome arms will remain constant from generation to generation and the arms will act as super genes in the meiotic recombination. *A. cepa* form randomized chiasmata (Figure 4a) while *A. fistulosum* form localized chiasmata (Figure 4b). The Beltsville Bunching shows a mixture of both. Table 6 summarizes the chromosome pairing relations as found in the parents used in this study.

Stickiness, multivalents, heteromorphic association and partial pairing were quite common in the early generations of the interspecific germplasm development (Ewart 1986). Most lines in the BC_4, BC_4 self and BC_5 generation still show occasional stickiness ring formation and multivalents at the metaphase I and anaphase I chromosome configurations. Because of poor separation of chromosomes, it was not possible to make exact chromosome counts in such cells.

The cells with reasonable separation of chromosomes, however, showed that the chromosome number was 16. Figures 5 to 8 show metaphase I or anaphase I chromosome configurations in various generations of the interspecific germplasm development. Figure 5 (a) shows the anaphase I stage of the line 1253-1. The pairing shows sticky chromosomes and the chromosome pairs are starting to separate. Unusual pairing formation, some multivalents as well as univalents are observed. Figure 5(b) shows stickiness, multivalents, ring formation and cruciform chiasma at metaphase I stage of 1255. Both BC_4 plant cells, 1253-1 and 1255, could contain 8 pairs of chromosomes but it is extremely difficult to be absolutely positive because of the overlapping and intermingling of the chromosomes. Figure 6 shows a relatively distinct metaphase I chromosome configuration of 89-4-1, a BC_4 plant. Multivalent chromosomes, ring and cruciform formations are observed. Figure 7 (a) shows a metaphase I chromosome configuration of 89-4-3, another BC_4 plant. This cell shows predominantly randomized chiasmata with various unusual pairing configurations. One bivalent is paired only at one end of the chromosome arm (chained chromosomes). Stickiness and univalents are also observed. Figure 7(b) is a metaphase I chromosomal configuration of a BC_4 self plant, 90-12. This cell contains two univalents. Pairing between most chromosomes is partial and only a few have complete pairing. Figure 8 is the metaphase I configuration seen in the BC_4 self plant, 90-14. The chromosome pairing of this cell is closest to a normal pairing chiasmata configuration seen in the interspecific derivative materials including BC_5 plants. The cell shows both randomized as well as localized chiasmata.

Multivalents, ring formation and stickiness are the results of interspecific meiotic irregularities resulting from translocations, inversions and duplications (Peffley et al., 1989). Plants used in this study were in the BC_4 generation. These meiotic

formations are still carried over in the BC_4, underscores the genetic exchange between initial *A. cepa* and *A. fistulosum* genomes. The severity of the irregularity, however, is decreasing with the increase of generations.

The stickiness and multivalent pairing indicates common DNA in the non-homologous chromosomes. Since Beltsville Bunching cells do show some bridges and fragments (Jones et al., 1942, Jones et al., 1963), it is reasonable to assume translocations and genetic exchanges have occurred between *A. cepa* and *A. fistulosum* genomes in the Beltsville Bunching nucleus. Thus, the interspecific triploid F_1 developed by crossing Beltsville Bunching with *A. cepa* inherited one *A. cepa* genome which already had genetic integration from the *A. fistulosum* genome. Depending on the extent of *A. fistulosum* DNA integration, the F_1 can be fertile as the male parent. When this triploid F_1 is backcrossed to *A. cepa*, the two *A. cepa* genomes, probably incompletely paired, recombined to reduce the chromosomal mutational effects and fertilization could be accomplished. It is further envisioned that the chromosomal architectural alterations were gradually reduced every generation as the interspecific DNA recombined with the normal *A. cepa* DNA. The stickiness and partial pairing observed in the current generations, will probably take many generations of selfing before little or none is observed. Nevertheless, if the pairing is good enough not to warrant a significant decrease in the fertility, one may consider such meiotic chromosomes as stabilizing.

The deleterious effects of the chromosomal mutations in the crosses between normal *A. cepa* and *A. fistulosum* were strong enough to affect fertility to a great extent. Early integration efforts (Emsweller et al., 1935, Emsweller et al., 1945, Cocharan 1950, Kehr 1957) did not succeed because of the sterility of the crosses. The sterility was due to a high level of meiotic irregularities caused by strong chromosomal mutations experienced in the interspecific hybrids. Since Beltsville

Bunching is an amphidiploid, the genetic integration between its genomes would be expected to be gradual and in small doses. Thus, the development of fertile interspecific hybrids using Beltsville Bunching was possible because of the presence of the original *A. cepa* genome in the Beltsville Bunching nucleus. If the speculation about gradual genetic integration between Beltsville Bunching genomes is correct, the *A. fistulosum* genome should also have reciprocal integration from *A. cepa*.

The stickiness was severe up to the BC_3 generation, it still persists, though the severity has decreased with the increase of generations. Some cells, however, were found to have near normal pairing (90-14, in figure 8).

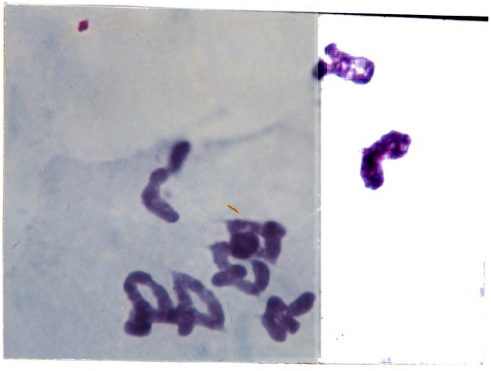
Two to four univalent chromosomes were found in some of the metaphase I cells of the interspecific plant materials. Occurrence of univalents does not necessarily mean that the chromosomes were unpaired, since the chromosomes may have separated prematurely (Swanson et al., 1981). Univalents, however, do increase the chances of nondisjunction and should be considered unfavorable structures to a normal diploidy development. Cells with univalent metaphase I and anaphase I stage chromosomes were counted in the BC_4, BC_4 self and BC_5 generations. Table 7 summarizes the observations. The results, by generation, show that 26% of the BC_4 cells had two to four univalents, 15% of the BC_4 self cells had two univalents and only 7% of the BC_5 cells had two univalents. It seems genetic recombination (from selfing and crossing with *A. cepa*) is decreasing the incidence of univalents probably by increasing homology between the interspecific chromosomes. This directly translates to progress towards development of diploids with regular meiotic behavior in the currently developed *Allium* germplasm.

Table 6: Chromosomal pairing relationships in the parents used in the Interspecific *Allium* Germplasm study.

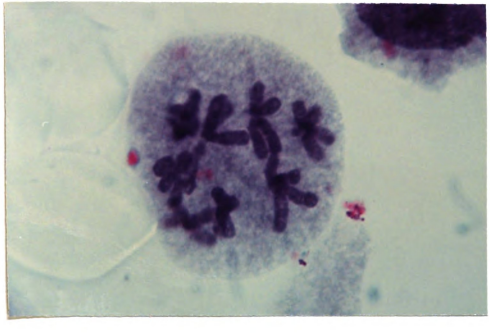
Plant	Paired chromosomes	Chiasmata
A. cepa	8 pairs	randomized
A. fistulosum	8 pairs	localized
Beltsville Bunching	16 pairs	mixture

Table 7: Univalents in some of the interspecific *Allium* germplasm metaphase I cells.

Line #	Generation	# of Cells	# of Univalents
1253	BC_4	5	0
		2	2
		1	4
1255	BC_4	6	0
		7	2
89-4-1	BC_4	3	0
		1	2
		1	4
89-4-2	BC_4	9	0
		1	2
89-4-3	BC_4	6	0
		2	2
90-3	BC_4 ⊕	7	0
		1	2
90-12	BC_4 ⊕	5	0
		2	2
90-14	BC_4 ⊕	5	0
90-18	BC_5	6	0
90-23	BC_5	8	0
		1	2



(a)



(b)

Figure 4: Metaphase I chromosomes of (a) *A. cepa* showing characteristic distal chiasmata (b) *A. fistulosum* showing localized (proximal) chiasmata.

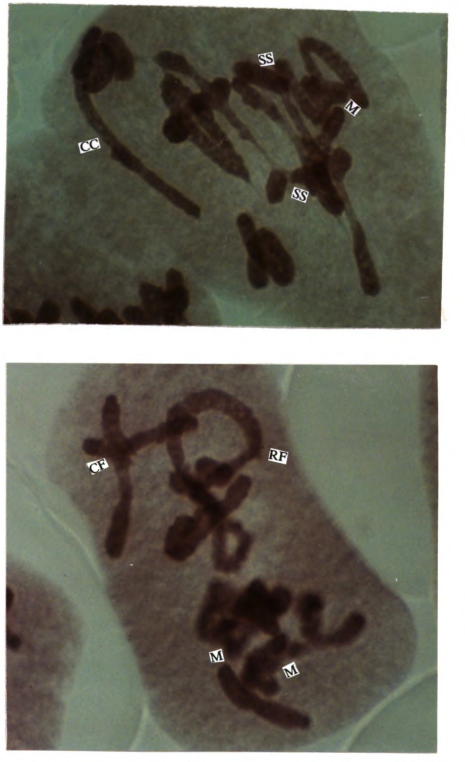


Figure 5: Chromosomes of the *Allium* interspecific derivatives, showing meiotic structures in (a) 1253-1 at anaphase I (b) 1255 at metaphase I. CC = chained chromosomes, CF = cruciform chiasmata, RF = ring formation, M = multivalent, SS = sticky spot.

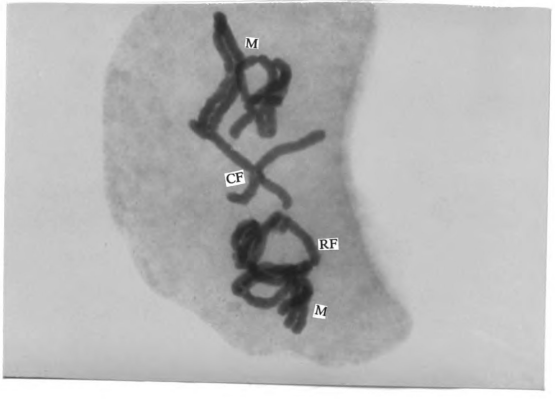


Figure 6: Metaphase I chromosomes in the *Allium* interspecific derivative plant, 89-4-1 (BC_4) showing meiotic formations at metaphase I. CF = cruciform chiasmata, RF = ring formation, M = multivalent.

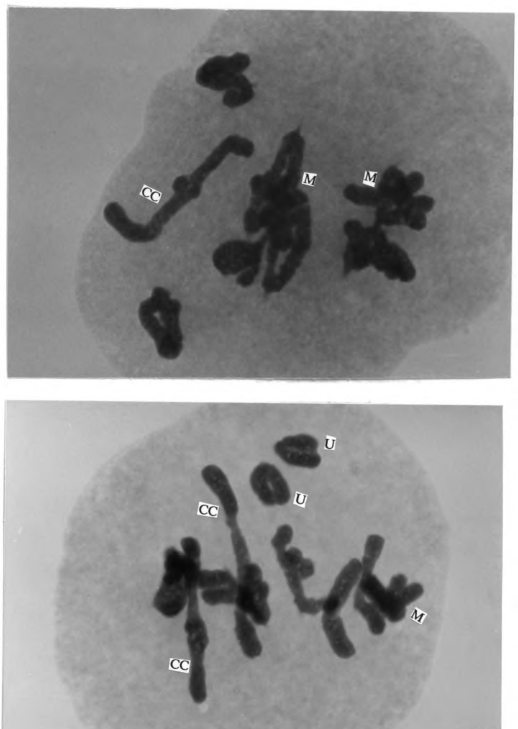


Figure 7: Metaphase I chromosomes in the *Allium* interspecific derivative BC_4 and self plants. (a) 89-4-3 showing majority of chiasmata being randomized type. and (b) 90-12 showing two univalents. CC = chained chromosomes, M = multivalent, U = univalent.

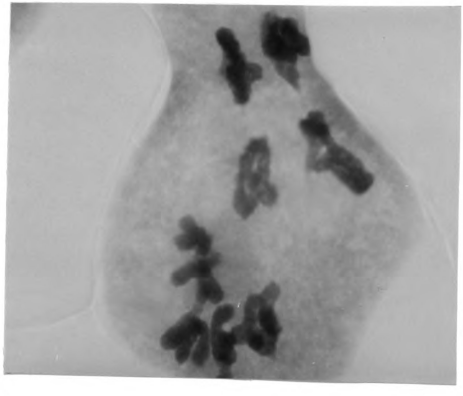


Figure 8: Metaphase I chromosomes in the *Allium* interspecific derivative, 90-14, a BC_4 self, showing complete 8 bivalents. with a combination of randomized and localized chiasmata.

RAPD Analysis

The original parents of the interspecific derivative lines (Beltsville Bunching and *A. cepa*) were from a European collection. Neither the actual parental plants nor their varietal representative plants were available for this study. An accession of Beltsville Bunching and an *A. fistulosum* cultivar Odessa, were used as the *A. fistulosum* DNA source. Six MSU inbreds, all of which were used in the disease evaluation experiment, were employed as the *A. cepa* DNA source. The assumption was that RAPDs common in *A. fistulosum* but unique to *A. cepa* could be used to document introgression in the interspecific lines. The populations used in this study are BC_4 selfs and theoretically carry over 96% *A. cepa* DNA.

The absence of a parental RAPD in an interspecific hybrid does not necessarily mean that the hybrid is not carrying the non-recurrent parental genetic materials; however, the presence of it does indicate that the hybrid contains a portion of the non-recurrent genome. In the process of genetic recombination, the region producing a specific RAPD may itself get recombined or get lost. But the likelihood of a region similar to the one present in the non-recurrent parent developing *de novo* in the hybrid is very small. So, if the interspecific germplasms display RAPDs from *A. fistulosum*, there is a very high probability that the germplasm also carries a portion of the *A. fistulosum* genome.

Figure 10 is a schematic drawn based on Figure 9 which shows the ethidium bromide gel for the primers RW2. This primer uncovered polymorphism in the parental DNA (*A. cepa* and *A. fistulosum* genome sources) and the unique RAPDs present in *A. fistulosum* (Odessa and Beltsville Bunching) were also found in the interspecific hybrids. Because the primer RW2 showed polymorphism between

A. cepa and *A. fistulosum* DNA, it shows strong potential as a genetic fingerprint of interspecific *Allium* germplasm.

In total, 17 RAPD marker loci were identified. Most of the *A. cepa* lines showed 6 unique RAPDs (3,5,8,10,12 and 13), although line 611 had RAPD 4 which was also present in *A. fistulosum* and the hybrid lines. Odessa, the other theoretical parental line (*A. fistulosum*), had 9 unique RAPDs. Interestingly, all of the RAPDs found in *A. fistulosum*, were missing in *A. cepa* with the exception of 2 and 14, which were present in both *A. cepa* and *A. fistulosum* lines.

Beltsville Bunching, the amphidiploid, carries both *A. cepa* and *A. fistulosum* genomes. As expected, it showed a high number of RAPDs, 12 out of the 17 found in the diploid species. Not surprisingly, 5 of its RAPDs were the ones found in *A. cepa*, another 5 were the ones found in *A. fistulosum* and two were common to both.

The interspecific lines, like *A. cepa* lines, showed very little polymorphism. They all contained 9 to 11 RAPDs. Lines 90-26-6 and 90-12-18 carried RAPDs 2, 3, 4, 5, 6, 8, 9, 12, 13, 14 and 16. The third interspecific line 90-11-9 did not have RAPDs 6 and 16.

Beltsville Bunching had 5 of the 9 unique RAPDs found in Odessa. The interspecific line also carried 5 of the 9 unique Odessa RAPDs. The interspecific derivative lines are BC_4, which is an advanced backcross generation. The presence of 5 out of 9 unique RAPDs in the BC_4, highlights the homogeneous integration of the *A. fistulosum* DNA into the *A. cepa* genome. Although the lines examined were not among the original parents, it is possible that the RAPDs appearing to be species specific in these studies would also be found in both species in a broader germplasm survey.

LE2, the other primer used in this study (Figure 11), produced only a single RAPD specific to the *A. fistulosum* genome. Unfortunately, not all lines of *A. cepa* were amplified by this primer.

The RAPDs are amplified 200-2000 base pair long DNA sequences. Such sequences may happen to be in the proximity of a morphological locus. The possibility that the amplified region may fall close to or within the sequence of an economically important gene provides us with an opportunity that the RAPD can be used to select the linked economically important genotype in a backcross program. This is specially valuable when the gene being introduced is a recessive one. Traditionally such a situation requires the breeder to self the cross (BC_1, BC_2 etc) in order to identify the individual carrying the gene. Linkage, closer than 25 cM has been shown to be identified by the amplification (Michelmore et al., 1991). Indirect selection using a closely linked RAPD will accelerate the breeding process by cutting the number of generations needed in half by avoiding the selfing generation.

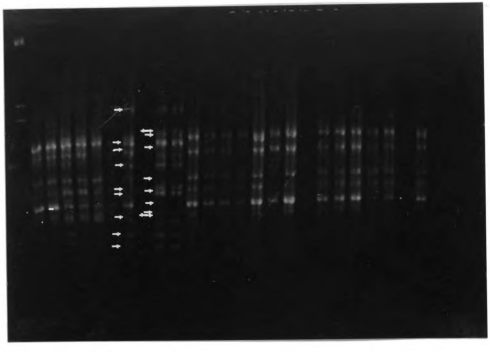


Figure 9: RW2 primer derived ethidium bromide gel showing polymorphic bands in *A. cepa*, *A. fistulosum* and interspecific derivative DNA. The left most lane is marker lambda DNA. Next six rows are interspecific derivatives (1-2:90-26-6, 3-4:90-12-18, 5-6:90-11-9), next two rows Beltsville Bunching (7-8), next two rows Odessa(9-10) and last fifteen rows are *A. cepa* DNA (11-12:8155, 13-14:1731, 15:611, 16-17:420, 18-19:2399, 20-21:1849, 22-23:611a, 24:Water, 25:611). Left lane arrows show *A. fistulosum* specific RAPDs while right lane arrows show *A. cepa* specific RAPDs. Bidirectional arrows show non-segregating (commom to both) RAPD loci.

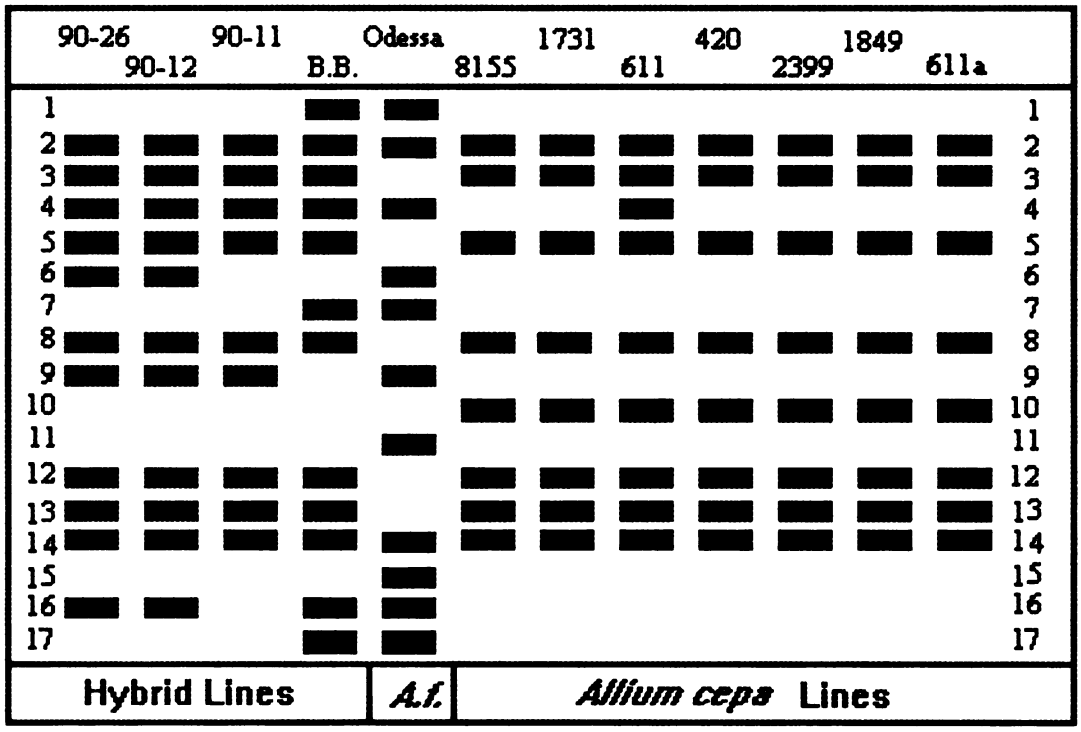


Figure 10: A schematic showing the banding pattern shown by RW2 primer in the RAPD analysis of the interspecific *Allium* germplasm. Only one replication of the two replications per sample is shown. Lane 611a is another DNA sample of line 611. While preparing the schematic, very faint bands were not considered.

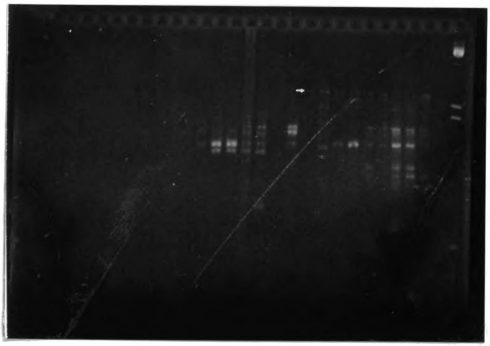


Figure 11: LE2 primer derived ethidium bromide gel showing polymorphic bands in *A. cepa*, *A. fistulosum* and interspecific derivative DNA. The right most lane is marker lambda DNA. Next six rows are interspecific derivatives (1-2:90-26-6, 3-4:90-12-18, 5-6:90-11-9), next two rows Beltsville Bunching (7-8), next two rows Odessa(9-10) and last six rows are *A. cepa* DNA (11-12:1731, 13-14:2399, 15-16:1849). The arrow shows the *A. fistulosum* specific RAPD.

Fertility Improvement

Because of low fertility in the interspecific F_1, F_2 and BC_1 generations of earlier *A. cepa* and *A. fistulosum* integration projects, they were abandoned without utilizing the developed materials in a breeding project. These interspecific hybrids greatly suffered from the sterility due to the meiotic irregularities of the crosses. The level of seed set in the earlier projects ranged from 0 to 0.1% in a controlled pollinated environment (Emsweller et al., 1935, Emsweller et al., 1945, Cocharan 1950, Kehr 1957 and Corgan 1985). The seed set even in the open pollinated environment would reach only up to 2% (Emsweller et al., 1945). In addition, in the course of the current research, various *A. cepa* lines were used to cross with *A. fistulosum* (var Odessa) in an attempt to obtain interspecific F_1 seeds. In spite of attempting hundreds of pollinations, no viable seeds were obtained.

For generation advancement, the interspecific germplasms were not only backcrossed but also selfed. In general, selfing produced more seeds than did crosses. Tables 8 and 9 show the seed set result of BC_4 selfs and BC_5 respectively. It seems by the BC_3 generation, that seed set begins to increase significantly. The point to note here is that the severity of the barriers which were expressed in the initial interspecific F_1, BC_1 and F_2 generations, are greatly decreased. In onions, traditionally artificial (emasculated) crossing does not yield a high percent of success. The crossing results with the current interspecific germplasms have become encouraging by the BC_4 generation. The seed set of BC_4 selfs, though not quite as good as for the *A. cepa* selfs, has shown much improvement.

Table 8: **Seed set in the 5th generation (BC_4 self) interspecific germplasm development between *A. cepa* and *A. fistulosum*.**

Cross	# of florets pollinated	# seeds	% success
89-4-2 ⊕	33	73	73.7
89-4-3 ⊕	33	49	49.9
89-4-4 ⊕	33	39	39.4
89-12-4 ⊕	17	4	7.8
Mean	-	-	47.4

Table 9: **Seed set in the 5th generation (BC_5) interspecific germplasm development between *A. cepa* and *A. fistulosum*.**

Cross	# of florets pollinated	# seeds	% success
611 x 89-3-2	10	2	6.7
611 x 89-4-2	10	2	6.7
611 x 89-4-4	10	1	3.3
611 x 89-12-4	10	12	40.0
2399 x 89-3-1	10	3	10.0
2399 x 89-4-1	10	14	46.7
2399 x 89-4-2	10	13	43.3
1731 x 89-3-2	10	1	3.3
1731 x 89-4-4	10	5	16.7
89-4-1 x 1849	10	11	36.7
Mean	-	-	21.3

The BC_4 self seed set ranged from 8% to 73% with a mean at 47%. There was a variation for seed set in different lines. In general, lines with the 89-4 pedigree produced more selfed seeds than line 89-12-4 which had only an 8% selfing success rate. Line 89-4-2 with a 73% success rate was the highest self seed producer.

The BC_5 generation was developed using *A. cepa* as the female parent. Only one cross was made with 89-4-1, a BC_4 plant, used as the female. The seed set rate for the crosses ranged from 3.3% to 46.7% with the mean at 21.3%.

Four *A. cepa* inbred lines were used in the BC_5 development. The mean success rate of the *A. cepa* lines was quite variable. The lines could be divided into two distinct groups. Lines 1849 and 2399 were among the high seed set group with 1849 yielding 36.7% and 2399 close behind at a 33.33% success rate. The low yielding group consisted of the 611 and 1731 inbreds. Line 1731 was last with a seed set rate of only 10% while 611 was not too far off at a 14.17% success rate. The high seed set level of the lines 1849 and 2399 indicate a possibility that the *A. cepa* lines used in early backcrosses (up to BC_3) may have had similar genetic background as these two *A. cepa* lines. It could partially explain why lines 1849 and 2399 scored dramatically better than the other two *A. cepa* lines used in the study.

In general, interspecific lines with the 89-4 background produced the best results, not only in selfing but also in crossing. The mean selfing success rate of these lines was 54.20% while the mean crossing success rate was 25.56%. Interspecific line 89-4-1 was not used for selfing but was used in crossing reciprocally. In either crossing, it had a high crossing success rate, 36.7% as the female and 46.7% as the male parent. It seems reasonable to assume that lines developed from 89-4 pedigree should have a relatively low level of aneuploidy and chromosomal mutation. Figures 5(b), 6 and 7(a and b) show chromosomal configuration of such individuals at

metaphase I or anaphase I. Though there seems to be some stickiness between non-homologous chromosomes, probably the chromosome architecture in the BC_4 generation has recombined and pairing properties have improved enough to provide the level of fertility obtained in this study.

Lines related to the 1253 pedigree did not do as well as lines with the 89-4 pedigree. Only one line of the 1253 pedigree, 89-12-4 was selfed and produce only 8% selfs. The mean crossing rate of such lines was 15%. Line 89-12-4 scored poorly in selfing but scored high when it was used as the male parent with a 40% seed set rate. This indicates the possibility that the plant 89-12-4 may be a trisomic and / or may have a significant chromosomal mutation which severely hampers its performance as the female gamete producer. Figures 5(a) and 8 show the metaphase I chromosomal configurations of some plants of 1253 pedigree. Some individuals of this pedigree show stickiness of non-homologous chromosomes and at the same time others show almost regular pairing structures (Figure 8). It seems some lines of this pedigree, as indicated by the their seed set, still have a high level of aneuploidy and / or chromosomal mutation that can affect their performance as female fertile gamete producers.

Disease Screening

Because of an insufficient number of seeds, only the selfed seeds of 89-4-2, 89-4-3 and 89-4-4 could be screened for disease resistance. The *A. cepa* lines, 6701, 1849 and 611 were used as checks in the *Fusarium* disease screening. In the pink root screening, the *A. cepa* lines 420, 8155 and 611 were used as inbred checks. Tables 10, 11 and 12 show the result of the *Fusarium*, pinkroot and control (without inoculum) screening respectively. The survival percent of the seedlings was adjusted for the germination percent assuming that germination is independent of inoculum effects. Thus, the survival is the ratio of the number of surviving seedlings on the 21st day to the number of seeds sown, and the adjusted survival is the ratio of the number of seedling surviving on the 21st day to the number of seedlings germinated on the 10th day.

All the analyses related to the *Fusarium* and pinkroot screening were performed on arcsine transformed data (Steel and Torrie 1980). Treatments with single replication were not included in the ANOVA calculation. Such treatments, however, are presented in the tables to facilitate comparison with other treatment means.

Table 12 shows the germination, survival and the adjusted survival percentage observation of all the *A. cepa* lines and a single interspecific derivative 89-4-3 self used in the study. The values are the observed values and not means as only a single replication of the control treatments was used due to too few interspecific seeds. No definite conclusions can be made because of lack of adequate replication, however, there was variation in the germination percent between lines. Even in the absence of any disease inoculum, some seedlings died after germination. An unexpected observation was that the interspecific derivative 89-4-3 self which consistently germinated 100% in the disease environment, germinated only 60% in the controlled benign environment.

Table 10: Means of *Fusarium* screening observations on selected *A. cepa* and interspecific germplasm lines[z].

Line	#Rows	Germtn.%[y]	Survival%[y]	Adj.Surv%[y]
6701	4	82.50	50.00 b	60.60 b
1849	4	77.50	2.50 c	3.23 c
611	4	90.00	80.00 a	88.88 a
89-4-2⊕	3	83.33	70.00 a	84.00 ab
89-4-3⊕[x]	1	100.00	80.00	80.00
89-4-4⊕[x]	1	40.00	10.00	25.00

z The calculations were done on arcsine transformed data and pairwise comparison was done using specific lsd for the pair. Single row treatments were not included in the ANOVA calculation.

y Means followed by the same letter are not significantly different at the 0.05 probability level.

x not included in the ANOVA calculation.

Table 11: Means of pinkroot screening observations on selected *A. cepa* and interspecific germplasm lines[z].

Line	# Rows	Germtn.%	Survival%[y]	Adj. Survival%
420	4	92.50 ab	92.50 a	100.00
8155	4	37.50 c	27.50 c	73.33
611	4	92.50 ab	85.00 ab	91.90
89-4-2⊕	2	75.00 b	60.00 b	80.00
89-4-3⊕	2	100.00 a	90.00 ab	90.00
89-4-4⊕[x]	1	50.00	30.00	60.00

z The calculations were done on arcsine transformed data and pairwise comparison was done using specific lsd for the pair. Single row treatments were not included in the ANOVA calculation.

y Means followed by the same letter are not significantly different at the 0.05 probability level.

x not included in the ANOVA calculation.

Table 12: The control screening observations on selected *A. cepa* and interspecific germplasm lines.

Line	# Seed Sown	Germination%	Survival%	Adj. Sur%
420	10	80.00	70.00	87.50
611	10	100.00	80.00	80.00
1849	10	90.00	60.00	66.67
6701	10	90.00	60.00	66.67
8155	10	50.00	50.00	100.00
89-4-3 ⊕	10	60.00	60.00	100.00

Tables 10 and 11 show *Fusarium* and pinkroot screening respectively. The data are presented as a mean percent germination, survival and adjusted survival of the lines in both the environments. For *Fusarium* it does not matter whether the data are adjusted or not. The ranking of the lines / derivatives in their expression of resistance remains the same. The lines 611, 89-4-2 self and 89-4-3 self showed the highest level of resistance. The line 6701 which was considered the best resistant line available, showed less resistance and lines 1849 and 89-4-4 self showed the lowest level of resistance. The interspecific line 89-4-4 probably does not carry the resistance gene.

The pinkroot screening result *is* affected by the germination adjustment. If the unadjusted data is used, lines 420 and 89-4-3 self performed the best, lines 611 and 89-4-2 self were next and as expected the susceptible line 8155 performed the worst as did the 89-4-4 self. Overall, the *A. cepa* lines behaved as expected. Nevertheless, if the survival is adjusted for germination, two things happen. The experimental error became higher and the range of means narrowed down. The test did not find a significant difference between the means. The adjusted survival percent data,

therefore, suggest that there was no difference in the expression of resistance to pinkroot between any of the lines / interspecific derivatives used in the study. The germination percent was closely reflected by the unadjusted survival percentage, and the lines showing better resistance also showed better germination. The original screening data is graphically presented in the figures 11-13.

Table 13 shows the overall germination percentage of the pooled observations from the three experiments. There was a significant difference in the germination percentage. The interspecific derivative selfs 89-4-2, 89-4-3 were among the best and the third 89-4-4 was among the worst germinators.

In conclusion, interspecific selfed lines 89-4-2 and 89-4-3 show excellent potential for resistance / tolerance to *Fusarium*. The interspecific derivative, 89-4-3 was one of the high tolerant lines to pinkroot and 89-4-2 showed tolerance equivalent to the one showed by the normal *A. cepa* line. When their score, however, was adjusted for germination, there was no significant difference between scores.

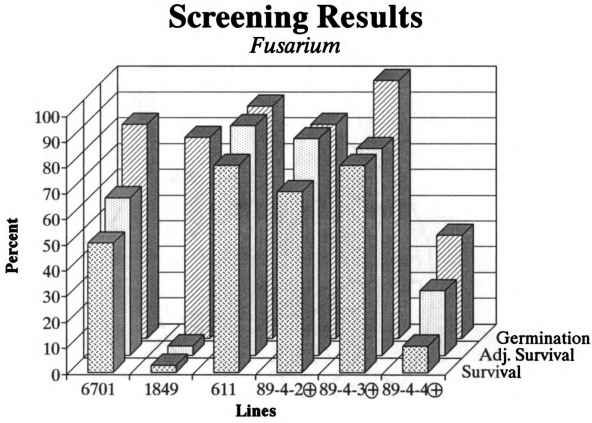


Figure 11: Bar graph showing survival and germination percentages of all the lines used in the basal rot screening study using *Allium* interspecific derivatives and normal *A. cepa* lines.

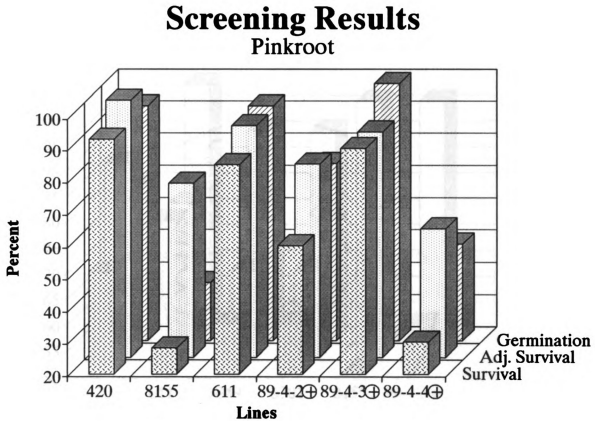


Figure 12: Bar graph showing survival and germination percentages of all the lines used in the pinkroot screening study using *Allium* interspecific derivatives and normal *A. cepa* lines.

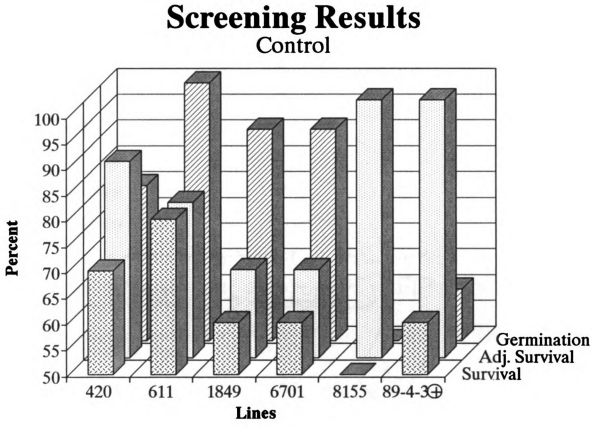


Figure 13: Bar graph showing survival and germination percentages of all the lines used in the control screening study using *Allium* interspecific derivatives and normal *A. cepa* lines.

Table 13: Overall germination of selected *A. cepa* and interspecific lines used in the *Fusarium*, pinkroot and control screening experiments.[z]

Line	# Rows	Germination%[y]
420	5	90 a
611	9	94 a
1849	5	80 b
6701	5	84 ab
8155	5	40 c
89-4-2 ⊕	5	80 b
89-4-3 ⊕	4	90 a
89-4-4 ⊕	2	45 c

[z] The calculations were done on arcsine transformed data and pairwise comparison was done using specific lsd for the pair.

[y] Means followed by the same letter are not significantly different at the 0.05 probability level.

Genetic Model: The screening data also provided an opportunity to speculate on genotypes of the parent plants of the interspecific derivatives used in the study. All the derivatives used were selfs and only two phenotypes could be considered for the resistance character - **alive** or **dead**. Genes providing the resistance are likely to have introgressed from the *A. fistulosum*, and the mode of genetic action of these genes may not be the same as found for the resistance genes in the *A. cepa* lines. For *Fusarium* resistance in *A. cepa* a two-gene incomplete dominant system has been suggested (Bacher 1989). Jones and Perry (1956) have reported a single recessive gene for resistance to pinkroot in the *A. cepa* system.

Table 14: Possible model of a single dominant gene (P) for pinkroot resistance in the *Allium* interspecific hybrid development study.

Line	Postulated Genotype	Observed Numbers of the selfed progeny Alive	Dead	Expected Ratio	X^2
89-4-2	Pp	12	8	3:1	2.40
89-4-3	PP	18	2	1:0	-
89-4-4	pp	3	7	0:1	-

Table 15: Possible model of a single dominant gene (F) for *Fusarium* resistance in the *Allium* interspecific hybrid development study.

Line	Postulated Genotype	Observed Number of the selfed progeny Alive	Dead	Expected Ratio	X^2
89-4-2	Ff	21	9	3:1	0.40
89-4-3	FF	8	2	1:0	-
89-4-4	ff	1	9	0:1	-

Table 16: Possible model of a single dominant gene (R) for overall resistance in the *Allium* interspecific hybrid development study.

Line	Postulated Genotype	Observed Number of the selfed progeny Alive	Dead	Expected Ratio	X^2
89-4-2	Rr	33	17	3:1	2.16
89-4-3	RR	26	4	1:0	-
89-4-4	rr	4	16	0:1	-

Tables 14 and 15 present parental lines of the interspecific selfs used in the disease screening and the observed number of selfed seedlings which were alive (and also which were dead) 21 days after sowing. The tables also present the assumed genotypes of the parental lines along with the calculated X^2 values for the expected ratio of the alive and dead phenotypes in the selfed progenies of the heterozygous parents. Table 14 presents the data for the pinkroot and table 15 the same for the *Fusarium* screening experiment respectively. A simple Mendelian model with a dominant resistance gene is assumed in both the cases. The tables also show data about assumed genotypes, dead and alive seedling at 21 days, and the expected ratio according to the model and the calculated X^2 values. The chi-square value for selfed homozygous parents could not be calculated for the reason that a ratio of 1:0 requires division by zero. The number in the zero class, however, is low enough to be attributed to chance alone. The calculated X^2 values in all the cases are not significant at one degree of freedom. The genotype for 89-4-2 is assumed to be heterozygous, 89-4-3 to be homozygous dominant and 89-4-4 to homozygous recessive. The resistance is assumed to be a dominant gene.

In nature, economic use of available resources is necessary in order to survive the natural competition. The survival of fittest theory implies that the one with the most efficient utilization system of resources will survive and produce progeny. With this axiom, it is possible that both the P and F genes are the same R gene and thus are pleiotropic. Table 16 shows the combined data for both the diseases. Again assumptions about the model, as well as, the individual genotypes are the same as above. The data fits well with the model, and the X^2 values are not significant. It is possible that the same gene is offering the resistance to both the diseases though the approach of imparting resistance in each case may not be the same.

The empirical experience and knowledge about the fungal disease infection physiology indicate that *Fusarium* and pinkroot diseases are multistep complex processes. A lot of information about the host pathogen interaction is still not clear. Nevertheless, current studies indicate that fungi, specially *Fusarium*, accumulate cutinase on their outer cell surface when attached to a host. This cutinase plays the key role in the penetration of the fungal haustoria (Kolattukudy 1985, Woloshuk et al., 1986). Plants respond to such attack by accumulating hydrolyzing enzyme chitinase in the surface cells (Mauch et al., 1989, Benhamou et al., 1990). Chitinase kills the fungus by hydrolyzing its cell wall. In the particular case of this experiment, the young seedling probably can not withstand the pathogen once it is penetrated. The only way for the seedling to survive would be avoid the pathogen by stopping it from penetration. The resistant / tolerant seedlings may have survived by either hydrolyzing the fungal cell wall and / or by producing one or more of known antifungal secondary metabolites like alkaloids, flavonoids or saponines (Wink 1988), which, when absorbed by the pathogen, kills it. Both these approaches are general plant defense mechanisms. Since *A. fistulosum* is known to be resistant / tolerant to a wide range of pathogens, it is not unreasonable to assume that it may

possess a few general resistance genes rather than many specific resistance genes. Since the same interspecific line shows resistance / tolerance to both *Fusarium* and pinkroot, 89-4-2 and 89-4-3 selfs; and the same line, 89-4-4 self, show susceptibility to both the diseases, it is hypothesized that a single gene could be pleiotropically imparting the resistance in both the cases.

SUMMARY AND CONCLUSIONS

The sterility of the interspecific F_1s between *A. cepa* and *A. fistulosum* has stopped many projects trying to introduce desirable characters from *A. fistulosum* into *A. cepa*. The current study is a part of a long term hybridization program which appears to have been successful in introgressing some part of the *A. fistulosum* genome into the *A. cepa* background. The initial *A. fistulosum* genome source in the diploid interspecific hybrid in this study, was the amphidiploid (2n = 32), Beltsville Bunching. It seems that the polyploid environment of Beltsville Bunching provided a better environment (probably by providing a buffered environment) for genetic exchange between *A. cepa* and *A. fistulosum* genomes than one provided by the direct cross of *A. cepa* and *A. fistulosum*. Cytogenetic studies show aberrations such as sticky chromosomes, heteromorphic chromosomes, fragments and chromatin bridges, all of which are gradually decreasing in subsequent backcross and selfed generations. These cytological formations which were important to verify the genetic introgression, adversely affect the fertility of the developed lines. Many developed interspecific lines show a high level of fertility indicating that the meiotic chromosomal aberrations have significantly decreased probably due to recombination by selfing and backcrossing with *A. cepa*. Some lines, however, do indicate strong chromosomal mutations by expressing reduced fertility when used as a female parent. The interspecifically developed plants morphologically resemble *A. cepa*. By the 4th generation, they could not be distinguished externally from *A. cepa* except for hybrid vigor. This study demonstrated that they are carrying *A. fistulosum* genetic materials and, in many cases, with significantly improved fertility.

The following conclusions are drawn from the study:

a. Interspecific derivatives between *A. cepa* and *A. fistulosum* have been developed which morphologically resemble *A. cepa*.

b. Many derivative lines show enhanced fertility and seem to approach the fertility of *A. cepa*.

c. Meiotic irregularities are decreasing with the increase in the generation level. Progress towards a regular meiosis is reflected by the increased seed set by many developed interspecific lines.

d. Some of the derivatives carry a high degree of resistance / tolerance to *Fusarium* and pinkroot.

e. The limited data indicates the mode of genetic action of the resistance genes to be dominant for both the diseases. It is possible that a single gene is pleiotropically imparting resistance in both the cases.

f. The derivatives are in an advanced stage ready to be directly used in a breeding program to develop inbreds.

g. The derived plants could possibly be used to transfer additional characters from *A. fistulosum*, as they should be easier to cross with both *A. cepa* and *A. fistulosum* because of the genetic integration of the two species.

The following further studies are suggested:

i. The seeds of the interspecific derived materials should be increased and a comprehensive screening study should be done for all the diseases *A. fistulosum* is known to show resistance. This will identify the potentials of different derivative lines which are already developed. Such a study would also find if further crossing with *A. fistulosum* is needed to transfer characters which were lost due to genetic drift / attrition in the interspecific recombination.

ii. With the results from section *i*, promising lines should be used to introduce their genes to the elite inbreds of onion. The crossing should be made in an open pollination block. Plants grown from the seeds harvested from the *A. cepa* lines could be screened with RAPD (using RW2 primer) for presence of *A. fistulosum* DNA.

iii. New *A. cepa* x *A. fistulosum* integration attempts using other selected Beltsville Bunching lines could be initiated. An open pollination crossing block procedure is recommended for possible maximum seed set. Then RAPD (with RW2 primer) could be used for screening of the selected plants from the crosses. With this procedure, accelerated development of interspecific hybrids could be achieved. Cytogenetic observations are not necessary, but they would provide useful information about the meiotic behavior of the cross.

APPENDICES

Appendix A: Picture of bulb types of *A. cepa*, *A. fistulosum* and the F_1. *A. fistulosum* does not produce bulbs, *A. cepa* is shown with regular onion bulbs and the F_1 has very slight bulbing. L to R : *A. fistulosum*, without bulb formation; two interspecific F_1 samples with only very slight tissue swelling (bulbing); *A. cepa*, with normal bulb formation. (Courtesy - Dr. Lowell C. Ewart).

Appendix B: Pedigree of the interspecific germplasm used in this study. (Source: Ewart 1986).

(a). *A. cepa* x Beltsville Bunching -> 73898 (F_1, triploid)

(b). *A. cepa* x 73898 -> 79353 (BC_1, occasional trivalents)

(c). 79353 x *A. cepa* -> 82148 (BC_2, diploid)

(d). 82148 x *A. cepa* -> 84462 (BC_3, diploid)

(e). *A. cepa* x 84462-2 -> 1253 (24 seeds obtained)

(f). 84462-2 x *A. cepa* -> 1255 (6 seeds obtained)

Appendix C: Arcsine transformed means, error and F-values for the treatments used in *Fusarium*, pinkroot screening and overall germination (*Fusarium*, pinkroot and control combined) in the disease screening of the *Allium* germplasm.

A. *Fusarium*:

Line	# Obs	Germ.	Surv.	Adj_Serv.
6701	4	68.785	45.000	53.060
1849	4	62.145	4.610	5.555
611	4	77.087	63.807	73.885
89-4-2⊕	3	66.147	56.790	66.830
Error		155.743	39.203	111.137
F-val		1.012	69.589	33.128
Probability		0.424	0.000	0.000

B. pinkroot:

Line	# Obs	Germ.	Surv.	Adj_Serv.
420	4	78.750	78.750	90.000
8155	4	37.442	31.390	64.742
611	4	76.172	67.867	78.362
89-4-2⊕	2	60.115	51.335	69.540
89-4-3⊕	2	90.000	71.560	71.560
Error		96.985	100.666	214.566
F-val		14.270	13.274	1.673
Probability		0.000	0.000	0.226

C. Overall Germination:

Line	# Rows	Germination
420	5	75.688
611	9	78.116
1849	5	64.030
6701	5	69.340
8155	5	38.954
89-4-2⊕	5	63.734
89-4-3⊕	4	80.139
89-4-4⊕	2	42.115
Error		139.549
F-val		7.655
Probability		0.000

Appendix D: Pairwise calculated least significant differences (lsd) to compare paired means in various *Allium* germplasm disease screening experiments.

A. *Fusarium* :

Paired Obs	Germination%	Survival%	Adj. Surv.%
3, 4	-	10.525	17.722
4, 4	-	9.745	16.407

B. pinkroot :

Paired Obs	Germination%	Survival%	Adj. Surv.%
2, 2	21.676	22.083	-
2, 4	18.772	19.125	-
4, 4	15.327	15.615	-

C. Overall Germination

Paired Obs	Germination%
2, 4	20.890
2, 5	20.182
2, 9	18.857
4, 5	16.182
4, 9	14.496
5, 5	15.256
5, 9	13.455

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