served with other techniques for assaying carbohydrates. Sugars determined by this method were observed to be basically of a free nature and not the hydrolytic products of either starch or cellulose.

There was no apparent evidence of anomerization between the different sugars extracted from plant tissues, although this phenomenon occurred when reagent grade carbohydrates were employed. Recovery of the trimethylsilyl derivatives was observed to be of a high order for all sugars analyzed.

LITERATURE CITED

1. ALBERSHEIM, P., D. J. NEVINS, P. D. ENGLISH, and A. KARR. 1967. A method for the analysis of sugars in plant cell wall polysaccharides by gas-liquid chromatography. *Carbohydrate Res.* 5:340–345.
2. KEULEMANS, A. I. M. 1964. Gas chromatography. Rheinhold Publishing Co. 234 p.
3. LUDLOW, J. C., T. M. HARRIS, and F. T. WOLF. 1966. An analysis of the free sugars of ferns by gas-liquid chromatography. *Phytochemistry* 5:251–257.
4. SWEELEY, C. C., R. BENTLEY, M. MAKETA, and W. W. WELLS. 1963. Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. *J. Amer. Chem. Soc.* 85: 2497–2507.
5. WILEY, R. C., M. TAVAKOLI, and M. D. MOORE. 1966. Determination of hexuronic acids in fruit by gas chromatography. *Proc. Amer. Soc. Hort. Sci.* 89:34–37.
6. WILLIAMS, M. W., and G. C. MARTIN. 1967. Quantitative gas chromatography of sugars and related polyhydroxy compounds in horticultural crops. *Hortscience* 2(2):68–69.

Karyotypic Analysis of Some *Allium* Species[1]

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Abstract. Allium galanthum, A. drobovii, A. pskemense, A. roylei, and *A. cepa* type have the same chromosome number (n = 8), as *A. cepa.* The karyotypes of these species have been described. Each species has seven V-shaped chromosomes. These can be grouped as metacentric, median, submedian, and one subterminal. The subterminal chromosome has a satellite attached to its short arm. There are no other conspicuous morphological markers on the chromosomes, such as knobs or heterochromatic blocks. Chromomeres in the several species are of the same size and are present along the entire length of the chromosomes.

Slight differences were found in the morphology of the genomes of these species. The genome in *A. roylei* is the longest—63.44 μ, while that of *A. cepa* type is the shortest—56.18 μ. *Allium cepa* and *A. pskemense* have the same genome length, but there are slight differences in the morphology of the individual chromosomes. *Allium cepa* and *A. cepa* type have a wider and bigger satellite than that present in the other species. The differences among the members of the complement in *A. cepa, A. cepa* type, and *A. galanthum* are less pronounced than in *A. pskemense, A. roylei,* and *A. drobovii.* It seems that these species may have had a common ancestor, and that chromosomal differences have arisen due to inversions, translocations, and pairing in unequal chromosomes.

ALLIUM species provide interesting material for cytological investigations, and a number of species have been studied by Schaffner (12), Taylor (13), Levan (6), (7), (8), Emsweller & Jones (3), Mensinkai (9), Feinbrun (5), Battaglia (1), and Bozzini (2). But *Allium pskemense* B. Fedtsch, *A. galanthum* Kar et Kir, *A. drobovii* Vved, *A. cepa* type and *A. Roylei,* Stearn have had a minimum of study. Since these species carry genes for resistance to pink root, downy mildew, and thrips (11), it was considered desirable to study their chromosomes and to compare them with those of *A. cepa* L.

MATERIALS AND METHODS

Allium pskemense, A. galanthum, A. drobovii, A. roylei, A. cepa type and *A. cepa* as described by Saini and Davis (11) were grown in the experimental plots of the Vegetable Crops Department of the University of California at Davis, during 1962 to 1966.

Flower buds for meiotic studies and studies of pollen mitosis were fixed for 24 hr in a freshly prepared solution of absolute ethyl alcohol, chloroform, and acetic acid (6:4:1). The material was then washed 3 times in 70% ethyl alcohol at intervals of 24 hr, after which it was stored in 70% ethyl alcohol until used. Separated and clearly visible chromosomes in 15 pollen cells at first mitosis were examined. Microphotographs were taken and enlarged and measurements were calculated in microns. To facilitate the karyotypic studies, tips from vigorously growing roots on bulbs sprouted in a beaker of water were fixed for 2 days in a mixture of absolute ethyl alcohol and glacial acetic acid (3:1). After several washings with 70% ethyl alcohol, they were stored in a like solution. These root tips were treated with a macerating solution (1 part of 95% ethyl alcohol to 1 part of concentrated hydrochloric acid) for 3 to 5 min, washed with water, and stained with acetocarmine.

RESULTS

Chromosome number and morphology. The chromosome number of each *Allium* species under investigation was ascertained from the configurations at diplotene, diakinesis, and postmeiotic division in pollen grains. The chromosomes in the first pollen division were often situated in one plane and were shorter, plumper, and more conveniently spaced for study than at other times. Their morphology was studied with ease because only one chromosome of each pair was present. However, smears of actively growing roots were also examined to facilitate karyotypic analysis.

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Table 1. Average total lengths and index numbers of chromosomes of *Allium* species studied.

Chromosome no.	*A. cepa*		*A. cepa* type		*A. galanthum*		*A. drobovii*		*A. pskemense*		*A. roylei*	
	Index no.	Length in μ	Index no.	Length in μ	Index no.	Length in μ	Index no.	Length in μ	Index no.	Length in μ	Index no.	Length in μ
1	.88	9.21	.75	8.22	.96	8.81	.92	9.01	.86	10.19	.78	10.58
2	.72	8.42	1.00	7.84	.91	8.23	.77	9.01	.95	8.03	.92	9.01
3	.91	8.23	.80	7.05	.40	8.23	.77	7.64	.66	7.84	.83	8.62
4	.77	7.64	.50	7.05	.80	7.05	.46	7.44	.36	7.45	.78	8.02
5	.81	7.44	1.00	7.04	.80	7.05	.95	7.24	.46	7.44	.46	7.44
6	.28	7.05	.89	6.65	.60	6.27	.42	6.66	.46	7.44	.84	6.85
7	.75	6.86	.78	6.26	1.00	6.26	.55	6.07	.89	7.05	.89	6.65
8	.74	6.46	.63	6.07	.56	5.87	.82	6.07	.67	5.87	.60	6.27
Total length		61.31		56.18		57.77		59.14		61.31		63.44

The length of a chromosome may vary, depending upon the stage of contraction, physiological factors, and cytological technique. It is difficult to measure chromosomal length at exactly the same stage in different species. Even the length of homologous chromosomes may vary in different nuclei (1). Consequently, chromosomal length cannot be used as the sole criterion for identifying chromosomes of different species. This has also been pointed out by Levan (7) and Emsweller-Jones (3).

Although chromosomes of *Allium* species are a favorable subject for cytological studies, the chromosomes of the species under study were not favorable for pachytene analysis. The chromosomes were entangled with one another, and were very difficult to follow individually. Moreover, there were no distinct physical markers (except the centromere), such as heterochromatin blocks or knobs, to distinguish one chromosome from the other. Even the chromomeres were uniform in size and spaced evenly along the entire length of the chromosomes.

Whatever the stage of contraction, the ratio between the 2 arms of the chromosomes was relatively constant. Therefore, an index number for each chromosome was determined by dividing the length of the short arm by the length of the longer arm. This method was used by Emsweller-Jones (3) in describing the chromosomes of *A. cepa* and *A. fistulosum* L. In the present studies this procedure was reasonably reliable, since the maximum deviation in the index number was 0.07. In each species,

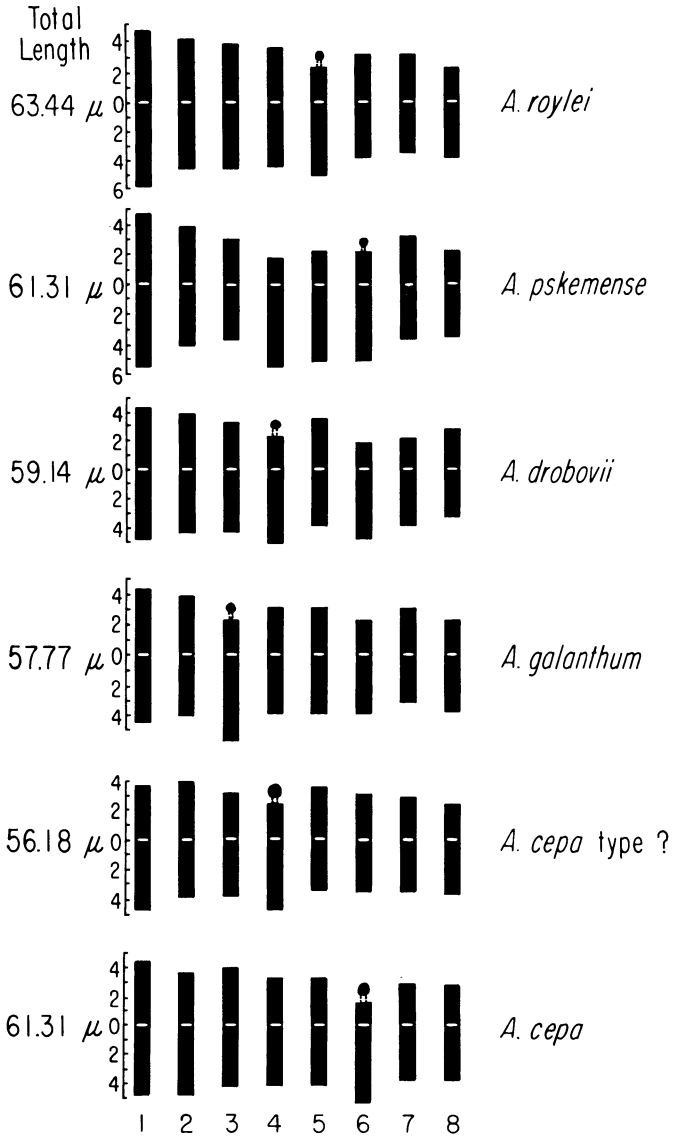

Plate 1. Idiograms of *Allium* species.

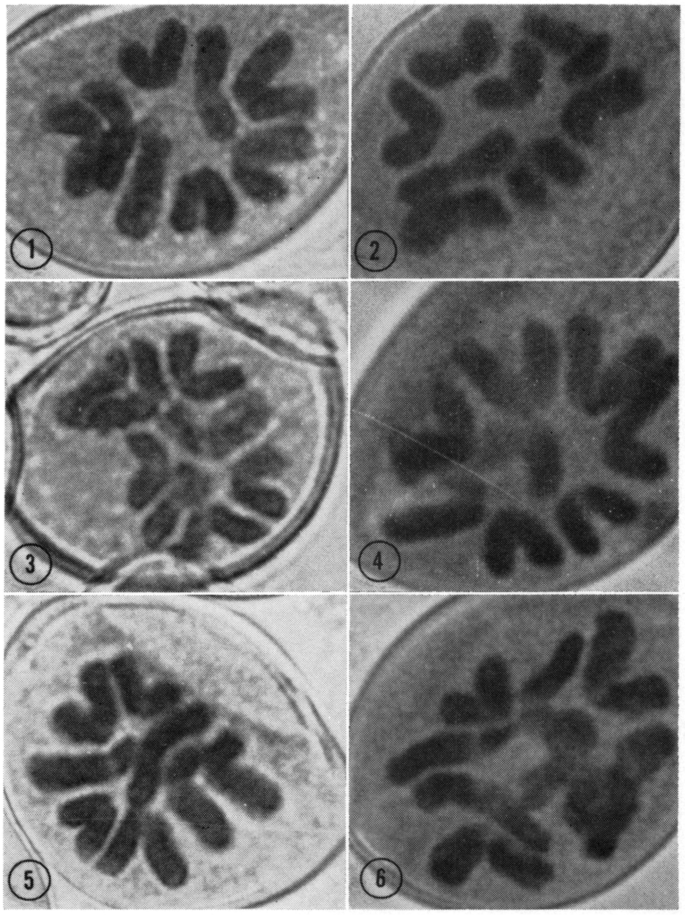

Plate 2. Microspores in I Mitosis × 2400. Fig. 1 *A. cepa,* Fig. 2. *A. cepa* type, Fig. 3. *A. galanthum,* Fig. 4. *A. drobovii,* Fig. 5. *A. pskemense,* Fig. 6. *A. roylei.*

enlarged photomicrographs of 15 cells with well-spread chromosomes in pollen mitosis were measured in microns. Chromosomal length and index number for each chromosome of the complement for the several species are given in Table I.

The chromosomes have been classified on the basis of index number as 1) median, 0.75 to 0.99, 2) submedian, with 0.51 to 0.74, and 3) subterminal, 0.50 or less. If the 2 arms of a chromosome were equal, the index number was one. Such chromosomes have been described as metacentric. On the basis of length, the chromosomes have been designated as 1) long, 8.50 μ and up, 2) medium, 6.50 to 8.49 μ, and 3) small, 6.49 μ or less.

All species studied had the same number of chromosomes, i.e., $n = 8$. There appeared to be good homology among the satellite chromosomes in these species. The satellite was on the distal end of the short arm of the subterminal chromosome. The satellite chromosomes in *A. roylei, A. pskemense* and *A. drobovii* were equal in length (7.44 μ) and had the same index number (0.46). The satellite in *A. galanthum* and *A. cepa* type was also situated on the short arm of the subterminal chromosome having the arm length of 2.35 μ though there were slight differences in the length of the long arm. In *A. cepa* the short arm of the satellite chromosome was the shortest, (0.28 μ), and had the lowest index number (0.28). From a perusal of the data in Table 1 and the karyotype in Plates 1 and 2, it can be inferred that morphological differences among the genomes of the species studied were slight. The differences among the members of the complement in *A. cepa, A. cepa* type, and *A. galanthum* were less pronounced than those among *A. pskemense, A. roylei,* and *A. drobovii.*

The chromosomes of the above species studied are described below. (Only one chromosome of each pair is mentioned.)

A. cepa:

1 long, median; one arm slightly shorter than the other.
4 medium, with median centromere.
1 medium, with submedian centromere.
1 medium, with subterminal centromere. A large satellite, with a long secondary constriction is attached to its shorter arm.
1 short, with submedian centromere. In contrast to the report by Mensinkai (9) no secondary construction was observed in the long chromosome. The karyotype agrees with that described by Battaglia (1).

A. cepa type:

3 medium, with median centromere.
2 medium, with metacentric chromosomes 2 and 5, which can be recognized because of differences in length.
1 medium, with subterminal centromere, with a satellite on its short arm.
1 short, median.
1 short, submedian.

A. galanthum:

1 long, median; one arm slightly shorter than the other.
3 medium, with median centromere; chromosomes 4 and 5 similar and not distinguishable.
1 median, with subterminal centromere, and a satellite on its shorter arm.
1 short, metacentric.
2 short, submedian; chromosomes 6 and 8 not distinguishable.

A. drobovii

2 long, median; in one chromosome the two arms are almost equal, but in the other the difference is greater. They can be recognized.
2 medium, median centromere.
2 medium, with subterminal centromere; and recognizable because of differences in arm length. Chromosome 4 has a satellite, smaller than that of *A. cepa,* attached to its shorter arm.
2 short, one with median and the other with submedian centromere. In some cells a secondary constriction in the long arm of chromosome 6 was observed. Two nucleoli could also be seen in pachytene.

A. pskemense:

1 long, median.
2 medium, with median centromere.
1 medium, with submedian centromere.
3 medium, with subterminal centromere; chromosome 6 has a satellite on its shorter arm. It is difficult to distinguish chromosome 4 from 5.
1 short, with median centromere.

A. roylei:

3 long, median; chromosome 1 and 3 can be distinguished because of longer arm length in chromosome 1 than in 3.
3 medium, median centromere; chromosomes 6 and 7 difficult to distinguish.
1 medium, with subterminal centromere, with a satellite on its shorter arm.
1 short, submedian. In some cells a secondary constriction was observed at the terminal end of the long arm of chromosome 5, where the nucleolus organizer was present.

Discussion

All *Allium* species investigated in this study have the same chromosome number, (n = 8). The chromosome number for the species other than *A. cepa* have not previously been reported. The general morphology of the chromosomes of the genomes of the species investigated fits well with that described by Feinbrun (5) for the Section *Cepa* and the Section *Melanocromyum*. The latter section comprises many desert and steppe species of the Near East and Central Asia, the region where the species studied other than *A. cepa* and *A. roylei* are endemic. The origin of *A. cepa* is also considered to be in the same region.

There are slight differences in morphology and possibly in gene arrangement of the chromosomes of the genomes of these species. The differences in the genomes of *A. cepa* type, *A. galanthum* and *A. cepa* are less pronounced than among *A. pskemense, A. roylei* and *A. drobovii.* Total length of the genome of *A. cepa* is equal to that of *A. pskemense,* but the differences among the individual members of the genome are more marked in *A. pskemense. Allium roylei* has the longest genome and *A. cepa* type the shortest, although the difference is only 7.26 μ.

All species studied have much in common. They possess seven V-shaped chromosomes which can be classed as metacenteric, median or submedian. One member of the genome in each species has a subterminal chromosome with a satellite on the short arm. Chromosomes with satellites appeared to be quite homologous although there are slight differences in their length in *A. galanthum, A. cepa* type, and *A. cepa.*

It appears that these species probably had a common ancestor and chromosomes differ in regard to gene arrangement and mutations. Pairing of chromosomes unequal in length in these species crosses has been observed (10). Such pairings, if between non-homologs, can give rise to chromosomes with different morphology. Emsweller and Jones (4) also reported the presence of morphologically different chromosomes in the mitosis of microspores of the species cross *A. cepa* × *A. fistulosum*. These chromosomes presumably arose from crossing over between the 2 parental genomes. Differences in chromosomes of a genome could arise by inversion, translocation, or crossing over in unequal chromosomes. It appears that inversions and pairings of unequal chromosomes have played an important role in the evolution of the species studied, and that speciation has proceeded on the background of an established chromosome number and slight changes in morphology. Differences in the karyotype of *A. cepa* have been pointed out by Battaglia (1), Bozzini (2), and Emsweller and Jones (3), in studies of various varieties. This further supports the observation that even in single species there are slight differences in the chromosomes, and that their morphology may change while the chromosome number remains the same.

LITERATURE CITED

1. BATTAGLIA, E. 1957. *Allium ascalonicum* L.; *A. fistulosum* L.; *A. cepa* L. Analisi cariotipica. *Caryologia* 10:1–28.
2. BOZZINI, A. 1964. On the karyotype of a viviparous onion, known as *Allium cepa* L. var. viviparum (Metzg) ALEF. *Caryologia* 17:459–464.
3. EMSWELLER, S. L., and H. A. JONES. 1935. Meiosis in *Allium fistulosum, Allium cepa* and their hybrid. *Hilgardia* 9:277–294.
4. ———— and ————. 1938. Crossing over, fragmentation, and formation of the new chromosomes in an *Allium* species hybrid. *Bot. Gaz.* 99:729–772.
5. FEINBRUN, N. 1954. Chromosomes and taxonomic groups in *Allium*. *Internat. Cong. Genet.* IX:1036–1041.
6. LEVAN, A. 1932. Cytological studies in *Alliums* II, Chromosome morphology. *Hereditas* 16:259–294.
7. ————. 1935. Cytological studies in *Allium* VI. The chromosome morphology of some diploid species of *Allium*. *Hereditas* 20:289–330.
8. ————. 1937. Cytological studies in the *Allium* paniculatum group. *Hereditas* 23:317–370.
9. MENSINKAI, S. W. 1939. Cytogenetic studies in the genus *Allium*. *J. Genet.* 39:1–43.
10. SAINI, S. S. 1966. Cytogenetic studies in some *Allium* species. Ph.D. Thesis, University of California, Davis. 107 pp. illust.
11. ————, and G. N. DAVIS. 1967. Compatibility in some *Allium* species. *Proc. Amer. Soc. Hort. Sci.* 91:401–409.
12. SCHAFFNER, J. H. 1898. Karyokinesis in the root tips of *Allium cepa*. *Bot. Gaz.* 26:225–238.
13. TAYLOR, W. R. 1925. The chromosome morphology of *Velthemia, Allium* and *Cyranthus*. *Amer. J. Bot.* 12:104–115.

Differential Tolerance of Several Inbreds of Onion *Allium cepa* L. to Certain Herbicides[1]

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Abstract. Considerable differences exist in the tolerances of onion inbreds to CIPC. Inbreds derived from 'Iowa Yellow Globe' were the most tolerant among the inbreds evaluated. Seedlings grown in the laboratory on agar containing the herbicide responded similarly to treated plants in field plots. The laboratory technique provides a fast, efficient method of screening large numbers of inbreds under controlled conditions.

CHEMICAL weed control is often unreliable in onion production. Preemergence herbicides, such as isopropyl N (3-chlorophenyl) carbamate (CIPC), have shown toxicity to the onion crop and occasionally have caused extensive damage (5, 7, 9). Other investigations have shown no injury from use of CIPC (8, 11, 12).

The objective of most onion-herbicide research is to develop new chemicals that control weeds and show minimum crop toxicity. Another approach would be to select onion lines that have higher tolerance to present herbicides. Genetic differences in response to certain herbicides have been reported in several other crops (3, 10, 14, 16). Differential response by onion varieties to herbicides has been reported. A post-emergence treatment of CIPC did not appear to damage plants of 'Brigham Yellow Globe,' but did reduce yields in 'Sweet Spanish' (1). Differences in varietal resistance may be important with nonselective herbicides (13). In transplanted onions, the variety 'Sweet Spanish' was considerably more tolerant to several herbicides than was 'Early Harvest' (4).

Numerous laboratory techniques have been reported for studying the growth-regulating properties of chemicals, but not for the selection of plant material tolerant to chemicals. The criterion generally used is root elongation (15, 2). The present investigation was designed to determine if tolerance to herbicides exists among onion inbreds and to develop a laboratory technique for evaluating onion inbreds for inherent tolerance.

MATERIALS AND METHODS

The plastic-box technique developed by Fults and Ross (6) for screening grass herbicides was modified for use with onion seedlings in the laboratory investigations (Fig. 1). Onion seedlings were grown on a nonnutrient agar in the presence of the herbicide being tested. Fifteen unselected seedlings, pre-germinated in petri dishes, were transferred to plastic boxes when the radicle was 2 to 3 mm long. Strips of filter paper impregnated with specific concentrations of the chemical solutions were placed on the surface of the agar, 44 mm from the young radicles. The chemical diffused through the agar toward the seedlings as they grew toward the treated area.

The boxes were tilted to 70° from the horizontal so that the roots would remain on the surface of the agar and grow toward the impregnated strip. The seedlings would not remain in place at a greater angle. Onion-skin

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