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PRODUCTION OF A COLCHICINE-INDUCED TETRAPLOID ASPARAGUS

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INTRODUCTION

As part of a breeding programme in execution at the Institute of Horticultural Plant Breeding at Wageningen, the writers obtained a colchicine-induced C_1-generation of tetraploid asparagus – *Asparagus officinalis* L. A number of these plants has been set out in the field, but at this moment it would be premature to give data about the horticultural value of the newly developed strain. However, in the process of obtaining the tetraploids some experiences were gained which seemed worth while communicating. They concern the response of young seedlings to treatment with a colchicine solution and the major differences observed between diploid and tetraploid asparagus. They also include fertility problems and sex heredity in the tetraploids. *Asparagus officinalis* L. has 2 n = 20 chromosomes. The somatic cells of tetraploid plants, therefore, contain 40 chromosomes.

References to colchicine treatment of asparagus are very scarce. CATH. L. BECKER (2), using a 1 per cent solution treated young seedlings and obtained plants with thicker shoots. HUNTER and DANIELSSON (5) treated seeds of the cultivar[1] Eden and found tetraploid C_0 plants, from which they harvested seeds and could thus start a population. JANAKI AMMAL treated seeds for 3 days, with a 1 per cent solution and

[1]) Cultivar. This term (abbreviated cv) is used for lines, clones and other groups of plants under cultivation. It is equivalent to the English "cultivated variety", the French "variété", the German "Sorte" and the Dutch "ras".

obtained several tetraploid plants as reported by WELLENSIEK (9). All these references consist of short notes without particulars about the technique employed, numbers of seeds treated or tetraploids obtained.

MATERIAL AND METHODS

Experiments were started in the autumn of 1950. We have worked with only one cultivar viz. Glory of Brunswick, seeds of which were harvested from plants growing in the garden of our Institute at Wageningen.

The growth habit of asparagus is very peculiar. The primary shoot is an unimportant part of the plant. It is a very tender shoot about 10 cm high. Before emerging from the seed it forms a lateral shoot primordium, visible as a small knob at its base. From this primordium a shoot grows in such a way that its apex does not emerge from the soil before it has formed a new lateral underground. This process is repeated, so that new shoots grow as laterals from the older ones. The subterranean parts of all these shoots form the rhizome.

Already in the earliest phase of germination numerous cell divisions were observed in the lateral primordia. Hence the treatment of lateral primordia on primary shoots looked the most promising method of obtaining tetraploid rhizomes. Two different methods were employed for administering the solution, viz. soaking and infiltration.

a. *The soaking method* (Experiment I)

The seeds were sown in petri-dishes on filter paper, adequately moistened with colchicine solution and stored for some days in an incubator at 30 °C. Then the seeds were sown in seed pans, which were placed in a greenhouse kept at a mean temperature of 20 °C.

b. *The infiltration method* (Experiments II and III)

This method was developed at Weibullsholm in Sweden and described by MRKOS (7). After the seeds had germinated for some days on moist filter paper in petri dishes in an incubator at 30 °C, they were put in small glass cups and immersed in a colchicine solution. The cups were transferred to an exsiccator from which the air was evacuated by means of a water vacuum pump. After about 10 minutes pumping the solutions started to boil. At that moment the exsiccator outlet was closed, the pump stopped and the vacuum was maintained for 10 minutes. Then the air was gradually admitted till the air pressure in- and outside the exsiccator was balanced again. The colchicine solution was thus forced into the tissues from which the air had been evacuated. Ten minutes later the remaining fluid was decanted, the seeds were washed with water and afterwards sown.

In judging the results we followed the same procedure for both methods employed. Seedlings which showed delayed germination were labelled; a number of them had a conspicuous dark green colour, and their "needles" were longer than those of the normal plants.

Due to lack of space in the greenhouse we were obliged to select the plants at a rather young age. All the plants which did not differ sufficiently from the controls were

discarded. The remaining plants were pricked off into small pots in the greenhouse and after some time when they had 2 or 3 shoots, were submitted to a cytological inspection.

The percentage of tetraploid cells in the slides prepared from shoot-apices was estimated. Those plants which had at least 80 per cent tetraploid cells were classified as tetraploids, the other plants were regarded as diploids. From the plants of the C_0-generation we had to take shoot apices; but when testing the C_1-generation root tips were taken if possible. Acetic-orcein squashes were used to make the chromosomes visible. For this purpose the second writer developed an improved serial squash-method already described earlier in this journal (10). With this method one can make more and clearer preparations within a given time than with the schedule given by DARLINGTON and LA COUR (3) or MRKOS (7).

All statistical tests of significance have been worked out on binomial probability paper: FERGUSON (4).

TABLE 1. EXPERIMENT I. SEEDS GERMINATED FOR 1, 2, 3 AND 4 DAYS IN COLCHICINE SOLUTIONS OF 0.2, 0.5, 1.0 AND 1.5 PER CENT

Germination period	Colchicine concentration	Number of seeds treated	Number of diploid plants after treatment	Number of tetraploid plants after treatment	Number of dead plants after treatment
a	b	c	d	e	f
1 day	0.2%	36	28	0	8
	0.5%	36	26	0	10
	1.0%	36	24	0	12
	1.5%	36	19	0	17
2 days	0.2%	36	28	0	8
	0.5%	36	28	0	8
	1.0%	36	18	2	16
	1.5%	36	7	1	28
3 days	0.2%	36	25	0	11
	0.5%	37	23	0	14
	1.0%	37	10	0	27
	1.5%	36	12	1	23
4 days	0.2%	36	25	2	9
	0.5%	36	29	0	7
	1.0%	37	15	0	22
	1.5%	36	20	0	16
4 days	control	36	32	0	4

RESULTS OBTAINED IN THE C_0-GENERATION

Experiment I

In this experiment seeds were treated using the soaking method. Batches of about 36 seeds were soaked in 0.2, 0.5, 1.0 and 1.5 per cent colchicine solutions. The seeds placed in an incubator on Dec. 11th 1950 were stored at 30 °C for 1, 2, 3 and 4 days.

One portion sown on filter paper moistened with tap water and stored for 4 days together with the other seeds, served as a control. Thus, 17 batches were sown in seedpans and stood in the greenhouse.

Table 1 shows the results of this experiment. The number of tetraploids obtained is very small. After one day's treatment there were no tetraploids at all. After 2 or 3 days a few were obtained with the higher colchicine concentrations and after 4 days with the lowest concentration.

Experiment II

This experiment, using the infiltration method, was started on December 12th, 1950. Seeds were germinated in petri dishes on filter paper moistened with tap water and stored in an incubator for 4, 6 or 8 days. Then the seeds were infiltrated with 0.2, 0.5, 0.7 or 1 per cent colchicine solution under vacuum. Simultaneously, control batches which had germinated for 4, 6 or 8 days too, were infiltrated with water. After treatment the seeds were sown in seedpans in the greenhouse.

The results of this experiment are shown in table 2.

A comparison with table 1 shows that in experiment II the largest number of tetraploid plants obtained was three times as large as in I (6 out of 37 plants treated compared with 2 out of 36 plants) and also, that the results obtained are more consistent. We may state that in this experiment a 6 days' germination previous to colchicine treatment was significantly better than 4 days, while 8 days resulted in a very high percentage of dead plants.

The tetraploid plants were kept for breeding purposes. One hexaploid was discovered among the plants tested. Probably it originated from a triploid embryo. Such embryos are not uncommon in asparagus. We encountered several in our experiments. A haploid plant was also obtained. It proved to be female, as was expected. It was a very weak plant with short "needles" of a pale green colour. It never produced any seeds.

Experiment III

On February 28th 1951 we started a third experiment. Seeds were germinated for 5, 6 and 7 days and then infiltrated with 0.4, 0.8, 1.2 and 1.6 per cent colchicine solutions. Simultaneously control groups were infiltrated with water. This experiment was carried out in duplicate. There were two groups of 36 seeds in each treatment. So we had 30 seed batches including the controls.

Table 3 gives the results of Experiment III. It shows that the yield of tetraploid plants is higher than in experiments I and II. Unexpectedly high numbers were obtained after 5 days germination. With 1.6 % colchicine 42 out of 72 seedlings treated proved to be tetraploid.

Two plants contained cells with aberrant chromosome numbers, viz. 44 and 50. All tetraploid plants were kept for breeding purposes.

REACTION OF THE PLANTS TO THE DIFFERENT TREATMENTS

Both experiments I and II were started in December 1950. They were comparable with respect to the age of the seeds and the environmental conditions during the growth of the seedlings in the greenhouse. The better results obtained in the second

TABLE 2. EXPERIMENT II. SEEDS INFILTRATED WITH 0.2, 0.5, 0.7 AND 1.0 PER CENT COLCHICINE SOLUTIONS AFTER HAVING BEEN GERMINATED FOR 4, 6 AND 8 DAYS

Pre-germination	Colchicine concentration	Number of seeds treated	Number of diploid plants after treatment	Number of tetraploid plants after treatment	Number of dead plants after treatment	Total tetraploid + dead plants
a	b	c	d	e	f	g (e + f)
4 days	control	36	32	–	4	4
	0.2%	36	26	0	10	10
	0.5%	36	26	0	10	10
	0.7%	36	27	2	7	9
	1.0%	37	22	3	12	15
6 days	control	36	29	–	7	7
	0.2%	36	17	2	17	19
	0.5%	36	15	4	17	21
	0.7%	37	11	2	24	26
	1.0%	37	18	6	13	19
8 days	control	36	36	–	0	0
	0.2%	36	4	0	32	32
	0.5%	36	1	1	34	35
	0.7%	38	1	2	35	37
	1.0%	37	4	0	33	33

TABLE 3. EXPERIMENT III. SEEDS INFILTRATED WITH 0.4, 0.8, 1.2 AND 1.6% COLCHINE SOLUTIONS AFTER HAVING BEEN GERMINATED FOR 5, 6 AND 7 DAYS

Germination period	Colchicine concentration	Number of seeds treated	Number of diploid plants	Number of tetraploid plants	Number of dead plants	Total tetraploid + dead
a	b	c	d	e	f	g (e + f)
5 days	control	72	69	–	3	–
	0.4%	70	60	10	0	10
	0.8%	72	38	33	1	34
	1.2%	72	38	31	3	34
	1.6%	72	25	42	4	46
6 days	control	72	62	–	10	–
	0.4%	72	47	10	15	25
	0.8%	67	17	26	24	50
	1.2%	71	5	15	51	66
	1.6%	71	11	18	42	60
7 days	control	72	71	–	1	–
	0.4%	70	29	18	23	41
	0.8%	69	31	18	20	38
	1.2%	70	15	19	36	55
	1.6%	70	7	17	46	63

experiment must therefore be ascribed to the difference in colchicine treatment. In experiment I dry seeds were soaked with colchicine. It is feasible that such an early uptake of colchicine inhibits the germination process to a certain degree before the colchicine can exert its specific action. In experiment II the conditions for the action of colchicine were more favourable. Cell divisions were in progress when the colchicine was administered, hence c-mitoses might be expected immediately afterwards.

In experiment I the yield of tetraploid plants was small, and no significant differences between the various treatments could be established. There was an effect, however, on the number of dead plants. This increased significantly with the increase in concentration of the colchicine solution, but it was not affected by the length of the germination period.

From the results of this first experiment we could not say definitely what was the most favourable concentration of colchicine or the best soaking period. However it proved possible to obtain tetraploid plants by germinating seeds in a colchicine solution for two or more days.

Our second experiment gave better results than the first. The total number of tetraploid plants obtained was 22 out of the 438 seeds treated, against 6 out of 579 seeds treated in the first. We observed only small differences due to the various concentrations used and these were not significant. But a significant difference proved to exist between the number of tetraploids obtained after 6 days germination and that obtained after 4 days and 8 days. Six days gave twice as many tetraploids as 4 and 8 days together.

With these results in mind experiment III was started in an attempt to define the optimal germination period and optimal colchicine concentration more accurately.

The outcome of this experiment (table 3) showed that a germination period of 5 days gave a significantly higher yield of tetraploids than 6 or 7 days (column e). The last 2 did not differ from each other in this respect. After 5 days germination almost all reacting plants became tetraploid, in other words the number of dead plants caused by colchicine treatment was so small that it compensated the larger number of reacting plants produced after a germination period of 6 or 7 days.

The question of the most favourable concentration could not be answered so easily. The shortest germination period (5 days) showed better results at higher concentrations, probably with an optimum at the highest concentration used, i.e. 42 tetraploids out of 72 seeds treated with 1.6 % colchicine. After 6 days germination the highest yield was at the much lower concentration of 0.8 % and after 7 days no difference between the different concentrations could be observed. It seems that a relation exists between the optimal colchicine concentration and the germination period, and that this optimum shifts to the lower concentrations as the age of the treated seedlings increases.

The explanation of this may be very simple. At a very young age the tissue of the seedlings is very dense, but gradually becomes looser. The intercellular spaces in particular become larger; hence with our infiltration method a relative larger quantity of colchicine solutions is given as the seedlings grow older. Thus the older stages receive more colchicine per seedling than the younger ones and therefore the former will more easily receive a lethal dose than the latter.

The results of experiment III suggest that the optimal number of days for germina-

tion is about five, the optimal concentration depends on the age of the seedlings treated. The data from experiment II partly support such a supposition and in no way contradict it.

It remains to be seen, however, whether experiments II and III are sufficiently comparable. At a glance it can be seen that in experiment III the yield of tetraploids is far better than in II (column e of tables 2 and 3). But there is also a feature which the two have in common. It is the total action of the colchicine. If a plant reacts to colchicine it can do this in two ways, i.e. it can become tetraploid, or it can die. The first action is specific, the second non-specific. The combined numbers of plants reacting in a specific and a non-specific way give the total of reacting plants. If the total number of reacting plants for each of the germination periods of both experiments is expressed as a percentage of the number of treated seeds and if for this calculation only the figures are used from a range of concentrations for which the experiments are comparable, i.e. 0.5 %, 0.7 % and 1.0 % in experiment II and 0.4 %, 0.8 % and 1.2 % in experiment III, the figures arrange themselves in the following natural sequence: 4 days – **31** % (exp. II), 5 days – **36** % (exp. III), 6 days – **60** % (exp. II) and **67** % (exp. III), 7 days – **64** % (exp. III), 8 days – **95** % (exp. II).

It is not easily explained, how an apparently equivalent treatment can give results, which show an agreement for the general colchicine action and a deviation for the specific action as we found in our experiments. Light conditions will have had an effect. These were less favourable for the plants of experiment II sown in December than for those of III sown in March. But also differences in conditions during infiltration e.g. fluctuation of room temperature, of air pressure may have played a part.

It has still to be investigated whether seedlings younger than 5 days cannot give higher percentages of tetraploids when stronger colchicine concentrations are given.

In summarizing the results we state that:

1. The infiltration method is preferable to the soaking method. It enables treatment of the seeds with a sufficient quantity of colchicine within a very short time, and administration of colchicine at the most favourable stage of development.
2. To get the best effect from colchicine treatment, it is imperative to look for a stage of development in which the lethal influence of the colchicine is as low as possible. At such a stage strong concentrations can be administered without harm.
3. The sensitivity of asparagus seedlings to colchcine treatment increases with age. In our experiments 8 days old seedlings nearly all died after the application of very weak solutions, but 5 days old seedlings could withstand solutions as strong as 1.6 %.
4. It is possible to obtain more than 50 per cent tetraploid plants after colchicine treatment of asparagus seedlings by using the infiltration method.

RAISING THE C_1-GENERATION

The tetraploid plants obtained in the 3 experiments were grown in the greenhouse until the winter of 1951. Several plants produced flowers in that period.

The first plants to flower were males. About a month later some female plants also produced flowers. As many female flowers as possible were pollinated by hand. This work was limited by the number of male flowers present at the moment of pollination.

TABLE 4. RESULT OF CYTOLOGICAL TEST OF C_1-PLANTS RAISED FROM SEED PRODUCED BY C_0-PLANTS IN 1951

Serial nr. of mother-plants	Number of seeds produced	Number of seedlings tested	Number of tetraploid seedlings
658	7	6	6
663	3	2	2
711	2	2	2
757	6	6	6
784	2	1	1
877	3	3	3
907	31	25	25
917	8	8	8
?	5	5	5

TABLE 5. RESULT OF CYTOLOGICAL TEST OF C_1-PLANTS RAISED FROM SEED PRODUCED BY C_0-PLANTS IN 1952

Serial nr. of mother plants	Number of seeds produced	Number of seedlings tested	Distribution of the plants over different cytological categories				
			No information	tetraploid	tetra/diploid	triploid	diploid
513	61	53	3	48[1]	–	1	1
662	296	44	1	42	–	1[2]	–
658[4]	520	50	1	–	–	–	49
659	60	39	26	–	–	–	13
682	11	8	–	5	–	2[3]	1
684	170	44	1	43	–	–	–
701	270	46	10	36	–	–	–
712	16	16	1	15	–	–	–
722	520	43	–	–	1	2	40
736	37	37	–	37	–	–	–
749	217	37	3	33	–	1	–
752	8	8	–	8	–	–	–
754	20	17	–	17	–	–	–
782	10	10	–	10	–	–	–
784[4]	?	40	2	37	–	1	–
1056	7	6	–	6	–	–	–
1070	38	35	–	35	–	–	–
1073	54	45	6	38	–	1	–
1102	35	33	3	30	–	–	–
1167	17	16	2	14	–	–	–
1172	32	30	2	28	–	–	–
1196	52	51	1	50	–	–	–
1205	7	7	–	7	–	–	–
1207	13	9	3	6	–	–	–
1226	42	40	3	37	–	–	–
1237	43	42	5	35	–	2	–
1253	17	17	1	16	–	–	–
1258	52	31	–	30	–	1	–
1266	37	37	6	31[1]	–	–	–
?	314	46	1	1	–	–	44

[1]) One plant with a few octoploid cells. [2]) Hypertriploid plant with 32 chromosomes.
[3]) One hypertriploid plant with 34 chromosomes. [4]) Already mentioned in table 4.

The seeds were harvested before the plants were set outside. They were sown in 1952. When the seedlings were about 3 months old roottips were fixed and chromosomes counted. The results of this test are given in table 4. All seedlings proved to be tetraploids.

At the end of the 1951 season the C_0-plants were transferred to the field where they passed through the winter. In the spring of 1952 after appearance of the first shoot, all plants with blue and loose shoot tops and with low lateral branches were discarded. The selected plants were isolated in one of the insectproof isolation-rooms, available at our Institute. Here they flowered without danger of pollination by agents other than the bees which had been put into the room (KRAAI, 6).

Seeds were harvested from 30 plants and another C_1-generation was raised. In the spring of 1953 small pots filled with a suitable soil were sunk in a greenhouse bed at about 20° and in each of the pots one seed was sown. During the summer the plants were examined cytologically.

The results of the tests are given in table 5. Of the 30 progenies tested 26 proved to consist of tetraploid plants. Some triploids and in two cases one diploid were encountered in a tetraploid progeny. Two years after the initial test it was found that 26 out of 30 plants were still tetraploid. Four C_0-plants, however, which proved to have at least 80 percent of tetraploid cells in 1951 produced a progeny consisting of only diploid plants in 1952. Hence these C_0-plants had reverted from tetraploid to diploid. In 1951 one of these four plants gave a progeny of 6 tetraploid plants from 6 seeds (see table 4, plant 658) but in 1952 it produced only diploid plants (see table 5). The few triploids found in the tetraploid groups probably originated from pollinations with pollen of diploid male flowers. In the diploid groups the reverse must have happened. In two cases we got tetraploid and some diploid seeds from the same plant.

Two tetraploid C_1-plants with some octoploid cells in the root tip preparations have been observed. This phenomenon, i.e. somatic doubling of chromosomes in root tips, is met with in various plant species. On the other hand we observed in the root tips of one plant, belonging to the progeny of no. 722, tetraploid cells mixed with diploid ones in such a ratio that we had to conclude that the plant in question was a mixoploid. The seedling must have developed apomictically from a nucellar embryo. Hence the C_0-plant which bore this seed must have been a mixoploid too. Except for this one no other mixoploid plants were encountered in the C_1-generations.

THE EFFECT OF TETRAPLOIDY ON SOME VEGETATIVE CHARACTERS AND ON SEGREGATION OF SEX

Morphological differences between tetraploid and diploid asparagus allowed us to select tetraploid plants in the C_0-generation soon after the plants had emerged. The tetraploids were distinguished by dark green and long phyllocladia (needles). The average length of the "needles" of 5 randomly chosen plants in the diploid was 18.9 and in the tetraploids 28.3 mm. Fig. 1 depicts 2 diploid and 2 tetraploid shoots. These shoots were lateral branches picked from plants grown in the greenhouse. The flowers of the tetraploid plants were distinctly larger than those of the diploid (fig. 2). This difference was most pronounced in the diameter of the cross section. This meant that the perianth leaves of the diploid plants are narrower than those of the tetraploid. The

shoots of the tetraploids are mostly thicker, which is shown in Figs. 3 and 4. In these two figures the characters of the shoot tips are also shown. The surface of the diploid is smooth and the tips taper towards the apex, the tetraploids are cylindrical, and the surface is provided with longitudinal ribs and furrows. The diameter of the shoots cannot be used in selecting for tetraploidy in young plants, as it increases with age and varies with environment. At the request of our colleague Mr. J. A. HUYSKES we made chromosome counts of wild asparagus, collected in the dunes near Scheveningen. This work revealed that the variety *Asparagus officinalis prostratus* is a tetraploid. This variety is described by ASCHERSON and GRAEBNER (1) as follows: "Plant small, shoots mostly thin and slack, often prostrate; phyllocladia generally thick and short". We should like to add: "flowers large".

This tetraploid variety differs from our colchicine-induced tetraploid asparagus in that its needles are short instead of long and that the shoots are prostrate and thin. But the leaf colour and flowers of the wild tetraploids do not differ from those of the induced ones.

Another wild form, supposed to have escaped from culture proved to be diploid.

We have observed in our numerous preparations that the tetraploids have larger nuclei in the meristimatic tissues of shoot and root than the diploids. In these tissues the cells are also larger. The larger diameter of the pollen of the tetraploid asparagus is also a character of tetraploidy. Tetraploid pollen is 30.6 to 34.0 μ in diameter, diploid pollen 23.8 to 27 μ. When comparing the quantities of seed harvested from tetraploids and diploids, which are shown in table 5 we see that 4 diploids bore 1414 seeds averaging 393 seeds per plant, the highest number was 520, the lowest 60; 25 tetraploids bore 1,453 i.e. an average of 58 seeds per plant, the highest number per plant harvested was 296, the lowest 7. This shows that fertility has decreased considerably. We have not investigated whether the number of seeds per flower or the number of flowers on a single plant are responsible for this decrease. Probably both phenomena are involved. The meiosis of the P.M.C. in the tetraploid asparagus is typical of autotetraploids. In metaphase I uni-, tri- and quadrivalents occur regularly. In anaphase I, laggards are usually seen as well as in anaphase II (Figs. 5 and 6). So it is not surprising that the tetrads mostly contain more than four nuclei. Figs. 7 and 8 give a picture of a diploid and a tetraploid mitosis. Attention should be payed to the SAT chromosomes; one visible at the half past six position in Fig. 7 and one above and slighly to the right of it. This chromosome has such a wide secondary constriction, that it may easily be counted as two. In Fig. 8 this chromosome lies at the one o'clock position.

In diploid asparagus the ratio of sex distribution is normally 1:1. In tetraploid asparagus the distribution may deviate from this ratio. We counted in a C_1-generation of 47 plants bred from C_0-plants in 1952, 36 male, 10 female and 1 andromonoecious, a ratio very unusual for asparagus. In his breeding work SNEEP (8, 9) could distinguish two types of male plants, one gave an offspring consisting of only males and the other male and female offspring in a 1:1 ratio. He indicated the genetic constitution of his plants by letters: *MM* for male plants of the first type, *Mm* for male plants of the second type and *mm* for female plants. According to the same system the tetraploid males of the C_0-generation have to be indicated by the symbols *MMmm* and the females *mmmm*. Hence the plants of the C_1-generation may be regarded as far

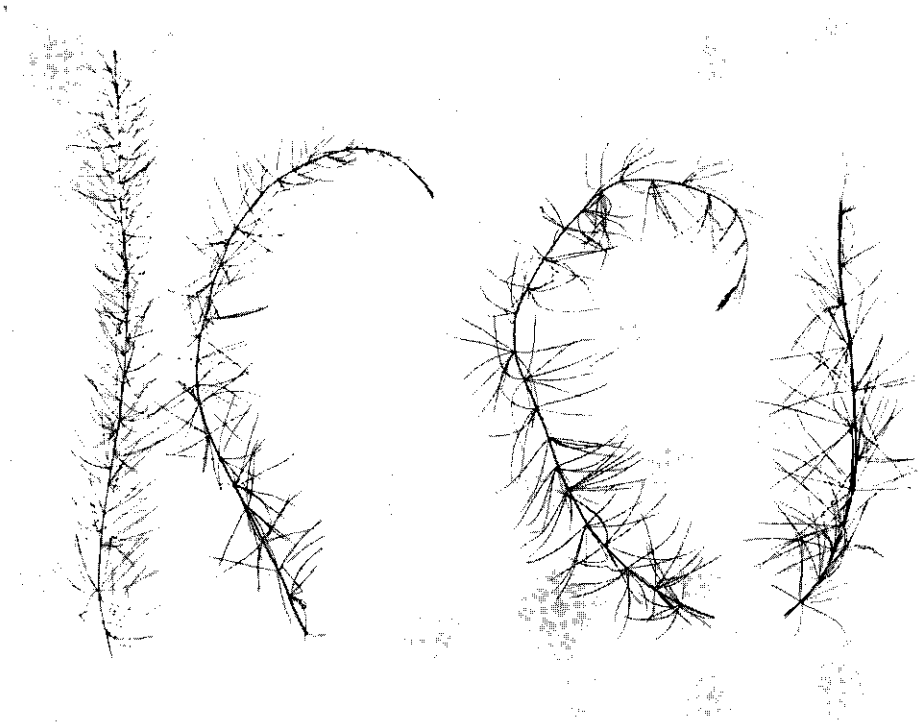

FIG. 1. YOUNG ASPARAGUS SHOOTS; LEFT DIPLOID, RIGHT TETRAPLOID

FIG. 2. ASPARAGUS FLOWERS, TOP AND SIDE VIEW; LEFT DIPLOID, RIGHT TETRAPLOID

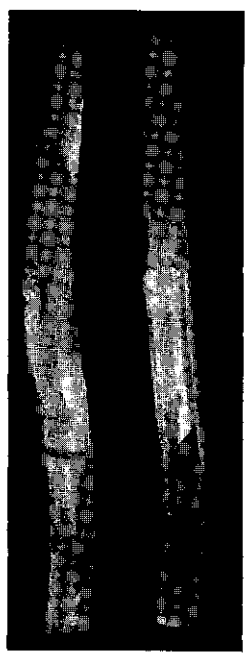

Fig. 3

FIG. 3. DIPLOID ASPARAGUS TIPS

Fig. 4

FIG. 4. TETRAPLOID ASPARAGUS TIPS

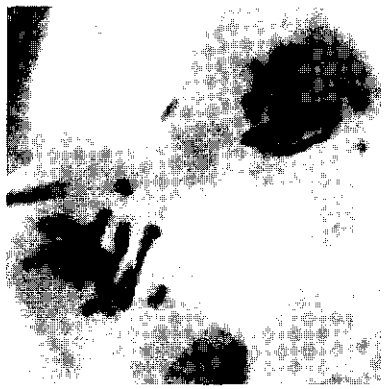

FIG. 5

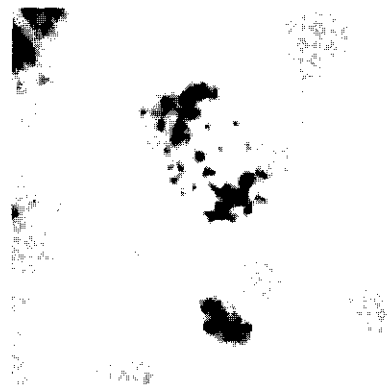

FIG. 6

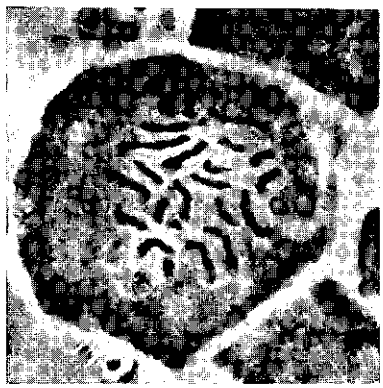

FIG. 7

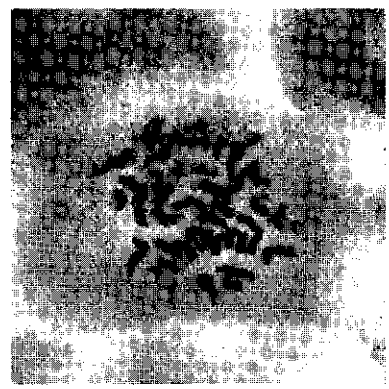

FIG. 8

FIG. 5. TETRAPLOID ASPARAGUS. MEIOSIS, MULTIVALENTS IN METAPHASE I

FIG. 6. TETRAPLOID ASPARAGUS. MEIOSIS. LAGGARDS IN ANAPHASE I

FIG. 7. DIPLOID ASPARAGUS. MITOSIS IN ROOT-TIP. TWO SAT-CHROMOSOMES: ONE IN THE SIX O'CLOCK POSITION AND ONE ABOVE

FIG. 8. TETRAPLOID ASPARAGUS. MITOSIS IN ROOT-TIP; NOTE THE SAT-CHROMOSOME IN THE ONE O'CLOCK POSITION

as sex is concerned as the offspring of a cross between a duplex (*MMmm*) and a nulliplex (*mmmm*) plant. Such a cross gives 1 homozygous recessive to 5 heterozygous dominants or in symbols 1 *mmmm* plant (nulliplex, female), 4 *Mmmm* plants (simplex, male) and 1 *MMmm* plant (duplex, male). Our segregation numbers were 37 males to 10 females, which do not differ significantly from the 5:1 ratio; 39.17:7.83 (D/m > 1) being the expected ratio. We were able to determine the sex of 105 plants of the C_1-generation raised in 1953; 84 of them were males, 17 females and 4 were andromonoecious plants. Here the numbers found were 88 to 17; the expected ratio was 87.5 to 17.5. We regret that we were unable to collect more data to enable us to descriminate between a chromosome- and a chromatide-segregation, and contribute to the question whether the sex determination in asparagus is localized in the autosomes or not. Now we can only state that our numbers agree with the chromosome segregation-ratio 5:1. Our C_0-generations contain only male plants of the duplex form (*MMmm*) but in the C_1 most male plants are simplex ones (*Mmmm*). The simplex/duplex ratio in the C_1-generation should be 4:1. Simplex plants give an almost normal sex segregation viz. 1:1. Hence without selection duplex plants will soon disappear from the next generations. From a commercial point of view male plants are more valuable (SNEEP, 8, 9). In order to get more males than females a breeder should therefore preserve the male plants of the C_0-generation or take care to select for duplex male plants, which can be done by means of test crosses.

SUMMARY

Some experiments on colchicine treatment of germinating asparagus seeds were undertaken with the object to obtain tetraploid plants for breeding purposes. Treating the seeds under vacuum gave excellent results. The length of the germination period previous to treatment proved to be important. Five days' germination at 30 °C gave the best results. At this age the seedlings could survive a 1.6 per cent solution and 60 per cent of tetraploids were obtained. On the other hand after 8 days germination most seedlings die after having been treated with a colchicine solution of only 0.2 per cent and only very few tetraploids were produced.

From 30 plants of the C_0-generation which on cytological examination had been classified as tetraploid, 26 gave tetraploid seeds and only four produced diploid offspring.

Data concerning the sex segregation in the C_1-generation were collected and a ratio of 5 males to 1 female was found. As from the grower's point of view male plants are preferred and only one out of 5 males from the C_1-generation is expected to give a 5 to 1 segregation in the next generation, selection of these individuals is advised. Otherwise a 1 to 1 segregating population will be the result.

SAMENVATTING

Het verkrijgen van tetraploide asperge door colchicine-behandeling

Met het doel tetraploide aspergeplanten te kweken zijn enkele proeven genomen met colchicine-behandeling van kiemende aspergezaden.

Zeer goede resultaten zijn verkregen met colchicine behandeling onder vacuum.

Hierbij is gebleken, dat de leeftijd van het kiemende zaad niet onverschillig is. Vijf dagen voorkiemen bij 30 °C bleek voor het doel de beste leeftijd. Op deze ouderdom verdroegen de plantjes zelfs 1.6 % colchicine zeer goed. Het aantal gewonnen tetraploiden was hierbij zeer hoog nl. ± 60 %. Na 8 dagen voorkiemen daarentegen stierf bij een behandeling van 0.2 % colchicine reeds het merendeel der planten. Het aantal tetraploiden was dientengevolge maar zeer gering.

De tetraploiden uit de C_0-generatie gaven voor het merendeel tetraploide nakomelingen, maar 4 van 30 planten bleken een diploide nakomelingschap te hebben. Eén van deze vier gaf het jaar te voren nog tetraploide planten.

De morfologische kenmerken van de tetraploiden zijn beschreven en vergeleken met de diploide vorm en met die van de in het wild voorkomende tetraploide *A. off. prostratus*.

Daar mannelijke aspergeplanten betere producenten zijn dan de vrouwelijke, zijn cijfers aangaande de geslachtsverdeling in de tetraploide generaties verzameld. In de 1e generatie, dus in de nakomelingschap van de behandelde planten, komen de geslachten voor in de verhouding 5 mannelijke op 1 vrouwelijk exemplaar. Maar deze verhouding heeft zonder doelbewuste selectie de neiging in de volgende geslachten terug te lopen naar 1 : 1. Hoe dit teruglopen kan worden vermeden wordt aangegeven.

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