CYTOMORPHOLOGICAL STUDIES IN AUTOTETRAPLOID *ASPARAGUS OFFICINALIS* L.

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Abstract

A comparative study of morphological and cytological characters of a diploid and artificially induced tetraploids *Asparagus officinalis* has been made. Vegetatively tetraploids are more vigorous than the diploids but cytologically they exhibit an unstable nature. A breakdown of tetraploidy has been noticed which was due to large number of irregularities in meiosis like quadrivalents, trivalents and univalents at metaphase and laggards and bridges at anaphase. In few cases multipolar spindle and nuclear breakdown was seen. Several PMC's showed reduced chromosome numbers probably due to chromosome elimination during the premeiotic division.

Introduction

Asparagus officinalis, a native of Europe, has been in cultivation for over 2000 years. Being a favourite spring vegetable of the Greeks and the Romans—it has wandered to all parts of the world and during this period several cultigens have arisen. An examination of a dozen commercial varieties has shown that cultivated asparagus has remained a diploid in all its long history. This is rather surprising when one considers that at least three tetraploids and two hexaploid species occur in nature (Janaki Ammal, unpublished). Tetraploidy was induced in two commercial varieties of asparagus Connovers colossal, Martha Washington of California (a rust resistant variety) as early as in 1952, at the Royal Horticulture Society's Garden, England, by the senior author. Triploids were obtained by controlled crossing of diploid males and tetraploid females and *vice-versa* and these are under trial in England and France.

These experiments were repeated in India in 1964, using the locally cultivated variety.

MATERIALS AND METHODS

Seeds of diploid local *Asparagus officinalis* ($2n = 20$) were freshly treated with 0·2% solution of colchicine for 24 hours at room temperature. They were germinated in sand boxes and the seedlings were transplanted in the seed bed. Two-year old seedlings were subjected to a chromosome count in order to separate tetraploids from diploids. The seedlings were either females, males or hermaphrodites (truly sub-andraceous and sub-gynaceous).

For cytological studies the buds of appropriate size were fixed in 1:3 acetic alcohol for 48 hours. The PMC squash was made in a drop of aceto-carmine to which traces of iron acetate were added. Morphological data were collected from the tetraploid plants and the diploids growing side by side in the field.

RESULTS

Some of the important morphological characters were studied in both the diploid and tetraploids. The data are presented in Table I. The cladodes of the tetraploids are larger, thicker and deep green in colour as compared with the diploid. On the whole, tetraploids are vegetatively more vigorous than the diploids.

TABLE I

Morphological characters of Diploid and Tetraploid Asparagus officinalis

	Diploid	*Tetraploid*
Habit	Moderate shurb	same
Ht. of plant in feet	3–5	4·5–6·5
No. of tillers ..	4–10	10–20
Length of the leaf	10–15 mm.	20–25 mm.
No. of leaves at a node	4–5	4–5
Colour of the leaves	Light green	deep green
Length of the flower	15 mm.	18 mm.
Pollen size ..	7·4 μ	9·3 μ
Seed setting ..	normal	moderate
100 seed weight (fresh)	15·0 gm.	20·4 gm.
Chromosome No.	20	40

Meiosis

Meiosis in diploids is regular, chromosome pairing is perfect and always 10 bivalents are formed. These bivalents may be either ring of rod-shaped. Ring-shaped are generally predominating, varying between 7–10 per nucleus. As against this meiosis in tetraploids is quite irregular Metaphase is conspicuous by the presence of quadrivalents, trivalents and univalents. The analysis of 30 cells at metaphase I is given below (Table II).

Table II

Chromosome associations at metaphase I Tetraploid Asparagus officinalis (*chromosomal configuration*)

	Quadrivalents	Trivalents	Bivalents	Univalents	Frequency of cells
	1	1	15	3	1
	1	...	18	—	2
	2	...	16	—	2
	3	...	13	2	2
	4	2	9	—	1
	4	...	9	6	1
	5	...	10	—	2
	5	...	9	2	2
	5	2	7	—	1
	6	...	7	2	2
	6	1	5	3	1
	6	...	8	—	2
	6	...	6	4	1
	6	...	8	—	5
	7	...	5	2	4
	8	...	4	...	1
Total	148	6	285	32	30
Average per cell	4·93	0·20	9·5	1·07	

Quadrivalents are present in all cells though their frequency varies. They range between 1 to 8 per cell. Trivalents were very few and are present only in six cells with a maximum of 2 trivalents per cell. The number of bivalents varied from cell to cell, being a minimum of 4 and a maximum of 18 per cell. Univalents however were observed in nearly 47% of the cells ranging between 1 to 6 per cell. These univalents were generally lying outside the metaphase plate. The chiasmata frequency varied from 30–36 per cell in tetraploids and 16 to 20 in the diploid. The bivalents at metaphase I were either ring-shaped (one chiasma in each arm) or a rod-shaped (a chiasma in only one arm). In the tetraploids the quadrivalents were either a closed ring, dumb-bell-shaped, open rod or a ziz-zag type.

In Anaphase I nearly 62% of the cells were possessing abnormality of one kind or another. Sixteen out of fifty cells were having lagging chromosomes, the most common type of irregularity. The other abnormalities included the formation of bridges with or without a fragment, unequal distribution of chromosomes to two poles and a single case of a tripolar spindle. In several cases chromosomes did not separate at the termination of anaphase I to opposite poles thus giving rise to restitution nucleus (Figs. 1–6) thus all the chromosomes were lying in a pool. At telophase there were two equal or unequal nuclei accompanied by one or four micronuclei. At metaphase II the chromosome number did not show any definite regularity, thus there were varying chromosome numbers in add tion to several laggards and stray chromosomes. At metaphase II lagging chromosomes and stickiness was quite often seen and the telophase was marked by the presence of micronuclei (Figs. 7–11).

At anaphase II several laggards were noticed. These may be due to the abnormalities in the spindle mechanism. In a few cases several loose grouping of the chromosomes were observed suggesting thereby a failure of spindle movement. These irregularities at anaphase II were reflected at telophase II by the production of 1–4 nuclei. The size of the nuclei varied from each other. Micronuclei were quite frequent, naturally due to the irregularities of meiotic division such as laggards and fragments, which ultimately got included as micronuclei.

SPORADS

Quite expectedly sporads were irrgular and had varying number of microspores. Microspores too were of different size. The lack of correlation in size and number of microspores can be understood in view of the irregular meiotic divisions.

POLLEN FERTILITY

The frequency of fertile pollen varied in all the plants studied. In the diploids the fertility of pollen was almost 100%, as tested by their stainability with acetocarmine. Pollen shape is generally round rather Iso-diametrical and size varied from 7–8 μ. In tetraploid the pollen fertility varied between 40–50% with an average of 47%. The remaining pollen grains were shrunken or empty. The pollen size too varied in tetraploids probably due to disrupted meiosis resulting in the unequal chromosomal distribution among the various gametes.

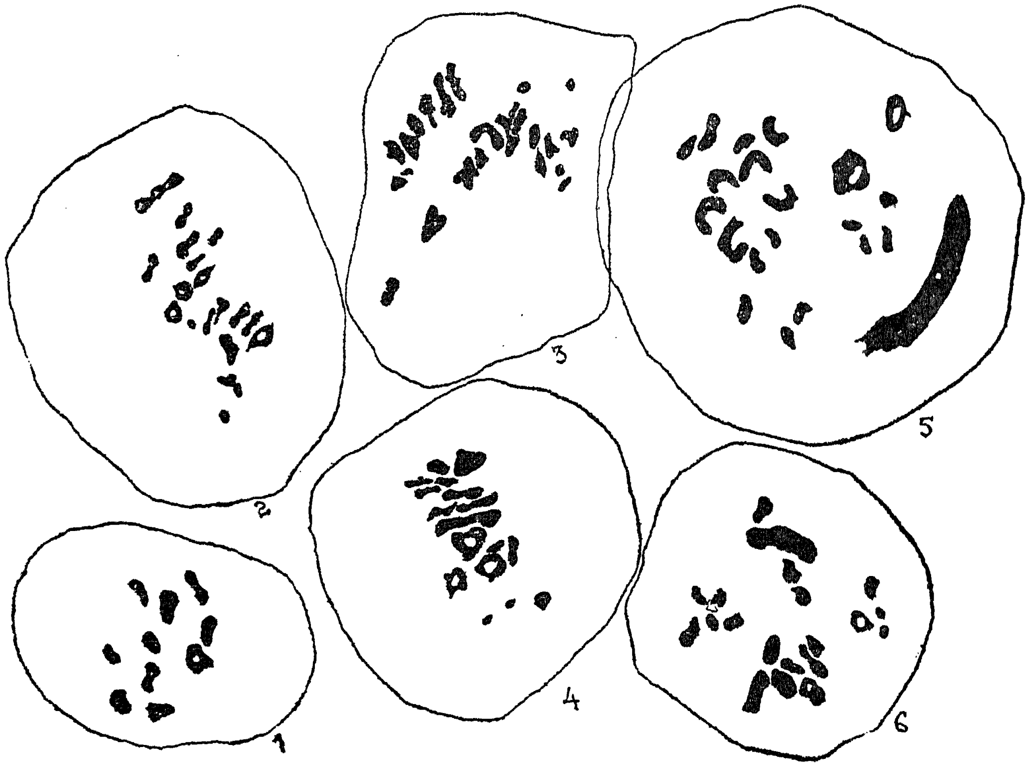
Discussion

Production of artificial tetraploids have two main applications in plant breeding. The first is to use these tetraploids as an improved strain and secondly, to use them in the production of hybrids. For either of these purposes polyploids have been used rather extensively. It is well established that autopolyploids are most useful in plants where vegetative characters are important. For example, plants where root and root products (Bannan, 1948; Peto and Hill, 1943; Nishiyama and Takasagi, 1950) or where green foliage is important (Levan, 1945; Schwanitz, 1951). In such plants where fruit size does not depend on the quantity of seed set as in Rubus and Fragaria, polyploids are equally useful (Schwanitz, 1951). Recently, polyploids of medicinal and aromatic plants have been shown to possess higher alkaloid content (Nogochi, 1940; Janaki Ammal, 1963).

When the polyploids are used by themselves the above holds good but when they are required in a breeding programme, their fertility is of much importance. Therefore, tetraploids should show cytological stability and the gametes be viable and effective in hybridisation. In the present paper cytological study of the autotetraploid asparagus is reported. The tetraploidy in asparagus was induced with the main aim of using this as a parent in the production of triploids.

From the results it is evident that the tetraploids are generally better than diploids. Tetraploid plants are taller with larger number of tillers and are having thicker stems. Therefore for commercial purposes they are more suitable than the diploids as the asparagus of commerce consists of young turions. Meiosis was perfectly normal in diploids. There being a regular formation of 10 bivalents and their orientation separation and distribution normal (Fig. 1).

Meiosis in tetraploids as already indicated, was highly irregular. At metaphase I, 40 chromosomes were observed to be regularly going into different multivalent configurations like quadrivalent, trivalents, and univalents. On an average 4·93 quadrivalents, 0·20 trivalents, and 1·07 univalents per



FIGS. 1–6. Fig. 1. Metaphase plate in a diploid plant showing 10 bivalents. Figs. 2 to 6. Metaphase I in an autotetraploid. Showing varying numbers of quadrivalents and univalents through Figs. 2–6. Note trivalent in Fig. 5 and a hexavalent in Fig. 6.

cell were observed. The data are in conformity with the behaviour of tetraploids reported earlier in Allium (Leavan, 1940), Agropyrin (Myers and Hill, 1942); Lycopersicum (Upcott, 1935); Oenothera (Linnert, 1948). Thus the cytological behaviour clearly indicates its unstable nature which seems to be due to disturbances at different stages of meiosis. Almost every cell carried a multivalent configuration. The lowest being I and maximum 8 per cell. These configurations are generally expected in an autotetraploid. Raju (1950) reported similar behaviour in a naturally occurring tetraploid of *Asparagus recemosus.*

TABLE III

		No. of cells having				
Cells scored	Normal	Lagging	Unequal distribution	Bridges	Tripolan spindle	Nuclear breakdown
50	19	16	7	4	1	3
				Total: 31		

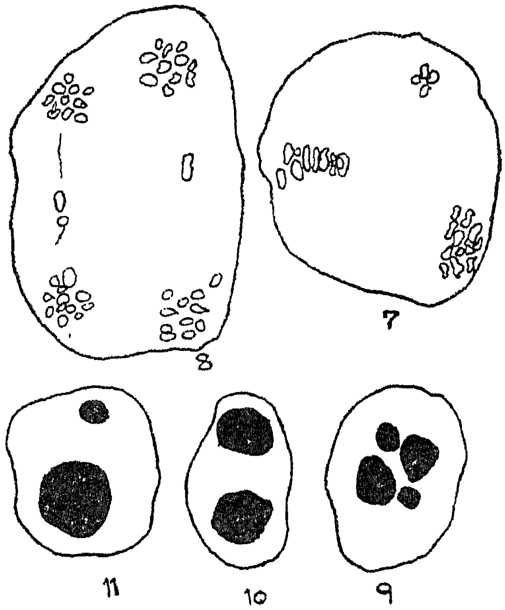
There is a regular division of the nucleus irrespective of the number of chromosomes it contained giving rise to the nuclei of varying sizes at telophase II. As already indicated in a few cases, non-disjunction of chromosomes giving rise to restitution nucleus was observed. As a rule, it should result in the formation of cell with two or more times the gametic number to give rise to the polyploid cells. As against this, unequal distribution was quite often observed. This is mainly due to the presence of univalents which were not quite often oriented at the equatorial plate. At metaphase they were lying scattered (Figs. 2–6).

We thus conclude that the tetraploids had several irregularities during the course of meiosis in the form of univalents, multivalents, multiple spindle and breakdown of spindle mechanism which lead to the production of gametes with unbalanced number of chromosomes. Two systems are seen generally working: (*a*) Production of reduced gametes by the chromosome elimination and (*b*) production of polyploids due to non-disjunction of chromosomes.

Although the meiotic abnormalities recorded were considerable yet the seed fertility was fair. Several authors have recorded complete to slightly reduced seed yield in different plant species. In wheat (Dorsey, 1956), cabbage Newcommer, 1941), cucumis melo (Hartman, 1950). However, whether the multivalent formation has any relation with the pollen or seed fertility is not yet clear. Sparrow *et al.* (1942) did not find any correlation between multivalent formation and sterility though lagging chromosomes at later stages did show some relationship. Grun (1951) found no relation between meiotic irregularity and frequency of aborted pollen in tetraploid *Medicago sativa.* Fischier reported by Ramanujam *et al.* (1956) recorded an interesting case in maize where more fertile lines had higher quadrivalent and less fertile had fewer. The reduced fertility of tetraploids thus is probably due to spindle abnormalities or due to lagging chromosome (Sparrow *et al.*, 1942; Mayer and Hill, 1941), etc.

In the present case, there is moderate frequency of multivalents associated with univalents. These univalents lag during anaphase I and II. It is possible that the reduced pollen fertility (pollen fertility is about 47%) is due to either of these. It seems more reasonable to believe that cytological irregularities resulting in unbalanced and less viable gametes and zygotes must be responsible to a certain extent for the sterility met with in autotetraploids (Ramanujam and Parthasarthy, 1955; Darlington, 1937).

It is evident from the above discussion that the autotetraploids of Asparagus are capable of producing gametes with different chromosome numbers. Their importance in evolution is obvious but they can be of good use in plant breeding, *e.g.*, in the production of aneuploids or other types of polyploids.



FIGS. 7–11. Fig. 7. Metaphase II showing laggards. Fig. 8. Telophase II with 2 chromatids of lagging chromosomes. Figs. 9–11. Telophase II showing varying number of nuclei. Note a micro-nucleus in Fig 11.

PRODUCTION OF TRIPLOIDS

In an attempt to produce triploids alternative rows of diploid and tetraploid plants were grown. It was contemplated that due to cross-fertilization some triploids will be produced. Cytological examination of the hybrid progeny showed several aneuploids, in addition to triploids. It is possible that gemetes with varying number of chromosomes have been effective in fertilization giving rise to hybrids with varying number of chromosomes. However, these aneuploids including triploids are vigorous in their vegetative characters and are therefore quite suitable for commercial exploitation through vegetative propagation, which is a regular practice in Asparagus cultivation. Trials on these lines are in progress, both at laboratory and large scale.

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