An electrophoretic and cytological study of hybridisation between *Aconitum napellus* ssp. *skerisorae* ($2n=32$) and *A. variegatum* ($2n=16$). II. Cytological evidence

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(Received: March 12, 1982)

Abstract

The number of chromosomes was determined in mitosis, the course of meiosis was analysed and the degree of pollen viability determined in plants with intermediate enzymatic phenotypes between *Aconitum napellus* and *A. variegatum*. One allotriploid plant was found with $2n=24$ and a group of plants with $2n=32$ showing disturbances in mitosis and meiosis. In mitosis somatic reduction and polysomaty were observed and in meiosis monads, diads, triads and polyads were present. These plants are considered as introgressive hybrids arising owing to a small scale gene flow from *A. variegatum* to *A. napellus*,

INTRODUCTION

On the basis of enzymatic investigations on plants from Tatra mountain populations of *A. napellus*, *A. variegatum* and mixed ones a group of putative hybrids was distinguished in view of the presence of intermediate phenotypes (Zieliński 1982). It seemed interesting to examine these plants from the point of view of cytology, the more so since among them were probably F_1 hybrids and introgressive ones.

MATERIAL

In the group of plants singled out on the basis of enzymatic results, with intermediate phenotypes between *A. napellus* and *A. variegatum* there was one plant with an over-all enzymatic pattern similar to that of the earlier discovered triploid hybrid 2413 (Szweykowski and Mendelak 1977)

and 28 plants with an intermediate peroxidase phenotype. A group of 19 plants with other rare peroxidase and glutamate oxaloacetate transaminase phenotypes were included, because in view of the low polymorphism which occurred in *Aconitum* each new phenotype was noteworthy. In these plants as compared with the control samples of 41 *A. napellus* and 21 of *A. variegatum* plants the number of chromosomes was determined on the basis of mitosis, meiosis was followed and the degree of pollen viability was determined.

METHODS

Mitosis. Young roots, about 10 from a single plant were taken. They were washed with water, treated with α -bromonaphthalene for 7 h at 16°C and fixed in AA (96% ethanol and glacial acetic acid, 3:1), then hydrolysed in 1 N HCl at 60°C for 9 min and stained with haematoxylin. The course of mitotic divisions was observed in at least three roots of each plant.

Meiosis. From each plant about 5-10 flower buds were taken fixed in AA and stored in 70 per cent ethanol. The anthers were prepared out and stained by the rapid orcein method. From one plant about 18 anthers were analysed in each of three buds.

Pollen viability determination. Flowers were collected shortly before their opening and fixed in 70 per cent ethanol. Pollen was stained with cotton blue in lactophenol. The percentage of viable grains for each plant was established on the basis of a 300-grain sample from nine anthers prepared out from three flowers.

RESULTS

Mitosis. In all 41 control *A. napellus* plants the somatic number of chromosomes was found to be $2n=32$ (Fig. 1). Only in one plant was a single $2n=64$ cell detected among $2n=32$ cells. In the control sample of *A. variegatum* consisting of 21 plants $2n=16$ (Fig. 2) one aneuploid plant with $2n=17$ was an exception (Fig. 3).

Plant no. 692 proved to be a triploid with $2n=24$ (Fig. 4). At the same time several polysomatic cells with $2n=48$ were revealed in this plant (Fig. 5).

Among the 28 plants with a heterozygotic peroxidase phenotype (PX^{ab}) in twenty four the following chromosome numbers were determined: in seventeen plants $2n=16$, in six $2n=32$ and $2n=64$ and in one $2n=32$ and $2n=16$. In the second category of plants the chromosome number $2n=32$ was most frequent and polysomatic cells with 64 chromosomes occurred much less frequently (Figs. 6-9). In the third category the frequency of $2n=16$ cells as compared with $2n=32$ ones was higher in some growth apices and lower in others (Fig. 10).

Meiosis. In the control samples consisting of 20 *A. napellus* and 20 *A. variegatum* plants tetrads of normally developed microspores were observed.

The triploid no. 692, produced, beside a small number of normally developed tetrads, mostly tetrads with nuclei of unequal size and numerous micronuclei as well as monads, diads, triads and polyads. Viable pollen consisted in 13 per cent of different-sized grains.

In the group of plants with phenotype PX^{ab} nine from among the sixteen plants had, beside tetrads with normally developed microspores, tetrads in which the microspores were different-sized or else contained additionally micronuclei and, instead of tetrads, monads (Fig. 11), diads (Figs. 12, 13), triads (Fig. 14) (Table 1) and polyads. For instance in one plant analysis of one anther showed the presence of 19 monads, 131 diads, 31 triads,

Table 1

List of plants with heterozygotic peroxidase phenotype (PX^{ab}) and phenotype PX^h together with their $2n$ chromosome numbers and meiosis products

Plant no. with phenotype PX^{ab} and PX^h	Mitosis $2n$	Meiosis products a, b
31 (PX^{ab})	—	—
48 "	32; 64	a
56 "	32	—
71 "	32; 16	b
73 "	32	b
77 "	32; 64	a
79 "	32	b
80 "	32	—
113 "	32	—
118 "	32	b
122 "	—	—
123 "	32; 64	b
127 "	—	—
209 "	—	—
365 "	32	a
403 "	32; 64	a
429 "	32; 64	a
434 "	32	b
476 "	32	—
557 "	32; 64	b
565 "	32	b
655 "	32	—
663 "	32	a
688 "	32	—
713 "	32	—
719 "	32	a
748 "	32	—
750 "	32	b
692 (PX^h)	24; 48	b

a—tetrads of normally developed microspores.

b—tetrads of normally developed microspores and tetrads with different-sized nuclei, monads, diads, triads and polyads

36 tetrads with nuclei of various sizes and 40 polyads. Not one normally developed tetrad was found in this anther.

Pollen viability in plants with normal course of meiosis was 83 to 99 per cent. Plants in which abnormal meiosis was observed exhibited a somewhat depressed pollen viability (75-98%) and, beside normal, small and large grains (Fig. 15). These grains were often twonucleate or contained one or more micronuclei (Figs. 16, 17).

Since in the control material no cytological disturbances were noted, whereas in more than half of the PX^{ab} plants they were detected, in several plants simultaneously polysomy and meiosis disturbances being noted, it may be supposed that these disturbances are closely connected with hybridisation.

DISCUSSION

In earlier enzymatic investigations a group of plants was isolated with intermediate phenotypes, this possibly indicating their hybrid origin (Zieliński 1982). One of these plants (no. 692) with a similar over-all pattern of several enzymes to that found earlier in the allotriploid no. 2413 also proved to be an F₁ hybrid with 2n=24 chromosomes.

Both the F₁ hybrids differed from each other in appearance. No. 692 was externally similar, particularly in the colour and structure of the flower, mainly of the galea, to *A. napellus* and no. 2413 to *A. variegatum*. Since there are no such external markers of hybridisation as intermediate morphology with normally developed generative organs (flowers, anthers) it is practically impossible to distinguish these hybrids in the natural life conditions.

The two hybrids found constitute 0.9 per cent of the sample of mixed populations. Thus, they are relatively rare, although the fact should be stressed that they were found on two different sites (Dolina Kondratowa (valley), source of Potok Miętusi (stream)). Both produce viable pollen in about 13 per cent, this indicating that back-crossing and gene flow between the parent species connected with it are potentially possible. The low abundance of triploids and, therefore, lower competition ability of their pollen the viability of which is, moreover, depressed, may cause this process to occur on an only small scale.

In spite of these limitations there exist circumstances favourable to back-crossing. Firstly, the triploids flower in the intermediate period between the two dates of intensive flowering of *A. napellus* and *A. variegatum*, thus, at the time when there are few other flowering plants. Secondly *Aconitum* hybrids as perennials participate repeatedly in crosses during successive vegetation seasons. Thirdly, on account of the high competition of foreign pollen the hybrids participated in crosses mainly as mother plants (Stebbins 1958).

In the light of the foregoing considerations most interesting seems the group of 28 plants which combines cytological disturbances with an intermediate

hybrid peroxidase phenotype (PX^{ab}) and a somatic chromosome number $2n = 32$. The enzymatic-cytological correlation occurring in these plants with its simultaneous absence in control material seems to be evidence that they are introgressive plants which stabilised at the ploidy level of *A. napellus*. The possibility of crosses between triploid hybrids and *A. napellus* and the gene flow direction connected with it from *A. variegatum* ($2x = 16$) to *A. napellus* ($4x = 32$) find confirmation in experimental studies (Vardi and Zohary 1967, Vardi 1970, 1974). The direction of gene flow depends, namely, to a great extent on whether the gametes produced by triploids are mostly reduced or not. Gametes produced by the allotriploid no. 692 are: reduced, containing various (lower than 24) chromosome numbers and unreduced, with 24 chromosomes. Each kind of these gametes in combination with the parent gamete of *A. napellus* ($n = 16$) and nearly each in combination with the gamete of *A. variegatum* ($n = 8$) may give a progeny with a number of chromosomes close to the tetraploid level, which will further cross with better efficiency with *A. napellus* than with *A. variegatum*. Hence the earlier stabilisation of hybrids beginning with the F_2 generation will occur at the tetraploid level.

It should be once more stressed that the mentioned group of 28 plants gave intermediate (hybrid) phenotypes only in the case of peroxidase. The enzymatic argument supporting the introgressive origin of these plants is based on the fact that the phenotype PX^{ab} was found in the triploid no. 2413 as well. It is, moreover, a phenotype to be expected in *Aconitum* hybrids, because it is a combination of the two most frequent phenotypes— PX^a in *A. napellus* and PX^b in *A. variegatum*. Since part of the plants with phenotype PX^{ab} were found exclusively in *A. napellus* populations, the question arises whether these are also introgressive forms or whether part of the *A. napellus* plants exhibits a variability of such character. More detailed investigations on the biochemical similarity of the bands belonging to plants with phenotype PX^{ab} have not been performed, therefore, it is difficult to give an unequivocal answer in this respect.

Nevertheless, cytological data rather univocally supporting the enzymatic arguments seem to point to the introgressive origin of plants with PX^{ab} since they show disturbances while none are noted in the control material. About 60 per cent of these plants showed cytological disturbances, 30 per cent of which in mitosis and 56 per cent in meiosis. In some plants mitotic and meiotic disturbances were found simultaneously. The character of these disturbances is interesting. In six plants cases of polysomy were noted ($2n = 32$ and $2n = 64$) and the same was observed in the triploid no. 692 ($2n = 24$ and $2n = 48$). Although polysomy is common in the plant world (Sen 1973), one of the causes of it is also considered to be hybridism in view of its connection with disturbances in the course of mitosis. The hybrid no. 692 is a good example of this. Somatic reduction observed

in one plant ($2n=32$ and $2n=16$) like polysomy has also been described in other hybrids (Sadasivaiah and Lesins 1974, Arutinova 1979).

Meiotic disturbances led above all to the formation of unreduced pollen grains. Their appearance has been observed in various plant groups (Harlan and de Wet, quoted after Ramanna 1979) and its mechanism consists in the formation of a restitutive nucleus in the first or second meiotic division. This mechanism is controlled by simple recessive mutations which may lead to an increase in the frequency of diploid pollen formation from 3 to 99 per cent (Mok and Peloquin 1975, Mashkina 1979). Unfavourable environmental conditions, however, as for instance high temperature during flowering (Ramanna 1979) or hybridisation (Gajewski et al. 1963, Bir 1971, Esen and Soost 1971, Noda 1971, Izmailov 1976, Maan and Sasakuma 1977) may modify the action of genes responsible for the normal course of meiosis and lead to the formation of diploid pollen. Gajewski (1957) found in various hybrids of *Geum* common appearance of diads which arose as the result of restricted chromosome conjugation, giving numerous univalents which delayed phase I and led to the formation of a restitutive nucleus. Diad formation observed in some *Aconitum* plants, in view of the existing association with the presence in these plants of the heterozygotic peroxidase phenotype (PX^{ab}), should also be interpreted as the influence of hybridisation.

In recapitulation it may be affirmed that between *A. napellus* ($4x$) and *A. variegatum* ($2x$) both crosses and gene flow occur. In view, however, of the low frequency of F_1 hybrids and their restricted fertility as well as chromosome barriers, introgression towards *A. napellus* occurs on but a small scale.

Acknowledgement

The author wishes to thank Professor Jerzy Szweykowski for his advice in the course of preparation of the present study, for his keen interest in it and critical reading of the manuscript. Thanks are also due to Dr Maria Mendelak for making available to the author the triploid hybrid no. 2413. The help of Mrs Grażyna Wojciechowska-Stefaniak in making part of the cytological preparations and photographic documentation is gratefully acknowledged.

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Elektroforetyczne i cytologiczne badania mieszańców między Aconitum napellus i A. variegatum. II Cytologia

Streszczenie

U roślin o pośrednich fenotypach enzymatycznych między *Aconitum napellus* i *A. variegatum* oznaczono liczbę chromosomów w mitozie oraz analizowano przebieg mejozy i określono stopień żywotności pyłku. Znaleziono jedną roślinę allotriploidalną o $2n=24$ oraz grupę roślin o $2n=32$, wykazujących zaburzenia w mitozie oraz w mejozie. U roślin tych zaobserwowano w mitozie redukcję somatyczną i polisomację, a w mejozie stwierdzono obecność monad, diad, triad i poliad. Rośliny te uważa się za mieszańce introgresyjne powstałe na skutek zachodzącego na niewielką skalę przepływu genów od *A. variegatum* do *A. napellus*.