Acta Bot. Neerl. 21(5), October 1972, p. 555-559

SOME NOTES ON THE DIPLOID Chromosome number of the genus Acorus L. (Araceae)

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SUMMARY

The meiotic chromosome number of a specimen of the genus *Acorus* L. from North Thailand proved to be 12. This confirms 24 as the diploid somatic number of the genus and not 18 as is sometimes supposed.

1. INTRODUCTION

The genus Acorus, as it is most often conceived, contains two rather closely allied species, A. calamus L. and A. gramineus Soland. According to Ohwi (1965) A. gramineus is smaller and narrower in all parts than A. calamus with the exception of the spadix (5-10 cm by 3-4 mm against 4-7 cm by 6-10 mm). The leaf of A. gramineus has no midrib whereas that of A. calamus has a prominently raised one.

Since it is not always known with certainty which species an investigator had at hand, and since the taxonomic subdivision of the genus is not of much importance for the contents of the present paper, I have chosen to consider the genus as a single entity.

The diploid chromosome number of *Acorus* does not seem to be a matter of common consent. The first chromosome count of *Acorus* is the one by NAKA-JIMA (1933; Japan: n = 12). This was followed by Dahl (ex DUDLEY 1937 and ex BUELL 1938; North America: 2n = 18), WULFF (1939, 1940; Europe and India: 2n = 36; 1946, cultivated *A. gramineus*: 2n = 24; 1950, North America: 2n = 24), and PALMGREN (1943; Europe: 2n = 36). Wulff also studied the meiosis of PMC's from European plants and found in MI many (up to 12) trivalents.

Nevertheless Malvesin-Fabres (1945 ex DELAY 1951; ex WULFF 1954) again reported 2n = 18. By that time the occurrence of plants with 2n = 44-48 was also known from Japan (KURAKUBO 1940 ex WULFF 1954; ITO 1942; WULFF 1954). WULFF (1954) stated that he had counted in some cells from Japanese material unequivocally 48 chromosomes. JANAKI AMMAL *et al.* (1964) reported three 2n = 36 plants from the Punjab and a 2n = 54 plant from Kashmir. They consider these as tetra- and hexaploids respectively, referring to MATSUURA & SUTO (1935) who should have counted 2n = 18. I was not able, however, to find a chromosome count of *Acorus* in the paper cited. RAQUIBUDDOWLA *et al.* (1967) reported 2n = 18 for a plant from Bangla Desh (presumably vicinity of Dacca) and LARSEN (1969) counted 2n = 24 (two plants) and 2n = 44 (one plant) for the Chiang Mai area (North Thailand).

From the data it can be understood that some authors consider 9 as the basic chromosome number (x) of the genus *Acorus* (e.g. Janaki Ammal *et al.*, Raquibuddowla *et al.*), whereas others (e.g. Wulff, Löve and Löve) accept x = 12 as the most probable basic number. So it seemed worthwhile to perform new chromosome counts, especially from meiotic divisions of fertile plants. When we received living rhizomes from Thailand (Nooteboom *et al.*)* we were in a position to start such an investigation.

2. MATERIAL AND METHODS

The rhizomes (Nooteboom *et al.* 871) are cultivated in jars filled with plastic grains (I.C.I. "Alkathene D47/04, Black 904") and watered every three hours during 15 minutes with a modified Hoagland nutrient solution. The plants were illuminated 17 hrs/day by 10 fluorescent tubes (Philips TL40W/33 011280.01.003) hanging 70 cm above the jars. The temperature and relative humidity could not be kept constant and fluctuated rather strongly. The temperature, however, did not fall below 10°C. Under these conditions the plants grew well and flowered frequently. Fruits, however, have not yet been produced.

In order to count the chromosome number a slice (c. 1/2 cm) from a young spadix was fixed in Carnoy's fluid (6:3:1) daily. After a few days the fixed slices were squashed according to the acetic-carmine method.

For the observation of ripe pollen, grains were stained for 24 hours in a 1/2% aqueous solution of malachite green and afterwards embedded in glyceroljelly. Grains were considered 'normal' if they did not clearly deviate in form or size and if a well stained content nearly filled the grain.

Voucher specimens are kept in the herbarium of the Laboratorium voor Experimentele Plantensystematiek. A voucher specimen from the original site of Nooteboom *et al.* 871 in is the collection of the Rijksherbarium at Leyden.

3. RESULTS

The meiosis in the PMC's of our plants proved to be normal. The haploid chromosome number is 12 (*fig. 1*). 95% of the pollen grains examined were found to be normal; this was compared with the pollen grains from a specimen from the Netherlands (LEP 4065; vicinity of Nieuwkoop), where only 1% of the grains was not obviously defective, the remaining 99% were empty. In each case 100 grains were examined.

^{*} Collected the 9th of January 1969, during an expedition to Thailand and Borneo sponsored by WOTRO.

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Fig. 1. Drawing of Metaphase I in meiosis of diploid *Acorus* from Thailand (Nooteboom *et al.* 871).



4. DISCUSSION

Apparently the meiosis in the PMC's of at least some representatives from Asiatic 2n = 24 populations is normal (NAKAJIMA 1933; present paper). Since WULFF (1950) has succesfully grown American 2n = 24 plants from seeds and since seeds received from Canada also germinated readily at Leyden, the same probably holds for the American 2n = 24 form.

Meiosis in the PMC's of the 2n = 36 form however has many uni- and trivalents in MI (WULFF 1940) and these plants never produce seeds.

For this reason I think it justified to consider 2n = 24 as the diploid and 2n = 36 as the triploid level in *Acorus* and hence x = 12 as the basic number of the genus. According to this reasoning the East-Asiatic 2n = 44-48 plants represent the tetraploid level. The counting of 2n = 54 from Kashmir (JANAKI AMMAL *et al.* 1964) is deviating and should be verified. I agree with WULFF (1940, 1950) that the repeatedly given number 2n = 18 does not seem to be correct. This may well be due to the fact that the chromosomes of *Acorus* are small and show a tendency to lie closely together in the mitotic metaphase.

Within the genus Acorus di-, tri-, and tetraploid plants are known at present. Only in South and East Asia all these levels of ploidy seem to occur (NAKAJIMA 1933; KURAKUBO 1940; ITO 1942; WULFF 1940, 1950; JANAKI AMMAL et al. 1964; RAQUIBUDDOWLA et al. 1967; LARSEN 1969).

In North America a diploid form (Dahl ex DUDLEY 1937 and ex BUELL 1938; WULFF 1950; LÖVE & LÖVE 1964) and possibly also a triploid form are found. The occurrence of the latter, which may well have been introduced from Europe, is yet to be confirmed (WULFF 1950). In Europe triploid populations are common (WULFF 1939, 1940; PALMGREN 1943; Vaarama ex WULFF 1954 and ex VON SCHANTZ 1959; KOZLOWSKI 1960; SOKOLOWSKA-KULCZYCKA 1961). Though quite common in large parts of Europe now, *Acorus* was introduced there only a few hundred years ago, most probably from Asia Minor or the Middle East (ENGLER 1905; MÜCKE 1908; VON SCHANTZ 1959)*. There are

* Prof. Dr. A. Baytop-Berk (in litt.) doubts whether *Acorus* occurs in the wild state in Turkey nowadays.

some indications that in the north-western part of Poland, and possibly also in other places in the Baltic area diploid *Acorus* exists (Vaarama in LÖVE & LÖVE 1948, ex WULFF 1950, 1954; KOZLOWSKI 1960). These diploids may well have been introduced by the Tartars (MÜCKE 1908; VON SCHANTZ 1959). In this connection ENGLER'S (1905) mentioning of the occurrence of narrowleaved plants in East and North China and East Siberia is of some interest. In the same connection it is to be regretted that the origin of the diploid plant from the botanical garden at Copenhagen is not known (WULFF 1940).

There seems to be a difference between the ecological preferences of diploid and tetraploid plants of North Thailand; diploids seem to prefer living water on stony ground, whereas tetraploids are more common in stagnant water on clayey soil (LARSEN 1969; note on voucher specimen of Nooteboom *et al.* 871).

ACKNOWLEDGEMENTS

The author is greatly indebted to Prof. Dr. R. Hegnauer for his encouragement and criticism; to Prof. Dr. J. K. Morton, Waterloo, Canada, who kindly provided seeds from Paris, Ontario; to Drs. H. P. Nooteboom, Leyden, for sending living rootstocks during his visit to Thailand; to Dr. C. E. Ridsdale, Leyden, for his help with the English text.

REFERENCES

BUELL, M. F. (1938): Embryogeny of Acorus calamus. Bot. Gaz. 99: 556-568.

- DELAY, C. (1951): Nombres chromosomiques chez les Phanérogames. Rev. Cytol. Biol. Végét. Paris. 12: 56.
- DUDLEY, M. G. (1937): Morphological and cytological studies of Calla palustris. *Bot. Gaz.* **98**: 556-571.
- ENGLER, A. (1905): Das Pflanzenreich IV (23b): 308-313. Leipzig.

ITO, T. (1942): Chromosomen und Sexualität von den Araceae. I. Somatische Chromosomenzahlen einiger Arten. Cytologia 12: 313–325.

JANAKI AMMAL, E. K., S. N. SOBTI, & K. L. HANDA (1964): The interrelationship between polyploidy, altitude and chemical composition in Acorus calamus. *Curr. Sci. Bangalore* 33: 500.

Kozlowski, J. (1960): Kariotypy tataraku (Acorus calamus L.) na terenie Polski. Biul. Inst. Rošlin Leczniczych. Poznań 6: 65-70.

*KURAKUBO, Y. (1940): Ueber die Chromosomenzahlen von Araceae-Arten. Bot. et Zool. Tokyo 8: 1492.

LARSEN, K. (1969): Studies in the flora of Thailand 54. Cytology of vascular plants. III. A study of Thai Aroids. Dansk Bot. Ark. 27: 39-59.

*LÖVE, Á & D. LÖVE (1948): Chromosome numbers of northern plant species. Reykjavik.

-- & -- (1964); in Á. Löve & O. T. SOLBRIG: IOPB Chromosome number reports. I. Taxon 13: 99-110.

*Malvesin-Fabres, G. (1945): Contribution à la caryologie des Aracées. Thèse Sci. Bordeaux.

MATSUURA, H. & T. SUTO (1935): Contributions to the idiogram study in phanerogamous plants. I. Jour. Fac. Sci. Hokkaido Univ. 5: 33-75.

MÜCKE, M. (1908): Über den Bau und die Entwicklung der Früchte und über die Herkunft von Acorus calamus L. Bot. Zeit. 66: 1–23.

NAKAJIMA, G. (1933): Chromosome numbers in some Angiosperms. Jap. Jour. Genetics 9: 1-5.

OHWI, J. (1965): Flora of Japan. Washington D.C.

PALMGREN, O. (1943): Chromosome numbers in angiospermous plants. Bot. Not. 348-352.

- RAQUIBUDDOWLA, M., M. SIDDIQUEULLAH, R. S. DEWAN, & A. HAQ (1967): Studies on the solvent extraction of oil from Acorus calamus Linn. Sci. Res. Dacca 4: 234–239.
- SCHANTZ, M. VON (1959): Über die Verbreitung und Herkunft von Acorus calamus L. Medd. Norsk Farm. Selskap 21: 13-21.
- SOKOLOWSKA-KULCZYCKA, A. (1961): in M. SKALINSKA et al.: Further additions to chromosome numbers of Polish Angiosperms. Acta Soc. Bot. Pol. 30: 463-489.
- *VAARAMA, A. (1948): in Á. LÖVE & D. LÖVE: Chromosome numbers of northern plant species. Reykjavik.
- WULFF, H. D. (1939): Chromosomenstudien an der schleswigholsteinischen Angiospermen-Flora. III. Ber. deut. bot. Ges. 57: 84-91.
- (1940): Über die Ursache der Sterilität des Kalmus (Acorus Calamus L.). Planta 31: 478-491.
- (1946): Der Ölgehalt verschiedenchromosomiger Rassen vom Kalmus (Acorus Calamus L.). Zeitschr. Naturf. 1: 600-603.
- (1950): Ölgehalt und Chromosomenzahl des nordamerikanischen Kalmus (Acorus Calamus L.). Arch. Pharm. 238: 155-161.
- (1954): Zur Zytologie, geographischen Verbreitung und Morphologie des Kalmus. Arch. Pharm. 287: 529-541.
- * Original literature not consulted.

POSTSCRIPTUM

Only after this paper went to press I saw the dissertation of Miss G. E. Jones (G. E. Jones (1957): Chromosomenumbers and phylogenetic relationships in the *Araceae*. Dissertation, Univ. of Virginia, Charlottesville Va). Miss Jones states that she has counted 2n = 36 in sterile plants from northern Virginia. This confirms the occurrence of triploid *Acorus* in North America.