## NUCLEOLUS-CHROMOSOME RELATIONSHIP IN *ALLIUM FISTULOSUM* VAR *VIVIPARUM* MAKINO

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ALLIUM FISTULOSUM var viviparum synonym of Allium cepa var viviparum, is a vegetatively reproduced form of onion bearing bulbils on the inflorescence. It resembles A. cepa morphologically. It is claimed to be a hybrid between A. cepa and A. fistulosum<sup>1,2</sup>. Bozzini<sup>1</sup> reported the presence of a single nucleolus in the cells of this variety. If var viviparum is a hybrid, then it should possess two nucleolar organizing chromosomes characteristic of the two species. The present work was therefore, undertaken to see what happens to the other nucleolar chromosome and the nucleolus in this hybrid and whether the ribosomal genes of one species can suppress the transcription of ribosomal genes of another species. Detailed karyotype study with special reference to the nucleolar chromosomes and the nucleoli were undertaken.

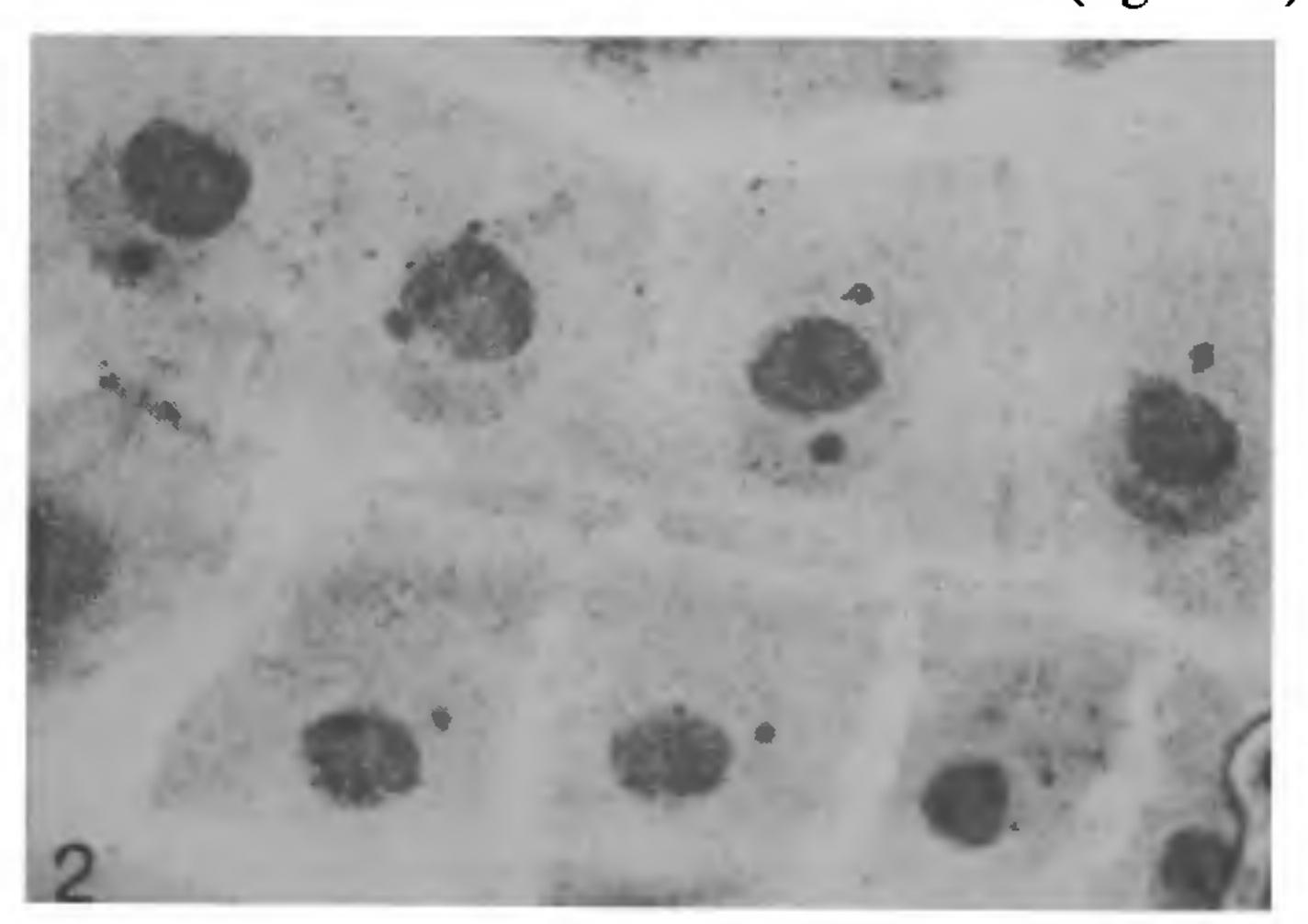
Actively growing root tips were pretreated in 0.05% colchicine for 4–5 hr followed by fixation in acetic-acid: alcohol (1:3) mixture. Usual aceto-orcein staining method was followed for karyotype study and air-dried slides were made after dry

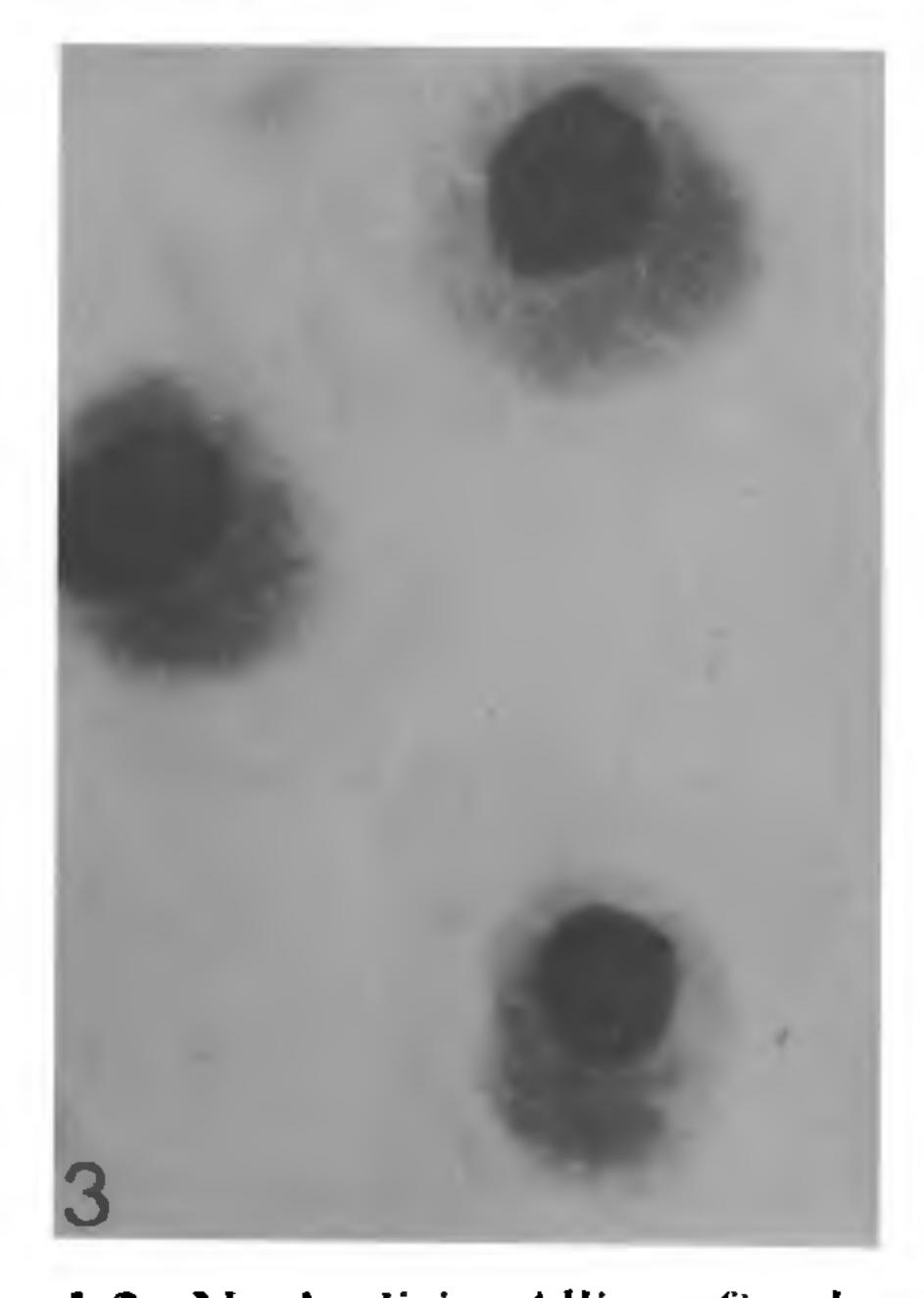
2 3 4 5 6 / 8 9 10 11 12 13 14 15 16

Figures 1 and 1a. 1. Metaphase plate from Allium fistulosum var viviparum root tip cell showing 2n = 16 chromosomes with a single nucleolar chromosome (arrow). Note the presence of a subterminal chromosome (arrow head). 1a. Karyogram of A. fistulosum var viviparum.

squash preparation. The methods of Howell and Black<sup>3</sup> and Varley<sup>4</sup> were followed for silver staining of nucleolus.

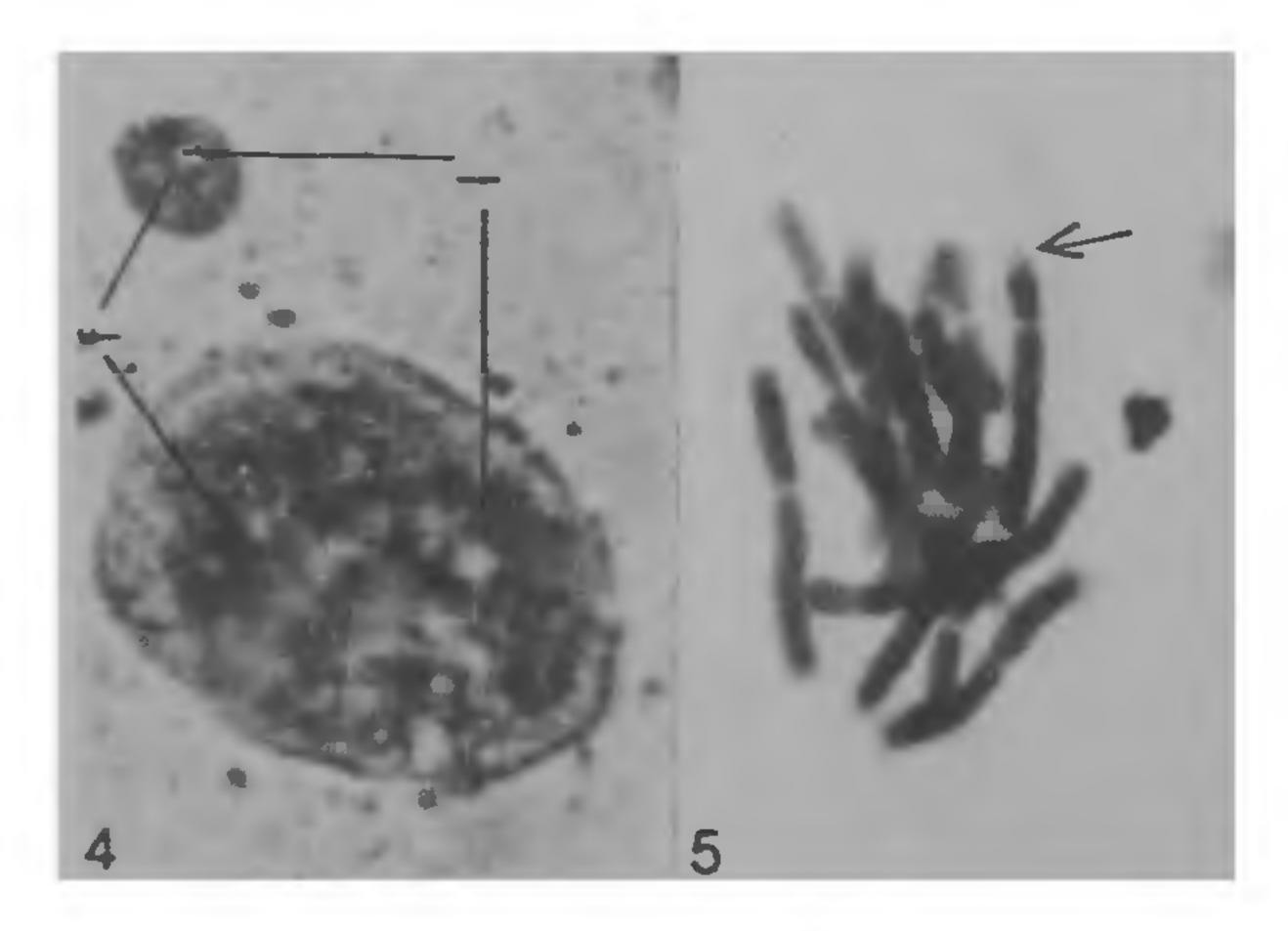
The karyotype analysis of A. fistulosum var viviparum shows 2n = 16 chromosomes. Almost all the chromosomes are meta- to submeta-centric and vary from 7.9 to 13.3  $\mu$ m in length with the centromeric index 22.4 to 47.1 (figure 1). Only a few chromosomes could be arranged in pairs and as such have been represented singly in the karyogram (figure 1a). The hybrid nature of the variety is evident from the karyotype. A similar observation was made earlier by Bozzini<sup>1</sup> and Battaglia<sup>5</sup> who reported the presence of a single nucleolar chromosome in the cells. This chromosome has almost submedian primary constriction and the secondary constriction is median to the short arm (figure 1).





Figures 2 and 3. Nucleoli in Allium fistulosum var viviparum cells after AgNOR staining. 2. following Howell and Black's method and 3. following Varley's method.

This type of nucleolar chromosome does not belong to any of the six types described in the genus Allium by Ved Bratt<sup>6</sup>. Bozzini<sup>1</sup> reported that the nucleolar chromosome present in the variety is of fistulosum type. It seems that the cepa type nucleolar chromosome is inactive in these cells. However, staining of the nucleolus by the silver staining technique<sup>3</sup> reveals the presence of a big nucleolus having the average area 2.01  $\mu$ m<sup>2</sup> and a very small nucleoli having the average area 0.19  $\mu$ m<sup>2</sup> (figure 2). Earlier Bozzini<sup>1</sup> reported the presence of only one nucleolus per ceil. Perhaps the small nucleolus could not be detected by the technique used by him. We also failed to detect the small nucleolus by using the AgNOR staining technique of Varley<sup>4</sup> (figure 3). Howell and Black<sup>3</sup> technique seems to be much more sensitive in staining the nucleolus. In some bigger cells, the small nucleolus is relatively bigger in size perhaps due to their polytenic nature<sup>7</sup> and clearly shows the typical nucleolar structure as lacunae, deep and light stained areas (figure 4) after silver staining. If we associate the origin of the big nucleolus with the fistulosum type of nucleolar chromosome, the origin of the small nucleolus should then be associated with a cepa-type nucleolar chromosome. Indeed in some of the less condensed chromosomes it was possible to detect the *cepa*-type nucleolar chromosome with a rudimentary satellite stalk (figure 5). It is clear that the satellite and possibly a part of the stalk is deleted from this chromosome. As such this chromosome cannot be detected readily (figure 1).



Figures 4 and 5. Nucleoli from polytenic cell after silver staining. Note the presence of lacunae (1), deeply-stained fibrillar regions (f), and light stained regions in both the large and small nucleoli.

5. Cepa-type of nucleolar chromosome with terminal NOR. Note the deletion of the satellite.

Earlier it has been established that ribosomal cistrons are located on the satellite stalk of A. cepa nucleolar chromosomes<sup>8</sup>. The development of a small-sized nucleolus may be caused by the deletion of a part of the satellite stalk. These observations on the karyotype, nucleolar chromosomes and nucleoli support the hybrid origin of A. fistulosum var viviparum. It also indicates that the ribosomal cistrons present in the cepa-type nucleolar chromosome are not repressed by the cistrons present in the fistulosum chromosomes.

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- 2. Vosa, C. G., Heredity, 1976, 36, 383.
- 3. Howell, W. M. and Black, D. A., *Experientia*, 1980, **36**, 1014.
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- 5. Battaglia, E., Caryologia, 1957, 10, 1.
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## CELL DIVISIONS IN NEWLY FORMED CELLS FROM LEAF MESOPHYLL PROTOPLASTS OF WHEAT (TRITICUM AESTIVUM CV SONALIKA)

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PLANT protoplasts have now become an extremely popular tool to plant scientists for their various and diverse nature of applications in studies encompassing both fundamental and applied aspects<sup>1, 2</sup>. But unfortunately only limited success has so far been achieved while culturing the cereal protoplasts, particularly the economically important ones due to their poor response to *in vitro* culture techniques<sup>3</sup>. The perusal of literature reveals that cereal protoplasts can swell, regenerate a cell wall and remain viable for a considerable period or divide occasionally in culture media<sup>4-6</sup>. Callus formation (though mostly from the cultured cells) has been