

**INVESTIGATION ON THE MEIOSIS OF DIPLOID (*M. ALBA* L.)  
AND 22-PLOID (*M. NIGRA* L.) MULBERRY SPECIES AND  
THEIR HYBRID RELATIVE TO THE PROBLEM  
OF THE ORIGIN OF SPECIES *M. NIGRA* L.**

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**Abstract**

In an attempt to trace out the origin of 22-ploid *M. nigra*, investigations on cytological behaviour of diploid *M. alba*, 22-ploid *M. nigra* and their hybrid revealed the occurrence of fairly regular meiosis in all the cases. In spite of a large chromosome number, the presence of bivalents and absence of multivalents in the 22-ploid *M. nigra* suggests that it has probably originated by an increase in ploidy of diploid *M. alba*.

In addition to the similarities in cytological behaviour, lack of apomixis and the tendency of *M. nigra* to propagate through seeds by artificial means also support the above conclusion.

**Introduction**

The genus *Morus* is represented both by diploid (Tahara 1909, 1910; Osawa 1920) and 22-ploid species (Thomas and Darlington 1942; Janaki-Ammal 1948). Besides artificially induced polyploids i.e. triploid, tetraploid, hexaploid and octoploid (Radjably 1962; Abdulaev 1963, '65, '67; Djafarev 1967) are also available. The basic chromosome number in this genus is 14 and the highest ploidy level is 22x. The species *M. nigra* ( $2n \pm 308$ ) probably originated in nature and exists at present only in cultivated form and is well known for its high vitality, drought resistance and immunity and adaptability to the soil. This species stands apart from diploid and tetraploids and differs considerably from both of them in morphological and anatomical structure of vegetative organs (Seki and Oshikane 1960) and in peculiarity of the growth biology (Makhmudbekova 1961; Abdulaev and Djafarev 1965). There is no information in literature on the origin of 22-ploid. It is, however, possible to throw light on the origin of such a polyploid form on the basis of its interrelationships with other species within the genus. In the present paper a study on the comparative cytological behaviour of diploid *M. alba*, 22-ploid *M. nigra* and their  $F_1$  hybrid has been presented with the object of understanding the way by which polyploid forms of the genus may have originated.

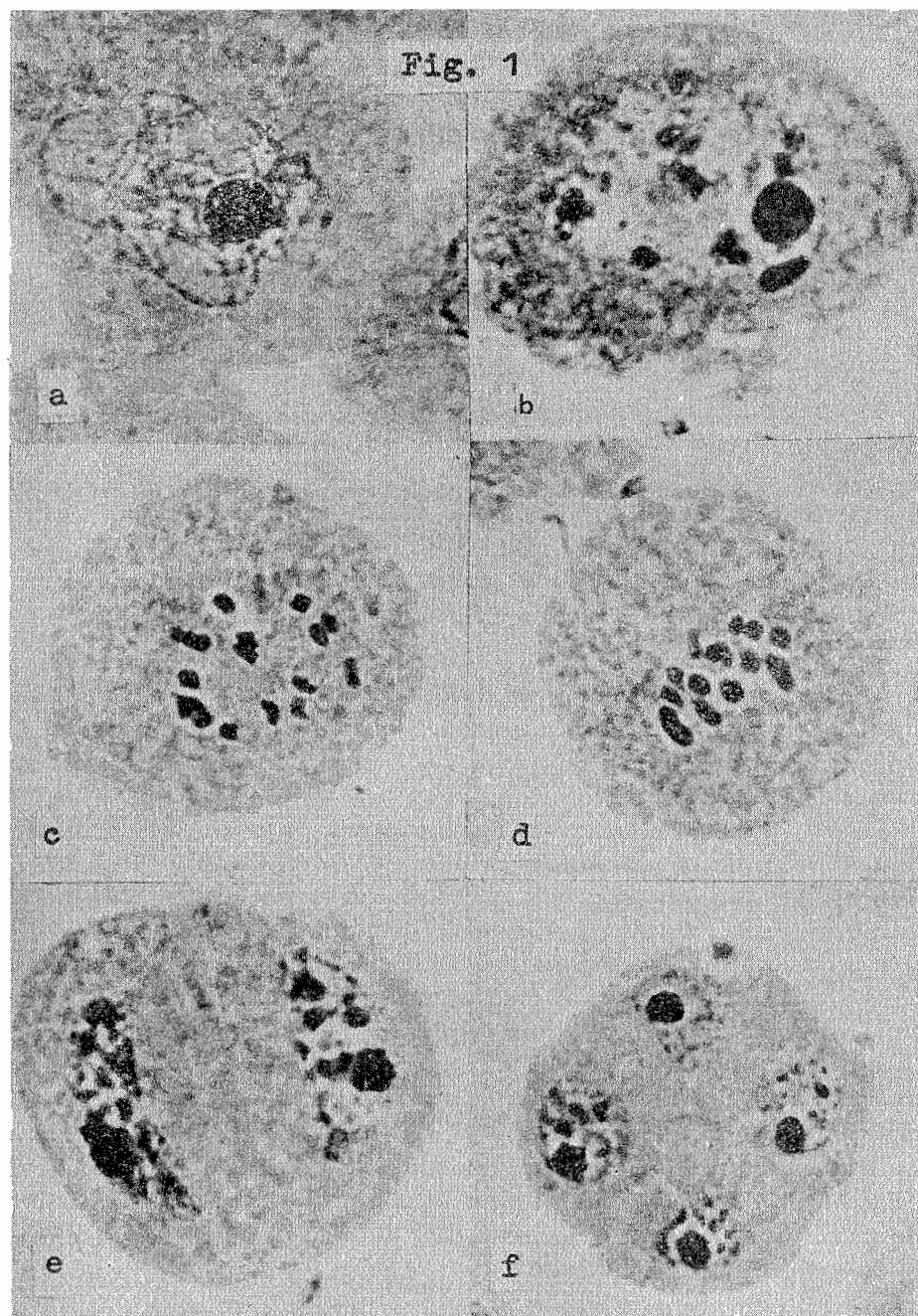


Fig. 1. The meiosis of diploid Zarif-tut variety (*M. alba*).  
a—zygonema; b—indistinct chromosomes at the transition stage from diplonema to diakinesis;  
c—diakinesis; d—metaphase I; e—interkinesis; f—tetrad of microspores.  
×1833.

### Materials and Methods

1. *Morus alba* L. var. Zarif-tut ( $2n=28$ ), bisexual.
2. *Morus nigra* L. var. Char-tut ( $2n=308$ ), usually known as female form.
3.  $F_1$  hybrid, *M. nigra*  $\times$  *M. alba* ( $2n=168$ ).

The material was fixed in Carnoy's fixative (6:3:1) for 24 hrs. Cell counts were made with temporary preparations in aceto-carminc according to the method suggested by Radjably (1963).

### Experimental Results

*M. alba* ( $2n=28$ ). All the stages of early prophase were normal. In early prophase chromosomes appeared as long filaments (Fig. 1 a) resulting from regular pairing of the homologues (Fig. 1 b). The chromosomes at diakinesis (Fig. 1 c) also appeared as regular bivalents.

In MI 14 bivalents (Fig. 1 d) were arranged in a normal fashion at the equatorial plate. During A I half bivalents separated to the poles simultaneously. Telophase I and II took place normally resulting in the formation of normal diads (Fig. 1 e) and tetrads respectively (Fig. 1 f).

*M. nigra* ( $2n=308$ ): In spite of its high ploidy level, the meiosis of 22-ploid Char-tut (*M. nigra*) form, is not significantly different from that of diploid Zarif-tut (*M. alba*) form. The principal steps of meiotic course of Char-tut form are presented in Fig. 2.

In telophase chromonemata appeared thin and granular (Fig. 2 a). Considerable shortening and thickening of chromosomes (Fig. 2 b) leads to more or less round shaped bivalents at diakinesis (Fig. 2 c). In some PMCs, 154 bivalents were seen at MI (Fig 2d). In others univalents together with bivalents were also observed and the chromosomes whether as bivalents or univalents showed more or less parallel arrangement to one another at the equatorial plate.

In spite of the presence of univalents and lagging chromosomes in AI (Fig. 2e), the two interkinetic nuclei do not show any departure from the normal. Although lagging chromosomes were observed at A II, no variation in chromosome grouping was observed. As a result normal tetrads were formed (Fig. 2f).

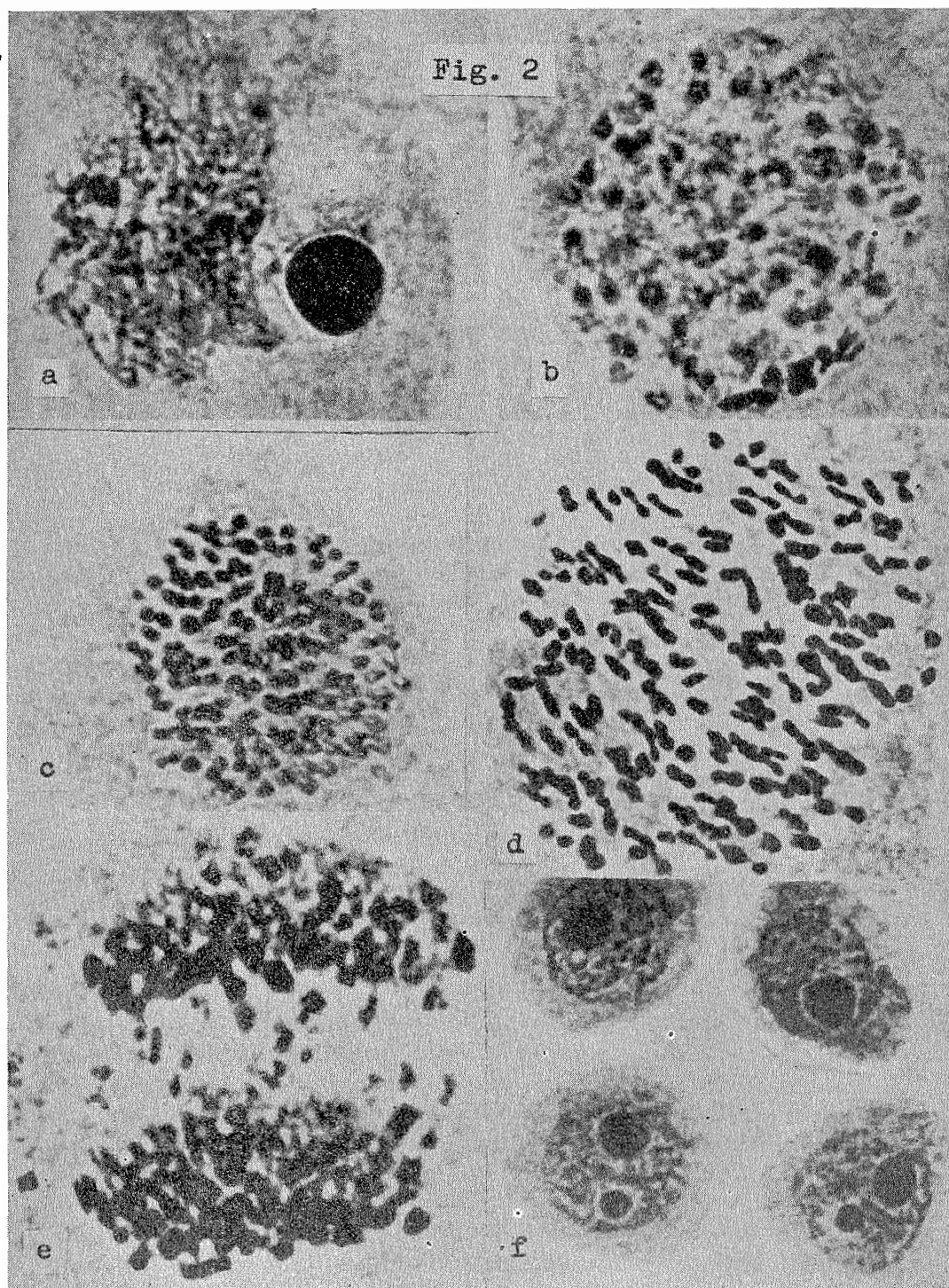


Fig. 2. The meiosis of 22-ploid Char-tut form (*M. nigra*).  
 a—zygonema; b—diplonema; c—diakinesis; d—metaphase I; e—anaphase I; f—tetrad of  
 microspores.  
 ×1833.

F<sub>1</sub> hybrid, *M. nigra* × *M. alba*: Early stages are similar to those of the parents (Figs. 3 a,b,c).

In M I the number of bivalents varies from 68-81, and that of univalents from 6-32. At times the cells were seen with 84 bivalents and no univalent. Univalents when present conform to the general pattern of chromosomal arrangement at the equatorial plate. At AI almost all the chromosomes diverge to the poles in spite of their some visible lag (Fig. 3d). A few bivalents were seen separated later than the rest.

At M II different chromosome numbers varying from 73-95 were seen at each plate.

Anaphase II and telophase II were usually normal resulting in the formation of normal tetrads. In some, the presence of univalents and laggards sometimes gave rise to daughter nuclei in excess of the usual four (Fig. 3e). Additional nuclei were of smaller size.

Similarities and differences between diploid, 22-ploid and the hybrid in respect of the size of PMCs and their nuclei are given in Table I.

TABLE I  
Size of PMCs and their nuclei in microns at the leptotene stage

Varieties	Size of PMCs		Sizes of nuclei	
	Width	Length	Width	Length
Zarif-tut (2x)	18.00 ± .22	21.67 ± .32	7.66 ± .12	8.71 ± .12
Char-tut (22x)	41.35 ± .54	50.45 ± .85	19.04 ± .21	20.80 ± .21
F <sub>1</sub> hybrid (Char-tut × Zarif-tut: 12x)	27.96 ± .20	35.75 ± .44	19.90 ± .01	22.84 ± .01

### Discussion

Sinoto (1929) and Datta (1954) reported that in *Morus alba*, 4 largest chromosomes associate either as trivalent and a univalent, or a quadrivalent. Unlike the findings of these authors it was observed in the present investigation that these chromosomes behave normally and like the remaining 24, paired regularly as bivalents. Meiosis in the natural 22-ploid *M. nigra* and in the hybrid with *M. alba* was regular resulting in the formation of 14 various types of bivalents



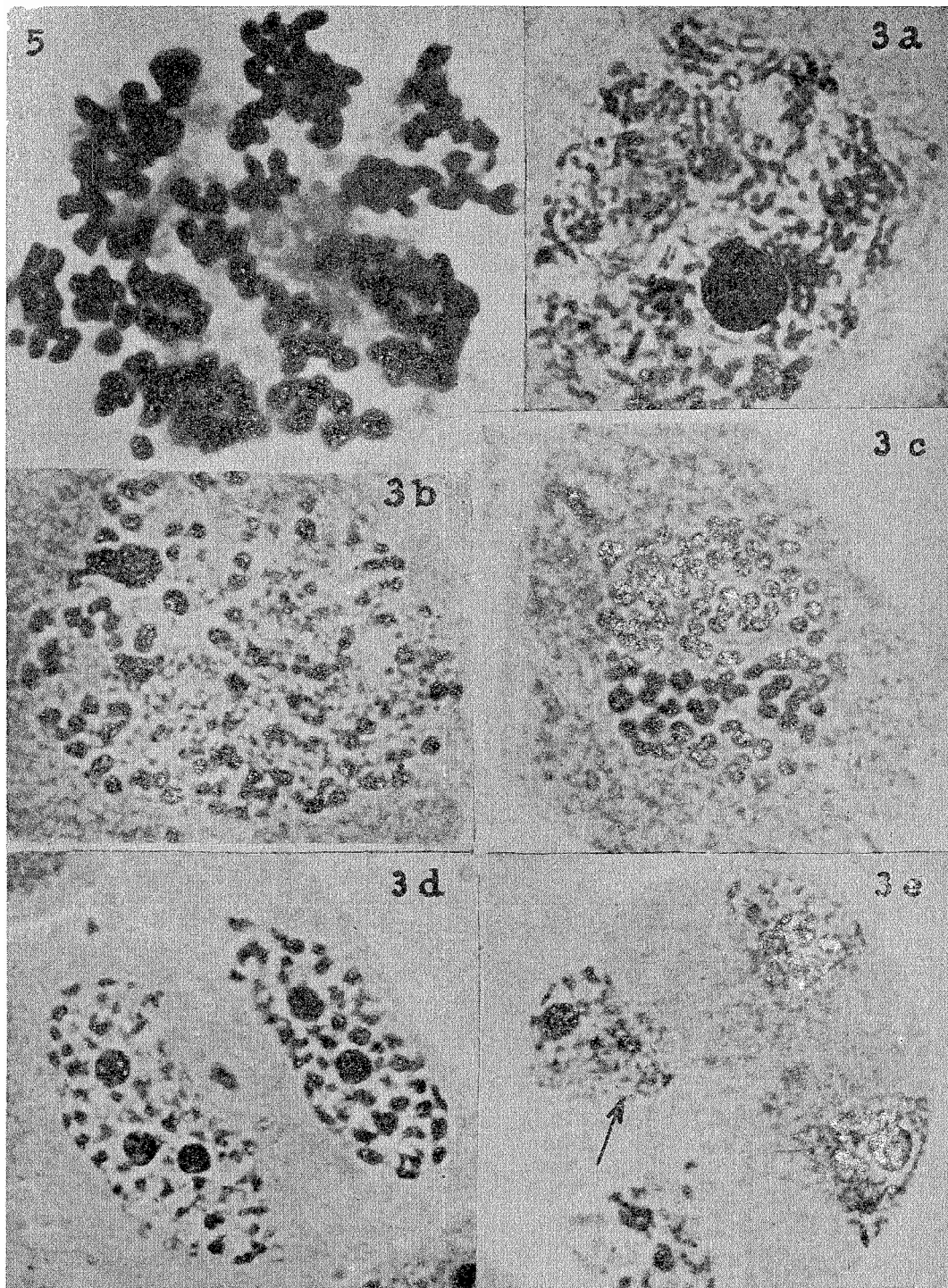


Fig. 3. The meiosis of the hybrid *M. nigra* (22x)  $\times$  *M. alba* (2x).  
 a—diplonema; b—transition stage between diplonema and diakinesis; c—diakinesis;  
 d—interkinesis; e—the formation of 4 primary and one additional nuclei at the telophase II  
 (additional nucleus separated from the primary by segmentation is pointed by an arrow).  
 $\times 1833$ .

Fig. 5. Metaphase plate with 308 chromosomes in a leaf cell of the 2-year old seedling of *M. nigra* species.  
 $\times 2200$ .

represented 11 times and 84 bivalents respectively and is accomplished with the formation of viable microspores.

The occasional presence of regular 84 bivalents in microsporocytes of the hybrid between *M. alba* and *M. nigra* probably is connected with the fact that 14 chromosomes of Zarif-tut variety *M. alba* conjugate with 14 chromosomes of Char-tut form. The remaining 140 chromosomes of Char-tut form made up by 10 haploid sets are grouped into 70 homologous pairs.

From the meiotic study of the hybrid and its parents it is evident that autosyndesis occurs. The presence of univalents side by side with the bivalents is not in contrast with our conclusion of autosyndesis because pairing between homologous chromosomes may be restricted due to some gene brought in through hybridization.

Such a situation may also arise if two or more species as shown below takes part in the origin of *M. nigra* assuming that each participating species has contributed even sets of genomes. In the first possibility 5 species A B C D E have been assumed to contribute two genomes each. In the second, 4 species have been assumed to take part, one contributing 4 sets and so on as follows:

- I—2A+2 B+2 C+2 D+2E
- II—4A+2 B+2 C+2 D
- III—4A+4 B+2 C
- IV—6A+2 B+2 C
- V—6A+4B
- VI—8A+2B
- VII—10A

In the first 6 possibilities we have to do with allopolyploidy, in the 7th with autopolyploidy. Such remarkable regularity can only occur if a certain genetic mechanism is assumed to control such a process similar to that discussed by Riley and Kempner in wheat etc. (1965).

Fairly regular meiosis of 22-ploid *M. nigra* and of the hybrid between *M. nigra* and *M. alba* suggests that this highly polyploid form with 308 chromosomes may have originated by an increase in ploidy level of one and the same genome, with concomitant mutation for restricting multivalent formation. The bivalent formation between the chromosomes of *M. nigra* and *M. alba* points to the same conclusion. Had there been different genomes involved in the origin of 22-ploid

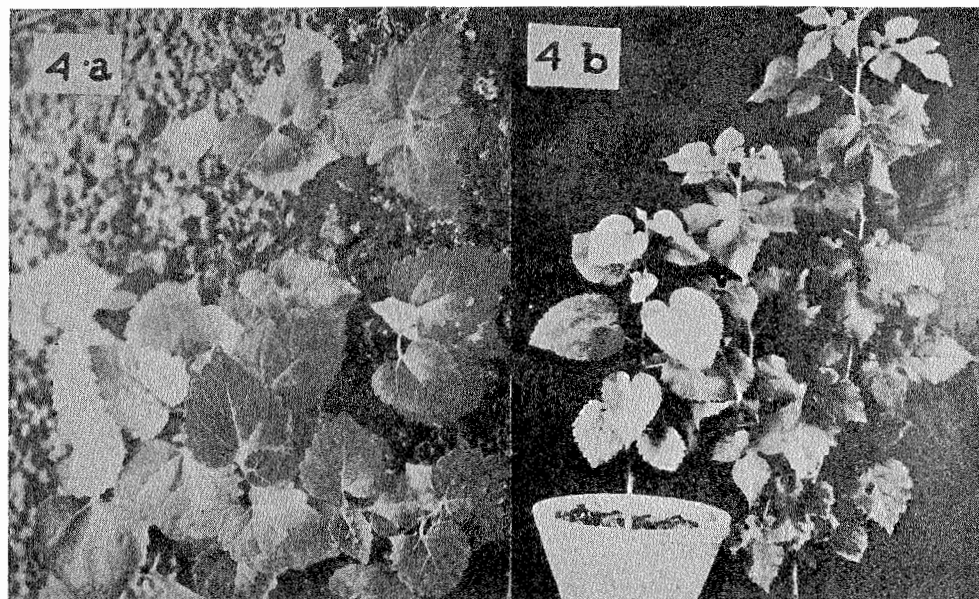


Fig. 4. Seedling of *M. nigra* (22x) from self-pollination.  
a—two months old sprout; b—two years old plant.



Char-tut form, there would have been much more disturbances in chromosome behaviour during microsporogenesis in the PMCs of *M. nigra* and its hybrid with *M. alba*. The presence of bivalents and the absence of multivalents (Breslavetz 1963) in the meiosis of Char-tut form, may be regarded as an evidence of antiquity.

Besides the cytological behaviour, studies on seed germination gives an important clue to the origin of the Char-tut form. This species at present is propagated only by vegetative means. Propagation through seeds in natural or artificial conditions is unknown (Janaki-Ammal 1948). However, Abdulaev and Djafarev (1962, 1965) succeeded in germinating self-pollinated seeds by artificial means. Seedlings raised in this manner are shown in Fig. 4a. Fig. 4 b shows a two-year old plant and Fig. 5 shows 308 chromosomes at mitotic metaphase in a young leaf of a 2-year old plant. This indicates that the seeds of Char-tut form arising through self pollination may give rise to a 22-ploid plant similar in every respect to the population raised through vegetative propagation. The fact that seeds may be induced to germinate supports indirectly the hypothesis of Janaki-Ammal (1948) that the origin of this species took place through recurrent polyploidisation. The capability of seeds of Char-tut form to germinate artificially suggests that in the past propagation took place through seeds. However ancient the species *M. nigra* may be, in all probability, it has originated from the diploid *M. alba* which is far more ancient than *M. nigra*.

In some polyploid groups the increase in ploidy level increases the tendency of seeds to develop through apomixis (Muntzing 1967). In order to circumvent irregular micro- and megasporogenesis some polyploids may gradually take the apomictic mode of reproduction while retaining the capability of reproduction through seeds. Such is not the situation in Char-tut in which apomixis does not seem to occur. The absence of apomixis in this species is also witnessed by such characteristics as normal meiosis with viable microspores, the presence of 308 and 168 chromosomes in somatic cells of seedlings obtained from seeds by self pollination of Char-tut form and by pollination with pollen of diploid varieties respectively.

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