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INTERRELATIONSHIPS AND TAXONOMIC STATUS OF SOME SPECIES OF *SOLANUM NIGRUM* COMPLEX

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ABSTRACT

Biosystematic studies have been carried out on some species of the *Solanum nigrum* complex, *viz*., diploid *S. nigrum*, *S. nodiflorum*, tetraploid *S. nigrum*, *S. luteum* and *S. villosum*. A comparison of fruit colour and chromosome number of the taxa divides them into two groups. Diploid *S. nigrum* and *S. nodiflorum* have shiny bluish black fruits and n = 12 chromosomes. In tetraploid *S. nigrum* the fruits are orange red while in *S. luteum* and *S. villosum* the fruits are yellow and the chromosome number in all the three taxa is n = 24. Meiosis was normal in all the species. The close relationship between diploid *S. nigrum* and *S. nodiflorum*, and among the species tetraploid *S. nigrum*, *S. luteum* and *S. villosum* has been confirmed by cytogenetical studies and statistical comparison of mean values of morphological characters.

In the light of the foregoing studies a taxonomic revision of the species studied is suggested.

INTRODUCTION

The species of the *Solanum nigrum* complex show considerable resemblances among themselves and, as a consequence, their identification and interrelationships have often been a puzzle to the traditional taxonomists. It was, therefore, considered desirable to study the biosystematics of the relevant taxa to remove the confusion. Although some aspects of interrelationships (D'Arcy, 1974) of the species of the *S. nigrum* complex have been studied biosystematically (Westergaard, 1948; Bhaduri, 1951; Tandon and Rao, 1964, 1966; Venkateswarlu and Krishna Rao, 1972; Usha and Kaul, 1974), a good deal remains to be done to understand the dynamic structure of the species of the complex, their relationships, and the genetical basis of their variability. The species included in the *S. nigrum* complex, selected for the present biosystematic investigations, are diploid *S. nigrum* Linn. (n = 12), *S. nodiflorum* Jacq. (n = 12), tetraploid *S. nigrum* Linn. (n = 24), *S. luteum* Mill. (n = 24) and *S. villosum* Mill. (n = 24).

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MATERIALS AND METHODS

The plants of the various species under investigations were obtained from our collections growing in the Botanical Gardens of the Aligarh Muslim University. Several reciprocal cross pollinations were made between diploid *S. nigrum* and *S. nodiflorum* and among the species tetraploid *S. nigrum*. *S. luteum* and *S. villosum*.

A statistical comparison of mean values of morphological characters was made between diploid *S. nigrum* and *S. nodiflorum* and among the species tetraploid *S. nigrum*, *S. luteum* and *S. villosum* by "Student" *t* test, to arrive at a better understanding of the relationships of the taxa.

Meiosis was studied in squashes of pollen mother cells fixed in Carnoy's fluid and made permanent with butyl alcohol (see Swaminathan, Magoon and Mehra, 1954: Bhaduri and Ghosh, 1954). Pollen fertility was determined as the stainability in acetocarmine.

OBSERVATIONS

Morphology of the parents

Diploid S. nigrum and S. nodiflorum:

Diploid *S. nigrum* and *S. nodiflorum* resembled each other in several morphological and cytological characters (Fig. 1). In both the species the fruits were spherical, shiny bluish black and identical in size and shape (Fig. 2), and the gametic chromosome number was 12. A detailed comparative account of morphological characters of the species is presented in Table 1.

A statistical comparison of mean values of morphological characters of diploid *S. nigrum* and *S. nodiflorum* showed a close resemblance between them as indicated by lack of significant differences in most of their morphological characters.

Tetraploid S. nigrum, S. luteum and *S. villosum :*

Tetraploid *S. nigrum, S. luteum* and *S. villosum* resembled each other in several morphological (Fig. 3) and cytological characters. The fruits of all the species were globose and almost identical in size (Fig. 4) but they differed significantly in colour. In *S. luteum* and *S. villosum* the fruits were yellow whereas in tetraploid *S. nigrum* they were orange red. The gametic chromosome number of all the species, as determined from pollen mother cells, was 24 A detailed comparative account of morphological characters of tetraploid *S. nigrum, S. luteum* and *S. villosum* is presented in Table 1.

A statistical comparison of mean values of morphological characters was made among the species tetraploid *S. nigrum, S. luteum* and *S. villosum* and the resemblances among them were found to be significant.

Hybridization :

Several interspecific reciprocal cross pollinations were made between diploid *S. nigrum* and *S. nodiflorum* and among the species tetraploid *S. nigrum, S. luteum* and *S. villosum.* The details of the number of flowers pollinated, the fruit-set, the number of seeds obtained from different crosses are given in Table 2. The Table also shows the number of hybrid seeds sown, the number germinated and the percentage of germination.

Morphology of the F_1 hybrids

Diploid S. nigrum × S. nodiflorum :

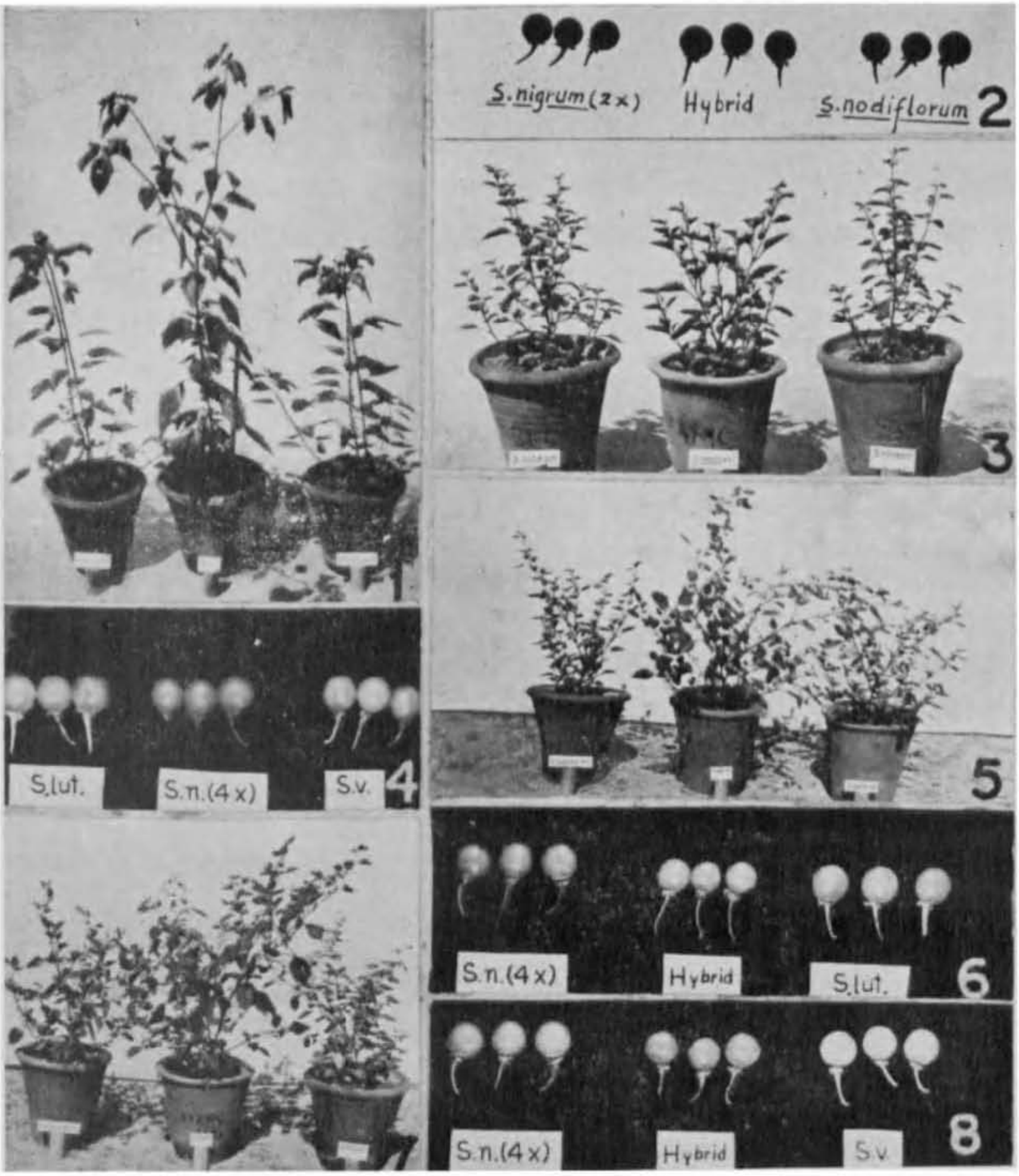
The hybrids of reciprocal cross pollinations were alike morphologically and cytologically. The hybrids were taller than the parents with large, thick, dark green, ovate leaves and large flowers (Fig. 1). They flowered profusely and produced shiny bluish black fruits (Fig. 2) with more seeds per fruit than the parents. The hybrids were late in flowering and continued to grow for a longer duration than the parents. The percentage of pollen fertility of the hybrids was 79.8 whereas that of the parents diploid *S. nigrum* and *S. nodiflorum* was 69.9 and 71.5 respectively. The gametic chromosome number of the hybrids as revealed from a study of pollen mother cells was 12. The hybrids resembled the parents in chromosome number and colour of fruit. A detailed comparative account of morphological characters of the parents and their F_1 hybrids is presented in Table 1.

Tetraploid S. nigrum × S. luteum :

The hybrids (n = 24) were more robust than the parents and flowered profusely. They exhibited hybrid vigour in respect of plant height (Fig. 5), size of leaf, thickness of leaf, number of flowers per inflorescence and number of seeds per fruit (Table 2). The hybrids produced orange red fruits with viable seeds. They resembled the parent tetraploid *S. nigrum* in fruit colour (Fig. 6) and size of pollen. The percentage of pollen fertility of the hybrids was 75.52 whereas in tetraploid *S. nigrum* and *S. luteum* it was 75.10 and 71.50 respectively.

Tetraploid S. nigrum × S. villosum :

The hybrids (n = 24) exhibited heterosis for characters such as height of plant, size of leaf, thickness of leaf (Fig. 7) and diameter of corolla (Table 1). They produced



Figs. 1-8 · 1. Plant of diploid *S. nigrum* (left), *S. nodiflorum* (right) and their F_1 hybrid (middle). 2. Fruits of diploid *S. nigrum* (left), *S. nodiflorum* (right) and their F_1 hybrid (middle). 3. Plant of *S. luteum* (left), tetraploid *S. nigrum* (middle) and *S. villosum* (right). 4. Fruits of *S. luteum* (left), tetraploid *S. nigrum* (middle) and *S. villosum* (right). 5. Plant of tetraploid *S. nigrum* (left), *S. luteum* (right) and their F_1 hybrid (middle). 6. Fruits of tetraploid *S. nigrum* (left), *S. luteum* (right) and their F_1 hybrid (middle). 7. Plant of tetraploid *S. nigrum* (left), *S. villosum* (right) and their F_1 hybrid (middle). 8. Fruits of tetraploid *S. nigrum* (left), *S. villosum* (right) and their F_1 hybrid (middle).

orange red fruits (Fig. 8) with a large number of viable seeds. The hybrids were intermediate between the parents with respect to pollen size but resembled the female parent with respect to fruit colour. The percentage of pollen fertility of the hybrids was as high as 68.26. The percentage of pollen fertility of tetraploid *S. nigrum* and *S. villosum* was 75.10 and 80.70 respectively.

S. luteum × *S. villosum :*

The hybrids (n = 24) exhibited heterosis in respect of plant height (Fig. 9), thickness of leaf and number of flowers per inflorescence (Table 1). They resembled the parents in number of fruits per inflorescence, size of fruit (Fig. 10) and colour of fruit. The hybrids were highly fertile and produced fruits of yellow colour with a large number of viable seeds. The percentage of pollen fertility of the hybrids was 85.50 whereas that of *S. luteum* and *S. villosum* was 71.50 and 80.70 respectively.

Comparison of morphological characters of the hybrids

The hybrids of the crosses, tetraploid *S. nigrum* × *S. luteum*, tetraploid *S. nigrum* × *S. villosum* and *S. luteum* × *S. villosum* were identical in general pattern of morphological and cytological characters (Table 1). They were late in flowering and continued to grow for a longer duration than the parents. The gametic chromosome number of the hybrids as indicated from a study of pollen mother cells was 24. The hybrids showed pronounced variability in pollen fertility and fruit colour. The percentage of pollen fertility of the hybrids tetraploid *S. nigrum* × *S. luteum*, tetraploid *S. nigrum* × *S. villosum* and *S. luteum* × *S. villosum* was quite high and it was 75.50, 68.26 and 85.50 respectively. The hybrids between tetraploid *S. nigrum* and *S. luteum* and tetraploid *S. nigrum* and *S. villosum* produced orange red berries with appreciably good number of viable seeds whereas the hybrids between *S. luteum* and *S. villosum* produced yellow coloured fruits with a large number of viable seeds.

Cytology of the parents

The parents diploid *S. nigrum* (n=12), *S. nodiflorum* (n=12), tetraploid *S. nigrum* (n=24), *S. luteum* (n=24) and *S. villosum* (n=24) behaved normally in meiosis, giving bivalents. Typical metaphase I plates from the parents are illustrated in figures 11, 12, 13, 14 and 15. Data on chromosome association at metaphase I are given in Table 3.

Cytology of the hybrids

Diploid S. nigrum × S. nodiflorum.

The meiotic behaviour of chromosomes was normal. Twelve bivalents were observed at both diakinesis and metaphase I (Fig. 16). At diakinesis and metaphase I, the chiasma frequency per bivalent was 1.76 and 1.34 respectively. Data on chromosome pairing at metaphase I are given in Table 3.

Tetraploid S. nigrum × S. luteum :

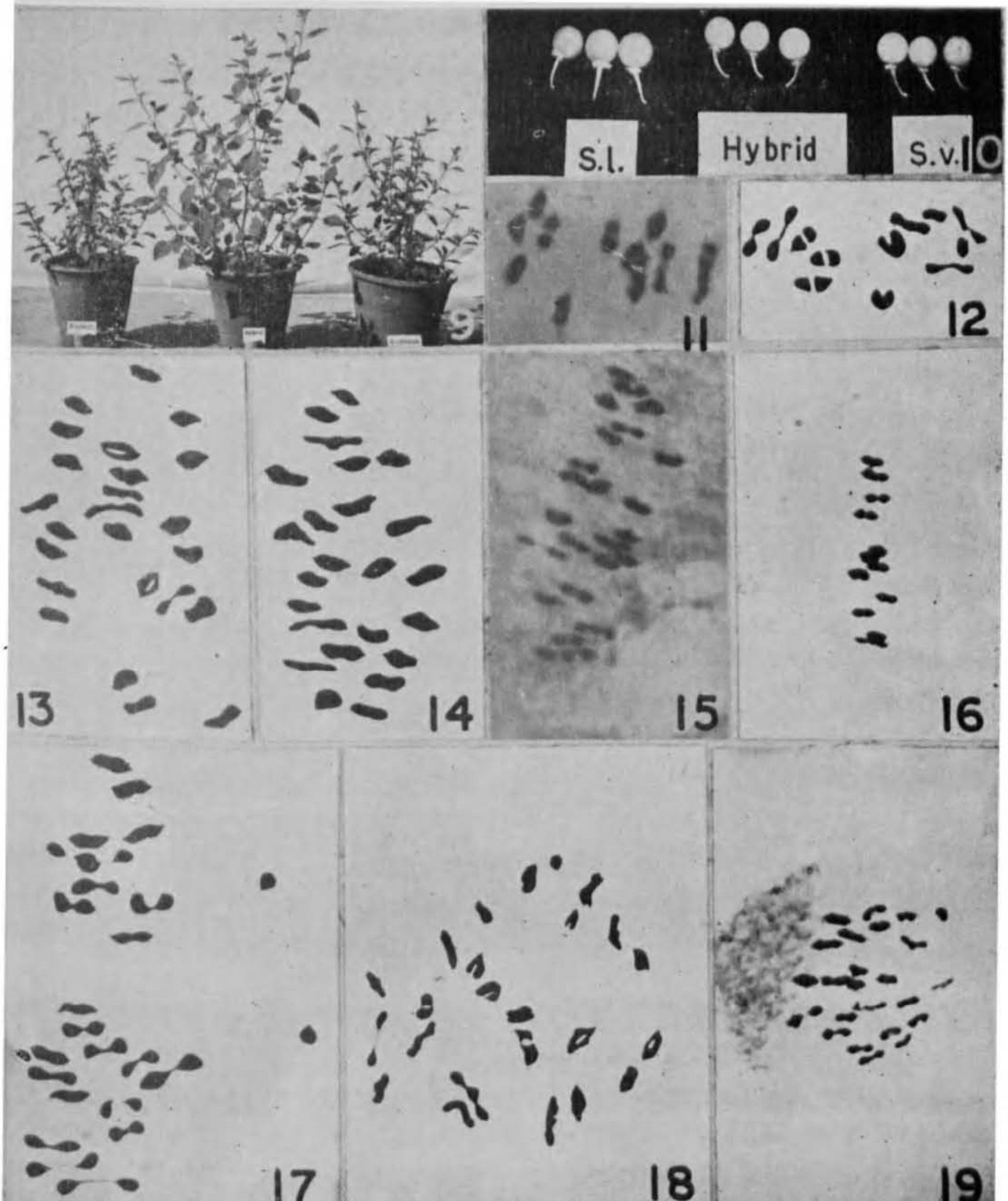
The course of meiosis was mostly normal. At diakinesis, in several pollen mother cells, 24 bivalents were observed. The mean number of univalents, bivalents and quadrivalents recorded per cell at diakinesis was 0.08, 23.16 and 0.40 respectively. The mean chiasma frequency per bivalent was 1.70.

At metaphase I, 80 per cent of the pollen mother cells showed 24 bivalents whereas the rest of the cells showed univalents (Fig. 17) and quadrivalents. The mean number of univalents, bivalents and quadrivalents estimated per cell was 0.24, 23.72 and 0.08 respectively (Table 3). The mean number of univalents and bivalents per cell increased from diakinesis to metaphase I with a corresponding decrease in the mean number of quadrivalents. At metaphase I, the chiasma frequency per bivalent was 1.09.

Anaphase I was normal in 96 per cent of the cells with 24 chromosomes at each pole. However, in a few cases the distribution was unequal. Laggards or bridges with or without fragments were not seen at anaphase I. Micronuclei were not observed at telophase I and II. Tetrads were normal.

Tetraploid S. nigrum × S. villosum :

Most of the pollen mother cells showed regular meiosis with 24 bivalents. In addition to bivalents, occasional occurrence, at diakinesis, of univalents, trivalents and quadrivalents was also recorded. The maximum number of univalents, trivalents and quadrivalents recorded was 2 whereas the



Figs. 9-19: 9. Plant of *S. luteum* (left), *S. villosum* (right) and their F_1 hybrid (middle). 10. Fruits of *S. luteum* (left), *S. villosum* (right) and their F_1 hybrid (middle). 11. and 12. M_1 in diploid *S. nigrum* and *S. nodiflorum* with 12II. 13-15. M_1 in tetraploid *S. nigrum*, *S. luteum* and *S. villosum* with 24II. 16-19. Meiosis in F_1 hybrids 16. Diploid *S. nigrum* × *S. nodiflorum*: M_1 with 12II. 17. Tetraploid *S. nigrum* × *S. luteum*: M_1 with 23II + 2I. 18. Tetraploid *S. nigrum* × *S. villosum*: M_1 with 22II + 1I + 1III. 19. *S. luteum* × *S. villosum*: M_1 with 23II+2I.

bivalents ranged from 17 to 24. At diakinesis, the chromosome association per cell was 0.48 univalents, 22.08 bivalents, 0.32 trivalents and 0.56 quadrivalents. The mean chiasma frequency per bivalent was 1.80.

The mean frequency of chromosome associations estimated at metaphase I was 0.56 univalents, 23.08 bivalents, 0.16 trivalents and 0.20 quadrivalents (Table 3; Fig. 18). The univalents ranged from 0 to 3 whereas the number of either trivalents or quadrivalents did not exceed one. At metaphase I, the frequency of univalents and bivalents per cell was more than at diakinesis and this increase was followed by a corresponding decrease of trivalents and quadrivalents from diakinesis to metaphase I. The chiasma frequency per bivalent was 1.17.

Anaphase I was normal in 76 per cent of the cells with 24 chromosomes at each pole. Lagging chromosomes ranging from 1 to 2 were observed in 20 per cent of the cells. Micronuclei were not seen at telophase I. Occasionally, they were recorded at telophase II (16 per cent of the cells) ranging from 1 to 3. The tetrads were mostly normal.

S. luteum × *S. villosum*: At diakinesis, in a great majority of the pollen mother cells, 24 bivalents were observed. Occasionally, quadrivalents were recorded and they ranged from 1 to 4. Univalents were absent. The mean chromosome pairing per cell, at diakinesis, was typified by 23.12 bivalents and 0.44 quadrivalents. The mean chiasma frequency per bivalent was 1.77.

At metaphase I, 84 per cent of the cells showed 24 bivalents whereas the remainder showed univalents and quadrivalents besides the bivalents (Fig. 19). The maximum number of univalents and quadrivalents was 2 and 1 respectively. At metaphase I, the mean number of univalents, bivalents and quadrivalents recorded was 0.08, 23.64 and 0.16 respectively (Table 3).

Anaphase I was normal with 24 chromosomes at each pole. Cells with laggards or unequal number of chromosomes at poles were not encountered. Micronuclei were not observed at telophase I and II.

DISCUSSION

Diploid *S. nigrum* resembles *S. nodiflorum* in several morphological and cytological characters. Their identity was further corroborated by a statistical comparison of mean values of morphological characters. The two taxa crossed readily with each other producing fertile hybrids with 12 bivalents at both diakinesis and metaphase I. Chromosome pairing in hybrids provide an index of the degree of homology between the genomes. The formation of 12 bivalents in the hybrids indicates the allosyndetic pairing of chromosomes. The mean chiasma frequency of the hybrids was comparable to the parents. Thus the karyomorphological studies, a statistical comparison of mean values of morphological characters of diploid *S. nigrum* and *S. nodiflorum*, and crossability with each other, producing fertile hybrids with normal meiosis, indicate that the two taxa are closely related to each other. In fact the relationship is so close that the two species together seem to constitute one taxon.

The species tetraploid *S. nigrum*, *S. luteum* and *S. villosum* resemble one another in several cytomorphological characters. The close similarity of the three taxa is corroborated by a statistical comparison of mean values of morphological characters. A close genetic relationship among the species is exemplified by their ready crossability with each other producing fertile hybrids with as many as 23 bivalents, at both diakinesis and metaphase I, in several pollen mother cells. The formation of such a large number of bivalents indicates the preponderance of allosynaptic pairing of chromosomes.

Thus the cytomorphological studies, a statistical comparison of mean values of

TABLE 1

Comparison of morphological characters of species of *S. nigrum* complex and their F_1 hybrids

Characters	Diploid *S. nigrum*	*S. nodiflorum*	Diploid *S. nigrum* × *S. nodiflorum*	Tetraploid *S. nigrum*	*S. luteum*	*S. villosum*	Tetraploid *S. nigrum* × *S. luteum*	Tetraploid *S. nigrum* × *S. villosum*	*S. luteum* × *S. villosum*
Habit	Erect and branched	Erect and branched	Erect and branched	Erect and branched	Erect and branched	Erect and branched	Erect and branched	Erect and branched	Erect and branched
Height (cm)	57.00-92.00	40.00-90.00	85.00-160.00	50.50-85.50	52.00-90.00	49.00-79.00	82.00-98.80	70.00-90.00	70.00-102.00
Stem	Slender and dark green without prominent ribs	Slender and dark green without prominent ribs	Slender and dark green without prominent ribs	Thick and green with purplish tinge. Ribs prominent	Thick and green without prominent ribs	Green without prominent ribs	Thick, green with prominent ribs	Green with less prominent ribs	Thick and green without prominent
Leaf	Thick and ovate with dentate margin. Petiole marginate	Thick and ovate with ill defined dentate margin. Petiole marginate	.Thick and ovate with ill defined dentate margin. Petiole marginate	Thick and ovate with dentate margin. Petiole marginate	Thick and ovate with sparsely dentate margin. Petiole marginate	Thick and ovate with dentate margin. Petiole marginate	Thick and ovate with dentate margin. Petiole marginate	Thick and ovate with sparseiy dentate margin. Petiole marginate	Thick and ovate with sparsely dentate margin. Petiole marginate
Length of petiole (cm)	1.00-5.00	1.40-4.50	2.50-7.00	1.50-4.50	1.20-3.50	1.60-3.00	1.50-5.00	2.00-5.50	1.00-3.90
Length of lamina (cm)	7.30-12.30	5.90-11.00	6.70-15.20	4.40-7.80	4.60-8.30	4.00-8.00	4.10-9.20	4.40-9.60	4.70-9.00
Breadth of lamina (cm)	3.90-7.60	3.50-7.00	4.50-9.20	3.30-5.40	2.70-5.00	2.40-4.40	2.70-6.40	2.70-6.90	2.80-6.00
Thickness of leaf (μ)	29.60-55.50	25.90-57.30	27.70-77.70	29.60-47.60	43.60-74.60	36.20-66.60	29.20-82.10	48.10-98.00	55.50-88.80
No. of flowers per inflorescence	3-5	3-5	2-5	2-5	2-5	2-5	2-6	3-5	2-5
Diameter of corolla	4.80-7.00	5.00-8.00	5.00-7.00	7.00-15.00	9.00-15.00	8.50-13.00	10.00-15.00	7.00-16.00	9.50-15.00
No. of fruits per inflorescence	1-5	2-5	2-5	2-5	2-5	2-5	2-5	2-5	2-5
Diameter of fruit (mm)	4.00-8.00	4.00-8.00	5.00-8.00	5.00-8.50	6.00-9.00	5.00-8.50	7.00-8.00	6.00-9.00	6.20-9.00
Colour of fruit	Shiny bluish black	Shiny bluish black	Shiny bluish black	Orange red	Yellow	Yellow	Orange red	Orange red	Yellow
No. of seeds per fruit	1-67	3-70	13-71	9-40	15-50	14-49	28-52	20-42	19-54
Diameter of pollen grain (μ)	12.60-27.00	12.60-25.20	16.60-22.90	14.40-30.60	14.40-32.40	18.00-36.00	10.80-30.20	14.40-32.40	12.60-34.20
Percentage of pollen fertility	69.90	71.50	79.80	75.10	71.50	81.70	75.50	68.26	85.50
Gametic chromosome number (n)	12	12	12	24	24	24	24	24	24

TABLE 2

Crossability within the species of *S. nigrum* Complex

Cross	Total no. of flowers pollinated	Total no. of fruits obtained	Total no. of seeds obtained	Total no. of seeds sown	Percentage of germination
S. nigrum (2x) × *S. nodiflorum*	52	15	203	100	77.00
S. nigrum (4x) × *S. luteum*	31	11	314	100	94.00
S. nigrum (4x) × *S. villosum*	23	7	223	100	36.00
S. luteum × *S. villosum*	65	29	815	100	79.00

TABLE 3

Chromosome association and chiasma frequency at metaphase I

Material	No. of PMCs studied	Mean frequency per cell of				Mean x per Cell	frequency per bivalent
		Uni-valents	Bi-valents	Tri-valents	Quadri-valents		
S. nigrum (2x)	50	—	12	—	—	13.20	1.10
S. nodiflorum	50	—	12	—	—	19.12	1.59
S. nigrum (4x)	50	0.16	23.92	—	—	30.12	1.25
S. luteum	50	—	24	—	—	31.52	1.31
S. villosum	50	—	24	—	—	25.32	1.05
S. nigrum (2x) × *S. nodiflorum*	50	—	12	—	—	16.20	1.34
S. nigrum (4x) × *S. luteum*	50	0.24	23.72	—	0.08	26.32	1.09
S. nigrum (4x) × *S. villosum*	50	0.56	23.08	0.16	0.20	27.56	1.17
S. luteum × *S. villosum*	50	0.08	23.64	—	0.16	29.92	1.08

morphological characters among tetraploid *S. nigrum, S. luteum* and *S. villosum,* and crossability with one another, producing fertile hybrids with a high degree of normal course of meiosis, indicate their close genetic relationship.

The morphological diversification of Indian tetraploid *S. nigrum* particularly with reference to its fruit colour from *S. luteum* and *S. villosum* is very weak. The fruits of *S. luteum* and *S. villosum* are light yellow whereas the fruits of tetraploid *S. nigrum* are orange red. The variation of fruit colour of tetraploid *S. nigrum* from *S. luteum* and *S. villosum* appears to be mostly due to genic differences, as indicated by the occurrence of a few univalents at diakinesis and metaphase I of the hybrids between tetraploid *S. nigrum* and *S. luteum* and *S. villosum.*

S. luteum and *S. villosum* cannot be separated morphologically and cytogenetically from each other and moreover, the species are interfertile and capable of gene exchange. It is obvious from literature (Jackson. 1946) that the same author has described the same species and named it as *S. luteum* and *S. villosum.* Since the meaning of the word luteum (luteus = deep orange yellow) is more meaningful than villosum, it is recommended that the name *S. luteum* should be retained whereas the name *S. villosum* should be abandoned.

In the light of the information now available it is suggested that (a) diploid forms of *S. nigrum* should be merged with *S. nodiflorum* and the entire group should be recognized as *S. nodiflorum,* and, (b) the species tetraploid *S. nigrum, S. luteum* and *S. villosum* should be collectively regarded as one species known as *S. luteum.* Since tetraploid *S. nigrum* shows heritable difference in fruit colour (orange red) from the other taxa (fruit yellow) it should be recognized as ecotype (Gregor, Davey and Lang, 1936) or subspecies of *S. luteum* (Bhaduri, 1951 ; Tandon and Rao, 1966).

The taxonomic position on the basis of bio-systematic information is summarised as follows:

Diploid *S. nigrum* Linn.	= *S. nodiflorum* Jacq.
S. luteum Mill. } *S. villosum* Mill. }	= *S. luteum* Mill.
Tetraploid *S. nigrum* Linn.	= Sub-species of *S. luteum* Mill.

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