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Polyploidy in a Cultivar of Black Pepper (*Piper nigrum* L.) and Its Open Pollinated Progenies

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Black pepper (*Piper nigrum* L., Piperaceae) is a predominantly self pollinated crop and propagated by vegetative cuttings, even though seeds are fully fertile (Dewaard and Zeven 1969, Sasikumar *et al.* 1991). Cultivated black pepper is a diploid of polyploid origin, with 2n = 4x = 52 (Mathew 1958, 1973). Variability in open pollinated progenies of black pepper was reported by Ibrahim *et al.* (1986). However reports on distinct morphological variants in open pollinated progenies of black pepper are lacking, except the one by Ravindran and Sasikumar (1992). They reported 18 distinct types of morphological variants in the seedling progenies of a black pepper cultivar (Collection No: 1344), and observed high amount of variation with respect to many seedling traits. The cultivar is characterised by unusually bold berries, loose spike, late maturity, large leaves and robust stem. A population of progenies of this cultivar raised from the open pollinated seeds is found to show variations in morphology and growth which is not frequent in the open pollinated progenies of the other cultivars of black pepper, being a predominantly self pollinated crop. In some of the progenies the initial variation sustained with growth, for leaf size and shape, leaf thickness, leaf tip, margin and leaf base, length of petiole and internodes, stem thickness, colour of petiole shoot tip and stem, besides variation in growth rate and vigour. A few of the variant progenies resembled wild species of *Piper*.

To ascertain cytological reasons for this unusual and distinct variation in the progenies, the mother plant along with 20 of its most distinct progenies including a few apparently normal ones were analysed cytologically. The present paper reports the result of the study.

Materials and methods

Actively growing root tips were collected from rooted cuttings of the mother plant and 20 of its progenies (Table 1). Roots were collected between 11:00 and 12:00 AM and treated with 1% solution of α-bromonaphthalene at 4–5°C for 4 hr. After that, the materials were washed thoroughly in distilled water and fixed in a 3:1:1 mixture of ethyl alcohol, acetic acid and chloroform for 24 hr.

The fixed root tips were hydrolysed with 1N HCl at 60°C for 4–6 min and stained in 2% lactopropionic orcein for 4 hr and squashed in 45% propionic acid. Five well spread mitotic metaphase plates each from two slides were used for counting chromosome numbers. Slides were made permanent after taking photomicrographs.

The mother plant and progenies have been maintained under uniform nursery conditions.

Observations and results

Considerable variations were observed in chromosome numbers in mother plant and its

Table 1. Somatic chromosome number and morphological characters of Coll. No: 1344 and its progenies

Plant No.	Somatic chromosome number	Important morphological characters						
		Shoot tip colour	Leaf shape	Leaf length (mean ± SD; cm)	Leaf breadth (mean ± SD; cm)	Petiole length (mean ± SD; cm)	Internode length (mean ± SD; cm)	Girth of stem (mean ± SD; cm)
1. Collection No-1344 (MP)	2n = 78	Purple	Cordate	15.3 ± 1.42	10.91 ± 0.92	7.09 ± 0.98	6.17 ± 0.66	1.04 ± 0.01
2. OP 3	2n = 52	White	Elliptical	13.44 ± 1.00	7.74 ± 0.65	4.40 ± 0.78	4.58 ± 1.47	1.29 ± 0.09
3. OP 5	2n = 104	Purple	Cordate	12.92 ± 1.23	8.6 ± 0.60	7.55 ± 0.68	4.67 ± 1.18	1.87 ± 0.08
4. OP 7	2n = 78	Purple	Elliptical	10.1 ± 1.14	5.86 ± 0.94	3.21 ± 0.73	3.07 ± 0.49	1.18 ± 0.22
5. OP 10	2n = 73	Light purple	Elliptical	7.29 ± 1.35	3.82 ± 0.59	2.62 ± 0.64	1.75 ± 0.81	0.97 ± 0.13
6. OP 13	2n = 65	Light purple	Elliptical	7.55 ± 0.86	3.78 ± 0.25	4.01 ± 0.76	2.07 ± 0.31	1.27 ± 0.19
7. OP 18	2n = 78	Purple	Elliptic-lanceolate	5.08 ± 0.14	1.94 ± 0.30	2.99 ± 0.59	4.58 ± 1.59	1.01 ± 0.03
8. OP 24	2n = 65	Purple	Ovate	5.78 ± 0.82	3.71 ± 0.60	2.56 ± 0.74	2.21 ± 0.65	0.85 ± 0.22
9. OP 26	2n = 65	White	Ovate	7.05 ± 0.21	3.64 ± 0.53	3.3 ± 0.36	4.11 ± 0.70	1.13 ± 0.12
10. OP 27	2n = 55	Purple	Cordate	14.07 ± 1.05	9.49 ± 1.38	4.71 ± 0.73	6.77 ± 2.47	1.49 ± 0.18
11. OP 30	2n = 65	Purple	Cordate	7.06 ± 0.47	3.86 ± 0.53	2.99 ± 0.32	2.31 ± 0.45	1.18 ± 0.13
12. OP 36	2n = 78	Purple	Ovate	9.49 ± 0.90	6.73 ± 0.71	4.85 ± 1.36	4.56 ± 0.97	1.3 ± 0.25
13. OP 38	2n = 78	Purple	Cordate	8.38 ± 1.38	6.75 ± 1.03	6.09 ± 1.23	3.14 ± 0.25	1.14 ± 0.18
14. OP 42	2n = 65	Purple	Ovate	9.03 ± 1.03	5.84 ± 0.99	3.87 ± 0.55	4.37 ± 1.03	1.23 ± 0.09
15. OP 59	2n = 82	Purple	Ovate-lanceolate	10.3 ± 1.76	4.77 ± 1.04	4.45 ± 0.29	5.20 ± 1.36	1.03 ± 0.08
16. OP 67	2n = 78	Green	Elliptical	6.98 ± 0.80	3.1 ± 0.24	2.6 ± 0.38	2.46 ± 0.42	1.02 ± 0.01
17. OP 77	2n = 72	Purple	Cordate	8.6 ± 0.99	6.56 ± 1.02	3.65 ± 0.58	2.21 ± 0.65	1.07 ± 0.02
18. OP 84	2n = 76	Purple	Elliptical	6.25 ± 0.63	2.82 ± 0.52	3.08 ± 0.90	2.17 ± 0.51	1.12 ± 0.11
19. OP 88	2n = 82	Purple	Elliptical	6.95 ± 1.08	3.63 ± 0.44	2.66 ± 0.55	3.34 ± 1.02	1.04 ± 0.05
20. OP 91	2n = 82	Purple	Ovate	5.02 ± 0.14	3.51 ± 0.19	2.04 ± 0.27	1.55 ± 0.10	1.5 ± 0.0
21. OP 117	2n = 78	Purple	Lanceolate	4.83 ± 0.52	1.77 ± 0.13	2.59 ± 0.32	1.39 ± 0.34	1.02 ± 0.04

MP—Mother plant. OP—Open pollinated progeny.

progenies (Table 1).

From the results it is clear that the cytological variation is only slightly smaller than the morphological variation in the progenies. (Figs. 1, 2).

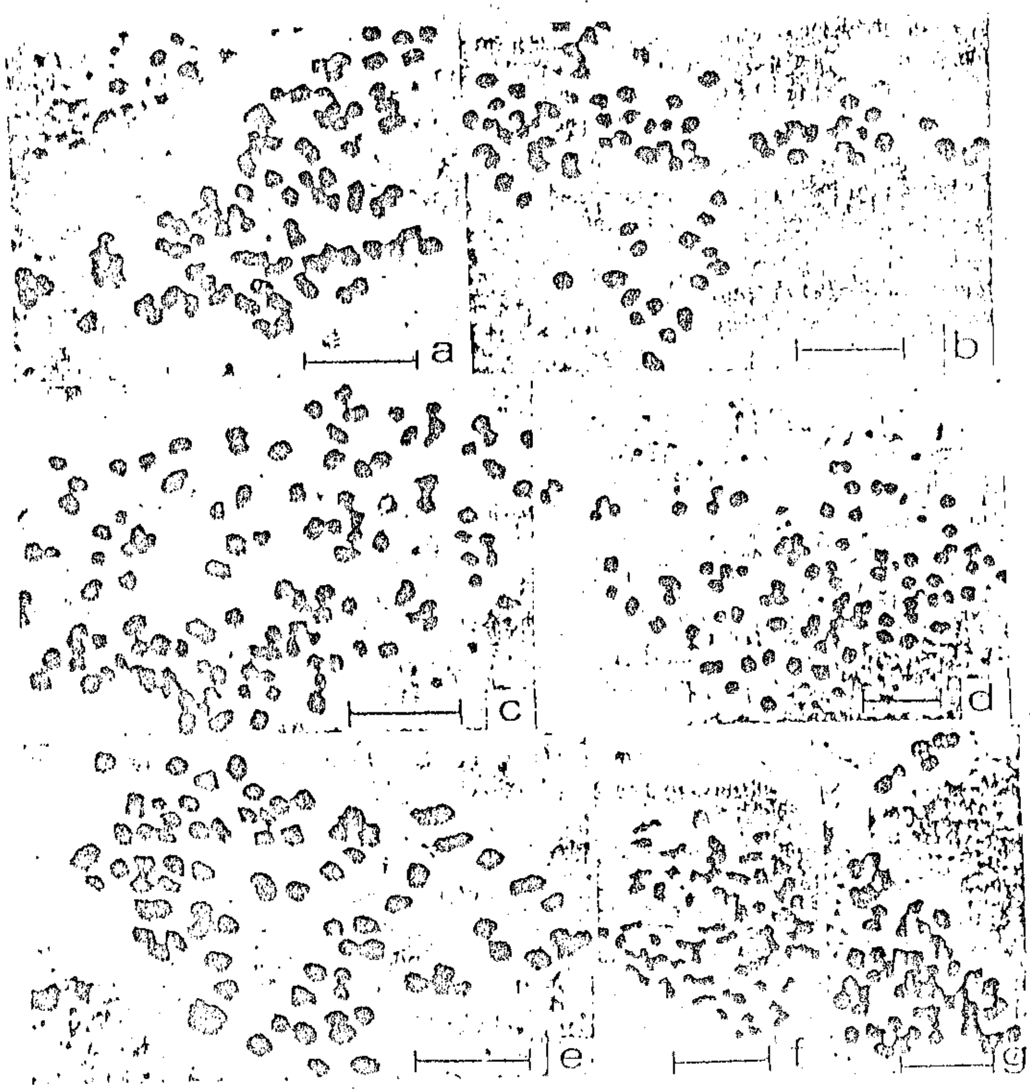

Fig. 1: Mitotic metaphase of Coll. No. 1344 (MP) and some of its progenies: a) Coll. No. 1344 (2n=78). b) OP.10 (2n=73). c) OP.5 (2n=104). d) OP.117 (2n=78). e) OP.18 (2n=78). f) OP.13 (2n=65). g) OP.27 (2n=55). Bar represents 4 μm

Discussion

The somatic chromosome number in all the cultivated, and in many of the wild, varieties of *Piper nigrum* is reported as 2n=52 (Mathew 1958, Martin and Gregory 1962, Mathew 1973, Samuel and Bavappa 1981, Jose and Sharma 1984, Okada 1986, Samuol and Morawetz 1989). Somatic chromosome number of 2n=104 was reported in a few wild varieties of *P. nigrum* by Mathew (1958, 1973) and Jose and Sharma (1984). Variable chromosome numbers of 2n=36 and 2n=60 in *P. nigrum* were also reported by Dasgupta and Datta (19[illegible]). In the present study, somatic chromosome number of 2n=78 was found in many of mitotic metaphase plates of the cultivar Coll. No. 1344, a number not previously reported, either in cultivated or wild varieties of *P. nigrum*. Progenies of the cultivar showed a range of chromosomal variation from 2n=52 to 2n=104. Out of the 20 progenies examined, six showed chromosome number of 2n=78, five 2n=65, three 2n=82, and the other six with chromosome number (2n) 52, 55, 72, 73, 76 and 104.

The genus *Piper* has a series of polyploid species having basic chromosome number x=13 (Mathew 1958, Okada 1986, Samuel and Morawetz 1989). Even though another basic number of x=12 was suggested by Dasgupta and Datta (1976) and Jose and Sharma (1984), it is restricted to two species, *P. cubeba* and *P. magnificum*. Mathew (1973) suggested that *P. nigrum* can be a diplodized tetraploid with 2n=4x=52. He observed secondary association of bivalents in metaphase I of meiosis in a wild variety of *P. nigrum* with 2n=52. He also reported somatic chromosome number 2n=104 in two wild plants of *P. nigrum*. It is inferred that the present cultivar with 2n=78 could have originated by natural crossing between 2n=52 and 2n=104 plants. The cultivar studied also showed a few features like loose spike (partial sterility), thick stem, large leaves, bold berries *etc.* suggesting hybridity. The variation in morphological characters of the progenies having the same chromosome number may indicate the allopolyploid nature of the parent. The morphological resemblance of a few of the progenies of the above cultivar to the wild varieties may indicate involvement of wild species in the origin of this cultivar.

Fig. 2: Morphology of black pepper Coll. No. 1344 (MP) and some of its progenies: a, Coll. No. 1344 b, OP.10. c, OP.5. d, OP.117. e, OP.18. f, OP.13. Bar represents 5 cm.

It is reported that autotriploids and inter-specific hybrids can produce pollen grains containing variable number of chromosomes, which result in progenies with variable chromosome numbers (Bezbaruah 1976, Sapre and Deshpande 1986, Tyagi and Ahmed 1989, Sapre and Mishra 1990). In autopolyploids it is due to random segregation of one of the chromosomes in trivalent and in interspecific hybrids it is due to random segregation of unpaired chromosomes. Cytomixis in pollen mother cells will also produce pollen grains with variable chromosome number (Murty and Tiwari 1986, Kundu and Sharma 1988, Sen and Bhattacharya 1988). Cytomixis is common in interspecific hybrids and it is one of the causes for pollen sterility in most cases, even though it produces viable pollen grains with aneuploid chromosome numbers (Murthy and Tiwari 1986, Sapre and Deshpande 1987, Bhal and Tyagi 1988, Kundu and Sharma 1988, Sen and Bhattacharya 1988).

In the present study, among 20 morphological variants analysed, eight were aneuploids of higher level. The morphological variation reflects presence or absence of one or a group of chromosomes which are to be identified by karyotypic analysis or chromosome banding. To reveal the mechanism by which the wide range of chromosome numbers arises among the progenies of this cultivar requires a detailed meiotic study.

Summary

Polyploidy in a cultivar (Collection No: 1344) of black pepper (*Piper nigrum* L.) and numerical chromosomal variation in its twenty progenies were identified by cytological analysis. The plant was a triploid with chromosome number 2n=78, and the progenies showed a range of variation from 2n=52 to 2n=104. Morphological types were observed with variation in chromosome number. Possible origin of the mother plant is also discussed.

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