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CYTOGENETIC STUDIES OF THE F_1 HYBRID *ABELMOSCHUS ESCULENTUS* x *A, TETRAPHYLLUS* AND ITS AMPHIDIPLOID

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Abstract: The F_1 hybrids, (*A.esculentus x A. tetraphyllus* resembled the wild parent and exhibited hybrid vigour. Its meiosis was abnormal snowing more of univalents and less of bivalents and was completely sterile. The amphidiploid *(A. esculentus-tetraphyllus)* obtained through colchicine treatment was fully fertile. It showed a low multivalent formation during meiosis and can survive as a new synthetic species. The practical value of the amphidiploid in okra breeding has been indicated.

INTRODUCTION

Cultivated varieties of okra are susceptible to a virus disease caused by yellow vein mosaic virus (YVMV). Intervarietal breeding programme thus was found to be of no value. At the same time, some of the wild related species of *Abelmoschus* were found to contain genes resistant to this disease. *A. tetraphyllus* is one of those species which possesses resistance to YVMV (Ugale *et al.*, 1976). The present sutudy reports about the morphological and cytological observations of the F_1 hybrid *A. esculentus* x *A. tetraphyllys* and its derived amphidiploid.

MATERIALS AND METHODS

The plant materials used in this study were cultivated okra *(A. esculentus)* variety IIHR 20-31, the interspecific hybrid of *A. esculentus* x *A. tetraphyllus* and their amphidiploid. The amphidiploid was obtained by treating the vegetative buds of the interspecific F_1 hybrid with 0.1 per cent colchicine solution for 3 hours.

For meiotic studies, the floral buds were first fixed in Carnoy's B fixative containing 6 parts of ethyl alcohol, 3 parts of chloroform and 1 part of glacial acetic acid between 7.30 am to 8.30 am for 24 hours. Later the flower buds were transferred into another fixative containing 3 parts of ethyl alcohol and 1 part of glacial acetic acid, the acetic acid portion was saturated with ferric chloride. Anthers were smeared in 1 per cent acetocarmine. The pollen was stained in a preparation as per Alexander (1980).

The F_1 hybrid was obtained by using *A. esculentus* as well as *A. tetraphyllus* as the pistillate parents. The ploidy level of the colchicine treated F_1 plants was checked by counting the chromosomes in the pollen mother cells (PMCs) and its configuration. Observations on morphological characters, meiosis and pollen were carried out in the parents, F_1 and amphidiploid.

RESULTS AND DISCUSSION

Cytological studies confirmed the chromosome number of *A. esculentus* line IIHR 20-31 as 2n = 130. The chromosome configuration at metaphase I was regular forming 65 bivalents. Pollen stainability was 96.01 per cent and its average diameter was 140.2 μm. The chromosome polymorphism in *A. esculentus* has been reported by several workers. The most frequent values of chromosome count come between 108 and 144 somatic chromosomes. After

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detailed cytological investigations, Kuwada (1966) decided on 2n = 124 and Joshi and Hardas (1956) on 2n = 130 as in the present studies. The course of meiosis in *A. tetraphyllus* was studied and confirmed its chromosome number 2n = 138. The chromosomal association consisted of 69 bivalents. The average pollen stainability was 94.75 per cent and diameter 143.3 μm. Reported chromosome number of *A. tetraphyllus* also varies. Ugale *et al,* (1976) stated it as 2n = 130, but in the present investigation, the chromosome count 2n = 138 was found for this species, which is in conformity with Gadwal (1976).

Interspecific hybridisation between *A. esculentus* and *A. tetraphyllus* was carried out in both directions successfully. The fruit set in the cross *A. esculentus* x *A. tetraphyllus* was 65 per cent and that of *A. tetraphyllus* x *A. esculentus* was 58.53 per cent. The seed set was normal in the reciprocal crosses and the seeds were well filled.

Meiosis in the interspecific hybrid was critically observed and its chromosome number was ascertained as 2n = 134. The chromosomal association in metaphase I consisted of univalents and bivalents (Table 1) in the 30 PMCs studied. The number of bivalents ranged from 24 to 46 and the maximum number of bivalents observed in 13.33 per cent of the PMCs. Pollen mother cells with 36 bivalents were more frequent and accounted for 56.66 per cent of the total. This observation is in agreement with Ugale *et al.* (1976) who observed that one genome is common between *A. esculentus* and *A. tetraphyllus.* The F_1 hybrid showed 76.42 per cent pollen fertility, still it did not set any seeds on selfing. Though Stebbins (1958) reported that in interspecific hybrids, the male gametes are more easily upset than the female ones, sterility in the female gametes had been observed in interspecific crosses by Greenleaf (1942), Davis (1955) and Ar-Rushdi (1956). So sterility in the female side might have rendered the F_1 hybrid completely sterile.

Meiosis in the C_2 generation amphidiploid was studied and its chromosome number was confirmed as 2n = 268. The chromosome configuration of metaphase I was more or less regular forming 134 bivalents. Pollen stainability was 94.14 per cent and the mean diameter of fertile pollen grains was 215.6 μm. In the amphidiploid, the frequency of multivalents was as low as three (Table 2), eventhough 46 bivalents were observed in the F_1 hybrid. This can be attributed to the preferential pairing of chromosomes (Darlington, 1937). Presumably, the parental chromosomes are similar enough to pair in the F_1, yet they differ in small segments, that is the cryptic chromosomal differences (Stabbins, 1950) of the species involved. Burnham (1962) observed that in genera having small chromosomes, there will be low frequency of crossing over and hence no multivalent association may occur. So the extremely small chromosomes of the parental species can also be a cause for the regular chromosome behaviour of the amphidiploid. The detailed sutudies on many amphidiploids in *Hibiscus* by Kuwada (1964) and in *Abelmoschus* by Jambhale and Nerkar (1982) have showed similar behaviour of chromosomes so that they can survive as fertile synthetic species.

Parental differences were seen for almost all the characters studied (Table 3). These included characters such as plant height, number of primary branches, length of petiole, number, length and width of epicalyx segment, length and girth of fruit, seeds per fruit,

Table 1. Chromosome associations at metaphase I in F_1 of *A. esculentus* x *A. tetraphyllus*

IV	HI	II	I	Frequency	
				No.	Per cent
--	--	46	42	4	13.33
--	--	42	50	3	10.00
--	--	36	62	17	56.66
----------	--	37	60	1	13.33
--	--	38	58	3	10.00
--	--	30	74	1	3.33
----------	--	24	86	1	3.33
Range		24-46	42-86	Total	
Mean/cell		37.56	58.86	No. of cells	30

Table 2. Chromosome associadons at metaphase I in the amphidiploid *A. esculentus x A. tetraphyllus.*

IV	III	II	I	Frequency	
				No.	Per cent
2	-	130	-	4	13.33
2	-	124	2	3	8.82
-	-	134	-	23	76.66
3	-	128	-	1	2.94
1	-	132	-	2	5.88
1	-	130	4	1	2.94
Range 1-3	-	124-134	2-4	Total	
Mean/cell 0.58	-	132.23	0.29	No. of cells	34

Table 3. Measurement of certain morphological characters of the parents, F_1 and amphidiploid (mean of 10 plants)

Characters	*A. esculentus*	*A. tetraphyllus*	F_1	*Amphidiploid*
Plant height (cm)	161.20	88.00	199.60	135.80
Days to flowering	39.67	42.60	41.60	45.53
Number of primary branches	1.40	6.40	5.40	5.00
Petiole length (cm)	18.50	8.20	19.60	20.60
Number of epicalyx segments	9.27	4.33	6.27	6.07
Length of epicalyx segment (cm)	1.76	1.19	1.62	1.91
Width of epicalyx segment (cm)	0.20	0.84	0.52	0.71
Length of fruit (cm)	19.45	4.17	7.45	0.91
Girth of fruit (cm)	8.17	7.51	7.65	9.23
Number of fruits per plant	9.73	17.40	21.93	14.87
Number of seeds per fruit	49.80	32.40	-	18.60
Field reaction to YVMV	Succeptible	Resistant	Resistant	Resistant

fruits per plant and field reaction to YVMV.

Morphologically all plants of the interspecific hybrid looked alike. The plants were erect in habit, robust and vigorous and represneted more towards the wild species. It recorded 23.82 and 20.03 per cent heterosis with respect to plant height and fruits per plant. These observations are in agreement with Ugale *et al*. (1976). The intermediate mean values were recoded for characters such as width of epicalyx segment, number of primary branches and furuit length. The expression of resistance to YVMV in the F_1 hybrid was similar to that of the wild parent.

The amphidiploid grossly resembled the F_1 hybrid for most of the morphological characters. The characteristics of stem, leaf, primary branches, epicalyx, fruit length and field reaction to YVMV were similar to those of F_1 hybrid. Unlike the F_1 hybrid, the amphidiploid was highly fertile and produced 18.6 seeds per pod and recorded 89.66 per cent germination. Mean value of amphidiploid for fruit girth was higher than parents and F_1 hybrid.

The progenies from the back cross with the amphidiploid combine YVMV resistance of the wild species and fruit characters of the cultivated species. This offers prospects for selection of economic types in okra breeding.

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