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Cytomarphological Studies of Interspecific Hybrid Between Cultivated Species of Finger Millet with Wild Species *Eleusine africana*

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Abstract

An investigation was carried out during 2005 and 2006 to study the cytomarphology of the interspecific hybrid *Eleusine coracana* × *E. africana* along with the parents. Results indicated that the hybrid showed intermediate for productive tillers, finger length, finger number and days to 50% flowering and exhibited reduced pollen fertility. The chromosome pairing was normal in parents and hybrid. However, one chain quadrivalent at diakinesis and one lagging chromosome at metaphase I was observed in hybrid in most of the meiotic cells scored. Further, recombination frequency was lower in the hybrid compared to their parents.

Key words : Interspecific hybrids, Cytomarphology, Finger millet.

The genus *Eleusine* to which finger millet (*Eleusine coracana* (L.) Gaertn) belongs, contain 9 to 10 species and the basic chromosome number is n = x =9. Among the reported species, three are tetraploids with 2n = 36 in *E. coracana* and *E. africana* and 2n=38 in *E. kageziensis*, while *E. indica*, *E. tristachya*, *E. floccifolia* and *E. intermedia* are diploid species with 2n = 18. Contrary to this, *E. multiflora* and *E. jeageri* are reported to possess 2n=16 and 2n = 20 chromosomes respectively (1). The studies pertaining to genome relations and molecular biology have not received much attention. Nevertheless, some attempts have been made to establish the chromosome relationship by crossing various species of genus *Eleusine*. Based on these studies it is concluded that the cultivated species *E. coracana* and others having 2n = 36 chromosomes are allotetraploid (2n= 4x = 36) and genomic composition is AABB which have originated by the hybridization of two diploid taxa having AA and BB genomes followed by chromosome doubling. Based on genome studies, it is perceived that the donor of A genome is *E. indica* while the identity of B genome donor species is still obscure (2). The interspecific hybridization studies so for attempted were made from the angle of knowing genome relationship. However, such evaluation and utilization of wild gene pool from the point of crop improvement has received scant attention. There have been no efforts in the past for introgression of genes from wild relatives and deriving lines for utilization in finger millet crop improvement. *Eleusine africana* is close relative of cultivated species of *E. coracana*. Earlier reports indicate that gene transfer from *E. africana* to *E. coracana* is feasible and useful in breeding (3). *E. africana* has more tillering ability (15—20) and high drought tolerance capacity. It matures early (95—100 days), also it has more fingers per ear and long finger length. So, utilization of *E. africana* as donor parent for present investigation is highly rewarding. In the present study one of the wild tetraploid species *E. africana* (2n=4x=36) was crossed with three varieties viz. Indaf 8, HR911 and PR 202 of cultivated species *E. coracana* with an objective of transferring some of the desirable characters from wild species to the popular cultivated varieties of finger millet and also to assess the chromosome behavior at bivalent formation in interspecific hybrids.

Methods

The material for present study comprised three

Table 1. Comparative morphological features of three interspecific hybrids *E. coracana* × *E. africana* and their parents. Where, P_1—Indaf 8, P_2—HR 911, P_3—PR 202, P_4—*Eleusine africana* (wild species).

	Characters	Parents P_1	P_2	P_3	P_4	F_1 $P_1 \times P_4$	$P_2 \times P_4$	$P_3 \times P_4$
1	Plant height (cm)	105.00	109.00	92.00	150.00	134.50	144.00	145.00
2	Culm thickness (cm)	1.20	1.10	1.00	0.60	1.10	0.70	0.80
3	Productive tillers / plant	2.40	3.00	4.00	17.00	8.00	8.00	10.00
4	Leaf number	12.00	13.50	13.00	16.00	13.00	14.00	12.00
5	Flag leaf length (cm)	26.00	25.00	26.00	40.00	35.00	42.50	30.00
6	Flag leaf width (cm)	1.10	1.10	0.90	0.76	1.20	1.00	0.80
7	Peduncle length (cm)	28.00	32.00	24.00	35.00	33.00	26.50	33.00
8	Finger number / ear	7.00	7.50	6.00	11.00	8.00	9.00	8.00
9	Finger length (cm)	7.25	7.00	5.10	13.00	12.50	11.50	10.00
10	Finger width (cm)	1.10	1.10	1.20	0.40	0.70	0.60	0.70
11	Days to 50% flowering	86.00	83.00	85.00	50.00	70.00	68.00	67.00
12	Days to maturity	125.00	120.00	117.00	95.00	120.00	115.00	112.00
13	Test weight (g)	2.67	3.33	3.20	1.03	1.92	1.97	1.43
14	Grain yield per plant (g)	18.00	25.40	26.00	4.70	8.20	7.50	8.75

crosses between wild species (*Eleusine africana*) with three cultivated popular varieties viz. Indaf 8, HR-911 and PR 202. The crossed seeds were obtained from the Project Coordination Cell, All India Coordinated Small Millets Improvement Project, Indian Council of Agricultural Research, Gandhi Krishi Vignana Kendra, Bangalore. The crossed seeds were sown during *kharif* of 2005 along with parents. The F_1 plants were identified based on morphological characters. At the same time crossing program was carried out to generate F_0 seeds.

Crossing Technique Employed. For crossing program three cultivated popular varieties viz. Indaf 8, HR 911, PR 202 were used as female parent while the wild species (*E. africana*) was used as male parent. Based on the days to flowering, sowing date of each parent was adjusted to achieve synchrony in flowering in all genotypes. Usually in finger millet, anthesis started at 0100 to 0400 h, but receptivity ends at 1100 h. Male parent was sown between female parents in 3 m row. In female, only two fingers were allowed to pollinate with male and remaining were removed before pollination. The ear heads of both the parents were joined together and tied with cotton thread before anthesis taking place. After pollination, ear heads were separated and seeds collected

Table 2. Mean superiority / inferiority (%) of the interspecific hybrids over its parents for various characters. Where, P_1—Indaf 8, P_2—HR 911, P_3—PR 202, P_4—*Eleusine africana.*

	Characters	Cross I P_1	P_4	Cross II P_2	P_4	Cross III P_3	P_4
1	Plant height (cm)	28.09	–10.33	32.11	– 4.00	64.13	0.66
2	Culm thickness (cm)	– 8.00	83.33	– 36.36	16.66	– 20.00	33.33
3	Productive tillers / plant	233.33	–52.94	166.66	–52.94	150.00	– 41.17
4	Leaf number	8.30	– 18.75	3.70	– 12.50	– 7.69	– 25.00
5	Flag leaf length (cm)	34.60	– 12.50	66.66	6.25	15.38	– 25.00
6	Flag leaf width (cm)	9.09	57.80	– 9.09	31.57	– 11.11	5.26
7	Peduncle length (cm)	17.85	– 5.71	– 17.18	– 24.28	37.50	– 5.71
8	Finger number / ear	14.28	– 27.27	20.00	– 18.18	33.00	– 27.77
9	Finger length (cm)	72.41	– 3.84	64.28	– 11.53	96.07	– 23.07
10	Finger width (cm)	– 36.36	75.00	– 45.45	50.00	– 41.66	75.00
11	Days to 50% flowering	– 4.65	64.00	– 6.02	30.00	– 11.76	50.00
12	Days to maturity	– 4.00	26.31	– 4.16	21.50	– 4.27	17.89
13	Test weight (g)	– 28.08	86.40	– 40.84	91.26	– 55.31	38.54
14	Grain yield / plant (g)	– 54.00	74.00	– 70.00	53.00	– 66.00	85.00

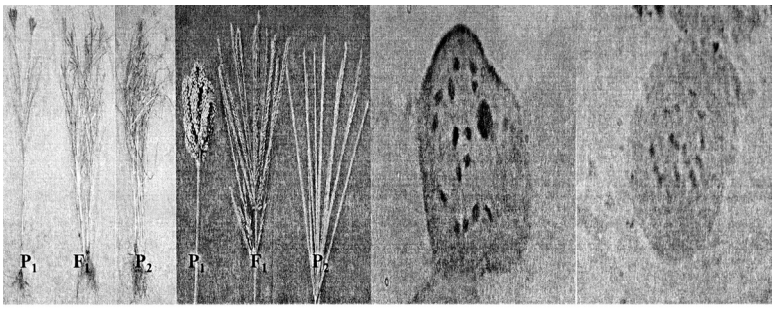

Fig. 1 Fig. 2 Fig. 3 Fig. 4

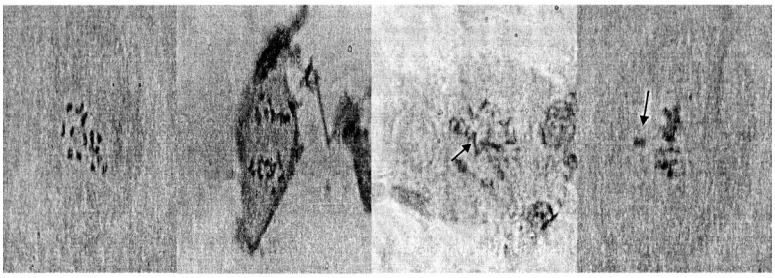

Fig. 5 Fig. 6 Fig. 7 Fig. 8

Figure 1. Comparison of productive tillers in interspecific hybrids and their parents.
Figure 2. Comparison of ear characters among *E. carocona,* wild species *E. africana* with F_1.
Figure 3. Diakinesis in *Eluesine caracona* var Indaf 8 with 18 II.
Figure 4. Diakinesis in *Eluesine africana* with 18 II.
Figure 5. Diakinesis in interspecific hybrid *Eluesine caracona* var Indaf 8 vs *Eluesine africana* showing norma 18 II.
Figure 6. Anaphase I showing normal movement chromosome in interspecific hybrid of *Eluesine caracona* var Indaf 8 vs *Eluesine africana.*
Figure 7. Diakinesis in interspecific hybrid *Elueaine caracona* var Indaf 8 vs *Eluesine africana* showing 16 II + IV arrow mark indicates chain quadrivalent.
Figure 8. Metaphase I showing lagging in interspecific hybrid of *Eluesine caracona* var Indaf 8 vs *Eluesine africana.* P_1—*Eluesine caracona* var Indaf 8, P_2—*Eluesine africana.*

only from female parent.

For assessing fertility, the pollen grains were stained in 2% acetocarmine and examined under the microscope. Deeply stained grains were counted as fertile and the unstained / partially stained pollens were recorded as sterile. From each slide, five fields were chosen at random and the percentage of pollen fertility was calculated.

For meiosis study flower buds of appropriate stage were fixed in the freshly prepared Cornoy's II mixture made of 6 parts of ethyl alcohol, 3 parts of acetic acid and 1 part of chloroform. The flower buds were collected during 0.008—1000 h on bright sunny days. The material was allowed to remain in the fixative for 24 hours and then transferred to 70% alcohol and stored for further use. The anthers were smeared in 1 or 2 drops of 2% acetocarmine and observed under microscope. Judicious warming over the flame before and after tapping helped to obtain good spread of chromosomes. Diakinesis and metaphase I for chromosome pairing and subsequently anaphase I for behavior of chromosomes were studied. Chiasma frequency was estimated at diakinesis and a total of 20 cells were scored for this purpose. Total chiasma per cell and per bivalent was calculated by using the following formula. Cytological observations were made on both temporary and permanent mounts. Photomicrographs were taken. Chiasmata per cell and chiasmata per bivalent were calculated (4) as follows.

$$\text{Chiasmata per cell} = \frac{\text{Total number of chiasma}}{\text{Number of cells scored}}$$

Table 3. Mean pollen fertility (%) of the interspecific hybrids in comparison with parents.

Parents and F_1 hybrids	Pollen fertility (%)
Indaf 8	82.00
HR 911	84.17
PR 202	80.00
E. africana	88.85
Indaf 8 × *E. africana*	35.50
HR 911 × *E. africana*	47.28
PR 202 × *E. africana*	42.25

$$\text{Chiasmata per bivalent} = \frac{\text{Total Chiasmata per cell}}{\text{Number of bivalents}}$$

Results and Discussion

Comparisons of morphological characters of different interspecific hybrids with their parents were presented in Table 1. It indicated that most of the characters were intermediate in interspecific hybrids. There was considerable difference in respects of productive tillers per plant between the two species. The cultivated species *E. coracana* var Indaf-8, HR911 and PR 202 showed less productive tillers (2 to 4) while *E. africana* had as many as 17 tillers. The F_1s was intermediate with 8—10 tillers per plant (Fig. 1).

With regard to finger number, the interspecific hybrid was intermediate between parents in all the three crosses. Finger length in interspecific hybrid is more towards *E. africana* (13 cm) than cultivated species (Fig. 2). It indicated significant contribution of male parent (*E. africana*) to the F_1 s in respect of this trait.

There was considerable difference for days to 50% flowering between the two species. The varieties viz. Indaf 8, HR911, PR202 took 86, 83, 80 days for flowering, respectively. Whereas *E. africana* flowered early in 50 days. The F_1 were intermediate between parents with70, 68, 67 days in cross I, cross II and cross III, respectively. Chennaveeraiah and Hiremath (3) observed interspecific hybrids were intermediate between the parent species for most of the characters.

With respect to grain yield per plant, the interspecific hybrid (8 g) was inferior to cultivated varieties (26 g), but they were superior to wild species (4.81 g) in all the crosses.

Table 4. Chaisma frequency in the interspecific hybrid and their parents.

	Mean bivalents/cell with				Mean xta per bivalent	Mean xta per cell
	1xta	2xta	3xta	4xta		
Parent 1 (*E. coracana* var Indaf 8)	3.0	15.0	–	–	1.83	33
Parent 2 (*E. africana*)	4.0	14.0	–	–	1.77	32.02
F_1	4.3	13.0	0.3	0.05	1.74	31.4

Table 2 indicated that interspecific hybrids were superior to cultivated species for the important traits like productive tillers, finger length, finger number and days to 50% flowering in all the crosses. This indicates that there is much scope for improve these traits in segregating generations.

Cytological Studies

The interspecific hybrids showed low pollen fertility in cross Indaf 8 × *E. africana* (35.50%), HR 911 × *E. africana* (47.25%) and 202 × *E. africana* (42.25%). On the other hand, the mean pollen fertility was 82.00, 84.17 and 80% in *E. coracana* var Indaf 8, HR911 and PR 202, respectively and 88.85% in *E. africana* (Table 3). The reduced fertility in the hybrid may be due to cryptic structural differences in the chromosome (5). Meiosis in the pollen mother cells of hybrid and parents was studied with special reference to pairing behavior of chromosome. Pairing between the homologous chromosomes was normal at diakinesis in cultivated species *E. coracana* variety Indaf 8, HR 911, PR 202 and *E. africana* (Figs. 3 and 4) and the bivalents mostly showed either one chiasma or two chaismata. The later stages of meiosis were normal resulting in high percentage of fertile pollen and good seed set. The hybrid also showed normal bivalents at diakinesis (Fig. 5) and normal movement of chromosomes during anaphase I (Fig. 6). It indicated the good chromosomal homology be-

tween *Eleusine coracana* and *Eleusine africana.* The most striking observation in the meiotic plates of hybrid was the presence of one chain quadrivalent (Fig. 7) at diakinesis in 90% of the pollen mother cell studied. A lagging chromosome during metaphase-I was also seen (Fig. 8).

Further, studies on chiasma frequency revealed that in the interspecific hybrid, chiasma frequency per cell (31.4) was lower compared to *E. coracana* (33.00) and *E. africana* (32.04) (Table 4). The lower chiasma frequency in interspecific hybrid suggests that the genome of both the species might be differing for cryptic structural changes in the form of deletions and inversions. Presence of chain quandrivalent also supports the occurrence of translocation, which may reduce the chiasma frequency.

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