

Cytomorphological studies in interspecific hybrids of finger millet.

Ratnakar Manjunath Shet^{1*}, Mallikarjuna N. M.¹, Naveen kumar K. S.², Jayarame Gowda¹ and Mohan Kumar M. V.¹

¹Department of Genetics and Plant Breeding, University of Agricultural Science, Bangalore 560065, India

²Department of Olericulture, College of Horticulture, Kerala Agricultural University, Vellanikara, Thrissur 680656, India.

*Corresponding author Email: ratnakar.ms@rediffmail.com

Abstract: Study of the cytomorphology of interspecific hybrid between *Eleusine coracana* × *E. africana* along with the parents indicated that the hybrids were intermediate for productive tillers, finger length, finger number and days to 50% flowering and exhibited reduced pollen fertility over the parents. Chromosome pairing was normal in parents and hybrid. However, one chain quadrivalent at diakinesis and one lagging chromosome at metaphase I were observed in hybrid in most of the meiotic cells scored. Further, chiasma frequency was lower in the hybrid compared to their parents. The lower chiasma frequency in interspecific hybrid is suggestive of the fact that the genome of both the species might be differing for cryptic structural changes in the form of deletions and inversions. Presence of chain quadrivalent also supports the occurrence of translocation, which may reduce the chiasma frequency.

Key words : Interspecific hybrids, cytomorphology, finger millet. chiasma frequency

INTRODUCTION

The genus *Eleusine* to which finger millet (*Eleusine coracana* (L.) Gaertn) belongs, contains 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 (Hiremath S. C. and Chennaveeraiah M. S., 1982).

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 (Hiremath S. C. and Salimath S. S., 1992).

The interspecific hybridization studies so far attempted were 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 a 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. *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 know the chromosome behavior at bivalent formation in interspecific hybrids.

MATERIALS AND METHOD

The material for the present study comprised of three 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* 2005 along with parents. The F_1 plants were identified based on morphological characters. At the same time crossing programme was carried out to generate F_0 seeds.

Crossing technique employed

For crossing programme three cultivated popular varieties viz., Indaf 8, HR 911 and 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 takes place from 1am to 4am and ends by 11 a.m. Male parent was sown in between female parents in 3m 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 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 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 8-10 am 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 behaviour 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 using following formulae (Manjula, 1998).

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

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

RESULTS AND DISCUSSION

Comparisons of morphological characters of different interspecific hybrids with their parents are 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 F₁ 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 F₁s in respect of this trait.

There was considerable difference for days to 50 per cent flowering between the two species. The varieties viz., Indaf 8, HR911 and PR202 took 86, 83 and 80 days for flowering, respectively whereas *E. africana* flowered early in 50 days. The F₁s were intermediate between parents with 70, 68 and 67 days in cross I, cross II and cross III, respectively. Chennaveeraiah and Hiremath (1974) also observed that interspecific hybrids were intermediate between the parent species for most of the characters. With respect to grain yield per plant, the interspecific hybrid (8g) was inferior to cultivated varieties (26g), but they were superior to wild species (4.81g) in all the crosses.

Table 2 indicate that interspecific hybrids are 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 shows that there is much scope for improvement of these traits in segregating generations.

Cytological studies

The interspecific hybrids showed low pollen fertility in the cross Indaf 8 × *E. africana* (35.50%), HR 911 × *E. africana* (47.25%) and PR 202 × *E. africana* (42.25%). On the other hand, 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 (Devarumath *et al.*, 2005). Meiosis in the pollen mother cells of hybrid and parents was studied with special reference to pairing behaviour of chromosomes. Pairing between the homologous chromosomes was normal at diakinesis in cultivated species *E. coracana* variety Indaf 8, HR 911, PR 202 and *E. africana* (Fig. 3 and Fig. 4) and the bivalents showed mostly either one chiasma or two chiasmata. 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 presence of good chromosomal homology between *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 per cent 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 is suggestive of the fact that the genome of both the species might be differing for cryptic structural changes in the form of deletions and inversions. Presence of chain quadrivalent also supports the occurrence of translocation, which may reduce the chiasma frequency.

Table 1. Comparative morphological features of three interspecific hybrids *E. coracana* × *E. africana* and their parents.

Sl. No.	Characters	Parents				F ₁		
		P ₁	P ₂	P ₃	P ₄	P ₁ × P ₄	P ₂ × P ₄	P ₃ × P ₄
1	Plant height (cm)	105.00	109.00	92.00	150.00	34.50	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	25.50	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

Where,

P₁ - Indaf 8 P₃ - PR 202 P₂ - HR 911 P₄ - *Eleusine africana* (wild species)

Table 2: Mean superiority / inferiority (%) of the interspecific hybrids over its parents for various characters

Sl. No.	Characters	Cross I		Cross II		Cross III	
		P ₁	P ₄	P ₂	P ₄	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

Where, P₁ - Indaf 8 P₃ - PR 202 P₂ - HR 911 P₄ - *Eleusine Africana*

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
<i>E. africana</i>	88.85
Indaf 8 × <i>E. africana</i>	35.50
HR 911 × <i>E. africana</i>	47.28
PR 202 × <i>E. africana</i>	42.25

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 (<i>E.coracana</i> var. Indaf 8)	3.0	15.0	-	-	1.83	33
Parent 2 (<i>E. africana</i>)	4.0	14.0	-	-	1.77	32.02
F ₁	4.3	13.0	0.3	0.05	1.74	31.4

REFERENCES

- Chennaveeraiah M. S. and Hiremath S. C., 1974. Genome analysis of *Eleusine coracana* (L.) Gaertn. *Euphytica* 23: 489-495.
- Devarumath R. M., Hiremath S. C., RamaRao S., Arunkumar and Sangeeta Bewal, 2005. Genome analysis of finger millet (*E. coracona*) by interspecific hybridization among diploid wild species of *Eleusine* (Poaceae). *Cytologia* 70: 427-434.
- Hiremath S. C. and Chennaveeraiah M. S., 1982. Cytogenetical studies in wild and cultivated species of *Eleusine*. *Caryologia* 35: 57-69.
- Hiremath S. C. and Salimath S. S., 1992. The “A” genome donor of *Eleusine coracana* (L.) Gaertn. (Graminae). *Theor Appl Genet.* 84: 747-754.
- Manjula T., 1998. Cytomorphology of interspecific hybrid *Helianthus annuus* X *H. argophyllus* T&G and evaluation of derived lines for their utility in sunflower improvement. *M.Sc (Agri.) Thesis*, UAS, Bangalore, India, p.108.



Fig. 1

Fig. 2

Fig. 3

Fig. 4

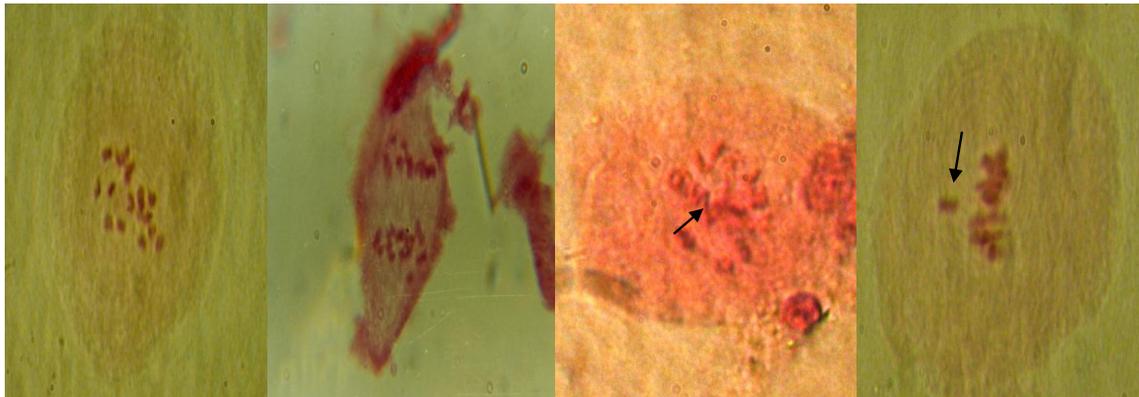


Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig.1: Comparison of productive tillers in interspecific hybrids and their parents

Fig.2: Comparison of ear characters among *E. caracona*, wild species *E. africana* with F_1

Fig.3 : Diakinesis in *Eluesine caracona* var Indaf 8 with 18 II

Fig.4 : Diakinesis in *Eluesine africana* with 18 II

Fig.5 : Diakinesis in interspecific hybrid *Eluesine caracona* var Indaf 8 \times *Eluesine africana* showing normal 18 II

Fig.6 : Anaphase I showing normal movement chromosome in interspecific hybrid of *Eluesine caracona* var Indaf 8 \times *Eluesine africana*

Fig.7 : Diakinesis in interspecific hybrid *Eluesine caracona* var Indaf 8 \times *Eluesine africana* showing 16 II + IV arrow mark indicates chain quadrivalent

Fig.8 : metaphase I showing lagging in interspecific hybrid of *Eluesine caracona* var Indaf 8 \times *Eluesine africana*

P_1 - *Eluesine caracona* var Indaf 8 , P_2 - *Eluesine africana*