African Crop Science Journal, Vol. 16, No. 4, pp. 237 - 241
ISSN 1021-9730/2008 $4.00

MEIOTIC BEHAVIOUR OF *ERAGROSTIS TEF* AND *ERAGROSTIS PILOSA*

SINTAYEHU ADMAS and KIFLE DAGNE[1]
Debre Berhan Agricultural Research Center, P.O. Box 112, Debre Berhan, Ethiopia
[1]Biology Department, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

(Received 9 March 2009; accepted 22 July, 2009)

ABSTRACT

Tef (*Eragrostis tef* (Zucc.) Trotter) is major cereal crop cultivated in Ethiopia. It occupies approximately two million hectares of land each year. However, little has been done on meiotic chromosome study of the interspes hybrid. So, studies were conducted to examine meiotic behavior and pollen fertility of the F_1-hybrid (*E. tef* x *E. pilosa*) and parental lines at Addis Ababa, Ethiopia in 200 7/08. The F_1-hybrid plant showed regular meiotic cell division and high level of pollen fertility (94.3%). It also produced better seed and biomass yield and number of tillers than the parental lines which are the manifestation of meiotic stability in the hybrid and the subsequent generations. Pollen fertility test of the parental lines and selected recombinant inbred lines of *E. tef* and *E. pilosa* cross was also high (90 - 95%), which are an indication of regular meiotic chromosome division. The result demonstrated chromosome stability of the hybrid plant, which is important for widening the genetic pool of *tef* germplasm through conventional crossing with *E. pilosa.* The study further strengthen the view that the two species are very closely related.

Key Words: *Eragrostis tef* , Ethiopia, pollen fertility

RÉSUMÉ

Eragrostis tef (Zucc.) Trotter) est la principale culture céréalière cultivée en Éthiopie. Il occupe environ deux millions d'hectares de terres chaque année. Cependant, peu d'études ont été faites sur le chromosome méiotique de l'hybride. Par conséquent, les études ont été réalisées afin d'examiner le comportement méotique et la fertilité de pollen de hybride F1 (*E tef x E. pilosa*) et les lignées parentales à Addis Abeba, Ethiopie en 2007/08. La plante F1-hybride avait montré division cellulaire méotique régulière et un niveau élevé de la fertilité du pollen (94,3 %). Elle avait également produit les meilleures semences et rendement en biomasse et nombre de talles que les lignées parentales qui sont la manifestation de la stabilité méiotique dans l'hybride et les générations ultérieures. Le test de fertilité du pollen des lignées parentales et des lignées recombinantes sélectionnées de *E. tef et E. pilosa* croisées était également élevé (90-95 %), qui est une indication d'une division chromosome méiotique régulière. Le résultat avait démontré une stabilité chromosomique de la plante hybride, qui est importante pour élargir le pool génétique du matériel génétique de *Eragrostis tef* (Zucc.) Trotter) par le biais de croisements classiques avec *E. pilosa.* Cette étude a aussi renforcer l'idée que les deux espèces sont très étroitement liées.

Mots Clés: *Eragrostis tef* (Zucc.) Trotter) , Ethiopie, fertilité du pollen

INTRODUCTION

Wild relatives of cultivated crops are good reservoir of desirable gene. Plant breeders have an opportunity of using these desirable genes to improve genetic make up. Crops like wheat (Jones *et al*., 1995a) and rice (Jones *et al.*, 1978) are the most benefited crops of genes from their wild relatives. In tef, many wild relatives were identified to be the source of desirable gene

(Jones *et al*., 1978b), but their applications are very limited. The reason is that difficulty in crossabihity and tiny chromosome size for detailed cytological studies.

The use of many wild species is limited by their poor crossability and high degree of F sterility in interspecific hybrids (Allard, 1960). Studies in different plant species have revealed that irregularities in meiotic chromosome division result in abnormal plant growth and decline in seed setting (Majumdar *et al*., 2004; Adamowski, 2008). If there were no crossability problem, interspecifc crossing would give a chance to introgress genes from wild relative to cultivated ones (Allard, 1960).

High pollen fertility and normal seed setting are the manifestation of regular meiotic chromosome division (Majumdar e*t al*., 2004; Adamowski, 2008). Pollen fertility is directly or indirectly dependent upon the efficiency of the meiotic process. In tef, recombinant inbred lines (RILs) were developed from B. f cv. Key Mum and . pilosa (accession 30-5) cross to create new gene pooi. Meiotic stability of the RILs has not yet been examined. Therefore, this study was conducted to examine the meiotic behavior of the hybrid.

MATERIAL AND METHODS

Meiotic study in the inter-specific hybrid. A successive cross was made between *E. tef* and *E. pilosa*. F_1 and 12 RILs obtained from Debre Zeit Agricultural Research Centre (DZARC) were grown in a pot under green house. When the inflorescence just emerged from the flag leaf, it was fixed in freshly prepared ethanol: chloroform: acetic acid (6:3:1 v/v) mixture for about 24 hr. The fixative was then replaced by 70% ethanol before the inflorescence was stored at 4°C. For meiotic analysis, the fixed inflorescence was hydrolysed using IN HC1 at 60°C for 3-5 mm florets stained in aceto-orcein or propionic-orcein for four hours at room temperature. Pollen mother cells (PMCs) were released in a drop of 45% acetic acid on a glass slide by macerating florets with forceps, and squashing under a cover slip. The preparation was then made semi-permanent by sealing around the edge of the cover slip with a nail varnish. It was then examined under a microscope for any aberrant meiosis such as laggards, bridges and micronuclei. Finally, photomicrographic pictures of appropriate division stages were taken.

Pollen fertility test. Pollen grains from freshly dehisced florets were released in a drop of cotton blue lactophenol on a glass slide. The dye was covered with a cover slip and the pollen grains were allowed to stain for three hours before scoring. The preparation was then examined under a microscope and the number of fully stained pollen grains scored as normal and the unstained or only partially stained pollen grains as aborted.

RESULTS

Meiotic analysis. In the study, no meiotic abnormalities were detected during metaphase I (Fig. 1 B), metaphase II (Fig. 1G and H), anaphase I (Fig. 1C), anaphase II (Fig. 11) and telophase I (Fig. 1D). The exception were two laggards in Telophase II observed in a single PMC (Fig. 1J). The total of 154 PMCs examined included 41 prophase I, 27 metaphase I, 26 metaphase II, 19 anaphase I, 8 anaphase II, 24 telophase I and 9 telophase II PMCs. The formations of tetrads were also found to be normal. A total of 160 PMCs at tetrad stage were examined and all produced normal tetrads with four microspores each (Fig. 1L). No micronuclei formation was detected during tetrad formations. Each PMC produced four equal sized microspores.

Pollen fertility test. Pollen fertility was examined for the parental lines, F_1-hybrid and 12 RILs. All showed high pollen fertilitys which ranged from 90 to 95.1%. Small and large sized pollen having circular or oval shapes appeared in mixture in all RILs and parental lines (Table I).

Morphology of the F_1-hybrid. Comparisons were done between F_1-hybrid and its parental lines (Fig. 2). The F_1-hybrid had loose panicle and brown seed colour like that of *E. pilosa* but purple lemma colour. The F_1-hybrid plants had similar stand establishment to Key Mum , except a little bit vigour of the former. Plant height (98 cm) and number of tillers (15) was observed which is higher

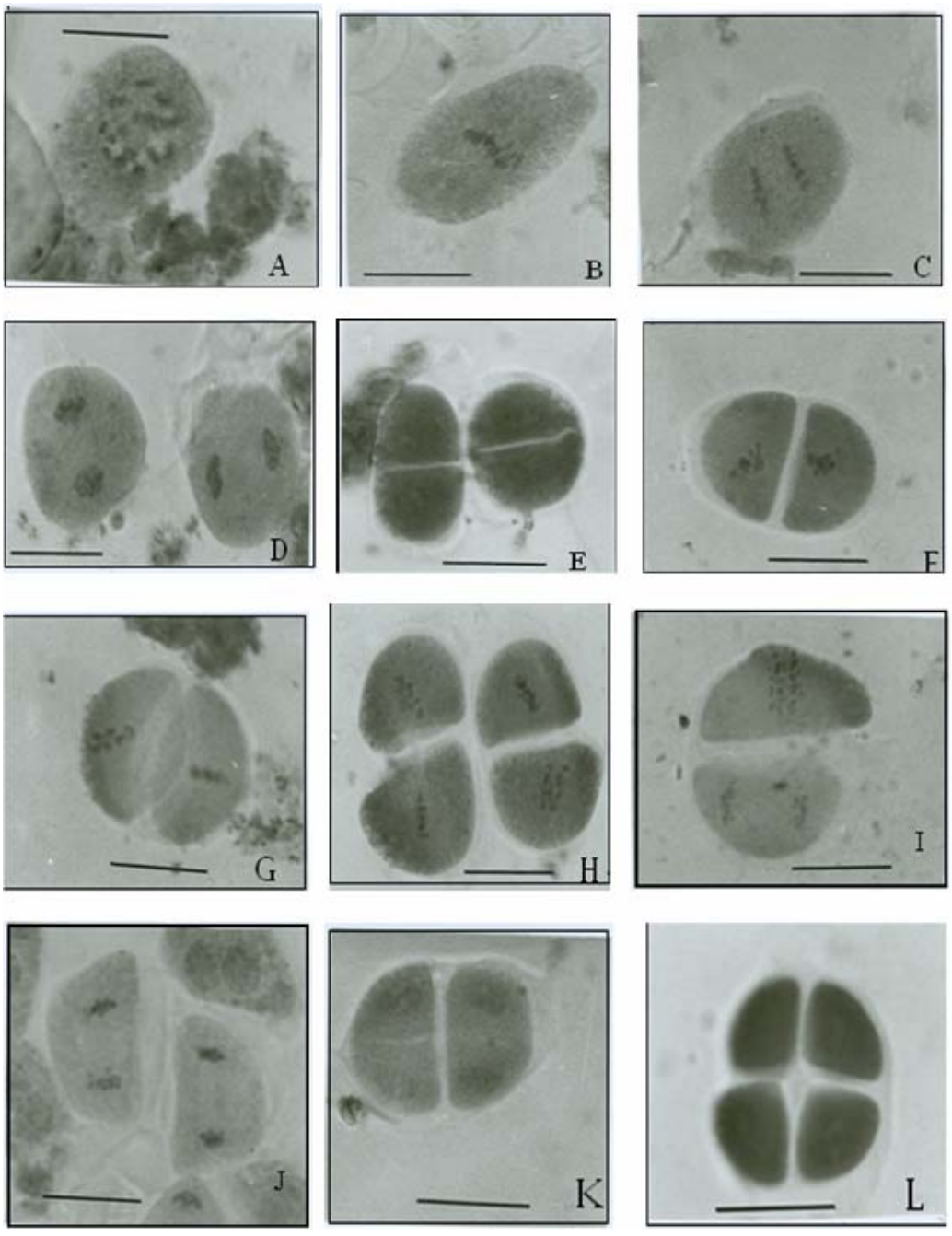

Figure 1. Meiosis in F_1-hybrid of *E. tef*, and *E. pilosa* A) Diakinesis, B) Metaphase I showing normal configuration, C) Anaphase I, D) Telophase I aggregated at the pole, E) Cytokinesis, F) Prophase I, G and H) Metaphae II showing normal configuration, I) Anaphase II, J) Telophase II with two chromosomes lagging behind, K) Anaphase II, L) tetrad formation. One bar represents 20 μm for all phases.

TABLE I. Pollen fertility percentage for *E. tef, E.pilosa* F_1-hybrid and some selected RILs

Variety	NLCP		NSCP		NLOP		NSOP		Fertility (%)
	Normal	Aborted	Normal	Aborted	Normal	Aborted	Normal	Aborted	
Key Murri	112	4	53	4	77	3	47	4	95.1
E. pilosa	99	5	42	3	76	3	42	5	94.2
F_1-hybrid	100	3	29	3	54	3	16	3	94.3
R1L317	89	7	21	2	37	4	23	5	90.4
R1L183	99	5	34	7	78	8	19	3	90.9
RIL118	105	6	31	3	42	3	21	4	92.6
R1L222	134	3	33	7	37	8	25	6	90.5
R1L317	74	3	23	6	27	5	29	3	90
R1L172	83	2	22	9	57	5	55	5	91.2
1UL16	65	4	35	5	46	7	39	3	90.7
R1L252	77	8	32	5	56	4	67	7	90.6
1UL275	97	6	25	3	52	7	37	4	91.3
JUL62	87	7	26	3	61	5	61	8	91.1
1UL290	79	6	39	3	30	4	35	2	92.4
1UL36	69	4	37	2	38	3	49	4	93.7

NLCP=Number of large circular pollen, NSCP=Number of small circular pollen, NLOP=Number of large oval pollen, NSOP=Number of small oval pollen

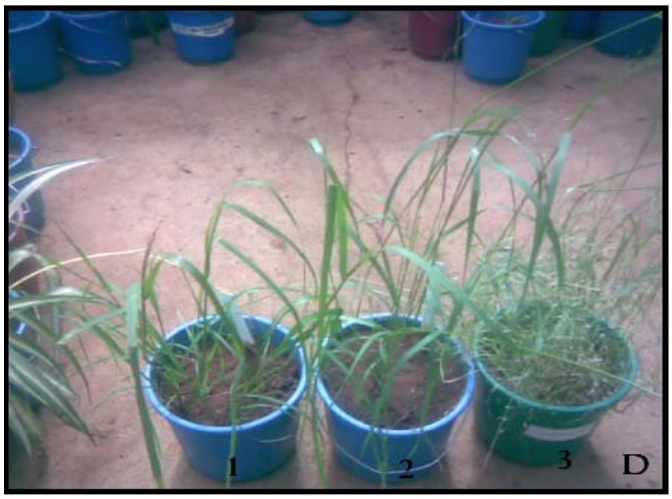

Figure 2. Stand establishment of Key Murri, F_1-hybrid and *E. pilosa* (A, D-1 = Key Murri, B, D-2 = F_1-hybrid and C, D-3 = *E. pilosa.*

in F_1 -hybrid plant than its parental lines (*E. pilosa* 56 cm, 7; Key Murri: 87 cm, 6). The F_1-hybrid gave better yield accompanied by excellent stand establishment than the parental (*E. pilosa* 0.46 g pl^{-1}; key murri:.0.61 g pl^{-1}).

DISCUSSION

The different stages of meiosis were examined in the F_1-hybrid except chromosome pairing and chiasmata formation which were not clearly

detectable due to the very minute size of tef chromosome (i.e., 0.8 - 2.9 µm; Mulu Ayele *et al*., 1996). The analysis indicated that all the bivalents moved and aligned on the equator of the spindle at equal pace and segregation of the bivalents in either pole was normal. This is an indicator of normal meiotic cell division exists. The F_1-hybrid showed regular meiotic cell division and high level of pollen fertility which confirmed the chromosome homology between the genomes of the two species. High levels of pollen fertility and seed setting in the F_1-hybrid and RILs were detected, which are additional evidences for meiotic stability in the hybrid exist.

The present findings about the meiotic regularity and seed fertility of the F_1-hybrid between *E. tef* and *E. pilosa* cross shows not only that gene transfer is possible from the latter to the former, but further strengthens the view that the two species are very closely related. Also, meiosis was found to be most frequent when the panicle just emerged from the flag leaf as opposed to 'at late-booting stage and proceeds through emergence (Tavassoli, 1986).

ACKNOWLEDGEMENTS

The authors acknowledge McNight Foundation and Debre Berhan Agricultural Research Center for financial support. Thanks also go to technical staffs of Tef National Genetic Improvement Project at DZARC for technical assistance.

REFERENCES

Adamowski, E.V., Pagliarini, M.S. and Valle, C.B. 2008. Meiotic behavior in three interspecific three-way hybrids between *Brachiaria ruzizensis* and *B. brizantha* (Poaceae: Paniceae). *J. Genet.* 87:33-38.

Allard, R.W. 1960. Principle of Plant Breeding. Jhon Wiley and Sons, New York.

Hailu Tefera, Kebebew Assefa and Getachew Belay 2003. Evaluation of interspecific recombinant inbred lines of *Eragrostis tef* x *E. pilosa*. *J. Genet. & Breed*. 57:21-30.

Jones, M.P., Dingkuhn, M., Aluko, G.K. and Semon, M. 1978a. Interspecific *Oryza sativa* L. x *O. glaberrima* Steud. progenies in upland rice improvement. *Euphytica* 92:237-246.

Jones, B.M.G., Ponti, J., Tavasoli, A. and Dixol, P.A. 1978b. Relationship of the Ethiopian cereal tef (*Eragrostis tef* (Zucc.) Trotter): Evidence from morphology and chromosome number. *Ann. Bot.* 42:1369-1373.

Jones, S.S., Murray, T.D. and Allan, R.E. 1995. Use of alien genes for the development of disease resistance in wheat. *Rev. Phytopathoi.* 33:429-43.

Kifle Dagne 1994. Cytology, phylogeny and oil quality of Guizotia cass. (compositae). Ph.D. Thesis, Addis Ababa University, Addis Ababa, Ethiopia.

Majumdar, S., Banerjee, S. and Kumar, K. 2004. Meiotic behavior of chromosomes in PMCs and karyotype of *Trifolium repens* L. from drajeeling Himalaya. *Acta Biologica Cracoviensia Series Botanica* 46: 217-220.

Mulu Ayele, Dolezel, J., Van Duren, M., Brunner, H. and Zapata-Arias, F.J. 1996. Flow cytometry nuclear genome of the Ethiopian cereal tef (*Eragrostis tef* (Zucc.) Trott. *Genetics* 98:211-215.