

ENVIRONMENTAL INFLUENCE ON CYTOMIXIS IN *CORCHORUS FASCICULARIS* LAMK. (TILIACEAE)

Aninda Mandal¹, Sudha Gupta² and Animesh K. Datta^{1*}

¹Department of Botany, Cytogenetics, Genetics and Plant Breeding Section, University of Kalyani, Kalyani - 741235, West Bengal, India

²Department of Botany, Pteridology-Palaeobotany Section, University of Kalyani, Kalyani - 741235, West Bengal, India

*Corresponding author e-mail: dattaanimesh@gmail.com

Abstract: Cytomictic behaviour (intensity assessed from the frequency of hypo- and hyperploid meiocytes formed) is noted in a single plant (to avoid any intra plant variations) of *Corchorus fascicularis* Lamk. from the natural population under a unit location (West Bengal plains, Kalyani, Nadia; latitude 22°50' to 24°11' N, longitude 88°09' to 88°48' E, altitude 9.75 m, sandy loamy soil, pH-6.89) from early June to early July 2012. Meiosis has been studied from 6 samples under the assessed period. High temperature has intensified the phenomenon of cytomixis as evidenced from the enhanced frequency of hypo- and hyperploid PMCs formation without affecting pollen fertility. Apart from cytomixis, differential condensation of chromosomes, meiocytes with 2 nucleoli, occurrence of minute fragments, high frequency of univalent formation and irregular anaphase I separation are also observed. Results obtained have been discussed.

Keywords: *Corchorus fascicularis*, Cytomixis, Temperature effect

INTRODUCTION

The unique phenomenon cytomixis has been defined as mixing of cytoplasm in somatic and reproductive cells through cellular bridging, thereby forming a syncytia involving 2 to many cells. Sharing of cytoplasmic constituents during cytomixis seems to be predominant in male reproductive cell lines and intercellular bridge formation is a key event during the process (Mandal *et al.* 2013). Gates (1911) first described cytomixis as migration of chromatin materials through wide cytomictic connections. The phenomenon was first reported in meiocytes of *Crocus sativus* by Körnicke (1901). Cytomixis involves intercellular transfer of the organelles and other cytoplasmic constituents during spermatogenesis of animals (Roosen-Runge 1977; Carlson and Handel 1988; Ventela *et al.* 2003; Guo and Zheng 2004) and in some lower group of plants (Carr 1976; Paolillo and Cukierski 1976; Dong and Junying 1988; Kwaitkowska *et al.* 2003; Dong *et al.* 2004; Guzicka and Wozny 2005; Heng-Chang *et al.* 2007). In flowering plants, cytomixis has been noted in a wide range of taxa (Mandal and Datta 2012) predominantly during meiosis (Datta *et al.* 2005; Boldrini *et al.* 2006; Singhal and Kumar 2008; Song and Li 2009; Li *et al.* 2009; Saggoo *et al.* 2011). The preponderance of cytomixis has been noted in cytologically imbalanced plants like haploids, polyploids, aneuploids, apomictics (Nirmala and Rao 1996; Peng *et al.* 2003) as well as in hybrids (Maity and Datta 2008; Li *et al.* 2009) and in some secondary polyploids (Mukherjee and Datta 2005; Iqbal and Datta 2007; Mandal and Datta 2011) among others.

Faulty fixation (Maheshwari 1950; Takats 1959), chemical and herbicide effects (Ajay and Sarbhoy 1987), mechanical injury (Tarkowska 1965; 1966), pathological consequences (Bobak and Herich 1978; Morisset 1978), nutritional deficiency (Miljajev 1967) and environmental stresses and pollution (Malallah and Attia 2003; Haroun *et al.* 2004; Kumar and Tripathi 2008) have been reported to be the possible causes for cytomixis. However, recent evidences suggest that it is a natural phenomenon under genetic control influenced by physiological and environmental factor(s) (Soodan and Wafai 1987; Zheng *et al.* 1987; Bellucci *et al.* 2003; Boldrini *et al.* 2006; Ghaffari 2006; Himshikha *et al.* 2010; Kaur and Singhal 2012). Soodan and Wafai (1987) presumed the involvement of specific genes for cytomixis, which express only under particular environmental conditions. Yun-sheng and Yong-ping (2006) reported that the phenomenon to be controlled by poly-gene or key-gene which exists in all three genomes of allopolyploid wheat.

Persistent cytomixis (24.33% in 2009 – Maity and Datta 2009; 14.57% in 2010 – Mandal and Datta 2011; 13.21% in 2012 – Mandal and Datta 2012) was recorded in *Corchorus fascicularis* Lamk. (Tiliaceae), a wild genetic resource of cultivated jute (Mahapatra and Saha 2008), under uniform agro-climatic conditions in an identical location in West Bengal plains. Mandal and Datta (2012) also reported inter- and intra-plant variation in cytomictic behavior of chromosomes in *C. fascicularis*. Present communication reports on the influence of environmental factor(s) on cytomixis in *C. fascicularis*. The objective of the work is to add more evidences under environmental control over the unique phenomenon.

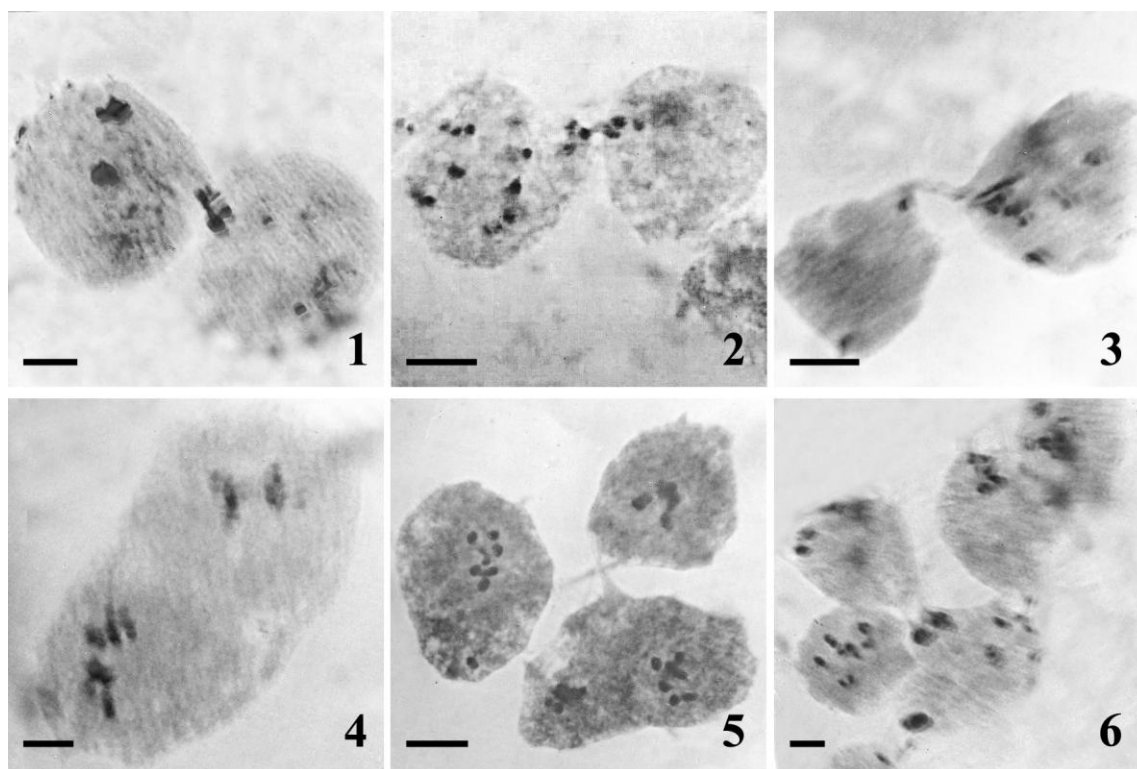


FIGURE LEGENDS

Figure plate I (1-6) showing cytotoxic behavior of chromosomes in *Corchorus fascicularis*. (1-3) cytomixis involving 2 meiocytes; (4) fusion of 2 meiocytes; (5) sticky bridge formation between meiocytes and fusion of PMCs; (6) four meiocytes in a cluster. Interesting to note that aberrant meiocytes are formed and chromosomes are differentially condensed. Scale bar = 10 μ m.

MATERIAL AND METHOD

A single randomly selected plant (to avoid any intra plant variation) from *C. fascicularis* ($2n=14$) population growing in the experimental field plots of University of Kalyani (West Bengal plain-latitude $22^{\circ}50'$ to $24^{\circ}11'$ N, longitude $88^{\circ}09'$ to $88^{\circ}48'$ E, altitude 9.75 m; sandy loamy soil, soil pH 6.85) was meiotically assessed from early days of June 2012 to early July 2012 (samples were collected in 6 different days – **Table 1**). Agro meteorological data was obtained from Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India. For meiotic analysis, floral buds were fixed uniformly at 6:00 AM in Carnoy's fixative in different dates of assessment (3 to 5 buds were scored in each data) and PMCs were squashed in 2% propino-carmin solution. The intensity of cytomixis was measured from the frequency of hypo- and hyperploid meiocytes scored at metaphase I (MI) and anaphase I (AI). Pollen fertility was assessed from pollen grains stability (Marks 1954) in 2% propinocarmin solution. Fully stained pollen grains were considered

fertile. Photomicrographs were taken from temporary squash preparation and subsequently magnified.

RESULT AND DISCUSSION

Meiotic data obtained has been presented in **Table 1**. Result suggested distinctive influence of temperature on the intensity of cytomixis; although, pollen fertility was nearly similar in different observations. Mostly 2 to 4 PMCs were in a cluster (**Figs. 1-6**). PMCs with hypo- and hyperploid chromosome numbers were formed more predominantly during enhanced temperature regime. Intercellular bridges, sticky in nature, and PMC fusions were evident (**Figs. 5-6**). During cytomixis, transfer of chromatin materials between meiocytes showed agglutination (**Fig. 1**) and were sometimes differentially condensed. PMCs with two nucleoli were also observed during the process, thereby suggesting transmigration of nucleoli. Apart from cytotoxic behavior of chromosomes, fragments of variable number (1 to 5) and sizes (0.04-0.09 μ m) with possible constrictions, enhanced univalent frequency including complete lack of pairing (14I formation) and irregular AI separation are also reported. Pollen size did not show any marked variations during the assessed periods and it ranged from $36.4 \mu\text{m} \pm 0.87 \times 29.5 \mu\text{m} \pm 0.17$ to $42.80 \mu\text{m} \pm 0.28 \times 32.4 \mu\text{m} \pm 0.91$. Rare often (3 out of 7398 – 0.07%) relatively small sized ($24.97 \mu\text{m} \times 17.83 \mu\text{m}$) pollen grains were observed but such type of pollen grains are rather not uncommon in *Corchorus* spp. where cytomixis has not been evident. Meiotic analysis in the marked plant thereafter (mid-July to early August

2012 – rainy season; temperature 26°-32°C, humidity 76-82%, rainfall 150-325 mm) showed incipient cytotoxicity without the formation of aneuploid and polyploid PMCs.

de Souza and Pagliarini (1997) reported that high temperature and water scarcity during summer season possibly accounted for cytotoxicity in *Brassica napus* and *B. campestris*, normally grown in winter. The high temperature has been also considered to be the cause of the high incidence of cytotoxicity in *Hemerocallis* (Narain 1979), *Ervatamia divaricata* (De and Sharma 1983), *Helicanthes elastic* (Soman and Bhavanandan 1993), Rose (Pécricx *et al.* 2011), among others. Malallah and Attia (2003) suggested that cytotoxicity observed in *Diplotaxis harra* may be associated with temperature and drought stress

phases prevailing during the growth season in Kuwait. On the contrary, Kaur *et al.* (2010) reported cytotoxicity in *Poa annua* while growing wild in higher altitudes but the species depicted normal meiosis in plains. In the present investigation, cytotoxicity studied irrespective of its intensity did not affect pollen fertility. However, formation of heterogenous pollen grains and reduction of pollen fertility and viability due to cytotoxicity have been reported in plant species (De and Sharma 1983; Baptista-Giacomelli *et al.* 2000; Bellucci *et al.* 2003; Rani *et al.* 2010; Saggoo *et al.* 2011; Srivastava and Kumar 2011). Gayen and Sarkar (1996) reported enhanced pollen fertility (15 to 20 folds) in maize haploids with extensive cytotoxicity.

Table 1. Cytotoxicity and pollen fertility in different studied periods of *C. fascicularis*

Samples	Climatic factors	Number of cells scored at diplotene and MI	Frequency (%)			Number of AI cells scored	Equal (7/7) separation (%)	PMCs with aneuploid chromosome number at AI (%)		Total number of pollen grains scored	Pollen fertility (%)
			Hypoploid	Hyperploid	Total			Hypoploid	Hyperploid		
1	T: 39°-30°C; H: 85.51%; R: 0.00 mm	123	8.94	13.82	22.76	87	88.51	0.00	3.44	1479	69.17
2	T: 39.4°-29°C; H: 94-61%; R: 0.00 mm	93	6.45	8.60	15.05	82	86.59	0.00	4.88	1234	72.93
3	T: 32°-25°C; H: 88-69%; R: 13.0 mm	138	2.90	3.62	6.52	68	88.24	0.00	0.00	1169	76.65
4	T: 32.2°-26°C; H: 91-69%; R: 20.0 mm	220	1.36	5.45	6.81	111	79.28	0.00	0.00	1081	74.01
5	T: 31.6°-26°C; H: 89-62%; R: 10.0 mm	77	0.00	6.49	6.49	73	95.89	0.00	0.00	1322	72.01
6	T: 38°-27°C; H: 91-68%; R: 7.00 mm	147	3.40	7.48	10.88	81	88.89	0.00	3.70	1113	71.34

1=05.06.2012, 2=11.06.2012, 3=17.06.2012, 4=22.06.2012, 5= 02.07.2012, 6=09.07.2012; T= Temperature, H= Humidity, R= Rainfall

CONCLUSION

High temperature has intensified cytotoxic behavior of chromosomes in *C. fascicularis*, and it is in accordance to the current concept that the phenomenon is influenced by the environmental factor(s). In the context, it would be relevant to accord the suggestion provided by Mandal and Datta (2012) that cytotoxicity is a natural process under gene control, and the gene(s) involved in the process may be differentially expressed or repressed under a given set of condition(s).

ACKNOWLEDGEMENT

The Research is grant-aided by University Grant Commission (India).

REFERENCES

- Ajay, K.J. and Sarbhoy, R.K. (1987). Cytogenetical studies on the effect of some chlorinated pesticides. II. Effect on meiotic chromosomes of *Lens* and *Pisum*. *Cytologia*, **52**: 55-61.
- Baptista-Giacomelli, F.R.; Pagliarini, M.S. and Almeida, J.L. de. (2000). Meiotic behavior in

- several Brazilian oat cultivars (*Avena sativa* L.). *Cytologia*, **65**: 371-378.
- Bellucci, M.; Roscini, C. and Mariani, A.** (2003). Cytomixis in pollen mother cells of *Medicago sativa* L. *Journal of Heredity*, **94**: 512-516.
- Bobak, M. and Herich, R.** (1978). Cytomixis as a manifestation of pathological changes after the application of trifluralin. *Nucleus*, **21**: 22-26.
- Boldrini, K.R.; Pagliarini, M.S. and do Valle, C.B.** (2006). Cell fusion and cytomixis during microsporogenesis in *Brachiaria humidicola* (Poaceae). *South African Journal of Botany*, **72**: 478-481.
- Carlson, J.G. and Handel, M.A.** (1988). Intercellular bridges and factors determining their patterns in the grasshopper testis. *Journal of Morphology*, **196**: 173-185.
- Carr, D.J.** (1976). Plasmodesmata in growth and development. In: Gunning BES, Robards AW (ed) Intercellular Communication in Plants: Studies on Plasmodesmata. Springer, Berlin, pp 243-288.
- Datta, A.K.; Mukherjee, M. and Iqbal, M.** (2005). Persistent cytomixis in *Ocimum basilicum* L. (Lamiaceae) and *Withania somnifera* (L.) Dun (Solanaceae). *Cytologia*, **70**: 309-313.
- De, M. and Sharma, A.K.** (1983). Cytomixis in pollen mother cells of an apomictic ornamental *Ervatamia divaricata* (Linn.) Alston. *Cytologia*, **48**: 201-207.
- de Souza, A.M. and Pagliarini, M.S.** (1997). Cytomixis in *Brassica napus* var. Oleifera (Brassicaceae). *Cytologia*, **62**: 25-29.
- Dong, L.Z. and Junying, Y.X.** (1988). By isolating single spore for determine genotype of *Pleurotus sapidus* and *Lentinus edodes*. *Journal of Agricultural University of Hebei*, doi: cnki:ISSN:1000-1573.0.1988-03-014
- Dong, W.; Li, W.; Guo, G.Q. and Zheng, G.C.** (2004). Ultrastructural aspects of plasmodesmata and cytoplasmic bridges during spermatogenesis in *Funaria hygrometrica*. *Acta Botanica Sinica*, **46**: 988-996.
- Gates, R.R.** (1911). Pollen formation in *Oenothera gigas*. *Annals of Botany*, **25**: 909-940.
- Gayen, P. and Sarkar, K.P.** (1996). Cytomixis in maize haploids. *Indian Journal of Genetics and Plant Breeding*, **56**: 79-85.
- Ghaffari, S.M.** (2006). Occurrence of diploid and polyploidy microspores in *Sorghum bicolor* (Poaceae) is the result of cytomixis. *African Journal of Biotechnology*, **5**: 1450-1453.
- Guo, G.Q. and Zheng, G.C.** (2004). Hypotheses for the functions of intercellular bridges in male germ cell development and its cellular mechanisms. *Journal of Theoretical Biology*, **229**: 139-146.
- Guzicka, M. and Wozny, A.** (2005). Cytomixis in shoot apex of Norway spruce [*Picea abies* (L.) Karst.]. *Trees*, **18**: 722-724.
- Haroun, S.A.; Al Shehri, A.M. and Al Wadie, H.M.** (2004). Cytomixis in the microsporogenesis of *Vicia faba* L. (Fabaceae). *Cytologia*, **69**: 7-11.
- Heng-Chang, W.; Li, J.Q. and He, Z.C.** (2007). Irregular meiotic behavior in *Isoetes sinensis* (Isoetaceae), a rare and endangered fern in China. *Caryologia*, **60**: 358-363.
- Himshikha, P.; Kumar, R.; Gupta, C.; Kumari, S. and Singhal, V.K.** (2010). Impact of chromatin transfer and spindle abnormalities on pollen fertility and pollen size in *Plantago lanceolata* L. *Cytologia*, **75**: 421-426.
- Iqbal, M. and Datta, A.K.** (2007). Cytogenetic studies in *Withania somnifera* (L.) Dun. (Solanaceae). *Cytologia*, **72**: 43-47.
- Kaur, D. and Singhal, V.K.** (2012). Phenomenon of cytomixis and intraspecific polyploidy (2x, 4x) in *Spergularia diandra* (Guss.) Heldr. & Sart. in the cold desert regions of Kinnaur district (Himachal Pradesh). *Cytologia*, **77**: 163-171.
- Kaur, H.; Gupta, A.; Kumari, S. and Gupta, R.C.** (2010). Meiotic studies in *Poa annua* L. from different altitudinal ranges of North India. *Cytologia*, **75**: 313-318.
- Körnigke, M.** (1901). Über ortsveränderung von Zellkarnern S B Niederhein Ges Natur-U Heilkunde Bonn A, pp 14-25.
- Kumar, G. and Tripathi, R.** (2008). Induced cytomictic variations through abiotic stresses in grasspea (*Lathyrus sativus* L.). *Indian Journal of Genetics and Plant Breeding*, **68**: 58-64.
- Kwiatkowska, M.; Popłońska, K. and Wojtczak, A.** (2003). *Chara tomentosa* antheridial plasmodesmata at various stages of spermatogenesis. *Biologia Plantarum*, **46**: 233-238.
- Li, X.F.; Song, Z.Q.; Feng, D.S. and Wang, H.G.** (2009). Cytomixis in *Thinopyrum intermedium*, *Thinopyrum ponticum* and its hybrids with wheat. *Cereal Research Communications*, **37**: 353-361.
- Mahapatra, A.K. and Saha, A.** (2008). Genetic Resources of Jute and Allied Fibers Crops. Jute and Allied Fibers Updates: Production and Technology, Barrackpore. p 327.
- Maheshwari, P.** (1950). An introduction to the Embryology of Angiosperms. McGraw-Hill, New York.
- Maity, S. and Datta, A.K.** (2008). Cytomorphological studies in F₁ hybrids (*Corchorus capsularis* L. x *Corchorus olitorius* L.) of jute (Tiliaceae). *Comparative Cytogenetics*, **2**: 143-149.
- Maity, S. and Datta, A.K.** (2009). Meiosis in nine species of Jute (*Corchorus*). *Indian Journal of Science and Technology*, **2**: 27-29.
- Malallah, G.A. and Attia, T.A.** (2003). Cytomixis and its possible evolutionary role in a Kuwaiti population of *Diplotaxis harra* (Brassicaceae). *Botanical Journal of the Linnean Society*, **143**: 169-175.

- Mandal, A. and Datta, A.K.** (2011). Secondary chromosome associations and cytomixis in *Corchorus* spp. *Cytologia*, **76**: 337-343.
- Mandal, A. and Datta, A.K.** (2012). Inter- and intra-plant variations in cytotoxic behavior of chromosomes in *Corchorus fascicularis* Lamk. (Tiliaceae). *Cytologia*, **77**: 269-277.
- Mandal, A.; Datta, A.K.; Gupta, S.; Paul, R.; Saha, A.; Ghosh, B.K.; Bhattacharya, A. and Iqbal, M.** (2013). Cytomixis—a unique phenomenon in animal and plant. *Protoplasma*, **493**: in press. DOI 10.1007/s00709-013-0493-z
- Marks, G.E.** (1954). An aceto-carmine glycerol jelly for use in pollen-fertility counts. *Biotechnic & Histochemistry*, **29**: 277-278.
- Miljajev, E.L.** (1967). Cytochimiceskoje I electron – mikroskopi ceskoje izucenje mikosporogeneza *Citrus sinensis*. Autoreferat Kandidaatske dizertacije.
- Morisset, P.** (1978). Cytomixis in the pollen mother cells of *Ononis* (Leguminosae). *Canadian Journal of Genetics and Cytology*, **20**: 383-388.
- Mukherjee, M. and Datta, A.K.** (2005). Secondary chromosome associations in *Ocimum basilicum* L. and *Ocimum tenuiflorum* L. *Cytologia*, **70**: 149-152.
- Narain, P.** (1979). Cytomixis in the pollen mother cells of *Hemerocallis* Linn. *Current Science*, **48**: 996-998.
- Nirmala, A. and Rao, P.N.** (1996). Genesis of chromosome numerical mosaicism in higher plants. *Nucleus*, **39**: 151-175.
- Paolillo, Jr. D.J. and Cukierski, M.** (1976). Wall developments and coordinated cytoplasmic changes in spermatogenous cells of *Polytrichum* (Musci). *Bryologist*, **79**: 466-479.
- Pécricx, Y.; Rallo, G.; Folzer, H.; Cigna, M.; Gudin, S. and Le Bris, M.** (2011). Polyploidization mechanisms: temperature environment can induce diploid gamete formation in *Rosa* sp. *Journal of Experimental Botany*, **62**: 3587-3597.
- Peng, Z.S.; Yang, J. and Zheng, G.C.** (2003). Cytomixis in pollen mother cells of new synthetic hexaploid amphidiploid (*Aegilops tauschii*×*Triticum turgidum*). *Cytologia*, **68**: 335-340.
- Rani, S.; Kumar, S.; Jeelani, S.M.; Gupta, R.C. and Kumari, S.** (2010). Effect of cytotoxic on male meiosis in populations of *Clematis grata* Wall. from Western Himalayas. *Chromosome Botany*, **5**: 61-64.
- Roosen-Runge, E.C.** (1977). The Process of Spermatogenesis in Animals. Cambridge University Press, London.
- Saggoo, M.I.S.; Gill, A. and Walia, S.** (2011). Cytotoxic during microsporogenesis in some populations of *Croton bonplandianum* of north India. *Cytologia*, **76**: 67-72.
- Singhal, V.K. and Kumar, P.** (2008). Impact of cytotoxic on meiosis, pollen viability and pollen size in wild populations of Himalayan poppy (*Meconopsis aculeate* Royle). *Journal of Bioscience*, **33**: 371-380.
- Soman, T.A. and Bhavanandan, K.V.** (1993). Temperature sensitive cytotoxic in *Helicanthes elastica* (Desr) Dans (Loranthaceae). *Cytologia*, **58**: 21-26.
- Song, Z.Q. and Li, X.F.** (2009). Cytotoxic in pollen mother cells of *Salvia miltiorrhiza*. *Caryologia*, **62**: 213-219.
- Soodan, A.S. and Wafai, B.A.** (1987). Spontaneous occurrence of cytotoxic during microsporogenesis in almond (*Prunus amygdalus* Batsch) and peach (*P. persica* Batsch). *Cytologia*, **52**: 361-364.
- Srivastava, P. and Kumar, G.** (2011). EMS-induced cytotoxic variability in safflower (*Carthamus tinctorius* L.). *Cytology and Genetics*, **45**: 240-244.
- Takats, S.T.** (1959). Chromatin extrusion and DNA transfer during microsporogenesis. *Chromosoma*, **10**: 430-453.
- Tarkowska, J.** (1965). Experimental analysis of the mechanism of cytotoxic. I. Cytotoxic in vegetative tissues. *Acta Societatis Botanicorum Poloniae*, **34**: 27-44.
- Tarkowska, J.** (1966). Experimental analysis of the mechanism of cytotoxic. II. Cytotoxic in the pollen mother cells of the lily - *Lilium candidum* L. *Acta Societatis Botanicorum Poloniae*, **35**: 25-40.
- Ventela, S.; Toppari, J. and Parvinen, M.** (2003). Intercellular organelle traffic through cytoplasmic bridges in early spermatids of the rat: mechanisms of haploid gene product sharing. *Molecular Biology of the Cell*, **14**: 2768-2780.
- Yun-sheng, W. and Yong-ping, C.** (2006). Study on cytotoxic on pollen-mother-cell (PMC) of "Arbo" wheat nullisomic lines. *Journal of Anhui Agricultural Sciences*, **34**: 25-26. doi: cnki:ISSN:0517-6611.0.2006-01-013
- Zheng, G.C.; Yang, Q.L. and Zheng, Y.R.** (1987). The relationship between cytotoxic and chromosome mutation and karyotype evolution in lily. *Caryologia*, **40**: 243-259.

