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First record of nucleus migration in premeiotic antherial cells of *Saccharum spontaneum* L. (Poaceae)

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Abstract. The occurrence of nucleus migration is reported for the first time in a clone ($2n = 64$) of ‘Thatch’ grass (*Saccharum spontaneum* L.) of the family Poaceae. Usually, its premeiotic antherial cells are thin walled, uninucleate and without any trace of chromosome individuality. However, the cells of those anthers that had been affected from flood water stress conditions were anucleated to hexanucleated in varying frequencies. Out of 2567 cells analyzed, two and three cells were noticed to be connected to each other through a well-defined cytoplasmic channel. The nuclei were observed at various stages of their migration in interconnected cells. The remaining cells exhibited a mosaic of anucleate to hexanucleate cells in varying frequencies with a dominance of binucleated condition (43.75%). The anucleate ‘ghost’ cells were much smaller in size than the uninucleate, binucleate and multinucleate cells showing insignificant variation among themselves. The anucleate, binucleate and multinucleate cells appeared to be resulted due to nucleus migration through cytoplasmic channels between two cells. The presence of a nucleus in donor cell united with recipient cell having four nuclei of different sizes, diminutive anucleate cell in the neighbourhood of uninucleate/trinucleate cell or connected with cytoplasmic channel/pentanucleate cell, and disorganizing cytoplasmic channel attached with binucleate/ tetranucleate cell witnessed the accomplishment of nucleus migration. This rare phenomenon of nucleus migration seemed to be triggered by flood water induced stress and facilitated by feeble cell wall. The variation in sizes of nuclei in multinucleate cells might be due to the transfer of nucleus/nuclei of different size(s). The prominent features of nucleus migration distinguishing it from the cytomixis have been discussed in detail. The syncytes resulted due to nucleus migration might have generated the pollen grains with different genetic constitution resulting into the origin of new intraspecific aneuploids/ polyploids for better adaptability.

Keywords: Cytomixis, Nucleus migration, Premeiotic cells, *Saccharum spontaneum*.

INTRODUCTION

Flooding stress has been considered as the strong driver of adaptive evolution (Jackson and Colmer 2005). In the disturbed habitat of *diara* land, it

seems to act as the main stress that can trigger syncyte formation leading to the production of new polyploids/aneuploids. Cytomixis (inclusive of nuclear migration) that operates as the most common pathway of syncyte formation is an important process of evolutionary significance (Kravets 2012, Mandal *et al.* 2013, Mursalimov *et al.* 2013). The phenomenon is considered to be an efficient mechanism for the production of unreduced (2n) pollen grains and thereby the origin of intraspecific polyploids in several plant species (Falistocco *et al.* 1995, Ghaffari 2006, Kim *et al.* 2009, Sheidai *et al.* 2009, Fadaie *et al.* 2010, Singhal *et al.* 2010, 2011, 2016, Kaur and Singhal 2012). The polyploids with more adaptability often thrive better in harsh and disturbed environments (Ramsey and Schemske 1998, Otto and Whitton 2000, Madlung 2013, De Storme and Mason 2014, de Peer *et al.* 2021). The occurrence of nucleus migration has been observed in the premeiotic cells of anthers of a clone of *Saccharum spontaneum* L. (English - Thatch grass, Vernacular - *Kans*, Family - Poaceae) growing under flood water stress conditions. This clone (2n=64) grows profusely in Bhagalpur (Bihar) *diara* land of Ganga basin, where flood is almost a perpetual annual feature (Singh *et al.* 2018). This is the favourite fodder of buffaloes. Besides, the species is very useful in thatching of roofs and in making various items such as cordage, ropes, mats, baskets, brooms, etc. (Singh 1997). The Indian sub-continent including Bihar is considered as the centre of greatest evolutionary activity of *S. spontaneum* L. (Panje and Babu 1960). The occurrence of nucleus migration in the premeiotic cells is the first record for this species, the most valuable germplasm in sugarcane breeding. Fifty-two years ago, chromosome and nucleus migrations were observed during microsporogenesis of some radiation-induced mutants of *Pisum sativum* L. (Gottschalk 1970). The present communication aims to clear, enrich and elaborate the concept of nucleus migration.

MATERIALS AND METHODS

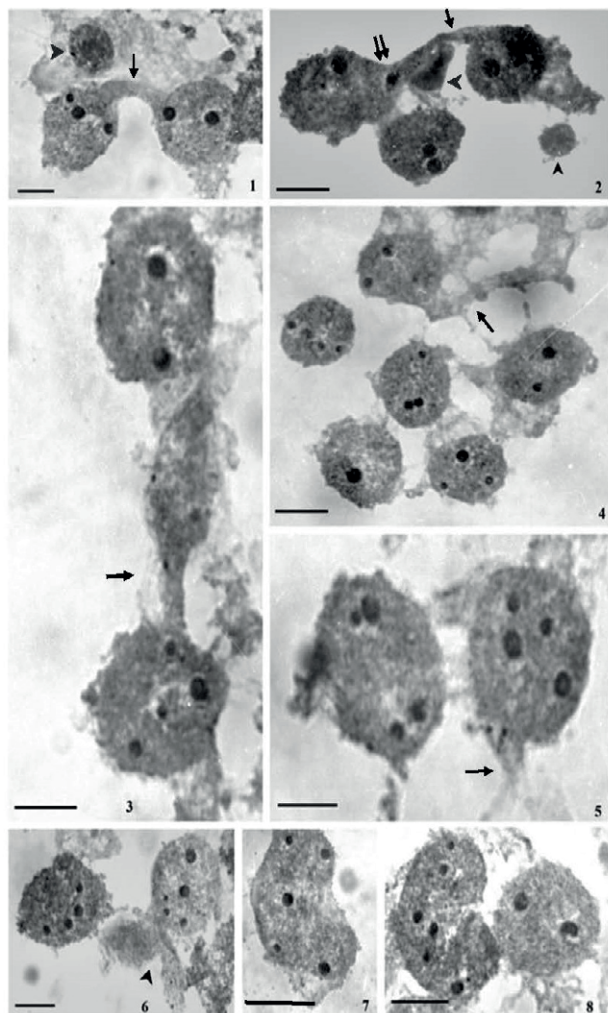
The spikelets of a clone of *Saccharum spontaneum* L. (Voucher specimen number 2180 of Herbarium of University Department of Botany, Tilka Manjhi Bhagalpur University, Bhagalpur) growing profusely in Bhagalpur *diara* land constituted the experimental material of the present investigation. These were collected every alternate day from both normal (flood free) and submerged conditions. The collected spikelets from these environments were fixed separately in Carnoy's fluid (6 ethanol: 3 chloroform: 1 glacial acetic acid). The fixative was

changed daily for 15 days to clear the cytoplasm. Subsequently, the material was preserved in 70% ethanol and refrigerated at 0°C until use. Anthers were squashed in 2% acetocarmine and preparations were studied to record abnormalities in premeiotic cells. Photomicrographs of unusual premeiotic cells were taken from the temporary preparations. The diameters of visible nuclei were determined from their enlarged photographs (at constant magnification) and the volumes of nuclei were calculated using the formula $\frac{4}{3}r^3$ for sphere; where r is the radius (i.e., half of diameter).

RESULTS

The premeiotic cells from young anthers of a *diara* clone of 'Thatch' grass (*Saccharum spontaneum* L.) had thin wall and a distinct nucleus. These cells were constantly uninucleate in the anthers of normal plants. However, wide variations in number of nuclei were observed in the cells from the anthers of flood affected plants. Out of a total 2567 cells analyzed, two and three cells were found to be connected to each other through a conspicuous cytoplasmic channel respectively, in nineteen (Figure 1) and two (Figure 2) instances. The nuclei were seen at various stages of their migration in the united cells (Figures 2-3). The remaining cells exhibited a mosaic of anucleate to hexanucleate cells (Figures 4-8) in varying frequencies (Table1) with dominance of binucleate condition (43.75%). Among the multinucleate cells, the trinucleate ones were the most frequent, while the pentanucleate were the least common. With respect to size, the anucleate 'ghost' cells were much smaller than the nucleate (uninucleate, binucleate and multinucleate) cells which did not show significant variation among themselves.

The anucleate, binucleate and multinucleate cells appeared to be the resultant products of nucleus migration, commencing with the development of a well-defined cytoplasmic channel between two cells (Figures 1-6). The possession of one nucleus in the donor cell, which is united with a recipient cell containing four nuclei of different sizes (Figure 3) showed that the cytoplasmic channel might have paved the way for gradual transference of nucleus/nuclei through it. The presence of a diminutive anucleate donor cell in the neighborhood of a trinucleate cell (Figure 1) or uninucleate cell (Figure 2) or connected prominently with a cytoplasmic channel (Figure 2) or pentanucleate cell (Figure 6) and the disorganizing cytoplasmic channel attached with a binucleate cell (Figure 4) or a tetranucleate cell (Figure 5), suggested towards the completion of nucleus migra-



Figures 1-8. Premeiotic antherial cells of *Saccharum spontaneum* L. showing nucleus migration and its products. 1. Two cells connected through a conspicuous cytoplasmic channel (arrowed) in between and a small anucleate ghost cell (arrowhead) present on upper side. 2. Two distinct uninucleate cells linked through a cytoplasmic channel (arrowed) and a small deformed anucleate ghost cell (arrowhead) united mid way with the channel – discharged nucleus (double arrowed) entering into a cell to make it binucleate; another small spherical free anucleate ghost cell (arrowhead) and a large binucleate cell discernible on lower side. 3. Two cells interconnected with each other through a cytoplasmic channel (arrowed): Donor cell possessing one nucleus and recipient cell containing four unequal sized nuclei; a binucleate cell visible above. 4. Uni, bi and trinucleate cells; a binucleate cell united with a disorganizing cytoplasmic channel (arrowed). 5. Tetranucleate cells showing marked variation in the size of nuclei and one tetranucleate cell showing attachment with a disorganizing channel (arrowed). 6. A tetranucleate cell in left and an elongated cum curved anucleate ghost cell (arrowhead) attached with a pentanucleate cell (having uneven sized nuclei) in right. 7. A deformed pentanucleate cell having nuclei of different sizes. 8. Bi and hexanucleate cells: Curved shape of hexanucleate cell remarkable. Bar = 20 μ m.

Table 1. Type, shape, and frequency of flood affected premeiotic antherial cells* of *Saccharum spontaneum* L.

Cell type	Shape	Number and Frequency (%)
Anucleate	Spherical	31(01.21)
Uninucleate	Spherical	234(09.12)
Binucleate	Spherical	1123(43.75)
Trinucleate	Spherical	954(37.16)
Tetranucleate	Spherical/ Ellipsoidal	165(06.43)
Pentanucleate	Spherical/ Irregular	16(00.62)
Hexanucleate	Irregular	44(01.71)

*Among 2567 cells, two and three cells connected to each other through a conspicuous cytoplasmic channel respectively in nineteen and two cases for nucleus migration.

tion. The phenomenon of nuclear migration seemed to be induced by abiotic stress due to flood water conditions and facilitated by feeble wall character of premeiotic cells resulting into syncyte formation.

The shapes of cells were noted to be specific to some extent to their nuclear status as exclusively spherical for anucleate (in free condition) to trinucleate; spherical or ellipsoidal for tetranucleate; spherical or irregular for pentanucleate and solely irregular for hexanucleate conditions (Table 1). The uninucleate and binucleate cells displayed the presence of only large sized nucleus and nuclei accordingly in them. Irrespective of their shapes, the multinucleate cells had nuclei of different sizes in them (Table 2). These nuclei were arbitrarily classified as large (above 50 μ m³), medium (above 20 μ m³ and up to 50 μ m³) and small (up to 20 μ m³). The presence of equal sized nuclei in binucleate cells and unequal sized nuclei in multinucleate cells seems to have been derived from similar and dissimilar types of cells. This became evident from the existence of an anucleate ghost cell and a binucleate cell that was connected with a trinucleate cell (having one nucleus of each category) through a cytoplasmic channel for nuclear migration (Figure 1). The size of nucleus contained in uninucleate cells showed approximation to the dimension of large sized nuclei possessed by tetranucleate cells, whose medium sized nuclei were almost of the magnitude of medium sized nuclei of trinucleate cells. Figure 5 indicates the presence of a tetranucleate cell possessing different sized nuclei, which might have resulted due to nucleus migrations from different types of donor cells.

DISCUSSION

Ever since the first report of syncytes by Gates and Reese (1921), these multinucleate cells have been report-

Table 2. Data on number of nucleus or nuclei per cell and dimension of different sized nuclei in flood affected premeiotic antherial cells of *Saccharum spontaneum* L.

Nucleus or nuclei/ Cell	Different sized nuclei / Cell	Diameter of nucleus Mean \pm S.D. (μm)	Volume of nucleus (μm^3)
1	Large - 1	6.01 \pm 0.20	118.89
2	Large - 2	5.04 \pm 0.68	67.06
3	Large - 1	5.68 \pm 0.70	95.99
	Medium - 1	3.47 \pm 0.18	21.89
	Small - 1	2.33 \pm 0.37	06.63
4	Large - 2	6.25 \pm 0.28	127.88
	Medium - 2	3.48 \pm 0.29	23.43
5	Large - 3	4.68 \pm 0.45	53.69
	Small - 2	2.17 \pm 0.21	05.35
6	Medium - 3	3.80 \pm 0.39	28.74
	Small - 3	2.26 \pm 0.33	06.05

ed in several plant species by different workers. The syncytes in plants are usually formed through arche-spore error, cell fusion and nuclear migration. Out of these, nuclear migration occurs through the transfer of whole chromatin material or nucleus from one cell to an adjacent cell. The migration of partial or total chromosome, referred to as “Cytomixis”, is a widespread phenomenon and has been reported in a large number of plant species (Kravets 2012, Mandal *et al.* 2013, Mursalimov *et al.* 2013, Rana *et al.* 2014, 2015, Kumar *et al.* 2015, Reis *et al.* 2015, Bhat *et al.* 2017, Mandal and Nandi 2017, Singhal *et al.* 2018, Paez *et al.* 2021). However, nucleus migration has been observed only in pollen mother cells of radiation-induced ‘pea’ (Gottschalk 1970). The occurrence of nucleus migration through a conspicuous cytoplasmic channel as observed in the present study appears to be closely allied with the well-known phenomenon of cytomixis through cytomictic channel. Both these phenomena seem to be homologous but indeed separate from each other at least in mode of operation. Cytomixis involves chromatin transfer between proximate cells (Kamra 1960, Omara 1976, Belluci *et al.* 2003, Singhal *et al.* 2009, 2018, Himshikha *et al.* 2010, Rana *et al.* 2013, Kumar and Singhal 2016, Kumar *et al.* 2016, 2017, Bhat *et al.* 2017, Mandal and Nandi 2017). Owing to this unique feature, it is also called as the phenomenon of intercellular chromatin transmigration (Kumar and Naseem 2013, Kumar and Choudhary 2016, Dwivedi and Kumar 2018, Khan *et al.* 2018, Kumar and Singh 2020). Akin to cytomixis, nucleus migration requires the passage of a whole nucleus from donor cell to recipient cell (Gottschalk 1970, Patra *et al.* 1987). According to Kihara and Lilienfeld (1934), the term “Cytomixis” should be used to designate only

the transit of structureless chromatin drops (“Uebertritte strukturloser chromatintropfen”). Keeping in view the above-mentioned limitation placed on the use of this term, Gottschalk (1970) designated the phenomenon of moving chromosome and nucleus with normal structure as “chromosome and nucleus migration” rather than cytomixis. The denotation of migration of chromosomes and nuclei, respectively as nuclear chromosome migration and migration of nucleus by Patra *et al.* (1987) may be a circumstantial consideration that cytomixis and nucleus migration are apart from each other. There appears no hitch in corroborating the event of shift of structured nucleus as evident in the present case as “nucleus migration”.

The uniformity in the size of uninucleate, binucleate and multinucleate cells in the presently studied grass suggests that only nuclei pass through cytoplasmic channels. Unlike this, cytomixis involves the transfer of both chromatin/ chromosome(s) along with cytoplasm as well as other cell organelles through cytomictic channels (Risueno *et al.* 1969, Romanov and Orlova 1971, Mursalimov and Deineko 2011, Kumar and Choudhary 2016, Kumar and Singh 2020). This becomes apparent from the increase in the size of cytomictic products/ recipient cells, as has also been recorded by Sarbhoy (1980) and Singh *et al.* (1989, 1990). Thus, nucleus migration has been treated here as a separate process different from cytomixis.

The possible causes of syncyte formation in plants include effect of chemicals, X-rays, temperature, moisture stress, viral infection, culture conditions or genetic factors. In the presently studied grass the syncyte formation could be attributed to flood water induced stress conditions prevailing in the *diara* land. The products of

such syncytes might have produced the pollen grains with different genetic constitution, which through chance fusion might have resulted in the production of polyploids/ aneuploids for better adaptability.

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REFERENCES

- Bellucci M., Roscini C., Mariani A. 2003. Cytomixis in pollen mother cells of *Medicago sativa* L. J Hered. 94 (6): 512-516.
- Bhat T.A., Gulfishan M., Wani A.A. 2017. Cytomixis: Causes and consequences as a case study in *Vicia faba* L. In: Chromosome Structure and Abberations, pp. 321-342. Springer, New Delhi.
- De Storme N., Mason A. 2014. Plant speciation through chromosome instability and ploidy change: Cellular mechanisms, molecular factors and evolutionary relevance. Curr Plant Biol. 1: 10-33
- Dwivedi H., Kumr G. 2018. Induced syncyte formation via cytomixis in *Trachyspermum ammi* (L.) Sprague (Apiaceae). Caryologia. 71(4)420-427.
- Fadaei F., Sheidai M., Asadi M. 2010. Cytological study the genus *Arenaria* L. (Caryophyllaceae) Caryologia 63(2): 149-156.
- Falisticco E., Tosti N., Falcinelli M. 1995. Cytomixis in pollen mother cells of diploid *Dactylis*, one of the origins of 2n gametes. J Hered. 86(6): 448-453.
- Gates R.R., Reese M. 1921. A cytological study of pollen development in *Lactuca*. Ann Bot 35(3): 365-398.
- Ghaffari S.M. 2006. Occurrence of diploid and polyploid microspores in *Sorghum bicolor* (Poaceae) is the result of cytomixis. Afr J Biotechnol. 5(16): 1450-1453.
- Gottschalk W. 1970. Chromosome and nucleus migration during microsporogenesis of *Pisum sativum*. Nucleus.13: 1-9.
- Himshikha, Kumar P., Gupta R.C., Kumari S., Singhal V.K. 2010. Impact of chromatin transfer and spindle abnormalities on pollen fertility and pollen size in *Plantago lanceolata* L. Cytologia. 75(4): 421-426.
- Jackson M.B., Colmer T.D. 2005. Response and adaptation by plants to flooding stress. Ann Bot. 96(4): 501-505.
- Kamra O.P. 1960. Chromatin extrusion and cytomixis in pollen mother cells of *Hordeum*. Hereditas. 46: 592-600.
- Kaur D., Singhal V.K. 2012. Phenomenon of cytomixis and intraspecific polypoidy (2x, 4x) in *Spergularia diandra* (Guss.) Heldr. & Sart. in the cold desert regions of Kinnaur district (Himachal Pradesh). Cytologia 77(2): 163-171.
- Khan N.A., Singhal V.K., Gupta R.C. 2018. First record of chromosome count and cytomixis in an endemic species of *Clematis ladakhiana* Grey-Wilson (Ranunculaceae) from cold deserts of Jammu and Kashmir. Caryologia. 71(3): 233-237.
- Kihara H., Lilienfeld F. 1934. Kerneinwanderung und Bildung syndiploider pollen mutterzellen be idem F₁Bastard. *Triticum aegelopoides* X *Aegilops squarrosa*. Jpn J Genet. 10: 1-28.
- Kim J.S., Oginuma K., Tobe H. 2009. Syncyte formation in the microsporangium of *Chrysanthemum* (Asteraceae): a pathway to intraspecific polyploidy. J Plant Res. 122(4): 439-444.
- Kravets E.A. 2012. Nature, significance and consequences of cytomixis. Cytol Genet. 46: 188-195.
- Kumar G., Choudhary N. 2016. Induced cytomixis and syncyte formation during microsporogenesis in *Phaseolus vulgaris* L. Cytol Genet. 50(2): 121 – 127.
- Kumar G., Naseem S. 2013. EMS induced intercellular chromatin transmigration in *Papaver somniferum* L. Czeck J Genet Breed. 49(2): 86-89.
- Kumar G., Singh S. 2020. Induced cytomictic crosstalk behaviour among micro-meicytes of *Cyamopsis tetragonoloba* (L.) Taub. (Cluster bean): Reasons and repercussions. Caryologia. 73(2): 111-119.
- Kumar P., Singhal V.K. 2016. Nucleoli migration coupled with cytomixis. Biologia 71(6): 651-659.
- Kumar P., Singhal V.K., Srivastava S.K. 2016. Chromosome counts and male meiosis in two species of *Pleurospermum* Hoffm. (Apiaceae): Additional comments on the cytogeographical pattern of the genus. Caryologia. 69(3): 273-282.
- Kumar P., Singhal V.K., Srivastava S.K. 2017. First detection of cytomixis and its consequences in *Thalictrum cultratum* Wall. (Ranunculaceae). Cytol Genet. 51(5): 384-390.
- Kumar R., Rana P.K., Himshikha, Kaur D., Kaur M., Singhal V.K., Gupta R.C., Kumar P. 2015. Structural heterozygosity and cytomixis driven pollen sterility in *Anemone rivularis* Buch – Ham. ex DC. from Western Himalaya (India). Caryologia. 68(3): 246-253.
- Madlung A. 2013. Polyploidy and its effect on evolutionary success: old questions revisited with new tools. Heredity. 110(2): 99-104.

- Mandal A., Datta A.K., Gupta S., Paul R., Saha A., Ghosh B.K., Bhattacharya A., Iqbal M. 2013. Cytomixis – a unique phenomenon in animal and plant. *Protoplasma*. 250: 985-996.
- Mandal G.D., Nandi A.K. 2017. Cytomixis with associated chromosomal anomalies and reproduction of *Chlorophytum borivilianum* Santapau and R.R Fern. Taiwan. 62(2): 211-225.
- Mursalimov S.R., Deineko E.V. 2011. An ultrastructural study of cytomixis in tobacco pollen mother cells. *Protoplasma*. 248(4): 717-724.
- Mursalimov S.R., Sidorchuk Y.V., Deineko E.V. 2013. New insights into cytomixis: Specific cellular features and prevalence in higher plants. *Planta*. 238(3): 415-423.
- Omara M. K. 1976. Cytomixis in *Lolium perenne*. *Chromosoma*. 55(3): 267 – 271.
- Otto S.P., Whitton J. 2000. Polyploidy incidence and evolution. *Annu Rev Genet*. 34: 401-437.
- Paez V. de los A., Andrada A.R., Kumar, P., Caro M.S. 2021. Cytomixis in angiosperms from Northwestern Argentina. *Bot Lett* 168(4): 536-545.
- Panje R.R., Babu C.N. 1960. Studies in *Saccharum spontaneum*. Distribution and geographical association of chromosome number. *Cytologia* 25: 152-172.
- Patra N.K., Chauhan S.P., Srivastava S.K. 1987. Syncytes with premeiotic mitotic and cytotoxic compartment in 'Opium' poppy (*Papaver somniferum* L.). *Indian J Genet*. 47 (1): 49-54.
- de Peer Y.V., Ashman T.L., Soltis P.S., Soltis D.E. 2021. Polyploidy: an evolutionary and ecological force in stressful times. *Plant Cell*. 33(1): 11-26.
- Ramsey J., Schemske D.W. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annu Rev Ecol Syst*. 29: 497-501.
- Rana P.K., Kumar P., Singhal V.K. 2013. Spindle irregularities, chromatin transfer and chromatin stickiness during male meiosis in *Anemone tetrasepala* Royle (Ranunculaceae). *Turk J Bot*. 37: 167-176.
- Rana P.K., Kumar P., Singhal V.K. 2014. Cytomixis and associated abnormalities during male meiosis in *Lindelofia longiflora* var. *falconeri* (Boraginaceae). *Cytologia*. 79(4): 535-540.
- Rana P.K., Kumar P., Singhal V.K. 2015. Chromosome counts, chromosomal pairing and pollen fertility in thirty-eight species of Asteraceae from Pangri Valley in district Chamba of Himachal Pradesh (India). *Braz J Bot*. 38(4): 837-850.
- Reis C.A., Souga S.M., Viccini L.F. 2015. High frequency of cytomixis observed at zygotene in tetraploid *Lippia alba*. *Plant Syst Evol*. 302(1): 121-127.
- Risueno M.C., Gimenez-Martin G., Lopez-Saez J.F., R-Garcia M.I. 1969. Connexions between meiocytes in plants. *Cytologia*. 34: 262-272.
- Romanov I.D., Orlova I.N. 1971. Cytomixis and its consequences in *Triticale* microsporocytes. *Genetika*. 7(12): 5-13.
- Sarbhojy R.K. 1980. Spontaneous occurrence of cytomixis and syndiploidy in *Cyamopsis tetragonoloba* (L.) Taub. *Cytologia*. 45(3): 375-379.
- Sheidai M., Jafari S., Taleban P., Keshavarzi M. 2009. Cytomixis and unreduced pollen grain formation in *Alopecurus* L. and *Catbrosa* Peauv. (Poaceae). *Cytologia* 74 (1): 31-41.
- Singh C.B. 1997. Notes on some little known uses of *Kans* (*Saccharum spontaneum* L.) from Bhagalpur diara land, Bihar. *J Non-Timber Forest Prod*. 4(3/4): 173-174.
- Singh C.B., Munshi J.D., Sinha S.P. 1989. Cytomixis in PMCs of *Saccharum spontaneum* L. *Curr Sci*. 58(13): 755-757.
- Singh C.B., Munshi J.D., Sinha S.P. 1990. A new basic chromosome number in *Saccharum spontaneum* L. *Cytologia*. 55(4): 645-648.
- Singh C.B., Singhal V.K., Kumar R. 2018. Genome elimination mediated pollen size dimorphism in clone of *Saccharum spontaneum* L. (Poaceae): A pathway to intraspecific polyploidy. *Eur J Phar Sci*. 5 (2): 379-383.
- Singhal V.K., Kaur S., Kumar P. 2010. Aberrant male meiosis, pollen sterility and variable sized pollen grains in *Clematis montana* Buch.- Ham.ex DC. from Dalhousie hills, Himachal Pradesh. *Cytologia*. 75 (1): 31-36.
- Singhal V.K., Khan N.A., Gupta R.C. 2016. Syncyte and 2n pollen grain formation in *Heracleum pinnatum* – a possible mechanism for the origin of intraspecific polyploids. *Cytologia*. 81(3): 335-339.
- Singhal V.K., Kumar P., Kaur D., Rana P.K. 2009. Chromatin transfer during male meiosis resulted into heterogenous sized pollen grains in *Anemone rivularis* Buch-Ham. ex DC. from Indian cold deserts. *Cytologia*. 74(2): 229-234.
- Singhal V.K., Kumar R., Kumar P. 2018. A new perception about cytomixis: causes, mechanism and uses. *Chromosome Sci*. 21: 61-66.
- Singhal V.K., Rana P.K., Kumar P. 2011. Syncytes during male meiosis resulting into 2n pollen grain formation in *Lindelofia longiflora* var. *falconeri*. *J Syst Evol*. 49: 406-410.