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NATURAL INCIDENCE OF CHROMOSOMAL CHIMERISM AND INTERCHANGES IN *TRICHOSANTHES CUCUMERINA VAR. ANGUINA* AND ITS IMPACT ON FRUIT SET

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Morpho-cytological tools carry great importance for identifying agronomically valuable and cytologically stable cultivars of vegetable crops like *Trichosanthes cucumerina* var. *anguina* (snake gourd). Presently, two cultivars of this cucurbit, one commercial and another locally grown in Jammu district, have been examined for phenotypic aspects. These correspond in most of foliar and floral attributes except for the yield, which is quite high for local cultivar but very low for commercial one. To identify bottlenecks accounting for limited fruit production in commercial cultivar, its six plants along with seven individuals of local form have been analysed for reduction division in male track. While pollen mother cells (PMCs) of local cultivar consistently contain 22 chromosomes showing regular behavior, commercial form has 7.2% hypo-/hyperploid cells (2x±2 chromosomes), with the remaining cells being diploid. Later cultivar is further unusual in having quadrivalents/ trivalents in 8.6% eu-and 30% hyperploid cells as well as irregular segregant patterns of 15:7, 13:9, 12:10, 10:3:9 and 11:1:10 type in 24% cells. These anomalies seem to have accounted for reduction in its pollen viability to 71.63%. In spite of having three-fourth fertile pollens, highly reduced fruit (10-15 per vine) and seed set (20-22 per fruit) in our commercial cultivar indicates that somewhat similar aberrations may be present in female tract. Apart from describing meiotic anomalies present, adverse impact of these on reproductive capacity of a diploid snake gourd cultivar has been discussed in this communication.

Keywords: aneuploids, quadrivalents, snake gourd, *Trichosanthes cucumerina* var. *anguina*, yield

Trichosanthes cucumerina var. *anguina* (Cucurbitaceae), commonly called snake gourd, is an annual, vegetable crop grown in tropical and subtropical regions of the world for vegetables (Schaefer and Renner 2013). In India, it is widely cultivated in West Bengal, Bihar, Uttar Pradesh and Jammu and Kashmir. Its highly nutritive vegetables are rich in proteins, vitamins and essential amino acids. The extracts of its fruits are used as a tonic while its roots and seeds are consumed by locals to treat indigestion and syphilis (Rahman *et al.* 2007, Ramachandran and Ramalingam 2017).

Despite importance, limited information on morpho-cytological variability of snake gourd is on record (Khatun *et al.* 2010) which, however, is required for identifying more productive and stable lines as well as for establishing factors leading to lesser fruit production in low yielding forms. Alam *et al.* (2011) noted lesser yield in two snake gourd varieties labeled as genetically pure by seed releasing firms and attributed the same to trisomy (2n=2x+1=23) and autotetraploidy (2n=4x=44) prevalent. Currently, preliminary phenotypic evaluation of a commercial and another local cultivar commonly grown in Jammu Province (Jammu and Kashmir, India) revealed high fruit and seed abortion in the former, with the latter exhibiting good seed set. Since reproductive efficiency of a taxon largely depends on chromosome behavior, for understanding the phenomenon leading to high sterility in commercial cultivar, its pollen mother cells (PMCs) were scrutinized for overall meiotic course. This study revealed manifold meiotic anomalies such as chromosomal chimerism, multivalent formation and unequal chromosomal segregations hitherto unreported for diploid snake gourd. These aberrations seem to have accounted for low pollen viabilty and large scale fruit abortion in commercial snake gourd cultivar.

MATERIALS AND METHODS

During 2017-18, 15-20 seeds of a commercial

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snake gourd cultivar labeled as genetically pure by the private seed company (S.Gourd-JKSPL marketed and packed by JK Agro Hybrid seeds) and a locally grown cultivar were sown in silty-clayey soil (pH=8, Relative Humidity=63-81%) of Botanical Garden of University of Jammu, Jammu (Jammu and Kashmir, India). Six to seven climbers raised from these were studied for morphological aspects like leaf size, petiole length, pedicel length, sepal size, petal size, fruit set per vine and seed set per fruit. Standard deviation and standard error for different plant parts was calculated using standard statistical procedures.

For studying meiosis, young male flower buds were collected in the morning hours, fixed in 1:3 acetic-alcohol solution for 24 hours, washed in tap water and preserved in 70% ethanol. For preparing slides, buds were washed with water and squashed in 1% propiono-carmine.

Pollen studies were made by squashing mature anthers in 1% acetocarmine. A total of 100-120 pollen grains were analysed for each case for pollen viabilty and pollen size. Filled and deep red pollen grains were considered fertile whereas shriveled, weakly stained and irregularly shaped grains were scored as non stainable. Besides, size of different pollen grains was measured using ocular micrometer. Pollen grains were further categorized into large (diameter of 67.20±0.37µm) and small (diameter of 58.05±0.23µm).

The plates showing good meiotic spreads revealing chromosome numbers and various associations were photographed from temporary mounts using Zeiss trinocular microscope with attached digital imaging system.

RESULTS AND DISCUSSION

Morphological Studies: Monoecious vines of commercial and local snake gourd cultivars under study correspond in most of the foliar and floral traits. They bore lobed, petiolated (petiole of 9.97±0.46 cm length) leaves with average size of 19.02±0.36cm×19.07±0.35cm. While its white male pedicellated (pedicels of 1.35±0.03cm length) flowers were born in a raceme, female pedicellated (pedicels of 1.4±0.32cm long) ones were solitary. Sepals of its flowers (both male and female) of 1.56±0.01x0.5 cm size formed a calyx tube whereas petals of 2.13±0.03cm×0.32±0.01cm size were peculiar in having reflexed segments. In so far as fruit production is concerned, these cultivars differed to great extent. Commercial cultivar exhibited high fruit abortion and produced only 10-15 fruits per vine, with each setting nearly 20-22 seeds. Local cultivar, on the other hand, formed 30-35 fruits per vine, with each exhibiting good seed set. Previously, low fruit production is also on record for Bangladeshi snake gourd cultivars studied by Khatun *et al.* (2010).

Meiotic Details: Since meiosis is the crucial stage in the life cycle of seed plants, any disturbance in its course may lead to genetically imbalanced gametes and low fruit and seed set. For determining relationship between reduced yield and disturbance in male meiosis, if any, PMCs of present commercial and local cultivars were studied for reduction division.

Because of the species having small sized, unamenable to stain chromosomes, only 237 PMCs of commercial cultivar and 126 PMCs of local cultivar could be analyzed for the meiotic course (Fig.1; Table 1).

A perusal of Table 1 reveals that PMCs of a local cultivar studied here consistently contained 22 chromosomes as per previous reports (Singh and Roy 1975, Datta and Basu 1978, Sarker *et al.* 1987, Beevy and Kuriachan, 1996). These chromosomes in outnumbering cells at metaphase I paired as 11 bivalents and in a few as 10 II and 2I, with all of these segregating as 11:11 at anaphase I. Present commercial cultivar, on the other hand, contained 92.8% PMCs with 22 chromosomes and 7.2% cells unusually having one to two additional/missing chromosomes. Like snake gourd, occurrence of PMCs with 2n±1-4

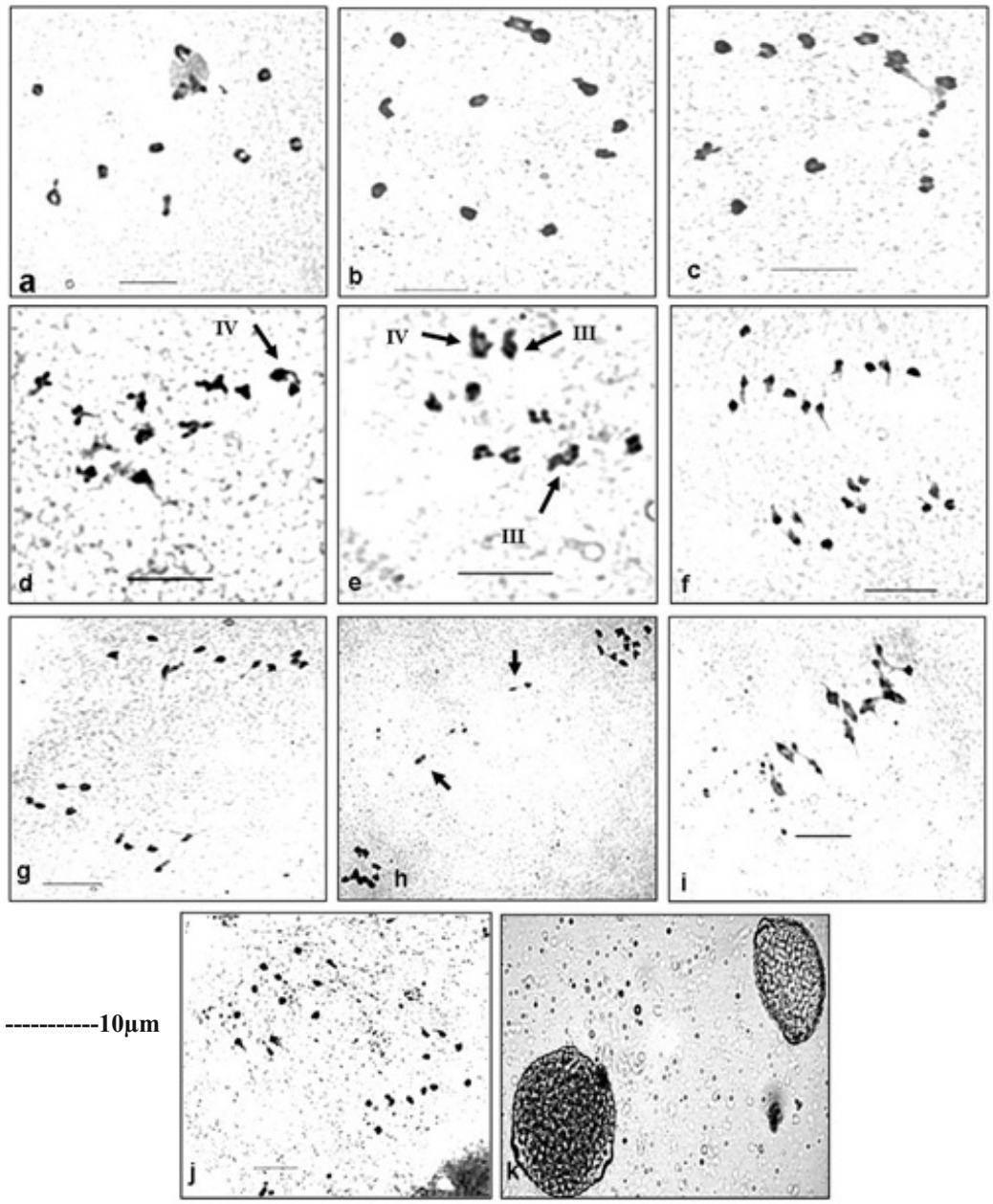

Figure 1 (a-k). Meiosis in male track of *Trichosanthes cucumerina* var. *anguina* (2n=22**). a.** A PMC at diplotene with 11 II (3 II attached with nucleolus), **b-c.** PMCs at metaphase I with 11 II and 10II+2I, **d-e.** PMCs at metaphase I with 1IV+9II and 1IV+2III+5II+2I, **f-h.** PMCs at anaphase I with 11:11, 12:10, 10:3:9 segregation of chromosomes, **i.** A hypoploid PMC at metaphase I with 20 chromosomes present as 10 II, **j.** A hyperploid PMC at anaphase I with 12:12 segregation, **k.** Stainable (left) and non-stainable (right) pollen grains.

chromosomes has been reported earlier in *Coix gigantea* (Sapre and Naik 1990), *Allium cepa* (Sharma and Gohil 2011), *Momordica charantia* (Bano *et al.* 2019) and *Luffa cylindrica* (Bano and Sharma 2020). Such aneuploid cells noted for the first time in snake gourd can result from non-disjunction of chromosomes during pre-meiotic mitosis, probably owing to spindle associated abnormalities. Though cells with abnormal spindles tend to be sieved out by spindle check-points at meiotic prophase, existence of aneuploid cells points towards the permissibility of these checkpoints in present

Table 1: Number of chromosomes and diplotene / metaphase I associations in local and commercial cultivars of *Trichosanthes cucumerina var. anguina.*

Local cultivar (126 cells)		**Commercial cultivar (237 cells)**		**Figs.**
Chromosome number	Associations	Chromosome number	Associations	
22	11 II (122)	22 (220)	11II (196)	1a-b
	10II+21 (4)		10II+2I (5)	1c
			1IV+9II (13)	1d
			1IV+2III+5II+2I (2)	1e
			2IV+7II (4)	-
		20 (7)	10II (7)	1i
		24 (10)	3IV+6II (1)	-
			2IV+8II (2)	-
			12II (7)	-

Table 2: Plant-wise (n=4) pollen viability and fruit set in two cultivars of *Trichosanthes cucumerina var. anguina*

Cultivar		**%age pollen viability**	**%age fruit formed**
Local	Plant 1	85	30.5
	Plant 2	90.5	33
	Plant 3	93	35
	Plant 4	94	35.2
	Mean	90.62	33.43
Commercial	Plant 1	68	10
	Plant 2	70.5	12.5
	Plant 3	73	13.5
	Plant 4	75	15
	Mean	71.63	12.75

species (Pacchierotti *et al.* 2007).

Euploid cells of present commercial diploid cultivar are further notable in having 8.63% cells with quadrivalents/trivalents. The remaining aneuploid cells, however, contained 12 II/11II+2 I, with 1 (30.18%), 2 (41.5%), 3 (20.75%), 4 (3.77%) and 5 (3.77%) of these bivalents being attached with nucleolus. Apart from diploid cells, two-three quadrivalents have been noted in 30% hyperploid cells. Percentage frequency of chromosomes present as quadri, tri-, bi- and univalents in euploid cells valued as 1.90, 0.24, 97.56 and 0.28, whereas those forming associations of four and two chromosomes in hyperploid cells figured 11.66 and 88.33. Both in quadrivalents and bivalents, chiasmata are randomly distributed with mean chiasmata frequency per cell and per bivalent being 18.5 and 7.5. Associations of four chromosomes noted in commercial cultivar seem to have formed due to exchange of segments between two non-homologous chromosomes. At the same time, tri- and univalents might have resulted from early terminalisation of one/two chiasmata of a quadrivalent. While Singh and Roy (1978) found quadrivalents and trivalents, as expected in colchi-tetraploid and triploid snake gourd produced by crossing experiments, such associations are not on record for any of its diploid cytotype. Apart from present cucurbit, multivalents have been reported in two other cucurbits namely, *M. charantia* (Bano *et al.* 2019) and *L. cylindrica* (Bano and Sharma 2020).

Though quadrivalents have the potential to segregate regularly as 2:2 at two anaphasic poles, in some cases these mis-segregate as 3:1. Similarly, univalents can mis-segregate owing to lack of physical basis of orientation. In

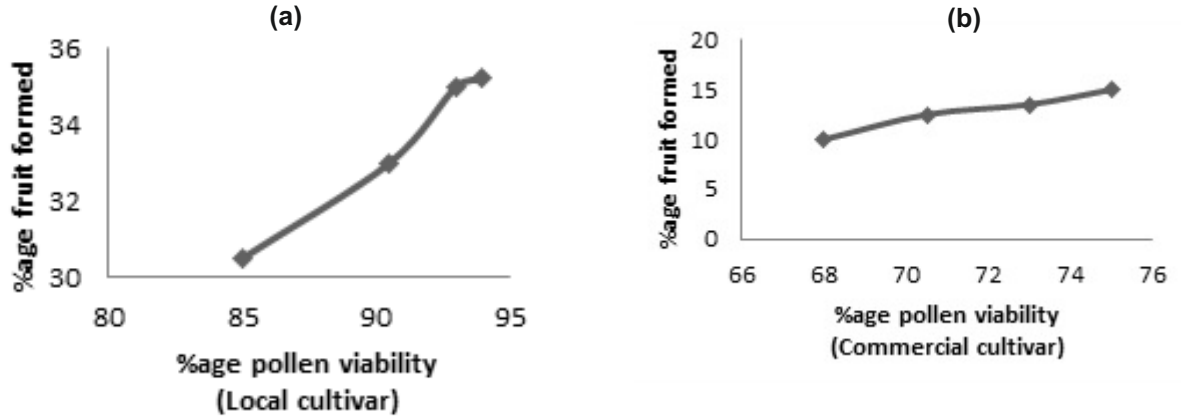

Figure 2(a-b); Scatter-plot of pollen viability (%age) vs. fruit set (%age) for local and commercial cultivars of *T. c.* var. *anguina*

commercial cultivar, mal-segregation of multivalents and univalents seems to have resulted in unequal disjunctional patterns of 15:7, 13:9, 12:10 (Fig. 1g), 10:3:9 (Fig. 1h) and 11:1:10 in 24% (6/25) euploid cells studied, with the rest (19/25) exhibiting equal disjunctions (Fig. 1f). PMCs with unequal chromosome segregations are likely to develop into aneuploid and non-viable pollen grains. Sterile pollens can result from cells having gametic chromosome number per pole but with some chromosomes having deficiencies/duplications for essential genes, later resulting from quadrivalents exhibiting adjacent orientations (Burnham 1956). To determine the effect of chromosomal anomalies on pollen viability, if any, pollen grains of four plants each of commercial and local cultivars were tested for stainability. While pollen viability of our commercial cultivar ranged from 68% to 75%, values of these parameters for local cultivar varied between 85% to 94% (Table 2).

Apart from differing in stainability, fertile and sterile pollens also depicted differences in size. Compared to fertile pollens being large having normal diameter of 67.20±0.37µm, sterile ones were small sized having diameter of 58.05±0.23µm (Fig. 1k).

As mentioned earlier, fruit setting capacity of current commercial snake gourd cultivar is very low, ranging from 10-15% compared to that (30.5-35.2%) witnessed in plants of local cultivar (Table 2). In order to determine whether percentage pollen viability and fruit set are correlated, values of these parameters for each cultivar were plotted (Figs. 2a-b).

Formation of a line almost going up in the two scatter diagrams indicates existence to positive correlation between pollen viability and fruit set. Apart from forming lesser fruit, commercial cultivars set few seeds (20-22 per fruit) as compared to local cultivar (35-36).

As fruit and setting ability of snake gourd is fertilization dependent, differentiation of few fruits (10-15 fruits per vine) with fewer seeds (20-22 per fruit) may be attributed to presence of one-fourth non-viable pollens having unbalanced male gametes, which on fertilization can give rise to unbalanced zygote.

CONCLUSION

Existence of meiotic anomalies, low fruit and seed set in our commercial cultivar indicates that either the claim of the seed-firm regarding the genetic purity of its seeds was incorrect or cytological aberrations might have arisen spontaneously after its release. Nevertheless, in view of present finding of cytological aberrations in *T. cucumerina* and previous reporting of these in *M. charantia* and *L. cylindrica (*Alam *et al.* 2011*,* Bano *et al.* 2019, Bano and Sharma 2020) it is concluded that each cultivar must be characterized morpho-cytologically and its genetic purity ascertained

by reliable experiments before releasing to the farmers.

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