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Study Of The Pollen Grain Development In *Lagenaria siceraria (* Mol.) Standl. infected by Cucumber Green Mottle Mosaic Virus (C.G.M.M.V.).

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Abstract
The pollen grain development followed the normal pattern as described for the Angiosperms. The pollen grain development occurs from meiocyte to the mature pollen grain. In the microsporogenesis, the microspore mother cells or the meiocytes underwent meiosis giving rise to the tetrads that were enclosed by the calose. Later, the tetrads were released by the dissolution of the calose by calase activity and microspores underwent mitosis. Microgametogenesis was characterized by asymmetrical mitotic division of each microspore giving rise to bi-nucleate pollen grains. Multivalent and asynchronismas laggard chromosome and chromosome bridges at metaphase 1 and later stage of division were observed more in diseased plants. Pollen viability reduces in diseased plants; sterile pollen may be empty or shrunken. The structures similar to the plastids were found in the cytoplasm and close to the nucleus of the generative cell.

Key words: Chromosome laggards, Microgametogenesis, Pollen grain,

Introduction-

Lagenaria siceraria (Mol) Standl. commonly known as bottlegourd belongs to family Cucurbitaceae have a world-wide distribution and provide maximum fruit vegetables of daily requirement. Since earlier days dry mature fruit wall is used as musical instrument and utensils by traditional people. In Uttar Pradesh it is commonly grown as summer season crop because of its best suitability to tropical condition. In winter it commonly trails on hut as well as in field. Different crop plant species were extensively cultivated for vegetable in India are found to be infected by viruses, resulting in the decrease of growth and development and ultimately reducing their yield. Only a few reports are available about the effect of viral diseases on the gametogenesis of plant.

Plant viruses are also known to produce some inclusion bodies in their hosts. Cytological abnormalities, including mitochondrial vesiculation have been reported in association with cucumber green mottle mosaic virus strain. The virus present in all plant tissues, including pollen, and occasionally in seed embryos. Meiotic abnormalities have been reported from prophase (Pollen mother cell) to pollen that leads to the reduction in fertility of pollen. However, no information is available on effect of CGMMV on meiosis in *Lagenaria siceraria*. The present study deals with comparative study of meiotic and post meiotic changes between healthy and diseased plants that leads to reduced pollen viability.

Literature Survey

[1]made a meiotic study of the mosaic infected *Nicotiana tabacum* var. *macrophyll*, and noticed that severe infection of the reproductive organs may initiate certain mutative process and this abnormalities of mosaic infected tobacco plants were undoubtlessly connected with metabolic disturbances caused by the virus. [2] reported that in certain varieties of *Solanum tuberosum* (L) pollen mother cells revealed a rapid increase in the irregularities of chromosome behavior with the result that most of the pollen grains formed were abortive.[3] also observed a complete breakdown of meiosis in tomato plants infected by virus. [4]observed nuclear abnormalities in *Lycopersicon esculentum* along with pollen sterility and disruption of reproductive mechanism due to viral infection. The effect of virus on *Capsicum annuum* studied by [5] and found reduced chiasma frequency, irregular anaphase separation and poor seed set. [6]studied different chromosomal anomalies in different crop plants.[7]studied the effect of infection with mosaic virus on the meiotic process of *Datura quarcifolia* and reported complete asynapsis at diakinesis and metaphase-1 and presence of 22 univalents. [8] recorded abnormalities in the arrangement of microspore mother cell and nonfunctional pollen grains in chili infected with cucumber mosaic virus.

Reduced size of pollen grain and reduced growth of pollen tubes from virus infected pollen may be regarded as hypoplastic effects. A variety of other effects on pollen grains has been described). Gamete development can be divided into three sequential and distinct stages: premeiosis, meiosis and post meiosis that are controlled and coordinated by a variety of genes [9]. It is known that increase in meiotic abnormalities caused an increase of pollen sterility.In view of these reports, the present investigation was undertaken to study the cytological effects of virus infection in meiotic cells of *Lagenaria siceraria* (bottle goured)..

Meiosis is an important phenomenon which occurs during the growth and development of sexually reproducing organism, comprises a perfectly integrated series of events which are linked in such a way that this process usually runs in a normal course. Any change or disturbances in any of these steps is likely to lead to an aberrant meiosis. Few workers have reported the effect of virus infection on the normal gametogenesis with different virus host combinations. The pollen viability estimate can be used an indication of possible interference from genetic or

Problem definition

environmental factors effect on this. The normal and harmonious course of meiosis (microsporogenesis) would guarantee viability to the gamete, but there are post meiotic genes, that is, genes that transcribe in microgametogenesis, that act in the interaction between the generative and vegetative cell of pollen. The present study will try to understand the relationship between host and virus physiology that will be helpful in regulating highly mutable pathogen, the virus.

Materials and Methods

To study the chromosome association, and meiotic abnormalities, young flower buds of suitable sizes from both normal and viral infected plants were collected during morning hours. These young flower buds were fixed in 6:3:1(alcohol: acetic acid: chloroform) to which a small amount of ferric acetate (1 g in 500 ml acetoalcohol) was added imparting a straw colour. After 48 hours of fixation young flower buds were rinsed with distilled water and they were reserved in 70% ethanol and kept in a refrigerator till used. Temporary slides were prepared from suitable anthers with 2% acetocarmine by using smear technique. To study pollen sterility, the mature anthers of both normal and virus infected plants were collected at the time of anthesis. The anthers were fixed in 70% ethanol directly and kept in refrigerator till used. Temporary slides were prepared from suitable anthers with 2% acetocarmine by using smear technique[10] The techniques proposed by [11]and improved by [12]was applied to stain young anthers.

To estimate the meiotic index (MI), ready-to-open flower buds were fixed in an ethanol solution with acetic acid (3:1); after 24 hours the solution was replaced by 70 % ethanol and the buds were stored in the refrigerator. Slides were prepared by the squashing technique, by staining the squashed anthers in a 1 % acetic carmine dye solution. Subsequently, the slides were observed under an optical microscope to count the number of normal (tetrad) and abnormal post-meiotic products. Tetrads with four cells of the same size were considered normal and any deviation was considered abnormal (monads dyad, triads, and polyads). Four slides/bud per plant were prepared and 500 cells per slide were counted. The meiotic index (MI) was estimated as the ratio of the total normal tetrads by the total post-meiotic products.Observations were made from both healthy as well as diseased plants. The number of monads, dyads, triads, tetrads and polyads were recorded for the meiotic index calculation.

Results/Discussion

Chromosome association and chiasma frequencies in normal and virus infected plants of *Lagenaria siceraria* were studied from their pollen mother cells (PMCs) at diakinesis. The normal plants of the species were found to have almost regular bivalent formation. But in case of virus infected plants all the species showed irregular chromosome association. In addition to bivalent, univalent and multivalent were observed. The findings of the present study revealed that in normal plants, meiosis as well as bivalents formation were regular at diakinesis. Chromosomal bridge,stickines of chromosome and laggards are more common in diseased plants in comparison to healthy ones at different stage of meiosis from metaphase to anaphase. On the other hand, in virus infected plants, asynapsis was noticed at diakinesis. The presence of univalent at diakinesis and M-I were reported by [7]. A complete breakdown of meiosis in plants infected with virus was reported by [1] in tobacco and Caldwell [3] in tomato.

The formation of the post-meiotic product different from perfect tetrads, such as monads, dyads, triads and polyads implies the formation of gametes with more chromosomes or with less chromosomes and the formation of unreduced or sterile pollen grains. The percentage, especially, of triads detected in this study is responsible for the variation in size (20-63micron) found in the viable and sterile pollen grains. The formation of two microspores of the same size and one larger microspores could usually be observed in the triads. The larger microspore may occur from two nuclei that did not segregate normally at meiosis II probably due to the irregularity in the spindle fiber orientation or even the absence of cytokinesis. In average pollen viability is 96.60% in healthy plants, where as it is nearly 70 percent in diseased plants.

In meiotic studies diseased plants show reduction in number of flower buds. In flower buds numbers of female flowers are less and most of them do not show further development. In male flower pollen mother cell shows abnormalities from differentiation to telophase II some mother cells do not differentiate and they die before onset of division. Multivalent and asynchronism at metaphase I and later stage of division observed more in diseased plants. Multipolar nuclei and spindle formation caused the formation of irregular pollen. Meiotic index and pollen viability reduces in the diseased plants. Sterile pollen may be empty or shrunken as observed in different repetitions. [3] Studied the effect of aspermy virus on the formation of mega and microspores in tomato plants and found obvious interference by the virus with the normal meiotic process.

Asynchronism in meiosis II was found in healthy and diseased plants both, but more in diseased plants. In such cells two groups of chromosomes dispersed in different times originating cells with three nuclei in telophase II, as only one group of chromosome segregated. This may be due to irregularity in spindle fiber orientation. [5] working with chilies infected with chili mosaic virus found significant reduction in chiasma frequency and partial asynapsis as compared to healthy ones.[8] recorded abnormalities in the arrangement of microspore mother cell and nonfunctional pollen grains in chili infected with cucumber mosaic virus. Similarly the effect of Tomato aspermy virus on meiotic recombination in *Lycopersicon esculentum* show increased contribution of viral agents, in generation of genetic

variability. [13], [14] has reported similar findings from *Lycopersicon esculantem* infected by tomao leaf cutl virus .

Table 01

Laggard chromosome (lag.) occurrence during meiosis I and II in diseased and healthy plants of *Lagenaria siceraria.*

U = univalent B = bivalent D = disease, H = healthy.

Meiotic phases.	Cells without lag		Cells with lag-U		Cells with lag-B		Total cells analysed		% of cells with lag	
	D	H	D	H	D	H	D	H	D	H
Metaphase I	140	160	16	9	9	5	165	174	15.15	8.04
Anaphase I	194	196	19	13	8	4	221	212	12.21	7.54
Telophase I	306	256	13	13	4	2	329	271	6.99	5.53
Metaphase II	118	115	5	3	3	2	127	120	6.29	4.16
Anaphase II	198	195	8	3	3	2	209	200	5.26	2.50
Telophase II	198	161	3	2	2	0	215	163	2.34	1.22

On an average in meiosis I, laggard chromosome were found in 11.45% and 7.03% of diseased and healthy cells respectively, while in meiosis II, laggard chromosomes were found in 4.89% and 2.62% cells of disease and healthy cells respectively and in overall diseased plants and healthy plants showed laggard chromosome 8.04% and4.83% respectively. Chromosome bridges were also found in anaphase I and II of diseased and healthy plants respectively. Lagging and eliminated chromosome at A-II formed micronuclei at T-II, being excluded from quartet of nuclei.

Table 2

Post –meiotic products and meiotic index (M.I) in healthy plants of *Lagenaria siceraria* (Mol.) standl.

Repelications	Products number resulting of meiosis-II					Total of cell analysed	M.I%
	Monads	Dyads	Triads	Tetrads	Polyads		
1.	1	3	7	460	-	481	95.63
2.	-	-	5	305	-	310	98.38
3.	2	3	6	389	1	402	96.76
4.	1	2	9	489	-	501	97.60
5.	1	3	7	490	-	501	97.80
6.	1	3	5	270	-	279	96.77
7.	-	2	10	480	-	492	97.56
8.	-	1	9	518	-	528	98.10
9.	1	4	9	488	1	502	97.21

10.	1	1	10	502	1	514	97.66
Total	8	22	77	4391	4	4510	97.36

According to data in above table, the meiotic index mean was 97.36% but monads, dyads triads and polyads were observed and quantity of triads was greater than the other irregularities.

Table No.3

Post –meiotic products and meiotic index (M.I) in diseased plants of *Lagenaria siceraria* (Mol.) Standl. Infected by Cucumber Green Mottle Mosaic Virus. (C.G.M.M.V.)

Replications	Products number resulting of meiosis-II					Total of cell analysed	M.I%
	Monads	Dyads	Triads	Tetrads	Polyads		
01.	-	15	144	607	1	767	79.13
02.	1	21	167	598	-	787	75.98
03.	2	23	133	483	1	642	75.35
04.	3	17	290	742	1	1053	70.53
05.	2	30	126	480	2	640	74.18
06.	3	35	106	400	-	544	73.52
07.	1	21	089	309	2	412	75.00
08.	2	14	062	452	-	530	85.53
09.	-	16	081	342	2	441	78.08
10.	1	13	98	278	1	381	72.96
Total	15	205	1296	4681	10	6207	75.41

Conclusion

The effect of CGMMV on pollen viability was studied in *Lagenaria siceraria (*Mol.) Standl**.** and abnormalities found in different meiotic stages.There was complete breakdown of synapses in diakinesis and metaphaseI.The sticknes of chromosome,univalent,,multivalent,laggards,chromosome bridge and micronuclei are more in diseased plants .The Meiotic index for diseased and healthy plants were observed 97.42% and 75.42 % respectively are due to effect of virus on cell division mechanism suggests that probably there was a checkpoint or nuclear restitution events during meiosis which leads to in viability. The frequency of pollen viability in healthy and diseased plants expressed a linear relationship with viral infection. The pollen viability decreases as severity of infection increases.

Future scope

The normal and harmonious course of meiosis (microsporogenesis) would guarantee viability to the gamete, but there are post meiotic genes, that is, genes that transcribe in microgametogenesis, that act in the interaction between the generative and vegetative cell. A break in the cell to cell communication can lead to gamete malformation, making it equally not viable. Thus a basic cytological relationship between host and viral infection will be studied to understand the physiology of parasitism for disease management

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