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EVALUATION OF POLLEN AND MEIOSIS VIABILITY BY IMAGE ANALYSIS IN *MIRABILIS JALAPA L.*

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ABSTRACT:

Mirabilis jalapa Linn. is an ancient medicinal plant, widely available in many parts of the world. The present day computational technique such as Image analysis provided good picture clarity for visualizing and used to analyze the architecture of pollen and the meiotic stages produced after staining of pollen grains at 100x and 1000x microscopy. The anther of *M.jalapa* is a two lobed structure attached to the stamen and is further subdivided into seven sub-lobes. The present experimentation on meiosis in *M.jalapa* provides good picturisation of various meiotic stages showing reductional division by dividing a cell into four cells. Replication in genomic DNA and growth in activity of cellular components play an important role in meiotic division of the male gametophyte of *M.jalapa*. Image analysis of pollen grain at 1000x micrograph provided more number of darker objects (12) than lighter objects (5). Pollen grains also play an important role in formation of phenotypic characters of the seed after fertilization.

Keywords: Image analysis, architecture of pollen grain, Meiosis, *Mirabilis jalapa*.

[I] INTRODUCTION

There is a growing interest among biologists and information technologists to study the cytology of angiosperm pollen using wet lab and various software's available [1, 2]. A number of botanical scientists have shown that the flowers of xenogamous taxa produce more number of pollen grains than the autogamous taxa [3]. Various reviews demonstrated and the experiments show that pollen and microspore development takes place within the anthers of angiosperm flowers [4].

Pollen grains play a key role as male gametophyte and contain extra cellular pollen wall- the exile, extra cellular lipid matrix - the pollen coat, which is particularly prominent in pollen dispersal and pollen-stigma recognition. The pollen coat precursors are under the control of the saprophytic genome [5]. The number and effects of genes influencing naturally occurring behavioral traits is an issue of great importance to studies of behavior and behavioral evolution, finally producing a descriptive classification based on these morphological features, which even to-day, with our modern microscopes, can hardly be bettered [6]. In *Mirabilis jalapa* pollen performance was influenced by the number of competing pollen grains or pollen tubes, but was not influenced by potential genetic differences with load diversity [7, 8].

The genus Mirabilis contains 350 species in 34 genera mainly occurring in the tropics and subtropics [9]. The common garden-variety four-o'clock (*Mirabilis jalapa*) is also known as Marvels of Peru [10]. Four o'clock received its name because of its habit of opening in the late afternoon, due to the drop in temperature. Very few reports are available on studies of architecture and meiotic division in pollen grains and application of image analysis in *Mirabilis jalapa* Linn.

Around 1900, Carl Correns used the *Mirabilis jalapa* as a model organism for his studies on cytoplasmic inheritance [11]. Sources that are visualized by humans to have color are generally referred to as pigments, have long fascinated chemists and biologists, who have examined their chemical and physical properties, their mode of synthesis, and their physiological and ecological roles [12]. *Mirabilis jalapa* has many kinds of variegated flowers, and Mendel reported many of its intriguing patterns [13]. The flowers are used in food colouring such as cakes and jellies [14, 15]. In herbal medicine, parts of the plant may be used as a diuretic, purgative, and for vulnerary (wound healing) purposes [16, 17].

Research on pollen architecture provides the properties of aggregates, such as size and shape, in exploring valuable information for pollen characterization and the optimization of production. For the last twenty years, image analysis techniques have been used for aggregate particle measurement where the stability of measurement methods is very important [18, 19, 20]. There are a number of image analysis methods for measuring aggregate particle size and shape, which increases speed and accuracy of analysis.

[II] MATERIALS AND METHODS

2.1 Study species

Mirabilis jalapa has tubular flowers and is diverge in color among plants. An individual flower have 5–6 stamens and a single-ovulate ovary, opens for one night in the early evening, the exact time depending on temperature and relative humidity, and closes early the next morning.

2.2 Plant Materials

M. jalapa (yellow, orange and pink flower cultivars) was collected from plants grown in the garden of the GITAM Institute of sciences, GITAM University, Visakhapatnam during winter season.

2.3 Micrographs of the Pollen Grains

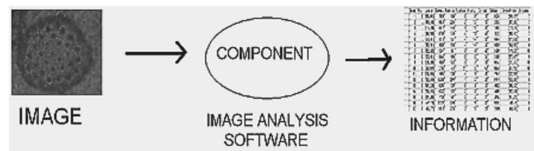
Photographs of the most copious pollen grains in the pollen masses were taken with an optical light microscope (Olympus BH-2). A standard magnification of 1000x has been used for most of the light micrographs. The pixel size of 2048X1536 has been adjusted in Olympus MODEL- FE-115, 5 megepixel cameras, at a distance of 25cm from object, equals to 0.1X0.075 mm at 1000x microscopic image. This permits an easy measurement of the size of a pollen grain in mega pixel.

2.4 Meiosis in flower buds (anther region) of *Mirabilis jalapa*

Experimentation on meiosis in anther of M.jalapa has been done and the methodology of this work is explained. Select the appropriate flower buds of different size from the inflorescence of *M.jalapa*. Fix them in Tween 80 fluid, which is used as fixative agent. Take a preserved flower bud and place it on a glass slide. Separate the anthers and discard the other parts of the bud. Put one or two drops of acetocarmine, squash the anthers and leave the material in the stain for five minutes. Place a cover slip over the squashed anthers and tap it gently with a needle or pencil. Warm it slightly over the flame of a spirit lamp for 1 minute. Put a piece of blotting paper on the cover slip and apply little and uniform pressure with the thumb. Observe the slide under the light microscope for different pollen grain meiotic stages in *M.jalapa* at 1000x visualization.

2.5 Image analysis software's

The analysis of the captured image was performed on a PC using image analysis softwares. The microscopy image was sent to a computer-assisted image analyzers such as GSA Image Analyzer and Pixcavator, which can provide changes in staining of pollen and Meiotic stages. The GSA Image Analyzer v 1.21 is a program for the scientific evaluation of 2D images of Photographic objects. Pixcavator IA-Image Analysis 4.3 is a light-weight program intended for scientists and engineers which implements image management and database capabilities.



[III] RESULTS

Mirabilis jalapa is an important, vulnerable medicinal plant having wide importance based on research. The plant is widely available in and around the regions of Visakhapatnam district. There is a huge molecular characteristic features in this plant and can be mutated easily due to the structural arrangement such as porous surface and condensed genome of pollen grain. Any changes during life, from genome formation to the denaturation of genome can have the capability to change the behavior of the cellular system. The top-to-bottom systems changes and the pathway in the organism will change and the new metabolites will be generated in the cytoplasmic regions. This can have affects in the cellular organelles such as mitochondria and chloroplast, where the plasmid DNA can also have minor changes.

Staining of pollen grain

Fig 1 provides the pollen grain is the male gametophyte generation of *M.jalapa*. Pollen is a mass of microscopic spores in a seed plant that appears usually as a fine dust. Each pollen grain is tiny, varies in shape and structure. The anther is a two lobed structure attached to the stamen and each lobe is further subdividing into seven sub-lobes. In appearance, the anther visualized as three different layers: outer layer as thick, middle cellular and inner slimmer. The middle layer which is cellular may be represented as pollen and there are more than 400 pollen grains per anther.

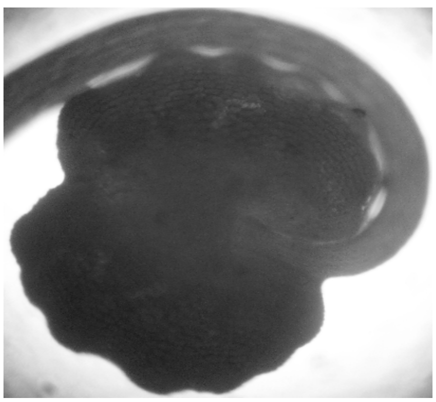


Fig 1: *Mirabilis jalapa* stamen and anther

Fig 2 has provided the architecture of pollen grain visualized at 1000x microscopy. The architecture is some what complex and has been adopted based on climatic factors. There is a thick outer layer, which is protecting the inner cellular components. The nucleus is clearly visualized, and the cytoplasm is containing various functional components such as mitochondria and endoplasmic reticulum.

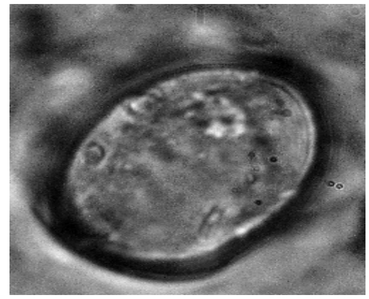


Fig 2: Internal Structure of pollen grain (stained) at 100x microscopy

Fig 3 provides the architecture of pollen grains. The surface of mature pollen grain is having larger pores which are arranged in serial fashion. In the anterior side may contain nucleus and the DNA is condensed. DNA is compressed as sphere, which is one of the characteristic feature for adaptation of pollen in nature. The large number of pores on the pollen grain may also be adopted for pollination where the sticky spores attach to the insects and will move from anther to stigma of the flower or neighboring flower, where fertilization takes place. The partial staining nucleolus of pollen grain is clearly visualized which is having condensed, globular DNA. The two outer layers of the pollen grains such as exine and intine are clearly visible. The internal organelles such as endoplasmic reticulum, chromoplasts, mitochondria and nucleus are also visualized after contrasting the image in Image Analyzer.

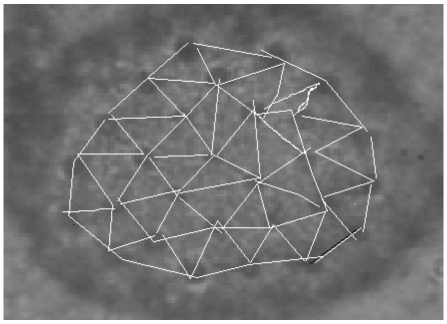


Fig 3: Stable architecture of pollen grain in M.*jalapa*

Fig 4 shows cellular process of pollen grains showing reductional division with meiosis. Two cell divisions separate the replicated chromosomes into four haploid male gametes, each with a haploid set of chromosomes. The images are provided with better visualization using Image Analyzer v 1.21.

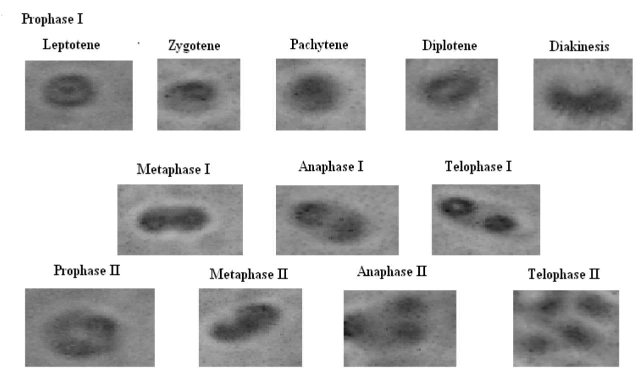


Fig 4: Meiosis in anther of M.*jalapa*

Fig 5 has provided the outputs of visualization of pollen grain by image analysis software - Pixcavator IA- v4.3 which shows clarity of the image before and after adjusting the image. The size of pollen grain showed 1500 X 1250 pixel, which is equal to 0.073X0.092 mm. The software provided better visualizations after adjusting brightness, contrast, Hue and saturation. The results also specified to be having more number of darker objects (12) than lighter objects (5). The further analysis can provide various sizes of pollen and the architecture which can make to be identifies in the next generations in various species and also in exopaleontology studies.

Objects	Type	Location	Size/area	Perimeter	Roundness	Intensity	Contrast	Saliency	Center of mass	Av. contrast
1	D	(855, 103)	7833	1926	3	87	33	55264	(859, 107)	7
2	L	(498, 356)	6870	2295	2	153	35	53030	(507, 360)	7
3	L	(514, 249)	5512	1455	3	153	35	51662	(512, 249)	9
4	L	(409, 460)	8709	1736	4	154	40	64222	(409, 463)	7
5	L	(176, 301)	5212	1327	4	145	32	45645	(177, 301)	8
6	L	(352, 581)	5002	1334	4	148	36	45834	(354, 582)	9
7	D	(422, 242)	5247	921	8	71	42	55437	(419, 229)	10
8	D	(178, 461)	8541	1974	3	73	34	81567	(177, 457)	9
9	D	(633, 280)	7009	1683	3	67	39	76542	(631, 272)	10
10	D	(360, 159)	2796	409	21	55	50	46644	(361, 158)	16
11	D	(635, 505)	4886	1220	4	66	35	47646	(637, 506)	9
12	D	(420, 684)	12503	3549	1	70	28	69417	(423, 683)	5
13	D	(667, 628)	6296	1269	5	68	30	44625	(668, 629)	7
14	D	(785, 495)	6525	1869	2	68	27	44487	(787, 494)	6
15	D	(697, 206)	7759	1417	5	63	31	54962	(701, 210)	7
16	D	(474, 75)	12226	2776	2	58	32	80560	(480, 75)	6
17	D	(65, 277)	8419	2208	2	59	28	52691	(64, 270)	6

Figure 5. Image analysis view from Pixcavator using Pollen of M.*jalapa*

Fig 6 and 7 has shown that the external shape and structure of the seed is structured due to the genome arrangement of pollen, which in turn backs in providing structure of pollen. The pollen grain of *M.jalapa* (Fig 6) has many pores with oval structure, transferred to the seed, which is also having oval structure. External structure in the Pollen grain of *Jatropa curcus*

(Fig 7) is smooth and somewhat bulged in the middle is also having similarity in seed.

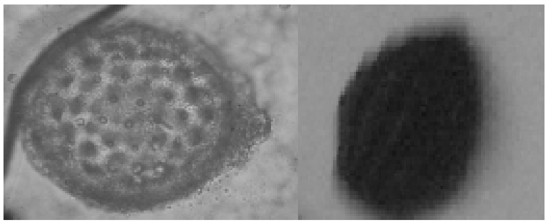


Fig 6. Structure of pollen grain and seed in M.*jalapa*

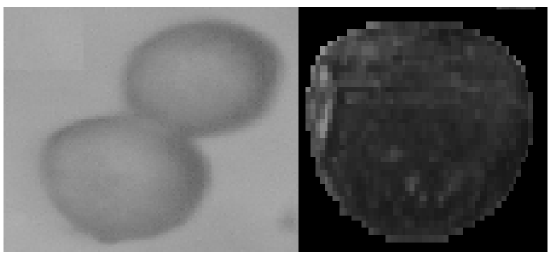


Figure 7. Structure of pollen grain and seed in J*atropa curcus*

[IV] DISCUSSION

Medicinal plants since times, are being used as medicines in the cure of diseases, Most of the animals are consuming these plants and being wild, tolerating the climatic changes. Most of the species and molecular data became extinct [21] and some are going to become extinct. The changes in phenotypic and genotypic characters are required for tolerating these sudden and sometime prolonged changes in the surroundings. The requirement of humans will not make the life to fit on the earth but the changes in them make themselves to fit to survive on the earth.

Life is constructed with molecules [22]. All the molecules will have certain properties which makes the cellular system to be survived and live for the basis of other cellular machinery. Hence the formation of one molecule to be utilized by destroying the other molecules is taking place in the earth's system. Due to this, the molecular energy is converting from one form to the other form. Biology of molecular conversions may be in microform such as ATP, NADP or as macroform such as viral DNA, prions and Bacterial DNA. Hence the Newton's second law [23], which specifies "no matter can be created or destroyed" applies well in biological systems also. There is no destruction in the life process but some molecular information will become extinct. Hence every organism is made up of chemicals as molecules.

The architecture of pollen and molecular construction in plants such as *M.jalapa* and *J.curcus* are specific and has been kept constant since era due to stable environment. Small variation in climatic changes can bring many changes in living organisms based on the construction of cellular molecules [24]. The mutational changes can vary from one species to other species. The ability of transformation the molecules [25] will be much faster in *M.jalapa* due to porous surface in pollen and the molecular construction in nucleus. Hence many kinds of variegated flowers are present and hybridization can be done easily in *M.jalapa* [26]. The pollen surface of *J.curcus* is smooth and visible pores on surface are absent. Hence few variations can be only seen in this species and the mutability in DNA is less in *J.curcus*.

The object or defect identification required in industrial vision applications, the operations of mathematical morphology are more useful than the complication operations employed in signal processing because the morphological operators relate directly to shape [27]. Model-based techniques have proven to be effective in segmenting, matching, and tracking anatomic structures by exploiting (bottom-up) constraints derived from the image data together with (top-down) a priori knowledge about the location, size, and shape of these structures. Evaluation of microscopy images can be used to analyse changes in staining and can also be used to show significant increase in blood pressure associated with marked myocardial inflammatory response [28]. Hence an interim analysis using data of pollen and spore monitoring found significant associations in pharmacy products [29].

[V] CONCLUSION

The present experimentation showed good results in application of software techniques along with wet lab methodologies. The architecture of anther is also complex with large number of sticky pores on surface in *M.jalapa* and smooth anther surface in *J.curcus*. Further research has to be conducted in future for analyzing the images and to construct phylogeny among various plant species.

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