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POLLEN MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES OF *RAPHANUS SATIVUS* LINN.

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ABSTRACT : *Raphanus sativus L. is an annual or biennial vegetable belonging to the family Brassicaceae (Cruciferae) and is a traditionally important vegetable in many countries. The enlarged root and hypocotyls of radish are consumed mainly as salted vegetables and also eaten fresh as grated radish, garnish and salad. Recent advances in cultivation techniques require more promising varieties of radish that can resist the problems of diseases and insects and can meet a more varied range of dietary demands.*

The direct and indirect roles played by pollen in various spheres of applied biological research will be found useful in view of the fact that pollen is a material to work which seems to be providing an easier and even better means of germplasm is experimentally controlling the genetic behaviors of the plants. For radish, breeding work has been carried out on ecological traits, resistance to diseases, and adaptability for different kinds of consumption. The ecological traits are productive and qualitative characteristics such as high yielding ability, early maturity, late bolting, edible quality (pungency) late pod formation, cold hardiness, drought resistance, heat and wet tolerance, and so on.

In the present study, Pollen phenology, pollen morphology, pollen physiological studies like pollen production, viability, germination – in vitro and in –vivo etc., total pollen protein and amino acid estimation of Raphanus was investigated.

Keywords: Brassicaceae, Phenology, Morphology, Physiology of pollen

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INTRODUCTION :

The radish, ***Raphanus sativus***, is an annual or biennial herb that belongs to the Mustard family Brassicaceae. It is specially grown for its fleshy roots, which vary in size from a few grams to kgs. The radish was developed from a wild plant that grew in the cooler regions of Asia; it is spread in the Mediterranean region before the Greek era and was introduced to India in the 16th century. The genus name comes from the Latin name, from the Greek word rhaphanis used for this vegetable which has been known from antiquity. The common name

radish comes from the Latin word radix meaning root. Radish roots are low in calories and are usually eaten raw: the young leaves can be cooked like spinach. The young fruits are also edible and are often eaten raw or sautéed. The small quick growing spring varieties have a mild, crisp, moderately firm flesh, whereas the large, slow-growing summer and winter types have pungent firm flesh. Summer varieties are eaten raw in a salad. Winter varieties are eaten cooked.

The plant grows 2 to 3 feet in height and spread 1 to 2 feet. Throughout India, the plant is cultivated. Flowers white or tinged with purple scented in lax racemes. Fruits erect cylindric inflated pods called silicle, yellow to pale purple, the beak is green, seeds 2-8 pendulous, avoid, light brown. Roots are acrid, bitter, appetizing, digestive, diuretic, laxative, and antibacterial which contain a glucoside, enzymes and methyl mercaptan. The leaves are antibacterial, diuretic, and laxative. Seeds are expectorant diuretic, carminative useful in cough, stagnancy and paralysis. Seeds of ***Raphanus*** yield non-drying fatty oils suitable for soap making also for edible purposes and as an illuminant. In Japan, hydrogenated oil is used in the manufacture of crayons. Seeds cake is rich in protein and used as manure and after the removal of isothiocyanate, it is used as a foodstuff.

Radish is an allogamous plant exhibiting a high level of self –incompatibility and shows inbreeding depression when self-propagation by bud pollination is repeated. It is difficult to obtain a large amount of seed mainly because of the limited seed numbers produced per pod. In the case of Raphanus F1 hybridization combined with self –incompatibility and Heterosis is a helpful breeding method. There are three methods of seed production in F1 hybridization i) single crossing ii) three-ways crossing and iii) double-crossing.

Fig.-1: Opened flowers with yellow pollen dust on anthers of *Raphanus*

Fig.-2: Beaked fruits of *Raphanus* (Silicle)

Pollen is a discrete, mobile male partner in the process of fertilization. No other plant part even though extremely tiny in size is packed with so much information and power. For the breeders, the pollen is a discrete mobile male partner of the fertilization process in higher plants. It carries the genotype of one partner of the pollination process, which is the process that the breeder manipulates.

The direct and indirect roles played by pollen in various spheres of applied biological research will be found useful because pollen is a material to work which seems to be providing an easier and even better means for experimentally controlling the genetic behaviours of the plants.

In the present study, Pollen phenology, pollen morphology, pollen physiological studies like pollen production, viability, germination – in vitro and in –vivo etc., total pollen protein estimation, Histochemical studies of germinated pollen grains of *Raphanus* was investigated.

MATERIAL AND METHODS :

Pollen phenology, pollen morphology, pollen physiological studies like pollen production, viability, germination – in vitro and in –vivo etc., pollen storage, total pollen protein estimation, Histochemical studies of germinated pollen grains of *Raphanus sativus* was carried out.

Pollen phenology - Anthesis took place nearly 9.00 am on the plant. Yellow coloured pollen dust deposited on another. Pollen grains are monads, triangular, yellow, and slightly sticky.

Pollen morphology - The morphological analysis comprises fresh material collected from the Botanical garden of the Institute of Science, Nagpur and the study was made with light microscopy and electron microscopy. Pollen morphology was studied by two methods first by acetolysis method in which the pollen was subjected to the chemical treatment and the pollen become able to clear its surface and provides useful information in reaching a conclusion of exine on variable taxonomy.

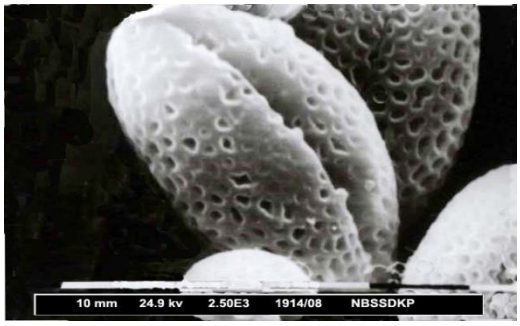
Fig.-3: Acetolyzed pollen grains of *Raphanus* 400X

However, scanning electron micrographs serves to elucidate further detail of exine sculpture such as pores and colpi and the external surface of the pollen grains as well as the diameter of pollen and measurement of the exine stratification.

Grains are 3 zonocolpate, Polar axis 33 µm, equatorial diameter 24 µm colpus length 30 µm. Grains subprolate, sexine reticulate. Lumen is successively smaller towards polar area, ungustimurate. The thickness of muri is 4.4 µm; muri ornamented at places.

Scanning Electron Micrographs of *Raphanus* Pollen with reticulate surface 1000X

Raphanus pollen with polar view 4000X



Equatorial view of *Raphanus* Pollen with deep long colpus 4000X

Pollen production- Pollen production was studied by two methods i) Simple method and ii) Haemocytometer method. It was noted that there were some differences in the total pollen output by both the method. Out of the two the Haemocytometer method appears to be more accurate as the number of pollen is counted for 0.1 cubic mm. of the solution. Whereas, in simple method it is counted from 0.05 ml. of the solution. Counting becomes easier by Haemocytometer and it appears to be the best suited instrument for the evaluation of pollen grains as the pollen size is small. In case of *Raphanus,* 13,340 pollens per flower by simple method and 8,526 pollens per anther were recorded by Haemocytometer meter.

Pollen viability- Pollen viability is an index of its quality and vigor. Pollen viability by stainability technique was studied using 2, 3, 5,-triphenyl tetrazolium chloride which is a vital stain used for the viability of tissues. The test is based on the presence of functional enzyme which converts the colorless solution of 2, 3, 5-triphenyl tetrazolium chloride into the insoluble red Triphenyl formazon. The viability of the pollen by TTC in the present study was 81.94%.

In-vitro germination study- Pollen viability by pollen germination *in vitro* method was carried out by 'Hanging Drop Technique'. The fresh material was sown in various artificial culture mediums like Sucrose, Boric acid along with sucrose and in Brewbaker's medium and observations were noted after 24 hours, so that they grow to their maximum limits. The

different media used for pollen culture were standardized by series of experiments. The different media used for studies were sucrose solution (from 5% to 40%), Boric acid medium with sucrose as a basal medium (from 10 ppm,25 ppm,50 ppm,100 ppm,200 ppm, 300 ppm, 500 ppm and 700 ppm) and in Brewbaker's medium which also known as 'Calcium complex'.

i) Sucrose :

Raphanus pollen showed best germination in 15 % sucrose solution with highest tube length of 500 micron with maximum germination of 68 %. In 5% and 10% of sucrose solution only protuberances of pollen tubes were observed which showed in some cases the bursting of the pollen grains. In 35% and 40% of sucrose no germination was recorded. When regular experiments were carried in 15 % sucrose solution, ***Raphanus*** pollen showed the maximum 68 % germination. The maximum pollen tube length was in the range of 360 to 500 micron.

ii) Boric acid with sucrose :

When ***Raphanus*** pollen grains were subjected to germination in different grades of boric acid along with 15 % of sucrose the pollen showed maximum germination in 50 ppm of boric acid with 15% sucrose showing 50 percent germination but often showing bursting ,healthy pollen tubes were occasionally seen . In the higher grades, no germination was noticed. When regularly ***Raphanus*** pollen was subjected to the germination in 50 ppm boric acid with 15 % sucrose, the germination percent varies from 28 to 50 percent with maximum tube length of 465 micron and average tube length varies from 276 to 428 micron. In most of the cases, the pollen tubes showed bursting of the contents; the healthy intact tubes were recorded in very few types of pollen.

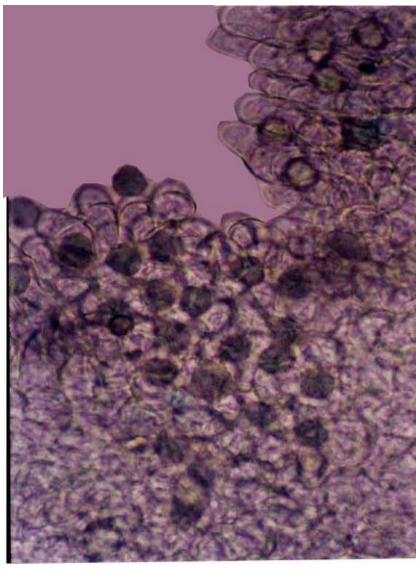
iii) Brewbaker's medium :

Response of ***Raphanus*** pollen in Brewbaker's medium was not very good. The pollen showed 34% maximum germination with 385 micron maximum pollen tube length and average tube length of 346 micron. The tubes showed uniformity in breadth except at the tips where a slight bulginess was noticed occasionally.

In vivo germination study- The breeding system of an angiosperm is multidimensional and covers, among other aspects, sequential processes such as pollen delivery, pollination, and pollen germination on the stigma, pollen tube growth down the style, fertilization of the ovules, seed development and dispersal of the seeds. Out of this broad field, the pollen germination and the competition of the tubes in the transmitting tract plays very important role in the fertilization that we studied in the laboratory by studying the stigmas.

In vivo studies was conducted on the stigma with some portion of styles on the first, second, third day of the anthesis till the drooping stage. All day's stigma showed the germination. *Raphanus* stigma was papillate type the upper surface having papillate hairs and showed deposition of pollens in intrapapillate cavities. Maximum germination of 78% was found on drooping stage with maximum length of 313 µ with all the ten stigma showing germination .Average tube length was found to be 248 µ in drooping stage. On the very first

day only 3 pollens showed germination out of 28 pollens which were deposited on the stigma with maximum length of 47 µ. On the second day comparatively larger pollen tubes were observed i.e. 168 µ with 67% of germination. On the third day 76% of germination on stigma was found with maximum tube length of 199 µ and average tube length of 187 µ.



Drooping stage papillate stigma of *Raphanus* with deposited and germinated pollen grains

1. Period after opening of flower.	Day 1	Day 2	Day 3	Dropping
2. Total number of stigma's observed.	10	10	10	10
3. Number of stigma's showing germination.	2	5	8	10
4. Mean number of pollen retained on stigma.	28	117	205	208
5. Mean number of pollen germinated on stigma	3	78	157	162
6. % of germinated pollen.	11	67	76	78
7. Average tube length in microns.	20	51	187	248
8. Maximum tube length in microns.	47	168	199	313

Table 1: *In vivo* pollen germination on stigma of *Raphanus*

Pollen storage - The artificial maintenance of the viability and fertilizing ability of pollen over a long period is an important problem from both the theoretical and practical point of view. The pollen longevity of different species varies between minutes and years depending primarily on the taxonomic status of the plant & abiotic environmental conditions.

The longevity of the pollen is governed by a number of factors. Temperature, Relative Humidity, light and the time of blooming govern the longevity of the pollen. Of special significance temperature & relative humidity and their effects are interdependent. Here, in the present study, the storage of pollen grains was studied using different parameters such as

providing the different temperature as well as the relative humidity. Pollen storage in different organic solvents at 4°C was also studied as a different parameter.). Low temperatures are generally found suitable for long term storage. At room temperature & 100% RH pollen lost viability within 24 hrs. Relative humidity between 0 – 20% had been reported to retain viability for several days. In the light of at available evidences, it is obvious that temperature alone cannot be the ideal storage condition, unless coupled with suitable levels of relative humidity.

In case of *Raphanus*, at the room temperature, we can store the ***Raphanus*** pollen up to 12 days in 0% RH with 2 percent germination and 95 microns tube length. In 10% RH, pollen remains viable for only 8 days with 1 percent germination and 56 microns maximum tube length. On the freeze temperature, pollen can be only stored on 0% RH up to 10 days with 14 percent germination and 59 microns maximum tube length of pollen grains. On the higher RH level i.e.10%, 20% and 3%, the pollen cannot be stored in viable conditions even one or two days.

Pollen viability in various organic solvents was studied, like Benzene, Isopropyl alcohol, Chlorophyll, Acetone and Xylene. In the present investigation Xylene was proved to be a bad solvent for storing the pollen grains. Isopropyl alcohol and chloroform showed maximum viability i.e. 48 and 59 days overall these solvents appear to be good for storing pollen for short term.

In case of ***Raphanus***, maximum 13 days viability was noted in benzene with 23 % germination and tube length of 534 µ. Acetone showed 10 days viability with 14% of germination with maximum pollen maximum tube length of 133 µm. Isopropyl alcohol showed only 8 days viability with 18 % germination and maximum tube length were 423 µm.8 days viability was also found in Chloroform showing 21 % of germination and 396 µm length. Here also, in case of Xylene, only 5 days pollen found viable with 5% of germination and 76 µm. maximum tube lengths.

	Organic solvent	**Viability (in days)**	**Maximum percent of germination**	**Maximum tube length (in µ)**
1	Benzene	13	23	534
2	Isopropyl alcohol	8	18	423
3	Chloroform	8	21	396
4	Acetone	10	14	133
5	Xylene	5	5	76

Table 2: Pollen viability of *Raphanus sativus* in different organic solvents

The major cause of the loss of viability during storage appears to be the deficiency of metabolites due to the continued metabolic activity of the pollen is going on at the much reduced rate had found that a higher moisture & temperature level reduces pollen quality by increasing metabolic rates & promoting microbial activities. Pollen storage conditions that

maintain fertility increases the efficiency of handling breeding & genetics material of any plant species.

Free amino acids estimation - Although the amino acids contained in pollen grains have been studied as a part of pollen pathology here we studied from the pharmacological point of view. Changes in free amino acid pattern and amino acid composition of pollen grains were recorded while studying pollen chemistry. Free amino acids are always found in relatively large amount. Altogether 16 different free amino acids were separated and identified by chromatographic methods.

Protein estimation- Proteins are the essential metabolic substance for activation of pollen, following germination were accompanied by the initiation of protein synthesis. The localization of proteins and enzymes has shown that the proteins occur most prominently in the cellulosic intine near the pore & in the cavities of exine. Protein synthesized or activated in the germination stage of pollen tube are apparently required for the early stage of tube development. 38.56% total pollen protein was estimated spectrophotometrically.

CONCLUSIONS :

i) The pollen is used as a convenient experimental system in genetic investigations, directed towards plant improvement. Pollen are irradiated to induce desired mutational variations and to overcome intraspecific incompatibility or to remove other fertilization barriers.

ii) The direct and indirect roles played by pollen in various spheres of applied biological research will be found useful in view of the fact that pollen is a material to work which seems to be providing an easier and even better means for experimentally controlling the genetic behaviors of the plants.

iii) In the eye of biochemist the pollen is a sac full of enzymes and substrates, locked up in compartments and filled with some types of cell organelles. In fact, if one carefully looks, one can find in pollen of different plant species nearly all physiologically important classes of substances, not only carbohydrates, proteins but all types of lipids, growth hormones, vitamins, pigments, sterols etc.

iv) India, with its vast biodiversity and potential for commercial exploitation, could become a world leader in the supply of raw material for the phytopharmaceutical industry.

v) Plant germplasm resources are one of the most important renewable natural resources of the world. Increasing exploitation coupled with natural calamities has led to rapid dwindling of important plant species. Nearly 20,000 to 25,000 species of vascular plants are currently facing threat their existence. This necessitates urgent measures to conserve the plant wealth of ecosystem, species and gene pool levels, to enable sustained use for present and future generations by establishing pollen banks and germplasm resources centers.

REFERENCES :

- **Agashe, S. N.** 2006. *Palynology and its Application*. Oxford IBH Publishing Co. Pvt. Ltd., New Delhi. pp. v - vii.
- **Rangari, J.S**. 2008. *Palynological studies in plants with anticancer activities*. Ph.D. Thesis. RTM Nagpur University, Nagpur.
- **Chatterjee, A. and Prakrashi, S. C.** 1991. *The treatise on Indian Medicinal Plants*, Publications and Information Directorate, New Delhi, Vol. I. pp. 155 – 158.
- **Ananymous,** 1992. *The Wealth of India, Raw Material*, CSIR, India, Vol. 3, pp. 210 – 213.
- **Asolkar, L. V. Kakkar, K. K. of Chakre, O. J.** 1992. *Secondary Supplement to glossary of Indian Medicinal Plants with active principles.* Publication & Information Directorate (CSIR), New Delhi. pp 150 – 215.
- **Dymack, W.** 1890. *Pharmacographia indica*, Humdard Foundation, Nizamabad, Karachi, Pakistan, Vol. II pp: 131 – 136.
- **Raychoudhary, S. P.** 1991. *Recent Advances in Medicinal, Aromatic and Spice Crops (Vol. I)* 63-72. Today and Tomarrow Publisher, New Delhi
- **Nair, P. K. K., Joshi, A. P. and Gangal, S. V.** 1986.*Airborne pollen, spores and other plant materials of India-A Survey*. CSIR centre for Biochemicals, Delhi. :41,56,181