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# A study on morphological and chemical characterization of pollen grains of gamma rays induced tomato (*Solanum lycopersicum)* mutants using SEM–EDS and FTIR analysis

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**Abstract**
Tomato (*Solanum lycopersicum L.*) is a most popular and widely grown vegetable crop in the world. Tomato (*Solanum lycopersicum L.*) is one of the most important vegetable crops under *solanaceous* group which can be grown both under open field conditions and greenhouse. Gamma rays induced early flowering (sample 1) and late flowering (sample 2) mutants pollens were used for the present study. SEM-EDS analyses were studied in the obtained tomato pollen grains. Scanning electron microscope (SEM) and Fourier transform infrared (FTIR) were employed to observe possible variation in the microstructures and spectral characteristics respectively. The FTIR spectral of control and processed samples showed slight variations in peak positions. The SEM images of the sample 1 and sample 2 tomatoes pollen grains showed some level of micro structural dissimilarities compared with control.

**Keywords:** *Solanum lycopersicum*, mutants, gamma rays, SEM, FTIR, Pollen grains

## Introduction

Tomato is a globally important vegetable crop with a total world production of 182 million metric tons harvested from 4,848,384 hectares in 2017 (FAOSTAT Database, 2018) [6]. Widely grown in tropics and subtropics. Tomato is an important source of vitamins A and C, antioxidants and carotenoids (Garcia Alonso *et al.*, 2009, Perveen *et al.*, 2015) [7, 14]. Tomato as a high value vegetable crop encourages small holders to switch from subsistence to commercial farming thereby improving their income and livelihood (Hanson *et al.*, 2016) [10]. Most resistance sources came from India, Philippines, Indonesia, Thailand, French West Indies and the United States (Boshou, 2005) [1] and have been frequently exchanged among the major tomato breeding groups worldwide (Daunay *et al.*, 2010, Ho *et al.*, 2013) [4, 11]. Tomatoes are also the excellent source of antioxidants, diet oxidants, dietary fiber, minerals and vitamins which are helpful for protecting cancers including colon, prostate, breast, endometrial lung and pancreatic tumors. It also contains very good levels of vitamin A and flavonoid anti-oxidants such as α and β carotenes, xanthenes which take part in vision maintain healthy mucus membranes, skin and bone health. Consumption of natural vegetables and fruit rich in flavonoids is known to help protect from lung and oral cavity cancers. Thus, tomato is an excellent crop for human consumption. The crop tomato requires good amount of nutrients for its growth and development. It is well responsive to micronutrients also. Micronutrients are required by plants in very small quantities, yet they are very effective regulating plant growth due to enzymatic action (Sathya *et al.*, 2010) [16]. Tomato is the second horticultural product cultivated and the first in industrialized volume in terms of area (Camargo *et al.*, 1996) [2]. It is consumed primarily for its color and the physical features it impacts on foods in addition to the provision of provitamin A. They contribute to a healthy and well-balanced diet as they are rich in dietary fibres and other nutrients. Due to the wide variety of nutrients and the many health related benefits, fresh tomato and tomato based food products provide to the body, They are eaten raw or most times included as an ingredient in a lot of dishes such as salads and sandwiches and as salsa while the processed ones are consumed dried or as pastes, sauces, soups, juices, and drinks (Ochida *et al.*, 2019) [13]. The perish ability nature of tomatoes allows quick deterioration which starts immediately after harvest and continue till they experience spoilage due to high moisture contents and enzymatic activities which leads to wastage (Ugonna *et al.*, 2015) [19].

The study was targeted at determining the effect of the chemical and morphological of tomato using some selected instruments. The tomato (*Solanum lycopersicum L.)* cultivars were obtained from traditional agriculture systems with the aim of evaluating their morphological characters. These features are important to supplement and promote the conservation. Pollen has a wall which is completely different from any other plant structures. The pollen wall is multilayered derived from both the developing pollen and sporophytic cells of the anther and consists of material that is highly resistant to degradation making it an important determinant of pollen viability (Taylor and Hepler, 1997, Wahid *et al.*, 2007) [18, 20]. The surface of the pollen wall also known as the pollen coat in some plant species, consists of materials like tryphine or protein-rich matrix, pollenkitt (Taylor and Hepler, 1997) [18].

This sticky substance may contain lipids, proteins and phenolic compounds, and can be present in substantial amounts in entomophilous species. The functions of the pollen coat are to facilitate pollen transfer through sticking to insects and protection against environmental stress. But the lipids and proteins of the pollen coat may also play an important role in the adhesion of pollen grains to the stigma (Dickinson *et al.*, 2000) [5].

## Experimental Analysis

### Plant Material

5, 10, 15 and 20KR doses Gamma rays treated tomato plants were used to study the SEM and FTIR analysis. The field experiment was conducted at the private agricultural farm Perambalur, Tamilnadu, during the year 2018 – 2019. Tomato (*Solanum lycopersicum L.)* In order to investigate the chemical composition, viability, and germination capacity of pollen, Pollen was collected from early flowering (sample 1) and late flowering (sample 2) tomato mutants. Anthers collected in eppendorf tubes from the opened flowers and stored at room temperature. The SEM and FTIR analysis studied were worked at National College, Tiruchirappalli, Tamil Nadu.

### Instrumental Analysis – Ftir and Sem – EDX Analysis

The various spectra or finger print of the samples were determined using FTIR spectrophotometer (FTIR-8400S Shimadzu Co, Ltd. Japan) (Mohie *et al.*, 2011) [12]. Fourier Transform- Infra Red (FT-IR) spectroscopy in order to determine the possible functional groups released during the different process, the FT-IR analysis was carried out. A scanning electron microscope, or SEM, is a powerful microscope that uses electrons to form an image. It allows for imaging of conductive samples at magnifications that cannot be achieved using traditional microscopes. Modern light microscopes can achieve a magnification of 1,000X, while typical SEM can reach magnifications of more than 30,000X. SEM - EDS was used to identify the chemical composition and relative distribution of macro and micro nutrients. The SEM is also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions (using EDS), crystalline structure, and crystal orientations.

### Result and Discussion

Some quality parameters such as compound analysis were carried out Scanning Electron Microscopy (SEM) and Fourier Transform Infrared (FTIR) was employed to observe possible variation in the structures and characteristics respectively. The FTIR spectral of samples showed slight variations in peak position.

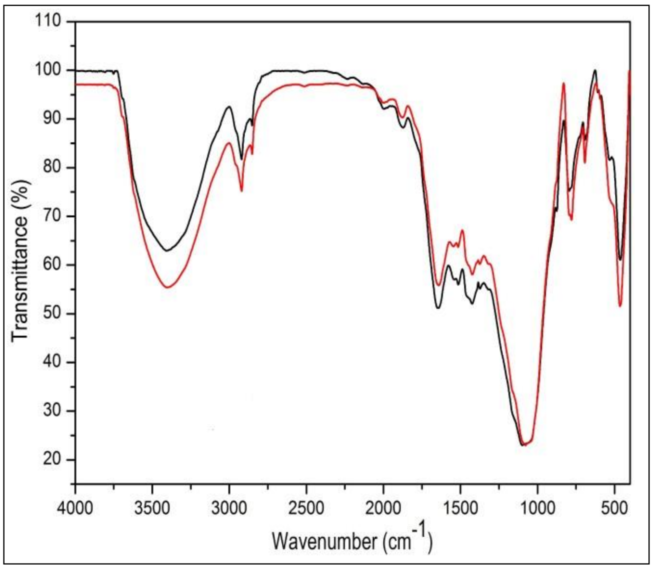


**Fig 1:** FTIR Spectrum of sample 1 (Tomato)

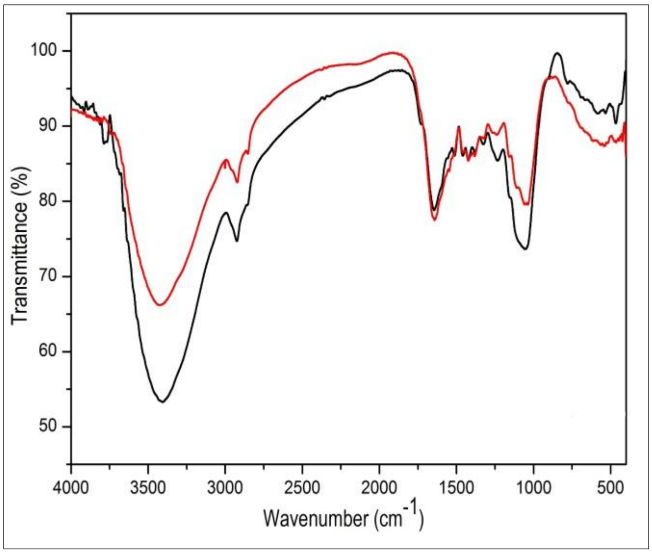


**Fig 2:** FTIR Spectrum of sample 2 (Tomato)

The FTIR spectral peaks of sample 1 and sample 2 in (Figures 1 and Figure 2) showed stretching OH of alcohols at (3600-3100 $cm^{-1}$), stretching C-H of aldehydes at (2830-2695 $cm^{-1}$), C-H bend of alkanes at (1470-1350 $cm^{-1}$), C-N stretching of nitriles at (2300-2200 $cm^{-1}$), C-N stretching of aromatic amines at (1335-1250 $cm^{-1}$) and CH out of plane deformation of aromatics at (900-675 $cm^{-1}$). Sample 1 and sample 2 showed H-C-H asymmetrical stretching of alkanes at (3000-2850 cm-1), Tomato pollens showed spectral peaks N-O symmetric stretching of nitro compounds at (1360-1290) while control, Oven dried and Tomato pollens showed spectral peaks C=C stretching of alkynes at (2260-2100 cm-1), C=C stretching of alkenes at (1680-1640). The FTIR spectral of control and processed samples showed slight variations in peak positions. There was a complete contrast in the peak position of the sample 1 when compared with the sample 2 and this could be attributed to the presence of some impurities. FTIR spectra of individual pollen samples are different, and the most visible differences are in the spectral regions corresponding to vibrations originating from lipids, sporopollenin and carbohydrate. Spectral variability enables sufficient differentiation of plant-related families and genera (Zimermann and Kohler 2014, 2015) [21], and even congeneric species (Bagcıoglu *et al.*, 2015, Zimmermann 2018) [23]. Furthermore, FTIR has been used in plant biology to differentiate between cell wall mutant plants (Stewart *et al.*, 1997, Chen *et al.*, 1997) [17, 3]. Those studies showed that FTIR could be used to identify structural and architectural alterations in cell walls.

### SEM-EDS analysis

The elemental composition and relative distribution of nutrients in tomato (*Solanum lycopersicum L.)* flower (Pollen grains) were analyzed using SEM-EDS. The EDS spectra showed an elemental composition and relative distribution of nutrients was increased in the sample 1 and the sample 2. (Table 1, Figure 3 and 4). The Scanning Electron Microscopy (SEM) is a test process that scans a sample with an electron beam to produce a magnified image for analysis. The method is also known as SEM analysis and SEM microscopy, and is used very effectively in microanalysis and failure analysis of solid inorganic materials. Scanning Electron microscopy is performed at high magnifications, generates high-resolution images and precisely measures very small features and objects. The signals generated during SEM analysis produce a two-dimensional image and reveal information about the sample, including external morphology (texture), chemical composition, when used with the EDS feature, and orientation of materials making up the sample. The EDS component of the system is applied in conjunction with SEM analysis to determine elements in or on the surface of the sample for qualitative information.

**Table 1:** SEM-EDS analysis of *S. lycopersicum* - Sample 1 and 2

| **Elements (%) sample 1** | **C K** | **O K** | **P K** | **S K** | **CL K** | **K K** | **Ca K** |
|---|---|---|---|---|---|---|---|
| Weight (%) | 65.85 | 24.57 | 1.74 | 1.93 | 0.79 | 4.34 | 0.79 |
| Atomic (%) | 75.23 | 21.07 | 0.77 | 0.82 | 0.31 | 1.52 | 0.27 |
| Elements (%) sample 2 | C K | O K | P K | S K | CL K | K K | Ca K |
| Weight (%) | 69.47 | 27.07 | 0.19 | 0.94 | 0.61 | 0.30 | 1.42 |
| Atomic (%) | 76.33 | 22.32 | 0.10 | 0.40 | 0.25 | 0.11 | 0.48 |

The elemental composition of the tomato pollen grains (*S. lycopersicum L.)* EDX analysis (Table 1 and figure 3 and 4) revealed the presence of seven compounds on included C- $CaCO_3$, $SiO_2$ -O, Gap- P, $FeS_2$ - S, KCl - Cl, MAD -K and Ca. In tomato sample 1 about 97% is accounted by atoms of C, K, Ca and O the major chemical constitutes of biomass. The remaining 3% is divided unequally among the other elements. The sample 2 analysis of the elemental composition of seven elements EDX scans from the tomato pollen grains (*S. lycopersicum L.)* is presented in figure 4, which indicated approximately 90% of the atomic and weight (%) was accounted for by atoms of Cl, K, C and O is accounted atoms (90%) and remaining percentage was accounted for the rest of the elements.

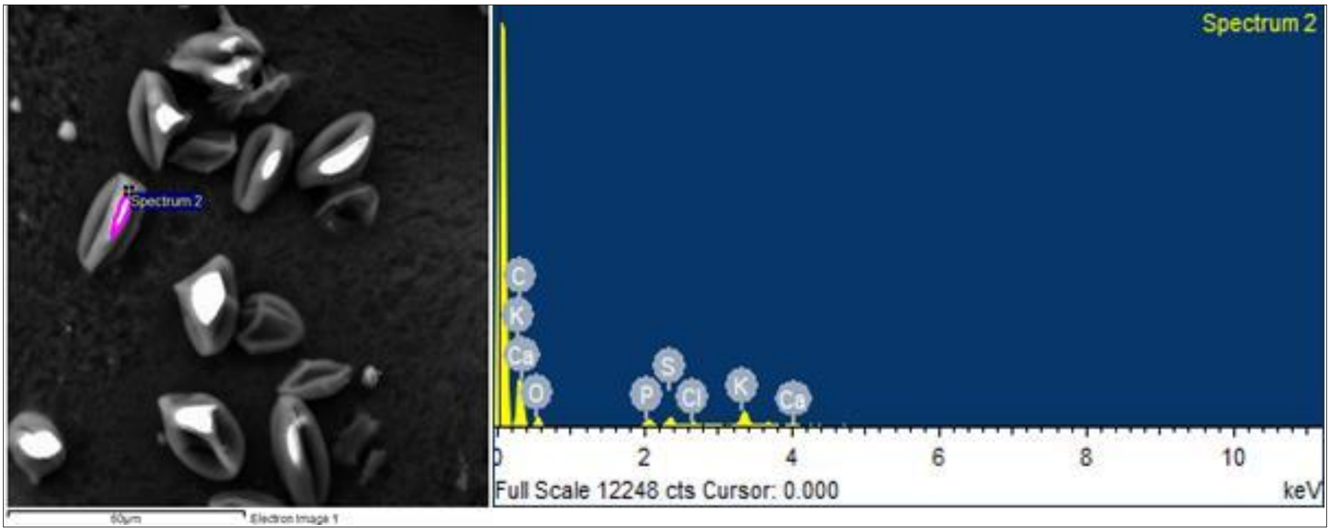


**Fig 3:** *S. lycopersicum L. pollen grains* using SEM-EDS analysis of Sample 1

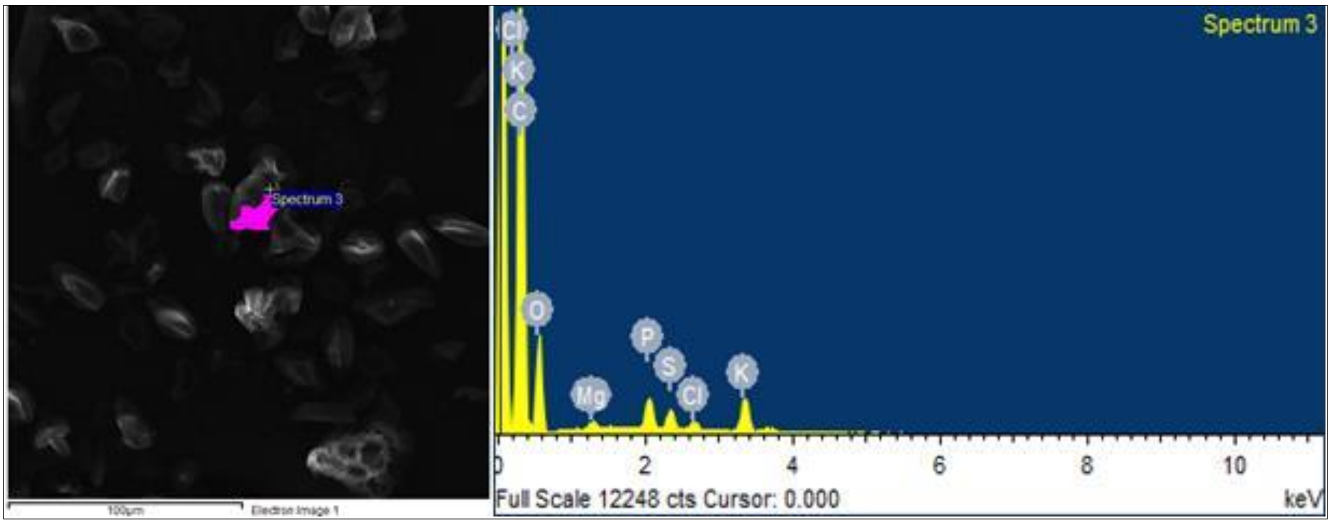


**Fig 4:** *S. lycopersicum L. pollen grains* using SEM-EDS analysis of sample 2

Scanning electron microscopy (SEM) coupled with energy-dispersive X-ray analysis (EDS) is an effective method that can yield both qualitative identification and quantitative elemental information (Goldstein *et al.* 2003) [8]. The SEM-EDS method was used to examine morphological changes and the relative distribution of macro- and micronutrients and chemical composition on the surface of REC3-inoculated plants (Guerrero *et al.* 2012) [9]. The sodium (Na) appeared to have a useful impact on the development of strawberry Cv. Korona under saline anxiety (Saied *et al.* 2005) [15], which could be identified with the substitution of K for Na, which helps in osmoregulation to keep up the water substance of plant tissues and at last increment of fresh weight (Turhan and Eris, 2004).

**Conclusion**

The tomato (*Solanum lycopersicum L.)* flowers (Pollen grains) were subjected to FTIR, SEM-EDS analysis. These instruments serve as good tools to analyze a specified chemical element or provide partial multi-element analysis. Some quality parameters such as compound analysis were carried out Scanning Electron Microscopy (SEM) and Fourier Transform Infrared (FTIR) was employed to observe possible variation in the structures and characteristics respectively. The FTIR spectral of samples showed slight variations in peak position among the two samples.

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