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Determination of pollen viability in some minicore accessions of cherry tomato (*Solanum* spp.)

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Abstract
The present work aimed to determine the pollen viability of ten minicore accessions of cherry tomato (*Solanum* spp.) was carried out at the Experimental Farm, Division of Vegetable Science, SKUAST-K, Shalimar, India during *Kharif* season 2020. The freshly opened male flower buds were collected in the morning between 7.30 am to 8.00 am. TZ test also known as Tetrazolium (2, 3, 5 triphenyl tetrazolium chloride) test was followed to determine the pollen viability of ten minicore accessions of cherry tomato. All living tissues, which respire, possess the ability of reducing a colour less chemical 2, 3, 5 triphenyl tetrazolium chloride into a red coloured compound formazan by H transfer reactions catalysed by the enzyme dehydrogenases (Norton, 1966). This method provides accurate results for many taxa as compared to other methods of staining (Shivanna and Johri, 1989) [30]. The concentration of tetrazolium salt, temperature and period of incubation needs to be standardized to get optimal and precise results in various pollen samples (Shivanna and Johri 1989) [30]. Based on the overall performance of the accessions, the maximum pollen viability percentage was showed by accession EC-520078 followed by VRT-02 and the least was showed by accession Suncherry followed by WIR-5032.

Keywords: cherry tomato, staining, pollen viability, tetrazolium

Introduction
Cherry tomato (*Solanum* spp., 2n=2x=24), one of the important botanical variety of the cultivated tomato (*Solanum lycopersicum* L.) is a member of nightshade family 'Solanaceae', consisting of 96 genera (Akhtar *et al*., 2013) [2] and over 3000 species (Melomey *et al*., 2019; Sharma *et al*., 2019) [28]. Cherry tomato is native to the Andean region encompassing Ecuador and Peru of South America and thereafter it spread around the world following the Spanish colonization of the Americas (Grandillo *et al*., 2011) [10]. It is perennial in its native habitat but is often grown as an annual crop in temperate climate. The growth habit of the plant is usually indeterminate and may reach up to 3 meters in height. Pollen quality is crucial for breeding and crop improvement, pollen storage studies, pollen-pistil interaction studies, understanding incompatibility and fertility studies and for seed industry (Shivanna and Rangaswamy, 1992) [31]. It is the quality of pollen that decides the pollen viability and vigour (Ottaviano and Mulcahy, 1989). Pollen viability and fertility have a paramount importance in hybridization programme. Successful pollination is a prerequisite for fertilization and seed set in most crop plants, and the insight knowledge on pollen biology, including pollen viability, pollen germination and pollen tube growth, is required for any rational approach to increase productivity (Bolat and Pirlak, 1999; Shivanna, 2003) [6, 29]. To distinguish aborted and non-aborted pollens, a simple and hasty technique is required for high throughput. An unambiguous procedure involves pollen deposition on receptive stigma followed by evaluation of seed set. These methods, however, are indirect and are universally preferred, associated with physical and physiological features of pollen with its ability to fertilize the ovule (Rodriguez-Riano & Dafni, 2000) [23]. Pollen germination assays frequently require optimization as well as are time consuming and difficult to reproduce (Boavida, 2007) [5]. Reproduction is a critical step in the life cycle of all organisms and pollination is one of the decisive stages (Piechowski *et al*., 2009; Jha and Dick, 2010) [15, 22] of reproduction process. The male gamete i.e., pollen is surrounded by sporoderm, which consists of the inner layer intine (which is composed of pectin, cellulose and protein) and outer layer exine (synthesized and secreted by the tapetal tissue of the anther and is composed mainly by sporopol protein). The glycoprotein present on the pistil generally interact with pollen coat protein (Zhang *et al*., 2008) [35].

In order to reach the micropyle region, pollen tube passes through four stages viz., a) imbibition stage b) Lag stage, c) tube initiation stage and, d) rapid tube elongation stage.

After getting deposited on stigma, pollen grain adheres to the stigma, hydrates and germinates by emitting pollen tube, which penetrate the cuticle of the stigma cells and grows through the extracellular matrix of the style.

The process culminates in the discharge of male gametes into the embryo sac. Pollen tube growth proceeds through tip extension and can be affected by many factors, including temperature, medium osmolarity and the availability of sucrose, calcium, zinc and boron (Sawidis and Reiss, 1995; Tylor and Hepler, 1997) [26].

In spite of taking proper care during pollination, breeders fail to get fertile seed during artificial pollination. Unless sterility is the main cause the failure of seed setting may be the result of slow growth of the pollen tube or its early degeneration in the style. To overcome these difficulties, it is necessary to study the pollen viability, physiology of pollen germination and pollen tube growth.

Numerous techniques are available to determine the pollen viability and the method of choice depends upon the crop species (Hanna and Towill 1995). The most accurate method of pollen viability is the ability of pollen to effect fertilization and seed set (Smith-Huerta and Vasek, 1984; Shivanna and Joshi 1989) [32, 30].

There are different methods to evaluate pollen viability; some noteworthy tests are dye and *in vitro* germination. The dye test has advantages as indicator of pollen viability because they are faster and easier compared with pollen germination, but they do tend to overestimate the viability and real germination of pollen grains. On the other hand, *in vitro* pollen germination depends on the genotype, environmental conditions, pollen maturity, composition and pH of the medium; thus, it is necessary to determine optimum conditions for pollen germination.

The literatures on different tests of pollen viability was reviewed by Dafni and Firmage (2000) [8] and they explained the advantages and disadvantages of each test. The ability to use chemical staining to discriminate aborted pollen grains from non-aborted pollen grains has well-known practical applications in agriculture.

It is observed that pollen staining methods are not reliable sometimes for most of the species (Barrow, 1981) [4], hence *in vitro* pollen germination is more practical used to determine the pollen viability.

The ability of pollen germination plays an important role not only on fruit set but also the flower-flower and flower-pollinator interaction. Pollen germination and pollen tube growth are very crucial for fertilization and seed development. Due to involvement of the pistillate tissue in the nature, physiological and biochemical investigations on pollen germination and pollen tube growth *in vivo* are rather difficult. Hence *in vitro* germination techniques generally been used extensively on a variety of pollen systems. Such studies have provided considerable information on the physiology and biochemistry of pollen germination and pollen tube growth (Shivana and Johri 1989; Heslop-Harrison 1987; Steer and Steer 1989) [30, 11, 33].

Materials and Methods

Study Material

The ten minicore accession of cherry tomato (*Solanum* spp.) viz., Suncherry, WIR-5032, EC-520074, EC-914115, EC-165690, EC-914092, EC-520078, WIR-3957, EC-914097, VRT-02 were used for pollen viability test. The plants have yellow flowers (< 2.5 cm in diameter) that occur in a complex inflorescence (racemose). The flowers are perfect and hermaphrodite and contain 5 green sepals, 5 yellow petals, 5 stamens alternate with petal position, protective anthers (fused to form an anther cone) that surrounds the stigma and short style. The anthesis starts at 6.30 am and continues up to 11.00 am. Dehiscence of anthers occurs from base to top (longitudinal) and one or two days after opening of corolla (Rolf, 2009) [24]. The stamen shed its pollens when the style grows up through anther tube, thus self-pollination occurs. Cross-pollination (0.07-10.0 %) also occurs through bees, when stigma protrudes outside the level of anther (exerted) (Accotto *et al.*, 2005) [1]. The optimum temperature for pollination is around 21^0C.

Study site and plant materials

Seeds of the ten minicore accession of cherry tomato were procured from Division of Vegetable Science, SKUAST-Kashmir. The seeds of all the accessions were first sown in nursery and then transplanted at 4-5 leaf stage to the insect proof polyhouse at a spacing of 60 x 60 cm between rows and plants respectively (Temperature-35-37^{0}C, RH 60-70%). Recommended package of practices were followed to raise a healthy crop. Freshly opened male flowers were used for pollen viability studies.

Pollen viability tests

The literature was reviewed to determine the types of tests used for pollen viability (Dafni and Firmage, 2000). Based on the literature and facility availability at laboratory condition, Tetrazolium staining method was chosen for pollen viability experiment.

Tetrazolium Test

Tetrazolium (2, 3, 5 triphenyl tetrazolium chloride) test is also known as the TZ test. All living tissues, which respire, possess the ability of reducing a colour less chemical 2, 3, 5 triphenyl tetrazolium chloride into a red coloured compound formazan by H transfer reactions catalysed by the enzyme dehydrogenases (Norton, 1966). This method provides accurate results for many taxa as compared to other methods of staining (Shivanna and Johri, 1989) [30]. The concentration of tetrazolium salt, temperature and period of incubation needs to be standardized to get optimal and precise results in various pollen samples (Shivanna and Johri 1989) [30].

Protocol

1% TTC was prepared by adding 200 mg of 2, 3, 5 triphenyl tetrazolium chloride and 12 g of sucrose in 20 ml distilled water. Two drops of this mixture were dropped on a microscope slide and the pollen were dusted over it and covered with a coverslip. The pollen viability counts were made after 1 hour of incubation under dark place. Pollen grains stained orange or bright red colour were counted as viable.

Results and Discussion

In this study, all the cherry tomato accessions showed wide range of pollen viability percentage as represented in Table 1. Based on the overall performance of various cherry tomato accessions, accession EC-520078 showed maximum pollen viability percentage followed by VRT-02 and EC-520074. The least pollen viability percentage was showed by accession Suncherry followed by WIR-5032 and EC-914115.

Table 1: Pollen viability (%) in minicore accession of cherry tomato (*Solanum* spp.) using TZ test.

Minicore Accession	Pollen viability (%)
Suncherry	81.26
WIR-5032	81.53
EC-520074	92.42
EC-914115	82.53
EC-165690	90.33
EC-914092	89.66
EC-520078	95.46
WIR-3957	85.53
EC-914097	87.53
VRT-02	94.21

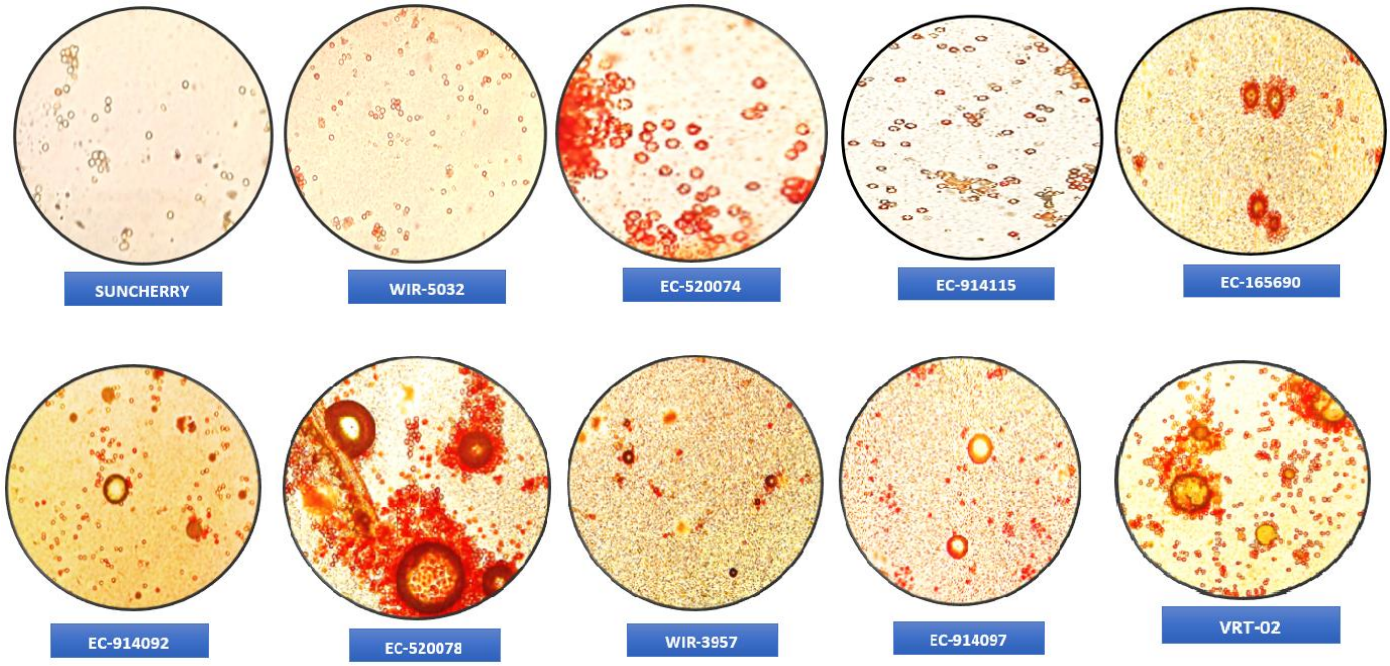

Fig 1: Tetrazolium staining method for pollen viability in cherry tomato accessions

Conclusion

Tetrazolium (2, 3, 5 triphenyl tetrazolium chloride) test, was more efficient for discrimination of viable and non-viable pollen grains in cherry tomato species. Accession EC-520078 showed maximum pollen viability followed by VRT-02 and EC-520074.

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