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Evaluation of Growth and Pollen Viability (Fertility) in Relation to Fruit Set among Five Varieties of Tomato

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The evaluation of pollen viability and fruit setting capacity are two essential criteria for pollinator's characterization. This study was carried out to evaluate pollen Fertility/ viability in five Varieties of Tomatoes. We analyzed the viability of pollen grains of five different varieties of tomatoes grown in ITM University, Gwalior, Madhya Pradesh, India. Aceto- carmine method is used to estimate pollen viability. The germination percentage, pollen viability and Fruit setting rate were recorded at different stages respectively. Pollen viability in relation to fruit set was analyzed while growth parameters and fruit yield per plant of each variety were also collected and analyzed. Results displayed that the highest pollen viability rate (100%) was obtained in SWEAKAR-448 Variety followed by Arka Vikas (95%), ARJUN-135 (93%), 9005-SIRI (90%) and PKM-1 (80%). The highest percentage of germination (95%) was shown by 9005-SIRI Variety and maximum fruit setting (15-20 Fruits per plant) was recorded in the SWEAKAR-448 Variety. The results showed significant vegetative growth variations and correlations between pollen viability and fruit setting characters. In addition, it was found a noticeable variation in Pollen viability/fertility, was not proportional to fruit

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setting percentage. In general Pollen viability was directly linked to fruit set, whereas formation and fruit set were not in the tomato varieties that were investigated. Pollen viability was high, which was accompanied through a reduction in the number of fruit sets, fruit size and pollen viability in the field is highly variable, indicating that differences in microenvironment may have a profound effect on fruit setting and pollen viability.

Keywords: Pollen fertility / viability; aceto-carmine test; germination rate; fruit setting.

1. INTRODUCTION

One of the most significant vegetable crops in the world is the tomato (*Lycopersicon esculentum* Mill.), a plant belonging to the Solanaceae family. Tomato crop is farmed in practically every part of the world, including tropical, sub-tropical, and temperate climates. The tomatoes need a constant temperature of 12-24^{o}C. Loamy soil, which is 6.0 -7.0 in pH, is ideal for the growth of tomatoes. Moreover, they grow best in a soil that is well-drained and loosely packed. These plants require a minimum of 6-8 hours of direct sunlight to grow well. Tomato is one of the most important "protective foods" because of its special nutritive value. It is rich in fiber and low in calories, supplying vitamins and minerals. Tomato fruit contain vitamins like 'A' and 'C' and antioxidant in abundance quantity. Due to the unique properties contained in its fruit, tomato sometime rightly referred as poor man's orange.

"The pollen grains of tomato have a highly specialized cell type that grows within the anthers of the flower through a complicated sequence of events, is the mature male gametophyte (micro gametophyte). Through pollen, plants deliver genetic material and expand genetic diversity by producing recombinant progeny in the subsequent generation" (Deveshwar *et al.,* 2011). Pollen fertility in flowering plants depends on proper cellular differentiation in anthers. In tomatoes the pollen shed normally occurs from morning to late afternoon, with midday being the ideal time for release and transfer. Tomatoes are self-pollinating plants that have the perfect flowers. This makes pollination easy. Despite taking extra precautions during pollination, breeders are unable to produce fertile seed when artificial pollination is used. Unless sterility is the primary cause, seed failure can be caused by the pollen tube's sluggish growth or early degeneration in the style. To determine pollen viability, a variety of approaches are available, and the method chosen is dependent on the crop species (Hanna and Towill 1995). The ability of pollen to mature, germinate, and transfer male gametes to the embryo sac is referred to as pollen viability. The vitality of pollen is what determines its quality. Before pollen granules lose their vitality, they must reach the stigma of the pistil to complete the fertilization. The duration of viability varies by species and is influenced by temperature and humidity. To measure pollen viability, two colorimetric methods were used *i.e.,* 2, 3, 5 triphenyl tetrazolium chlorides (TTC) and the Acetocarmine test. In these two methods the colorant is added to pollen and viewed under a microscope.

Acetocarmine test is one of the most well-known pollen stain processes. Acetocarmine stains the nuclei of pollen grains and slightly stains the cytoplasm, resulting in a good contrast between the grain and the surrounding medium. Any nuclear asymmetry is easily observable. Fresh pollen (or anther loculi or germinated grains) is spread on a microscope slide, then suspended in a drop of aceto carmine for the aceto carmine staining process, heated over a sprit flame, shredded, and evaluated after 15-30 minutes. Slides that are both permanent and semi-permanent can be built. The percentage of pollen viability was calculated by dividing the number of viable grains by the total number of grains. The degree of staining was utilised to measure pollen viability. (pollen with bold red color are considered as viable and colorless are considered as nonviable).

2. MATERIALS AND METHODS

The present experiment was conducted at Crop Research Center, Department of Genetics and Plant Breeding, School of Agriculture, ITM University, Gwalior, Madhya Pradesh in *Rabi* Season (2021-2022). The five different varieties of tomato used in the experiment are 9005-SIRI (Hybrid), Arka Vikas (Variety), ARJUN-135 (Hybrid), PKM-1 (Variety) and SWEAKAR-448 (Hybrid). During the tomato growth the average monthly temperature ranges from 21°C to 26°C and the relative humidity ranged between 55 and 82 per cent. In a year average rainfall is noted as 910mm. Generally in December and January the temperature ranges from 14 - 20^{o}C. The experimental blocks soil is a well fertile sandy

loam soil with pH values ranging from 4.8 to 5.3. The field was well drained with proper irrigation.

Planting the seeds involves sowing the seed into five different trays to obtain the seedlings; the trays were filled with a mixture of coco peat and vermi compost with two holes to enhance drainage. The seedlings were transplanted 34 days after sowing. The experiment was laid out in RBD (randomized block design), each plot size was 3m × 7 m, spacing between rows and plants is 60 X 50 cm. Each plot acquired 50 plants. The data recorded during the experiment were on germination and mortality percentage, growth characters of the varieties, floral and fruit characteristics, Fruit setting based on pollen viability percentage, and correlation coefficient of growth characters. The varieties were tested for pollen fertility status by using the aceto-carmine staining method. From each variety of tomato 3-5 randomly selected flowers were collected. Flower buds of these varieties were collected from research field and were analyzed for viability, using dissecting forceps, scalpel and a needle. The anthers of five varieties were opened to allow extraction and subsequent transfer of pollen dust to a microscopic glass slide in a drop of aceto-carmine stain solution and observed under the microscope. Each slide was observed under the 10 X and 40 X objective lens (100 X & 40 X magnifications). For each plant, five slides were prepared. The slides were covered with cover slips which were gently placed and pressed with the thumb. About 100 pollen grains from each flower bud were observed and percentage pollen viability was determined.

To determine pollen fertility, deeply stained pollen grains were recorded as fertile and viable, and unstained or very lightly stained ones were considered as sterile or non-viable. Five samples for each variety are considered to calculate the average. Pollen fertility was calculated by dividing the number of viable pollen grains by the total number of pollen grains counted in the region of view on slide and averaging them for all plants in that varieties. Pollen viability was expressed as percentage pollen fertility in each tomato plant variety. The data obtained for various parameters evaluated were subjected to Analysis of Variance (ANOVA) and the means were separated at level of significance $P< 0.05$.

3. RESULTS AND DISCUSSION

Among the five tomato varieties germination percentage and mortality percentage was recorded and from the tenth to the thirty-first day after sowing, seed germination was measured until maximum germination was noticed in seeds. The percentage of germination ranged from 70% (PKM-1) to 95% (9005- SIRI). Whereas, the highest mortality rate after plant transplantation in to the field was shown by the varieties Arka Vikas, ARJUN-135 and PKM-1 as 10% and the lowest mortality rate was observed by the varieties 9005-SIRI (6%) and SWEAKAR-448 (5%). Table 1 shows the comparison between the germination percentage and mortality rate percentage of the five tomato varieties.

Table 1. Percentage of germination and mortality in five varieties of tomato

Variety Name	Germination (%)	Mortality (%)
9005-SIRI	95	6
Arka Vikas	90	10
ARJUN-135	85	10
PKM-1	70	10
SWEAKAR-448	93	5

Low germination percentage and mortality percentage in the tomato varieties were caused by the presence of endogenous cyanide or physiologically immature embryo which could have resulted in poor germination. The mortality percentages after the transplantation of plants into the field was due to the shock and stress that effect rooting and nutrient uptake from which the young plants could not recover [1].

At maturity the analysis of quantitative morphological parameters were evaluated (Table 2). The highest plant height was recorded by the variety PKM1 (55-65 cm) had the tallest plants while ARJUN-135 (40-45 cm) had the shortest plants. The plant high mean values ranged from 41 to 57.5 cm.

The tomato variety PKM1 had the highest mean value (57.5) and 9005-SIRI (41) had the lowest mean value.

In line with the findings of Akindele et al. [2] study of quantitative morphological parameters evaluated at maturity revealed considerable variances. However, in some varieties character differences were not very peculiar from one another. The ARJUN-135 variety developed the shortest plants (55-65 cm), while variety PKM1

Table 2. Magnitude of growth parameters

Variety Name	Plant Height (cm)	Branches per Plant (number)	Days to Flowering (number)	Flowers per Plant (number)	Fruits per Plant (number)
9005-SIRI	41	9.5	67	22.5	7.5
Arka Vikas	44	9.5	63.5	22.5	3.5
ARJUN-135	42	12.5	71	24.5	9.5
PKM-1	57.5	10.5	72	16	4.5
SWEAKAR-448	51.5	15.5	62	27.5	16.5

Table 3. Correlation coefficient of quantitative growth characters among five varieties of tomato

	Branches per Plant (number)	Flowers per Plant (number)	Plant Height (cm)	Fruits per Plant (number)
Branches per Plant	1			
Flowers per Plant	0.587^{*}	1		
Plant Height	0.258^{NS}	-0.448^{NS}	1	
Fruits per Plant	0.791^{**}	0.776^{**}	-0.120^{NS}	1

** Correlation is significant at 0.05 level*
*** Correlation is significant at 0.05 level*
NS Correlation is not significant at 0.05 level

developed the tallest plants (40-45cm). The examined variants SWEAKAR-448 and 9005-SIRI have branches in varying numbers, ranging from 15.5 to 9.5 of mean values. This showed increased growth factor production, which promotes vegetative growth. The tendency in the varieties growth patterns is explained by the positive connections between leaf characteristics and plant height (Table 3). Significant relationships between a few of the vegetative development properties have also been found by quantitative character analysis. A similar result was reported by Nwosu et al. [3], that significant correlations existed in the vegetative parameters of the tomato varieties studied.

Table 4 shows the mean values for the fruit characters. The variety SWEAKAR-448 shows highest mean values than other varieties in every character of the fruits *i.e.,* fruits cluster (6), fruit cluster per plant (4.5), average fruit weight (86), average fruit yield per plant (2.2), harvest duration (21). The minimum fruits per cluster (2.5) and lowest fruit cluster per plant (1) were observed in the variety Arka Vikas. The Variety 9005-SIRI showed the minimum fruit weight (21g), lowest average fruit yield per plant (0.75kg) and lowest harvest duration (15.5). The mean values recorded for the Harvest duration ranged from 21 days (SWEAKAR-448) to 15.5 days (9005-SIRI). Whereas, the highest harvest duration was recorded in SWEAKAR-448 (20-25) followed by PKM-1 (18-22), ARJUN-135 (16-20), Arka Vikas (16-17) and the lowest Harvest duration was observed in 9005-SIRI (15-16) respectively. This finding is in agreement with earlier reports that different factors are responsible for plant vegetative growth and fruiting in plants [4,5].

“Fruit set generally varies from plant to plant, and fruit set percentage probability can relate better the changes than fruit set number. Additionally, fruit set is a dynamic process that varies depending on the crop's stage of growth, with cyclical abortions being noticed in sweet pepper” [6]. “Early fruiting, high fruit productivity, and marketable fruit size traits should be screened to enable the selection of high-fruit yielding varieties easier with a view to commercial production; As Bernousi et al. [7] suggested, selection should not, be made solely on the basis of vegetative development”.

Pollen fertility results obtained using the aceto-carmine method and the percentage of fruit set for the varieties are presented in Table 5. Out of the five varieties analyzed for pollen viability, SWEAKAR-448 showed a higher pollen viability percentage whereas PKM-1 variety displayed lowest pollen viability percentage.

Table 4. Mean values for the fruit characters of five varieties

Name of the Variety	Fruit per Cluster (number)	Fruit Cluster per Plant (number)	Average Fruit Weight(g)	Average Fruit Yield per Plant (kg)	Harvest Duration (number of days)
9005-SIRI	3.5	3	21	0.75	15.5
Arka Vikas	2.5	1	52	0.8	16.5
ARJUN-135	5.5	4	73.5	1.25	17
PKM-1	4	2	80.5	2.15	19
SWEAKAR-448	6	4.5	86	2.2	21

Table 5. Percentage of fruit setting according to pollen viability percentage

Name of the Variety	Pollen Viability/Fertility (%)	Fruit Setting (%)
9005-SIRI	90	75
Arka Vikas	95	70
ARJUN-135	93	85
PKM-1	80	78
SWEAKAR-448	100	95

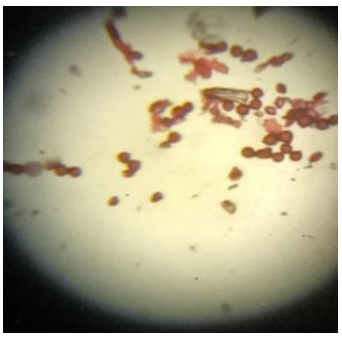

Plate 1. Pollen Fertility Status of 9005-SIRI

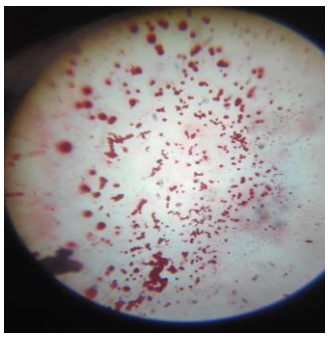

Plate 2. Pollen Fertility Status of Arka Vikas

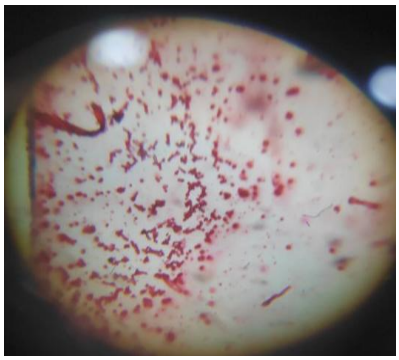

Plate 3. Pollen Fertility Status of ARJUN-135

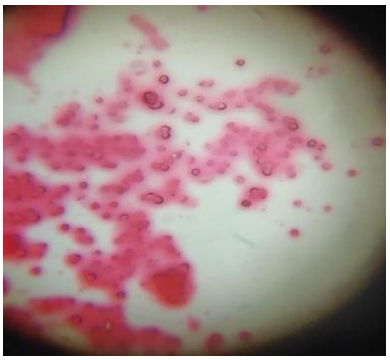

Plate 4. Pollen Fertility Status of PKM-1

Plate 5. Pollen Fertility Status of SWEAKAR-448

Pollen grains can be considered viable if they are able to achieve fertilization in a natural setting of fruit. Therefore, analyzing pollen viability would ideally involve analyzing seed set after natural pollination in the field. However, fertilization is not only dependent on pollen viability, but on other factors too, such as pollen dispersal and ovule development in the mother plant. Various methods exist to determine pollen viability that bypass the need for analyzing seed set.

"Pollen viability was directly proportional to fruit set among the varieties studied, a number of factors have been described to influence pollen viability and subsequent fruit set in crop plants" [8,9,10]. The highest fruit production was achieved by the variety SWEAKAR-448 that had the most viable pollen. According to Ozores Hampton et al. [11] blossom drop and post-pollination disorder can diminish the flower-fruit set ratio in tomatoes. The investigation supports these findings because there was a significant variation in the ratio of produced fruit to blossoms. The lack of a direct correlation between flower counts and fruit set percentages indicates that tomato fruiting is influenced by a variety of mechanisms and influences. Low fruit set is probably caused by inadequate pollination and poor embryo growth in the variety PKM-1 [6,1] .

The percentage of tomato genotypes that developed fruit under high-temperature stress dropped, as found by Dane et al. [8], which significantly reduced pollen viability and favored blossom drop. Similar to this, Sato et al. [12] and Stephenson [13] have clarified additional elements that influence fruit set. They include the following: stigma, stylar exertions, pollen viability, pollen dehiscence, ovule viability and pollen production. Reduced fruit set and tomato production could be caused by any one of these elements or by a combination of them. The use of pollen viability knowledge as a selection factor for high yield tomato production could therefore provide crucial information for efficient breeding programs, it is worth emphasizing.

"In this experiment pollen grains were germinated immediately after collection from flowers in the field, without the need to transport the pollen grains to the laboratory thus, in this case, germination percentages may accurately reflect pollen viability in the field. Pollen viability rates are determined by factors such as water balance, temperature stress and UV-B radiation. The effect of these factors on viability varies with species, and various adaptations that reduce damage can be found in pollen grains. Such adaptations may include the dehydrated state of mature pollen grains" (Sugandha G, 2013). Our results indicate that the pollen viability of the variety SWEAKAR-448 was higher than the other 4 studied varieties and the pollen viability of the variety PKM-1 was the lowest.

4. CONCLUSION

The germination percentage was recorded before and after transplanting, whereas pollen fertility and fruit set were recorded at the time of anthesis and physiological maturity respectively. Results showed that the highest pollen viability rate (100%) was obtained in SWEAKAR-448 Variety, followed by Arka Vikas (95%), ARJUN-135 (93%), 9005-SIRI (90%) and PKM-1 (80%). The highest percentage of germination (95%) and maximum fruit setting (15-20 Fruits /plant) were also recorded in the SWEAKAR-448 variety respectively. In addition, it is found that pollen viability in the field is highly variable, indicating that differences in microenvironment may have a profound effect on pollen viability [14]. This is noted that there is a noticeable variation in vegetative growth and relationships between plant growth parameters and fruit parameters. Pollen viability/fertility, on the other hand, was not proportional to fruit setting percentage. Pollen viability was directly linked to fruit set, whereas formation and fruit set were not in the five tomato varieties that were investigated. Pollen viability was high, which was accompanied through a reduction in the number of fruit sets, fruit size and number of fruits per plant number may be influenced by biotic or abiotic factors [1,15-20].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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