Advances in Research

15(1): 1-9, 2018; Article no.AIR.41145
ISSN: 2348-0394, NLM ID: 101666096

Effect of Sucrose and Boric Acid on *in-vitro* Pollen Germination of Guava (*Psidium guajava*) Varieties

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Authors' contributions

This work was carried out in collaboration between all authors. Author TS collected the data, wrote the protocol and wrote the first draft of the manuscript. Author SKS designed the study and reviewed the manuscript. Authors SV managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AIR/2018/41145
Editor(s):
(1) Slawomir Borek, Department of Plant Physiology, Adam Mickiewicz University, ul. Umultowska, Poland.
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(2) Mohammed Mesnoua, Algeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/24722

Original Research Article

Received 5th March 2018
Accepted 16th May 2018
Published 22nd May 2018

ABSTRACT

The Premise of Research: To reproduce, higher plants utilize a unique multicellular microorganism: The male gametophyte, or pollen grain. It is known fact that pollen has a strong impact on physical and biochemical quality of fruit. Pollen germination and pollen tube growth are prerequisites for fertilization and seed development. Information about pollen germination will be helpful in the production of hybrids, which is an important step in a breeding program. In guava, such work on the pollen growth under the in-vitro condition is not clearly reported. So the aim of the study is to find the suitable media for in-vitro pollen germination of guava.
Methodology: We used four different concentrations of sucrose and boric acid like 10%, 15%, 20% and 25% and 0.2, 0.4, 0.6, 0.8 g/l respectively. Using a brush, we evenly distributed the pollen over the culture medium in order to achieve the most homogeneous distribution of the material possible.

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After inoculation, the dishes were maintained in the dark at controlled temperature conditions (27±1ºC). These processes took place 2-4 h after inoculation, within the culture medium, by means of observation through a binocular stereomicroscope at 10× magnification.

Results: The pollen germination was maximum 81.52% in Hissar Surkha at 10% sucrose solution and the boric acid concentration at 0.8 g/l shows the maximum germination rate (87.00%) in L-49 variety. The germination rate increases with increasing the boric acid concentrations. The boric acid concentration at 0.8 g/l shows the maximum germination rate (87.00%) in L-49 variety and the lowest was 75.73% in Kafri after three hours and with the same medium, highest pollen tube growth 42.25 µm in L-49 variety.

Conclusion: The results suggest that the suitable media for pollen germination and pollen tube growth of guava under the *in-vitro* condition is boric acid (0.8 g/l) as well as sucrose (10%) solution.

Keywords: Growth; media; Psidium guajava; pollen tube.

1. INTRODUCTION

Guava (*Psidium guajava*) is known as “apple of the tropics” is a popular fruit tree of the tropical and subtropical regions and native to tropical America. It has been adopted in India so well that it appears to be an Indian fruit. Guava is cross-pollinated crop but self-pollination also occurred. The wide genetic variation could be obtained by employing hand pollination and using the external sources of variation [1]. In West Bengal, two important periods of bloom is April- May and September-October [2]. The initial fruit set in nature is quite high, around 80-86% of the flowers set fruits, however, due to severe fruit drop, only 34-56% of set fruits reach maturity. Pollen germination and pollen tube growth are prerequisites for fertilization and seed development. Knowledge of the pollen germination will be helpful in the production of hybrids, which is an important step in a breeding program. However, there is no available information about ideal conditions for testing pollen germination through in vitro tests, only a few studies with some species of *Psidium* were found in a literature review [3,4].

The development of reliable methods for determining the functional quality of pollen helps in monitoring pollen vigour during storage, genetics and pollen-stigma interaction studies, crop improvement and breeding, and incompatibility and fertility studies. The factors such as temperature and relative humidity have a decisive role in pollen longevity [5]. Germination ability and pollen tube growth which carry the male gamete cells, leading them toward the embryo sac, are a sign of compatible pollination [6,7]. *In vitro* pollen germination is not quite the same as germinating pollen on the stigma surface. However, it is considered as an index of pollination and ovule fertilization [8]. Pollen could be considered as germinated if its length is at least equal to the diameter of a pollen grain. Pollen germination in in-vitro is a good way to determine germination percentage, however finding a suitable media for each cultivar is the main problem.

The mature pollen grain represents the male gametophyte and consists of two or more cells enclosed within the exine. Pollen performance criteria (pollen vitality, germination and pollen tube growth rate) are critical for discharging male gametes in the embryo sac and are a prerequisite for fertilization and fruit set [9]. After pollination with compatible pollen, only the fastest growing, most vigorous pollen grain will result in fertilization. Seeds which are produced after double fertilization by relatively fast-growing pollen will be more vigorous than those whose pollen donor had slow-growing pollen tube [10]. It is a unique structure and plays an important role in the post-pollination growth of the ovary/ ovule, resulting in the formation of the fruit and the development of the seed. During their growth *in vivo*, the pollen tubes are surrounded by the tissues of the stigma and the style and their complex metabolic products. This makes it almost impossible to follow the growth of pollen tubes in vivo and to study their metabolism, nutritional requirements, and other growth characteristics. The technique of the culture of pollen in vitro, therefore, allows one to germinate and study the growth of pollen in chemically defined media under strictly controlled and known experimental conditions, without interference from the tissues of the pistil. Pollen grains of most species can be cultured easily in vitro, and much of our present understanding of the growth characteristics of pollen is based on pollen grains grown on nutrient media by various techniques. It is true that the extent of pollen tube elongation in vitro does not often equal that

obtained in vivo, and there is also evidence that certain aspects of the growth of pollen tubes in vitro-particularly the ultrastructure of the growing tip region of the tube are not comparable to pollen tube growth after compatible pollination in nature. However, for most practical purposes the germination and growth of pollen in vitro and in vivo can be favourably compared. In this context, the present worked aimed at evaluating the pollen germination of 24 guava genotypes in in-vitro pollen germination methods by using boric acid and sucrose media.

2. MATERIALS AND METHODS

The experiment on the Effect of sucrose and boric acid on in-vitro pollen germination of guava (*Psidium guajava*) varieties were evaluated in 24 guava varieties, in the department of fruits and orchard management, B.C.K.V. Mohanpur, India. In order to evaluate in vitro germination, newly opened flowers in good condition are necessary. So this analysis can only be carried out by completely randomized design. Pollen grains were distributed onto Petri dishes containing culture medium consisting of sucrose concentration with 10%, 15%, 20%, 25% and 0.2, 0.4, 0.6 and 0.8 g/l boric acid. Using a brush, we evenly distributed the pollen over the culture medium in order to achieve the most homogeneous distribution of the material possible. A sample consisting of the pollen from one flower of each genotype was distributed among the dishes. Three slides, with 500 pollen grains each, were evaluated for each genotype. After inoculation, the dishes were maintained in the dark at controlled temperature conditions (27±1ºC) before proceeding with the counting process of the germinated grains of pollen and measurement of the pollen tube length. These processes took place 2-4 h after inoculation, within the culture medium, by means of observation through a binocular stereomicroscope at 10× magnification. Germination was assessed by the percentage of germinated pollen, in that grains showing pollen tube with a length similar to or greater than its own diameter being considered viable. To assess the significant differences between the Sucrose and Boric acid concentrations, generalized linear models were applied (SPSS 17.0, SPSS Inc., Chicago, IL, USA). Significant differences between the treatments were determined using Duncan's New Multiple Range Test at $P \leq 0.05$, and a difference was considered to be statistically significant at 95%.

3. RESULTS

The pollen germination in different guava genotypes varied significantly (Table 1) and different growth stage of pollen tube was showed in Fig. 1A, B,C. In this experiment, various concentrations of sucrose solution viz. 10%, 15%, 20% and 25% were used for pollen germination and it was found that the maximum percentage of pollen grain germinated at 10% sucrose solution for most of the cultivars. Among the genotypes, means of pollen germination percentage and pollen tube length ranged between 49.58-81.52% and 41.41-19.84µm, respectively. Based on the data (Table 1), the pollen germination was maximum in 81.52% in Hissar Surkha at 10% sucrose solution whereas minimum pollen germination in 49.58% in Hissar Safeda at 25% sucrose solution. In Table 2, the highest pollen tube growth 41.41µm in Lucknow-49. In all varieties, the pollen germination decreased with the increasing percentage of sucrose except in Lucknow-49 where the germination increased (76.65%) in 15% sucrose solution over 10% sucrose solution (72.86%). The addition of boric acid significantly increases the pollen germination rate (Table 3). The boric acid concentration at 0.8 g/l shows the maximum germination rate (87.00%) in L-49 variety and the lowest was 75.73% in Kafri after three hours (Table 3). In Table 4 the highest pollen tube growth 42.2 µm in L-49 in the concentration of 0.8 g/l and the lowest in SRD-1 (15.30 µm) variety (Fig 1D). Differences among sucrose and boric acid means were found statistically significant and the lowest germination and pollen tube growth was observed in sucrose media as well as boric acid media in all genotypes.

4. DISCUSSION

The study of In-vitro pollen germination is a powerful tool for improving genetically, physiologically and biochemically for a wide range of plant species belonging to different families [11]. Pollen grains of a most of species germinate successfully in sugar solutions. Sucrose is probably the best and most commonly used source of carbon and energy for pollen. Sugars play two major roles in pollen germination viz., osmotic regulation and nutrition. Several workers believed that exogenous sugars are required only for osmotic control and not for nutrition [12]. Others have supported the view that apart from having an osmotic role, the externally supplied sugars, whether in vivo or *in vitro*-serve as important sources of nutrition

Table 1. Effect of different concentration of sucrose solution on pollen germination rate (%) of different guava varieties

Varieties	10%	15%	20%	25%
Pant prabhat	73.25±1.00^{efgh}	59.44±5.49^{g}	51.21±0.93^{l}	51.16±0.58^{jkl}
Lalit	73.51±2.09^{efg}	53.79±1.24^{h}	51.41±3.70^{l}	51.47±1.24^{ijkl}
Allahabad safeda	68.74±4.75^{ijk}	65.73±1.91^{de}	64.65±1.74^{bcd}	58.07±0.67^{def}
L-49	72.86±1.43^{fghi}	76.65±1.78^{a}	63.52±1.92^{cde}	54.66±3.41^{fghijk}
Sweta	73.40±5.07^{efg}	70.60±1.91^{bc}	60.70±1.65^{fgh}	57.39±1.50^{defg}
Phillipines	69.73±1.17^{hijk}	71.67±1.78^{b}	65.34±1.40^{abc}	61.35±2.25^{bcd}
China	76.76±1.39^{bcde}	70.02±3.43^{bc}	66.90±1.51^{ab}	63.64±2.77^{ab}
Kohir safeda	78.56±0.57^{ab}	68.52±0.81^{bcd}	63.67±1.26^{cde}	64.29±4.95^{ab}
Arka amulya	75.19±1.91^{bcdef}	75.44±3.71^{a}	67.54±1.62^{a}	62.41±6.42^{bc}
Hissar surkha	81.52±1.45^{a}	70.66±3.12^{bc}	66.36±0.99^{ab}	66.57±3.30^{a}
Safed jam	76.53±2.53^{bcde}	65.98±1.95^{de}	59.07±0.63^{gh}	53.25±2.00^{ghijkl}
Hissar safeda	71.83±1.94^{fghijk}	61.62±1.58^{fg}	54.25±1.00^{ijk}	49.58±1.53^{l}
Arka mridula	68.31±1.05^{k}	59.43±1.07^{g}	52.17±1.01^{kl}	50.95±0.51^{jkl}
Arka kiran	71.99±1.59^{fghij}	63.95±2.52^{ef}	55.62±1.18^{i}	48.92±0.58^{l}
Kohir round	75.35±1.05^{bcdef}	68.62±1.52^{bcd}	61.25±1.00^{efgh}	55.25±1.00^{efghij}
Kohir red	77.32±0.95^{bcd}	61.58±1.53^{fg}	52.92±1.53^{jkl}	50.66±0.51^{kl}
SRD-1	78.65±0.6^{1ab}	63.95±1.47^{ef}	55.58±1.15^{i}	51.94±0.60^{ijkl}
Kohir long	78.50±1.40^{abc}	70.57±0.59^{bc}	64.58±0.58^{bcd}	61.25±1.00^{bcd}
Kafri	74.29±0.94^{defg}	67.28±1.05^{cde}	62.58±0.57^{def}	59.25±1.00^{cde}
Md. khaja	68.38±1.03^{k}	58.58±0.58^{g}	51.40±1.04^{i}	50.95±0.61^{jkl}
Baruipur local	71.25±1.00^{ghijk}	64.25±1.00^{ef}	61.58±1.5^{efg}	56.28±1.67^{efgh}
Dudh khaja	69.32±0.94^{ijk}	58.68±0.58^{g}	55.28±0.9^{ij}	50.57±0.60^{kl}
Khaja	74.90±0.57^{cdefg}	69.40±1.03^{bcd}	62.25±1.01^{def}	55.52±1.11^{efghi}
Taiwan red	72.25±1.00^{fghij}	64.62±0.55^{ef}	58.76±0.52^{h}	52.18±0.88^{hijkl}

Different small letters on the columns showed significance differ by Duncan test (P=0.05)

[13]. The evidence for the utilization of exogenous sugars by pollen is both direct and indirect. Pollen grains of *Hippeastrum aulicum* produce 17-22 mm long tubes when grown in 1% sucrose, and only 7-8 mm long tubes in 0.2545% sugar [14]. Pollen grains of *Crofalaria juncea, and Dolichos lablab* [15] also produce long tubes in sucrose solutions of suitable concentrations. In cherry, 10% sucrose concentration gave the highest germination percentage and increasing concentration of sucrose affected negatively the germination rate of the pollen [16]. The appearance of starch was observed in pollen grains and tubes of several species of gymnosperms and angiosperms, when these were incubated in sucrose solutions [17,18,19,20], while pollen tubes of *Typha lutifolia* growing in sucrose gradually accumulated lipid droplets [21]. The length of the pollen tubes grows well for the medium containing sucrose (5%) solution in date palm [22]. Pollen tube growth varies with genotypes. Most pollen grains will germinate when placed in calcium, boron and an osmoticant solution. The modified concentration of solution must be performed in vitro germination among different species and even among cultivars of the same species, although it provides a controlled in vitro condition for pollen germination [23]. Other researchers like Rodrigo et al. [24], also observed that pollen germination to the high in 10% sucrose solution in feijoa guava.

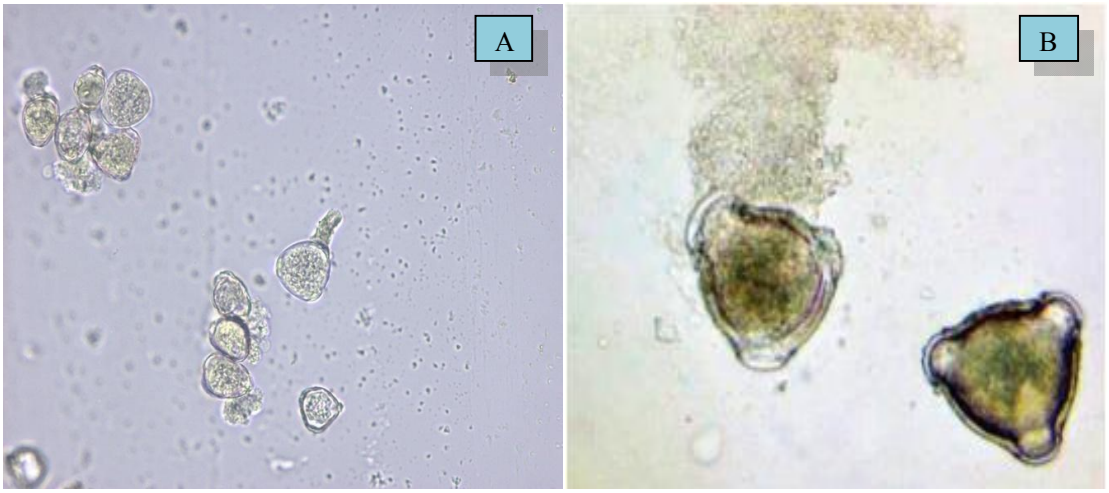
The stimulating effect of boron on pollen germination and pollen tube growth was discovered by Schmucker [25]. He observed that Nymphaea species pollen hardly germinated in glucose, but satisfactory germination could be obtained by supplementing the sugar medium with stigmatic extract, which was shown to contain appreciable quantities of boron. The pronounced effect of boron on the germination and growth of pollen grain has been extensively investigated and discussed by several earlier workers. Schmucker [26] reported that boron regulates the hydration of colloids, is associated

with polyhydroxy compounds of the pollen membrane and is involved in the synthesis of pectic substances for the tube wall. Several other workers have also come to similar conclusions [27]. The excessive bursting of pollen grains and pollen tubes so often encountered in the absence of boron from the nutrient media may be due to a close negative correlation between tissue hydration and the supply of boron, and retarded deposition of new wall materials in the growing tip of the pollen tube.

Table 2. Effect of different concentration of sucrose solution on pollen tube length of different guava varieties

Varieties	10%	15%	20%	25%
Pant prabhat	36.16±2.02^{def}	35.71±2.20^{bc}	30.29±0.94^{b}	26.39±0.96^{bcdef}
Lalit	33.75±1.66^{fgh}	32.37±2.66^{def}	28.37±1.06^{bcde}	26.71±1.30^{bcd}
Allahabad safeda	40.28±0.90^{ab}	38.55±0.70^{ab}	34.01±1.65^{a}	30.58±1.30^{a}
L-49	41.41±1.38^{a}	39.99±1.18^{ab}	35.49±1.09^{a}	31.49±0.48^{a}
Sweta	38.89±0.41^{abc}	29.62±1.52^{fghi}	27.92±0.75^{bcdefg}	24.99±1.46^{defgh}
Phillipines	35.02±1.77^{efg}	28.25±1.00^{hij}	25.82±1.30^{fghi}	23.70±2.22^{fgh}
China	38.11±1.55^{bcd}	31.59±1.53^{efg}	30.09±1.32^{bc}	28.16±0.72^{b}
Kohir safeda	39.54±1.53^{abc}	29.58±1.53^{fghi}	27.91±1.23^{bcdefg}	25.47±0.96^{cdef}
Arka amulya	36.55±0.99^{cde}	31.29±1.0^{6efgh}	27.86±1.40^{bcdefg}	25.12±1.24^{cdefg}
Hissar surkha	40.40±0.60^{abc}	35.32±1.05^{cd}	29.78±2.00^{bcd}	25.89±1.14^{bcdef}
Safed jam	34.51±1.00^{efgh}	28.38±1.20^{ghij}	28.20±.041^{bcdef}	25.44±0.73^{cdef}
Hissar safeda	39.78±0.94^{abc}	32.97±1.56^{cde}	28.61±0.95^{bcde}	24.66±1.58^{defgh}
Arka mridula	30.58±1.15^{ij}	26.62±4.05^{ij}	25.92±2.041^{fgh}	24.62±1.56^{defgh}
Arka kiran	28.62±1.18j^{k}	25.25±1.00^{j}	23.59±1.22^{ij}	19.84±1.02^{i}
Kohir round	27.29±0.95^{k}	25.74±1.35^{j}	23.31±0.97^{j}	22.89±0.44^{gh}
Kohir red	32.58±3.21^{ghi}	26.62±0.55^{ij}	25.52±0.67^{ghij}	24.59±0.67^{defgh}
SRD-1	38.27±1.05^{bcd}	31.67±2.47^{efg}	27.75±0.85^{cdefg}	26.63±1.50^{bcde}
Kohir long	33.26±1.73^{gh}	30.40±0.84^{efgh}	28.83±0.78^{bcd}	20.24±1.23^{i}
Kafri	34.28±1.95^{efgh}	33.58±30.6^{cde}	28.87±1.17^{bcd}	27.37±0.94^{bc}
Md. khaja	27.28±1.05^{k}	25.92±1.53^{j}	24.65±1.00^{hij}	24.25±0.61^{efgh}
Baruipur local	33.03±1.55^{ghi}	31.35±1.15^{efgh}	28.58±0.64^{bcde}	27.89±1.73^{b}
Dudh khaja	38.29±1.00^{bcd}	32.67±1.36^{cdef}	27.58±1.22^{defg}	26.05±1.16^{bcdef}
Khaja	32.08±1.64^{hi}	29.45±1.21^{fghi}	26.17±1.54^{efgh}	22.74±1.73^{h}
Taiwan red	35.32±1.05^{efg}	33.16±1.3^{2cde}	28.06±1.75^{bcdef}	20.14±0.56^{i}

Different small letters on the columns showed significance differ by Duncan test (P=0.05)



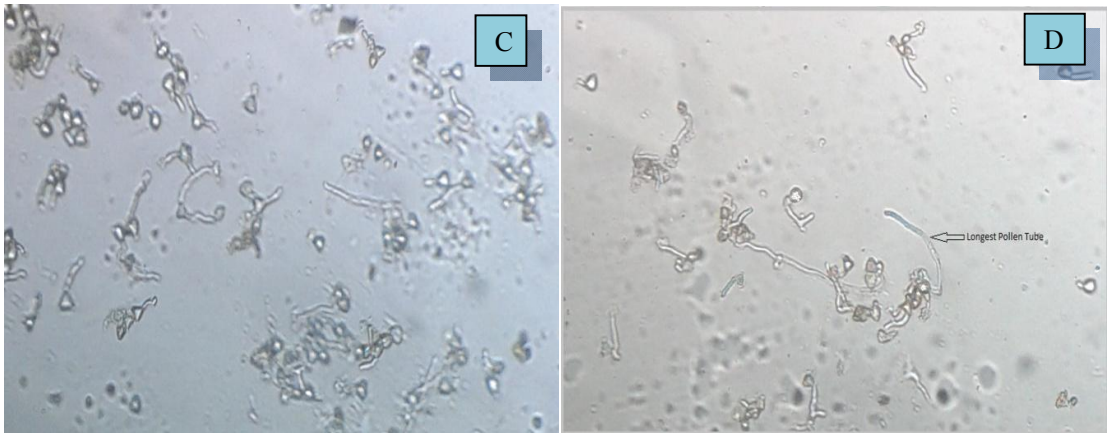


Fig 1. A (40x magnification) & B (100x magnification). At initial satge of pollen germination. C. The maximum pollen germination. D. The mature growth stage of pollen and the arrow indicate highest pollen tube length (10× magnification) under boric acid solution

Table 3. Effect of different concentration of boric solution on pollen germination of different guava varieties

Varieties	0.2	0.4	0.6	0.8
Pant prabhat	71.33±1.53^{cde}	74.00±1.00^{bc}	79.00±1.00^{bcd}	82.00±1.00^{c}
Lalit	70.67±1.53^{de}	73.00±1.00^{cde}	80.00±1.00^{bc}	86.00±1.00^{a}
Allahabad safeda	72.00±1.00^{bcd}	74.67±1.53^{bc}	80.07±1.90^{bc}	84.33±0.58^{b}
L-49	75.67±0.58^{a}	77.00±1.00^{a}	83.08±1.01^{a}	87.00±1.00^{a}
Sweta	68.00±1.00^{ghi}	70.33±0.58^{ghi}	76.67±1.53^{efg}	79.33±1.15^{efghi}
Phillipines	70.00±1.00^{defg}	71.00±1.00^{fgh}	76.00±2.00^{fgh}	80.67±0.58^{cdef}
China	66.67±1.53^{ij}	69.33±0.58^{hij}	73.84±1.77^{hij}	78.67±0.58^{ghij}
Kohir safeda	71.33±0.58^{cde}	73.00±1.00^{cde}	78.33±0.58^{bcde}	80.00±1.00^{defgh}
Arka amulya	72.00±1.00^{bcd}	73.67±0.58^{cd}	78.00±1.00^{cdef}	81.33±0.58^{cd}
Hissar surkha	67.00±1.00^{ij}	68.67±1.15^{ij}	72.67±1.53^{ij}	77.67±0.58^{ij}
Safed jam	69.23±0.58^{efgh}	71.33±0.58^{efg}	77.75±.1.56^{cdefg}	79.33±0.58^{efghi}
Hissar safeda	69.33±1.00^{efgh}	72.78±0.70^{cdef}	77.42±0.73^{defg}	79.07±1.10^{fghi}
Arka mridula	71.17±1.00^{bcd}	73.33±0.58^{ccd}	73.00±1.00^{ij}	77.11±0.84^{jk}
Arka kiran	73.67±1.08^{b}	74.67±0.58^{bc}	78.67±0.58^{bcde}	80.47±0.64^{cdefg}
Kohir round	67.67±1.53^{hi}	71.42±0.52^{defg}	75.67±0.58^{gh}	78.13±1.01^{ij}
Kohir red	70.33±1.04^{def}	74.33±0.58^{bc}	80.33±0.58^{b}	80.87±0.76^{cdef}
SRD-1	72.82±1.53^{bc}	75.33±0.58^{ab}	78.67±0.58^{bcde}	81.10±1.01^{cde}
Kohir long	67.00±1.53^{ij}	71.00±1.00^{fgh}	74.00±1.00^{hi}	78.48±0.67^{hij}
Kafri	68.33±0.32^{fghi}	69.33±1.53^{hij}	71.67±1.53^{j}	75.73±1.55^{k}
Md. khaja	70.33±1.00^{df}	74.00±2.00^{bc}	79.33±1.53^{bcd}	82.03±1.27^{c}
Baruipur local	72.88±1.15^{bc}	71.67±1.15^{defg}	73.84±1.77^{hij}	76.83±0.57^{jk}
Dudh khaja	69.67±0.58^{efgh}	71.67±1.15^{defg}	76.00±1.00^{fgh}	78.40±1.06^{hij}
Khaja	68.00±1.00^{ghi}	70.00±1.00^{ghi}	72.67±0.585^{ij}	77.52±1049^{ij}
Taiwan red	65.33±0.58^{j}	67.67±1.53^{j}	72.33±0.58^{ij}	76.91±1.50^{jk}

Different small letters on the columns showed significance differ by Duncan test (P=0.05)

The effects of boric acid on the germination and growth of pollen were reported [28,29]. Boron may affect the ductility of cell walls by changing the polysaccharide reticular formation of these walls. It can also promote pollen germination and help the pollen tube rapidly enter the ovary. Thus, boron is favourable for fertilization and seed formation. Anther and filament atrophy occur in boron deficient conditions, a phenomenon that causes difficulties in the pollen tube formation, thereby hindering fertilization. The pollen germination and growth can be considerably improved through the addition of boric acid an appropriate concentrations. *In vitro*

Table 4. Effect of different concentration of boric acid solution on pollen tube length of different guava varieties

Varieties	0.2	0.4	0.6	0.8
Pant prabhat	23.25±1.00^{bcd}	23.92±2.08^{bcd}	30.92±1.15^{bcd}	35.92±1.53^{fg}
Lalit	24.66±1.15^{abc}	26.25±1.00^{ab}	28.59±0.57^{a}	35.38±1.03^{fgh}
Allahabad safeda	25.55±1.74^{a}	25.59±1.15^{ab}	34.92±0.58^{ab}	41.58±0.58^{ab}
L-49	26.26±0.91^{a}	26.74±0.47^{ab}	36.29±1.00^{a}	42.25±1.02^{a}
Sweta	25.09±1.65^{ab}	25.25±1.00^{ab}	31.51±.40^{ab}	38.92±0.58^{de}
Phillipines	21.92±1.53^{def}	21.17±1.00^{fg}	32.72±1.86^{fg}	35.92±0.58^{fg}
China	22.92±0.70^{cde}	22.98±0.45^{cdef}	31.59±1.52^{cdef}	39.58±0.58^{cd}
Kohir safeda	20.71±0.84^{fgh}	21.09±0.74^{fg}	33.92±1.53^{fg}	40.38±0.23^{bc}
Arka amulya	18.46±0.6^{ij}	23.88±0.55^{bcd}	30.95±1.47^{bcd}	38.12±0.76^{e}
Hissar surkha	19.68±0.9^{ghi}	25.25±1.00^{ab}	34.26±1.01^{ab}	40.88±0.75^{bc}
Safed jam	18.58±0.63^{ij}	24.38±1.20^{bc}	32.68±1.51^{bc}	36.22±0.95^{f}
Hissar safeda	19.97±0.39^{fghi}	24.25±1.00^{bc}	35.34±1.1^{bc}	40.82±0.40^{bc}
Arka mridula	20.64±1.06^{fgh}	23.82±0.69^{bcde}	26.92±0.58^{cde}	34.65±.087^{ghi}
Arka kiran	21.11±0.67^{efg}	21.94±1.49^{efg}	26.25±1.00^{efg}	32.25±1.00^{j}
Kohir round	19.00±1.74^{hij}	20.58±0.59^{g}	24.92±1.15^{fg}	30.31±0.18^{k}
Kohir red	17.16±1.08^{ikl}	18.42±0.21^{h}	24.01±0.42^{h}	34.39±1.20^{hi}
SRD-1	15.30±1.06^{l}	23.28±0.58^{cdef}	31.69±0.90^{cde}	38.75±0.52^{de}
Kohir long	15.32±0.84^{l}	23.92±1.00^{bcd}	32.39±1.03^{bcd}	36.18±0.47^{f}
Kafri	18.36±0.95^{ij}	22.25±1.53^{efg}	33.62±0.55^{defg}	38.12±0.16^{e}
Md. khaja	16.28±1.13^{kl}	21.92±1.16^{efg}	26.32±0.95^{efg}	30.65±0.76^{k}
Baruipur local	15.96±0.55^{l}	21.91±1.16^{efg}	27.72±0.50^{efg}	34.59±0.58^{ghi}
Dudh khaja	18.14±0.55^{ijk}	22.54±0.50^{cdef}	31.28±1.00^{cdef}	38.50±1.98^{de}
Khaja	16.98±0.68^{ikl}	18.05±0.79^{h}	24.25±1.01^{h}	33.95±0.51^{i}
Taiwan red	18.03±0.68^{ijk}	21.17±1.12^{fg}	31.05±0.72^{fg}	36.62±0.55^{f}

Different small letters on the columns showed significance differ by Duncan test (P=0.05)

pollen germination would be hindered if boric acid is not added or if high concentrations of boric acid are added. Thus the main effect of boron may not necessarily be the translocation of carbohydrates into the pollen, but rather on the rate of their metabolism. The sugars translocation increased of observed in meristematic tissues in the presence of boron could thus be a consequence of increased cellular activity and growth, rather than the formation of sugar-borate complexes, which facilitate the translocation of sugars [30,31]. Cavusoglu A and Sulusoglu [32] found that the media prepared with sucrose and boric acid play a key role for in vitro germination of pollen in medlar. Gupta et al. [33] also found the similar result in Longan crop.

5. CONCLUSION

With this current investigation, it has been concluded that ideal sucrose and boron concentrations for most of the guava cultivars for in-vitro pollen germination studies could be 10% and 0.8 g/l respectively.

ACKNOWLEDGEMENT

The authors are grateful to Bidhan Chandra Krishi Viswavidyalaya, India for providing necessary technical support during the course of the investigation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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