

## IN-VITRO STUDIES ON VIABILITY AND GERMINATION OF POLLEN IN VARIOUS CITRUS SPECIES

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Various media containing 0, 5, 10, 15, 20 and 25% glucose + 0.07% bacto agar were tested and acetocarmine test was carried out to evaluate the pollen viability of different species of citrus, i.e., *Citrus sinensis*, *C. reticulata*, *C. limittoides* and *C. paradisi*. In-vitro pollen viability was highest in the acetocarmine test (87.3–96.3%) as compared with pollen germination in different glucose media, i.e., up to 59%. The maximum germination of 59% and longest pollen tube (59.2 microns) in sweet lime were found in the medium having 15% glucose + 0.07% bacto agar failed to induce germination of pollen in all cases. Variety wise, kinnow, Feutrell's Early, grape fruit and pine apple got their place in descending order after sweet lime (55, 43, 38 and 30% respectively). The pollen size was highest in grape fruit (5.44 microns) and smallest in kinnow (3.2 microns).

**Key words:** In vitro, Pollen, Glucose, Bacto agar, Citrus species.

### Introduction

Citrus is the prized fruit of orient and held in great esteem by all (rich or poor), since time immemorial. It is major fruit crop of Pakistan, grown on an area of 1,58,800 hectares with a total production of 14,11,300 tonnes [1]. Many workers have noted that morphology of pollen has direct influence on the productivity and quality of the fruit. Pollen germination and growth of the pollen tube are greatly influenced by different phenolic compounds [2]. It has long been observed that some monoecious plants in citrus bear a small amount of pollen and in some cases a considerably large percentage of pollen has been found nonviable. This phenomenon, consequently, has resulted in poor yield and low profit.

Acetocarmine test showed pollen from 78.40% in Florida Rough and 91.66% in Nepali Oblong among all other cultivars, studies by Venkateswarlu and Lavania [4]. Meanwhile, nine cultivars studied, differed in frequency of callus induction from anthers, cultured on improved MS medium.

Wuyi orange (35.84%) and Szpusiang (*C. sinensis* x *C. reticulata*) (29.14%) gave the highest induction rate [5]. For optimum germination of different lemon and citrus pollen, Kasha [3] observed best results with 15% sugar solution at 28°. The study of Benoit [6] revealed 20% germination of pollen of citrus in 10% sugar media. Ahmed and Shah [7] found favourable results of pollen viability in sweet lime in media having 15% glucose and sucrose with the addition of bacto agar that stimulates the growth of pollen tube. Jicinska [8] compared the viability of Roso-pollen using 2,3,5-triphenyl tetrazolium chloride with actual germination on sucrose agar medium and found both methods gave similar results. Rao and Ong [9]

found that bursting of pollen grain was more common when the sucrose concentration was low. Pollen germination in *Rosa hugonis* was studied at various temperatures and in different agar-sucrose media by Koncalova [10]. The best results were obtained at 28° with 30, 35 and 40% sucrose in 1.5% agar and up to 90% germination was achieved. Clapham [11] while working on barley anthers observed better germination on media with 6-12% sucrose. Similarly, Khan [12] found successful germination of six cultivars of *Rosa* pollen on 1% agar medium, containing 30% sucrose.

There is very little information available for the improvement of different horticultural crops on the viability, on the germination of pollen and on pollen tube elongation in our local conditions. The purpose of the present study was to determine accurate methods for testing pollen viability and germination of various citrus species.

### Materials and Methods

The experiment was conducted in the laboratory of Department of Horticulture, University of Agriculture, Faisalabad. Pollen was obtained from flowers of uniform trees of *Citrus reticulata* (kinnow and Feutrell's Early), *C. sinensis* (pine apple), *C. paradisi* (grape fruit) and *C. limittoides* (sweet lime). Pollen germination was tested in different concentrations of glucose solution, viz., 5, 10, 15, 20, 25 and aqua with 0.07% bacto agar.

The petals of unopened but mature flowers were removed in the laboratory. By using the technique of one stroke of camel brush, the pollen were obtained and dispersed in sterilised petri dishes which contained media of various concentrations. The petri dishes were covered and placed at 17° for 12 hrs and then examined under a microscope. Germination of pollen was

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recorded as percentages and the length of the pollen tube was calculated in microns. The viability of pollen of all citrus species was also tested using acetocarmine and after staining, the pollen grains were examined under a microscope.

The data obtained was fed to an IBM AT compatible computer and by using M-STAT-C package the analysis of variance was applied. The means were compared by employing DMR test.

**Results and Discussion**

Viability of pollen, as affected by acetocarmine, was maximum in kinnow (96.3%) and minimum in grape fruit and pine apple, i.e. 87.33-88.84% (Table 1). The pollen, received from trees of sweet lime and Feutrell's Early showed good viability response, ranging from 91-93%. The percentages were slightly higher than Vankateswarlu and Lavania [4], who found pollen viability from 78.40-91.66% which might be attributed to the difference of species. The largest pollen grains in grape fruit and pine apple were measured 5.44 and 5.1 microns, respectively. The pollen size of Feutrell's Early and sweet lime was medium, i.e. from 4.32-4.16 microns while smallest pollen, having size of 3.2 microns, were observed in kinnow.

The media containing 15% glucose gave highest response (Table 2) and showed superiority over all other concentrations. Almost no germination of pollen of any species was observed in 25% glucose solution and aqua + bacto agar. The germination of pollen decreased with the change in glucose concentration (15%) on any side. There was apparent differ-

ence of pollen germination in the plants under test. The germination rate was highest at 15% glucose + 0.07% bacto agar in sweet lime and kinnow, 59 and 55%, respectively, followed by Feutrell's Early (43%), whereas the pollen of pine apple and grape fruit could hardly germinate from 30-38%. These results agree with the findings of Rajput and Haribabu [3]. Ahmed and Shah [7], who recommended 15% glucose media for maximum germination of pollen in citrus but contradicted the recommendations of Benott [6], who favoured the use of 10% sugar media. The difference in latter case could be attributed to the use of different media for testing pollen germination.

It can be seen that 15% concentration of glucose makes the bar highest but it shows fall trend for 0,20 and 25% of glucose solution (Fig.1). Similarly addition of bacto agar stimulated the growth of pollen tube [4]. The 15% glucose solution have proved most effective compared to other con-

TABLE 1. PERCENTAGE OF POLLEN VIABILITY THROUGH ACETOCARMINE TEST AND POLLEN SIZE OF DIFFERENT CITRUS SPECIES.

Sl. No.	Parameters	Pine apple	Kinnow	Sweet lime	Feutrell's Early	Grape fruit
1.	Viability(%)	88.8 d	96.3 a	93.0 b	91.0 c	87.3 e
2.	Pollen size (micron)	5.1 b	3.2 d	4.2 c	4.3 c	5.4 a

TABLE 2. GERMINATION PERCENTAGE OF POLLEN AND AVERAGE LENGTH OF POLLEN TUBE OF CITRUS SPECIES IN DIFFERENT CONCENTRATIONS OF GLUCOSE.

Sl. No.	Name of plant	Pollen germination (%) media concentrations					Pollen tube length (micron) media concentrations						
		0	5	10	15	20	25	0	5	10	15	20	25
1.	Pine apple	0e	17c	20b	30a	12d	0e	0e	30.8c	40.7b	47.3a	19.0d	0e
2.	Kinnow	0f	27c	39b	55a	17d	2e	0e	35.2c	39.2b	52.0a	17.0d	0e
3.	Sweet lime	1e	25c	41b	59a	19d	0f	0e	53.6b	53.1c	59.2a	25.1d	0e
4.	Feutrell's Early	0e	15c	29b	43a	11d	0e	0e	36.8b	43.2a	31.6c	21.0d	0e
5.	Grape fruit	1f	18c	27b	38a	13d	1.5e	0.25e	30.4c	40.0b	56.7a	27.0d	0f

Note: Means followed by the same letter in a row do not differ significantly at P<0.05 according to DMR test..

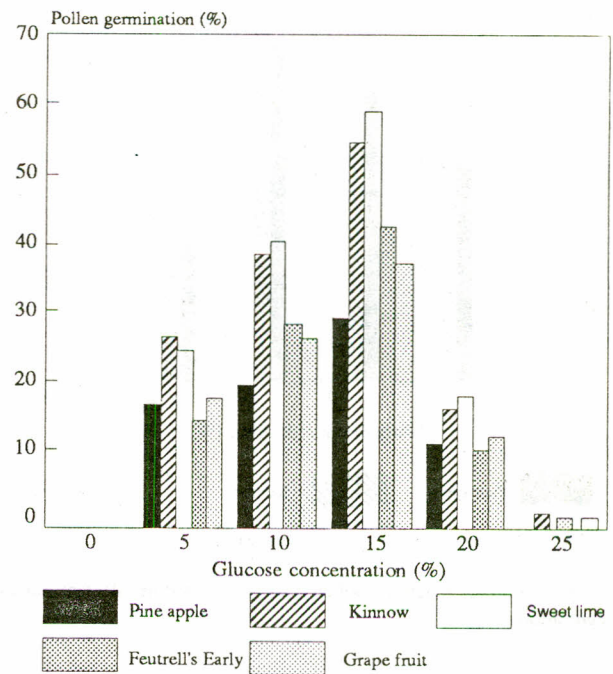


Fig. 1. Percentage of pollen germination in different concentrations of glucose.

centrations which resulted maximum length of pollen tube in all species except cultivar Feutrell's Early, measuring 31.6 microns, while its length was 43.2 microns, when germinated in the media having 10% glucose solution. Sweet lime excelled all other species with a pollen tube length of 59.2 microns, followed by grape fruit (56.7 microns). In general, pollen tube of sweet lime have shown excellent growth (53.6, 53.1 and 59.2 microns) and germination (25, 41 and 59%) in media having 5, 10 and 15% glucose, respectively (Fig. 2).

### Conclusion

The present study revealed the fact that acetocarmine

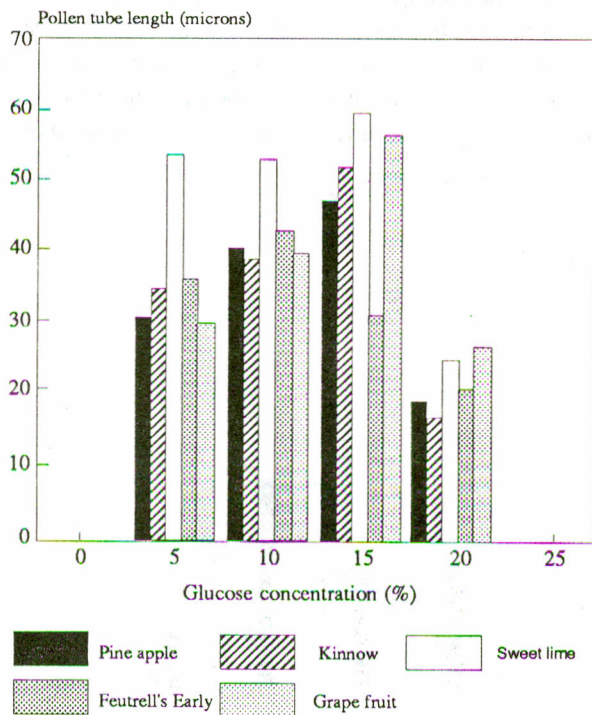


Fig. 2. Average length of pollen tube germinated in different glucose concentrations.

used for viability test of pollen in citrus was proved to be a better staining technique than glucose media. While the glucose media is a better test for determining the pollen germination. Pollen viability in kinnow was highest among all the species of citrus. Moreover, germination media with 15% glucose solution and 0.07% bacto agar was optimum for evaluating germination percentage or pollen tube length in sweet lime, grape fruit, kinnow, pine apple and Feutrell's Early. The media, containing 0 and 25% glucose showed no response in either case.

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