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GENETICS & PLANT BREEDING

Effects of Various Carbohydrates on the *in vitro* Pollen Germination of *Vinca rosea* and *Cucumis melo* var. *utilissimus*

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ABSTRACT

Pollen germination is a crucial stage of plant development that significantly depends on the presence of carbohydrates as a primary energy source. In this study, we analysed the differential effects of four sugars (Glucose, Sucrose, Galactose, Fructose) with varying concentrations on the pollen germination of *Vinca rosea* Linn. and *Cucumis melo* var. *utilissimus* Duth and Fuller using Brewbaker and Kwack medium as germination medium and hanging drop method after incubation of an hour. Sucrose and glucose aided the pollen germination but galactose and fructose significantly inhibited the pollen germination of *Vinca rosea*. In *Cucumis melo* var. *utilissimus*, all four types of sugars supported pollen germination. The study suggests that 15% sucrose, for *Vinca rosea*, and 12% galactose, for *Cucumis melo* var. *utilissimus*, depict the highest pollen germination percentage when used in the pollen germination medium.

HIGHLIGHTS

- Among all the carbohydrates with various concentrations used, 15% sucrose in *Vinca rosea* and 12% galactose in *Cucumis melo* found to have significant ameliorative effect on *in vitro* pollen germination.

Keywords: Brewbaker and Kwack medium,Carbohydrates, *Cucumis melo* var. *utilissimus*, *In vitro* pollen germination, *Vinca rosea*

The development of pollen grains on stigma or in the *in vitro* conditions is a time-bound and high energy requiring process (Selinski and Scheibe 2014). The recognition of compatible pollen and the growth of the pollen tube mostly depends upon the receptivity of stigma and other recognition factors as well as the relative humidity of the environment which improves the adhesion of pollen in stigma surface. The further growth of the pollen tube, till it reaches the embryo sac, is facilitated by the environment of the stylar canal (Lora *et al.* 2016). Carbohydrates act both as an osmotic regulator and substrate for primary energy source, thus ameliorating the pollen germination and the pollen tube growth in stylar canal (Reinders 2016).

Most of the studies conducted on *Vinca rosea* are based on its medicinal and therapeutic properties. In Ayurveda, extracts of both roots and shoots of this plant are used to treat several diseases *viz.* alkaloids like vinblastine and vincristine are used for treating leukaemia and Hodgkin's lymphoma (Moudi *et al.* 2013). *Cucumis melo* var. *utilissimus* is commonly called American cucumber or "kakri" in India. Extract from this fruit used for suppressing cough, reducing fever and act as digestive aid (Fahamiya *et al.* 2016).

Despite the medicinal importance of these two plants and the significant role of carbohydrates in

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pollen germination, no work has yet been reported on the effect of various carbohydrate sources onto the pollen germination of *Vinca rosea* and *Cucumis melo* var. *utilissimus*. The present study aims to investigate the effects of various carbohydrates (Glucose, Sucrose, Fructose, Galactose) with varying concentrations on the *in vitro* pollen germination of *Vinca rosea* and *Cucumis melo* var. *utilissimus* by using a pollen germination medium and calculating pollen germination percentage.

MATERIALS AND METHODS

Pollen collection: Flowers of *Vinca rosea* and *Cucumis melo* var. *utilissimus*, at anthesis, were collected from the botanical garden of Miranda House, University of Delhi during daytime. Pollen grains were carefully collected on separate clean petri dishes by tapping and brushing the anthers of each flower.

Preparation of Pollen Germination Medium: Standard solution of Brewbaker and Kwack medium (Brewbaker and Kwack 1963) was prepared using 0.01g of boric acid, 0.01g of potassium nitrate, 0.02g of magnesium sulphate heptahydrate and 0.03g of calcium nitrate. The medium was supplemented by using four different sugars: sucrose, glucose, fructose and galactose, separately with varying concentrations of 5%, 10%, 12% and 15%. Pollen grains kept in distilled water served as control.

Experimental Set-up: Hanging drop method (Shivanna and Rangaswamy 1992) with slight modifications was used for performing the *in vitro* pollen germination. A small drop of pollen germination medium was taken on a glass slide and the pollen grains were transferred onto it with the aid of a nylon brush. The glass slide was inverted and placed on a glass cavity block. The edges were sealed with the help of petroleum jelly and incubated in light with temperature, set at 30°C.

Observation of pollen germination: Observations for pollen germination were taken four times under light microscope, with 15 mins intervals. Three microscopic field views per replicate, that contained a minimum of 30 pollen grains, was observed. Pollen grains were considered as germinated when the length of the pollen tube was greater than the diameter of the pollen grain. The total number of pollen grains that germinated was calculated and the percentage germination was calculated by dividing the number of pollen grains germinated by the total number of pollen grains.

Statistical analysis: Three biological replicates for the experiments were performed using the above methodology to determine mean and standard deviation for pollen germination percentage. One-Way Analysis of Variance (ANOVA) was used to determine the significant differences in pollen germination percentage among different sugar concentrations.

RESULTS AND DISCUSSION

Pollen germination in *Vinca rosea*: The pollen germination percentage for *Vinca rosea* under the effect of various sugars with varying concentrations was obtained as shown in Table 1. As presented, pollen grains germinated in every media after a certain time interval except in the fructose supplemented one (Table 1).

Importantly, at 5% concentration, inhibition in germination observed in *Vinca rosea*, despite different carbohydrates types (Supplementary Fig. 1). Lower concentrations of both glucose and galactose had an inhibitory effect on the pollen germination of *Vinca rosea*. Fructose, in any concentration, inhibited pollen germination (Supplementary Fig. 2).

Pollen germination in *Cucumis melo* var. *utilissimus*: The pollen germination percentage for *Cucumis melo* var. *utilissimus* under the effect of various sugars with varying concentrations was obtained as shown in Table 2.

As depicted, every germination medium irrespective of the type of sugar and its concentration used, was successful in initiating pollen germination (Table 2). Pollen grains showed the highest germination percentage (65.33%) when 12% galactose was used in the germination medium(Supplementary fig.3) and the lowest germination percentage (17.71%) was obtained when 5% galactose was used in the germination medium after an incubation of an hour (Supplementary Fig. 4).

The results of this study allow us to compare the effects of different sugar concentrations on pollen germination in *Vinca rosea* and *Cucumis melo*, allowing us to select an appropriate medium to ameliorate pollen germination rates.

Different carbohydrates have been reported to have diverse effects on pollen germination in various

Table 1: Effect of various sugars with varying concentration on the pollen germination of *Vinca rosea* Values (in %) are represented as Mean ± S.D. Within columns values of each parameter with the same superscript are not significantly different at (P< 0.05) Duncan's multiple range test (DMRT)

Time	Sucrose Concentration				
(min)	5%	10%	12%	15%	Control
15	0 ± 0a	0 ± 0a	0 ± 0a	25.7936 ± 0.8361a	1.6269 ± 1.4102a
30	0 ± 0a	2.0634 ± 1.8028b	3.4555 ± 1.2370b	31.7272 ± 0.5662b	1.7105 ± 1.4827a
45	0 ± 0a	9.1547 ± 0.7364c	15.1532 ± 0.7780c	42.1530 ± 0.6243c	3.5776 ± 1.2426b
60	0 ± 0a	12.5216 ± 1.1040d	23.2478 ± 0.4986d	67.996 ± 1.1547d	4.4014 ± 1.2714c
Time	**Glucose Concentration**				
(min)	**5%**	**10%**	**12%**	**15%**	**Control**
15	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	1.6269 ± 1.4102a
30	0 ± 0a	0 ± 0a	0 ± 0a	7.4853 ± 0.7090b	1.7105 ± 1.4827a
45	0 ± 0a	0 ± 0a	0 ± 0a	10.4323 ± 0.9278c	3.5776 ± 1.2426b
60	0 ± 0a	0 ± 0a	0 ± 0a	23.4508 ± 0.8336d	4.4014 ± 1.2714c
Time	**Galactose Concentration**				
(min)	**5%**	**10%**	**12%**	**15%**	**Control**
15	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	1.6269 ± 1.4102a
30	0 ± 0a	0 ± 0a	3.0786 ± 0.2806b	0 ± 0a	1.7105 ± 1.4827a
45	0 ± 0a	0 ± 0a	5.6903 ± 0.4949c	0 ± 0a	3.5776 ± 1.2426b
60	0 ± 0a	3.0693 ± 0.1905b	12.8835 ± 1.2903d	0 ± 0a	4.4014 ± 1.2714b
Time	**Fructose Concentration**				
(min)	**5%**	**10%**	**12%**	**15%**	**Control**
15	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	1.6269 ± 1.4102a
30	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	1.7105 ± 1.4827a
45	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	3.5776 ± 1.2426b
60	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	4.4014 ± 1.2714c

Table 2: Effect of various sugars with varying concentration on the pollen germination of *Cucumis melo* var. *utilissimus*. Values (in %) are represented as Mean ± S.D. Within columns values of each parameter with the same superscript are not significantly different at (P<0.05) Duncan's multiple range test (DMRT)

Time (min)	Sucrose Concentration				
	5%	10%	12%	15%	Control
15	10.932 ± 0.6062a	7.9194 ± 0.5337a	16.4781 ± 0.7762a	12.6515 ± 0.6200a	2.7329 ± 0.1515a
30	25.0221 ± 1.1519b	12.4689 ± 0.9166b	25.4757 ± 1.1330b	20.3921 ± 0.3396b	5.6198 ± 0.3202b
45	33.9432 ± 0.5295c	23.6438 ± 0.7673c	36.8434 ± 0.5884c	29.9636 ± 0.8164c	10.0637 ± 1.1559c
60	36.6987 ± 0.5306c	28.0373 ± 0.8773d	53.4214 ± 0.9768d	34.2811 ± 0.9045d	12.9308 ± 0.7233d
Time (min)	**Glucose Concentration**				
	5%	**10%**	**12%**	**15%**	**Control**
15	15.6545 ± 0.2337a	18.9507 ± 0.5462a	9.0535 ± 0.8325a	2.7834 ± 0.1548a	2.7329 ± 0.1515a
30	26.6025 ± 1.0612b	30.2455 ± 0.7299b	14.2483 ± 0.7924b	9.5750 ± 1.0785b	5.6198 ± 0.3202b
45	32.4059 ± 0.0459c	46.1841 ± 1.0849c	21.1655 ± 1.0173c	18.6612 ± 0.695c	10.0637 ± 1.1559c
60	41.7055 ± 0.7001d	56.3911 ± 1.1185d	31.9536 ± 0.6112d	25.7282 ± 0.7353d	12.9308 ± 0.7233d
Time (min)	**Galactose Concentration**				
	5%	**10%**	**12%**	**15%**	**Control**
15	5.5814 ± 0.4667a	13.7774 ± 0.5721a	28.828 ± 0.6515a	18.4244 ± 0.9229a	2.7329 ± 0.1515a
30	8.1438 ± 0.6624b	21.5488 ± 0.5831b	41.6864 ± 1.0166b	24.5261 ± 1.1647b	5.6198 ± 0.3202b
45	12.6119 ± 0.4348c	40.2146 ± 0.7107c	53.5372 ± 0.8779c	37.7841 ± 0.8211c	10.0637 ± 1.1559c
60	17.7137 ± 0.6764d	54.8933 ± 0.6366d	65.3399 ± 0.7144d	48.9583 ± 1.8042d	12.9308 ± 0.7233d
Time (min)	**Fructose Concentration**				
	5%	**10%**	**12%**	**15%**	**Control**
15	11.6799 ± 0.7277a	19.2885 ± 0.8009a	5.8207 ± 0.6638a	9.6757 ± 0.8514a	2.7329 ± 0.1515a
30	18.0022 ± 1.1831b	33.3505 ± 0.9267b	10.6516 ± 0.7224b	12.7688 ± 0.2327b	5.6198 ± 0.3202b
45	22.87 ± 0.5724c	46.9229 ± 0.5268c	16.0279 ± 1.0168c	18.1152 ± 0.5700c	10.0637 ± 1.1559c
60	35.7376 ± 0.6145d	52.2535 ± 0.7847d	25.9454 ± 0.3239d	25.9726 ± 0.4390d	12.9308 ± 0.7233d

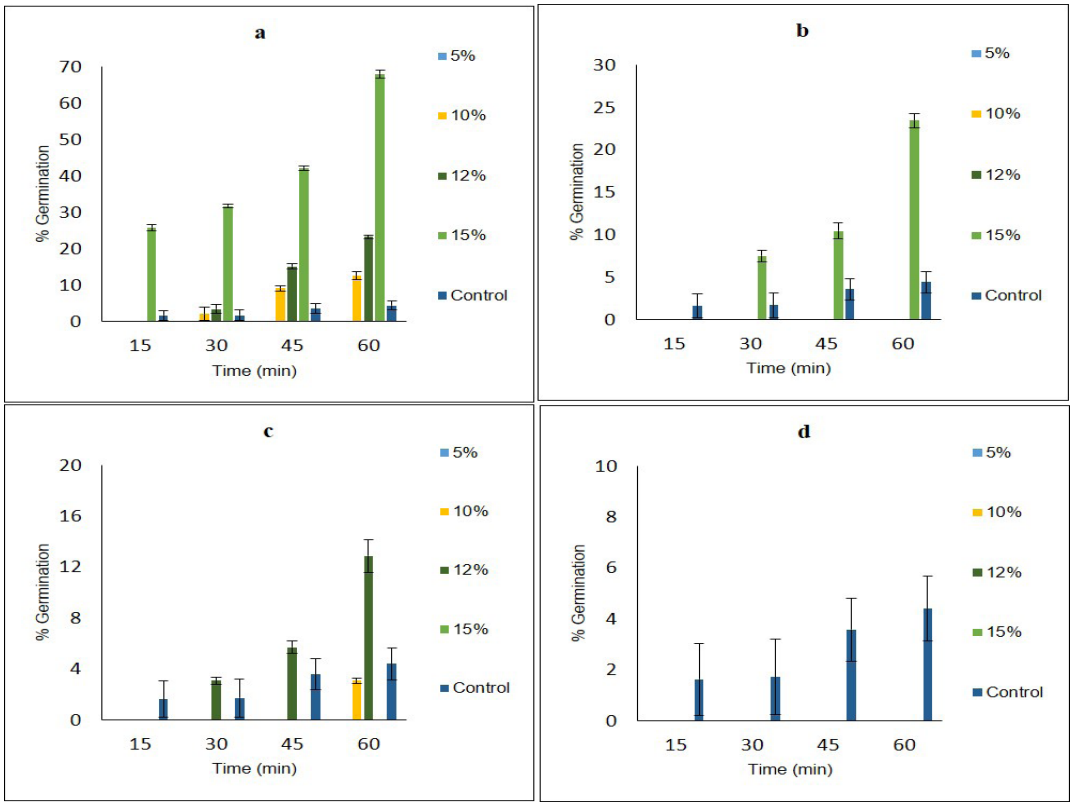

Fig. 1: Effect of varying concentrations of **(a)** Sucrose, **(b)** Glucose, **(c)** Galactose and **(d)** Fructose on the *in vitro* pollen germination of *Vinca rosea*. Data are shown by Mean ± S.D.

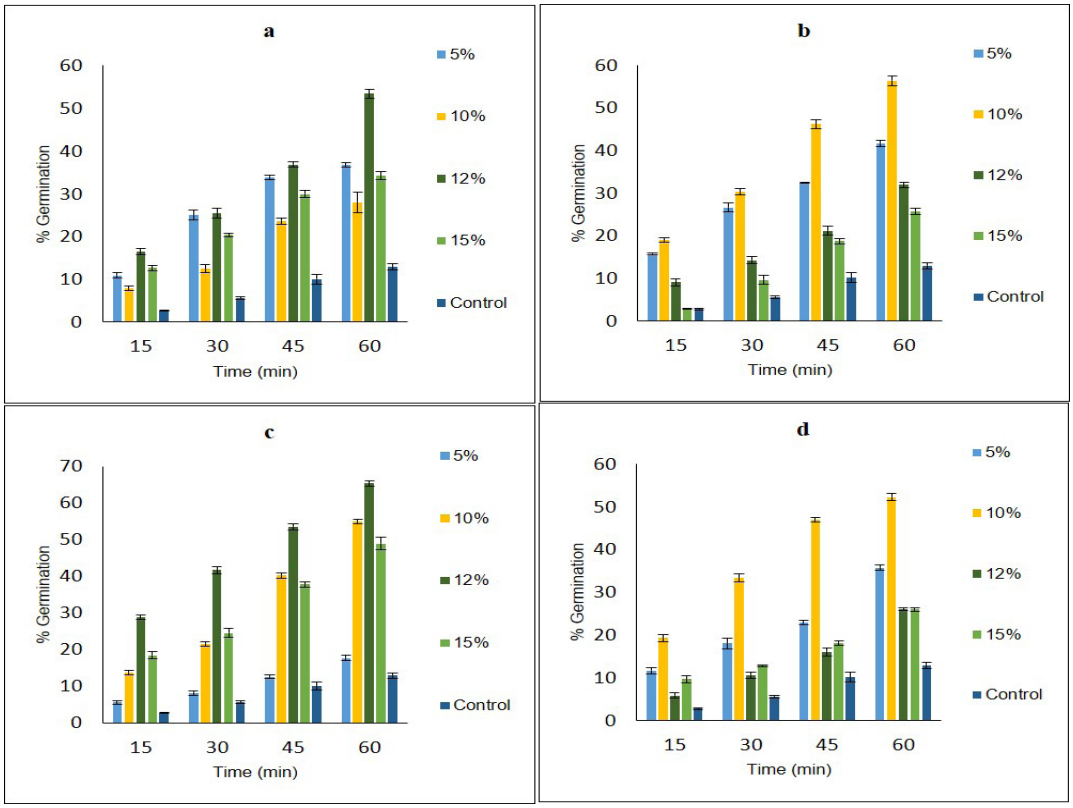

Fig. 2: Effect of varying concentrations of **(a)** Sucrose, **(b)** Glucose, **(c)** Galactose and **(d)** Fructose on the *in vitro* pollen germination of *Cucumis melo var. utilissimus*. Data are shown by Mean ± S.D

plant species (Ismail 2014). Numerous studies have shown that sucrose exhibits stimulatory action on the growth of pollen grains (Patel 2017). Higher concentration of sucrose (Fragallah *et al.* 2019) and glucose (Waniale *et al.* 2021) promotes the in vitro pollen germination of *Vinca rosea*. Although, in our experiments, the lower concentrations of glucose inhibit the pollen germination which may be due to the involvement of hexokinase mediated pathway of inhibiting sucrose mediated pollen germination (Hirsche *et al.* 2017). Sucrose promotes pollen germination in both *Vinca rosea* and *Cucumis melo* var. *utilissimus* may be due to its translocation to non-photosynthetic tissue, such as flower, and also excreted from the stigma and the stylar canal to hydrate the pollen and support the pollen tube growth (Reinders 2016). Glucose supports the pollen germination minimally in *Vinca rosea* as well as in *Cucumis melo* var. *utilissimus*. This observation may be supported by the reports of Rottmann *et al.* (2018) that glucose inhibits *Arabidopsis* pollen grain elongation and maturationby hexokinase mediated pathway via inhibiting the SUGAR TRANSPORT PROTEINs (STPs).

Fructose promotes the germination in *Cucumis melo* var. *utilissimus* as it already reported that the presence of fructose in the stigmatic fluid of plants promotes pollen germination (Patel 2015). But surprisingly it inhibits the pollen germination in *Vinca rosea*. This observation can be supported by the report of Hirsche *et al.* (2017), that due to inability to utilize the fructose from the media, pollen grains exploitthe stored sugars to maintain the viability and thus fails to germinate. Like that of fructose, the same effect can be clearly seen with galactose which inhibits the pollen germination in *Vinca rosea* but largely promotes the pollen germination in *Cucumis melo* var. *utilissimus*. The inhibitory effect of galactose can be can be explained by the report of Wang *et al.* (2022), proved that galactose inhibits the pollen germination via galactokinase mediated pathway.

This study establishes the fact that the influence of various sugars on the germination of pollen grains also depends on the physiology of the pollen grains. This study adds to the information of developmental biology of pollen grains of *Vinca rosea*, which is a known ornamental and medicinal plant, and *Cucumis melo* var. *utilissimus*, which is a popular vegetable crop and medicinal plant of India. This study can further be used for extensive cultivation of these plants around the world.

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