1 **Effects of various carbohydrates on the *in vitro* pollen germination of** 2 ***Vinca rosea* and *Cucumis melo* var. *utilissimus***

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7 **Abstract**

8 Pollen germination is crucial for the process of plant development which strongly
9 depends on the presence of carbohydrates as a primary source of energy. In this study,
10 we analyzed the differential effects of four sugars with varying concentrations on the
11 pollen germination of *Vinca rosea* Linn. and *Cucumis melo* var. *utilissimus* Duth. &
12 Fuller. using Brewbaker and Kwack's medium as germination medium and hanging
13 drop method after an incubation period of 1h. Sucrose and glucose supported the
14 pollen germination but galactose and fructose did not support and even considerably
15 inhibited the pollen germination of *Vinca rosea*. Whereas in pollen germination of
16 *Cucumis melo* var. *utilissimus*, all the four sugars supported the pollen germination.
17 The study suggests that 15% sucrose, for *Vinca rosea*, and 12% galactose, for
18 *Cucumis melo* var. *utilissimus*, supports in achieving the highest pollen germination
19 percentage when added to the pollen germination medium.

20 **Keywords:** Pollen grains, carbohydrates, *in vitro* pollen germination, *Vinca rosea*,
21 *Cucumis melo* var. *utilissimus*, Brewbaker and Kwack's medium, Hanging drop
22 method.

23

24 Introduction

25 The development of pollen grains on stigma or in the *in vitro* conditions is a time-
 26 bound and high energy requiring process (Selinski and Scheibe, 2014). The
 27 recognition of compatible pollen and the growth of pollen tube largely depends upon
 28 the receptivity of stigma and other recognition factors. The further growth of pollen
 29 tube, till it reaches embryo sac, is facilitated by the environment of stylar canal
 30 (Boavida et al., 2005). Carbohydrates, act as an osmotic regulator and also as a
 31 substrate for primary energy source, supporting pollen germination and the pollen
 32 tube growth in stylar canal (Reinders, 2016).

33 Most of the studies conducted on *Vinca rosea* are based on its therapeutic properties.
 34 In Ayurveda extracts of both roots and shoots are used to treat several diseases. Many
 35 vinca alkaloids including vinblastine and vincristine are used for treating leukaemia
 36 and Hodgkin's lymphoma (Moudi *et al.*, 2013). *Cucumis melo* var. *utilissimus* is
 37 commonly called as American cucumber or "kakri" in India. The fruits are used for its
 38 juice or made into powder for cooking (Khare, 2007). Extract from the fruits are
 39 medicinally used to promote skin hydration, treat light burns and act as a stomach
 40 tonic. Seeds are used for suppressing cough, reducing fever and act as digestive aid
 41 (Gill *et al.*, 2010).

42 The present study investigates the effects of various sugars with varying
 43 concentrations on the *in vitro* pollen germination of *Vinca rosea* and *Cucumis melo*
 44 var. *utilissimus* by using a pollen germination medium and calculating pollen
 45 germination percentage. Different effects of sugars and their respective concentrations
 46 were observed on the pollen germination of *Vinca rosea* and *Cucumis melo* var.
 47 *utilissimus*. 15% sucrose and 12% galactose prove to be the best sugar concentrations
 48 along with the pollen germination medium to achieve the highest pollen germination

49 percentage after an incubation period of 1h for *Vinca rosea* and *Cucumis melo* var.
50 *utilissimus* respectively.

51 **Materials and Methods**

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

56 **Preparation of Pollen Germination Medium:** Standard solution (100ml) of
57 Brewbaker and Kwack's medium (Brewbaker and Kwack, 1963) was prepared by
58 using 0.01g of boric acid, 0.01g of potassium nitrate, 0.02g of magnesium sulphate
59 heptahydrate and 0.03g of calcium nitrate. The medium was prepared by using four
60 different sugars: sucrose, glucose, fructose and galactose. For each sugar, four
61 different concentrations were made: 5%, 10%, 12% and 15%. The germination
62 medium was autoclaved to maintain the sterility.

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

69 **Observation of pollen germination:** Observations for pollen germination was taken
70 by microscopy four times, each with 15 mins interval. Three microscopic field views
71 per replicate, that contained a minimum of 30 pollen grains, was observed. Pollen
72 grains were considered 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. The experiment was replicated thrice.

Statistical analysis: Descriptive statistics was used 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

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 except when fructose was used (Fig. 1a.-1d.). 5% sucrose concentration had an inhibitory effect on pollen germination

Using 15% sucrose in germination medium resulted in highest pollen germination percentage (67.99%) while the lowest pollen germination (3.06%) was obtained by using 10% galactose in germination medium after an incubation period of an hour. Lower concentrations of both glucose and galactose had an inhibitory effect on the pollen germination of *Vinca rosea*. Fructose, in any concentration, inhibited the pollen germination.

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.

96 As depicted, every germination medium irrespective of the sugar and its concentration
 97 used, was successful in pollen germination (Fig. 2a.-2d.). Pollen grains showed the
 98 highest germination percentage (65.33%) when 12% galactose was used along with
 99 the germination medium and the lowest germination percentage (17.71%) was
 100 obtained when 5% galactose was used along with the germination medium after an
 101 incubation period of an hour.

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Table 1. Effect of various sugars with varying concentration on the pollen germination of *Vinca rosea*

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

Values (in %) are represented as Mean ± S.D, P-value ≤ 0.05 is represented by *.

136 Table 2. Effect of various sugars with varying concentration on the pollen germination of Cucumis melo

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

137 Values (in %) are represented as Mean ± S.D, P-value ≤ 0.05 is represented by *.

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142 Discussion

143 The present study demonstrates that the germination of *Vinca rosea* and *Cucumis*
144 *melo* var. *utilissimus* pollen grains is largely influenced by the exogenous supply of
145 various sugars with varying concentrations.

146 Numerous studies have shown that sucrose exhibits a strong stimulatory action on the
147 growth of pollen grains (Patel, 2017). Both glucose and fructose have reported to
148 show varied effects on pollen germination of various plant species (Okusaka and
149 Hiratsuka, 2009). A higher concentration of sucrose as well as glucose promotes the
150 in vitro pollen germination of *Vinca rosea* (Bandyopadhyay and Mukherjee, 1977).
151 Although, in case of *Vinca rosea*, the lower concentrations of glucose inhibit the
152 pollen germination which supports the involvement of hexokinase mediated pathway
153 of inhibiting sucrose mediated pollen germination (Godt *et al.*, 1995; Sinha *et al.*,
154 2002). Sucrose promotes the pollen germination both in case of *Vinca rosea* and
155 *Cucumis melo* var. *utilissimus* and this can be supported by the fact that sucrose is the
156 most common form of sugar that is translocated to non-photosynthetic tissue, such as
157 flower, and thus is also excreted by the stigma as well as the stylar canal for hydrating
158 the pollen and supporting pollen tube growth (Hopkins and Hunner, 2009). Glucose
159 supports the pollen germination minimally in *Vinca rosea* as well as in *Cucumis melo*
160 var. *utilissimus*. This could be due to the weak expression of AtSTP9, a glucose-
161 specific monosaccharide transporter, in the mature pollen grain (Schneidereit *et al.*,
162 2003, 2005).

163 Fructose inhibits the pollen germination of *Vinca rosea* and not *Cucumis melo* var.
164 *utilissimus*. Presence of fructose causes the pollen grains to completely utilise their
165 stored sugars as respiration substrates and thus fail to develop (Okusaka and

166 Hiratsuka, 2009). On the other hand, in case of *Cucumis melo* var. *utilissimus*,
 167 fructose promotes the pollen germination which can be supported by the studies
 168 which states the presence of fructose in the stigmatic fluid of plants promote pollen
 169 germination (Portnoi and Horovitz, 1977). Like that of fructose, the same effect can
 170 be clearly seen with galactose which inhibits the pollen germination in *Vinca rosea*
 171 but largely promotes the pollen germination in *Cucumis melo* var. *utilissimus*. The
 172 inhibitory effect of galactose can be explained by the hexokinase mediated pathway of
 173 sucrose mediated pollen germination inhibition (Godt *et al.*, 1995; Sinha *et al.*, 2002).
 174 This study establishes the fact that the influence of various sugars on the germination
 175 of pollen grains also depends on the physiology of the pollen grains. This study adds
 176 to the information of developmental biology of pollen grains of *Vinca rosea*, which is
 177 a known ornamental and medicinal plant, and *Cucumis melo* var. *utilissimus*, which is
 178 a popular vegetable crop and medicinal plant of India. This study can further be used
 179 for extensive cultivation of these plants around the world.

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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 $\pm$ S.D. Mean values with the same letter are not significantly different at $P=0.05$ by Tukey's multiple-range test.

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 $\pm$ S.D. Mean values with the same letter are not significantly different at $P=0.05$ by Tukey's multiple-range test.