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EFFECT OF pH ON *IN VITRO* POLLEN GERMINATION OF FOURTEEN CULTIVATED AND WILD SPECIES OF CUCURBIT

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Abstract

Context: The pH of the *in vitro* germination medium is the important factor controlling pollen germination and pollen tube development in different plant species

Objectives: The aim of the present study was to assess the effect of pH levels of germination medium on the germinability 14 species of cucurbit.

Materials and Methods: *In vitro* pollen germination of 14 cucurbit species was examined at five different levels of pH using Brewbaker and Kwack's medium. The pH of the medium was adjusted to 7.0, 7.5, 8.0, 8.5 and 9.0 by using a digital pH meter with the help of 0.1 N HCl and 0.1 N NaOH. *In vitro* pollen germination was tested using sitting drop culture technique. Five sitting drop cultures of pollen grains of five different pH for each species were raised on five different microslides. Then the slides were placed in a humidity chamber and incubated at 22 $\pm$ 2 °C for 1 to 2 hrs. All the cultures were fixed by adding a drop of a 1-2% acetocarmine before start scoring and the germinated and non-germinated pollen grains were scored using a microscope.

Results: The pollen germination rate was higher in increased pH level in most of the cucurbit species studied and at 9.0 pH the maximum germination was observed in pumpkin (98%), snake gourd (98%), ridge gourd (96%), pointed gourd (89%), cucumber (98%), bitter gourd (96%), sweet gourd (98%) and sponge gourd (93%). The highest percentage of germination was observed in bottle gourd (98%) cucumber short (96%), watermelon (98%), muskmelon (97%) and ivy gourd (87%) at 8.5 pH level. The only cucurbit species wax gourd showed highest germination (97%) at the lower pH level of 7.5. Analysis of variance for the 14 cucurbits at five different pH levels showed that the differences between the germination rates of pollen grains of 14 cucurbit species at different pH level were highly significant ($F=12.49$, $P<0.001$, at 13/52 df).

Conclusion: In most of the investigated cucurbit species pH level 8.5 to 9.0 was found optimum for the highest number of *in vitro* pollen germination, while wax gourd responded maximum pollen germination at pH 7.5.

Key words: cucurbit, pollen, germination, pH.

Introduction

Cucurbits are present in both the New and Old World and are among the most important plant families that supply human with edible products and useful fibers. Although cultivated cucurbits are very similar in above ground development and root habit, they are extremely diverse for fruit characteristics. Fruits are eaten when immature (summer squash) or mature (watermelon). Fruits can be baked (squash), pickled (cucumber), candied (watermelon), or consumed fresh in salads (cucumber) or dessert (melon). Also, seeds, flowers (squash and pumpkins) and roots (chayote) are consumed by humans. Cucurbits are also produced for other uses than food. Fruits (bottlegourd) are used for storage, drinking containers, bottles, utensils, smoking pipes, musical instruments, gourdcraft decoration, masks, floats for fish net, and other items. The fiber of a mature loofah fruit can be used as a sponge for personal hygiene, household cleaning and various other

purposes, including filtration. Seeds or fruit parts of some cucurbits are reported to possess purgatives, emetics and antihelmintics properties due to the secondary metabolite cucurbitacin content (Robinson and Decker-Walters 1997, Bisognin 2002).

The pH of the *in vitro* germination medium is the important factor controlling pollen germination and pollen tube development in different plant species (Therios *et al*. 1985, Henny 1977, Bellani *et al*. 1997, Abraitiene *et al*. 2003, Munzuroglu *et al*. 2003, 2005, Burke *et al*. 2004, Qiu *et al*. 2005, Mbogning *et al*. 2007). Once released from anthers, pollen grains act as independent functional units and are exposed to the ambient environment.

Although pH ranges and optimum values are known to vary for pollen germination and pollen tube growth among and within species (Proctor 1983, Rinallo 1989, Bellani *et al*. 1997, Qiu *et al*. 2005, Munzuroglu *et al*. 2003), no previous study could be identified which examined the effect of varying substrate pH on cucurbit species pollen germination. The aim of the present study was to assess the effect of pH levels of germination medium on the germinability 14 species of cucurbit.

Materials and Methods

The 14 cucurbit species were collected from natural population, commercial and homestead gardens used for the present work and they are pumpkin (*Cucurbita maxima* Duch ex Poir.), wax gourd (*Benincasa hispida* (Thunb.) Cogn.), bottle gourd (*Lagenaria siceraria* (Mol) Standl.), snake gourd (*Trichosanthes cucumerina* L.), ridge gourd (*Luffa acutangula* (Roxb.) L.), pointed gourd (*Trichosanthes dioica* Roxb.), cucumber (*Cucumis sativus* L. and *Cucumis anguina* L.), bitter gourd (*Momordica charantia* L.), sweet gourd (*Momordica cochinchinensis* Spreng.), watermelon (*Citrullus lanatus* Thunb.), muskmelon (*Cucumis melo* L.), sponge gourd (*Luffa cylindrica* Roem.) and ivy gourd (*Coccinia cordifolia* (Voigt) L.).

Brewbaker and Kwack's medium (Brewbaker and Kwack 1963) was used for the testing of effects of pH on *in vitro* pollen germination. The pH of the medium was adjusted to 7.0, 7.5, 8.0, 8.5 and 9.0 by using a digital pH meter with the help of 0.1 N HCl and 0.1 N NaOH. *In vitro* pollen germination was tested using sitting drop culture technique. Five sitting drop cultures of pollen grains of five different pH for each species were raised on five different microslides. To prevent the contamination of different medium with different pH, the needle was rinsed in distilled water each time after use. Then the slides were placed in a humidity chamber. A pair of petri plates with a moist filter paper or tissue paper lining the lower plate was served as an improvised humidity chamber. Two glass rods placed parallel at about 4 cm apart on the moist filter paper facilitated the humidity of the pollen germination. Then the humidity chambers containing cultures were incubated at 22 ± 2 °C for 1 to 2 hrs.

As the scoring of pollen germination takes considerable time, all the cultures were fixed by adding a drop of a 1-2% acetocarmine (i.e. fixative) before start scoring and the germinated and non-germinated pollen grains were scored using a microscope to calculate the percentage of pollen germination of the five different pH media.

Results

The effects of pH on *in vitro* pollen germination of pollen grains of 14 cucurbits in Brewbaker and Kwack's medium having five different pH are presented in Table 1. The pollen germination rate was higher in increased pH level in most of the cucurbit species studied and at 9.0 pH the maximum germination was observed in pumpkin (98%), snake gourd (98%), ridge gourd (96%), pointed gourd (89%), cucumber (98%), bitter gourd (96%), sweet gourd (98%) and sponge gourd (93%). The highest percentage of germination was observed in bottle gourd (98%) cucumber short (96%), watermelon (98%), muskmelon (97%) and ivy gourd (87%) at 8.5 pH level. The only cucurbit species wax gourd showed highest germination (97%) at the lower pH level of 7.5.

Analysis of variance for the 14 cucurbits at five different pH levels showed that the differences between the germination rates of pollen grains of 14 cucurbit species at different pH level were highly significant (F=12.49, P<0.001, at 13/52 df). The differences between the germination rates of pollen grains of each cucurbit at different pH level were also highly significant (F=16.74, P<0.001 at 4/52 df).

Table 1. *In vitro* pollen germination rates of different cucurbit species at different pH levels.

Species	Germination rate (%) at					Mean ± S.E.
	7.0 pH	7.5 pH	8.0 pH	8.5 pH	9.0 pH	
Pumpkin	88.00	93.00	95.46	98.00	98.00	$94.49^{a} \pm 1.67$
Wax gourd	96.00	97.00	94.30	90.00	87.00	$92.86^{abc} \pm 1.69$
Bottle gourd	91.00	95.00	97.19	98.00	97.00	$95.64^{a} \pm 1.13$
Snake gourd	85.00	87.00	82.55	96.00	98.00	$89.71^{abc} \pm 2.75$
Ridge gourd	86.00	89.00	85.51	93.00	96.00	$89.90^{abc} \pm 1.81$
Pointed gourd	69.00	72.00	77.98	80.00	89.00	$77.40^{d} \pm 3.12$
Cucumber	85.00	91.00	95.53	94.00	98.00	$92.71^{abc} \pm 1.99$
Cucumber (small)	91.00	94.00	94.63	96.00	95.00	$94.13^{ab} \pm 0.76$
Bitter gourd	90.00	93.00	91.19	95.00	96.00	$93.04^{abc} \pm 1.01$
Sweet gourd	83.00	88.00	92.05	97.00	98.00	$91.61^{abc} \pm 2.51$
Watermelon	89.00	96.00	95.03	98.00	96.00	$94.81^{a} \pm 1.37$
Muskmelon	78.00	82.00	88.29	97.00	90.00	$87.06^{c} \pm 2.94$
Sponge gourd	80.00	84.00	88.01	92.00	93.00	$87.40^{bc} \pm 2.18$
Ivy gourd	72.00	75.00	80.11	87.00	81.00	$79.02^{d} \pm 2.32$
Mean ± S.E.	$84.50^{c} \pm 2.02$	$88.29^{b} \pm 2.05$	$89.85^{b} \pm 1.67$	$93.64^{a} \pm 1.37$	$93.71^{a} \pm 1.38$	-

ANOVA

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square	F	P<0.001
Species	2046.42	13	157.42	12.49*	3.61
pH levels	843.91	4	210.98	16.74*	6.01
Error	655.44	52	12.60		
Total	3545.77	69			

The means having common letters are statistically similar as per DMRT. *** P<0.001

From the result it is observed that there is no significant difference between the germination rates of pollen grains of bottle gourd, watermelon, pumpkin, cucumber (short), bitter gourd, wax gourd, cucumber, sweet gourd, ridge gourd and snake gourd at different pH level. Similarly the differences between the germination rates of pollen grains of bitter gourd, wax gourd, cucumber, sweet gourd, ridge gourd, snake gourd, sponge gourd and muskmelon at different pH level were insignificant. Again in ivy gourd and pointed gourd there was no significant differences between the germination rates of pollen grains at different pH level. But significant differences were observed between the germination rates of pollen grains of bottle gourd, sponge gourd, muskmelon and pointed gourd at different pH level (Table 1). Germination rate at five different pH levels shows that there was no significant differences between the germination rates of pollen grains of the 14 investigated cucurbits at pH 7.5 and pH 8.0. Similarly the differences between the germination rates of pollen grains of the 14 cucurbits at pH 8.5 and pH 9.0 were insignificant. But significant differences were observed between the germination rates of pollen grains of the 14 cucurbit species at pH 7.0, pH 8.0 and pH 9.0 (Table 1).

Discussion

The pollen tube is a highly specialized cell type that delivers the sperm cells to the ovule for fertilization. Pollen tube growth is thus crucial for the process of plant sexual reproduction and food production; it is also of fundamental interest because it is one of the fastest growing plant cell types known (Holdaway-Clarke and

Hepler 2003). The pH of the germination medium has been shown to affect pollen germination of several plant species (Henny 1977, Bellani *et al*. 1997, Abraitiene *et al*. 2003, Munzuroglu *et al*. 2003, Burke *et al*. 2004, Qiu *et al*. 2005, Mbogning *et al*. 2007).

Pollen from the different species tested in this work behaved differently, and they were obviously affected by pH value of germination media. According to Kwack (1964), the optimal pH for pollen germination of several species was rather narrow, with the best results generally at pH 7.3 or 8.3 and the poorest results at pH 5.3. Qiu *et al*. (2005) reported that injury symptoms could be observed in pollen treated at pH 3.5, and pollen germination was severely reduced.

Burke *et al*. (2004) tested the effect of pH in the range of 6-8 in steps of 0.5 on *in vitro* germination of cotton pollen, as well as the elongation of the pollen tube and stated no differences between the tested pH values. On the other hand, Salles *et al.* (2006) verified differences between the pH levels in the range from 3.5 to 6.5 in three citrus varieties, as well as an interaction between varieties and pH levels, influencing *in vitro* pollen germination. The importance of the determination of the ideal pH in the physiological processes that involve pollen grains is linked to the resulting higher germination percentage, which increases the chances of fertilization (Salles *et al*. 2006), aside from influencing the nutrient availability, plant regulators and the degree of agar solidification (Pasqual *et al*. 2002). The pH of the cultural medium has been shown to affect pollen germination of several plant species (Brink 1925, Sisa 1930, Henry 1977). There was no significant difference in germination of *Spathiphyllum* pollen within the pH range of 5.0-7.0, whereas *Vriesea* pollen germinated equally well from pH 4.0-8.0. Germination of *Spathiphyllum*, pollen was drastically reduced at pH 3.0, 8.0 and 9.0, while *Vriesea* barely germinated at pH 3.0 or 9.0. Previous work with several species (Kwack 1964) indicated the optimal pH for pollen growth was rather narrow with best results generally at pH 7.3 or 8.3 and poorest results at pH 5.3.

In the present study, the acidity or alkalinity of germination media had an effect on pollen germination. This is the first study on the effect of a wide range of pH on pollen germination of different cucurbit species. In most of the cucurbit species studied it was observed that germination rates of pollen grains were increased when the pH level was increased. While in some of the species germination rates of pollen grains were remain constant at increasing level of pH. Again in some of the species germination rates of pollen grains were decreased when the pH level was increased.

Conclusion

In most of the investigated cucurbit species pH level 8.5 to 9.0 was found optimum for the highest number of *in vitro* pollen germination, while wax gourd responded maximum pollen germination at pH 7.5. Cucurbit pollen was generally more tolerant of a basic medium than acidic; however, their pollen was adaptable to a wide range of pH levels. Those species with higher pollen germination *in vitro* can be evaluated as wide pH range tolerant. The results shows that the pH level of *in vitro* germination media is the important factor controlling pollen germination for cucurbit species.

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