POLLEN GERMINATION, VIABILITY AND TUBE GROWTH IN FOURTEEN CULTIVATED AND WILD SPECIES OF CUCURBIT GROWN IN BANGLADESH

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Abstract

Pollen germination, viability and tube growth in fourteen species of cucurbit were examined. All the male flowers had three anthers each. The highest number of pollen grain (917.00 ± 52.69) per anther was recorded in pumpkin (Cucurbita maxima Duch ex Poir.) whereas the lowest (479.00 ± 13.14) per anther was recorded in snake gourd (Trichosanthes cucumerina L.). Viable pollen was highest (98.51%) in bitter gourd (Momordica charantia L.) and lowest (86.87%) in pointed gourd (Trichosanthes dioica Roxb.). Pollen grains of all species were starchy in nature. The highest percentage of pollen germination (97.19%) was recorded as in bottle gourd (Lagenaria siceraria (Mol) Standl.) and lowest (77.98%) in pointed gourd. Rate of increasing lengths of the tubes was very fast between first two hours and became very slow in last six hours. After 12 hrs. of germination the longest pollen tube (1649.20 ± 15.05) was found in pumpkin and the shortest (766.08 ± 19.03) was found in pointed gourd.

Key words: Pollen germination; viability, tube-growth, cucurbit.

Introduction

Flower structure is important in relation to pollen distribution and reception and in many of the hermaphroditic plant species flowers have evolved dramatically to suit particular pollination mechanisms (Ainsworth, 2000). In wind pollinated plants (where there is a strong correlation with dioecy; Renner and Ricklefs, 1995), sexual specialization of male and female flowers is common. Intraspecific variation in size of the pollen loads deposited on stigmas may influence both the number and quality of the eventual progeny (Herrera, 2002). Threshold effects, nonlinear dose-response relationships, and maternal and paternal identity, act for determining the number of seeds produced (Bertin, 1990; Waser and Price, 1991; Holm, 1994; Melser et al., 1997; Mitchell, 1997; Bosch and Waser, 1999; Dieringer and Cabrera, 2002). In addition, the amount of pollen deposited may also influence the quality of the progeny through the action of prefertilization (microgametophyte competition: Snow, 1986; Schlichting et al., 1987; Winsor et al., 1987, 2000) and/or postfertilization mechanisms (selective abortion: Niesenbaum and Casper, 1994; Rigney, 1995; Havens and Delph, 1996; Niesenbaum, 1999; Melser and Klinkhamer, 2001).

Important role played by pollen in sexual plant reproduction has motivated a multitude of observational and experimental investigations regarding patterns
and consequences of variation in size of stigmatic pollen loads. This contrasts sharply less number of investigations that have so far documented patterns of variation in the size of pollen tube populations for naturally pollinated wild plants (Levin, 1990; Honig et al., 1992; Aizen and Feinsinger, 1994; Niesenbaum, 1994; Plitmann and Levin, 1996; Herrera, 1997; Quesada et al., 2001; Herrera, 2002). However, because of the increasing realization of the importance of pollen in both fundamental and applied areas, there has been an explosion of knowledge on pollen biology (Mulcahy and Ottaviano 1983; Shivana and Johri 1985; Mulcahy et al. 1986; Giles and Prakash 1987; Cresti et al. 1988; Iwanami et al. 1988; Quesada et al., 1995; Delph et al., 1997; Corff et al., 1998).

Pollen germination and pollen tube growth are prerequisites for fertilization and seed development. Due to involvement of the pistillate tissue in the nature, physiological and biochemical investigations on pollen germination and pollen tube growth in vivo are rather difficult. In vitro germination techniques have therefore been used extensively on a variety of pollen systems. Such studies have provided considerable information on the physiology and biochemistry of pollen germination and pollen tube growth (Shivana and Johri 1985; Steer and Steer 1989). Pollen germination and tube growth are generally divided into four phases: imbibition phase, lag phase, tube initiation phase, and rapid tube elongation phase (Linskens and Kroh, 1970). The time taken for different phases varies greatly, from species to species, depending on the type of reserve food material in the pollen and the external factors.

Cucurbits form an important group of vegetable crops comprising both cultivated and wild species. This group includes mostly seed propagated ones, besides few vegetatively propagated ones like pointed gourd and also few perennials like ivy gourd. From nutritional point of view bitter gourd is rich for vitamin C, pumpkin contains high carotenoid pigments, sweet gourd is high in protein and pointed gourd is fairly high in calcium (Bhuiya et al., 1977; Gopalan et al., 1982). A few cultivars of squashes and pumpkins are relatively high in energy and carbohydrates (Seshadri, 1993). Cucurbits are of tremendous economic importance as food plants and it has been difficult to estimate or quantify them because of the inadequate information regarding their area and production in Bangladesh. No work on pollen biology of this important group of plants has been done in our country. So, an endeavor has been made to study the pollen biology of some wild and cultivated cucurbits grown in Bangladesh.

Materials and Methods

The 14 cucurbit species collected from natural population, commercial and homestead gardens used for the present work and they are Pumpkin (Cucurbita maxima Duch ex Poir.), Ash 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 C. anguina L.), Bitter gourd (Momordica charantia L.), Sweet gourd (M. cochinchinensis Spreng.), Watermelon (Citrullus lanatus Thunb.), Musk melon (Cucumis melo L.), Sponge gourd Luffa cylindrica Roem.), and Ivy gourd (Coccinia cordifolia (Voigt) L).

The fresh male flowers at blooming stage were collected for counting the number of anthers of each flower and for estimating the pollen viability. The anthers were stained with 1% acetocarmine solution and the stained pollen was considered as viable and non-stained as non-viable pollen. To determine the starchy and non-starchy pollen grain the anthers were stained with potassium iodide solution where non-starchy pollen did not take stain.

Brewbaker and Kwack’s media as suggested by Brewbaker and Kwack (1963) and Roberts’ media as per schedule of Roberts et al. (1983) were used to study in vitro germination rate of pollen grains by sitting drop culture method. The culture was maintained in a humidity chamber to prevent evaporation. Ten humidity chambers were prepared in the same procedure with ten pair of petri plates for each experiment. Cultures along with humidity chambers were incubated under desired temperature (22 ± 2°C is optimal) for 1 to 1.5 hrs. Respond to germination of the cultured pollen grains was expressed in percentage.

The prepared slides were observed under a microscope and the time required for germination of the pollen from each chamber was recorded with the
help of a stopwatch. The tube lengths of in vitro germinating pollen grains were recorded after 1-, 2-, 6- and 12 hours of setting the experiment.

**Results**

All the male flowers of the 14 species had three anthers each. Anther size varied proportionately based on the size of flowers. Average value for highest number of pollen grain per flower was found to be 917.00 ± 52.69 in pumpkin and the lowest value was 479.00 ± 13.14) in snake gourd. The microphotographs showing pollen morphology of different cucurbit species are presented in Fig. 1.

Highest number of viable pollen (98.51%) was observed in bitter gourd while the lowest (86.87%) in pointed gourd. Pollen grains of all the 14 species were found to be starchy in nature as it was determined by potassium iodide solution. Pollen grains were found to germinate frequently in Brewbaker and Kwack’s medium. But Roberts’ medium was found not suitable for in vitro pollen germination and none of the pollen grain was found to germinate in that medium. The highest percentage of pollen germination was recorded as 97.19% in bottle gourd whereas the lowest was 77.98% in pointed gourd. The time required for germinating pollen tube was maximum (101.60 mins.) for snake gourd and it was minimum (13.20 mins.) for cucumber.

Tube lengths of in vitro germinating pollen grains were recorded after 1, 2, 6 and 12 hrs of germination. The rate of increasing lengths of the tubes was very fast between first two hours and became very slow in last six hours. After 12 hrs of germination the longest pollen tube (1649.20 ± 15.05) was found in pumpkin and the shortest (766.08 ± 19.03) was found in pointed gourd.

Statistically the differences between different values for pollen grains per anther, germination rates, times required to germinate pollen tube and lengths of pollen tube (after 12 h) among 14 cucurbits were found to be highly significant (P<0.001).

**Table 1. Pollen characters in different cucurbit species.**

<table>
<thead>
<tr>
<th>Cucurbits</th>
<th>Mean No. of pollen/ anther ? S.E.</th>
<th>Pollen viability (%)</th>
<th>In vitro germinated pollen grain (five focuses)</th>
<th>Pollen germination (%)</th>
<th>Time required to germinate pollen tube (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pumpkin</td>
<td>917.00&lt;sup&gt;abc&lt;/sup&gt; ± 52.69</td>
<td>96.84</td>
<td>348.80&lt;sup&gt;de&lt;/sup&gt; ± 15.51</td>
<td>95.46</td>
<td>51.80&lt;sup&gt;b&lt;/sup&gt; ± 1.42</td>
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<tr>
<td>Ash gourd</td>
<td>776.67&lt;sup&gt;cd&lt;/sup&gt; ± 11.94</td>
<td>96.79</td>
<td>496.60&lt;sup&gt;e&lt;/sup&gt; ± 18.17</td>
<td>94.30</td>
<td>13.40&lt;sup&gt;b&lt;/sup&gt; ± 0.83</td>
</tr>
<tr>
<td>Bottle gourd</td>
<td>656.00&lt;sup&gt;de&lt;/sup&gt; ± 53.13</td>
<td>97.22</td>
<td>388.60&lt;sup&gt;abcd&lt;/sup&gt; ± 14.91</td>
<td>97.19</td>
<td>27.00&lt;sup&gt;c&lt;/sup&gt; ± 1.09</td>
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<tr>
<td>Snake gourd</td>
<td>479.00&lt;sup&gt;ef&lt;/sup&gt; ± 13.14</td>
<td>91.08</td>
<td>173.20&lt;sup&gt;bc&lt;/sup&gt; ± 3.73</td>
<td>82.55</td>
<td>101.60&lt;sup&gt;bc&lt;/sup&gt; ± 1.28</td>
</tr>
<tr>
<td>Ridge gourd</td>
<td>553.33&lt;sup&gt;ef&lt;/sup&gt; ± 13.51</td>
<td>97.93</td>
<td>297.40&lt;sup&gt;def&lt;/sup&gt; ± 58.45</td>
<td>85.51</td>
<td>19.80&lt;sup&gt;c&lt;/sup&gt; ± 0.44</td>
</tr>
<tr>
<td>Pointed gourd</td>
<td>581.67&lt;sup&gt;ef&lt;/sup&gt; ± 30.45</td>
<td>86.87</td>
<td>165.80&lt;sup&gt;e&lt;/sup&gt; ± 4.56</td>
<td>77.98</td>
<td>51.60&lt;sup&gt;b&lt;/sup&gt; ± 1.69</td>
</tr>
<tr>
<td>Cucumber</td>
<td>761.33&lt;sup&gt;cd&lt;/sup&gt; ± 25.25</td>
<td>96.29</td>
<td>410.00&lt;sup&gt;abcd&lt;/sup&gt; ± 25.59</td>
<td>95.53</td>
<td>13.20&lt;sup&gt;b&lt;/sup&gt; ± 0.44</td>
</tr>
<tr>
<td>Cucumber (short)</td>
<td>890.00&lt;sup&gt;abc&lt;/sup&gt; ± 38.18</td>
<td>97.82</td>
<td>373.60&lt;sup&gt;bcd&lt;/sup&gt; ± 35.76</td>
<td>94.63</td>
<td>18.80&lt;sup&gt;def&lt;/sup&gt; ± 0.44</td>
</tr>
<tr>
<td>Bitter gourd</td>
<td>945.00&lt;sup&gt;ab&lt;/sup&gt; ± 16.68</td>
<td>98.51</td>
<td>149.00&lt;sup&gt;f&lt;/sup&gt; ± 6.19</td>
<td>91.19</td>
<td>21.40&lt;sup&gt;e&lt;/sup&gt; ± 1.04</td>
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<tr>
<td>Sweet gourd</td>
<td>956.67&lt;sup&gt;ef&lt;/sup&gt; ± 15.33</td>
<td>98.22</td>
<td>245.40&lt;sup&gt;f&lt;/sup&gt; ± 11.66</td>
<td>92.05</td>
<td>27.20&lt;sup&gt;d&lt;/sup&gt; ± 2.63</td>
</tr>
<tr>
<td>Watermelon</td>
<td>850.33&lt;sup&gt;abc&lt;/sup&gt; ± 13.24</td>
<td>94.17</td>
<td>478.00&lt;sup&gt;ab&lt;/sup&gt; ± 36.24</td>
<td>95.03</td>
<td>44.20&lt;sup&gt;c&lt;/sup&gt; ± 0.82</td>
</tr>
<tr>
<td>Muskmelon</td>
<td>778.33&lt;sup&gt;cd&lt;/sup&gt; ± 14.53</td>
<td>97.72</td>
<td>422.22&lt;sup&gt;ab&lt;/sup&gt; ± 39.76</td>
<td>88.29</td>
<td>15.20&lt;sup&gt;f&lt;/sup&gt; ± 0.99</td>
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<tr>
<td>Sponge gourd</td>
<td>669.33&lt;sup&gt;de&lt;/sup&gt; ± 44.71</td>
<td>89.03</td>
<td>367.00&lt;sup&gt;bcd&lt;/sup&gt; ± 8.95</td>
<td>88.01</td>
<td>16.40&lt;sup&gt;ef&lt;/sup&gt; ± 0.61</td>
</tr>
<tr>
<td>Ivy gourd</td>
<td>794.33&lt;sup&gt;bcd&lt;/sup&gt; ± 13.46</td>
<td>91.91</td>
<td>228.80&lt;sup&gt;f&lt;/sup&gt; ± 11.99</td>
<td>80.11</td>
<td>13.40&lt;sup&gt;f&lt;/sup&gt; ± 0.61</td>
</tr>
</tbody>
</table>

*Means followed by same letter are statistically similar as per Duncan’s multiple range test
**Fig. 1.** Microphotographs showing morphology of the pollen indifferent cucurbit species.
14 species. Stanley and Linskens (1974) correlated cucurbits and no germination occurred in any of the be suitable for Kwack’s medium. Roberts’ medium was not found to recorded for pointed gourd in Brewbaker and for bitter gourd whereas the lowest (86.87%) was percentage of pollen viability (98.51%) was recorded found to differ from species to species. Highest number of pollen was recorded in the snake gourd. gourd, pumpkin and cucumber (short). The lowest was found higher in sweet gourd followed by bitter present experiment the number of pollen per flower is variable. In watermelon and pumpkin, pollen production is good while in crops like muskmelon the production is scanty (Seshadri, 1993). In the flowering in cucurbits normally starts at 40-45 days old plants, although it varies due to changes of weather. In typical monoecious sex form, as 14 species used in the present study, the numbers of male flowers are sometimes produced in greater proportion than the pistillate flowers in the same plant. Thus intraspecific variation in the size of pollen loads deposited on stigmas may influence both the number and quality of the eventual progeny (Herrera, 2004). Pollen production in different genera is variable. In watermelon and pumpkin, pollen production is good while in crops like muskmelon the pollen production is scanty (Seshadri, 1993). In the present experiment the number of pollen per flower was found higher in sweet gourd followed by bitter gourd, pumpkin and cucumber (short). The lowest number of pollen was recorded in the snake gourd.

The pollen viability in the present study was found to differ from species to species. Highest percentage of pollen viability (98.51%) was recorded for bitter gourd whereas the lowest (86.87%) was recorded for pointed gourd in Brewbaker and Kwack’s medium. Roberts’ medium was not found to be suitable for in vitro pollen germination of cucurbits and no germination occurred in any of the 14 species. Stanley and Linskens (1974) correlated decrease in viability with the stage of development of the male gametophyte and found binucleate pollen to survive long time than trinucleate. Pacini et al. (1997) found pollen viability to be decreased with time. The rapid decrease in pollen viability of Cucurbita pepo is related to the fact that the flowers of both sexes of this monoecious plant are accessible to insects (bees and bumble bees) for only 6 hrs and only the pollen is partially dehydrated (Nepi and Pacini, 1993, 2001; Winsor and Stephenson, 1995). After that period the flower closes and wilts. There is no adaptive advantage to be gained by the pollen for remaining viable for longer time than the pollinators, which have access to the flower. Polyploid pollen grains of Cucumis melo showed a lower germination percentage in vitro and a slower germination rate than haploid pollen (Susin and Alvarez, 1997).

The duration of pollen viability has been also studied by Stanley and Linskens (1974) and found not to be correlated with the type of pollination. However, Ottaviano and Mulcahy (1989) stated that pollen must be programmed to have high viability in the habitat of the species. The duration of pollen viability varies greatly between species, and is related to the type of pollination. In general, plants with entomophilous pollination have pollen with longer viability than those with anemophilous pollination (Bassani et al., 1994). Pollen carried on the wind is

<table>
<thead>
<tr>
<th>Cucurbits</th>
<th>Mean length (μm)</th>
<th>S.E. after germination time of 1 hour</th>
<th>2 hours</th>
<th>6 hours</th>
<th>12 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pumpkin</td>
<td>308.56 ? 38.07</td>
<td>649.04 ? 27.75</td>
<td>1448.84 ? 18.72</td>
<td>1649.20 ? 15.05</td>
<td></td>
</tr>
<tr>
<td>Ash gourd</td>
<td>106.40 ? 15.05</td>
<td>498.44 ? 17.80</td>
<td>989.52 ? 24.26</td>
<td>1223.60 ? 15.05</td>
<td></td>
</tr>
<tr>
<td>Bottle gourd</td>
<td>329.89 ? 27.75</td>
<td>766.08 ? 19.03</td>
<td>1287.44 ? 23.31</td>
<td>1447.04bcde ? 43.61</td>
<td></td>
</tr>
<tr>
<td>Snake gourd</td>
<td>95.76 ? 9.52</td>
<td>585.20 ? 30.09</td>
<td>745.60 ? 60.75</td>
<td>820.08g ? 32.96</td>
<td></td>
</tr>
<tr>
<td>Pointed gourd</td>
<td>106.40 ? 15.05</td>
<td>446.88 ? 24.26</td>
<td>744.80 ? 30.09</td>
<td>766.08g ? 19.03</td>
<td></td>
</tr>
<tr>
<td>Cucumber</td>
<td>266.00 ? 15.05</td>
<td>872.48 ? 19.03</td>
<td>1255.52 ? 19.03</td>
<td>1276.80ef ? 33.65</td>
<td></td>
</tr>
<tr>
<td>Cucumber (short)</td>
<td>287.28 ? 19.03</td>
<td>872.48 ? 32.27</td>
<td>1244.88 ? 38.66</td>
<td>1500.24bc ? 17.80</td>
<td></td>
</tr>
<tr>
<td>Sweet gourd</td>
<td>127.68 ? 19.03</td>
<td>829.92 ? 44.13</td>
<td>1351.28 ? 32.27</td>
<td>1564.08ab ? 19.03</td>
<td></td>
</tr>
<tr>
<td>Watermelon</td>
<td>436.24 ? 17.80</td>
<td>1074.64 ? 17.80</td>
<td>1340.64 ? 34.97</td>
<td>1415.12cde ? 28.55</td>
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</tr>
<tr>
<td>Muskmelon</td>
<td>659.68 ? 38.66</td>
<td>1127.84 ? 17.80</td>
<td>1393.84 ? 40.93</td>
<td>1372.56de ? 27.75</td>
<td></td>
</tr>
<tr>
<td>Ivy gourd</td>
<td>74.48 ? 11.65</td>
<td>521.36 ? 23.31</td>
<td>893.76 ? 23.31</td>
<td>1181.04f ? 27.75</td>
<td></td>
</tr>
</tbody>
</table>

*Means followed by same letter are statistically similar as per Duncan’s multiple range test.

Table 2. Pollen tube growth in different cucurbit species using Brewbaker and Kwack’s medium.

Discussion

Flowering in cucurbits normally starts at 40-45 days old plants, although it varies due to changes of weather. In typical monoecious sex form, as 14 species used in the present study, the numbers of male flowers are sometimes produced in greater proportion than the pistillate flowers in the same plant. Thus intraspecific variation in the size of pollen loads deposited on stigmas may influence both the number and quality of the eventual progeny (Herrera, 2004). Pollen production in different genera is variable. In watermelon and pumpkin, pollen production is good while in crops like muskmelon the pollen production is scanty (Seshadri, 1993). In the present experiment the number of pollen per flower was found higher in sweet gourd followed by bitter gourd, pumpkin and cucumber (short). The lowest number of pollen was recorded in the snake gourd.

The pollen viability in the present study was found to differ from species to species. Highest percentage of pollen viability (98.51%) was recorded for bitter gourd whereas the lowest (86.87%) was recorded for pointed gourd in Brewbaker and Kwack’s medium. Roberts’ medium was not found to be suitable for in vitro pollen germination of cucurbits and no germination occurred in any of the 14 species. Stanley and Linskens (1974) correlated
dispersed as soon as the anther open, and does not have to await for arrival of the pollinating insect.

Highest percentage of in vitro pollen germination (97.19%) was recorded for bottle gourd and the lowest (77.98%) for pointed gourd. Germination time of pollen tube also found to differ among the cucurbit species. The shortest time taken by cucumber was only 13.20 ± 0.44 against 101.60 ± 1.28 minutes by snake gourd. Rapid growth of pollen tube depends on constant fusion of vesicles forming the plasmalemma, and continuous secretion of cell wall material (Blevins and Lukaszewski, 1998). Jackson (1991) stated that capture of the secreted pollen proteins for membrane and wall building, proceeds through borate complexes with sugar residues. The pollen tube is a cellular extrusion of the pollen grain, and forms after germination of the pollen on stigma of a receiving flower. The function of the pollen tube is to transmit gametes from pollen grain to the ovary, and this can occur over long distances (several centimetres in some cases) also (Cresti et al., 1992).

Because of its fundamental importance to the process of fertilization in higher plants, the pollen tube has recently been subjected for intensive study, with the aim of understanding the cell biology involved and regulating it through biotechnology (Cai et al., 1997; Stephenson et al., 2003).

References


