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Differences in Style Length and *In vitro* Germinated Pollen Tube Length and Other Reproductive Structures of Tetraploid and Diploid Cottons

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Abstract: It was suspected that differences in style length and other reproductive structures of tetraploid and diploid cottons may contribute towards failure of crossing between two ploidies. Style length, stigma depth and pollen grain size of tetraploid were 20.5, 60.0 and 10.0 mm against 8.5, 2.0 and 7.0 mm of *G. harknessii* and 9.0, 2.5 and 7.0 mm of *G. arboreum* respectively. *In vitro* germinated pollen tubes of both the ploidy levels also varied significantly where tetraploid produced pollen tubes upto 20.5 mm long against 8 to 9.0 mm pollen tubes of both diploid species. It was therefore confirmed that style length is highly correlated with pollen tube length. Thus, shorter pollen tubes of diploid species are not expected to grow long enough in the longer styles of tetraploids and reach the ovary to fertilize it. Since, pollen grain size and stigma depth are also considered as food reserves for pollen tubes to grow into the styles and the differences in these structures can also be accountable to the failure of fertilization between the ploidy levels. Based on these results, it can be predicted that differential reproductive structure could at least be partially responsible for crossing failure and reciprocal crosses may be tried for successful fertilization.

Key words: *In vitro* Cotton pollen germination, ploidy differences in relation to tube length, Reproductive structures

Introduction

Cytogeneticists are usually interested to transfer desirable genes such heat, insect, disease resistance and others of wild relatives (most of them diploids, 2n = 26) to the cultivated cotton (tetraploids, 2n = 52) through hybridization. Crossing problems however are frequently encountered that render unsuccessful fertilization. Failure of successful hybridization is usually thought to be either due to impairing of chromosomes between two species, at meiosis, that cause embryo to abort or due to incompatibility of S alleles in both the stigma or the style tissues that hinders the pollen tubes to grow and reach the ovary (Poehlman, 1983). Besides these two reasons that crosses between wild and tetraploid, usually fail, embryologists have yet a third opinion. They noticed the differences in pollen grain sizes, stigma depth, style length and pollen tube length thus reasoned that these differences can also cause crossing problems in plants. Cruden and Lyon (1985) reported only a significant correlation (r = 0.740) between stigma depth and pollen grain volume. This type of study does not support our hypothesis that style length differences, consequently pollen tube sizes of diploid and tetraploid cotton may pose the problem of unsuccessful fertilization. Though previous studies (Motten, 1992) have already suggested that *in vitro* germinated pollen tubes are not only very difficult to germinate as in case of cotton (Philomena and David, 1984) but are much shorter than the tubes that grow *in vivo* (styler tissues). Choudhry and Akhmedova (1982) also reported difference in tube length of cotton pollen grains. In the present study we still expect that even though the *in vitro* pollen tubes are shorter than the *in vivo* tubes but we could yet get some idea as how far the style lengths and pollen tubes of diploids and tetraploids differ. If style length and pollen tube length of diploid and tetraploid vary significantly, then one can argue that if diploids grow shorter pollen tubes than the tetraploid cottons (due to any reason or hypothesis mentioned earlier), then generative nucleus from diploid may not complete the travel of whole the style of tetraploid, eventually will render unsuccessful hybridization. Studies as done by Motten (1992) can further guide us whether or not shorter styled diploid tubes germinate *in vitro* actually reaches the ovary of tetraploid cotton. The long term aim in these type of studies are therefore made with the interest that in interspecific crosses, using shorter styled species as the female parent, the crossing may be successful when the reciprocal crosses fail.

Materials and Methods

A medium containing Calcium nitrate [$(CaNO_3)_2$], magnesium sulphate ($MgSO_4.4H_2O$), Boric acid (H_3BO_3) and sucrose each in quantities weighing 300 mg, 140 mg, 50 mg and 40% respectively were dissolved in 100 ml of distilled water. The only modification of our method from Taylors (1972) was that instead of manganese sulphate ($MnSO_4$), we have used $MgSO_4.4H_2O$. Taylors (1972) used hanging droplet procedure where the Petri dishes were inverted and the pollens were suspended in hanging droplet of medium. In our procedure, we made circular droplets of medium on microscope glass slides and left in Petri plate on wet filter papers.

A drop or two of medium was taken by camel hair brush, placed on clean glass slides. Ten Petri plates each with one glass slide for each ploidy, tetraploid (4x = 52) and diploid (2x = 26) were prepared. Wet filter papers with two to three additional drops of water were left in the Petri plates so as to provide required humidity to the germinating pollen grains during incubation. At about 10.00 a.m. which seemed an appropriate time for anther dehiscence, about ten flowers were collected from each species of cotton and brought in the laboratory. Pollen grains from only one flower was shed onto each glass slide, scattered with camel brush so as to saturate the pollens completely with the medium. The inoculated slides were then carefully placed in Petri plates. The plates were covered half a way

Table 1: Mean differences in style and *in vitro* germinated pollen tube length and other reproductive structures of diploid and tetraploid cottons.

Ploidy level	Style length (mm)	*In vitro* pollen tube length (mm)	Stigma depth (mm)	Pollen grain size (mm)
Tetraploid cotton (2n = 4x = 52)	20.5	20.0	6.0	10.0
Diploid cotton (2n = 2x = 26)				
i. *G. Harknessii*	8.5	8.0	2.0	7.0
ii. *G. arboreum*	9.0	9.0	2.5	7.0
S.E.	2.0	2.5	2.0	1.5

The average were declared significntly different when the differences were twice as great or greater than the standard error (S.E.)

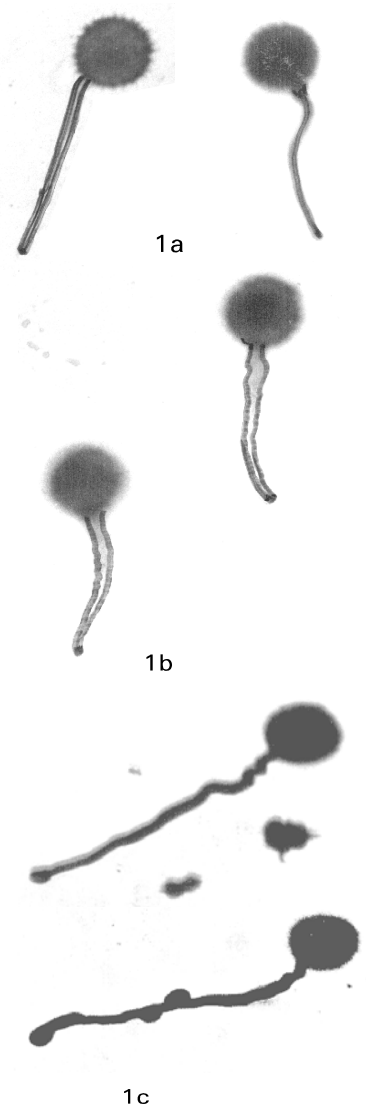

Fig. 1a: Pollen tubes of *G. arboreum* (diploid cotton) 1b, Pollen tubes of *G. harknessii* (diploid cotton). 1c, Pollen tubes of tetraploid (*G. hirstum*) cultivated cotton

in order to let the air flow freely into the Petri plates. After inoculation, the plates were kept at room temperature of 30°C. The pollen tubes were observed at five random microscopic fields under 10x magnification after 3.0 hours of incubation. Thus average tube lenght of 50 fields were calculated. A drop or two of 0.5% acetocarmine was added to germinating pollen tubes for staining. The tube lengths were measured in millimeters from negative films. The observations on 50 style length from each ploidy were taken from the tip of stigma to the tip of ovary in millimeters. The pollen grain sizes and stigma depths of both the ploidies were also recorded in millimeters. The pollen tube lengths were measured in millimeters from the negatives.

Results and Discussion

Cotton wild species nurture in natural environments for many years. Thus, nature has gifted these species with many desirable characteristics so as to survive in many adverse natural conditions. Cytogeneticists and plant breeders devote lot of efforts to transfer their desirable traits into the cultivated cotton, mainly through conventional hybridization procedures. However, crossing wild species with cultivated cotton is usually accompanied with various crossability barriers, including hybrid sterility mainly due to incompatibility between chromosomes of two species to pair at meiosis. In other cases, pollen grains even fail to germinate on the stigma due to incompatible S alleles residing in pollen grains and the styler tissues of different species (Poehlman, 1983). Embryologists still reason that failure of crossing may be contributed to pollen tubes that may not grow long enough in the styles and reach the ovary to fertilize it.

Several hypothesis have been put forth so as to determine if the differences in style length, stigma depth and pollen grain size of different species are accountable to the failure of crossing. *In vitro* germination studies of cotton pollen grains of two ploidy levels have therefore been carried-out to see if the differences in style length, stigma depth and pollen grain size are responsible for failure of crossing between two ploidies of cotton.

The results presented in Table 1 suggest that style length of tetraploid and diploids vary significantly where the average style length including stigma of tetraploid is more than twice longer than the diploid species. The stigma depth of two ploidy levels also varied where tetraploid produced stigma averaged 6.0 mm as compared to 2.0 and 2.5 mm of diploids, *G. harknessii* and *G. arboreum* respectively. Similar were the results for pollen grain size. The pollen grains of tetraploid were 10.0 mm in diameter as compared to 7.0 mm of diploids. These types of variation in reproductive structures of both the ploidies better explain the reason as why interspecific crosses between tetraploid and diploid usually fail. As suggested by embryologists that pollen grains had to retain sufficient food reserve to sustain the pollen tube growth to reach an ovule. Other scientists are of the opinion that not the pollen grain but the stigma depth contributes the resources to the pollen tubes to grow into the style and fertilizes the ovary. There is yet a third opinion that styler tissue serves as the transmission force and also provide nourishment to the pollen tube while it grows (Hopping and Jerram, 1979). To test these three hypothesis, Cruden and Lyon (1985) made studies on the relationship among style length,

stigma depth and pollen grain volume of 14 unrelated species of umbelliferae family. They found significant positive correlations between stigma depth and pollen grain volume only. They also reported significant differences in reproductive structures of two ploidies that definitely can pose problems in crossing. For example, if we assume that pollen grain is food source for pollen tubes to grow, then smaller pollen grains of diploids (Table 1) may not contain enough food reserves to nourish the pollen tubes to reach the longer styles of tetraploid and fertilize its ovary. Similarly, if the stigma depth is considered as the food reserve, then it can easily be speculated that shorter stigma of diploids may not again contain enough material to nourish the pollen tubes to grow into the longer styles of tetraploid cotton and travel all the way to fertilize their ovary. Thirdly, if the styles are considered as transmission tissues to nourish the pollen tubes, it again raises a question that, since pollen tubes of diploids are naturally shorter than the tetraploids, thus it becomes beyond the reach of diploid pollen tubes to fertilize to the ovary of tetraploid cotton. One can still argue that what could be the reasons if crosses between diploid with diploid also fail. Of course, it may be attributed to impairing between the chromosomes of two distinct species at meiosis, thus pollen formation or fertilization fails. However, supporting studies on the *in vitro* germination of pollen tube lengths of diploid and tetraploid were also carried-out so as to investigate if the differences in pollen tube length is accountable to the failure of crossing between the two ploidies. The *in vitro* geminated pollen tubes (Fig.1a, 1b, 1c) clearly demonstrate that pollen tubes of tetraploids measuring 20.0 mm were more than twice longer as compared to diploids (8.0 mm of *G. harknessii* and 9.0 mm of *G. arboreum* L.). Thus, quite substantial pollen tube lenght differences occur in two ploidy levels of cotton.

Very interestingly, our actual style length of tetraploid was 20.5 mm and diploids i.e., 8.0 and 9.0 mm respectively for *G. harknessii* and *G. arboreum* were approximately equal to *in vitro* germinated pollen tubes measuring 20.0 mm for tetraploid and 8.0 for *G. harknessii* and 9.0 mm for *G. arboreum*. The differential pollen tube length between tetraploid and diploid by *in vitro* germination studies suggested that failure of crosses could partially be explained to the differences in pollen tube length of two ploidies. It could further be expected that reciprocal crosses may be successful where shorter pollen tubes growing species is used as female parent instead of male parent with the assumptions that other reproductive structures do not impose any problem in the fertilization process of two ploidies. Grant and Grant (1960) also presumed that species with shallow stigma might not contain enough food material to support the pollen tubes to grow in the style of a species with a deep stigma. They further observed that stigma of *P. glaberrima* sp. interior wherry are nearly as long as the styles of *P. pilosa* sp. (Levin and Kerster, 1967) when *P. glaberrima* sp. interior was used as female parent, the cross failed, whereas the reciprocal cross produced 8 percent seed set.

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