

## STUDIES ON PALYNOLOGICAL FEATURES AND *IN VITRO* POLLEN TUBE GROWTH IN SOME *GOSSYPIUM* SPECIES

M.GUNASEKARAN and T.S. RAVEENDRAN

Department of Cotton,  
TNAU,  
Coimbatore - 641 003.

### ABSTRACT

Area, equivalent diameter, perimeter and roundness of the pollen grains were studied in seven diploid wild species and two tetraploid species of the genus *Gossypium*. The size of the pollen grain is correlated with ploidy level and pollen grains of the tetraploid species are larger than diploid species. Significant difference for the above characters were noticed in the pollen grains of the different species belonging to the same ploidy level. The germinated pollen grain in artificial medium produced longer pollen tube at initial period. The relationship between pollen size and breeding value are discussed.

**KEY WORDS:** Palynology, *Gossypium*, Ploidy level, Breeding value

The successful fertilization involves a matching genetic system in the parents involved in the gene regulated stepwise physiological and biochemical process and intimate pollen-pistil relationship. Therefore, the biology of the pollen grains is of great concern to the plant breeders because of its implication in crop breeding (Mulcahy and Ottaviano, 1983). Studies on pollen grain characters will be useful to characterise the species. This would be useful for selection of appropriate species for the wide hybridisation programme. Campell *et al.* (1993) observed difference in pollen size between diploid and tetraploid genotypes in *Hordeum*. But such studies and its relationship to initial pollen tube growth has not been previously described in *Gossypium*. The significance of genetic selection at the pollen level has been reported for various qualitative and quantitative traits in several compatible plant species (Pfahler *et al.*, 1996) This has been associated with difference among pollen grain containing different alleles at specific loci which compete in fertilizing the same ovule. The specific mechanism are not completely understood, but this competition must be associated with germination rate and the tube growth at the pollen level as well as with the genotype of the pistil through which the pollen tube grows. Variation for such characteristics would influence consistency and interpretation of pollen genotype selection useful for the wide crosses.

### MATERIALS AND METHODS

Area, equivalent diameter, perimeter and roundness of the pollen grains were measured on seven wild diploid species belonging to different

genomic group *viz.*, *Gossypium thurberi* (D1), *G. armourianum* (D2-1), *G. davidsonii* (D3-d) *G. gossypoides* (D6), *G. trilobum* (D8) *G. triphyllum* (B2) and *G. barbosanum* (B3) and two cultivated tetraploid species, namely, *G. hirsutum* (AD1) var MCU-5 and MCU-9 and *G. barbadense* (AD<sub>2</sub>) var, Suvin. The seed of wild and cultivated species were obtained from the Department of Cotton, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore.

Pollen grains were collected from freshly dehisced anthers by gently squeezing the anthers on a glass slide containing a drop of glycerol. Then coverslips were placed over the pollen and slides were examined under Leica microscope in ordinary light (visible light range). The observation were recorded from atleast 2.5 pollen grains for each field. The data were analysed in Q 500 MC Win software programme.

Studies on *in vitro* Pollen generation were carried out using Brewbaker and Kwack (1963) artificial medium with modified composition described by Gunasekaran (1997). The pollen used for this investigation was preconditioned.

Pre-conditioned pollen grains of cultivated and wild species were dusted on a drop of medium placed on a cavity slide and observed under the microscope. The length of the pollen tube was measured from the base (Point of emergence) of the pollen tube emerged from the pollen to the tip of the pollen tube and expressed in microns. The measurements were made at 5.10 and 15 minutes after incubation.

Table 1. Area, Equivalent diameter, Perimeter and Roundness of the pollen grains of *Gossypium* species

| Species                         | Area ( $\mu\text{m}^2$ ) |                              |               | Equivalent diameter ( $\mu\text{m}$ ) |               |                           | Perimeter ( $\mu\text{m}$ ) |                         |       | Roundness |       |         |
|---------------------------------|--------------------------|------------------------------|---------------|---------------------------------------|---------------|---------------------------|-----------------------------|-------------------------|-------|-----------|-------|---------|
|                                 | Range                    | Mean SE                      | Range         | Range                                 | Mean SE       | Range                     | Range                       | Mean SE                 | Range | Mean SE   | Range | Mean SE |
| <i>G. thurberi</i>              | 5997.01-7087.65          | 6650.21 79.99 <sup>b</sup>   | 87.38-95.00   | 91.99 0.55 <sup>b</sup>               | 292.26-324.93 | 310.87 2.11 <sup>cd</sup> | 1.04-1.15                   | 1.09 0.01 <sup>b</sup>  |       |           |       |         |
| <i>G. armourianum</i>           | 6466.96-9132.45          | 7805.87 403.57 <sup>b</sup>  | 90.74-107.84  | 99.82 2.69 <sup>b</sup>               | 304.30-364.47 | 335.83 9.58 <sup>bc</sup> | 1.01-1.10                   | 1.08 0.00 <sup>bc</sup> |       |           |       |         |
| <i>G. davidsonii</i>            | 6014.75-6939.86          | 6393.28 62.03 <sup>b</sup>   | 87.51-94.00   | 90.21 0.43 <sup>bc</sup>              | 297.42-314.81 | 301.97 1.21 <sup>cd</sup> | 1.02-1.10                   | 1.07 0.01 <sup>cd</sup> |       |           |       |         |
| <i>G. gossypoides</i>           | 4525.10-5813.76          | 5303.70 161.14 <sup>c</sup>  | 75.90-86.04   | 82.11 1.20 <sup>c</sup>               | 256.16-293.88 | 273.60 4.53 <sup>c</sup>  | 1.03-1.11                   | 1.06 0.01 <sup>c</sup>  |       |           |       |         |
| <i>G. trilobum</i>              | 3523.13-7628.53          | 6484.69 372.32 <sup>b</sup>  | 66.97-98.55   | 90.45 2.90 <sup>b</sup>               | 228.65-326.65 | 300.86 9.18 <sup>bc</sup> | 1.03-1.11                   | 1.06 0.01 <sup>c</sup>  |       |           |       |         |
| <i>G. triphyllum</i>            | 5323.12-7202.92          | 5959.85 235.76 <sup>b</sup>  | 82.33-95.76   | 87.00 1.68 <sup>bc</sup>              | 273.35-330.09 | 290.30 7.28 <sup>de</sup> | 1.02-1.13                   | 1.08 0.01 <sup>b</sup>  |       |           |       |         |
| <i>G. barbosanum</i>            | 6803.90-7826.56          | 7410.80 253.36 <sup>b</sup>  | 93.07-99.82   | 97.09 1.68 <sup>b</sup>               | 314.61-347.28 | 332.38 7.79 <sup>cd</sup> | 1.09-1.15                   | 1.12 0.01 <sup>a</sup>  |       |           |       |         |
| <i>G. hirsutum</i> var. MCU 5   | 9941.00-12321.64         | 11020.23 210.21 <sup>c</sup> | 114.45-128.00 | 118.31 1.12 <sup>c</sup>              | 371.50-422.65 | 390.51 3.97 <sup>c</sup>  | 1.03-1.11                   | 1.06 0.01 <sup>d</sup>  |       |           |       |         |
| <i>G. hirsutum</i> var. MCU 9   | 9930.98-12691.56         | 10960.23 208.30 <sup>c</sup> | 112.45-127.12 | 118.06 1.11 <sup>c</sup>              | 376.50-417.77 | 393.70 3.96 <sup>c</sup>  | 1.03-1.11                   | 1.06 0.01 <sup>d</sup>  |       |           |       |         |
| <i>G. barbadense</i> var. Suvin | 9333.94-10956.69         | 10326.54 214.77 <sup>c</sup> | 109.01-118.11 | 114.63 1.20 <sup>c</sup>              | 361.03-398.85 | 383.38 5.19 <sup>de</sup> | 1.03-1.10                   | 1.06 0.01 <sup>d</sup>  |       |           |       |         |

In a column, means followed by common letters are not significantly different at the 5% level by DMRT.

The materials were replicated three times with five slides per replication. The data were analysed in completely randomised design (CRD) after transforming the percent values into angles and transformed means were compared using Duncans Multiple Range Test. The slides were photographed using builtin camera of Leica microscope.

## RESULTS AND DISCUSSION

Significant difference among the species means were found for all the characters studied (Table-1). The pollen grains of tetraploids had significantly larger area, equivalent diameter and perimeter than that of diploid wild species. Larger size of tetraploid pollen grains may be due to increased ploidy level. Bothmer *et.al.*, (1993) noticed similar difference in pollen size between diploid and polyploid of *Hordeum sp* and attributed this due to increased volume of the nucleus. The influence of ploidy level on the pollen grains has also been discernible in crops like maize (Pfahler and Wilcox, 1983), rice (Anwar and Reddy, 1980) wheat and barley (Franke, 1981). Among the two tetraploid species, *G.hirsutum* var.MCU-9 had higher mean area (10960.23  $\mu\text{m}^2$ ), perimeter (393.70  $\mu$ ) and equivalent diameter (118.06  $\mu\text{m}$ ) than *G.barbadense*.

The diameter of the pollen grain was reported

Table 2. *In vitro* pollen tube growth of parents in artificial medium

| Parents                        | Length of pollen tube ( ) min. after incubation |                  |                  |
|--------------------------------|---|------------------|------------------|
|                                | 5   | 10               | 15               |
| <i>G.thurberi</i>              | 633 <sup>f</sup>                                | 643 <sup>b</sup> | 643 <sup>f</sup> |
| <i>G.armourianum</i>           | 622 <sup>e</sup>                                | 633 <sup>i</sup> | 633 <sup>b</sup> |
| <i>G.davidsonii</i>            | 662 <sup>c</sup>                                | 674 <sup>f</sup> | 623 <sup>i</sup> |
| <i>G.gossypoides</i>           | 612 <sup>b</sup>                                | 622 <sup>i</sup> | 623 <sup>i</sup> |
| <i>G.trilobum</i>              | 694 <sup>d</sup>                                | 715 <sup>c</sup> | 725 <sup>d</sup> |
| <i>G.triphyllum</i>            | 694 <sup>d</sup>                                | 705 <sup>f</sup> | 705 <sup>c</sup> |
| <i>G.barbosanum</i>            | 716 <sup>c</sup>                                | 735 <sup>d</sup> | 735 <sup>c</sup> |
| <i>G.hirsutum</i> var. MCU 5.  | 788 <sup>a</sup>                                | 806 <sup>e</sup> | 807 <sup>a</sup> |
| <i>G.hirsutum</i> var. MCU 9   | 765 <sup>b</sup>                                | 794 <sup>b</sup> | 809 <sup>a</sup> |
| <i>G.barbadense</i> var. Suvin | 765 <sup>b</sup>                                | 777 <sup>c</sup> | 778 <sup>a</sup> |

In a column, means followed by common letters are not significantly different at 5% level by DMRT.

to be influenced by gametophytic genotype in maize (Johnson *et al.*, 1976 ). But later it was demonstrated that the pollen grains diameter is a fairly stable and highly heritable character influenced primarily by the genotype of the mother plant.(Kumar and Sarker,1983). However Johanson and Bothmer (1994) reported that polyploidy level alone did not determine the absolute size of the individual pollen grains in *Hordeum*. Significant variation for area, equivalent diameter and perimeter were also recorded among the diploid wild species. *G.armourianum* had significantly highest mean value for area (7805.87  $\text{m}^2$ ), equivalent diameter (99.82  $\text{m}^2$ ) and perimeter (335.83  $\text{m}$ ) while *G.gossypoides* recorded the lowest value for the above characters.

The variation among the pollen grain of different species belonging to same ploidy level may be attributable to the differences in the genome constitution of the species. Hence this will be a useful parameter for characterisation of the species. Within the species, variation may however be attributed to the environment that prevailed during the development of the pollen.

It has been found that the pollen grain size was controlled by polygenes which are sensitive to the environment and pollen diameter was mainly controlled by additive components followed by dominance effects with greater influence of environmental variation in maize (Kumar and sarkar, 1984) and sesame (pfahler *et al.*, 1996). The present investigation revealed that pollen grain was not perfectly round in any of the species and the ploidy level did not have any influence on the shape of the pollen grain. However, the shape of the pollen grain has not been reported to have any breeding value.

The relationship between pollen grain size and competitive ability to grow in artificial medium under *in vitro* condition was studied (Table 2 ). The germinated tetraploid pollen grain grew faster and produced significantly larger pollen tube than diploids. The effect was maximum during the initial period of growth and declined thereafter. Such a correlation would appear logical since larger pollen grains contain more energy reserves for improved germination rate at initial period. This is in concurrence with the studies made by Neuffer *et.al.* (1968) in wheat.

Thus, the influence of ploidy level in the size of the pollen grain is evident from the present study in cotton.

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## CORRELATION AND PATH ANALYSIS IN COWPEA

(*Vigna unguiculata* (L.) Walp)

R.KALAIYARASI and G.A.PALANISAMY

Centre for Plant Breeding and Genetics  
Tamil Nadu Agricultural University  
Coimbatore - 641 003

#### ABSTRACT

Correlation and path analysis was carried out using 12 cross combinations of cowpea in  $F_1$  and  $F_2$  generations for nine component characters including seed yield. Number of branches per plant, number of pods per plant and plant height had positive correlation with seed yield both at genotypic and phenotypic levels in  $F_1$  and  $F_2$  generations. Path analysis showed positive direct effects of number of branches per plant, plant height, pod length, hundred seed weight on seed yield in  $F_1$  generation while the traits hundred seed weight and seeds per pods had positive direct effect on seed yield in  $F_2$  generations. These traits should be given more emphasis while selecting for improvement in seed yield per plant.

KEY WORDS: Cowpea, Correlation, Path analysis