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Impact of Pollen and Intergenetic Crosses Between Graminaceous (Poaceae) Plants

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Abstract

The pollen grains belonging to 17 different species of Gramineae were collected from Punjab University, New campus, Lahore during the spring/summer of 1984. The preservation and mounting media were farmer's fluid, cornoy's solution and molten jelly respectively. The size of pollen ranges from 17.50 ± 1.96 μ (width)/17.97 ± 1.67 μ (length) in *Imperata cylindrica* to 58.13 ± 1.96 μ (width)/64.49 ± 0.68 μ (length) in *Triticum aestivum*. The pollen of tetraploid and hexaploid species were larger in size as compared to those of diploids. The post pollen mitotic divisions do not occur at the site of production of pollen. Meiotic stages were observed in PMCs of some of the 17 plants. The results are discussed in relation to breeding programme leading to the formation of at least another " man made genus" Tritipoa or "Tritipogon".

Introduction

Palynology is the science of pollen and spore morphology. It has application in plant taxonomy, geology, climatology and geography. Pollen analyses is used as a means of tracing the history of cultivated cereals. It is an index tool to investigate asthma, alergies, medicine, oil and gas exploration. Hyde and William (1944) worked on both fossil and living pollens. Meo *et al.* (1988) described pollen morphology of seven different graminaceous species for comparison. Meo *et al* (1989) worked on pollen grain described with reference to allergies in 17 graminaceous species and reported that four species viz. *Poa annua. Imperata cylindrica. Avena sativa and Triticum aestivum* cause pollenosis while rest of the 13 species do not play any role in pollenosis. Ellis (1986) confirmed that pollen irradiation in barely crosses can cause deviations from expected segregation ratios for certain characters. The reduced fertility observed in these crosses could be problematical in breeding programmes. Hashemi *et al* (1986) observed that chromosome pairing and pollen fertility in Parthenium (Asteraceae) is striking because there are many morphological differences between the parents. A technique for artificially culturing detached wheat tillers has been developed through physiological research on immature seed vernalization (Inagaki and Tahir, 1995). Inagaki (1997) found that stored pollen and detached-tiller culture was useful as a strategy for reducing the time required to obtained homozygous breeding lines and improving selection efficiency of wheat breeding programs. Inagaki (1997) also concluded that stored pollen can be used for crossing on wheat when fresh pollen is not available. The aim of present investigation was to study the i) post-pollen division from uninucleate condition ii) established the chromosome number of a particular plant species even when roots and immature flowers were not available, and iii) to determine the size of pollens and attempt to find out possible parents that could take part in a cross involving the bred wheat *(Triticum aestivum)* and a grass species thus producing one or another synthetic genus, similar to man-made genus Triticals.

Materials and Methods

The studies were conducted in the Department of Botany, University of the Punjab, Lahore. The plants were collected from the Botanical Garden and the other adjoining areas, for the purpose of cytomorphological studies of pollens. The collection was made throughout the year. The seventeen species from the Sub-families poaideae and panicoideae of Gramineae (poaceae) were selected. The flowering season for all the species ranges between August and March. The study was divided into the following:

Pollen Isolation and their morphology: The spikelets of these species were collected at mature stage from fresh, undehised anther between 7 to 9 a.m in farmer's fluid. The pollen grains were acetolyzed using the technique presented by Erdtman (1960). The pollen were isolated by centrifugation and then these were treated with 5-7 per cent KOH solution and one part concentrated HCl and then distilled water rinse was given by centrifugation after each treatment. Finally molten glycerine jelly was added into the centrifuged tube and mixed with pollen grains by warming slightly. The terminology follows that of Praglwski and Raj (1979). The prepared slides were palynologically studies under Polish Research Microscope, at high power 40X. The data of twenty five pollen grains of each species were recorded and average was calculated for breadth and length of the pollen grain. The recorded data was analyzed by chi-square.

Meiotic and post-pollen Division: For the purpose of cytological examination the immature as well as mature panicles/spikes of different species of Gramineae were fixed separately in cornoy's solution (6:3:1 fixative) to which a few drops of ferric chloride in 45 percent acetic acid were added as mordant. The material was fixed at 6 a.m. After 24 hours the spikes/panicles were transferred separately in 70 per cent alcohol. For cytological examination one of the anthers was used till the required stage (metaphase I/anaphase I/telophase I and II) were recorded. The slides for pollen mother cell (PMCs) was prepared by putting the

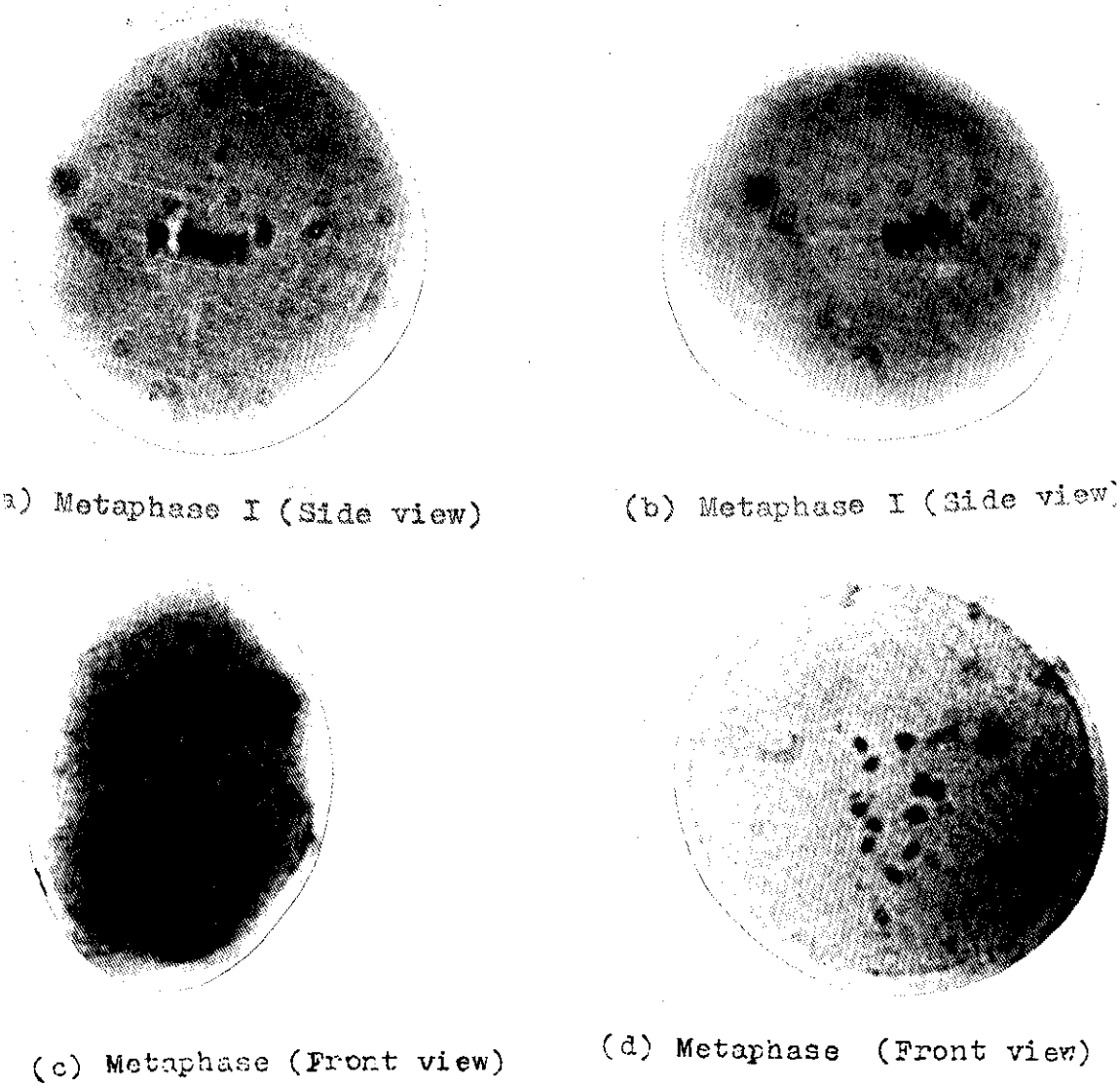


Fig. 1: *Zea mays*

anther on the slide in a drop of acetocarmine and cutting it into two halves with a needle. The cover slip was placed and the slide was warmed gently. In order to observe the chromosomes separated out from one another and exhibiting a particular phase, such anther were kept in snow's solution for about 2 hours of 60°C and their squashes were prepared in 45 per cent acetic acid (Snow, 1963).

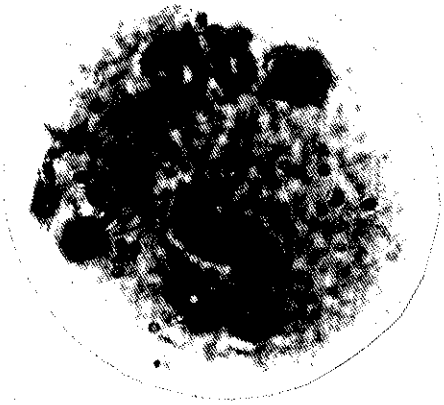
Results and Discussion

The results regarding the size (length and breadth) of the pollen and the chromosome number are presented in Table 1 which shows that pollen length in Gramineae ranges from 17.97 ± 1.67μ in *Imperata cylindrica* to 64.49 ± 0.68 μ in *Triticum aestivum*. Similarly the pollen width ranges from 17.50 ± 1.31 μ in *Imperata cylindrica* to 58.13 ± 1.96 μ in *Triticum aestivum*. The Table 1 also indicate that larges pollens were of *Triticum aestivum* and smallest of *Imperat cylindrica*. These results are in agreement with finding Chapman and Riley (1970). Gupta (1971), Zahur *et a* (1975-78). The largest size of the pollen grains of *Triticu aestivum* and *Avena sativa* and smallest size in *Imperat cylindrica* could be due to ploidy level. The pollens tetraploid and hexaploid species are larger as compared that of diploid species. In no case the post-pollen mitos was observed in the preserved material and also wh pollen were subjected to germination. However, during th process the pollen mother cells were observed with vario meiotic stages Fig. 1 and 2 in *Zea mays* and *Pennisetu typhoides*. The non-observance of post pollen divis points to the fact that germination of the pollen grains w a-vis the activity of the nucleus does not initiate as long

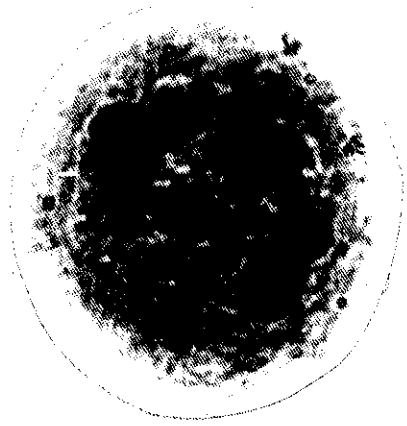
(e) Anaphase (Side view)

(f) Anaphase (Side view)

Plate II Pennisetum typhoids



(g) Metaphase (Side view)



(h) Metaphase (Front viwe)

Fig. 2: *Pennisetum typhoids*

the pollen is present on the site of its production i.e. pollen chamber. This was the reason that author was not able to locate and observe that post pollen division. However, the basic chromosome number equal to 7 coupled with similarities of others in morphological characters indicate that species like *Lolium termulentum, Pennisetum typhoides, Avena, sativa, Koeleria phalloids, Hordeum vulgare, Polypogon, monspeliensis* and *Poa annua* can be used in breeding programme. Similar studies are also in progress in other parts of the world (Gupta, 1972). The sub-tribe Triticinae possess X = 7 as the basic chromosome number. The homologous relationship that exists between individual chromosomes from different genera of hexaploid wheat can also be extanded to other members with basic chromosome number equal to 7. The best man made genus "Triticale" has assumed the status of cash fodder crop. In our country *polypogan monspeliensis* and *Poa annua* could be used in breeding projects as both have seven basic chromosome number and they occur in and around wheat fields. These results lead to the formation of another man made genus "Tritipogon or Tritipoa". The research on pollen germination both in "Vivo" and "Vitro" coupled with studies on morpho-logy and cytology of grasses possessing seven as the basic chromosome number should be able to

Table 1: Comparative data showing size of the pollen with basic, diploid and polyploid chromosome number of some graminaceous species.

Name of Plants	Size Range	Chromosome Number
Poa annua	24.13 ± 0.56 μ (24.88) ± 0.13 μ	X = 7, 28
Eragrastis poaeoids	26.63 ± 0.99 μ (28.93) ± 1.26 μ	
Eleusine flagellifera	30.61 ± 2.41 μ (32.83) ± 1.33 μ	X = 9, 45
Koeleria phalleoides	25.40 ± 2.11 μ (29.21) ± 1.64 μ	X = 7
Avena sativa	53.02 ± 3.22 μ (57.17) ± 2.82 μ	X = 7, 42
Lolium temulentum	41.77 ± 1.97 μ (42.32) ± 2.24 μ	X = 7, 14
Triticum aestivum	58.13 ± 1.96 μ (64.49) ± 0.63 μ	X = 7 (21) (63)
Hordeum vulgare	47.50 ± 1.31 μ (49.31) ± 0.63 μ	X = 7, 14 28
P. monspeliensis	34.63 ± 1.26 μ (36.40) ± 0.90 μ	X = 7, 28
Sporobolus pallidus	30.15 ± 1.04 μ (33.09) ± 0.56 μ	X = 9, 10, 12
Oryza sativa	27.38 ± 0.66 μ (30.52) ± 0.91 μ	X = 12, 24 (36, 48)
Cenchrus setigerus	31.78 ± 0.24 μ (39.12) ± 1.09μ	X = 9, 17 34 36
C. penisetiformis	24.72 ± 0.23 μ (31.31) ± 3.69 μ	X = 9, 17
P. typhoides	35.41 ± 0.77 μ (37.99) ± 0.85 μ	X = 7,9 14-17
Imperata cylindrica	17.50 ± 1.31 μ (17.97) ± 1.67 μ	X = (5) 10 20
Sorghum helapense	23.93 ± 0.65 μ (28.56) ± 0.90 μ	X = 5,20,40 40
Sorghum vulgare	37.42 ± 1.13 μ (39.94) ± 1.26 μ	X = 5,20

Results. N.S
N.S = Non-significant
μ = Microne
± = Standard error of the means
Value within brackets pertain to lenght and those outside brackets represents the width of the pollen.

determine not only the chromosome number of a particular species even when the roots and young flowers etc. are not available but also some useful members for crossing with Triticum and arrivest but also some useful members for crossing with Triticum and arrivest a goal of another man made genus corresponding to Tritical. The plant breeding and research worker undertaking such studies would have to surmount all types of difficulties and most important of which is homologous relationship between the likely parents.

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