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PN Jagadev
Department of Plant Breeding and Genetics, OUAT, Bhubaneswar, Odisha, India

VK Khanna
Department of Genetics and Plant Breeding, GBPUAT, Pantnagar, Uttarakhand, India

Corresponding Author
PN Jagadev
Department of Plant Breeding and Genetics, OUAT, Bhubaneswar, Odisha, India

Role of Obturator in pollen tube entry into the ovary in wheat-rye crosses

PN Jagadev and VK Khanna

Abstract
The Obturator, a transmitting tissue between the stigma and the ovary plays a critical role in controlling the direction of pollen tube entry into the ovary and was related to the release of chemotactic substances like carbohydrates, proteins and callose at the base of the style and in the ovary wall. Immediately after Selfing of the wheat cultivars *viz*. Chinese Spring (CS) and Hope, insoluble carbohydrates were produced in the extra-ovarian region and in the Obturator, through which the pollen tube had to pass. In CS x rye, the situation was similar to that in selfings. But the crosses between Hope and rye, during the initial hours after pollination, there was no staining in the Obturator region although there was uniform light staining of the extra-ovarian region resulting in loss or reduction of seed set. The secretion of protein was delayed in the Obturator of Hope x rye crosses as compared to CS x rye and the selfings of CS and Hope. It thus seems that once pollen tube movement is delayed in the initial period after pollination, fertilization may be prevented due to lack of secretion of proteins resulting in reduced or no seed set in Hope x rye. In selfings and CS x rye crosses, Callose was not present till 72 h after pollination, but callose was present in the entire ovary region in Hope x rye crosses, which prevents the pollen tube growth.

Keywords: Wheat, rye, pollen tube, obturator, carbohydrates, protein, callose
Abbreviations: EC-egg cell, en-endosperm, ES-embryo sac, FU-funicle, ob-obturator, OV-ovule, OY-ovary, PN-polar nuclei, PT-pollen tube

Introduction
It is known that Chinese Spring has often been selected as a standard cultivar in the genetic study of wheat (*Triticum aestivum* L. em. Thell.) Primarily for its easy crossability with rye (*Secale cereale* L.). But it would be very useful to study some material which shows no crossability or very little crossability, as it may be having some important genes. Hence, it is important to know the causes of pre-fertilization barriers as to why pollen tubes cannot go beyond the base of the style and into the ovary wall. The obturator is a protuberance of tissue on the carpellary wall facing the micropyle and played a major role in pollen tube guidance (Peterson *et al*., 1979) [8]. Arbeloa and Herrero (1987) [1] investigated the changes in the obturator of the peach (*Prunus persica*) and related these to pollen tube growth. According to them, pollen tube growth along the obturator surface depends on the secretions from the cells of the obturator. The discontinuous secretion may provide a mechanism that controls the entrance of the pollen tubes into the ovary in peach. Santosh and Khanna (1996) [10] studied the role of the obturator in pollen tube entry into the ovary in wheat-barley crosses. Carbohydrates, proteins and callose were found to be responsible in preventing or permitting pollen tube growth towards the egg for fertilization. However, a detailed study of pollen tube growth in this area is so far lacking. Information is also missing on any possible changes in this region accompanying pollen tube growth. Hence, the present investigation was undertaken to study the relationship of the changes in the obturator to pollen tube growth occurred in this region with respect to poor seed set in wheat–rye crosses.

Materials and Methods
The experimental material consisted of two genotypes of hexaploid wheat, *viz*. Chinese Spring (CS) and Hope and a diploid rye genotype (Rye 8461). The seeds were sown on two different dates during December. Emasculation was done during the morning and the evening hours on randomly selected plants when anthers were still pale green. Two days later, when the stigmas were feathery and receptive, the pollinations were performed in the month of March by hand pollinating the pistils with dusting of the pollens from dehiscing anthers of the male parents. To study the process of fertilization and early development of the seed, the pistils were collected at 2, 8, 24, 48 and 72 h after pollination and fixed immediately for 2 h and then preserved in 70 percent ethanol (Morrison *et al.*, 1959) [5].

Dehydration, infiltration and embedding were performed according to the ethanol tertiary butanol paraffin schedule of Sass (1958) [11]. For microtomy, a Spencer rotary microtome was used. The material embedded in wax was cut to 10 µm thickness. The paraffin ribbons affixed with Haupt's adhesive (Prasad and Prasad 1975) [8] were stained with periodic acid Schiff's reagent (Feder and 0' Brien, 1968)[2] for insoluble carbohydrates, with coomassie brilliant blue (Fisher, 1968) [3] for proteins, with aniline blue for callose and with safranin, crystal violet and light green (Gerlach, 1969) [4] for embryosac development. The permanent slides were prepared with DPX mountant and the photomicrographs were taken with the help of an olympus trinocular microscope equipped with an automatic camera.

Results and Discussion

In this study, the emphasis has been given to the obturator, an organ of special significance, which facilitates the entry of the pollen tube into the ovule. This tissue is continuous with and of the same cytohistology, as the transmitting tissue from the style (Sterling, 1964) [11]. Usually, it is a swelling of the placenta, which grows towards the micropyle and fits like a hood or canopy over the nucellus, serving as a sort of bridge for the pollen tube. Often, the obturator cells may be greatly elongated or may have a glandular appearance. After pollination in wheat and wheat-rye crosses, the secretions produced in the cells of the style, the obturator and the ovule were studied at 2, 8, 24, 48 and 72 h after pollination in longitudinal sections of the ovary.

PAS Staining for Insoluble Carbohydrates

Periodic acid-Schiff's (PAS) reagent stains violet, showing the presence of insoluble carbohydrates. In the case of selfing of wheat (CS and Hope), at 2-24 h after pollination, the extra –ovarian portion along with the obturator was stained (Plate-1). At 48-72 h after pollination, the extra-ovarian portion including the obturator took a dark stain, but some parts of the central portion of the ovule were also lightly stained. In the case of CS x rye, the situation was similar to that in selfings. At 2-8 h after pollination and the extra-ovarian portion along with the obturator and some inner parts of the ovule were stained. From 24-72 h after pollination, there was a consistent presence of insoluble carbohydrates at the extra-ovarian portion and the obturator. There was an increase of crease (space between ovary and ovule) and empty spaces in the ovule with the passage of time. When Hope was crossed with rye, different results were obtained. From 2-24 h post-pollination, the extra-ovarian tissues were lightly stained, but the obturator was not stained (Plate-2). At 48-72 h post-pollination, there was a complete absence of insoluble carbohydrates. At 72 h of pollination, there was a complete degeneration of endosperm and embryo in the ovule.

Coomassie brilliant blue staining for protein

This reagent stains dark blue showing the presence of protein. In the case of selfing of wheat (CS and Hope), from 2-24 h after pollination, a constant and uniform stain was observed in the extra-ovarian tissues including the obturator and the ovule (Plate-3). At 48-72 h post-pollination, the obturator and the ovule took a darker stain than the adjoining extra-ovarian tissues. When CS was crossed with rye, the trend of staining was almost like the selfing in wheat, but there was degeneration of endosperm and an increase of crease and the empty space size in the ovule with the increase in period after pollination from 24-72 h post pollination. In the case of Hope x rye cross, there was no staining in the extra-ovarian portion including the obturator and the ovule from 2-8 h after pollination. At 24-48 h post-pollination, the obturator and the ovule were stained and by the end of 72 h of pollination, the obturator and the inner wall of the degenerated ovule got stained and the extra-ovarian portion did not take the stain (Plate-4). Hence, once the pollen tube movement was delayed at the initial period after pollination for want of protein, then the fertilization might be prevented, resulting in less or no seed set.

Aniline blue staining for callose

Aniline blue indicates the presence of callose, a cell wall polysaccharide consisting mainly of β-1,3 glucan, but which may also contain β-1,4 glucosidic linkages. In the case of selfing of wheat (CS and Hope) and in CS x rye cross, there was no staining in the extra-ovarian tissues, but a light staining was observed in the ovule and the nearby obturator region from 2-72 h post-pollination (Plate-5). In CS x rye cross, there was an increase in the size of the crease (space between ovary and ovule) with the increase in time of pollination. But, in Hope x rye, from 2-48 h after pollination, the extra-ovarian portion along with the obturator and the ovule got stained (Plate-6). At 72 h of pollination, the obturator along with the entire ovary took a dark stain and there was a complete degeneration of endosperm in the ovule.

Safranin-crystal violet-light green staining for embryo-sac development

In the case of CS selfing, at initial period after pollination, the ovule was normal and internal cells of the ovule were at the dividing stage. By the end of 72 h, a clear-cut and well-developed embryo was seen. This might result in normal and high seed set (Neeraj and Khanna, 1992) [6]. In the case of selfing of Hope, the results were quite similar to those after CS selfing, but by the end of 72 h of pollination, there was no embryo formation and post-fertilization stage was still continuing. In CS x rye, from 2-8 h after pollination, the ovule development was normal, but at 24-48 h post-pollination, there was an increase in the empty space outside the central cells of the ovule and at the end of 72 h of pollination, a degeneration and shrunken embryo was seen. In the case of Hope x rye, at 2 h after pollination, the ovule development was normal, but at 8-48 h after pollination, there was an increase in the crease and empty space outside the ovule central cells. At 72 h of pollination, there was a complete degeneration of endosperm and no embryo was seen (Plate-8). In the case of CS selfing and CS x rye cross, the time taken to form the embryo, irrespective of the normal and the shrunken one, was almost the same. This might be due to the fact that the female gametophyte of both were the same and they were responsible for the timing of the embryo formation after pollination. As regards to Hope selfing and Hope x rye cross, the entire process of post –fertilization development seemed to be delayed as there was no clear cut embryo formation even after 72 h.

The growth of the pollen tubes on the obturator depends on the presence of secretions. The dependance of the pollen tube on secretion, together with fact that the secretion is not continuous, gives the obturator a significant role in controlling communication from the style to the ovary in wheat and rye and their crosses. Thus, the obturator will act as a temporary bridge connecting the ovary with the style.

Connection is established only during the secretion phase, because, before it takes place, the obturator is unable to support pollen tube growth. No growth is possible later when the obturator gets filled with callose or degenerates, isolating the ovary region again. Hence, the obturator played a significant role in the regulation of fertilization to form the seeds in wheat-rye crosses.

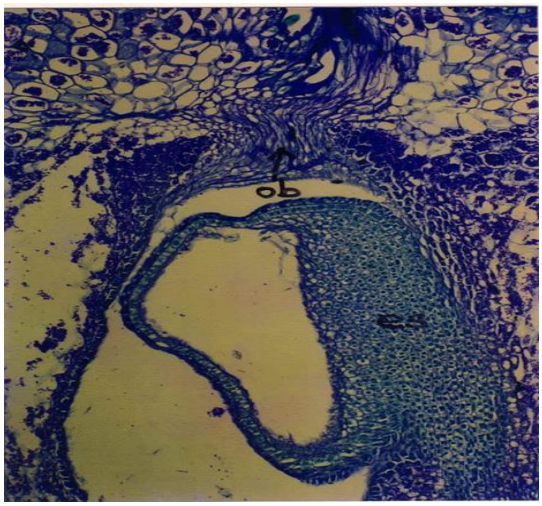

Plate 1: 24 h after Selfing in CS (100 X) (PAS stain)

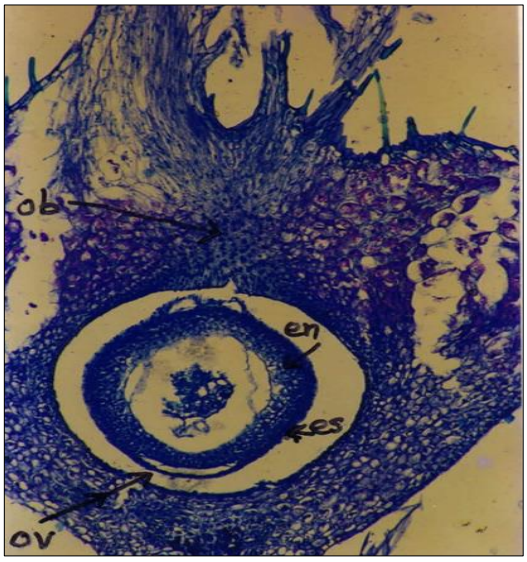

Plate 2: 2 h after pollination in Hope x Rye (40 X) (PAS stain)

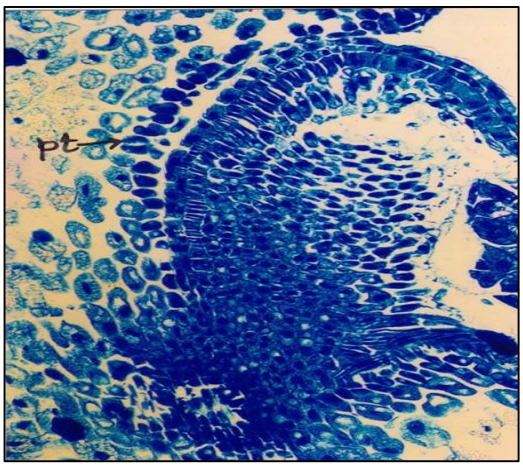

Plate 3: 8 h after Selfing in CS (200 X) (Coomassie brilliant blue stain)

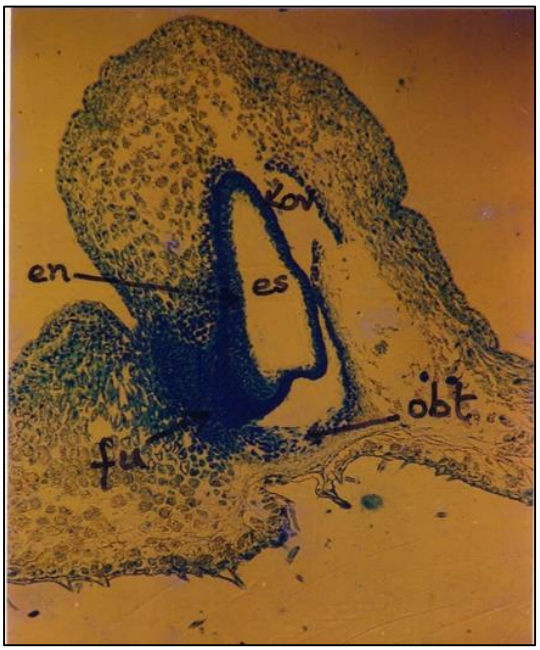

Plate 4: 72 h after pollination in Hope x Rye (Coomassie brilliant blue stain)

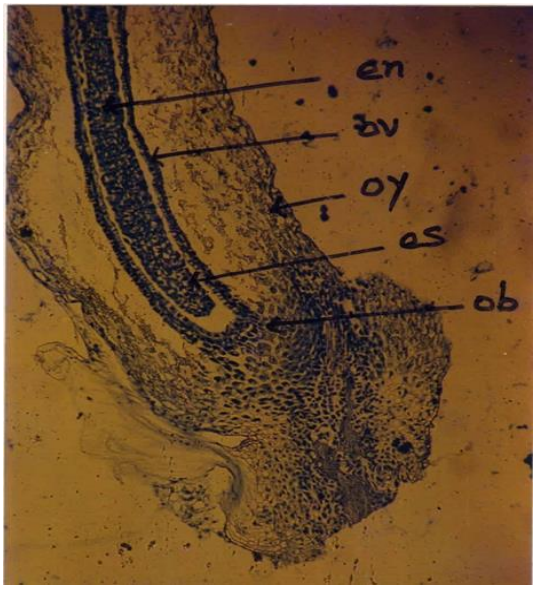

Plate 5: 8 h after Selfing in Hope (40 X) (Aniline blue stain)

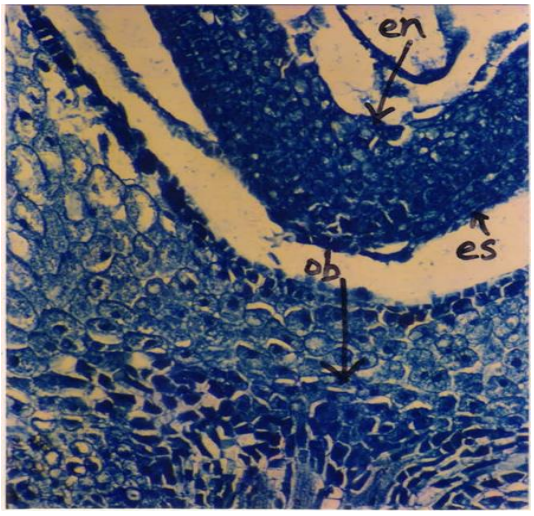

Plate 6: 24 h after pollination in Hope x Rye (100 X) (Aniline blue stain)

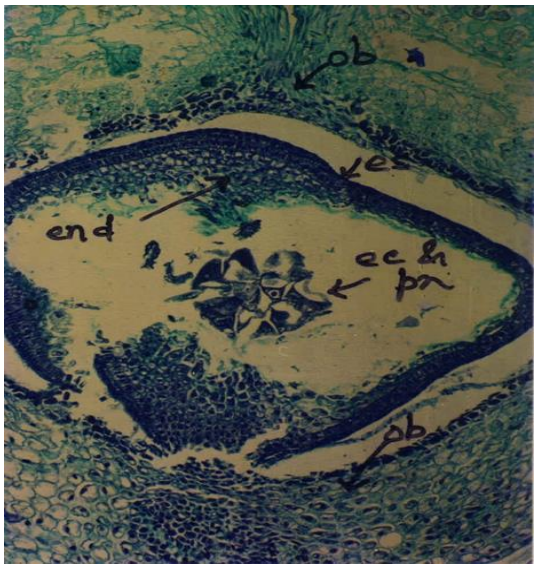

Plate 7: 24 h after selfing in CS (100 X) (Safranin-crystal violet-light green stain)

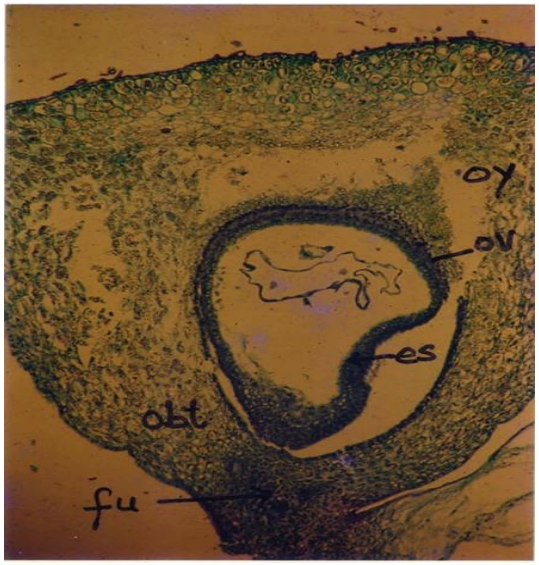

Plate 8: 72 h after pollination in Hope× Rye (40 X) (Safranin-crystal violet-light green stain)

Plates 1-8: A portion of the ovule and the obturator.

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