International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(4): 2398-2402

Received: 16-05-2018
Accepted: 18-06-2018

Usha Pant
Department of Genetics and Plant breeding, College of AgricultureG. B. Pant University of Agriculture and Technology, Pantnagar, USN, Uttarakhand, India

AnupreetKour
Department of Genetics and Plant breeding, College of Agriculture G. B. Pant University of Agriculture and Technology, Pantnagar, USN, Uttarakhand, India

VK Khanna
Department of Genetics and Plant breeding, College of Agriculture G.B. Pant University of Agriculture and Technology, Pantnagar, USN, Uttarakhand, India

Correspondence
Usha Pant
Department of Genetics and Plant breeding, College of Agriculture G. B. Pant University of Agriculture and Technology, Pantnagar, USN-263 145, Uttarakhand, India

Study of Post-fertilization developments in wheat-maize system of haploid production

Usha Pant, Anupreet Kour and VK Khanna

Abstract
To study the post hybridization events in wheat-maize system wheat f_1's were crossed with the maize (pollen parent). Three types of staining viz; Coomassie Brilliant Blue staining for protein was done to observe the embryo development and growth stages of haploid embryos. In crossed seeds the amount of protein started depleting and degenerated endosperm, antipodal and egg nuclei were observed. The insoluble carbohydrates were absent after 48hr of pollination. Crease was observed along with deformed pre-embryos. In case of crosses again deformed pre-embryo, degenerated antipodal cells and endosperm were observed. In some of the cases only the pre-embryo was found. The empty space was observed outside of the ovule and central cells due to degeneration of inner cells.

Keywords: Wide hybridization, wheat, haploid embryo, microtomy, Wheat x maize crosses

Introduction
Wheat is the premier food crop of worldwide importance. It is also a crop where conventional plant breeding has paid rich dividend, as epitomized by the green revolution. Common wheat is a self-pollinated hexaploid (2n=6x=42) species. Polyploidy of wheat (hexaploid and tetraploid) is another advantage of this crop, which is considered to contribute to its worldwide adaptability. Haploid production involves chromosome elimination during inter-specific or inter-generic hybridization followed by chromosome doubling of haploid plantlets. Laurie and Bennett (1986) [15] used maize as the pollen parent to produce wheat haploid plants. Double haploid (DH) system is a biological tool, which has been widely applied in wheat breeding programmes (Moieni *et al*., 1997) [17]. The system is developed through haploid production; followed by chromosomal doubling, to produce homozygous plant in a single generation. Haploid production is important in genetic manipulation and crop improvement because it offers a means to rapidly advance selected lines to complete homozygosity and increase efficiency of selection. Wheat and maize crosses have been studied since Zenikteler and Nitsche (1984)[26] described that embryo were formed after pollination of wheat with maize. Laurie and Bennett (1986)[15] found that the embryos were initially the hybrids between wheat and maize, but the embryos lost the maize chromosomes during the early cell divisions and became haploid embryos of wheat. Several technical problems affected the efficiency of haploid embryo production. Some key problems must be addressed before large-scale generation of haploid plants becomes feasible. Embryogenesis was studied in wheat - maize hybrids using paraffin sectioning (Berlyn and Miksche, 1976) [2]. Nerling and Morris (1979) [18] observed that there is reduced cytokinin biosynthesis in endosperm of the hybrid as compared to the parents, which is responsible for the abortion of embryos. Majority of haploid embryos resulting from wheat × maize crosses are poorly developed or completely lack an endosperm (Laurie and Bennett, 1986)[15]. Pollen tube growth and early embryogenesis was analyzed on paraffin sections after histochemical staining (Wedzony and Lammeren, 1996) [25]. Zhang *et al.* (1996) [28] studied different stages of embryonic development in wheat × maize crosses by using different treatments. Carbohydrates, proteins and callose were found to be responsible for preventing or permitting pollen tube growth towards the egg for fertilization in wheat × barley crosses (Santosh and Khanna, 1996)[20], and in wheat × rye crosses (Jagadev and Khanna, 2002) [10].

Material and Methods
Thirteen drought tolerant (VL 804, VL 802, UP 2572, PBW65, PBW 175, WH 730, JOB 666,

NI 5439, NP 846, NIAW 34, HI 385, PBN 51, Halna) and three drought susceptible (UP 2338, PBW 337, UP 2425) varieties of wheat were crossed to produced F_1's. Following twenty-nine F_1's were produced for wheat- maize crossing.

1. F_1 = VL 804 × UP 2425
2. F_2 = VL 804 × PBW 373
3. F_3 = VL 802 × UP 2338
4. F_4 = VL 802 × PBW 373
5. F_5 = VL 802 × UP 2425
6. F_6 = UP 2572 × PBW 373
7. F_7 = UP 2572 × UP 2425
8. F_8 = UP 2572 × UP 2338
9. F_9 = PBW 65 × PBW 373
10. F_{10} = PBW 65 × UP 2425
11. F_{11} = PBW 65 × UP 2338
12. F_{12}= PBW 175 × UP 2425
13. F_{13}= PBW 175 × PBW 373
14. F_{14}= WH 370 × UP 2425
15. F_{15} = WH 370 × UP 2338
16. F_{16} = JOB 666 × UP 2338
17. F_{17} = JOB 666 × UP 2425
18. F_{18} = NI 5439 × UP 2425
19. F_{19} = NI 5439 × UP 2338
20. F_{20} =NP 846 × UP 2338
21. F_{21} =NP 846 × UP 2425
22. F_{22} = NIAW 34 × PBW 373
23. F_{23} = NIAW 34 × UP 2425
24. F_{24} = HI 385 × PBW 373
25. F_{25} = HI 385 × PBW 373
26. F_{26} = PBN 51× UP 2425
27. F_{27} = PBN 51× UP 2338
28. F_{28}= Halna× UP 2425
29. F_{29} = Halna× PBW 373

Maize varieties Pragati, Pearl Pop Corn, New composite were used to make intergeneric crosses. To observe fertilization and early development of the embryo, pistils were collected at 8, 24, 48 and 72 h after pollination and fixed immediately for microtome preparations.

Botanical Microtechnique

The technique by which the plant tissues are prepared for microscopic examination is called microtechnique. Killing and fixation, dehydration, clearing, infilteration, embedding, sectioning, staining and mounting of the tissues on a slide were the key steps.

Results

Post- fertilization studies to observe the events of embryo development in wheat- maize crosses

Post-fertilization studies were performed in wheat F_1 to study post-pollination events and changes in the embryo-sac during its development. The longitudinal section in wheat x maize indicated that the antipodal cells degenerated similar to the self-pollinated wheat having seed with embryos and in sections showing endosperm nuclei. This suggested that fertilization has occurred after pollination with maize. The embryo or endosperm nuclei in wheat x maize hybrid seed must have been derived from a maize fertilized egg or maize fertilized polar nuclei.

Based on the longitudinal sectioning of seeds, three types of seeds were seen in wheat- maize hybrids, i.e. seeds with embryo, but endosperm was absent; seeds with endosperm only and unfertilized seeds with antipodal cells, polar nuclei and egg apparatus. Hybrid seed, with embryo and endosperm, from wheat- maize crosses was previously reported to occur at a low frequency (Laurie, 1989)[16]. Embryo development in wheat- maize hybrids was not accompanied by the endosperm development. In contrast, in self-pollinated seeds, the early accumulation of a nutritive endosperm around the embryo ensured its initial growth**.** However, the degenerative antipodal cells were the major source of food material for the growing embryo. Pre - embryos were obtained when wheat was pollinated with maize.

The presence of abundant endosperm nuclei in the embryo sac suggested that the polar nuclei fused with a sperm nucleus. The endosperm nucleus remained free and clustered in the cytoplasm 3 days after pollination. In contrast, in the early stages of self-pollinated seed, the endosperm nuclei were distributed along the edge of the embryo sac. The cellular endosperm had already formed along the perimeter of the embryo sac 2 days after self-pollination. Further, endosperm nuclei and unfertilized egg cell were present in one of the sectioned seed 3 days after pollination with maize. This suggested that a single fertilization may occur in wheat-maize crosses.

Change in the ovary

The secretions produced in the cells of the style, the ovule and obturator were studies at 8 hr, 24 hr and 48 hr after pollination in the longitudinal sections of the ovary, after pollination in wheat and wheat × maize crosses. Obturator is the organ of special significance, which facilitates the entry of the pollen tube into the ovule. The obturator is the protuberance of tissue on the carpellary wall, facing the micropyle. This tissue is continuous with and of the same cytohistology as the transmitting tissue from the style **(**Sterling, 1964) [15].

Coomassie brilliant blue staining for protein

Dark blue color confirmed the presence of protein in ovule and extra ovarian portion. In selfing, constant and uniform dark color was observed in the extra-ovarian tissues including the obturator and the ovule (Fig.1a).

In case of crosses a dark stain was observed initially and with time the crease increased. Degeneration of endosperm and egg cell apparatus took place (Fig.1d). At 24-72 hr the obturator and the inner cell wall of the degenerated ovule got stained but the extra-ovarian portion took a relatively light stain (Fig.1b, c).

Periodic acid schiffstaining for insoluble carbohydrate

Periodic acid schiff's (PAS) reagent stains violet, showing the presence of insoluble carbohydrates. In case of selfing, presence of insoluble carbohydrates was noticed even at 48 hr after selfing (Fig. 2c). Extra- ovarian portion along with the obturator was stained. The extra- ovarian portion including obturator took a dark stain, but some parts of central portion of the ovule were also lightly stained (Fig. 2b).

In case of crosses extra- ovarian portion along with the obturator and some of the inner part of the ovule were stained at 2-24 hr after pollination. The insoluble carbohydrates were present but the amount was less as compared to the selfings. There was an increase in the size of the crease and empty space in the ovule with the passage of time (Fig. 2d).

At 48 hr very less or no insoluble carbohydrates were present. The degeneration of endosperm and embryo of ovule was also detected. In some crosses, at 48 hr after pollination a pre-formed embryo was also seen (Fig. 2e, 2f). In some cases extra- ovarian portion and other apparatus did not take any stain but they showed the degenerated egg cell apparatus (Fig. 2a).

Safranin, gentian violet, fast green staining for embryo development

In case of selfing, at initial stage the ovule was normal and internal cells of the ovule were at the dividing. In crosses from 2-8 hr after pollination, the ovule development was normal (Fig. 3a, 3b and 3c). There was an increase in the empty spaces outside the central cells of the ovule and at the end of the 48 hr the degenerated antipodal cells and endosperm were observed (Fig. 3d and 3f) and a pre-formed embryo was noticed at 72 hr(Fig. 3g). In some cases the embryo also degenerated. In some other cases even at 8 hr the empty space was observed but no pre-embryo and egg cell apparatus were found, while well-developed pre- embryo was found in few of the cases.

Discussion

Different types of staining provide a clear picture of the post-fertilization events in wheat-maize crosses. These data suggested that the embryos or endosperm nuclei in a fertilized seed might be derived from a single fertilization. The endosperm nuclei in fertilized seed fail to form cellular endosperm and subsequently degenerate.

Coomassie Brilliant Blue Staining for protein

In selfing uniform presence of protein was observed in the extra-ovarian portion of the obturator and the ovule. At 48-72 hr of pollination, the obturator and the ovule took a dark stain than the adjoining portion. This suggested that after selfing in wheat, the flower was immediately prepared to permit pollen tube growth and fertilization (Fig.1). Arbeloa and Herrero (1987) [1] reported similar results in peach.

In case of a cross, the staining is different from selfing. At 8 hr after pollination the amount of protein in the extra-ovarian portion was very less. Due to late secretion of protein pollen tube might not grow towards the egg, hence resulting in prevention of fertilization. Endosperm got degenerated due to late / slow growth of pollen tubes. After 24 hr degeneration of endosperm started that lead to the formation of empty spaces in the ovule which resulted in the formation of shrivelled grain. Sirohi (2003)[22], Jagadav and Khanna (2005)[8] and Kour (2005)[13] have reported similar findings.

Periodic Acid Schiff's reagent staining for insoluble carbohydrates

In case of selfing the extra-ovarian portion along with the obutrator took the stain. That means immediately after pollination insoluble carbohydrates were produced. Pollen germinated within 5 min after reaching the stigma in *Hordeumdistinchum* and reached the micropyl in 40 min. Results indicated that after selfing, the pistil and the ovule were immediately ready for pollination and fertilization process. Santosh and Khanna (1996) [20] reported the presence of insoluble charbohydrates on selfing in wheat and barley.

In case of cross, the obturator along with the extra-ovarian region and some inner point of the ovule took a very light stain. This indicated very less amount of insoluble carbohydrates. The degenerative endosperm and antipodals were the source of nutrition for the developing embryo. At 48-72 hr of pollination, there was a complete absence of insoluble carbohydrates on entire ovarian portion including obturator so there was no staining (Fig.2). The results were in conformity with the results of Kour *et al.* (2009)[12]. Pollen tube growth in wheat-maize crosses was slower than in selfing. That might be the reason why the obturator was not showing any secretion and was not ready to permit entry of the pollen tube. In crosses only pre-embryo was observed after 48 hr of pollination that leads to the formation of haploid embryos. Jagadav and Khanna (2005)[8] found similar results in wheat and rye crosses. Kour (2005)[13] showed the presence/absence of insoluble carbohydrates in wheat – maize crosses.

Safranin, crystal violet and light green staining for embryo development

After 48 hr well developed embryos could not be seen i.e. the embryo development was still underway. In case of the crosses, ovule development was normal from 2-4 hr after pollination. There was a continuous increase in empty spaces as the time passed (Fig. 3b, 3c and 3a). At 48 hr the antipodal nuclei and in some cases endosperm nuclei were also observed along with the embryo. After 48 hr degeneration of antipodal and endosperm was observed (Fig. 3d and 3f).

The degeneration of the apparatus took place to provide nutrition to the embryos. Khanna *et al.* (1994)[11] reported abnormalities in wheat × barley crosses. The hybrid plantlets had cytological abnormalities, which may be responsible for abnormal embryo development. That is why embryo rescue of wheat hybrids is necessary.

Tilton *et al*. (1984)[24] suggested that a significant function of transmitting tissue is to provide an opportunity for pollen tube competition to occur and to thereby align to forces of natural selection to eliminate all but the most vigorous and rapidly growing male gametophytes. Dang *et al.* (2017)[3] attempted inter-specific crosses among jasmine species and on the basis of microtomy results he concluded that the crossability in jasmine was affected mostly by the pre-fertilization barriers.

The regulation of fertilization has been proved to be remarkably complex involving various kinds of controls imposed at different levels including various direct interactions between the male and the female gametophytes that determine whether fertilization will or will not be affected (Heslop Harrison and Helsop Harrison, 1985) [7]. Zhang *et al*. (1996) [28], Sirohi (2003) [22] and Jagadav and Khanna (2005) [8] also reported similar findings. The embryo development in a wheat- maize hybrid was not accompanied by endosperm development as in a self-pollinated seed. The main source for the embryo development in wheat-maize hybrid might be from degenerated antipodal cells and the nuclear cells. The nutritional shortage might be the cause of embryo abortion at early stages when the embryos were not rescued. Kumar *et al.* (2010) [14] demonstrate the development of embryo under cold stress by the microtomy. Piosik*et al*. (2016) [19] did microtomy studies for monitoring the development of embryo in inter-generic cross of *Lactuca sativa* and *Helianthus annus*.

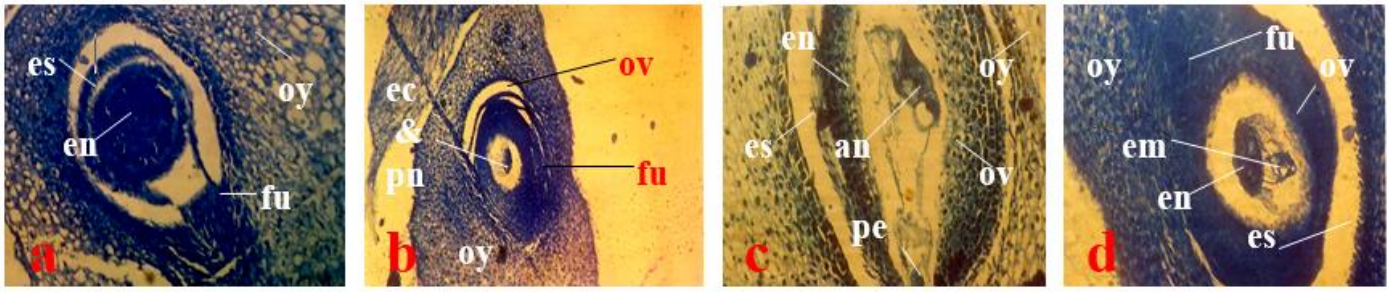

Fig 1: Coomassie Brilliant Blue staining showing events of post fertilization after selfing of wheat (a), 8 hr after pollination and in wheat × maize (b), 24 hr after pollinationin wheat × maize (c), and 48 hr after pollination in wheat × maize (d)

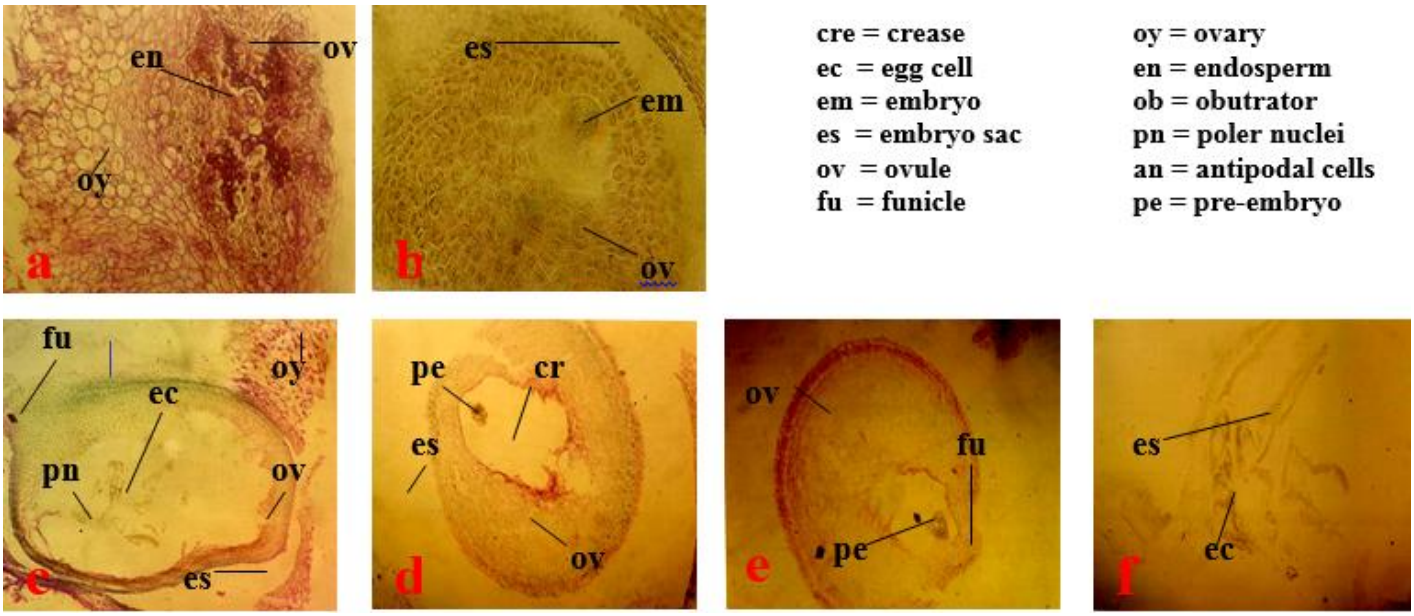

Fig 2: Stages of embryo development after Periodic Acid Schiff's reagent staining onselfing (a, b), after 48 hr of pollination (c) and in wheat × maize cross after 48 hr (d) and 72 hr after pollination in wheat × maize cross (e, f).

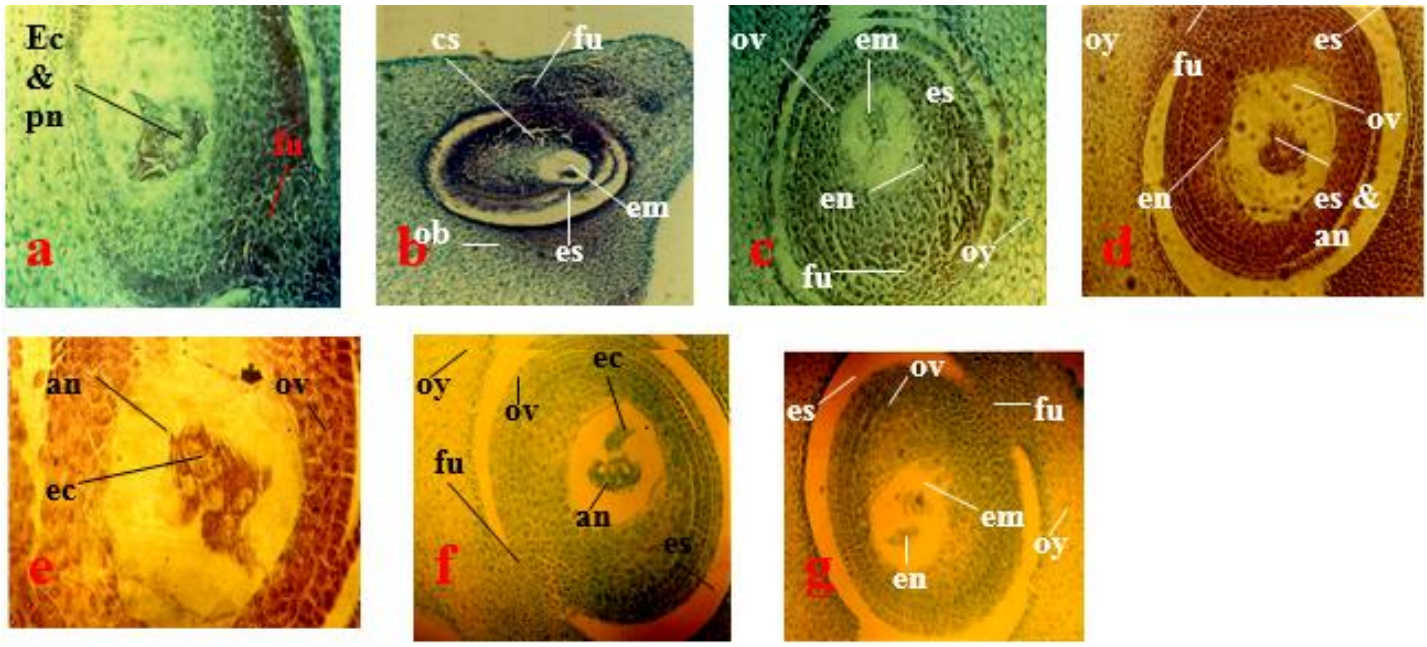

Fig 3: Safranin-Crystal Violet-Light Green staining showing stages ofembryos development after selfing (a and b), after 24 hr of crossing of wheat with maize (c, d), after 48 hr (e, f) and 72 hr (g) after pollination of wheat with maize.

References

1. Arbeloa A, Herrero M. The significance of the obturator in the control of pollen tube entry into the ovary in peach (Prunus persica). Ann. Bot. 1987; 60:681-685.
2. Berlyn GP, Miksche JP. Botanical microtechniques and scytochemistry. The Iowa State University Press Ames, 1976, 24-85.
3. Deng Yanming, Sun Xiaobo, GuChunsun, JiaXipn, Liang Lijian, Su Jiale. Identification of pre-fertlization

reproductive barrier and the underlying cytological mechanism in crosses among three petal types of jasminumsambac and their relevance to phylogenetic relationships. PLoS one. 2017; 12(4).
4. Feder N, Brien TP. Plant microtechniques: Some principles and methods. Amer. J Bot. 1968; 55:123-142.
5. Fisher DB. Protein staining of ribboned open sections for light microscopy. Histochemie.1968; 16:92-96.
6. Gerlach D. A rapid safranin-crystal violet-light green staining sequence for paraffin sections of plant materials. Stain Tech. 1969; 44:210-211.
7. Heslop-Harrison J,Heslop-Harrison Y. Surface and pollen secretions in the pollen stigma interactions: a brief review. J Cell. Sci.1985; 2:287-300.
8. Jagadev P N and Khanna V K. A study of genetic distances among wheat-rye hybrids and their parents by isozymic analysis. Journal of Research, Orissa University of Agriculture & Technology 2005; 21(2): 90-95.
9. Jagadev PN, Khanna VK. A study of genetic distances among wheat-rye hybrids and their parents by isozymic analysis. Journal of Research, Orissa University of Agriculture & Technology.2003; 21(2):90-95.
10. Jagadev PN, Khanna VK. A study of crossability mechanism, isozyme analysis and organogenesis from wheat embryos in wheat-rye crosses. Proceedings of the 5th International Symposium, June 30 to July 5, 2002, Radzikow, Poland. 2002; 1:105-108.
11. Khanna VK, Rohilla JS, Singh M. Crossability of wheat, triticale, barley and rye and pollen tube growth after gamma radiations. In: Proceedings of SABRAO seventh international congress and WSAA symposium held at Academia Senica, Nankang, Taipei, Taiwan. 1994; 1:325-331.
12. Kour A, Bhatt Usha, Khanna VK. Study of pre-fertilization parameters in wheat-maize crosses. Indian. J Genet. 2009; 69(2):87-92.
13. Kour A, Khanna VK. Pre-fertilization studies and embryo rescue for the production of wheat haploids by wheat-maize crosses. Thesis, Doctor of Philosophy (Department of Genetics and Plant Breeding), G.B.P.U.A. & T Pantnagar. 2005, 205.
14. Kumar Sanjeev, Nayyar Harsh, Bhanwara RK. HD 2 Chilling stress effects on reproductive biology of chickpea. An Open Access Journal published by ICRISAT. 2010; 8:4-14.
15. Laurie DA, Bennett MD. Wheat x maize hybridization. Can. J Genet. Cytol. 1986; 28:313-316.
16. Laurie DA. Factors affecting fertilization frequency in crosses of Triticum aestivum cv. Highbury x Zea mays cv. Seneca 60. Plant Breeding. 1989; 103(2):133-149.
17. Moieni A, Dvallarieille P, Sarrafi A. Potential use of doubled haploid lines for the screening of resistance to yellow rust (*Puccinia striiformis*) in hexaploid wheat. Euphytica. 1997; 100:207-217.
18. Nerling FAV, Morris DA. Cytokinin level and embryo formation in interspecific phaseolous crosses. ZP flanzenphysiol. 1979; 91:345-358.
19. Piosik L, Zenkteler E, Zenkteler M. Development of haploid embryos and plants of Lactuca sativa induced by distant pollination with Helianthus annus and H. tuberosus. Euphytica. 2016; 208:438-451.
20. Santosh K, Khanna VK. Role of the obturator in pollen tube entry into the ovary in wheat × barley crosses. Acta Agron. Hung. 1996; 44:339-346.
21. Sirohi M, Khanna VK.RAPD of parental wheat genotypes, post fertilization studies and in vitro culture of embryo derived from wheat × maize crosses. Thesis, Doctor of Philosophy (Department of Genetics and Plant Breeding), G.B.P.U.A. & T., Pantnagar, 2004, 153.
22. Sirohi M, Vijaipal, Khanna VK. Pollen tube behaviour and haploid wheat production via embryo rescue in wheat x maize crosses. Ind. J Genet. 2003; 63(1):67-68.
23. Sterling C. Comparative morphology of carpel in the rosaceae. I Prunoidea Prunus. Am. J Bot. 1964; 51:36-44.
24. Tilton VR, Wilcox LW, Palmer RG, Albertsen MC. Stigma, style and obturator of soybean. *Glycine max* (L.) Herr. (Leguminosae) and their function in the reproductive process. Am. J Bot. 1984; 71:676-686.
25. Wedzony M, Van Lammeren AAM. Pollen tube growth and early embryogenesis in wheat-maize crosses influenced by 2, 4-D. Ann. Bot. 1996; 77:639-647.
26. Zenkteler M, Nitzsche W. Wide hybridization experiments in cereals. Theor. Appi. Genet. 1984; 68:311-315.
27. Zhang Y, Zhangfu N, Rongqi L, Jigangi L, Baoyoni L, Jian LG *et al*. Composition of high molecular weight glutenin subunits in synthesized hexaploid wheat introduced from CIMMYT. J China Agric. Univ. 2001; 6(3):38-43.
28. Zhang J, Friebe B, Raupp WJ, Harrison SA, Gill BS. Wheat embryogenesis and haploid production in wheat × maize hybrids. Euphytica. 1996; 90:315-324.