2. REVIEW OF LITERATURE

The first recognition of haploids in plants was exercised by A.D. Bergner in 1921 in *Datura stramonium* L. which was reported in the journal Science by Blakeslee *et al.* (1922). Gaines and Aase (1926) discovered haploid plants in wheat. Subsequently haploids were reported in many species but at low and variable frequencies. Kimber and Riley (1963) in a review pointed out that the two major problems limiting the use of haploids were their low frequencies and genotype differences in response. Thus, research was directed at finding methods of producing haploids in crop species to address these problems.

The first attempt to use haploidy in breeding appears to be Chase (1951) who selected the low frequency of parthenogenic haploids (egg cell develops into an embryo without fertilization) in maize and then applied chromosome doubling treatments to produce inbred lines.

Guha and Mahashwari (1964 and 1966) brought renewed interest to haploidy breeding by remarkable discovery that haploid embryos and plants can be produced by culturing anthers of *Datura*. Later on, a number of haploid production techniques have been developed *viz.*, the *bulbosum* technique (Barclay 1975), the Salmon method (Kobayashi and Tsunewaki 1978 and Tsunewaki *et al.* 1984), wheat x maize system (Laurie and Bennett 1988) and wheat x *I. cylindrica* system (Chaudhary *et al.* 2005). Keeping in view the utility of haploidy breeding, the possibility to widen its horizon by utilizing the approach in other crops and scope for improvement in haploid production efficiency, the relevant literature pertaining to the present investigation has been reviewed under following heads:

2.1 Induction of haploids through wide hybridization

2.2 Enhancement of doubled haploid production efficiency

2.1 Induction of haploids through wide hybridization

2.1.1 Wheat

Doubled haploidy breeding is the fastest route to achieve superior homozygous cultivars within shortest span and time and eliminating the
need to raise segregating population generation after generation. The production of wheat haploid plants through wide hybridization followed by chromosome elimination was first used successfully by crossing wheat with *Hordeum bulbosum* which is commonly known as *bulbosum* technique (Barclay 1975). However, this technique has a limitation that is presence of *Kr* inhibitor genes which express in the style of most of the wheat genotypes and inhibited the growth of pollen tube of *Hordeum bulbosum*. Later on, Laurie and Bennett (1988) gave wheat x maize system of wheat haploid production by hybridizing wheat with maize. The maize was reported to be insensitive to the action of dominant genes *Kr1* and *Kr2*, located on the long arms of chromosome 5B and 5A respectively (Sitch et al. 1985). This intergeneric hybridization of wheat with maize results in the formation of haploid wheat embryos because the maize chromosomes in the hybrid zygote are rapidly eliminated in the first few cycles of cell division. The maize chromosomes are lost because their centromere fails to attach to the spindle during the mitosis (Laurie and Bennett 1987). They also studied the effect of crossability loci *Kr1* and *Kr2* on fertilization frequency in wheat x maize crosses and found that fertilization occurred in wheat when pollinated with maize and developed haploid embryo without endosperm or the endosperm was highly aberrant. A higher yield of haploid plants was achieved when the spikelets also contained a normally developing seed arising from self pollination (Laurie and O’Donoughue 1989).

Wang *et al.* (1991) studied the frequency of fertilization and embryo formation in wheat x maize crosses. Hybrid embryos and endosperms obtained from wheat x maize hybridization were karyotypically unstable and were characterized by rapid elimination of the maize chromosomes to produce haploid wheat embryos. Hence, the reduced genotypic specificity, absence of albinism and ease of application, make the wheat x maize hybridization technique more efficient than the anther culture and the *bulbosum* technique for the production of haploids in common wheat.
Accordingly, Inagaki and Tahir (1991); Sun et al. (1992) and Kasha et al. (1995) advocated the use of this technique for breeding purpose by raising a large number of wheat haploids. Recently, Chaudhary et al. (2005) identified the *I. cylindrica* as an efficient alternative pollen source for doubled haploid production performing superior over maize-mediated haploid plant production. Pratap et al. (2005) reported that among all the *Graminae* genera tested for haploid plant production, *I. cylindrica* produced more embryos and haploids over others. Cytological investigation of the wheat x *I. cylindrica* chromosome elimination system has shown that there is no endosperm formation and the elimination of chromosomes of *I. cylindrica* takes place in the first zygotic division in seed development thus allowing the production of embryo-carrying seeds (Komeda et al. 2007). Chaudhary (2007) reported that combination of *I. cylindrica*-mediated doubled haploid production and molecular cytogenetic techniques like GISH and FISH can accelerate the alien introgression mediated wheat breeding programmes in various farming systems. Chaudhary (2008 a and b) reported that *I. cylindrica* performed significantly better than maize for all the haploid induction parameters in wheat and triticale and their derivatives.

Kishore et al. (2010) used maize and *I. cylindrica* for haploid induction in spring and winter wheat cross with Himalayan rye derivatives and observed that the *I. cylindrica* produced more haploid embryos whereas maize did not produce any haploid embryo.

Chaudhary (2009, 2010a and b) reported that *I. cylindrica* performed better than maize in all the crosses of winter x winter wheat, spring x spring wheat, winter x spring wheat derivatives, whereas it exhibited par excellence in triticale and wheat x rye derivatives.

### 2.1.2 Rice (*Oryza sativa L.*)

Till now, no any report has been made so far regarding haploid and doubled haploid production in rice following *I. cylindrica*-mediated
chromosome elimination approach and wide hybridization. But, many workers had attempted to produce various haploids and doubled haploids in rice either mostly through anther culture or by following combination of both conventional and biotechnological approaches. The doubled haploid breeding programme in India in rice was initiated in 2001 by the Indian Council for Agricultural Research (ICAR) for development of new technologies for genetic improvement in popular rice varieties to boost rice production in the country. CR dhan-10 is a rice hybrid variety developed through the haploid breeding method.

No effort in rice has ever been made anywhere in India and abroad for doubled haploid production through chromosome elimination mediated approach especially following maize and *Imperata cylindrica* pollen sources.

### 2.1.3 Maize (*Zea mays L.*)

It takes 7-8 generations to obtain a maize inbred line and the plants will still not be 100% pure in traditional breeding. Doubled haploid technology improves breeding efficiency by generating inbred lines with 100% purity and genetic uniformity in just two generations. Maize (*Zea mays* L.) is a typical diploid plant (2n = 20), but haploid individual (2n =10) occur naturally at a rate of one per 1,000 kernels (Chase 1949). Haploid can be derived either via anther culture or interspecific crosses. Reports on haploid production using interspecific crosses/ chromosome elimination are negligible in maize. The first method to produce DH in maize was proposed by Chase (1951) using monoploids or synonymously haploids. During the past two decades, big efforts have been made to establish anther culture in maize. Nevertheless, response to anther culture seems to be a complex trait and is highly genotype dependent with most genotypes being non-responders (Wan and Widholm 1993). In addition, anther culture is affected by many factors, very expensive and gametic selection can lead to a distorted segregation after anther culture (Buter 1997).
Heslop-Harrison et al. (1985) carried out wide hybridization by pollinating maize with Sorghum pollen. They showed that sorghum pollen tubes readily reach the ovary after pollination. Although Sorghum tubes have been traced across the inner ovary wall, they have not been seen to enter the micropyle, and hybrid embryos could not be obtained. Nowadays, *in vivo* haploid induction using inducer lines such as UH400 have been developed that warrant induction rates of 10% haploids and higher and it has been adopted as a routine method in many commercial hybrid maize breeding programme in Europe and North America (Rober et al. 2005 and Seitz 2005).

Tang et al. (2006) induced haploid maize plants by *in vitro* culture of pollinated ovaries. From a total of 26,400 cultured ovaries 24 haploid plants were obtained and two of them were doubled after colchicine treatment. The maximum frequency of gynogenesis was 0.17% at 19.5 hour post-pollination. Their results showed that post-pollination time was an important factor affecting plant induction from ovaries.

Zhang et al. (2008) produced haploids by stock 6-derived indices line HZI1 following chromosome elimination. The average frequency of haploid induction in four inbred lines was above 10%. Above 56.4% of the radicals from the kernels with purple aleurone and colourless embryos were mixoploid (2n = 9-21) and more than 42.22% cells were haploid cells (n = 10) in three crosses H 285 X HZI1, Ha 24 x HZI1, HZ124b x HZI1 and HZ141 x HZI1. More than 62.5% of the radicals from the kernels with purple aleuronic and purple embryos were mixoploid (2n = 9-21) having 54.27% cells with 2n = 20.

Chromosome elimination-mediated approach DH breeding has never been tried anywhere in the world to induce haploids in maize. Although *Imperata cylindrica* is an efficient pollen source for haploid induction in wheat yet it has yet to be explored for developing DHs in maize.
2.1.4 Oat (Avena sativa L.)

Doubled haploids (DHs) are becoming increasingly important in oat breeding programmes. Haploids in oat (Avena sativa L., 2n=6x=42) have been produced by crossing oat with maize following the procedure similar to ones described for the production of wheat (Triticum sp.) haploids from wheat x maize hybridization (Laurie and Bennett 1988 and Rines et al. 1997). However, in oat the plant recovery frequencies reported is 1-2% of florets maize-pollinated and likewise for oat haploid production in oat anther culture (Kiviharju et al. 2000), not yet adequate for routine use in breeding.

There are also important differences in the types and in the reproductive behaviour of plants recovered in oat x maize compared to wheat x maize hybridization, which include occasional maize chromosome retention in the recovered oat plants and partial self-fertility in oat haploid plants (Riera–Lizaraju et al. 1996).

Sidhu et al. (2006) studied factors affecting oat haploid production following oat x maize hybridization. They investigated the factors influencing the rate of caryopsis and haploid embryo production which included genotype, post-pollination plant growth regulator application and temperature. The four growth regulators tested showed significant differences in their capacity to induce caryopsis formation with dicamba producing the higher number of caryopses, followed by picloram, 2,4-dichlorophenoxyacetic acid and gibberrellic acid (GA_3). No significant differences were observed between these growth regulators in respect of embryo formation. The concentration of dicamba was also important and was found to influence caryopsis but not embryo production, with 50 and 100 mg/L dicamba producing significantly more caryopses than 25 to 5 mg/L. Temperature had a significant input on both caryopsis and embryo production with the magnitude and direction of response depending on genotype. Rates of haploid embryo production observed were between 0.8% and 6.7% of the pollinated florets. The proportion of haploids, which
summed and were successfully doubled with colchicine following transfer to soil, was between 72% and 81%.

Riera-Lizarazu et al. (1996) had crossed hexaploid oat (2n=6x=42) and maize (2n=2x=20) and recovered 90 progenies through embryo rescue. Fifty two plants (58%) produced were oat haploids (2n=3x=21) following maize chromosome elimination. Twenty eight plants (31%) were found to be stable partial hybrids with 1.4 maize chromosomes in addition to a haploid set of 21 oat chromosomes (2n=21+1 to 2n=21+4). Ten of the ninety plants produced were found to be apparent chromosomal chimeras, where some tissues in a given plant contained maize chromosomes while other tissues did not, or else different tissues contained a different number of maize chromosomes. DNA restriction fragment length polymorphisms (RFLPs) were used to identify the maize chromosome(s) present in various oat-maize progenies. Maize chromosomes 2, 3, 4, 5, 6, 7, 8, and 9 were detected in partial hybrids and chromosomal chimeras. Maize chromosomes 1 and 10 were not detected in the plants analyzed to-date. Furthermore, partial self-fertility, which is common in oat haploids, was also observed in some oat-maize hybrids. Upon selfing, partial hybrids with one or two maize chromosomes showed nearly complete transmission of the maize chromosome to give self-fertile maize-chromosome-addition oat plants. They also recovered fertile lines that contained an added maize chromosome or chromosome pair representing six of the ten maize chromosomes. Four independently derived disomic maize chromosome addition lines contained chromosome 4, one line carried chromosome 7, two lines had chromosome 9, one had chromosome 2, and one had chromosome 3. One maize chromosome-8 monosomic addition line was also identified. They also identified a double disomic addition line containing both maize chromosomes 4 and 7. This constituted the first report of the production of karyotypically stable partial hybrids involving highly unrelated species from two subfamilies of the Gramineae (Pooideae — oat, and Panicoideae — maize) and the subsequent recovery of fertile oat-maize chromosome addition lines.
These represent novel material for gene/marker mapping, maize chromosome manipulation, the study of maize gene expression in oat, and the transfer of maize DNA, genes, or active transposons to oat.

Although maize has been used by few researchers in India and abroad for DH production in oat yet the results are not much encouraging. The present investigation is unique as the *Imperata cylindrica* has never been used as a pollen source anywhere in the Indian and foreign labs to generate haploids in oats.

- **2.1.5 Barley (Hordeum vulgare L.)**

  In barley, haploid plants were reported as early as 1934 (Johansen 1934), but the frequency of such spontaneous or induced haploids is too low to be considered in breeding programmes. The first report of a haploid production method having potential for barley breeding was made by Kasha and Kao (1970). This method was refined and improved by many researchers, led by K.J. Kasha; E. Reinbergs and their associates at Guelph, Ontario, Canada. This technique, called the *bulbosum* method, leads to production of haploids through selective elimination of *bulbosum* chromosomes in the interspecific hybrids between cultivated barley (*Hordeum vulgare* L.) and a wild species ( *H. bulbosum* L.). Using the *bulbosum* method, Ciba-Geigy Seeds in Canada developed the first DH cultivar, ‘Mingo’. (Ho and Jones. 1980)

  Chen and Hayes (1989) reported modifications of the *Hordeum bulbosum* technique that led to greater efficiencies and less germplasm specificity.

  However to ensure production of DH lines from every possible barley hybrid in a breeding programme, anther culture can be used in parallel, since recalcitrant genotypes to both methods have been identified (Devoux *et al.* 1996). After *bulbosum* approach, no endeavour has ever been made in India and abroad to utilize the genotype non-specific pollen sources like maize and *Imperata cylindrica* for haploid induction in barley. The present investigation registers its uniqueness in this regard.
2.2 Enhancing doubled haploid production efficiency

2.2.1 *In vivo / in vitro* colchicine application

The relatively high frequency of doubled haploids needed by plant breeders, requires the application of efficient techniques to induce doubling which must involve high doubling rates, low frequency of damage and minimum plant mortality less time consuming and easy to handle on a practical scale.

2.2.1.1 *In vivo* colchicine application

Koksal *et al.* (2002) investigated the efficiency of different *in vivo* methods for chromosome doubling viz. colchicine application to lateral buds by dropper, colchicine containing agar and colchicine in imbibed cotton. They found that colchicine application by immersing shoot tips is the best method of chromosome doubling.

Sood *et al.* (2003) developed two chromosome doubling strategies for producing wheat doubled haploids from wheat x maize crosses. First was *in vitro* colchicine application to haploid embryos and second was colchicine treatment through post pollination tiller injections. Different concentrations of colchicine (0.5, 0.75 and 1.0%) which also contained 2, 4-D (100 ppm) were injected into uppermost internodes of crossed tillers 48 and 72 hours after pollination. The chromosome doubling efficiency varied from 33 to 100% with 10% treatment being the most effective. No chimeras of doubled haploid sectors were observed and all the florets showed seed set in doubled plants.

2.2.1.2 *In vitro* colchicine application

Stefanowaska (1989) studied the action of colchicine on F₁ hybrids of *Triticum aestivum* x *Secale cereale* and observed that the combination of dimethyl sulphonate (2.0%) and colchicine (0.3%) treatment was most effective (36.8%) with spike setting at least two grains/spike.

Hassawi and Liang (1991) stated that colchicine is one of the most effective antimitotic agents for chromosome doubling.
Matzk and Mahn (1994) cultured the plantlets with one or two leaves and roots *in vitro* on medium containing 0.02% colchicine for 30 hours for doubling the chromosome number.

Shilko *et al.* (1994) investigated the effect of colchicine treatment on F₁ of wheat and rye and found that colchicine effectiveness depends upon genotypes of hybrids treated and weather conditions.

Navarro-Alvarez *et al.* (1994) found that addition of colchicine to wheat anther culture medium increased the doubled haploid plant production. Increasing the colchicine concentration reduced the number of embryoids produced from 77.4 to 29.9 embryoids/100 anthers but did not significantly affect the frequency of plant regeneration and increased the frequency of doubled haploid plants (19.0 to 72.3 DH plants/100 green plants).

Hensen and Andersen (1998) isolated microspore culture of two doubled haploid lines of wheat and treated with 0, 3, 10, 30, 1000 and 3000 µM of colchicine for 24 hours and 48 hours during microspore culture. Highest number of embryos regenerated from 24 hours colchicine treatments while highest frequencies of green plants and fertile plants were obtained from 48 hours colchicines treatment. The highest number of DH plants per spike resulted from treatment with colchicine concentrations of 300-1000 µM.

Zamani *et al.* (2000) studied the effect of colchicine added to induction medium for the production of doubled haploid plants after *in vitro* anther culture in one winter and two spring wheat genotypes. They reported that in case of winter wheat variety colchicine treatment resulted in 100% completely fertile plants.

Chen *et al.* (2002) investigated the effect of colchicine treatment on doubled haploid production efficiency. They reported that MS media supplemented with 100 ml/L colchicine gave maximum chromosome doubling frequency (100%) while in case of colchicine treatment by
immersing leaves and roots of plants in colchicine solutions (500 mg/L) yielded 98.2% doubling frequency.

Klistov and Artemeva (2004) utilized two methods for doubled haploid production in spring and winter wheat ecotypes and their hybrids obtained with *Aegilops speltoides* and *Agropyron erectus*. In the first method they utilized wheat x maize system in which emasculated wheat and hybrid heads were hand pollinated with maize pollen. Embryos were excised 12-14 days after pollination and cultured on B5 medium. The resulting seedlings were immersed in 0.1% colchicine solution, rinsed and transplanted in pots which were kept in green house. In second method, they used anther and microspore culture technique for doubled haploid production. Wheat and hybrid anthers were isolated immersed in P11 nutrient agar medium for 20-30 days. Embryo like structures were transplanted into medium with 0.5 mg/L Kinetin. They obtained four doubled haploid lines from *in vitro* culture and 102 doubled haploid lines through wheat x maize system.

Redha *et al.* (2004) reported that colchicine at concentration of 100-1000 mg/L when added to induction medium of wheat anther culture for 1-5 days, beneficial effects were obtained.

Suriano *et al.* (2007) elucidated the effect of colchicine application on chromosome doubling androgenic response in anther and microspore culture of different bread wheat genotypes. A significant increase in doubling was observed with 300 mg/L in low androgenic responding cultivar Caramba. Colchicine application during the first hour of culture improved percentage of doubling in all genotypes, in both anther culture and microspore culture. Application of 300 mg/L colchicine improved the percentage of doubling in the two low responding genotypes to 118% of control in DH 24033 and 75% in Caramba in microspore and anther culture, respectively. In the cultivar, Pavan a two and a threefold increase in percentage of embryogenesis and green plants, respectively were obtained with 300 mg/L colchicine in microspore culture.
Mohammadi et al. (2007) studied *in vitro* androgenesis of *Zea mays* L. *via* anther culture. Combination of two embryo induction media (IMSS & YPm) in presence of different colchicine concentrations (0, 100, 200, 250, 300 and 400 mg/L) in the pre-treatment medium (IML) and pre-treatment duration (0, 3, 6 and 9 days) in two genotypes (DH 5 × DH 7 and ETH-M82) were tested. It was found that in the genotype DH 5 × DH 7, colchicine at a concentration of 100 mg/L significantly induced the number of embryo like structures (19.6 %). The control (without colchicine) and 400 mg/L of colchicine resulted in lower levels of embryo like structures (5.8% and 5.7%, respectively). In ETH-M82 genotype, 6 days of pre-treatment with 300 mg/L with colchicine, produced highest frequency of embryo like structure (25%). The frequency of spontaneous chromosome doubling in control group was very low for both genotypes (7%), but these genotypes were able to produce doubled haploid plantlets from the embryo like structures (63% doubled haploid) using a low concentration of colchicine in the pre-treatment medium (250 mg/L for 6 days). At high concentrations of colchicine (300 and 400 mg/L), more morphological and chromosomal aberrations were observed.

Although some efforts for *in vivo* and *in vitro* application of colchicine have been made by various workers in India and abroad yet more concerted well planned experiments are required to be executed repeatedly by including a large range of colchicine doses applied at varied intervals at the *in vivo* and *in vitro* level so as to draw concrete inferences. No such efforts has ever been made in the *Imperata cylindrica*- mediated chromosome elimination approach of doubled haploidy breeding in bread wheat for enhancing the DH production efficiency. Hence, the current endeavour made through the present investigation is unique in this direction.