INTRODUCTION

Chapter I

The term “Androgenesis” is originated from the Greek word “androgen” means male and “genesis” means creation or production. In androgenetic development, the organism develops at the expense of cytoplasm of the mother egg cell and male nuclear material (Astaurov, 1957). Androgenesis has been proved to be a very useful breeding tool to obtain the homozygous individuals in both plants (Naleczynska, 1991; Datta, 2005) and animals (Parsons and Thorgaard, 1985; Komma and Endow, 1995).

Extensive studies on androgenic development have been carried out in a wide group of plant species by in vitro culture in rice, Oryza sativa (Coulibaly and Demarly, 1979), in vivo induction in sweet pepper, Capsicum annuum (Vagera and Havranek, 1985) and tobacco, Nicotiana tabacum (Novak, 1984), through gamma radiation (Yoon et al., 1990) in maize, Zea mays (Nikoilic et al., 1989), barley, Hordeum vulgare (Li-wenze, 1991), wheat, Triticum aestivum (Pershina et al., 1993). In addition, androgenic development has been studied in various plants like egg plant, Solanum melongena (Rotino et al., 1992), tomato (Zhang-Xiongang, 1990), Coriander, Coriandrum sativum (Egorova and Reznikova, 1982), pea nuts, Arachis hypogaea and A. villosa (Bajaj et al., 1981), potato (Singsit and Veilieux, 1989). Flax (Straathof, 1989), citrus, (Germana et al., 1992) and mulberry, Morus alba (Ogure, 1989). Attempts have been made for the improvement of various plants through androgenesis such as asparagus (Falavigna et al., 1983) strawberry (Sayegh and Hennerty, 1989) and wheat (Schmid et al., 1991).
Androgenesis has been induced in several animals through various inducing agents like gamma rays (Ye et al., 1989; Nagoya et al., 1996), X-rays (Whiting, 1955). Androgenetic development has been studied in the rainbow trout, *Salmo gairdneri* (Parsons and Thorgaard, 1984; 1985; Thorgaard et al., 1990), brook trout, *Salvelinus fontinalis* (May et al., 1988), carps (Grunina et al., 1990; 1991; Bongers et al., 1995), bovine (Iwasaki and Hamano, 1991), fishes (Ye et al., 1989; Clifton and Pandian, 2008), albino rats (Ghosh et al., 1995), amago salmon, *Oncorhynchus masou* (Nagoya et al., 1996), mouse (Latham and Rambhatia, 1995) and in several insects like *Drosophila* (Komma and Endow, 1995), stick insects *Bacillus rossius* (Mantovani and Scali, 1992; Tinti and Scali, 1996).

In the mulberry silkworm, *Bombyx mori* L., sex control is an important aspect as male silkworms have higher viability than the females and produce more amount of silk (Xia and Tang, 1980). Xiang et al. (1982) have found that the silk yield of males turn out to be 20% higher than that of the females. It has been estimated that 10 – 15% more silk can be obtained if only male silkworms are reared (Huang, 1980). Androgenesis opens the possibility to obtain homozygous lines in a short period (Strunnikov, 1983; Malinova et al., 1996; Nacheva et al., 1998).

Androgenetic development was induced in the silkworm by warming freshly laid eggs at 40 °C hot air for 135 minutes and at 46 °C in hot water for 18 minutes (Hasimoto, 1934). Astaurov (1937) reported androgenetic individuals with complete development up to adult stage by combined effect of heat shock and heavy doses of X-rays irradiation. The nuclei of diploid androgenetic individuals induced by heat shock treatment were due to fusion of two sperm nuclei (Astaurov, 1967). Strunnikov
(1983) obtained androgenetic individuals by warming the eggs for 200 minutes at 38 °C. Higher yield of androgenetic individuals following warming the eggs at 40 °C for 135 minutes than 60 minutes was reported (Astaurov, 1968). Importance of androgenesis in the development of pre-dominantly homozygous males in the silkworm has been reviewed by many workers (Tazima, 1964; Astaurov, 1967; Strunnikov, 1975; Chowdhury, 1989; Ravindra Singh et al., 2001a).

Androgenetic individuals have been produced following treatment of silkworm eggs with CO₂ at various developmental stages (Tazima and Onuma, 1967; Li et al., 1988), X-ray treatment of females fertilized by normal males following heat treatment of eggs after 90 minutes of oviposition (Chowdhury, 1989), by laser microbeam irradiation (Xu et al., 1990), gamma irradiation (Xu et al., 1997) and supercooling of eggs at –11 °C (Tamazawa, 1977a;b). More than 80 % androgenetic development was obtained when 20 - 40 minutes old eggs were exposed to 0 °C for 4 – 9 days and 70 % androgenic development was recorded following exposing the eggs to hot water (40 °C) for 60 -135 minutes (Sugai et al., 1987). When freshly laid silkworm eggs were cooled at –11 °C, 7 % androgenetic larvae were observed (Astaurov, 1967). Ravindra Singh et al. (1991) have obtained several androgenic larvae in F₁ hybrids between multivoltine Pure Mysore Chocolate and bivoltine NB₁ by exposing the eggs to hot air (40 °C) for 135 minutes. Strunnikov et al. (1982) have observed low phenotypic variability of quantitative characters in isogenic F₁ hybrids from genetically different homozygous lines. Attempts were made to isolate bisexual lines of mulberry silkworm by means of androgenesis (Xu et al., 1997; Nacheva et al., 1999). Malinova et al. (1996) have developed bisexual homozygous silkworm lines
through androgenesis and treatment of eggs at 42 °C for 210 minutes was found ideal for androgenetic development.

Astaurov and Ostriakova-Vasrshaver (1957) obtained hybrid adult androgenetic males from a cross between domesticated silkworm, *B. mori* and wild silkworm, *B. mandarina* and its reciprocal, where the specific characters were of parental type. Strunnikov (1958) constructed a silkworm line balanced for two non-allelic Z lethals. During oviposition, several sperms enter inside the egg leading to the development of androgenetic individual with monospermic, dispermic or bi-paternal origin (Chowdhury, 2005). In monospermic androgenesis, the diploidy status of the zygote has been restored by fusion of the products of the first division of the male pronucleus and resulted in the development of purely homozygous males whereas in dispermic androgenesis, no female pronuclei are formed and fusion of two different male pronuclei arises in the formation of a diploid zygote (Tazima *et al.*, 1967). The possibility of androgenetic development by inseminating a female by different males (bipaternal androgenesis) showed viable androgenetic offspring following the fusion of sperm nuclei from different fathers (Strunnikov, 1983). Recently, Nirupama and Ravindra Singh (2007a) have observed pronounced androgenetic development following exposure of eggs at 38 °C for 200 minutes.

In Japan, rearing of F1 hybrids was first advocated by Toyama (1906). Increased hybrid vigour in silkworm has been reported by several workers (Hirobe, 1957; Harada *et al.*, 1961; Kobayashi *et al.*, 1968). Bhargava *et al.* (1992) have reported that the heritability of characters in silkworm is very high for larval
duration, cocoon shell weight, filament length, cocoon yield and cocoon shell percentage indicating that these characters are influenced by environmental factors.

In India, rearing of hybrids started during 1920s. Since then, extensive studies were made on the utilization of hybrid vigour in silkworm (Sengupta et al., 1971; Tayade, 1987; Subba Rao and Sahai, 1989; Nagaraju et al., 1989; Bhargava et al., 1993; Ravindra Singh et al., 1990; 1992; 1994; Rajanna and Puttaraju, 1998; Narayanswamy et al., 2002). Manifestation of hybrid vigour has been studied in silkworm (Rao et al., 2001a; 2002a; 2003; Ravindra Singh et al., 1998a; 2000; 2001b; 2002; Nazia Choudhary and Ravindra Singh, 2006a; Dandin et al., 2007). Cross breeding has been extensively used in silkworm improvement as a means of exploiting heterosis (Harada et al., 1961; Krishnaswami et al., 1964, Singh and Hirobe, 1964; Hirobe, 1985, Datta and Pershad, 1988: Nagaraju et al., 1996; Rao et al., 1997).

Indian silk is known for its luster and elegance and is mainly produced either from multivoltine × multivoltine or multivoltine × bivoltine hybrids. It is essential to determine the combining ability of the silkworm breeds in order to produce superior hybrids for commercial exploitation. Krishnaswami et al. (1964) have studied the expression of economic characters in a diallel analysis involving five multivoltine silkworm breeds. Chatterjee et al. (1993) have studied genetic variability in multivoltine silkworm breeds. Sen et al. (1995) have observed the importance of both additive and non-additive gene action for the amelioration of multivoltine silkworm breeds. Datta and Pershad (1988) have indicated that additive genes play an important role in the inheritance of several economic characters. Extensive studies have been carried out on the identification of promising silkworm hybrids utilizing combining
ability (Bhargava et al., 1995; Datta et al., 2001; Rao et al., 2002b; Ravindra Singh et al., 2000b; 2001c; 2005; Gangopadhyay and Ravindra Singh, 2006a; Nazia Choudhary and Ravindra Singh, 2006b).

Identification of silkworm breeds / hybrids based on cumulative effect of several characters is necessary (Narayanaswamy et al., 2002). Among various evaluation methods used in silkworm, Multiple Trait Evaluation Index (E.I.) method of Mano et al. (1993) has been extensively employed for short-listing and selection of multivoltine × bivoltine hybrids (Singh and Subba Rao, 1998; Vidyumnala et al., 1998; Mal Reddy et al., 2002), bivoltine hybrids (Rajalakshmi et al., 2000; Rao et al., 2001; Ramesh Babu et al., 2002), multivoltine breeds (Kariappa and Rajan, 2005) and multivoltine and bivoltine breeds (Nazia Choudhary and Ravindra Singh, 2006c; Gangopadhyay et al., 2006; Rao et al., 2006; 2007; Nirupama and Ravindra Singh, 2007b). Subordinate function index method suggested by Gower (1971) has also been employed for the identification of promising silkworm breeds and hybrids (Ramesh Babu et al., 2002; Rao et al., 2006; Lakshmi and Chandrashekharaiah, 2007).

Cocoon size is an important parameter from the standpoint of silk production, evolution as well as evaluation of commercial hybrids and has direct bearing on the raw silk production and variation in cocoon shape and size results in variation in the quality of raw silk reeled and size of silk filament (Mano, 1994). Studies have been made on cocoon size variability in multivoltine × bivoltine hybrids (Ravindra Singh et al., 1998b; 2001b; 2002; 2004; Mal Reddy et al., 2002; Rao et al., 2002a; 2003; 2005; Umadevi and Rao, 2006) and in bivoltine hybrids (Suresh Kumar et al., 2003).
Androgenesis in silkworm acquires special significance in the development of outstanding homozygous genotypes with low phenotypic variation, increased hybrid vigour, viability and combining ability (Ohkuma, 1971; Strunnikov et al., 1982; Strunnikov, 1986; 1995). In conventional breeding, offspring receives only a random half of alleles from each parent and therefore results are not completely predictable especially in the case of low heritable characters (Seidel and Brackett, 1981). Thus, androgenesis is an important tool in silkworm breeding to regulate the sex ratio in one direction (Astaurov, 1957) and to increase the efficiency of hybridization (Sarikisyan and Giroryan, 1985).

Perusal of literature reveals that no work has been carried out in India on the development of silkworm breeds utilizing androgenesis as a breeding strategy. The present study was carried out at Central Sericultural Research and Training Institute, Mysore to develop superior multivoltine breeds and multivoltine × bivoltine hybrids with high survival, hybrid vigour, combining ability and less phenotypic variability coupled with conventional breeding technique with the following objectives-

- Standardization of techniques for the induction of androgenesis.
- Development of multivoltine homozygous lines through androgenesis.
- Evaluation of newly developed line.