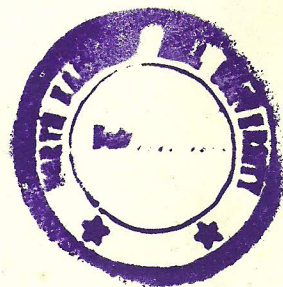


POLLEN PHYSIOLOGY OF ALKALOID YIELDING SOLANUMS (S. KHASIANUM CLARKE AND S. MARGINATUM L. f.) AND FLOWER BUD DEVELOPMENT OF S. KHASIANUM IN VITRO

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THESIS SUBMITTED IN FULFILMENT OF THE DEGREE OF
DOCTOR OF PHILOSOPHY



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I certify that the thesis entitled Pollen Physiology of alkaloid yielding Solanums (S. khasianum Clarke and S. marginatum L.f.) and Flower bud development of S. khasianum in vitro, submitted by Mr. Vituo Belho for the Degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by him under my supervision. The thesis, presented is worthy of being considered for the award of the Ph.D. Degree. This work has not been submitted for any Degree of any other University.

SHILLONG
The 6. 3. 1992

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Chapter-I

GENERAL INTRODUCTION

The social and economic importance of steroidal drugs were emphasized by Bradley et al. (1979). Steroidal hormones comprise nearly 6% of present pharmaceutical preparations and are the active ingredients in drugs used for the cure of Addison's disease, rheumatoid arthritis, leukaemia, chronic cases of asthma and obesity. These hormones are also the active principle of oral contraceptive.

Diosgenin, a steroid sapogenin of Dioscorea is used commercially for the synthesis of steroidal drugs. Due to paucity of Dioscorea raw material availability attempts were made to find out alternate sources of raw material

for steroid industry. Like diosgenin, alkaloid solasodine a nitrogenous analogue of diosgenin obtained from the berries of Solanum species, also yields pregnadienolene acetate, a key intermediate for the synthesis of steroidal drugs (Sato et al., 1951). For the above reason, solasodine yielding solanums have received increasing attention as a convenient and most promising alternate source of raw material for the commercial synthesis of hormonal steroids (Schreiber, 1979). Solasodine occurs as glycosides in more than 100 species of Solanum (Schreiber, 1968). Both Solanum khasianum and S. marginatum yield solasodine in commercially exploitable amount (Chaudhuri and Rao, 1964; Saini et al., 1965; Chaudhuri and Hazarika, 1966; Maiti et al., 1964, 1965; Cruz and Proana, 1970; Chauhan and Joshee, 1985). Solanum khasianum, native to India, is widely distributed in the sub-continent while S. marginatum, a native of Ethiopia introduced in Meghalaya (India) by Chauhan and Joshee (1985), has economic potential as a source of solasodine (Cruz and Proano, 1970). Berries of S. marginatum contain solasodine much higher than any other Indian species (Chauhan and Joshee, 1985). Among the solanums indigenous to India S. khasianum berries contain the highest percentage of solasodine (Kaul and Zutshi, 1977).

The commercial cultivation of these species, however, is hampered due to difficulty in harvesting of

berries because of presence of sharp spines on the aerial parts of the plant, asynchronous flowering resulting in unsynchronized berry maturity and low berry yield due to andromonoecy. Therefore, the need to genetically improve S. khasianum has been emphasized, but attempts to improve S. khasianum by conventional hybridization methods have not succeeded (Kaul and Zutshi, 1974). Thus there is a need to try alternate methods for genetic improvement of solasodine yielding species of Solanum. Indeed mutations are considered an alternative to hybridization and recombinations in plant breeding. Using mutagens attempts were made to synthesize new types of S. khasianum rich in solasodine content, less spiny and high berry yielder (Matsunaga et al., 1969; Bhatt, 1975, 1977). However, attempts have not met with much success.

Ionizing radiations are increasingly used to induce mutations in plants and many medicinal and aromatic plants have been improved by inducing mutations with gamma-rays (Kapoor and Datta, 1967). Gamma rays have been tried for the genetic improvement of S. khasianum and although Bhatt (1972) could get a mutant having curved and blunt spines success has not been spectacular (Bhatt, 1972; Chauhan et al., 1975a,b, 1976; Ravindran, 1981).

Stair and Mergen (1964) suggested that pollen irradiated with low level of radiation could be used to

obtain mutations. Further, the mutants thus obtained are rarely chimeric (Briggs, 1970). Pfahler (1983) emphasized that gametophytic selection is more effective than sporophytic selection in plant breeding. However, for successful crop improvement programme a thorough understanding of the radiobiology of the species being improved is essential since great differences (more than 100 fold) exist in the radiosensitivity of different species (Sparrow et al., 1961a,b). Rudolph (1971) considers that information on relative radiosensitivity of different species under comparable conditions is of value and helps in understanding the radiobiological responses of the species. However, for the success of plant breeding programme employing radiated pollen the first pre-requisite is to understand pollen physiology and pollen radiobiology of the species concerned. Investigations of in vivo pollen germination are not easily feasible due to involvement of complex pistillate tissue. Therefore, in vitro studies are used for investigating physiology of pollen germination and pollen tube growth. Since information on these aspects are lacking for pollen of S. khasianum and S. marginatum an attempt was made to investigate these aspects in S. khasianum and S. marginatum.

Stylar heteromorphism, that is, the occurrence of flowers with different style length in the same inflorescence of an individual is common in many species of Solanum (Hossain, 1973). Depending on the style length, two types of flowers can be recognised, viz. (i) long-styled flowers which are female fertile and bear fruit, and (ii) short-styled flowers which are female sterile flowers and do not bear fruit. Such stylar variation is also found in S. khasianum (Murty and Abraham, 1975). Several attempts were made to find out the factors responsible for stylar heteromorphism in solanums. The variations in style size has been attributed to weather and season (Pal and Singh, 1943), nutrition (Wakhloo, 1972; Hossain, 1973). In S. khasianum occurrence of long- and short-styled flowers could be altered by exogenous application of growth hormones (Chauhan and Ravindran, 1980; Chauhan and Joshee, 1987). Using tissue culture techniques De Jong and Bruinsma (1974b,c) found the involvement of growth hormones in the development of pistil in Cleome flowers. Thus in vitro flower bud culture studies can be used as a tool to investigate hormonal involvement in flower development. Although the flower buds of some plant species have been cultured successfully, the potentiality of the flower buds for growth, development, and differentiation in in vitro

experiments varies from species to species (Konar and Kitchlue, 1982). In a number of studies young flower buds of diverse species have been cultured in vitro (Konar and Kitchlue, 1982). However, the success in obtaining normal growth of buds to maturity in both dicotyledons (Konar and Nataraja, 1964; Mohan Ram and Wadhi, 1966; Ganapathy, 1969) and monocotyledons (Guha and Johri, 1966) was limited. Tepfer et al. (1963, 1966) used complex medium for the growth of young floral buds of Aquilegia and although initiation and early development of various floral organs was achieved, the petals did not grow to maturity and stamen primordia aborted in later stages. In Viscaria, the floral apices with sepal or petal primordia could be reared to maturity only if a pair of leaves was included with the floral buds (Blake, 1966, 1969). Only in a few cases the normal development of stamens and ovules and the differentiation of pollen grains (Black, 1966, 1969; Porath and Galun, 1967; Hicks and Sussex, 1970; Polowick and Greyson, 1982) and embryo sac (Hicks and Sussex, 1970) has been achieved. In Lycopersicon esculentum, however, normal development of young floral buds occurred in vitro (Rastogi and Sawhney, 1986, 1988). In all these studies nutritional and hormonal requirements vary from species to species. But no attempt has been made so far to culture young floral buds of S. khasianum

to determine its nutritional and hormonal requirements. Therefore, an attempt is made to investigate these aspects.

Thus it is evident from the above that not much work has been done on physiology of pollen germination and pollen radiobiology of S. khasianum and S. marginatum and in vitro flower bud development of S. khasianum. Therefore, in the present study an attempt was made to investigate the following aspects of pollen physiology, radiobiology and in vitro flower bud culture :

- I. Pollen germination :
 - A. Nutritional requirement
 - B. Effect of growth hormones
 - C. Radiosensitivity
 - D. Modulation of radiation response
 - E. Cytochemistry.
- II. Fertilizing ability of irradiated pollen.
- III. M_1 generation of S. khasianum.
- IV. Flower bud development of S. khasianum in vitro.