GENERAL INTRODUCTION

Sexuality in plants is somewhat neglected field of plant science though it is a direct issue in the economic sphere. Our knowledge of the control of sexuality in plants is in complete contrast to that of sexuality in animals, which has been a subject of active and highly fruitful research for decades. In plants, indeed, it is a field that had its roots in the remote past, but unfortunately attracted little attention from modern researchers although it bears closely on the practical applications of agricultural and horticultural science. In recent years, there has been a renewed interest in the mechanism of sex determination, sex differentiation, sex expression, sexual dimorphism and sex conversion in plants (Negrutiu and Sala, 1991).

For deep understanding of various aspects of sexuality in plants, the study of dioecious plants with heteromorphic chromosomes is absolutely essential. On the other hand, development of methods for sex conversion, characterization of sexual dimorphism and selective use of female plants can be utilized for successful agriculture. However, in plants the occurrence of dioecious species that has strong male and female individuals are limited. Heterochromosomes related to the sex determination have been detected in about 20 species (Chattopadhyay and Sharma, 1991). Of these *Melandrium album* is the most studied plant, with sharp correlation between phenotypic sex and the presence of heteromorphic sex chromosomes (Ye et al., 1991). In many plants like *Momordica dioica*, *Trichosanthes dioica*, and *Asparagus officinalis* eventhough dioecy is absolute, recognizable sex chromosomes reported to be absent (Trivedi and Roy, 1972; Roy et al., 1982; Bracale et al., 1991).

The present interest in *Coccinea grandis* (L.) Voigt., a member of Cucurbitacea is derived from its strong dioecious nature with male and female plants. With regard to sexuality, various species of the family Cucurbitaceae exhibits gradations of monoecism and dioecism including gynomonoecious and andromonoecious, polygamous and dioecious plants. *Coccinea grandis* is one of the few flowering plants that has a chromosomal basis of sex determination. The dioecious nature of this plant is exhibited in the form a heteromorphic pair of chromosomes, designated as sex chromosomes. The diploid chromosome number is

2n = 24. The female and male plants carry XX and XY chromosomes respectively (Kumar and Vishveshwaraiah, 1952; Roy and Roy, 1971). Moreover, the sweet variety of *Coccinea grandis* is economically important for its non-bitter fruits and ayurvedic uses as a medicinal plant (Hossain et al., 1992). Though it is a promising plant to study different aspects of sexuality, very few attempts so far have been made and it is yet to be fully exploited.

Although sexuality is fully developed in plants, the manifestation of sexual dimorphism is rather poor. Sexual dimorphism, is defined as morphological differentiation of sexually mature male and females, is a conspicuous feature of dioecious plants (Wilson, 1991) The identification of sex at early stages of development in plants would not only be of theoretical interest but also important for agricultural practices. In discerning sexual dimorphism or identification of sex, conventional methods are found to be not satisfactory as they have their own shortcomings. Application of molecular markers and emerging techniques of artificial intelligence may be immense potential in discerning sexual dimorphism in plants. Reports on sex determination with the aid of molecular markers are now being gradually accumulated but no attempt has yet been made with image processing and artificial neural networks. The present study describes the extent of sexual dimorphism in *C. grandis* primarily with the conventional methods and elaborates further the status with plant tissue culture, image analysis and artificial neural networks.

Male and female plants may differ from one another in the duration of growth and development as well as in other respects. Morphometric evaluation of betelvine (*Piper betle*) a dioecious plant showed sexual dimorphism in leaf shape (Maity and Biswas, 1991). Correlation between seed morphology and physiology on the sex expression was reported in papaya (Dhara and Verannah, 1998). In case of *C. grandis* flowering is the only means to recognize sexual dimorphism and so far no attempt has been made to study morphological marker in this species though strict male and female plants are available. It is essential to look for morphological markers for sexual dimorphism in this plant. An attempt has been made to identify the sex on the basis of sexual dimorphism at morphological level without examining the flowers for stamens and gynoecia.

The determination of sex in dioecious plants has long been a matter of discussion especially in relation to the sex chromosomes. The importance of karyotype study in establishing phyletic relationships, evolutionary trends is well recognized. The discovery of heteromorphic sex chromosomes in a handful of dioecious species (Chattopadhyay and Sharma, 1991) provides a morphological marker of the difference in the phenotypic sex and also a basis of sex determination. In *C. grandis* conflicting reports exist with regard to the heteromorphic nature of chromosomes. Kumar and Deodikar (1940) reported the heterogametic nature of *C. grandis* in female. In contrast, Bhaduri and Bose (1947); Chakravorti (1948); Kumar and Vishveshwaraiah (1952); Roy and Roy, (1971); Sen and Datta (1977) noted typical X-Y mechanism with heterogamesis on the male side. In the present study a detailed chromosomal analysis has been made to find out a reliable cytological marker for male and female.

The potential of plant cell and tissue culture for exploring fundamental as well as applied problems is demonstrated in many cases. Regeneration of plants and expression of sex particularly in dioecious plants are extremely limited. Attempts were restricted for vegetative propagation (Gulati, 1988, Josekutty et al., 1993; Roussos et al., 1999). Culafic et al., 1987) obtained in vitro organogenesis in two dioecious species *Rumex acetocella* and *Rumex acetosa.* In the reported experiments flowering was not induced in culture and the sex of regenerated male and female plants was not altered by culture conditions. Differential hormonal requirements have been suggested for clonal propagation of male and female plants of jojoba, a dioecious shrub (Agrawal et al., 1999). In spinach, the expression of sex in the regenerated plants and the effect of exogenous growth regulators on sex expression were examined (Komai et al., 1999). It has been suggested that the sex expression of spinach is manifested as a flexible phenotype, converged from dioecy to andro- or gynomonoecy by the tissue culture procedure and the exogenous GA_3 enhances convergence.

C. grandis being dioecious, is suitable for studies on hormonal regulation of sex expression and further sexual dimorphism. The present investigation is aimed at to develop a protocol for in vitro regeneration of plants from explants derived from known sex and to distinguish growth regulator requirements, if any for regeneration of male and female plants.

Anther culture is used to attain haploidy and further to get homozygosity in a short span of time. Studies on androgenesis of members of Cucurbitaceae and dioecious plants are very limited (Sinha et al., 1979). Anther culture for production of dihaploid inbreeds in economically important dioecious plants merit more attention (Manshardt et al., 1998). In case of *Coccinea grandis* besides the possibility of reaching of homozygosity, anther culture provides a very specific and original advantage. It is possible to produce two different kinds of homozygous plants one of them, the super male plants may allow the creation of F_1 hybrid only with male plants. Such plants are useful for fundamental research in the area of sexuality in plants (Doré, 1990). In the present work attempt has been made to obtain haploid plants by anther culture.

Apart from the above mentioned approaches the application of image processing and artificial neural networks may also be helpful to discern sexual dimorphism with clear identification of male and female sex. A method of identification of sex of a dioecious plant at juvenile stage prior to transplanting from nursery to field can save the agriculture sector from wastage of lot of resources and money. In dioecious species seed progeny yields 50% males and 50% females (Bhojwani and Razdan, 1983) and the identification is only possible when plant comes to flowering. Early detection of sex is essential when plants of one of the sexes are more desirable. Artificial neural networks with its amazing speed of understanding intricacies in complex patterns enable plant scientists to differentiate between objects or organs or organelles which otherwise look alike. Image analysis (Computer vision) with its powerful capacity for extracting concrete detailed information from images has numerous diverse applications in biology (Smith, 1995).

A neural network is a computational structure inspired by the study of biological neural processing. Artificial neural networks cover the way to organize synthetic neurons to solve the same kind of difficult, complex problems in a similar manner as we think the human brain may. The networks are used as vehicles for adaptively developing the coefficients of decision functions via successive presentation of training sets of patterns. Neural networks can be trained to recognize patterns (Rao and Rao, 1996).

Image analysis and artificial neural network techniques are now being used, for various biological applications for example automated sex separation in broiler chicks (Tao and Walker, 2000), the shape analysis of leaf and plant identification (Franz et al., 1991a,b; Humphries and Simonton, 1993; Guyer et al., 1993; Fiorino and Mancuso, 1998), the identification of seed varieties (Dehghan-Shoar et al., 1998; Luo et al., 1999); the quality measurement of vegetables, fruits and nuts (Ghazanfari et al., 1996; Nielsen and Paul, 1998; Beni et al., 1998; Rapisarda et al., 1998); the separation of embryogenic callus from non embryogenic callus (Honda et al., 1999) and the separation of normal embryos from abnormal embryos (Zang et al., 1996).

In view of the potential applications of image processing and artificial neural networks, one of the aims of the present work was to develop a method using machine vision for the detection of sex in *C. grandis* during vegetative stage of growth.

Taking into account the aforesaid the specific objectives of the present programme are:

1) To explore the dimorphic criteria in morphological and cytological features
2) To establish an in vitro regeneration protocol from explants derived from known sex
3) To induce androgenesis and morphogenesis in anther culture
4) To develop a machine vision system for early detection of sex