ABSTRACT

Trichosanthes dioica Roxb. popularly called pointed gourd, is a dioecious, perennial, viny, herbaceous plant showing morphological difference between the male and female plants expressed only during the flowering stage. The plant belongs to the family Cucurbitaceae. Its fruits, highly nutritious and wholesome, are consumed as a cooked vegetable. And, the plant is known to possess medicinal properties that have not been systematically investigated. The species is distributed in the tropical and sub-tropical regions of the world. It is considered to have originated in the South East Asian region and DeCandolle (1882) considered India to be its center of origin.

A deep understanding of the mechanism of sex determination is a pre-requisite for the genetic improvement of a dioecious crop species. Sex determination is primarily under genetic control and sexual dimorphism is not merely an adaptive character developed in the process of evolution. Rather, it is also a consequence of physiological and biochemical differences between male and female organisms. Secondary sexual distinctions are coded by different genotypes in the opposite sexes and are genetically linked to the primary sexual characters. Studies have shown that variations between the male and female of a dioecious species are found in a whole array of morphological and biochemical indices, such as dimensions of various vegetative parts, the contents of pigments, vitamins, carbohydrates, proteins, difference of type and number of amino acids occurring in their proteins, nucleic acid content, DNA sequence complexity, mRNA activity, activities of enzymes, hormone level, occurrence of isoenzymes etc.

The pointed gourd crop is propagated by vegetative methods, through vine cuttings or root suckers. Propagation through seeds is avoided due to a poor seed germination rate, very short seed viability, emergence of 50% male (non-fruiting) plants and the longer time (2-3 years) required for flowering in the plants raised from seeds. This important and highly nutritious vegetable crop also suffers from low yield. The breeding methods outlined are clonal selection, controlled hybridization and further maintenance of F1 seedling clonally selection of seedling segregates and alteration of ploidy. A single elite plant selected from a population or from among seedling segregates could form the basis of a new variety and a rapid clonal propagation through micropropagation is of great practical importance. The *in vitro* selection of elite clones may be made easier if the linkage of single gene traits with biochemical loci (for e.g. isoenzyme markers) is known. Somatic hybridization and gene cloning may offer exciting variety improvement strategies. No molecular biological probes or *in vitro* studies have been reported in *T. dioica* in relation to dioecy.

The present study was, therefore, undertaken with the following objectives:

(i) To explore the dimorphic criteria in morphological features.

(i) To study the expression of dimorphism during pollen grain development.

(iii) To determine whether isoenzyme markers for sexual dimorphism are available in the plant.

(iv) To develop a micropropagation protocol for the rapid clonal propagation of *Trichosanthes dioica* Roxb. and to study the isozyme profile *in vitro*.

Quantitative study of the following morphological characters was made from a large sample from the farm population of a local variety of *Trichosanthes dioica*. Six leaf characters (viz. leaf length, breadth, area, length/breadth ratio, dry weight and dry weight/area from the male and female plants), internodal lengths of male and female plants, number of stomata from the lower epidermis of the leaves of male and female plants, and seed weights were recorded. Gross morphological features of male and female plants showed no difference. Biometric evaluation of quantitative leaf characters revealed a significant difference between the two sexes for many of the parameters. Anatomy of the male and female flowering apices and young flower buds were studied under light microscope.

For the study of pollen morphology and development, the following experiments were conducted: (i) Staging of the anthers by pollen nuclear staining with acetocarmine (ii) Image processing of pollen grains, to determine their projected area as viewed under the light microscope, followed by cluster analysis (iii) Tests for Pollen viability (iv) Study of pollen germination in different media (v) Study of pollen tube mitosis, (vi) Scanning Electron microscopy, and (vii) Fluorescence microscopy to ascertain the nuclear status of the mature pollen. The presence of two clusters for the seed weights, and pollen grain dimension detected by digital image analysis technique corroborates the earlier findings on pollen size groups of *Trichosanthes dioica* indicating a possible heterogametic nature of male. This is in conformation with the hypothesis that there may be sex-linked traits in pollen grains that may be expressed at a very early stage such as a dimorphism of size (or size-range).

The isoenzyme polymorphism was studied with respect to seven enzyme systems viz. catalase, peroxidase, esterase, malate dehydrogenase, phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase and aspartate aminotransferase by Polyacrylamide Gel Electrophoresis (PAGE) and *in situ* staining of the gels for enzymatic activity, followed by scanning densitometry and image analysis. All the seven enzymes showed clear and consistent isoenzyme pattern as revealed by their activity staining. Definite isozyme polymorphism was observed for peroxidases, during floral development, between the two sexes and for aspartate aminotransferase in tissues of male and female plants during all stages. Densitometric quantification for comparison of the enzyme activity staining bands revealed quantitative variation between the tissues, and between male and female.

A protocol was developed, for the first time, for *in vitro* propagation of *Trichosanthes dioica* through single node culture with simultaneous rooting. Cultures had been maintained continuously through 20 passages. Isoenzyme patterns from the leaves of the male and female plants (raised *in vitro*) for the two enzymes peroxidase and aspartate aminotransferase were examined and were found to be similar to that of the field grown plants.

The current study successfully demonstrates for the first time, several non-chromosomal markers for sexual dimorphism, both morphological and isozymic, in *Trichosanthes dioica* Roxb. The protocol developed for the rapid clonal propagation of *T. dioica* can be used for large-scale plantlet production for field cultivation of selected clones. *In vitro* grown tissues also display similar type of isoenzymic patterns. The work indicates that *Trichosanthes dioica* can be used as a model system for the study of sexual development and differentiation without discernible morphological sexual dimorphism in a dioecious species.