INDUCTION OF POLYPLOIDY IN *PHLOX DRUMMONDI*II

R.C. VERMA, RAMESH AHIRWAR AND PREETI DAS
School of Studies in Botany, Vikram University, Ujjain (M.P.) 456010

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**SUMMARY**

Polyploidy was induced in *Phlox drummondii* (Polemoniaceae) by the treatment of shoot apices of seedling with 0.2% colchicine solution. Polyploid plants were found to show gigantism. The leaves, flowers, and seeds were larger as compared to diploids. PMCs of autotetraploids showed on average 1.75 quadrivalents, 9.63 bivalents and 1.98 univalents per cell. The overall purpose of inducing autotetraploidy was achieved as the flowers were bigger, brighter, and had longer shelf life and thus increasing the ornamental value.

**Key Words:** Polyploidy, *Phlox drummondii*

**INTRODUCTION**

Polyploidy has played an important role in the improvement of many plant species. The field of floriculture has probably benefitted the most because polyploidy can increase genetic variability. Polyploidization is known to affect various aspects of morphology, anatomy, physiology and genetics. The most immediate effect is on morphological characters particularly in the leaf and flower size. In India, the most commonly grown ornamental species of *Phlox* is *P. drummondii* an annual, herbaceous plant which completes its life cycle in 4-5 months. The chromosome number is 2n=14. The present work was undertaken to induce polyploidy by colchicine treatment, with the possibility to develop a form better suited for ornamental purpose.

**MATERIALS AND METHODS**

*Phlox drummondii* was selected for the induction of colchitetraploidy. Seeds were sown in the pots in first week of November. After 10-12 d, seedlings came out. When seedlings were 8-10 d old, they were treated with 0.2% colchicine solution for 5-6 h per day for 2-3 d. For meiotic study, young flower buds were fixed in 1:3 glacial acetic acid: absolute ethanol mixture for 24 h. Anthers were squashed in 2% iron acetocarmine. For mitotic study, excised root tips were pretreated with 0.02% colchicine for about 3 h and then fixed in 1:3 glacial acetic acid (Ethanol), mixture for 24 h. Fixed root tips were hydrolyzed in 1N HCL at 60°C for 10 min. Root tips were stained in leucobasic fuchsin in the usual manner and squashed in 2% iron acetocarmine. Photographs were taken from temporary preparations.

**OBSERVATIONS**

208 seedlings (18 maroon, 16 maroonstar, 14 magenta, 16 orange, 12 orangewhite, 13 pink, 15 pinkstar, 18 red, 16 redstar, 14 voilet, 12 lightviolet, 16 white, 12 whitetstar, 16 whiteblue) were...
treated with 0.2% colchicine, of which only 153 (12 maroon, 12 maroonstar, 10 magenta, 12 orange, 10 orangewhite, 11 pink, 12 pinkstar, 12 red, 12 redstar, 10 violet, 8 lightviolet, 12 white, 10 whitestar, 10 whiteblue,) survived. Flower buds of 89 plants (8 maroon, 8 maroonstar, 6 magenta, 6 orange, 8 orangestar, 9 pink, 4 pinkstar, 6 red, 4 redstar, 8 violet, 6 lightviolet, 6 white, 5 whitestar, 5 whiteblue, ) were analyzed for meiotic behavior but colchitetraploidy was found only in 6 plants (2 violet, 1 light violet, 2 white, and 1 whiteblue). Percentage success was 25% violet, 16.5% lightviolet, 33% white and 20% for whiteblue.

In general, there was an increase in overall size of the colchitetraploid plants. The stem had thicker diameter and hairs over them were more prominent as compared to control. The average plant height at maturity is 37.8 cm in colchitetraploids as compared to 30.2 cm in control plants. The leaves of colchitetraploid plants were darker, thicker and bigger in size; the shape also changed to ovate as compared to lanceolate in diploid. The total area (lengthxwidth) of a leaf ranged from 16.2 to 17.8 cm as compared to 14.8 cm in diploid (Fig. 1). The general increase in leaf area was a distinguishing feature of colchitetraploids from diploid. The flowers in colchitetraploids were bigger and had deeper colour (Fig. 2). The longevity of the flowers in colchitetraploid was also 5-7 d more than the diploids. Individual petals of the flowers were thicker and fleshier. There was an overall improvement in the important features which are of ornamental value. All the colchitetraploids could be very well distinguished in the field from the diploids on the basis of flower characters. Flower diameter of colchitetraploids was about 1.5 times more as compared to control.

Number of stomata per microscopic field was less in colchitetraploids which ranged from 72 to 96 as compared to 148 in control. Size of stomata was about 1.5 times more in colchitetraploid than control (Fig. 3). Pollen stainability/fertility was observed in diploid and all colchitetraploid. Pollen stainability/fertility ranged from 72 to 78.8% in colchitetraploid as compared to 97% in control. Average pollen stainability was 76.2%, minimum (72) in PC03 and maximum (78.8) in PC06. Pollen size also varied in diploid and colchitetraploid. In diploid, pollen grains were of equal size (16.2µm). Whereas in colchitetraploids, slight variation was seen in pollen size; the largest pollen grains ranged from 22.2 to 26.4µm while the small pollen grains were 16.8 to 19.3µm. Capsules per plant in colchitetraploid ranged from 52 to 76 as compared to 96 in control. Average capsules per plant ranged from 65.3-76. Seeds per capsule in colchitetraploids ranged from 1.70 to 1.96 as compared to 3.04 in diploid. Average seeds per capsule range from 1.8 to 1.96.

Chromosome number. of diploid is 2n=14. The chromosome complement of colchitetraploids consists of 28 chromosomes. Each chromosome of colchitetraploids was represented four times (Figs. 11-14), the karyotypic formula was 28J.

PMCs in diploids (Figs. 8-10) have 7 bivalents. The number of chiasmata per cell ranged from 14-18, mean number being 15.5, out of which 14.0 were terminalized giving terminalization coefficient of 0.90 and 1.5 chiasmata were unterminalized. The meiotic studies have been done in 89 colchitreated plants and were analyzed at diakinesis/metaphase-I. Various cytological features recorded at diakinesis/metaphase-I were average and range of associations like quadrivalents,
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bivalents, univalents, chiasma frequency per cell, chiasma terminalization, and terminalization coefficient etc. Average number of quadrivalents in colchitetraploids were 1.75 per cells, minimum was 1.1 in PC04 and maximum 2.4 in PC02 (Figs. 15-16). The average number of bivalents were 9.6 in all colchitetraploids, minimum was 7.2 in PC02 and maximum 11.4 in PC04 was compared with 7.0 in control (2X). The average number of univalents were 1.9 in all colchitetraploids, minimum 1.0 in PC03 and PC04, and maximum 4.0 in PC02. Mean number of chiasma being 28.7 out of which 19.5 were terminalized giving terminalization coefficient of 0.67 and 9.1 chiasmata were unterminalized. The minimum average number of chiasmata per cell was 27.9, out of which 19.3 were terminalized giving terminalization coefficient of 0.69 in PC02 and maximum average number of chiasmata per cell was observed in PC04 whereas 29.5 out of which 21.3 were terminalized giving terminalization coefficient of 0.72. The chromosomes were distributed equally (14:14) at anaphase-I (Figs 17-18).

DISCUSSION

Induced polyploidy has been used with much success in the improvement of ornamental plants all over the world. In India, induction of autoploids in ornamental like Linaria was done (Bali & Tandon 1957). Arora (1975) induced tetraploids in Verbena which had vigorous growth habit, broader leaves, larger flower and sterile pollen. Verma & Raina (1991) reported morphological changes in colchitetraploids as the leaves were broader and thicker, had increased internodal length, thicker stem, increased flowers size and longer blooming in Phlox drummondii.

The tetraploids showed considerable increase in cell size which was evidenced by the increase in pollen grains and stomata. Higher multivalent frequency might have resulted sterility of colchicine treated colchitetraploids in the present investigation. Seed fertility can be correlated too with their pollen fertility. Raghuvanshi & Pathak (1975) concluded that the seed setting in Phlox drummondii colchitetraploids may be the result of genetic factor (s). However, observations by Rao et al. (1982) indicated that it may also be the result of cytological factors.

The chromosome count of colchitetraploids was also observed at mitotic metaphase and was found to be 4x = 2n = 28 which also is in confirmation with earlier reports (Verma & Raina 1991). Seven bivalents were observed in diploids as observed by previous workers (Verma & Raina 1991, Rama Rao et al. 1982). In colchitetraploids, the number of quadrivalents varied from cell to cell and plant to plant. The average number of qudrivalents per cell in the present study was 1.75 which was lower (2.13) as compared to the observation by Raghuvanshi & Pathak (1975) and higher (1.36) as compared to Verma & Raina (1991) in the same species.

Increased fertility was accompanied by an increase in the frequency of bivalents and decrease in the frequency of quadrivalents. In the present case, although enhanced frequency of bivalents may have significantly contributed to enhanced fertility, some improvement may also have been brought about by selection of genotypes leading to greater fertility without any effect on meiotic behavior of chromosomes.
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CYTOTOXIC EFFECT OF A LECTIN FROM CYMBIDIUM ALOIFOLIUM ON HUMAN COLORECTAL CANCER CELL LINES

ANANYA SARKAR, M.C. GAYATRI AND *SARNGI, S.K.
Department of Botany, Bangalore University, Bangalore 560 056
* Department of Microbiology & Biotechnology, Bangalore University, Bangalore 560 056.
sarkarananya2@gmail.com

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SUMMARY

Cymbidium aloifolium (Orchidaceae), an orchid with anti-viral properties was found to contain a novel mannose-specific lectin CAA (Cymbidium, aloifolium Agglutinin) with potential biological activities. C.aloifolium is an economically and medicinally important species occurring mostly in Eastern Himalayas and the Western Ghats region. The lectin was prepared from the leaves of the mature plant collected during the flowering season. The crude extract was subjected to affinity purification with an overall yield of 37 mg. A cell viability assay was performed using three human cell lines, viz., fibroblast cells, colorectal Aden carcinoma cell line (SW 480) and colorectal orectal carcinoma cell line (HCT 116) at different time points. It was found that 10µg/ml concentration of CAA being the most effective in killing the cancer cells specifically (LD50). The degree of CAA cytotoxicity was found to be comparatively stronger on SW480 than HCT 116.

Key word: Anti-cancer Properties, Cymbidium aloifolium, lectin, Orchidaceae, colorectal carcinoma, adenocarcinoma.

INTRODUCTION

The study of lectins (called lectinology) dates back to 1888, when extracts from castor beans were found to agglutinate red blood cells (Still mark, 1888). Since then hundreds of different lectins have been isolated mostly from plants both from monocotyledonous and dicotyledonous members of the plant kingdom. In recent times, lectins have attracted attention, not only for their occurrence but also for their varied properties and biomedical importance.

The word “lectin”, derived from the Latin verb “lager” meaning “to select”, was introduced only in the first half of the 19th century. Prior to this, the lectins were known as ‘haemagglutinins’ because of their agglutinating actions on red blood cells (Elf strand, 1898). Evidences, based on purification and characterization, indicate that lectins are a very heterogeneous group of proteins and are classified into four major types, and this categorization is based primarily on the structure of the subunits. The four classes are merolectins, hololectins, chimerolectins and superlectins.

Plant lectins occur predominantly in the seeds, although their occurrence has been well documented in other vegetative tissues such as leaves, stems, barks, bulbs, tubers, corms, rhizomes,
roots, fruits, flowers and even in nectar (Peumans et al., 1995). Due to significant progress made in the study of biochemistry and molecular biology of plant lectins, the structural and evolutionary relationships between the different members have become evident. The super family of mannose-binding lectins (MBLs) is a relatively new group of proteins. The first of such lectins was isolated from the bulbs of snowdrop (Galanthus nivalis) only in 1987 (Van Damme et al., 1995). Since then structurally related lectins have been isolated from five different monocot families viz. Amaryllidaceae, Alliaceae, Araceae, Orchidaceae and Liliaceae. All the lectins belonging to this super family not only have a marked sequence homology, but also exhibit an exclusive specificity towards mannose.

Lectins from the orchids are poorly known and the first orchid lectin to be isolated is an MBL from the leaves of Listera ovata (Van Damme et al., 1987). This lectin was named as Listera ovata Agglutinin (LOA). Since then lectins have been isolated from a number of species of orchids viz., Epipactis helleborine, D. findleyanum and Cymbidium hybrid (Balzarini et al., 1982 and Sudmoon et al., 2008). The present study aims to isolate purified fraction of a lectin from an orchid species, Cymbidium aloifolium, found in Western Ghat region of Karnataka, India and to ascertain its potential biological activity against human colorectal cancer cell lines. Cymbidium aloifolium, the orchid species under present study is also known for its medicinal attributes. It is used as a paste, which is tightly plastered on bone fractures and also on “crack foot” (Reddy et al., 2005). Furthermore, the natives of certain areas of Eastern Ghats use the seeds of Cymbidium sp. as oral contraceptive (Mazumder et al., 2010). Moreover, it has also been observed that several orchid species have the potential to become effective pesticides and fungicides, which indirectly emphasizes the presence of abundant quantity of defense proteins like lectins in them. The present investigation is, therefore, an attempt to unravel the medicinal importance of lectin of C. aloifolium, which may emerge, in future, as a “green remedy” for the treatment of several human ailments like colorectal carcinoma and also adenocarcinoma. This has encouraged the author to perform the cell viability assay of these novel molecules against both normal and cancer cell lines to illuminate not only their biological significance but also humanitarian attributes as potential remedy for colorectal carcinoma and colorectal adenocarcinoma.

MATERIAL AND METHODS

Collection of samples

Leaves of C. aloifolium were collected during flowering season (October to March) from the plants maintained in vivo in the orchidarium, Plant Biotechnology Unit, Annexe-Department of Botany, Bangalore University, Bangalore, Karnataka, India. The fresh leaves were used immediately and the extra leaves were stored at -20°C for future use.

Purification by Affinity chromatography

Purification of the dialyzed crude fraction of lectin sample was done by using a Con A Sepharose 4B (GE Healthcare UK Ltd., Amersham, UK) affinity matrix. Con A Sepharose™ was
Concanavalin A coupled to Sepharose 4 B by the cyanogens bromide method. The medium had a ligand density of 10 - 16 mg ConA/ml drained medium. The available capacity data as determined in 0.1 M Phosphate buffer pH 7.0 was 20 - 45 mg porcine thyroglobulin/ml medium. The maximum linear flow rate was 75 cm/hour at 25°C on a 5 cm bed height. After reading the absorbance at 280 nm, the eluted fractions of 0.5 ml were assayed for lectin activity by hemagglutination assay. All the fractions showing lectin activity were pooled and concentrated using polyethylene glycol (PEG) and freeze dried for later use. The purity of the CAA was continuously testified with SDS-PAGE (Laemmli, 1970) till a single band was obtained.

**Hemagglutination assay**

The serial two-fold dilutions were prepared from the crude extracts of CAA in 0.9% saline, and were incubated with different volumes of 4% erythrocyte suspension (20µl, 40µl, 60µl, 80µl and 100µl) in a microtiter plate for one hour at room temperature. The assay also included ammonium sulfate and whole blood as the controls. The titer strength of the samples was determined as the reciprocal of the lowest dilution, which showed detectable agglutination and expressed as hemagglutination unit (HAD). The agglutination was determined visually with the unaided eye after one hour at room temperature. The minimum amount of each sample (EO, expressed as µg/ml) required for agglutinating the RBCs is calculated.

**Demonstration of biological activity of CAA**

**Source of cells and culture medium**

Fibroblast primary cell culture - established in the Cell Biology Lab, Enzene Biosciences Pvt., Ltd. was used as the control healthy cell; SW 480 (Human colorectal adenocarcinoma) and HCT-116 (Human colorectal carcinoma), were obtained from ATCC for the anti-cancer assay of CAA. DMEM, Fetal Bovine Serum, Trypsin - EDTA, Antibiotic - Antimycotic mixture, obtained from Invitrogen were different components for the cell culture. Tissue culture flasks and plates were specifically procured from Nunc.

**Culturing of normal and cancer cells in vitro**

All the cell lines were grown in monolayer in appropriate media [DMEM supplemented with 10% FBS, containing antibiotic - antymycotic] and were maintained at 37°C in an atmosphere of humidified air with 5% CO₂. Trypsin-EDTA was used to detach the monolayer for subculturing or for preparing cells for experiments. SW480 and HCT116 were colorectal carcinoma cells, whereas Fibroblast is representative of a normal epithelial cell. The fibroblast cell selected at the 8th passage stage whereas the two immortal cancer cell lines were revived and cultured for two weeks before conducting the assay. All the cells used for the as say were adherent cell lines. The cells were seeded on the 96 well plate with approximately 105 cells well and left overnight. Fresh medium was supplied to the cells after overnight incubation and were subsequently assayed for cytotoxicity with CAA treatment.
Cytotoxicity assay

Cells in the log phase of growth were trypsinized and washed in serum free DMEM. Cells were counted using a Neubauer chamber (Hemocytometer). 10^5 cells/well of all three cell types were plated in individual wells of a sterile 96well T/C plate in 105 cells/well of complete growth medium. Plate was then incubated at 37°C with 5% CO₂, overnight incubation, which allowed cells to adhere. CAA was used at the following final concentrations of 0.1, 1 and 10 µg/ml. Al lectin was used as the control at the same volume as 10µg/ml of test lectin. Phosphate buffer was used as control. Dilutions of the controls and test lectins were made in DMEM.

Plate containing cells was removed from incubator and spent media was then replaced with complete DMEM. Diluted CAA samples and controls were then added to the cells, in a final total volume of 200µg/well Cells were then incubated at 37°C for 6 and 24hrs. At each time point, cells were separately trypsinized, washed once in PBS and resuspended in PBS. An equal volume of trypan blue was added, and after 2 min cells were scored for live and dead cells using a Neubauer chamber. Dead cells take up trypan blue and appear blue, whereas live/healthy cells exclude trypan blue and appear clear. Results were noted down and percentage of cell death calculated as follows:

\[
\text{Percentage of dead cell} = \frac{\text{Number of dead cells}}{\text{Total number of cells}} \times 100
\]

\[
\text{Percentage of living cell} = \frac{\text{Total number of cells} - \text{Number of dead cells}}{\text{Total number of cells}} \times 100
\]

RESULTS AND DISCUSSION

The *C. aloifolium* leaves were extracted with ammonium sulfate and PMSF to obtain the crude CAA. The crude CAA was then applied on a desalting column to remove the extra salts which generally causes hindrance during protein purification.

The increasing use of lectins in chemical and biological research has prompted the development of many methods for their purification. Because of its specificity, affinity chromatography has been used most widely, and several types of ligands and supports have been employed (Baues and Gray, 1977). The ligands utilized have either been glycoproteins or synthetically prepared glycosides. In the present study, the mannose specificity of *C. aloifolium* leaf lectin ensured easy selection of affinity column for further purification of the crude protein. Con A Sepharose 4B column, employed in the present study is Cocanavalin A coupled to Sepharose 4B by the cyanogen bromide method. Con A is a tetrameric metallo-protein isolated from *Canavalia ensiformis* (jack bean). Con A binds molecules containing a D-Mannopyranosyl, D-Glucopyranosyl and sterically related residues.
The binding sugars requires the presence of C-3, C-4 and C-5 hydroxyl groups for reaction with Con A. Con A coupled to Sepharose is routinely used for separation and purification of glycoproteins, polysaccharides and glycolipids. When crude extract was loaded onto a Con A Sepharose 4B affinity column, the CAA fraction gets selectively bound to Con A through their mannose residues as both are mannose specific in nature. Therefore, on elution with an increasing gradient (linear or step) of 2-D-methylmannoside containing equilibration buffer, the purified CAA is obtained. The overall yield of CAA after affinity purification was found to be 0.37 mg/ml (Fig.1). The purity of CAA was repeatedly tested on SDS-PAGE. The affinity purification was carried out till a single band of purified CAA was obtained.

The marker proteins were also run to find out the molecular weight of the purified CAA. The molecular weight was found to be 25 kDa (Fig. 2). Cell - cell interaction in multicellular organisms is
a fundamental phenomenon which includes cell-cell recognition, communication, adhesion and rearrangement, etc. Most of these interactions are mediated by the cell-surface which contains a number of surface membrane proteins or glycoproteins responsible for cellular recognition (Sharon and Lis, 1972). Indeed RBC agglutination is the usual method for detecting the presence of lectins in a solution and has contributed to the discovery of many lectins (Boyd and Reguera, 1949). However, in the present study, leaf extracts of both crude and purified fraction of CAA showed a strong ability to agglutinate rabbit RBCs. This could be visually anticipated from the amount of precipitate formed after reaction with both types of plant samples. Positive results of the agglutination reaction formed a red carpet, which covered the bottom of the wells of the microtiter plate. Further, the ED was calculated and found to be 12.968 µg/ml.

The biological activity of plant lectins has attracted the attention of researchers of different fields. A number of MBLs including Orchidaceae lectins are found to have an anti-viral activity mostly against HIV(2). These lectins interfere with HIV gp 120, a viral envelope glycoprotein, thus preventing the viral binding with the target cells. In the present investigation, the cytotoxicity of CAA was performed against two human cancer cell lines, SW 480 and HCT 116 and a normal human fibroblast cell line. SW480 is a double mutant of p53 gene responsible for causing human adenocarcinoma, a tumor of the glandular epithelial cells, whereas HCT 116 cells are epithelial like in morphology and used as an in vitro model for colorectal cancer to study tumor markers, CEA (Carcinoembryonic Antigen) production, biochemistry of tumorigenicity, pharmacodynamics, hormone sensitivity and as a positive control for the nuclear protein Beta catenin. HCT-116 cell line has a mutation in codon 13 of the ras protooncogene. When subjected to a 10µg/ml concentrations of CAA, SW 480 and HCT 116 cell lines experience more percentage of cell death (51.5 % and 26.4% respectively) (LD50) after 24 hours time point with the fibroblast cells showing negligible mortality (2.2 %). On the contrary, the three cell lines were observed to be completely unaffected at 6 hours time point even with 10µg/ml concentrations of CAA. Moreover, the mortality rate was not noticeable at 0.1 and 1µg/ml concentrations of CAA. Therefore, it is evident that 10µg/ml concentrations of CAA is effective against both human colorectal carcinoma and adenocarcinoma cell lines (Table 1; Figs.3,4a 4b and 5).

<p>| TABLE 1: Cell viability assay at 24 hours time point. Numbers denote percentage of dead cells |
|----------------------------------|----------------------------------|----------------------------------|</p>
<table>
<thead>
<tr>
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<th>% cell death</th>
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<tr>
<td>Fibroblast</td>
<td>SW 480</td>
<td>HCT-116</td>
<td></td>
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<tr>
<td>Media control</td>
<td>1.8</td>
<td>1.1</td>
<td>2.34</td>
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<tr>
<td>Buffer control</td>
<td>3.1</td>
<td>1.2</td>
<td>1</td>
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<tr>
<td>0.1µg/ml of lectin</td>
<td>2.9</td>
<td>0.9</td>
<td>1.6</td>
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<tr>
<td>1.0 µg/ml of lectin</td>
<td>1</td>
<td>1.2</td>
<td>2.14</td>
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<tr>
<td>10µg/ml of lectin</td>
<td>2.1</td>
<td>31.5</td>
<td>26.4</td>
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<td>Al lectin 30 µg/ml</td>
<td>2.2</td>
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CYOTOXIC EFFECT OF CYMDIBUM LECTIN

Fig. 3: At 24 hrs time point normal Fibroblast after treatment with 10µg/ml of CAA

Figs. 4a-b : (a) At 24 hrs time point HCT-116 after treatment with 10µg/ml of CAA. b) At 24 hrs time point SW480 after treatment with 10µg/ml of CAA

Fig. 5: Percentage of cell death in different concentration of CAA at 24 hours time point
The cell death caused by CAA cytotoxicity may be due to apoptosis and not by necrosis. Should it be due to necrosis, cell death would have set in within 4 to 6 hours. The remarkable aspect of the CAA-induced cytotoxicity in this study is that it does not affect the normal cells and selectively kills the cancerous ones. Therefore, it can be assumed that CAA can effectively get metabolized after inducing cytotoxicity to the cancer cells, leaving the normal cells unaffected within 24 hrs under in vivo condition (Fig.5). This is a first report on anti-cancer properties of an orchid lectin, CAA.

CONCLUSION

In the present investigation, a mannose-specific lectin (CAA) has been isolated, purified and characterized for the first time from a pure species of *Cymbidium, C.aloifolium* which can agglutinate rabbit erythrocytes readily. This novel lectin, CAA of 25 kDa is found to have potent anti-cancer activity against human colorectal carcinoma and adenocarcinoma cell lines without affecting the normal fibroblast cells. Therefore, deciphering how specific CAAs exert their biological effects should provide insights into how they can be exploited for therapeutic interventions, and potentially have major clinical implications for the treatment of neoplastic growth.

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VIRULENCE OF NUCLEAR POLYHEDROSIS VIRUS OF *SPODOPTERA LITURA* (F.) (LEPIDOPTERA: NOCTUIDAE) ON DIFFERENT HOST PLANTS

B.S. RAVISHANKAR AND M.G. VENKATESHA*
Department of Zoology, Bangalore University, Bangalore 560056
*E-mail: venkatmelally@gmail.com

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SUMMARY

*S. litura* is a very serious polyphagous pest on various economically important crops, which is controlled by nucleo polyhedro virus. Various bioassay methods were employed to screen *SINPV* against *S. litura* reared on different host plants. In the first experiment *S. litura* larvae were reared on different host plants such as *Arachis hypogaea* L., *Brassica oleracea* L., *Gossypium hirsutum* L., *Rosa indica* L. and *Solanum tuberosum* L. When they completed second instar, they were transferred to semi-synthetic diet which was treated with different doses of *SINPV*. The range of \(LT_{50}\) values at different concentrations on different host plants was as follows: cabbage 4.877-7.382, cotton 5.623-8.606, groundnut 4.579-6.557, potato 5.455-7.806, and rose 5.800-9.032. In the second experiment, *S. litura* larvae were reared on semi-synthetic diet up to second instar and subsequently they were fed on leaf disc of different host plants, which were treated with *SINPV*. The range of \(LT_{50}\) values on different host plants was as follows: cabbage 4.933-7.954, cotton 6.135-9.394, groundnut 4.757-7.155, potato 5.584-9.164, and rose 6.312-9.816. In the third experiment, *S. litura* larvae were reared on different host plants till they complete second instar and they were screened against *SINPV* in the laboratory on respective host plants by using leaf disc method. The range of \(LT_{50}\) values on different host plants was as follows: cabbage 5.442-8.273, cotton 6.465-11.076, groundnut 5.167-7.867, potato 6.055-9.731, and rose 6.776-12.208. Among the different host plants, there was a significant difference in \(LT_{50}\) between groundnut and cabbage. In all the experiments, the \(LT_{50}\) values were highest in lower concentrations and visa versa.

Key words: Bioassay, host plants, Nuclear polyhedrosis virus, *Spodoptera litura*, \(LT_{50}\)

INTRODUCTION

*Spodoptera litura* (F.) (Lepidoptera: Noctuidae) is a major polyphagous pest attacks various economically important crops such as cotton, groundnut, rice, tomato, tobacco, citrus, cocoa, potato, rubber, castor, millets, sorghum, maize and several vegetables (Hill, 1993). The pest occurs in India, Pakistan, Bangladesh, Sri Lanka, S. E. Asia, China, Korea, Japan, Philippines, Indonesia, Australia, Pacific Islands, Hawaii and Fiji (Hill, 1993). *S. litura* has already developed resistance to several organic pesticides resulting severe crop losses in India (Singh & Singh, 1998). Of late, environment friendly methods of pest control have come into practice due to increasing failures of
chemical pesticides and their indiscriminate use in the field. The insect viruses are of immense utility owing to their often pronounced host specificity and their high virulence to susceptible insect hosts. Baculoviruses are considered to be one of the most efficient biocontrol agents for insects (Ignoffo & Couch, 1981). The virions are occluded in Polyhedral Occlusion Bodies (POBs) and are protected against environmental conditions for years. Today, at least 1300 insect viruses are known to infect insects (Moscardi, 1999). Among these, Nuclear Polyhedrosis Viruses (NPVs) are the most suitable and effective pest control agents due to their desirable attributes like infectivity, lethality, storage stability and environmental safety (Cunningham et al., 1999). They are particularly attractive as bioinsecticides because of two factors. They are safe for vertebrates and other non-target fauna and they are generally highly pathogenic, thus host death being the most likely outcome of an infection. Today, several baculoviruses have been commercialized all over the world and used as possible alternative for toxic and environmentally disruptive chemicals. As the selection of virulent strain of NPV is key to the development of effective bioinsecticides, local strains are always preferred for sustainability, adaptability and efficacy under a given set of agro-ecosystem and hold an ample scope for their wide spread multiplication and commercial use as novel biopesticides in a particular region (Gupta et al., 2007). Phenotypic variation among different isolates of insect viruses recovered from members of the same host species from different geographical locations has been reported in terms of survival time and pathogenicity and their DNA differed in restriction endonuclease and protein patterns (Toprak et al., 2006).

Fortunately, S. litura is highly susceptible to its NPV and studies have shown that the virus can be used effectively as biopesticide in the field (Jayaraj et al., 1980). S. litura nucleo polyhedro virus (SINPV) is the most promising biocontrol agent and its efficacy has been established successfully against the pest in India (Jayaraj & Rabindra, 1990; Muthuswami et al., 1993). Considering the reliability, suitability and effectiveness of SINPV in terms of economic and ecological reasons, its utilization in the pest management has received a great deal of significance. Among the ecofriendly pest management tools, the use of insect viruses remains the most promising, considering the fact that they can be used in a manner similar to the common chemical pesticides. Unlike other natural enemies, insect viruses can be produced and stored and made available to the farmers at short notice due to their longer shelf life. Of the various insect viruses, nucleo polyhedron virus (NPV) has been more successful in pest management (Roberts et al., 1991).

Although SINPV has been reported as a very effective microbial biocontrol agent against S. litura, greater amount of variations in the efficacy of SINPV across the host plants hindered its practical utility in the field condition as reported in other lepidopteron pests (Young et al., 1976; Felton et al., 1987; Felton & Duffey, 1990; Keating et al., 1988; Rabindra et al., 1994). The information related to the direct effect of host plants and/or host plants effect through the host insect on the efficacy of SINPV at the same concentration under identical conditions is incomplete. Therefore, experiments on screening of SINPV were undertaken to understand the effectiveness of SINPV against S. litura on different host plants.
MATERIALS AND METHODS

Source of insect and virus

Field collected larvae of Spodoptera litura on castor, Ricinus communis L. in Bangalore were reared on semi-synthetic diet in the laboratory following the method of Shorey & Hale (1965) at constant temperature (25±1°C), humidity (65±5%RH) and photoperiod 14L:10D. When these larvae were pupated, the pupae were sexed and kept in nylon cages (30x30x30cm) at 5:5 ratio (Male: Female) per cage. The emerged male and female moths were allowed to mate in the cages and females were provided with muslin cloth for egg deposition. Freshly hatched larvae were mass reared on semi-synthetic diet. The initial culture of Spodoptera litura nuclear polyhedrosis virus (SiNPV) was obtained from Biological Control Research Laboratory, Pest Control India Pvt. Ltd., Bangalore and infected the early fourth instar larvae of S. litura by diet surface contamination method. The infected larvae exhibiting typical symptoms of polyhedrosis virus were collected and stored in stopper flask with 1ml of water per larva and they were left to putrefy for about 15 days enable them to release polyhedra from infected tissues. The putrefied suspension of diseased larvae was homogenized using the tissue homogenizer and left undisturbed for 2-3 days to facilitate the polyhedra to settle as a whitish layer at the bottom of flask. Then the dead tissue on the upper layer of the flask was removed gently without disturbing the polyhedra at the bottom and the remains of the polyhedra was filtered by two-layered filter cloth and was centrifuged at 6000 rpm for 1 hr. The pallets containing pure polyhedral inclusion bodies were collected and re-suspended in distilled water and stored at 4°C till further use. The concentrations of the polyhedra were estimated with the help of a double ruled improved Neubaur Haemocytometer of depth factor 0.1 mm (Weber, England) under a phase contrast microscope. Appropriate SiNPV dilutions were made using stock preparation of 1×10^9 POB/ml.

Methodology followed to count POB

Serial dilution

Suspension of 10µl stock was transferred to sterile microfuge tube and the volume made up to 100µl with distilled water. Serial dilution was repeated twice, enumeration and calculation of the number of POB’s were performed thrice as described by Rabindra et al. (2001).

Bioassay procedure

Disease-free colony of S. litura was maintained on semi-synthetic diet in the laboratory. In the first experiment, to understand the influence of various host plants on SiNPV efficacy against S. litura larvae without directly coming into contact, freshly hatched larvae of S. litura from the laboratory culture were reared on different pot cultured host plants such as Arachis hypogaea L. (groundnut), Brassica oleracea L. (cabbage), Gossypium hirsutum L. (cotton), Rosa indica L. (rose) and Solanum tuberosum L. (potato) in the green house. The newly emerged female and male moths of S. litura from these cultures were paired and caged separately for egg laying. When these eggs
hatched the second generation neonate larvae were transferred to the respective host plants and reared up to the end of second instar. When they reached third instar, the larvae of uniform size were selected and introduced singly into vial (6x25cm) containing semi-synthetic diet, which was treated with S/NPV by diet surface contamination method following the procedure of Ignoffo (1966). The mortality of the larvae was recorded at 24 hrs intervals after treatment till tenth day.

In the second experiment, to understand the effect of host plants on S/NPV efficacy by direct contact, uniform sized fresh third instar larvae of S. litura reared on semi-synthetic diet were selected and fed separately on S/NPV treated fresh leaf discs (5mm diameter) of cabbage, cotton, groundnut, potato and rose for one day in Petri dishes (21×2.5cm diameter). Subsequently, these treated larvae were fed with respective untreated fresh leaves of host plants at every 24 hrs.

In the third experiment, to understand the direct effect of host plants on S/NPV as well as through S. litura larvae, which were reared on the different host plants even after the treatment, newly emerged S. litura adults on semi-synthetic diet were paired and caged for egg laying. Newly hatched S. litura larvae were reared on cabbage, cotton, groundnut, potato, and rose plants in the green house and allowed to complete their generation. Newly emerged the female and male moths of S. litura from these cultures were paired and caged separately for egg laying. When the eggs hatched neonate larvae of second generation were transferred to the respective pot cultured host plants and reared up to the end of second instar. When they reached third instar, the larvae of uniform size were selected and reared on fresh leaf discs of different host plants treated with S/NPV in the Petri dishes for one day and later they were fed with untreated leaves till completion of their development or death.

In all the above experiments, to force the larvae to ingest the S/NPV treated food, they were kept starved for 2 hrs before the treatment as followed earlier (Trang & Chaudhari, 2002). In all the experiments, the larvae reared on semi synthetic diet were treated as control. For each treatment, there were five replications with ten larvae per replication. Bioassay of S/NPV using 1×10^6, 2×10^5, 4×10^4, 8×10^3, 1.6×10^3 and 3.2×10^2 concentrations were conducted.

**Data analysis**

LT_{50} of S/NPV against the pest on different host plants was assessed using Probit analysis (Finney, 1952).

**RESULTS AND DISCUSSION**

In the case of S. litura larvae reared on different host plants and subsequently fed with S/NPV treated semi-synthetic diet, the range of LT_{50} values at different concentrations on different host plants in the ascending order was as follows: groundnut 4.579-6.557, cabbage 4.877-7.382, potato 5.455-7.806, cotton 5.623-8.606 and rose 5.800-9.032. The lowest LT_{50} value was recorded in groundnut and the highest LT_{50} value was in rose at the same concentration (Table 1).
TABLE 1: LT₅₀ values of S/NPV against *Spodoptera litura* assayed on semi-synthetic diet

<table>
<thead>
<tr>
<th>Concentration (POBs/ml)</th>
<th>Host plants</th>
<th>LT₅₀</th>
<th>95% limit</th>
<th>Slope</th>
<th>Intercept</th>
<th>x²</th>
<th>Df</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Upper</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1×10⁶</td>
<td>Groundnut</td>
<td>4.579</td>
<td>4.043</td>
<td>5.071</td>
<td>7.618</td>
<td>-5.034</td>
<td>14.117</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>5.455</td>
<td>5.131</td>
<td>5.801</td>
<td>6.132</td>
<td>-4.518</td>
<td>7.539</td>
</tr>
<tr>
<td></td>
<td>Rose</td>
<td>5.800</td>
<td>5.427</td>
<td>6.230</td>
<td>5.410</td>
<td>-4.130</td>
<td>8.722</td>
</tr>
<tr>
<td></td>
<td>Cabbage</td>
<td>5.215</td>
<td>4.674</td>
<td>5.784</td>
<td>6.265</td>
<td>-4.494</td>
<td>11.043</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>5.606</td>
<td>5.268</td>
<td>5.975</td>
<td>5.951</td>
<td>-4.455</td>
<td>8.645</td>
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<td>Cotton</td>
<td>5.951</td>
<td>5.598</td>
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<td>-4.692</td>
<td>5.957</td>
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<tr>
<td></td>
<td>Rose</td>
<td>6.134</td>
<td>5.728</td>
<td>6.632</td>
<td>5.304</td>
<td>-4.178</td>
<td>7.041</td>
</tr>
<tr>
<td></td>
<td>Cabbage</td>
<td>5.754</td>
<td>5.128</td>
<td>6.559</td>
<td>5.245</td>
<td>-3.986</td>
<td>10.637</td>
</tr>
<tr>
<td>8×10³</td>
<td>Groundnut</td>
<td>5.587</td>
<td>4.808</td>
<td>6.627</td>
<td>4.914</td>
<td>-3.672</td>
<td>15.527</td>
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<tr>
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<td>Cabbage</td>
<td>6.610</td>
<td>6.203</td>
<td>7.140</td>
<td>6.090</td>
<td>-4.995</td>
<td>2.549</td>
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<td></td>
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<td>5.247</td>
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<td>1.6×10³</td>
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<td>5.187</td>
<td>7.154</td>
<td>4.677</td>
<td>-3.628</td>
<td>13.264</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>7.122</td>
<td>6.611</td>
<td>7.873</td>
<td>5.525</td>
<td>-4.711</td>
<td>3.742</td>
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<td>Cotton</td>
<td>7.387</td>
<td>6.821</td>
<td>8.271</td>
<td>5.381</td>
<td>-4.674</td>
<td>3.843</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>7.806</td>
<td>7.151</td>
<td>8.916</td>
<td>5.350</td>
<td>-4.774</td>
<td>3.006</td>
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<tr>
<td></td>
<td>Cotton</td>
<td>8.606</td>
<td>7.675</td>
<td>10.458</td>
<td>4.703</td>
<td>-4.397</td>
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<tr>
<td></td>
<td>Rose</td>
<td>9.032</td>
<td>7.969</td>
<td>11.315</td>
<td>4.710</td>
<td>-4.502</td>
<td>2.347</td>
</tr>
</tbody>
</table>

*Control.
The range of LT$_{50}$ values at different concentrations with respect to S. litura larvae reared on semi-synthetic diet and subsequently fed with SlNPV treated leaf discs of various host plants in the ascending order was as follows: groundnut 4.757-7.156, cabbage 4.933-7.954, potato 5.584-9.164, cotton 6.135-9.394 and rose 6.312-9.816. The lowest LT$_{50}$ value was recorded in groundnut and the highest LT$_{50}$ value was in rose at the same concentration (Table 2).

**TABLE 2: LT$_{50}$ values of SlNPV against Spodoptera litura reared on semi-synthetic diet and assayed on different host plants**

<table>
<thead>
<tr>
<th>Concentration (POBs/ml)</th>
<th>Host plants</th>
<th>LT$_{50}$</th>
<th>95% limit</th>
<th>Slope</th>
<th>Intercept</th>
<th>X$^2$</th>
<th>DF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>5.584</td>
<td>5.235</td>
<td>5.970</td>
<td>5.650</td>
<td>-4.220</td>
<td>8.707</td>
</tr>
<tr>
<td></td>
<td>Cotton</td>
<td>6.135</td>
<td>5.743</td>
<td>6.612</td>
<td>5.533</td>
<td>-4.374</td>
<td>6.419</td>
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<tr>
<td></td>
<td>Rose</td>
<td>6.312</td>
<td>5.873</td>
<td>6.877</td>
<td>5.050</td>
<td>-4.041</td>
<td>7.029</td>
</tr>
<tr>
<td>2x10$^7$</td>
<td>Groundnut</td>
<td>4.992</td>
<td>4.311</td>
<td>5.695</td>
<td>5.835</td>
<td>-4.075</td>
<td>15.898</td>
</tr>
<tr>
<td></td>
<td>Cabbage</td>
<td>5.416</td>
<td>4.843</td>
<td>6.066</td>
<td>5.693</td>
<td>-4.177</td>
<td>10.689</td>
</tr>
<tr>
<td></td>
<td>Cotton</td>
<td>6.594</td>
<td>6.135</td>
<td>7.211</td>
<td>5.232</td>
<td>-4.286</td>
<td>5.538</td>
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<td>4x10$^4$</td>
<td>Groundnut</td>
<td>5.707</td>
<td>5.046</td>
<td>6.562</td>
<td>5.149</td>
<td>-3.895</td>
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<td></td>
<td>Potato</td>
<td>7.010</td>
<td>6.449</td>
<td>7.843</td>
<td>4.740</td>
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<td>8x10$^3$</td>
<td>Groundnut</td>
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<td>5.654</td>
<td>6.564</td>
<td>5.135</td>
<td>-4.019</td>
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<td>1.6x10$^4$</td>
<td>Groundnut</td>
<td>6.492</td>
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<td>4.881</td>
<td>-3.965</td>
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<td>7.130</td>
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<td>Potato</td>
<td>8.350</td>
<td>7.461</td>
<td>10.033</td>
<td>4.460</td>
<td>-4.111</td>
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<td>8.688</td>
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<td>11.629</td>
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<td>Rose</td>
<td>9.816</td>
<td>8.497</td>
<td>13.228</td>
<td>5.018</td>
<td>-4.978</td>
<td>2.346</td>
</tr>
</tbody>
</table>

*Control.
VIRULENCE OF NUCLEAR POLYHEDROSIS VIRUS OF SPODOPTERALITURA

The ascending range of LT$_{50}$ values in the case of S. litura larvae reared on different host plants and subsequently fed with SlNPV treated leaf discs of various host plants was as follows: groundnut 5.167-7.867, cabbage 5.442-8.273, potato 6.055-9.731, cotton 6.465-11.076 and rose 6.776-12.208. Again the lowest LT$_{50}$ value was recorded in groundnut and the highest LT$_{50}$ value was in rose as noticed in the above experiments (Table 3).

**TABLE 3:** LT$_{50}$ values of SlNPV against Spodoptera litura reared and assayed on different host plants

<table>
<thead>
<tr>
<th>Concentration (POBs/ml)</th>
<th>Host plants</th>
<th>LT$_{50}$</th>
<th>95% limit</th>
<th>Slope</th>
<th>Intercept</th>
<th>X$^2$</th>
<th>dF</th>
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<tbody>
<tr>
<td>Cabbage</td>
<td>5.442</td>
<td>4.848</td>
<td>6.121</td>
<td>5.734</td>
<td>-4.219</td>
<td>11.481</td>
<td>6</td>
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<tr>
<td>Potato</td>
<td>6.055</td>
<td>5.653</td>
<td>6.543</td>
<td>5.244</td>
<td>-4.102</td>
<td>9.020</td>
<td>6</td>
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<tr>
<td>2x10$^6$</td>
<td>Groundnut</td>
<td>5.760</td>
<td>5.401</td>
<td>6.169</td>
<td>5.640</td>
<td>-4.289</td>
<td>7.682</td>
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<td>Cabbage</td>
<td>5.778</td>
<td>5.146</td>
<td>6.508</td>
<td>5.266</td>
<td>-4.012</td>
<td>10.828</td>
<td>6</td>
</tr>
<tr>
<td>Rose</td>
<td>7.400</td>
<td>6.734</td>
<td>8.474</td>
<td>4.422</td>
<td>-3.844</td>
<td>5.806</td>
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<tr>
<td>8x10$^6$</td>
<td>Groundnut</td>
<td>6.575</td>
<td>6.262</td>
<td>7.438</td>
<td>5.087</td>
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<tr>
<td>Cabbage</td>
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<td>8.008</td>
<td>4.559</td>
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<td>Potato</td>
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<td>8.005</td>
<td>11.647</td>
<td>4.374</td>
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<tr>
<td>Rose</td>
<td>11.064</td>
<td>9.116</td>
<td>17.153</td>
<td>4.223</td>
<td>-4.409</td>
<td>0.740</td>
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<tr>
<td>3.2x10$^7$</td>
<td>Groundnut</td>
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<td>9.067</td>
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<td>2.668</td>
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<tr>
<td>Cotton</td>
<td>11.076</td>
<td>9.103</td>
<td>17.166</td>
<td>4.064</td>
<td>-4.244</td>
<td>1.212</td>
<td>6</td>
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<tr>
<td>Rose</td>
<td>12.208</td>
<td>9.675</td>
<td>23.474</td>
<td>4.510</td>
<td>-4.901</td>
<td>0.279</td>
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</table>

*Control.
In all the three experiments viz., a) without direct contact between host plants and SlNPV, b) with direct contact between host plants and SlNPV and c) with direct contact between host plants and SlNPV as well as host plants influence through the host insect the lowest LT$_{50}$ value recorded was in groundnut and the highest LT$_{50}$ value was in rose at all concentrations. Similarly, varying LT$_{50}$ values were also observed at the same concentration of SlNPV screened against S. litura in direct contact with different host plants (Kulkarni and Hugar, 2000). Moreover, LT$_{50}$ values were varied in the case of fungal pathogen, Beauveria bassiana L. tested on Helicoverpa armigera (Hubner), S. litura and Spilosoma obliqua, which was reared on different host plants (Haseeb, 2007).

From our experiment it could be made out that the different host plants influence the virulence of SlNPV through the host insect as well as by direct contact with the virus. It is known that the leaf exudates from glandular hairs of the cotton plants inactivate the NPV of Heliothis spp. (Falcon, 1971; Young and Yearin, 1974). Further, it is reported that plants mediate interactions between insect and its pathogens, which increase or decrease the impact of the pathogen (Rabindra et al., 1994; Meade et al., 1995).

Further, the increasing larval mortality was noticed from fourth to tenth day after treatment. Moreover, the lowest LT$_{50}$ value was found at higher concentrations and visa versa. In addition, larval mortality was also increased with increasing concentrations of SlNPV irrespective of the host plants.

From the foregoing results it could be made out that the different host plants influence the effectiveness of SlNPV by direct or indirect means. Further investigations on the leaf pH, larval midgut pH and secondary metabolites of the different host plants could provide a vital clue about the factors that interfere with the virulence of SlNPV.

ACKNOWLEDGEMENTS

The authors thank Dr. K. P. Jayanth, Vice President, Bio-control Research Laboratory (BCRL), Pest Control of India Private Limited (PCI), Bangalore 561 203 for providing the virus culture and laboratory facilities for conducting the experiments. Thanks are due to Dr. Gangadhar B. Narabenchi and Dr. S. S. Gayathri Devi, BCRL for their help and discussions during the experiment.

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MICROSPOROGENESIS IN GENETIC MALE STERILE AND FERTILE VN-2 LINES IN CAPSICUM ANNUUM L.

R. B. MAHESHWARI, C. G. P. A. TIL, AND O. SRIDEVI

1Department of Botany, Karnatak Science College, Dharwad - 580 001
2Department of Genetics and Plant Breeding, College of Agriculture, University of Agricultural Sciences, Dharwad - 580 005

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SUMMARY

A comparative histological study of VN-2 male sterile and fertile anthers of chilli (Capsicum annuum) revealed shriveling of microspores due to failure of post-meiotic mitosis leading to disintegration of nucleus, shrinkage of microspore cytoplasm and unusual vacuolation. Further, due to hypertrophied tapetum, there was crushing of microspores which appeared as thick black undifferentiated mass ultimately leading to sterility.

Key Words: Capsicum annuum, genetic male sterility, microsporogenesis.

INTRODUCTION

Male sterility has gained more interest because of its potential practical value in producing commercial hybrid seeds as it produces a means of genetic emasculation in hybrid seed production (Edwardson, 1970, Laser & Lersten, 1972). Use of heterotic hybrids is an important option to improve the yield potential. Utilization of male sterility permits hybrid seed production and commercial exploitation of heterosis in crops in which emasculation on a large scale is not possible. Considerable histological basis of male sterility has been reported in different crops such as wheat (Chauhan & Singh, 1966), cotton (Soddi et al., 1995) and soybean (Dubey, 1970). Male sterility in chilli was reported by Peterson (1958) and Shifriss (1997). However, the histological basis of genetic male sterility has not been well understood in Capsicum annum. Therefore, in the present investigation, an attempt was made to analyze the comparative histological events accompanying genetic male sterility in VN-2 lines of chilli with a view to understand the nature of irregularities during microsporogenesis and pollen development.

MATERIALS AND METHODS

The genetic male sterile and fertile VN-2 lines were grown at Agricultural Research Station, Dharwad during kharif 2009. For comparative microsporogenesis study, different sized flower buds of both sterile and fertile lines were collected and fixed separately in Carnoy’s - A for 48 hr and histological studies were carried out by standard methods of micro-technique (Jensen, 1962). Hydrated sections were incubated for 15 min in 0.05% amido black 10B at room temperature. Sections were rinsed in 7% acetic acid for 1 minute, air dried, cleared in xylol and mounted in DPX. The stained sections were photomicrographed using ‘Axiostar plus’ (Carl Zeiss) Bright field/Fluorescent Modular microscope and Cannon’s Power Shot G2 digital camera with higher resolution and optical zoom of 4.3X.
RESULTS AND DISCUSSION

A male sterile plant was observed in the normal population of hot chilli VN-2 lines with distinct difference in morphological characters from normal fertile plant. The plant had greater vegetative growth and was bushy with broad dark green leaves. The flowers were white with bluish anthers while in normal the anthers were whitish. Microsporogenesis in anthers of GMS line was indistinguishable from its fertile counterpart until the release of microspores from tetrads. Six to eight archesporial cells in each anther lobe give rise to an outer layer of primary parietal cells and an inner layer of primary sporogenous cells. The primary parietal cells by further divisions give rise to an endothecium, a middle layer and the secretary tapetum (Fig. 1). Tapetum is the inner most wall layer of anthers wall which is in direct contact with the developing meiocytes. Embryologically, tapetum has been regarded as secretory tissue and therefore supplies nutrients to the developing meiocytes and their derivatives, microspores and pollen grains (Maheshwari, 1950). Its cells disintegrate after the release of microspores from tetrad condition in fertile lines (Fig. 4). Differential number of sporocytes has been observed in both male fertile and GM sterile anthers (Table.1)

<table>
<thead>
<tr>
<th>Stage of anthers</th>
<th>Fertile/Sterile anthers</th>
<th>Number of sporocytes ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early pollen mother cell</td>
<td>Male fertile</td>
<td>6.0±0.3</td>
</tr>
<tr>
<td>Early pollen mother cell</td>
<td>Male sterile</td>
<td>5.8±0.2</td>
</tr>
<tr>
<td>Late pollen mother cell</td>
<td>Male fertile</td>
<td>8.6±0.7</td>
</tr>
<tr>
<td>Late pollen mother cell</td>
<td>Male sterile</td>
<td>8.4±0.5</td>
</tr>
<tr>
<td>Tetrads</td>
<td>Male fertile</td>
<td>6.8±0.6</td>
</tr>
<tr>
<td>Tetrads</td>
<td>Male sterile</td>
<td>5.6±0.4</td>
</tr>
</tbody>
</table>

During different stages of anther development the primary sporogenous tissue by further divisions gave rise to pollen mother cells. Later through meiosis resulted in the formation of dyads, trihedral and tetrahedral tetrads in both GMS and male fertile anthers by furrowing (Fig. 2). The secretory tapetum disintegrates soon after the release of microspores in (Fig. 3).

The microspores in male fertile line had large nucleus and dense cytoplasm (Fig. 4) but later they started moving towards one side of the microspores. Each microspore has a thin intine and a thick exine wall. The pollen grains at 3 celled stage were released after the breakdown of endothecium layer. Each pollen at this stage showed prominent nucleus either in centre or little away from the centre with intact wall. The anther dehisces and the pollen grains were released from the locules.

The microspores in GMS line though enlarge initially (Fig. 6), but later shrivel and show vacuolation accompanied by the disintegration of nucleus and shrinkage of cytoplasm. Sometimes, microspores exhibit an abnormal shape (Fig. 5). The anther sacs were completely collapsed and compressed towards inside (Fig. 10), enclosing degenerated microspores which formed a thick, black undifferentiated mass along the anther cavity. Just before anthesis, the anther sacs were empty with remnants of dead microspores in the form of a dark mass of tissue (Fig. 10).
However, the tapetum in sterile line remained intact for a longer duration showing radial elongation and hyperptrophy of its cells indicating its malfunction (Fig. 8). Thus, the abortion of pollen grains in GMS lines was brought about by abnormal behaviour and malfunctioning of tapetum. Similar results were observed by Warmke and Overman (1972) in cytoplasmic male sterile sorghum. Novak (1977) observed male sterility in chilli was due to the hypertrophic activity of tapetum followed by crushing of PMCs which was noticed in chilli. Abnormal changes in tapetum during the formation of microspores resulted in disintegration of pollen grains. Thus, the anther sacs were empty with dead remains of collapsed tissue.

**CONCLUSION**

Genetic male sterility in VN-2 lines of chilli may be due to considerable shrivelling of microspores associated with disintegration of nucleus, shrinkage of cytoplasm, formation of vacuoles which lead to the failure of post meiotic mitosis and also due to the radial elongation and hypertrophied tapetum cells which ultimately caused crushing and abortion of pollen grains.

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Fig 1-4: Transverse section of fertile anthers showing - 1. Early PMC 2. Late PMC 3. Microspores with degenerating tapetum 4. Fertile pollen grains Fig 5-10: Transverse section of sterile anthers showing - 5. Early PMC 6. Late PMC 7. Degenerating microspores 8. Degenerating meiocytes with hypertrophied tapetum 9. Completely degenerated microspores 10. Abnormal shape enclosing deformed late microspores
MICROSPOROGENESIS IN GENETIC MALE STERILE

- Tapetum (T)
- Pollen mother cell (PMC)
- Degenerating tapetum (DT)
- Anther wall (AW)
- Hypertrophoid tapetum (HT)
- Pollen grain (P)
- Vacuolation (V)
- Degenerating anther (DA)
- Anther sac (AS)
- Empty anther sac (EA)
CONSANGUINITY STUDY IN THE MUSLIM COMMUNITY FROM KOLLM, KERALA

DEVIPRIY A V1, APSARA S1 AND MATHEW P M2 1 Department of Botany, Sree Narayana College, Kollam.  
2 Department of Botany, University of Kerala, Kariavattom.  
e-mail:devipriyav@hotmail.com  
(Received 9 December 2010, accepted 15 March 2011)

SUMMARY

Consanguinity study was carried out in a sample of 1500 families of Muslims in the Kollam district (Kerala). The pattern of close-kin alliance in the community was predominantly first cousin matrilateral type followed by 1 C patrilateral. The overall frequency of related marriages in the group was 16.87%. Factors which influence the choice of mates were examined which included socio-economic variables and demographic determinants. The time trend of consanguinity was also examined. The risk effects associated with consanguinity in the community was estimated in terms of pre-reproductive mortality and morbidity. The mortality rate was found to be highly significant in the consanguineous group of families (10.51 %) and so also the morbidity profile. The family income, social status, spousal age at marriage, level of occupation of wife, marital distance between spouses etc. were observed to be negatively correlated with level of inbreeding and the risk effects thereof. However, an increase in the incidence of consanguinity was observed among the present day Muslim youth despite the increasing levels of literacy in the state. The recent trend of ‘going back to the age old tradition’ spurred by incidence of communal violence, might have contributed to this. Literacy promotion, awareness regarding employment opportunities and initiation of steps to promote communal harmony are suggested to be very essential in enhancing the financial stability, social status and emotional well-being of the members of this community for countering the practice of related marriages and thereby minimizing the harmful effects of the phenomenon.

Keywords: consanguinity, Muslim community, Kollam, mortality, morbidity, genetic counselling

INTRODUCTION

Consanguineous marriages are marriages contracted between blood relatives. Earlier definitions for consanguineous marriages have stressed on the possession of common ancestors in the preceding few generations of the ascending line leading to the bringing together at fertilization of two alleles that are identical by descent (Mathew et al. 2006). The homozygosity of recessive alleles has been found to be associated with impairment of function leading to many harmful traits or diseases. This has been predicted from the reduced early survival of offspring in first cousin marriages and from similar results in other organisms (Bittles & Neel, 1994). Diverse social customs and cultural practices rampant in the Indian scenario have imposed several reproductive barriers, leading to endogamous isolates based on castes, tribes, races etc. The joint

1 Sarasi, Sreemoolam Road, Medical College Po, Thiruvananthapuram-695011  
2 Perakathusseril, Muttada Po, Thiruvananthapuram,. Kerala-695025
family system and custom of arranged marriages have resulted in a high degree of consanguinity, especially in the rural and illiterate communities (Abraham & Mathew, 1969). The Hindu and Muslim communities of Kerala have encouraged consanguineous marriages to an appreciable degree for economic, social or other benefits. Although marriages between close biological kins are preferential in many parts of the state, there still is a great lack of knowledge of this central feature of human kinship structure. The exposure to foreign culture and customs through the easily accessible media like the television and the internet, have accelerated the advancement of socio-economic changes, leading to an increased tendency among the youth to defy the existing social norms and culture. This is evidenced by the increased rates of inter caste marriages, which may in turn succeed in tearing away the existing binds of caste and community, thereby fading or blurring the present day clear-cut boundaries between the various endogamous isolates in the near future. This calls for a speedy and comprehensive exercise of consanguinity study of the inbreeding groups in the state. It is in this context that the present consanguinity study and the effects there of in the muslim population of kollam was conducted.

MATERIALS AND METHODS

The present study focuses on the members of the Muslim community from different parts, in and around Kollam, Kerala. The sample size was 1500 Muslim families from urban and suburban regions of Kollam, which is around 30% of the community in the region. The data was collected by visiting and interviewing the members of each family with the aid of a comprehensive questionnaire which included a variety of socio-economic variables and demographic determinants. Some families were visited twice or thrice in order to collect information from other family members or to examine children who were absent during the first visit. The help of a local person was sought for gaining introduction to the subjects selected. The temporal changes in consanguinity levels were examined by disaggregating the data into six periods of marriage like before 1950, 1950-1960, 1960-1970, 1970-1980, 1980-1990 and after 1990. The age of spouses were grouped under five classes from 18-30 years to above 60 and the age at marriage under four classes from below 18 years to above 35. The degrees of spousal relationships include Uncle-niece (UN), Double first cousin (DFC), First cousin (IC), First cousin once removed (1.5C) and second cousin (2C). First cousin pattern comprises four subtypes such as patrilateral parallel cousin (PPC), patrilateral cross cousin (PCC), matrilateral parallel cousin (MPC) and patrilateral cross cousin (MCC). Inbreeding coefficient (F) for various degrees of spousal relationship was determined by the path coefficient method. The coefficient of non-consanguineous mating was taken as F=0. The mean coefficient of inbreeding was computed following Wright (1922). Various parameters were used for assessing the reproductive outcome such as pregnancy wastage (abortion and still birth), postnatal mortality and juvenile mortality. Total mortality or pre-reproductive mortality is the sum of all losses from abortion to juvenile death. The morbidity data was collected under two types such as congenital defects and diseases, both systemic and genetic. The data were hand-coded and analyzed in a personal computer. Statistical methods were used to find out the significance of association between consanguinity and influencing parameters. Tests of proportion were used to find out the significance of the effect of consanguinity.
CONSANGUINITY STUDY IN THE MUSLIM COMMUNITY

RESULTS AND DISCUSSION

A preponderance of non-consanguineous marriages (83.13%) was observed in the Muslim community of Kollam, with only 16.87% of the total 1500 marriages coming under the category of consanguineous types (Table 1). Among the consanguineous marriages, majority (241 out of the total 253) was of the first cousin type, with only three uncle-niece and nine first cousin once removed marriages. No second cousin marriages were observed. Among the first cousin marriages, cross cousin types were more frequent, with a preference for the matrilateral (8.13%) over the patrilateral (7.34%). The mean coefficient of inbreeding was calculated as 0.009425. The current observation of highest frequency of consanguineous mating for first cousin is in agreement with the global trend. The highest rates of consanguineous marriage in the global scenario have been associated with low socioeconomic status, illiteracy, and rural residence. (Bittles 1994). In the present study, no significant relationship was found between the level of education and the incidence of consanguinity (Table 2). The middle education group showed the highest percentages of consanguinity for both husband (18.16%) and wife (18.09%). Increased rates of consanguinity among the upper education group may be attributed to the desire for socio-economic benefits, wherein both the spouses being better employed, would opt for consanguineous marriage to hold together their family assets as well.

No significant relationship was observed between the level of occupation and consanguinity in the case of husband. Here the middle occupation group showed the highest percentage (25.99%) of consanguinity. However, the level of occupation of the wife was found to be negatively correlated to the incidence of consanguinity, and this was observed to be significant at 1 % level. The existing system of dowry and other financial needs, which the society demands from the family of the bride, force many a female to opt for spouses with lower occupation or background. Hence, the occupation of the husband might have less impact on him being chosen as the potential partner. However, the negative correlation observed in the case of the wife, may be attributed to the female psychology of taking more precaution to avoid future calamities.

The family income was observed to be negatively correlated to the percentage of consanguinity, and the relation was found be significant at 5% level. The percentages of consanguinity were the highest for the low-income group (19.13%). The value then declined and, although an increase was observed in the upper middle-income group, it declined sharply in the high-income group. Mathew et al (2006) opined that economic consideration is the foremost influencing factor of blood-related marriages in many communities belonging to different social classes of Kerala.

A significant negative correlation (p-value = 0.033; Table 2) was observed between the social status and percentage of consanguinity at 5% level, with the percentage of consanguinity being the highest (20.56%) among the group with lower social status. This could be attributed to the reasons mentioned above.

The age at present was found to be significantly correlated in a negative fashion with the percentage of consanguinity at 5% level in the case of the husband (p-value = 0.041; Table 2). Here
the spouses of older generation (above 60 years) showed the least value for incidence of consanguinity (11.01 %), while the highest values were observed for the younger group between 18 and 30 years of age (25%). The current observation appears to be due to an increase in the incidence of consanguinity among the present day Muslim youth, despite the increasing levels of literacy in the state. However, no such correlation was observed in the case of the wife, despite a sharp decline after the age of 60.

The age of the husband at the time of marriage was found to be negatively correlated with consanguinity percentages, and this was observed to be significant at 5% level. The age group below 18 years showed 50% consanguinity, with the values diminishing to less than half in the next group viz. 18-25 years. The age group above 35 years showed the minimum value (5.26%). The relative high incidence of early marriages in the community is reflected here. The age of the wife at the time of marriage was found to be negatively and significantly correlated with consanguinity percentages at 5% level, although an increase was observed in the 25-35 years of age.

A significant positive correlation was observed between the year of marriage and the percentage of consanguinity (Table 3), at 5% level. No consanguineous marriages were observed before 1950 in the present study, and this may be due to the lesser number of subjects belonging to this group obtained in the present study. However, after 1950, the frequency of consanguineous marriages increased steadily, and in the 1970s the maximum value was observed. Despite a slight decrease in the 1980s, the values appear to have picked up again since the 1990s. The recent trend of ‘going back to the age old tradition’ spurred by incidence of communal violence might have contributed to this.

A significant negative correlation (Table 2) was observed between marital distance between spouses and the percentage of consanguinity. Maximum value for consanguinity (18.65) was obtained at a marital distance of less than 5 kilometers. The values gradually decreased with increasing distance. Similar observations were made by Imaizumi (1986) for marital distance, who noticed the highest F-value for the shortest distance (0.00252), as against those for other distance classes (0.00072-0.00097). Closer distances enable more interactions between the families, the strengthening of family ties, the ease of marital arrangements and a closer relationship between the wife and her in-laws, leading to greater marriage stability and durability (Bittles 1994; Hussain 1999).

Among the parameters presently studied, education of the husband and wife and the occupation of the husband did not show any significant correlation with the percentage of consanguinity. The year of marriage showed a significant positive correlation at 5% level, while a host of other parameters viz. family income, social status, marital distance, age at marriage for both husband and wife and age at present for wife were negatively correlated with the incidence of consanguinity at 5% level. The age at present of the wife, although negatively correlated, was found to be not significant. But the negative correlation observed for the occupation level of wife was found to be significant at 1% level.
MORTALITY

Mortality refers to the ratio of the total number of deaths to the total population. It includes pregnancy wastage, infant mortality, child mortality and juvenile mortality. Pregnancy wastage includes abortions and stillbirth. Infant mortality has been divided into neonatal mortality and postneonatal mortality depending on the time at which death occurs. Neonatal mortality occurs within the first 27 days of life and postneonatal mortality from 28 days to 365 days. Neonatal deaths are typically attributed to endogeneous causes of death related to pregnancy, while postneonatal deaths are more likely to be classified as being from exogeneous or environmental causes. Mortality between one and ten years of age is considered as child mortality, while juvenile mortality refers to deaths occurring between 10 and 20 years of age. The data regarding various types of mortality for each group are provided in Table 3. No mortality was observed among the offspring of uncle-niece marriages, while the first cousin once removed group showed only neonatal mortality (12.5%). The first cousin group showed an almost doubled incidence of abortions (4.78%) in comparison to the non-consanguineous group (2.87%), although no stillbirths were observed here. Stillbirths were observed only in the non-consanguineous group (0.41).

The values of postnatal mortality for first cousin marriages were almost double or triple when compared to the non-consanguineous group, with the percentages of neonatal mortality and post neonatal mortalities being 2.87 and 0.96 respectively as against the corresponding values of 1.23% and 0.41% for non-consanguineous types. Dawodu et al. (2005) have reported multisystem malformations as the commonest congenital malformations leading to neonatal mortality. The values pertaining to child mortality (1.44%) and juvenile mortality (0.48%) for the first cousin marriages were almost triple that of those for the non-consanguineous group (0.49 and 0.16 respectively). A 4-5% increase in child mortality has been found in the offspring of first cousin marriages, and similar results have been reported in other studies (Bittles and Makov 1988; Bittles et al. 1991).

The total pregnancies numbered to about 4317, of which 3660 were from the non-consanguineous marriages. Among the consanguineous marriages, the maximum number of pregnancies was obtained for first cousin mating (657). Sterility was observed in only thirty-six cases, of which nine were from first cousin marriages. It has been proposed that fertility may be lower in consanguineous couples due to a failure to initiate pregnancy when the couple share specific HLA haplotypes (Ober et al. 1992), or because of the expression of deleterious genes acting during early embryonic or foetal development that result in periconceptual losses (Ober et al. 1999). Conversely, it could be argued that greater genetic compatibility between the mother and developing foetus in a consanguineous pregnancy would lead to reduced rates of involuntary sterility and prenatal losses. Additionally, there is a strong possibility that greater fertility may be observed in consanguineous unions as a compensatory mechanism for infant and childhood losses (Bittles et al. 2001). The higher fertility rates reported for consanguineous marriages have been considered partially due to the generally lower parental age at marriage and the age at the first birth of couples who are close relatives (Bittles et al. 1991).
TABLE 1: Frequency of consanguinity by type among the Muslims of Kollam

<table>
<thead>
<tr>
<th>Type of marriage</th>
<th>Number of marriages</th>
<th>Consanguinity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UN/AN</td>
<td>3</td>
<td>0.20</td>
</tr>
<tr>
<td>First Cousin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matrilateral Cross</td>
<td>122</td>
<td>8.13</td>
</tr>
<tr>
<td>Matrilateral Parallel</td>
<td>6</td>
<td>0.40</td>
</tr>
<tr>
<td>Patrilateral Cross</td>
<td>110</td>
<td>7.34</td>
</tr>
<tr>
<td>Patrilateral Parallel</td>
<td>3</td>
<td>0.20</td>
</tr>
<tr>
<td>Total</td>
<td>244</td>
<td>16.27</td>
</tr>
<tr>
<td>First Cousin Once Removed</td>
<td>9</td>
<td>0.60</td>
</tr>
<tr>
<td>Second Cousin</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Consanguineous</td>
<td>253</td>
<td>16.87</td>
</tr>
<tr>
<td>Non-Consanguineous</td>
<td>1247</td>
<td>83.13</td>
</tr>
<tr>
<td>Grand Total</td>
<td>1500</td>
<td>100</td>
</tr>
</tbody>
</table>

Mean Coefficient of inbreeding: 0.009425

TABLE 2: Correlations of socio-economic variables and demographic determinants with consanguinity

<table>
<thead>
<tr>
<th>Correlation with consanguinity</th>
<th>Significance (p-Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Education Husband</td>
<td>0.696</td>
</tr>
<tr>
<td>Education Wife</td>
<td>0.821</td>
</tr>
<tr>
<td>Occupation Husband</td>
<td>0.491</td>
</tr>
<tr>
<td>Occupation Wife</td>
<td>-0.999</td>
</tr>
<tr>
<td>Family Income</td>
<td>-0.899</td>
</tr>
<tr>
<td>Social Status</td>
<td>-0.999</td>
</tr>
<tr>
<td>Marital distance</td>
<td>-0.882</td>
</tr>
<tr>
<td>Age at Marriage Husband</td>
<td>-0.953</td>
</tr>
<tr>
<td>Age at Marriage Wife</td>
<td>-0.921</td>
</tr>
<tr>
<td>Age of Husband</td>
<td>-0.894</td>
</tr>
<tr>
<td>Age of Wife</td>
<td>-0.518</td>
</tr>
<tr>
<td>Year of Marriage</td>
<td>0.901</td>
</tr>
</tbody>
</table>

* significant at 5% level  ** significant at 1% level
Morbidity refers to a diseased condition and includes any departure, subjective or objective, from a state of physiological or psychological well-being. In this sense, sickness, illness, and a morbid condition are synonymous. In the present study, polio, mental retardation, squint eye, asthma, clubfoot, mental retardation and dwarfism were observed in a few cases in the non-consanguineous group, which constituted 83.13% of the total study population. Of the total 253 consanguineous matings, 241 belonged to the first cousin category - mostly matrilateral and patrilateral cross cousins. In both these cases several instances of morbidity in the form of artificial uvula, polio, mental retardation, cleft palate, mongolism, deafness, dumbness, limb abnormalities, vitiligo, epilepsy, pigeon chest and cleft lip were observed. The first cousin once removed group included only nine marriages, of which two produced female offspring with brachydactyly. Only three cases of uncle-niece marriage were observed in the current study. But three female offspring resultant from this marriage showed limb abnormalities. Jaber et al. (1998) have reported that the rate of congenital malformations among the offspring is 2.5 times higher than that among the offspring of unrelated parents. According to Khanum et al. (2004), musculoskeletal system was the most commonly involved system in morbidity. A population specific genetic liability for multi-factorial inheritance was suggested for epilepsy by Nair and Thomas (2004).

The high incidence of mortality and morbidity observed in the offspring from consanguineous marriages calls for a large scale campaign against this social evil, with a view to create an awareness.
among the public as to the harmful effects associated with it. This is particularly significant in view of the present finding of an increase in the percentage of consanguinity in the study population with regard to marriages effected from 1990 onwards. Further the failure of the current system of education to effectively deliver the seriousness or gravity of the situation and the decreased concern in the community regarding the occupation of the husband in relation to the wife, serve only to aggravate the problem. Proper remedial measures include genetic counselling, inclusion of topics related to consanguinity in the school curriculum, house-to-house campaign by health personnel, appeal through ‘madrasas’ and propaganda through the audio-visual media.

ACKNOWLEDGEMENT

The first author gratefully acknowledges the Principal, Sree Narayana Colege, Kollam for providing the facilities required for this work.

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A STUDY ON THE EFFECT OF GAMMA IRRADIATION ON ANDROGRAPHOLIDE PRODUCTION IN ANDROGRAPHIS ECHIOIDES

SUSILA KURUVILLA1 AND P.R.UNNIKRISHNA PILLAI2
1. Department of Botany, St. Joseph’s College for Women, Alappuzha, Pin-688001, Kerala, India
2. Department of P.G. Studies and Research in Botany, S.D.College, Alappuzha, Pin688003, Kerala, India.

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SUMMARY

The effect of gamma-irradiation on secondary metabolite production in callus cultures of A. echoides was carried out in the present study. The significant result of this study indicated that γ-irradiation induced andrographolide production in the callus cultures. The callus cultures produced from non-irradiated seedlings showed the absence of andrographolide. An enhanced rate of andrographolide in the callus was found in all the doses of γ-irradiated samples in HPLC analysis.

Key words : Andrographis echoides, γ-irradiation, andrographolide.

INTRODUCTION

Andrographis echoides belonging to the family Acanthaceae is an important ayurvedic herb. It is distributed throughout India in the plains, and also in forests as undergrowth mostly in the crevices of rocks. The plant is an erect, branched, annual herb with hairy stems and leaves. The leaves are simple, with serrate margins. The flowers are small with brown and purple spreading with racemose inflorescence.

In spite of numerous medicinal values, these plants are not being grown on a commercial scale anywhere in India. At present they are collected generally from the forest areas. In order to combat the shortage and adulteration some alternate methods such as in vitro production of secondary metabolites are currently being tried. Low doses of radiation have been found to have a stimulatory effect in the production of secondary metabolites. The supply of an exogenous precursor to culture medium may also increase the yield of the desired component. The irradiation of the seedlings with various doses of gamma rays together with selected growth regulators triggered the production of andrographolide. Concentration and combination of growth regulators directly influence the differentiation. Ramawat (2004) observed that the type of growth regulators also affects the production of secondary metabolite. Ramawat & Arya (1979) attained a high yielding callus strain in Ephedra plant giving 0.6% ephedrine by supplementing the medium with L-Phenylalanine. When cell suspension cultures of Salvia officinalis were supplemented with phenylalanine, it stimulated the production of rosmarinic acid. Chang et al., (2006) reported the secondary metabolite production in plant cell cultures treated with low and high doses of gamma radiation. Suspension cultures of Lithospermum erythrorhizon were irradiated to 2,16 and 32 Gy. The gamma radiation significantly stimulated the shikonin biosynthesis of the cells and increased the total Shikonin yields by 400% at 16
Gy and by only 240% and 180% at 2 and 32 Gy. The objective of the present work is to study the effect of gamma irradiation on andrographolide production in *Andrographis echiodes*.

**MATERIALS AND METHODS**

Certified seeds of Andrographis *echiodes* were procured and verified at KFRI, Peechi. Seedlings were raised and Thirty-day-old seedlings were used for γ-irradiation. Murashige and Skoog (MS) medium was used as basal media in this study. Basal media were used in different combinations and the most suitable combination was selected by trial and error method. The explants tried for callus initiation were nodal segments, internodal segments and third leaf taken from the greenhouse grown plants. The seedlings were subjected to four doses of gamma irradiation. The irradiation doses were 0.5kR, 1kR, 1.5kR and 2kR. The seedlings after irradiation were allowed to grow in greenhouse. After 10 days, the third leaf from the apical portion was selected for *in vitro* inoculation.

The seedlings of *A. echioides* were not viable above 0.5kR gamma-irradiation. So the explants were taken from the seedlings exposed to 0.5 kR of gamma-irradiation. The proliferated calli, after 15 days, were sub-cultured into callus proliferating medium containing basal MS medium supplemented with various concentrations of cytokinins (BA/KIN) or in combination with auxins (NAA, 2,4D). The medium was also enriched with vitamins, phenylalanine and the additive polyethylene glycol in various concentrations of auxins and cytokinins. The medium used for callus proliferation was used as medium for callus andrographolide accumulation. The calli kept in the proliferating medium were grown for 70 days and were taken out and allowed to dry in the hot air oven at 33°C. Before drying, the fresh weight of the calli was taken. The dry weights of the calli were also taken after proper drying and they were stored at 4°C, after proper labeling. The andrographolide content was estimated by HPLC analysis.

**OBSERVATIONS**

The andrographolide accumulation in callus established on MS medium fortified with 1mg/l kinetin, 2mg/l NAA and 50mg/l L-phenyl alanine was 0.005% w/w. When 20mg/l PEG 6000 was supplemented together with 1mg/l kinetin and 0.5 mg/l 2,4 D no andrographolide accumulation was observed. Stock calluses were transferred to MS medium fortified with 50mg/l phenyl -alanine, 1mg/l 1 kinetin and 2mg/l NAA and the impact of auxins and cytokinins together with L-phenylalanine on andrographolide synthesis was analysed after a period of seventy days. The fresh weight observed after forty days was 5437mg and after seventy days was 8109mg. The presence of andrographolide was 0.0038% w/w. (Chromatogram 1). When compared to the control plants 7 times increase in andrographolide accumulation was noted in 0.5kR- gamma - irradiated seedlings. Gamma irradiation promoted the alkaloid production when the seedlings of *A.echioides* were irradiated at 0.5kR of γ-rays. (Table 1)

When 20 mg/l PEG 6000 was supplemented together with 1mg/l kinetin and 0.5 mg/l 2,4 D the andrographolide accumulation was 0.001% w/w. When the callus were transferred to full MS medium fortified with 0.5mg/l 2,4 D, 0.5 mg/l BA and 0.1mg /l kinetin, produced a fresh weight of
A STUDY ON THE EFFECT OF GAMMA IRRADIATION

Detector A Ch1 223nm

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>Height%</th>
<th>Mark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.088</td>
<td>444647</td>
<td>38458</td>
<td>26.616</td>
<td>22.612</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>19.069</td>
<td>188890</td>
<td>23402</td>
<td>11.307</td>
<td>13.760</td>
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<tr>
<td>3</td>
<td>21.589</td>
<td>701232</td>
<td>72861</td>
<td>41.975</td>
<td>42.841</td>
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</tr>
<tr>
<td>4</td>
<td>24.327</td>
<td>335830</td>
<td>35352</td>
<td>20.102</td>
<td>20.786</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1670599</td>
<td>170074</td>
<td>100.000</td>
<td>100.000</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 1**: Data Showing The Presence Of Andrographolide in 0.5kr Gamma Irradiated Seedlings Of Andrographis Echiodes

<table>
<thead>
<tr>
<th>Control</th>
<th>Fresh Wt After 70 Days</th>
<th>Andrographolide After 70 Days</th>
<th>Fresh Wt After 70 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 kr</td>
<td>Andrographolide</td>
<td>Fresh Wt</td>
<td>Andrographolide</td>
</tr>
<tr>
<td></td>
<td>%/W/W</td>
<td>After 70 Days</td>
<td>Contect</td>
</tr>
<tr>
<td>MEDIUM C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5MG/12,4 D</td>
<td>0.0003%w/w</td>
<td>5432mg</td>
<td>0.0031%w/w</td>
</tr>
<tr>
<td>0.5mg/IBA</td>
<td>0.1mg/l Kinetin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEDIUM H</td>
<td>20%PEG 6000</td>
<td>1mg/Kinetin</td>
<td>0.0</td>
</tr>
<tr>
<td>0.5mg/1.2,4 D</td>
<td>0.0005%w/w</td>
<td>5896mg</td>
<td>0.038%w/w</td>
</tr>
<tr>
<td>MEDIUM PA</td>
<td>50mg/l Phenyl alanine</td>
<td>1mg/l Kinekin</td>
<td>2mg/l NAA</td>
</tr>
</tbody>
</table>

5234mg after seventy days and the andrographolide accumulation was 0.0031%/w/w.

(Chromatogram 2).

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Maximum andrographolide production was found in the callus produced from seedlings irradiated with 0.5kR of γ-irradiation proliferated in a medium supplemented with the precursor-phenylalanine. In A.echiodes the andrographolide content in callus produced from nonirradiated explants raised in a medium containing phenylalanine was negligible 0.0003%w|w . But a significant increase in andrographolide accumulation (0.0038%w|w) was noticed in the callus produced from explants after 0.5kR of γ-irradiation. This result was in agreement with the reports of Hua Z et al (1999). They studied the influence of gamma ray on Shikonin formation on cultured cells of Onasma paniculatum. The optimum gamma radiation dose was 1500 R, which gave a 144.6% increase in Shikonin content compared to the control (unirradiated) callus gamma irradiation together with the presence of higher level of PEG. The report of the present study was in agreement with the study on Solanum tuberosum done by Li et al, 2005. When plantlets were irradiated with 4Gy a significant increase in the fresh mass and low doses of irradiation increased the starch content of micro tubers and also significantly increased the protein content of micro tubers. Tarasonko (1993) had reported the stimulative effect of gamma rays at low doses in S.tuberosum.

**CONCLUSION**

Medicinal plants are used for several purposes. Essentially plant cells contain primary and secondary metabolites. Secondary metabolites are biologically active principles accumulated by the plant cells at particular developmental stage. Since the secondary metabolites have high economical and pharmacological importance, search must be continued for the successful exploitation of new tissue culture technique, which includes genetical and molecular technique for large-scale production of useful metabolites.
A STUDY ON THE EFFECT OF GAMMA IRRADIATION

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TARASONKO N D 1993 Effect of gamma rays fast neutrons and ethylene amine on the variability and chromosome aberrations of potato seedlings Radiobiologia 3 427-430.
CHROMOSOME NUMBER AND SEX-DETERMINING MECHANISM IN FORTY-ONE SPECIES OF POLYPHAGAN COLEOPTERA (INSECTA) FROM INDIA

PARAMJEET KAUR AND ABHAY SINGH YADAV*
Department of Zoology, Kurukshetra University, Kurukshetra- 136119
*E-mail: abyzkuk@gmail.com, Phone(M): 09416173289
(Received 18 November 2010, accepted 30 May 2011)

SUMMARY
Chromosomal data on 41 species, representing 21 genera, 12 subfamilies and 5 families of sub order Polyphaga have been tabulated. Of these 19 species are of new cytological record.

Keywords: Coleoptera, Chromosomes, Meioformula

INTRODUCTION
Karyologically, Coleoptera is an extensively investigated group of insects and data of more than 3500 species and subspecies and parthenotes are recorded (Yadav et al. 1989). The major contribution in context with Indian fauna on Polyphaga are due to Joneja (1960), Agarwal (1960, 1962), Lahiri and Manna (1969), Saha and Manna (1971), Manna and Lahiri (1972), Saha (1973), Yadav and Pillai (1975, 1976 a, b, 1977 a, b). The present investigation was undertaken to add some more species in this group.

MATERIALS AND METHODS
A classified list of 41 species investigated, the localities from which the specimens were

| TABLE 1: Chromosomal analysis of species under study from Kurukshetra (Haryana) |
|---|---|---|---|
| Species name with Classification | Locality & period of collection | Diploid number | Meioformula |
| Family: SCARABAEIDAE |
| Subfamily: Geotrupinae |
| 1. Bolboceras indicum Westw. | Seonti forest, 2010 | 20 | 9+Xyp |
| 2. B. quadridens F. | Seonti forest, 2010 | 20 | 9+XyP |
| Subfamily: Hybosorinae |
| 3. Hybosorus orientalis Westw. | Kirmichi Village, 2009 | 22 | 9+Xyp |
| Subfamily: Dynamopinae |
| 4. Dynamopus athleta Sem. | Masana Village, 2010 | 22 | 10+Xyp |
| Subfamily: Aphodiinae |
| 5. Aphodius moestus F. | Kirmich Village, 2009 | 22 | 10+Xyp |
| 6. *A. testaceus (Germ.) | Kirmich Village, 2009 | 20 | 9+Xyp |
| Subfamily: Scarabaeinae |
| 7. Catharcius molosus L. | Seonti Forest, 2009 | 20 | 9+Xyp |
| 8. C. pithecus F. | Seonti Forest, 2010 | 20 | 9+Xyp |
9. *Copris signatus* Walker  
Seonti Forest, 2010  
20  
9+Xyp

10. *Gymnopleurus miliaris* (F.)  
Seonti Forest, 2008  
36  
17+Xyp

11. *G. parvus* MacL.  
Seonti Forest, 2008  
18  
8+Xyp

12. *G. mundus* (F.)  
Seonti Forest, 2009  
14  
6+Xyp

13. *Oniticellus pallipes* F.  
Kirmich Village, 2009  
20  
9+Xyp

14. *O. pallens* (Olivier)  
Kirmich Village, 2009  
20  
9+Xyp

15. *O. spinipes* (Roth.)  
Piple Village, 2008  
24  
11+Xyp

16. *O. cinctus* (F.)  
Piple Village, 2008  
20  
9+Xyp

17. *Onitis philemon* F.  
Piple Village, 2008  
20  
9+Xyp

18. *Onthophagus catta* F.  
Kirmich Village, 2008  
20  
9+Xyp

19. *O. bonasus* F.  
Kirmich Village, 2008  
20  
9+Xyp/Xyr

20. *O. dama* F.  
Seonti Forest, 2010  
20  
9+Xyp/Xyr

21. *O. falsus* (Gill.)  
Seonti Forest, 2010  
20  
9+Xyp

22. *O. mopsus* (F.)  
Seonti Forest, 2010  
20  
9+Xyp

23. *O. ramosellus* Bates  
Seonti Forest, 2010  
20  
9+Xyp

24. *O. fasiatus* (Bouc.)  
Seonti Forest, 2010  
20  
9+Xyp

25. *O. unifasciatus* (Schall.)  
Seonti Forest, 2010  
20  
9+Xyp

26. *O. bifasciatus* F.  
Seonti Forest, 2010  
20  
9+Xyp

27. *O. spinifex* (F.)  
Seonti Forest, 2010  
20  
9+Xyp

28. *O. bengalensis* (Har.)  
Seonti Forest, 2010  
20  
9+Xyp

29. *Caccobius ultor* Sharp  
Seonti Forest, 2010  
20  
9+Xyp

30. *Sisyphus neglectus* Gory  
Seonti Forest, 2010  
16  
7+Xyp

Subfamily: Rutelinae

32. *Anomala bengalensis* Blanch  
Kurukshetra University Campus, 2010  
20  
9+Xyp

33. *Adoretus* sp.  
Seonti Forest, 2010  
22  
10+Xyp

Family: CHRYSOMELIDAE

Subfamily: Galerucinae

34. *Aulacophora oveicollis* (Lucas)  
Kurukshetra University Campus, 2008  
30  
14+Xyp

Subfamily: Chrysomelinae

35. *Zygogramma bicolorata* Pallister  
Kurukshetra University Campus, 2008  
24  
11+Xyp

Family: ATTELARIDAE

Subfamily: Apoderinae

Kurukshetra University Campus, 2008  
30  
14+Xyp

Family: MELOIDAE

Subfamily: Meloinae

Seonti Forest, 2010  
22  
10+Xyp

Family: COCCINELLIDAE

Subfamily: Coccinellinae

38. *Coccinella septempunctata* (L.)  
Kurukshetra University Campus, 2008  
20  
9+Xyp

39. *C. repanda* (transversalis) (F.)  
Kurukshetra University Campus, 2008  
20  
9+Xyp

40. *Chilomenes sexmaculata* (F.)  
Kurukshetra University Campus, 2009  
28  
13+Xyp

Subfamily: Epilachninae

41. *Epilachna circularis* (Cors.)  
Kurukshetra University Campus, 2009  
20  
9+Xyp

* showed new cytological records
collected, their diploid chromosome number and the chromosomal formulae are presented in Table 1. All the specimens were collected from Kurukshetra (Haryana) during the period of Ph.D research work. Karyological preparations were made according to Yadav and Lyapunova (1983).

RESULTS AND DISCUSSION

These 41 species are from 21 genera belonging to 12 subfamilies and 5 families. Of these 19 species of 9 genera belonging to 3 families are new determinations. The karyological data of other species were earlier recorded by other Indian Scientists (Yadav and Pillai 1975, 1976 a, b, 1977 a, b; Yadav et. al 1977 a, b, 1990, 1993 a, b; Lyapunova et. al 1984; Yadav and Dange 1989; Yadav and Gahlawat 1994).

The diploid number of chromosomes in the investigated family Scarabaeidae varies from 14 to 36. However, the commonest number found in 25 species is 20. Whereas three species possessed 22, one species 16, one species 24, one species 36, one species 14 and one species 18 chromosomes in their diploid complement. The predominant sex - determining mechanism found was 9+Xyp in the male. Whereas Xyr type was also found in Onthophagus bonasus and Onthophagus dama.

Only two species of Chrysomellidae were investigated with diploid chromosome number 24 in Zygogramma bicolorata and 30 in Aulacophora foveicollis with Xyp type of Sex mechanism. One species each from families Attelabidae and Meloidae were recorded with diploid complement 30 in Hoplapoderus gemnatus and 22 in Mylabris pustulata, respectively.

Among Coccinellidae under present investigations, the diploid number of chromosomes ranges from 20 in three species to 28 in one species. Xyp is the predominant male sex chromosome system in this family.

Details of structure and behaviour of chromosomes in the species under report will be published elsewhere. In addition to this, the chromosomal anomalies like polypody, stickiness, and decondensation were also observed in natural population of Gymnopleurus mundus and Gymnopleurus miliaris.

ACKNOWLEDGEMENT

Our sincere thanks are to the authorities of Kurukshetra University, Kurukshetra for providing laboratory facilities, member of Forest Entomology Laboratory, Dr. Sudhir, Forest Entomologist, F.R.I., Dehradun and Dr. Nidhi Kakkar, Department of Zoology, Kurukshetra University, Kurukshetra for their help in identification of beetles.
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EFFICACY OF ANTIBIOTICS ON PRODUCTIVITY OF ERI SILK WORM
‘PHILOSAMIA CYNTHIA RICINI’ (LEPIDOPTERA: SATURNIDAE)

MAHABOOB BASHA P*, SHABANA BEGUM 2 AND DEVARAJU R3
1.Department of Zoology, Bangalore University, Bangalore 560056, India 2.Maharanis Science College for Women,
Bangalore, 560001, India 3.Seshadripuram PU College, Bangalore, 560020, India
*Corresponding author: Email:pmbashabub@rediffmail.com

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SUMMARY
The effect of antibiotics such as amoxicillin, norfloxacin, ciprofloxacin and hostacycline on the economic characters of eri silkworm Philosamia cynthia ricini was studied. They were fed on castor (Ricinus communis) leaf supplemented with 0.001%, 0.0015% and 0.002% concentrations of all the antibiotics to assess their influence on larval weight, cocoon weight, shell weight and shell ratio of each treatment. Treatment of antibiotics has brought considerable increase in larval weight, cocoon weight, shell weight and shell ratio of eri silkworm. Out of the four antibiotics tested, only norfloxacin and amoxicillin showed higher response in improving the commercial qualities of silk. The beneficial action of antibiotics can be attributed to their activity in conditioning the composition of intestinal bacteria, their role as possible growth factors, biological efficiency in increased turn over of feed into body weight and potential disease control activity, hence may be used in routine rearing practice on a regular basis for more silk production.

Keywords: Antibiotics, eri silkworm, economic characters, silk production.

INTRODUCTION
Growth, development and economic characters of silkworms are influenced to a great extent by nutritional content of their host plants (Grimaldi & Engel, 2005). In recent years, many attempts have been made to improve the quality and quantity of silk (Hazarika, 2006); through enhancing the leaf nutrients, spraying antibiotics and botanicals. Antibiotics are extensively used in the nutrition of domestic animals for increasing the productivity, which acts as growth stimulators and known to improve both the growth of the larvae and to some extent the silk production in silkworms (Krishnaswami et al., 1980; Radha Krishna Rai & Devaiyah, 1988; Dechu, 1995).

Antibiotics are specific in their function and show varied effects on different races of silkworm (Upadhyay & Tripathi, 2006). Studies conducted so far by many researchers using different larval instars especially IV & V have noticed differences in the effects of antibiotics. Studies of Murthy and Sreenivasaiah (1954) reported increased pupal weight, and decreased silk output on supplementation of aureomycin and chloromycetin. No effect on the larval duration, pupal weight and shell percentage was reported as a consequence of antibiotic exposure like subamycin,
aureomycin and ladermycin in *Bombyx mori* (Verma & Kushwaha, 1971). Krishnaswami et al. (1980) reported augmentation in larval weight on chloromphenicol supplementation to bivoltine hybrid NB, D X KA. Radha et al. (1981) noticed no significant influence of antibiotics chloromphenicol and tetracycline on filament length. Banuprakash et al. (1999) have shown the significant effects of antibiotics on cocoon weight and shell weight and filament length. The highest survival rate of larvae was observed on gentamycin exposure than erythromycin and neomycin (Banuprakash et al., 1999). Thus, information available clearly indicates influence of antibiotics on economic traits of silkworm. Though the use of antibiotics on silkworm has been reported, pertinent information is lacking on the effects of antibiotics like amoxicillin, norfloxacin, ciprofloxacin and hostacycline on silk production in non-mulberry silkworms. Keeping in view the economic importance of silk, the present study was undertaken in *Philosamia ricini* to investigate the impact of antibiotics on economic traits and efficiency in the production of quality silk.

**MATERIAL AND METHODS**

**Silkworm rearing:**

Disease-free egg layings of eri silkworm, *Philosamia cynthia ricini* B (*Lepidoptera: Saturniidae*) were obtained from the grainage of National Silkworm Seed Project, Central Silk Board, Bangalore and were incubated in BOD incubator at 28 ± 2°C and 80 ± 5% relative humidity until the emergence of larvae. The rearing techniques followed were that of Krishnaswami et al. (1973). Silkworms were made into four batches immediately after 2nd moult and 50 worms were taken for each replication of every antibiotic treatment. The treatment was started after the emergence of worms from 2nd moult. When the larvae attained spinning stage, larvae from each replication were mounted to separate mountages. The cocoons were harvested after 5 days of moulting.

**Antibiotic treatment:**

Commercial grade antibiotics viz., ciprofloxacin, amoxicillin, norfloxacin, and hostacycline were chosen for the present study and obtained from Glaxo Smith Kline, Cipla and Aventis pharmaceuticals. The 0.001 %, 0.0015 %, 0.002 % solutions of above antibiotics were prepared in distilled water (w/v) and uniformly smeared on the castor leaves at the rate of 0.1ml per leaf (approximately leaf area being 280 sq meter). Once in every 4 days a fresh stock of the above antibiotic solution was prepared and stored in refrigerator for use on subsequent days. The smeared leaves were shade dried and fed to the larvae after 3rd and 4th moult along with the control separately. The treatment had three replications having 50 larvae in each. Fresh untreated castor leaves were provided to the control group. Weight of the larvae was taken before and after the antibiotic treatment along with the weight of the controls, similarly during spinning and emergence. Cocoon weights were recorded. Statistical analysis was done using analysis of variance (ANOVA); critical difference of the treatment significance was calculated at P < 0.05 as per the procedure of Sundarajan et al. (1972).
Efficacy of Antibiotics on Productivity

Economic Characters:

Observations were made for economic characters in terms of larval length (III, IV and V instars) and larval weight was studied from each replication of every treatment. The cocoon weight, pupal weight, shell weight, shell ratio percentage, was recorded and the data was statistically analyzed. The cocoon yield per replicate of each treatment in terms of number and weight was recorded. A sample of ten cocoons was drawn from each replicate of every treatment and individual cocoon weight was taken from all the ten cocoons separately to calculate average weight of single cocoon. Further, they were cut open without causing any damage to pupa and shell weight was calculated. Similarly, shell ratio percentage was calculated by using the following formula:

\[
\text{Shell ratio percentage} = 100 \times \frac{\text{Shell weight}}{\text{Cocoon weight}}
\]

Mortality due to disease was recorded daily from each replicate and percentage incidence of grasserie (viral disease), flacherie (bacterial disease), pebrine (protozoan disease), muscardine (fungal disease), cytoplasmic poly-hedrosis and nuclear poly hedrosis was recorded by counting the number of infected larvae.

RESULTS

It was observed in the present study that differential concentration of antibiotic supplementation to III instar larvae caused an elevation in the larval weight. Maximum elevation was recorded in the group fed with 0.0015% ciprofloxacin followed by amoxicillin and hostacycline, and minimum elevation noticed in larvae fed with norfloxacin, whereas amoxicillin exposure in 0.002% and 0.001% concentrations had no influence on larval weight (Table-1). Similarly a progressive larval growth was observed in IV and V instar larvae as a consequence of antibiotic supplementation. A dose at 0.0015% found to be beneficial compared to other doses. However no change in larval weight was observed in IV instar larvae fed with 0.002% hostacycline. Likewise ciprofloxacin supplementation at 0.002% concentration showed a negative influence in affecting the body weight in V instar larvae (Fig. 1 & 2).

Supplemented antibiotics showed profound effect in improving cocoon weight significantly (Fig. 3). Maximum cocoon weight (7.56g) was recorded with 0.001% norfloxacin and 0.002% hostacycline (7.267g), and least with 0.001% of amoxicillin. The analysis of shell weight and ratio revealed that all the four antibiotics showed increased shell weight and maximum shell percentage was observed in 0.001% norfloxacin supplementation followed by amoxicillin and hostacycline (Fig. 4). Similarly, a higher shell ratio (11.3%) was noticed with 0.002% norfloxacin and lowest shell ratio (6.628%) with 0.002% hostacycline.
### MAHABOOB BASHA ET AL...

<table>
<thead>
<tr>
<th>ANTIBIOTICS</th>
<th>CONCENTRATION (%) (W/V)</th>
<th>3rd instar Weight (g)</th>
<th>4th instar Weight (g)</th>
<th>5th instar Weight (g)</th>
<th>Cocoon Shell Weight (g)</th>
<th>Shell Shell Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amoxicillin</strong></td>
<td>0.001 %</td>
<td>0.3159±0.019</td>
<td>1.094±0.076*</td>
<td>5.63±0.394</td>
<td>6.3±0.441*</td>
<td>0.696±0.0487*</td>
</tr>
<tr>
<td></td>
<td>0.0015 %</td>
<td>0.325±0.023</td>
<td>1.172±0.066*</td>
<td>5.97±0.418*</td>
<td>4.97±0.298*</td>
<td>0.480±0.036*</td>
</tr>
<tr>
<td></td>
<td>0.002 %</td>
<td>0.328±0.022*</td>
<td>1.023±0.078*</td>
<td>5.41±0.079***</td>
<td>6.44±0.440*</td>
<td>0.574±0.0402</td>
</tr>
<tr>
<td><strong>Norfloxacin</strong></td>
<td>0.001 %</td>
<td>0.320±0.024</td>
<td>1.065±0.092+</td>
<td>5.57±0.308***</td>
<td>7.56±0.529*</td>
<td>0.791±0.0497*</td>
</tr>
<tr>
<td></td>
<td>0.0015 %</td>
<td>0.321±0.025+</td>
<td>1.107±0.067*</td>
<td>6.13±0.429*</td>
<td>6.0±0.300*</td>
<td>0.562±0.0399</td>
</tr>
<tr>
<td></td>
<td>0.002 %</td>
<td>0.321±0.025*</td>
<td>1.110±0.081*</td>
<td>6.51±0.391*</td>
<td>5.61±0.392*</td>
<td>0.630±0.045*</td>
</tr>
<tr>
<td><strong>Ciprofloxacin</strong></td>
<td>0.001 %</td>
<td>0.343±0.023*</td>
<td>1.140±0.071*</td>
<td>5.48±0.384*</td>
<td>7.25±0.508*</td>
<td>0.426±0.338*</td>
</tr>
<tr>
<td></td>
<td>0.0015 %</td>
<td>0.314±0.217*</td>
<td>1.122±0.060*</td>
<td>6.22±0.435*</td>
<td>6.80±0.426*</td>
<td>0.491±0.0344*</td>
</tr>
<tr>
<td></td>
<td>0.002 %</td>
<td>0.321±0.025*</td>
<td>1.118±0.080*</td>
<td>6.03±0.422*</td>
<td>5.60±0.392*</td>
<td>0.480±0.032*</td>
</tr>
<tr>
<td><strong>Hostacycline</strong></td>
<td>0.001 %</td>
<td>0.324±0.028*</td>
<td>1.123±0.064**</td>
<td>6.44±0.451*</td>
<td>5.71±0.399*</td>
<td>0.476±0.0333*</td>
</tr>
<tr>
<td></td>
<td>0.0015 %</td>
<td>0.324±0.028*</td>
<td>1.147±0.067**</td>
<td>6.28±0.432*</td>
<td>5.32±0.372*</td>
<td>0.504±0.0399</td>
</tr>
<tr>
<td></td>
<td>0.002 %</td>
<td>0.324±0.022*</td>
<td>1.083±0.0386**</td>
<td>5.52±0.386*</td>
<td>7.26±0.509*</td>
<td>0.456±0.0319*</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td>0.320±0.019</td>
<td>0.982±0.068</td>
<td>5.25±0.67</td>
<td>4.59±0.321</td>
<td>0.430±0.030</td>
</tr>
</tbody>
</table>

Value are mean ± S.D. of 10 observations and expressed in grams

Value in parenthesis indicate % change over controls ‘+’ sign indicates increase and ‘-’ indicates decrease over Controls * P<0.001

---

**Fig (1) Effect of Antibiotics on Larval Weight**

<table>
<thead>
<tr>
<th>Weight of 4th Instar Larva</th>
<th>Control</th>
<th>Amoxicillin</th>
<th>Norfloxacin</th>
<th>Ciprofloxacin</th>
<th>Hostacycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of 5th Instar Larva</td>
<td>Control</td>
<td>Amoxicillin</td>
<td>Norfloxacin</td>
<td>Ciprofloxacin</td>
<td>Hostacycline</td>
</tr>
</tbody>
</table>

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**Fig (2) Effect of Antibiotics on Larval Weight**

<table>
<thead>
<tr>
<th>Weight of 4th Instar Larva</th>
<th>Control</th>
<th>Amoxicillin</th>
<th>Norfloxacin</th>
<th>Ciprofloxacin</th>
<th>Hostacycline</th>
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<tbody>
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<td>Control</td>
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</tr>
</tbody>
</table>

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The nutritional performance and success of diét is dependent upon a number of factors (Grimaldi & Engel, 2005) and metabolic processes vary during developmental stages of holometabolous insects (Agrell, & Lundquist, 1973) due to the high metabolic activity and cellular differentiation (Locke, 1985). Earlier reports indicate considerable increase in silk production, by using antibiotics like chloromycetin, erythromycin, tetracycline etc., on mulberry silk (Verma & Atwal, 1963; Anonymous, 1981; Radha Krishna Rai & Devaiah, 1988; Banuprakash et al., 1999; Rahmathulla et al., 2006).

Antibiotics are specific in function; various types of antibiotics may have differential effects. Influence of antibiotics on economic characters was studied and results indicate increased larval growth rate followed by cocoon size. Antibiotic supplementation in small quantities was found to stimulate growth as seen in the present study. On day-1 of the III instar stage; mean larval weight for both control and antibiotic fed group were not significantly different. However the mean larval weight differences were evident from day 4 of the IV instar and these differences continued until spinning. The effectiveness of various antibiotic concentrations was between 0.001% and 0.0015% and showed progressive larval weights. These results concur with those of earlier reports of Shashidharan et al. (1993), who observed an increase in moulting and larval weight upon antibiotic supplementation. The most striking property associated with antibiotics is their remarkable stimulatory effect on growth under favorable dietary conditions. Antibiotics have a significant effect in stimulating the general metabolism of the silk, particularly nitrogen building up in the body tissues. Increased larval weights can be attributed to efficient utilization of nitrogen, mineral constituents, crude fat, increased deposition of minerals and shifts in nitrogen metabolism.

The use of antibiotics in the nutrition of silkworms helps in increasing the productivity. In this study norfloxacin supplementation at 0.001% dose significantly increased the cocoon weight and

**DISCUSSION**

The nutritional performance and success of diét is dependent upon a number of factors (Grimaldi & Engel, 2005) and metabolic processes vary during developmental stages of holometabolous insects (Agrell, & Lundquist, 1973) due to the high metabolic activity and cellular differentiation (Locke, 1985). Earlier reports indicate considerable increase in silk production, by using antibiotics like chloromycetin, erythromycin, tetracycline etc., on mulberry silk (Verma & Atwal, 1963; Anonymous, 1981; Radha Krishna Rai & Devaiah, 1988; Banuprakash et al., 1999; Rahmathulla et al., 2006).

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The use of antibiotics in the nutrition of silkworms helps in increasing the productivity. In this study norfloxacin supplementation at 0.001% dose significantly increased the cocoon weight and
shell weights (up to 64.7% and 64.91% respectively), whereas hostacycline, ciprofloxacin and norfloxacin supplementations at 0.002 %, 0.001 % and 0.002 % dose increased only cocoon weights (+58.32%, +58.08%, +40.30% respectively). Taken together, the findings of this study indicate that 0.002% dosage of antibiotics found to be effective in raising the better productivity of the cocoon than the other doses tested. The results observed were in agreement with the previous findings of earlier reports which indicate considerable increase in silk production, by the use of other antibiotics like chloromycetin, erythromycin, tetracycline etc on mulberry silk (Verma & Kushwaha, 1971). In addition studies of Savithri et al. (1999) confirmed that use of high protein diet effectively increases the quality of cocoon shell. However in this study significant differences were noted for shell weights, which could be attributed to the high pupal weights observed in the antibiotic fed group as opposed to low pupal weights in the control group. Further, a significant elevation observed in cocoon weight on exposure of various antibiotics indicates their ameliorative role of antibiotics. Their supplementation in low concentrations favoured the silkworm to attain maximal cocoon weight. Thus the beneficial action of the antibiotics can be attributed to (a) their activity in regulating the composition of intestinal bacteria; (b) their potential role as possible growth factors; (c) their biological efficiency in increased turn-over of the feed in to body weight; and (d) their potential disease control activity.

In conclusion, it can be noted that treatment of silkworm larvae with antibiotics elicits favourable response in improving the commercial qualities of silk fiber and can be used in sericulture for yield enhancement.

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HARI PRASAD. T.P.N. AND SHETTY. N. J.
RELATIVE AND ATTRIBUTABLE RISKS OF INBREEDING AND GENETIC LOAD IN THE MUSLIM COMMUNITY FROM KOLLAM, KERALA

DEVIPRIYA V1, APSARA S1 AND MATHEW P M 2
1Department of Botany, Sree Narayana College, Kollam. 2Department of Botany, University of Kerala,Kariavattom.
e-mail:devipriyav@hotmail.com

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SUMMARY

The relative and attributable risks of inbreeding and genetic load are evaluated and estimated from the data of consanguinity-associated mortality effect in the Muslim community of Kollam (Kerala). The impact of inbreeding is evaluated in terms of 2 measures of association, the relative risk (RR) and attributable risk (AR). The relative risk against total mortality (RR3) is 1.87 and attributable risk (AR3) 0.128. The attributable risk was significant only for postnatal mortality (21.1%). The genetic loads were estimated following the MCM model in units of lethal equivalents based on the values of A and B statistics. The B/A ratio was relatively low being 14.6886, segregational that the load element in the community could be largely segregational. The estimated levels of lethal equivalents are also low, ranging from 0-1, segregational that the community may be loaded with few numbers of highly lethal recessive genes operating.

Keywords: Relative, attributable risks, genetic load, Muslims, Kollam

INTRODUCTION

Consanguineous marriages enhance the likelihood of spouses possessing the same genes inherited from the common ancestor(s), and hence higher frequencies of homozygous offspring are expected in consanguineous families. Many harmful traits in man are known to be recessives and hence recessive homozygous offspring are often associated with impairment of function, leading to many harmful defective conditions and diseases. Reports on the occurrence of such conditions, across a wide range of traits suggest a large number of deleterious alleles in the human genome. This has been predicted from the reduced early survival of offspring in close-kin marriages and from similar results in other organisms (Bittles & Neel, 1994; Charlesworth & Hughes, 1999). The mortality profile of a population can yield clues for understanding the relative influence of epidemiological factors on human health (Freymann 1982). Khoury et al (1987) proposed an epidemiological approach in terms of 2 measures of association such as relative risk (RR) and attributable risk (AR) for evaluating the public health impact of inbreeding. The method is considered to give an insight into the overall influence of inbreeding on the public health problems and prospects of human populations, which is of great value in genetic counselling programmes. The concept of genetic load in the popular sense was introduced by Muller (1950) as one of the approaches to explain

1 Sarasi, Sreemoolam Road, Medical College Po, Thiruvananthapuram-695011
2 Perakathisseril, Muttada Po, Thiruvananthapuram,. Kerala-695025
the relationship between inbreeding and its effects. Attempts for analyzing the element of genetic load in human populations have relied on the possibility that the expressed load can be estimated from the data of consanguinity-associated mortality. Morton et al (1956) suggested that inbreeding effect on mortality can provide a means of estimating the measure of genetic load in units of lethal equivalents. This paper aims at evaluating the public health impact of inbreeding in terms of the relative and attributable risks arising there from, and also estimating the genetic load in the Muslim community of Kollam based on the consanguinity-related mortality risk data.

MATERIALS AND METHODS

The history of Muslim community in Kerala dates back to the first visit made by Sulaiman, a Muslim merchant in 851AD. Since then trade between Kerala and Muslim countries increased, and more Arab Muslims came and settled down in the Malabar coast, where they were welcomed by the Zamorin of Calicut, who encouraged them to marry local women and serve on his armed forces. The first Muslim mosque in Kollam was established by Ibn Dinar, a devout Arab Muslim, which could be responsible for the spread of ‘Islam’ in Kerala. According to the 2001 census data, Muslims form 24.7% of the total population of Kerala. The sample size in the present study was 1500 Muslim families from urban and suburban regions of Kollam, which is around 30% of the community in the region. The data was collected by visiting and interviewing the members of each family, with the aid of a comprehensive questionnaire which included a variety of socio-economic variables and demographic determinants. Some families were visited twice or thrice in order to collect information from other family members or to examine children who were absent during the first visit. The help of a local person was sought for gaining introduction to the subjects selected. Two measures of association, the relative risk (RR) and attributable risk (AR) were used to assess the public health impact of inbreeding in the community. The RR denotes the ratio of mortality risk in offspring of consanguineous marriages to the risk in offspring of non-consanguineous unions, while AR refers to the fraction of risk that can be prevented if inbreeding were dispensed with; and this is computed following Levin & Bertell’s (1978) formula – AR = P (RR-1) / [1+p(RR-1)]. This equation expresses the attributable risk as the function of the relative risk associated with the prevalence of inbreeding in the population (P). The RR values of prenatal (RR₁), postnatal (RR₂) and total (RR₃) mortality, and also the corresponding AR values (AR₁, AR₂ and AR₃) were computed. The genetic loads were estimated from the data of consanguinity effect on mortality in units of lethal equivalents based on the A and B statistics obtained by the weighted regression analysis following the MCM formula, \(-\log S = A + BF\) (Morton et.al. 1956), where S is the proportion of survivors, F the coefficient of inbreeding, A the estimate of deaths that occur in non-inbred offspring and B the measure of the hidden genetic damage that would be expressed by inbreeding. The measure of total genetic damage is the quantity which is equal to the sum of B and A, and hence the number of lethal equivalents per gamete lies between B and B+A. The B/A value provides critical information about the relative importance of mutational and segregational loads, the former if B/A is more than 10 and the latter if less.

RESULTS

Data on the levels of inbreeding, inbreeding-related mortality, relative risk (RR) and attributable
TABLE 1: Mortality due to consanguinity and the respective RR and AR by degree of inbreeding among the Muslims of Kollam

<table>
<thead>
<tr>
<th>Type of mating</th>
<th>Prenatal mortality (%)</th>
<th>Postnatal mortality (%)</th>
<th>Total mortality (%)</th>
<th>RR1</th>
<th>AR1</th>
<th>RR2</th>
<th>AR2</th>
<th>RR3</th>
<th>AR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>3.28</td>
<td>2.3</td>
<td>5.58</td>
<td>1</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>UN</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-0.002</td>
<td>0</td>
<td>-0.002</td>
<td>0</td>
<td>-0.002</td>
</tr>
<tr>
<td>1C</td>
<td>4.78</td>
<td>5.74</td>
<td>10.52</td>
<td>1.46</td>
<td>0.068</td>
<td>2.50</td>
<td>0.194</td>
<td>1.89</td>
<td>0.125</td>
</tr>
<tr>
<td>1.5C</td>
<td>0</td>
<td>12.5</td>
<td>12.5</td>
<td>0</td>
<td>-0.006</td>
<td>5.43</td>
<td>0.026</td>
<td>2.24</td>
<td>0.007</td>
</tr>
<tr>
<td>Total cons.</td>
<td>4.5</td>
<td>5.94</td>
<td>10.44</td>
<td>1.37</td>
<td>0.059</td>
<td>2.58</td>
<td>0.211</td>
<td>1.87</td>
<td>0.128</td>
</tr>
</tbody>
</table>

NC – Nonconsanguineous  UN – Uncle – Niece  1C - First Cousin  1.5C - 1.5 Cousin  Con.- Consanguineous

The inbreeding frequency was 16.07%. The mortality rate in the consanguineous families was 10.44%. The relative risk against total mortality (RR₃) was 1.87 and attributable risk (AR₃) 0.128. The B statistics (0.8454) was much higher than the A statistics (0.0576). The B/A ratio was 14.6886 and the number of lethal equivalents per gamete ranged from 0 – 1.

DISCUSSION

The relative risks for total mortality being estimated directly from the total mortality rate, are consistently less than the sum of the corresponding values of prenatal and postnatal mortality (Freire – Maia, 1990). Mathew et al. (2007) observed that the total mortality profiles among an assemblage of 35 communities in Kerala revealed a wide spectrum, ranging from 5.75% (Parayan) to 39.04% (Paniya Tribe). They arbitrarily considered three levels each for both, for recognizing the association between levels of inbreeding and mortality, such as low inbreeding (<25%), medium inbreeding (25-50%), high inbreeding (>50%) and low mortality (<10%), medium mortality (10-20%), high mortality (>20%). Based on this, they categorized the 35 communities under eight association types, and the Muslim community of Kerala was included under the medium inbreeding-medium mortality group. However the Muslims of Kollam have shown a relatively lower frequency of inbreeding (16.07%) and mortality rate (10.44%). So they may be included under the low inbreeding-medium mortality.
mortality category. The reduced rates of inbreeding might be attributed to their socio-economic development in recent times. By reviewing and analyzing numerous world data, Khoury et al. (1987) pointed out that the differential interaction of socio-economic and demographic variables with inbreeding might lower the impact of inbreeding among communities with lower socio-economic levels in comparison with those having higher. They also evaluated the impact of inbreeding in terms of RR, and have observed that the levels of inbreeding and mortality are independent events and mutually exclusive, and have indicated that in the world scenario, cases of low–low association type displayed the highest inbreeding impact and conversely those under the high–high type the least. This negative trend observed by Khoury et al. (1987) by and large applies to the present observation of low relative risk (RR\text{\textsubscript{\text{3}}} = 1.87) in the community which belongs to the low-medium type.

The attributable risk for total mortality was also low (AR\text{\textsubscript{\text{3}}} = 0.128), which indicates that the risk of mortality exclusively due to inbreeding is low. This is in contrast with earlier reports in several of the Tribal and Scheduled Caste communities (Muthuvan, Pallan, Karuvazhipulaya, Parayan, Irular etc.) of Kerala, who have all registered very high inbreeding frequencies (70-91%) and high estimates of ARs (0.40 -0.48) (Mathew et al. 2007). This positive association between inbreeding rate and attributable risk is similar to the one reported by Khoury et al (1987) in several world population groups in which the estimated ARs ranged from 1-50% with great bulk showing very low values. They are of the opinion that this is due to only minor role being played by consanguinity in determining mortality, which is possibly due to the declining levels of inbreeding as a sequel to socio-economic development and advanced literacy in those population groups. This is also suggestive that long-term inbreeding has not played significant role in eliminating the deleterious recessive alleles from their gene pool.

Genetic load is the proportion by which the fitness of the average genotype in a population is reduced in comparison with the optimum genotype (Crow 1958). The deleterious recessive genes are maintained in a population by recurrent mutations which balance the elimination due to selection (Crow & Kimura 1965). Most of them are often revealed by inbreeding, with some being semilethals or sublethals (Morton 1958). A precise estimate of such genes in a population, therefore, should need weighing genes by their lethal effect. Morton et al. (1956) have proposed a statistical model based on weighted regression for quantifying the load factor making use of the A and B statistics. The estimated values of A in the present community is very low (0.0576). An inverse relationship between As and public health condition has been suggested in the world scenario (Cavalli-Sforza & Bodmer 1999) which implies that low value of A is an indicator of better public health exposure and vice versa. Mathew et al (2007) have also opined that low values of A predominate in the socio-economically more developed communities in contrast to high values in the least developed Scheduled Castes and Scheduled Tribes.

The estimates of B value which represents almost all the causality by recessive lethals, was also relatively low (0.8454). Reddy & Naidu (1978), based on the results in a few communities in Andhra Pradesh, have suggested that environmental and socio-economic conditions may positively influence the B estimates. Mathew et al (2007) observed a trend of negative association between
inbreeding ratio and B estimates in which those communities with very high inbreeding levels (>70%) displayed very low Bs and vice versa. The B/A ratio has been suggested to be an index for determining the nature of the load element (Crow & Kimura, 1965) such that very low values (<10) suggestive of segregational load and high, mutational in origin. Mathew et al (2007) had observed very high B/A values (>100) in the Vellala, Reddiar and the Malaiarayas of Kerala. The B/A value observed in the present study was low (14.6886) suggesting that the load element in them could be largely segregational.

The measure of genetic load is estimated in units of lethal equivalents which is either a single gene that is fully lethal in a homozygote or a group of mutant genes in different loci of such number that, if dispersed in different individuals, would cause on the average one death. One lethal equivalent may comprise several detrimental mutant genes, and hence every individual must be heterozygous for many genes which would be seriously detrimental in the homozygous state (Murthy & Jamil 1972). The values of lethal equivalents per gamete deduced from the difference between B and B+A in the present community was 0.1. This is suggestive of the high lethality of the gene concerned in bringing about mortality in the population. A single such gene may be considered sufficient enough to bring about the extent of mortality observed in the population. It has been suggested that if the genes are fully lethal, the number of lethal equivalents would be the same as the number of lethal genes in a haploid complement (Cavalli –Sforza & Bodmer 1971), and here the selection intensity against the recessive homozygote is complete (s=1). As the selection intensity (s) decreases, the actual number of deleterious genes is roughly the number of lethal equivalents divided by the average value of s, and hence in such instances the number of lethal equivalents per gamete could increase and the corresponding homozygote would have selection intensity less than unity (Mathew et al 2007). According to this postulation the Muslim community of Kollam with very low number of lethal equivalents should be endowed with less number of highly lethal recessive genes.

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GENOTOXIC EFFECT OF THE LEAF EXTRACT OF EUPATORIUM TRIPLINERVE VAHL. (ASTERACEAE)

ASHA RAMACHANDRAN1 AND JOHN E. THOPPIL2
1Bishop Moore College, Department of Botany and Biotechnology, Mavelikara-690 110, Alappuzha, University of Kerala, India. 2Cell and Molecular Biology Division, Department of Botany, University of Calicut, Pincode - 673 635, Kerala, India.
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ABSTRACT

Cytotoxic effect of leaf extracts of Eupatorium triplinerve Vahl., a potential medicinal plant, was studied. The leaf extract induced a number of chromosomal abnormalities (CA) on Allium cepa root tip meristem, both of the clastogenic and non-clastogenic types. Clastogenic abnormalities observed were nuclear lesion, chromosome bridge, chromosome breakage and pulverization of chromosomes whereas non-clastogenic abnormalities observed were ball metaphase, chromosome clumping, early movement of chromosomes, disturbed metaphase, star metaphase, chromosome lagging etc. Mitotic index (MI) decreases when compared with the control, indicating mitotic inhibition.

Key words: Eupatorium triplinerve, Asteraceae, Allium cepa, Cytotoxicity, Clastogenic, Non-clastogenic

INTRODUCTION

Eupatorium triplinerve Vahl. (Asteraceae) is a small aromatic plant with profuse branching, prostrate, lanceolate, three nerved leaves (Sivarajan & Balachandran 1994). It is a potential medicinal plant used for treating haemorrhages, haematuria, wounds, ulcers, poison bites, inflammations, cough and asthma. Hence an investigation has been conducted to determine the mitoclastic effects induced by E. triplinerve leaf extract on Allium cepa root meristem.

MATERIALS AND METHODS

Germplasm collection of E. triplinerve was made from the Calicut University campus and were herbarized (CALI 123719). Crude extract was prepared from the leaves of E. triplinerve. Cytotoxic effect of various concentrations (0.025%, 0.05%, 0.1%,0.3% & 0.5%) of the aqueous extract was analyzed by Allium cepa assay. From the extract containing both polar and non-polar compounds, the extract containing polar compounds alone was prepared after the selective removal of non-polar compounds using diethyl ether as solvent in a separating funnel. Distilled water was used as the control. After each treatment (1h, 2h & 4h), a few healthy root tips were taken, washed thoroughly with distilled water and fixed in Carnoy’s solution for 1 h. Mitotic squash preparations were made with the help of improved techniques (Sharma & Sharma 1980) using 2% acetoorcein. Preparations were scanned under Olympus CX 21 binocular microscope and the photomicrographs were taken with an attached Olympus Camedia C-4000 Zoom digital compact camera.
RESULTS AND DISCUSSION

Treatment with leaf extracts of *E. triplinerve* on *A. cepa* root meristem showed many clastogenic and nonclastogenic mitotic effects and also lowered the mitotic activity significantly than the control. Comparative analysis reveals that the chromosomal aberrations were found to be pronounced in the extracts containing polar compounds alone, when compared with the extracts containing both polar and non-polar compounds (Table 1). The mitotic index decreased with increase in the concentration of the extract in both cases. Clastogenic abnormalities observed were nuclear lesion, chromosome bridge, chromosome breakage and pulverization of chromosomes whereas nonclastogenic abnormalities observed were ball metaphase, chromosome clumping, early movement of chromosomes, disturbed metaphase, star metaphase, chromosome lagging etc. Frequency of aberrations showed a positive correlation with the increase in concentration of the extract. Mitotic index also varies with the increase in concentration and duration of exposure. Clumping of chromosomes at anaphase, scattering of chromosomes at metaphase, diagonal orientation of chromosomes, pulverization of chromosomes at metaphase etc were the most frequent kinds of aberration induced by the *E. triplinerve* extract.

Bizarre nucleus (Fig. 1) may occur due to the disintegration of proteins and unequal clumping of the nuclear material (Badr & Ibrahim 1987). Diagonal orientation of chromosomes (Figs 2 & 8) were due to the failure and improper functioning of the spindle apparatus (Das et al. 1968). Clumping of chromosomes (Fig. 3) is attributed to the increased concentration of the cytotoxicant. This physiological effect is manifested by cells which are already in division at the time of treatment (Somil et al. 1982). Chromosome pulverization (Fig. 4) is due to the premature condensation of chromosomes (Sakari et al. 1981). Scattering of chromosomes (Fig. 5) occurs due to the disturbances in the mitotic spindle. Scattered and clumped metaphases are the partial and full effects respectively of a C- mitotic agent (Hadder & Wilson 1958). Stickiness (Fig. 6) may result from the enlargement of the chromatin fibre which fail to condense properly during the initial stages of mitosis (Mc Gill et al. 1974). Stellate arrangement of chromosomes (Fig. 7) may be due to the clumping of daughter chromosome groups in a star like manner at anaphase. Amer (1965) considered stellate arrangement of chromosomes as being a fore-step of the complete disturbance of the spindle. Chromosome bridge (Fig. 9) may arise due to stickiness or due to the formation of dicentric chromosomes by breakage and reunion (Raj & Rao 1972). Mitotic poison may cause metabolic imbalance which may interfere with the synthesis and structure of nucleic acids, inducing physiological effects and structural changes in the chromosomes during cell division which may lead to mitotic inhibition (Somil et al. 1982).

*Allium* test has been widely used to determine the clastogenic effects of different chemicals due to relative simplicity (Kihlman 1996). Results from the cytotoxic studies are relevant because the toxicological target is DNA, which exist in all living forms. Thus it can be concluded that the polar and non-polar components of the leaf extracts of *E. triplinerve* directly or indirectly disturbs the genetic material by which it regulates the nucleic acid metabolism and thereby generating a wide spectrum of both clastogenic and non-clastogenic aberrations.
Table 1. Cytotoxic effects of *E. triplinerve* leaf extract

<table>
<thead>
<tr>
<th>Duration of treatment</th>
<th>Control</th>
<th>0.025%</th>
<th>0.05%</th>
<th>0.1%</th>
<th>0.3%</th>
<th>0.5%</th>
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<tbody>
<tr>
<td></td>
<td>CA%</td>
<td>MP%</td>
<td>CA%</td>
<td>MP%</td>
<td>CA%</td>
<td>MP%</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1h</td>
<td>44.58±0.32</td>
<td>26.3±0.96</td>
<td>25.3±3.76</td>
<td>33.7±0.80</td>
<td>27.3±0.36</td>
<td>40.1±1.40</td>
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<tr>
<td>viti</td>
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</tr>
<tr>
<td>2h</td>
<td>44.35±0.54</td>
<td>35.64±1.30</td>
<td>27.81±0.68</td>
<td>59.69±0.62</td>
<td>24.2±0.65</td>
<td>44.7±0.76</td>
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</tr>
<tr>
<td>4h</td>
<td>37.57±0.67</td>
<td>43.7±0.90</td>
<td>25.4±3.78</td>
<td>46.8±8.81</td>
<td>25.96±0.33</td>
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</tr>
<tr>
<td>1h</td>
<td>44.58±0.32</td>
<td>29.67±1.05</td>
<td>22.54±0.46</td>
<td>56.34±0.32</td>
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<td>42.67±0.74</td>
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</tr>
<tr>
<td>2h</td>
<td>44.35±0.54</td>
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<tr>
<td>4h</td>
<td>37.57±0.67</td>
<td>45.89±1.13</td>
<td>24.10±0.82</td>
<td>48.93±0.95</td>
<td>23.01±0.90</td>
<td>50.27±1.35</td>
</tr>
</tbody>
</table>

Figures indicated the mean values ± Standard Deviation (SD)

Bar=10µm
GENOTOXIC EFFECT OF THE LEAF EXTRACT

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