# BIOSYSTEMATIC STUDIES OF WATER HYACINTH (*EICHHORNIA CRASSIPES* (MART.) SOLMS)

BY

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# CERTIFICATE

This is to certify that the research work embodying the results reported in this thesis has been carried out under our guidance in the experimental field and Plant Breeding and Tissue Culture Laboratory of Department of Botany, University of Dhaka, Department of Biochemistry, University of Dhaka, Bangladesh University of Engineering and Technology, Dhaka and BCSIR Laboratories, Dhaka. It is further certified that the research work presented here is original and suitable for submission for the award of Ph.D.

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The author

# BIOSYSTEMATIC STUDIES OF WATER HYACINTH (*EICHHORNIA CRASSIPES* (MART.) SOLMS)

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# ABBREVIATIONS

| | |
|---|---|
| ADH | Alcohol dehydrogenase |
| AOV | Analysis of variance |
| BOD | Biochemical oxygen demand |
| cf | Confer, compare with |
| cm | Centimetre (s) |
| chloropt | Chloroplatinate |
| COD | Chemical oxygen demand |
| conc | Concentrated |
| contd. | Continued |
| df | Degree of freedom |
| dia | Diametre |
| DO | Dissolved oxygen |
| EDTA | Ethylene diamine tetra acetic acid |
| *et al.* | Et alii, and others |
| Fig. | Figure (s) |
| gm | Gram (s) |
| hr | Hour (s) |
| i.e. | id est. in other words |
| inf | Inflorescence |
| lb/lbs | Pound/Pounds |
| l | Litre |
| loc.cit. | Loco citato, before cited |

| | |
|---|---|
| LP | Long petioled form of water hyacinth |
| MDH | Malate dehydrogenase |
| ms | Mean square |
| µ | Micron |
| µl | Microlitre |
| µm | Micrometre |
| mg | Milligram |
| mg/l | Milligram per litre |
| ml | Millilitre |
| mm | Millimetre |
| mM | Milli molar |
| min | Minute (s) |
| M | Molar |
| MS | Murashige and Skoog medium |
| nm | Nanometre |
| N | Normal |
| No. | Number |
| NTU | Nephelometric turbidity unit |
| obs | Observed |
| PDB | Paradichlorobenzene |
| % | Percentage |
| pH | Negative logarithm of hydrogen ion concentration |
| post | Posterior |
| pt | Platinum |
| S.E. | Standard error |

| | |
|---|---|
| soln | Solution |
| SP | Short petioled form of water hyacinth |
| ss | Sum square |
| TBE | Tris-borate EDTA |
| TE | Tris-EDTA |
| temp | Temperature |
| Viz. | Videlicet, namely |
| wt | Weight |

# INTRODUCTION

# INTRODUCTION

Eichhornia crassipes (Mart.) Solms, commonly known as water hyacinth, is a floating hydrophyte, belonging to the monocot family, Pontederiacae. The taxon is native to Brazil of tropical America. It has spread many other tropical and subtropical regions, India, Pakistan, Bangladesh, Sri Lanka, Africa, Australia and in some parts of North America, namely, Florida and California. In these regions the plant often creates critical problems in agriculture, navigation, irrigation, fishing and public health. This is a pest in rice fields in Bangladesh and every year subsistence farmers in the wet low lands face disaster due to its invasion. Rafts of water hyacinth weighing up to 300 tons/hectare are floated over the paddy fields by flood water. Even when the flood recedes, the population of this weed remains in the fields, adversely affecting the crops to a great extent (National Academy of Sciences, USA, 1976).

Water hyacinth spreads very quickly, mainly by vegetative means. Penfound and Earle (1948) found that the plants' number doubled fortnightly. Wolverton (1979) reported that plants doubled their mass every nine to ten days. In growing season 25 plants can produce enough biomass to cover 10,000 square metres of water surface with approximately two milli-

on plants (Barrett 1989). In certain parts of the world water hyacinth propagates by seeds also (Das 1969; Tag El Seed and Obeid 1975). The seeds of water hyacinth have been reported to remain dormant up to 20 years (Myers 1964). Westlake (1963) reported a maximum biomass of 29 Kg/$m^2$ fresh and 1.5 Kg/$m^2$ dry in growing season with daily productivity of 7.4-22 g/$m^2$ dry, with an average biomass of 1473 g/$m^2$ dry weight. In Bangladesh, annual productivity of water hyacinth was found to be as follows:short petioled form of water hyacinth, 1700 Kg/ha fresh wt, 117 Kg/ha dry wt and long petioled form, 16000 Kg/ha fresh wt, 1120 Kg/ha dry wt (Majid 1983).

The heavy growth of this weed causes a multitude of problems. A single plant or a part thereof, if left behind during a cleaning operation,gives birth to a full population in no time. The extensive vegetative reproduction and water retention capacity help the plant invade wide territories. Total elimination of the plant by chemical, mechanical or biological means has so far been a failure. The scientists therefore gradually changed their attitude towards the plant. They tried to turn this troublesome pest into a useful product.

Research so far carried out indicates that, if properly utilized, it can prove to be of great potential value, benefiting the human race as cattle feed (Joint project report of BAU, Mymensingh and DANIDA),soil additive, compost, mulch

or ash (Philipp *et al.* 1983), fibre (Haider 1989; National Academy of Sciences, USA, 1976), water purifier (Wooten and Dodd 1976; Soerjani 1984;Indra and Krishnamurthy 1984; Majid 1986; National Academy of Sciences, USA, 1976), source of energy (NASA; Philipp *et al.* 1983; Haider 1989), source of paper, pulp (Indra and Krishnamurthy 1984) and human food (Majid 1986; Burkill 1966). It is also used in mushroom cultivation as well (Soerjani 1984; Baruah 1984). Some research has been conducted in different parts of the world on the physiological, ecological morphological and cytological aspects of water hyacinth as well as on the incidence of its diseases and their control measures. However, extensive biosystematic studies on this very important weed have been meagre, with hardly any work ever being undertaken in Bangladesh.

Due to many contradictions regarding the morphological status of the water hyacinth plant, so far there has been no agreement among biologists on taxonomic status of the short petioled form and long petioled form of water hyacinth. According to some workers,the large and small type represent different stages of the same taxon (Backer 1951). According to others (Avadhani *et al.* 1984) the floating form appear to be associated with deeper water. The third group considers that the chemical contents of the water determine the ultimate phenotype of the plant (Haider 1984; Thomas 1984; Balasooriya *et al.* 1984). According to still others the

crowded condition of the plants leads to the formation of the type with non-swollen petioles (Wolverton 1979).

In Bangladesh,two forms of water hyacinth (short petioled and long petioled) are found. The short petioled form floating in water, is much more smaller in size compared to the long petioled form; while the long petioled form is much taller, usually anchored in the soil or free floating.

No serious attempts have so far been made in Bangladesh to ascertain the taxonomic status of the long petioled form and short petioled form of water hyacinth.

The present investigation was undertaken in order to gain an insight as to the factors which determine the form of water hyacinth, found in the water bodies of a locality. Experiments were undertaken to determine whether the observed differences are gene-mediated or dependent on the environment in which the plant in question is found to occur.

To achieve the above goal, biosystematic studies covering the following parameters were carried out : (i) ecological, (ii) morphological, (iii) pollen chracteristics, (iv) growth characteristics, (v) seed development and germination, (vi) cytological, (vii) estimation of protein, calcium, phosphorus and moisture content, (viii) chemical composition of water supporting the growth of the different forms, (ix) water purifying capacity, (x) Study of protein bands and isoenzyme patterns,(xi) comparative morphological study of lilac-blue flowered and white-flowered water hyacinth and (xii) DNA pattern.

# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

Eichhornia crassipes. popularly known as water hyacinth, grows abundantly in tropical and subtropical regions of the world. There exists great controversy in the scientific world regarding this widely growing wild plant, involving its morphological and genetical status and also its utility. According to some authors,E. crassipes consists of different varieties and according to others there exists only one variety, morphological differences being induced by the depth of water, nutrient content of water,seasonal variation or density of its population.According to still another school of thought, they are the different stages of the same variety. As for their economic importance, some regard these as a menace,while others consider them to be of considerable economic importance.

According to Jamil (1990) Eichhornia plants could be classified into three types, depending on the environmental conditions: (i) plants tall with elongated petioles, (ii) short with bulbous petioles and (iii) medium sized with bottle-neck petioles. Jamil (loc.cit.) also mentioned that morphogenic characters of the plants depended on the amount of sunlight received by them. The plants growing on the shaded side of the pond were tall, those in the partly

shaded part were medium sized, and those growing in full illumination of the sun were short with bulbous petioles.

Eichhornia has more roots when floating on deep water than on moist soil (Oki and Ueki 1984). More profuse flowering occurred in emerged plants than in floating ones (Ueki and Oki 1979).According to Backer (1951), petioles are spongy and short in young specimens and also much swollen in or below the middle, in adult plants, petioles are much longer, up to 30 cm long. In Malaysia, only a single form occurs having flowers with three very short anterior filaments and three much longer exterior ones; stigma located at medium height between the anthers of the long and the short filaments. Fruit was never reported to be produced in Malaysia. During ecological survey of aquatic weeds in different parts of Kerala, India, Thomas (1984) came across three ecotypes of E. crassipes. showing distinct variations in size and morphology. The length of leaf of these three varieties were: (i) 2 to 3 cm, (ii) 10 to 15 cm and (iii) 40 to 60 cm respectively, while the commonly available plants had leaves, 20 to 25 cm long. The species shows considerable variation in morphological and ecological features with adaptability to a wide spectra of habitats. Three ecotypes were so pronounced that some of these appeared to be distinct species, while the small type had thick bulbous inflated petiolar base, the tallest type possessed thick long petioles. Reza and Khan (1981) stated

that there are three types of water hyacinth: Type 1 is very big having long and thin stem. Leaves are extremely green, big and erect. Roots are long (10 to 90 cm or more). They multiply very rapidly. The average height of the plant is about 45 cm in mature stage but generally it ranges from 30 to 70 cm. Type 2 plant is medium, stem provided with swollen petiole. Leaves are medium in size and green. Roots are generally 10 to 30 cm long and the height of the plant is 20 to 30 cm. Type 3 plant is dwarf having high rate of multiplications; stems more or less rounded, slightly reddish, leaves are small, round and less green than the other two types. Roots are short in length (5 to 15 cm). The plant is normally less than 20 cm long. Avadhani *et al.* (1984) stated that normally Eichhornia occurs in two morphological forms, one is the floating form and the other is anchored form. The floating form appears to be associated with deeper water and/or crowded conditions. Oki and Ueki (1984) reported that the results of the comparison of three different types of water hyacinth are of much interest. All plants of the floating type could recover even after 30 days, while this characteristic of emerged type was already reduced after 18 days, the plants of land type exhibited the highest survival capacity of all. According to Oki and Ueki (loc.cit.), the maximum growth occurred under emerged conditions. Haigh (1936) also observed better growth in 15 cm deep water with the plant roots anchored in the mud.

The effect of fluctuation in water level and desiccation has been studied by several workers, and most of these reports indicate that the change in water level does not effect the growth (Gopal and Sharma 1981). It was also found that, emerged plants produced more inflorescence and seeds than floating plants (Ueki and Oki 1979).

Barrett (1982) stated that E. crassipes (Mart.) Solms exhibits the rare genetic polymorphism tristyly. In a survey of style morph representation in 198 new world populations, 77% were monomorphic, 18.4% dimorphic and 4.6% were trimorphic. Water hyacinth of Texas, USA was composed of 69.6% long-styled morphs and 39.6% mid-styled morphs; a population at Palo Verde,Guanacaste Province, Costarica,contained 80.2% mid-styled morphs and 19.8% long-styled morphs. The short-styled morphs was common in the Amazon Basin and Mato Grosso. These data are consistent with the suggestion that E. crassipes is native to low land tropical south America and in particular, the Amazon Basin of Brazil (Holm *et al.* 1977; Barrett 1979). Barrett (1989) stated that by the early 1970's Botanists had reached the conclusion that short-style morph was extinct and E. crassipes had two floral forms, not three. On the contrary, in 1974 Barett (1977a) found Eichhornia flowers with short-styles.

Jamil (1990) stated that Balasooriya *et al.* (1984) monitorted the physio-chemical properties of various water bodies supporting the growth of Eichhornia and found that

the amount of phosphates, nitrates and ammonia present in water had direct effect on morphogenic expression of Eichhornia plants. With supply of the optimum nitrogen, dense mats of water hyacinth were formed, while due to its reduced supply growth was retarded resulting in the development of varying size of plants. However, the same organism, placed in a restrictive environment,adjusts itself to it for survival. Ueki (1978) reported that maximum growth of mature plants occurred in the pH 7.0. Chadwick and Obeid (1966) and Santiago (1973) also reported that water hyacinth yielded best at pH 7.0. Haller and Sutton (1973) reported that water hyacinth plant growing in either acidic or alkaline water had a tendency to change the pH towards neutrality.Similar results was also observed by Ueki (1978).

Thomas (1984) reported that the chemical content of the water in which the plant grows,significantly affects the size of the plant. This observation has been confirmed by Thomas (loc.cit.)during a comparative study of the different ecotypes, during his investigation. The extremely small size of the ecotype may be due to the nutrient deficiencies, mainly $N_2$, in the water. Thomas (loc.cit.) observed that if considerable quantities of nutrients, especially $N_2$, are supplied, growth is very rapid and dense mats are soon formed. Naturally it follows that in the absence of optimum quantity of nutrients, especially $N_2$, growth is retarded resulting in small size of the plant.

Das (1969) stated that according to Baker (1965) virtually sterile clones are produced as a result of vigorous vegetative growth. Although this makes the species handicapped in harnessing the genetic variability, it plays a role of great ecological significance in making such species more aggressive and better colonizers.

Different workers studying the breeding system concluded that the long-styled form is a recessive character and mid-styled form a dominant character. Francois (1970) considered the inheritance to be based on single allel while Mulcahy (1975) and Reddy and Bahadur (1977) held two alleles responsible for the inheritance. The long-styled plant is considered homozygously recessive (mmss) and the mid-styled form is either heterozygous or homozygous for the dominant allele (MmSs). Reddy and Bahadur (1977) considered the inheritance to be based on two genes each with two alleles, exhibiting a sporophytic incompatibility. Barrett (1977) has studied the crosses with the short-styled plants but did not get the inheritance of floral form in $F_1$ generation. It is however possible that, short-styled form is dominant over the long-styled form and the mid-styled condition is heterozygous.

Barrett (1977a) in his survey found that inheritance of style form in plants obtained from Stockton and Jari populations indicate that these plants are heterozygous for genes controlling style length.

Barrett (1977) stated that the absence of the short-styled form in the adventive range is probably due to chance and the genetic determination of style form. Partial information on the inheritance of tristyly in E. crassipes suggests that a two-locus system (S and M) with S epistatic to M is involved (Francois 1964; Barrett 1977b). Neither long nor mid-styled form, can segregate short-styled plants, the only means by which this form could enter the adventive range is by the chance introduction of short-styled plants. Apparently this process has not occurred.The only certainity is that the mid-styled form was introduced to the adventive range.If the mid-styled genotype (S) introduced were heterozygous for M gene, then long-styled form could be produced by self-or cross-pollinations. Alternatively if only homozygous mid-styled genotypes were involved a separate introduction of long-styled form (s) would be necessary to account for their presence in the adventive range. Bock (1966) in California, Backer (1951) in Malaysia, McLean (1922), Bruhl and Sengupta (1927) in India,reported little or no seed set or seedling establishment and thus there are minimal possibilities for the segregation of long-styled plants, assuming that heterozygous mid-styled plants are present in these areas. It is noteworthy that in these regions, long-styled plants have not been reported. Barrett (1989) stated that in the water hyacinth two genes determine the length of the style. One gene decides whether or not the style will be

short. If the short trait is not expressed, a second gene decides whether the style will be medium or long. The second gene has two alleles, dominant (M) and recessive (m). If a plant is of homozygous form with both pairs of its genes being recessive, its flowers will have a long-style. If a plant has at least one copy of the dominant gene (mM or Mm Or MM), its flowers will have a medium-style. Wain and Martin (1980) compared three groups of water hyacinth by using horizontal starch gel electrophoresis. Electrophoresis has become a well-established and powerful method for analysing genetic variation within and among populations at the molecular level. Extremely little or no differentiation has occurred at the structural gene level. High level of self-fertilization again reduce genetic variability within populations. The study of Wain and Martin (1980) indicates that the character differences which separate the water hyacinth morphs do not seem to be associated with a major genetic divergence in response to different environmental pressure. These differences may however, still have a genetic basis. For example, differentiation may be controlled by only one or a few structural genes or by certain non-structural regulatory genes.

A white variety of water hyacinth has been reported by Wolverton (1979) and Mahabuba Halim (National Herbarium of Bangladesh, personal communication), however, no detailed information was available about this plant.

As has been mentioned before, according to some, water hyacinth is a menace,as it has been found to create problems in agriculture,fisheries,navigation and public health. Hence attempts have been made to control E. crassipes by biological means (Harley 1989; Gunasekara and Balasooriya 1982; Indra and Krishnamurthy 1984; Baruah 1984; Freeman and Charudattan 1974) and also by chemical process (Bakar *et al.* 1984; Ramaprabhu *et al.*1984; Menn and Charles 1965; Hammouda 1968). However, water hyacinth once considered to be the world's worst, fast growing troublesome,indestructible weed, now finds many possibilities of use in human welfare in the present world. Anon (1966) stated that water hyacinth mulch improves soil structure, reduces soil erosion. Water hyacinth has been used as a fertilizer in rice field (Subramanyam 1976) and also in maize, soybeans, sweet potato (Sluyters *et al.* 1979). Jamil (1990), Finlow and McLean (1917) and Venkataraman *et al.* (1984), Backer (1951), Soerjani (1984), Indra and Krishnamurthy (1984) recommended the use of water hyacinth as a fertilizer. Water hyacinth ash has a high potash content (Philipp *et al.* 1983). Finlow and McLean (1917) used water hyacinth ash in jute field and found that about 25% yield was increased. About 98% of potassium oxide content could be extracted from water hyacinth ash with hot water (Day 1918). Water hyacinth is also used in biogas production (Philipp *et al.* 1983; Haider 1989; Jamil 1990; Soerjani 1984 and Majid 1986). Water

hyacinth fibre has been used for producing board and other indigenous materials (Haider 1989). It is also used as raw materials for paper, paste boards and cement boards (Indra and Krishnamurthy 1984; Haider *et al.* 1984; Majumder *et al.* 1984; Joedodibroto *et al.* 1983). Water hyacinth has been used for manufacturing paper, fibre-reinforced cement board and carbon block (Solly 1984) and also as human food (Majid 1986 and Burkill 1966). The long stem of water hyacinth plant is suitable for fabrication of various domestic items such as baskets, bags, containers, mats, hats etc (Monsod 1975 and Majid 1986). According to Chakraverty (1984) and Majid (1986), water hyacinth is reported to have positive effect on the growth and development of plants and has a potential for increasing crop production. Its extracts have also been used as medium for fungal and bacterial growth (Chakraverty 1984). Water hyacinth biomass can be used for mushroom cultivation (Soerjani 1984; Baruah 1984), and also as livestock feed (Soerjani 1984; Chatterjee and Hye 1938; Hossain 1959; Soewardi 1974; Vaas 1951; Osman *et al.* 1975; Philipp *et al.* 1983). Folliar application of root extract of water hyacinth on rice and jute plant stimulated height of plants and production of tillers (Chakraverty 1984). Water hyacinth has been reported to be effectively used in purification of water, polluted by industrial waste or domestic sewage (Wooten and Dodd 1976; Soerjani 1984; Majid 1986; Indra and Krishnamurthy 1984).

# MATERIALS AND METHODS

# MATERIALS AND METHODS

3.1 The present investigation was conducted with two morphologically different specimens of Eichhornia crassipes (Mart.) Solms, collected from Dhaka city and its suburbs. One form possessed long petioles and the other short petioles. The long potioled form is mostly rooted to the ground, while the short petioled one floats in water. Hereinafter they will be designated as LP and SP respectively.

## 3.2 Collection of samples

Samples were collected from ponds of different localities of Dhaka city and its suburbs.

## 3.3 Experimental sites

Experiments were carried out in the Bangladesh Council of Scientific and Industrial Research Laboratories (BCSIR), Dhaka, Departments of Botany and Biochemistry in the University of Dhaka, Bangladesh University of Engineering and Technology (BUET), Dhaka and International Centre for Diarrhoeal Disease Research (ICDDR), Dhaka.

## 3.4 Ecological study

For ecological study a total number of 287 ponds were surveyed in Dhaka city and its suburbs. Out of these ponds,

277 ponds were surveyed from March 1986 to February 1987. Abundance of LPs and SPs in these ponds were determined and expressed in percentage of the total population.

For detailed ecological study, ten ponds were selected in Dhaka city and its suburbs. The selected ponds were studied throughout the year to observe the manner and cycle of growth of the two forms of water hyacinth, LPs and SPs. Abundance and percentage of LPs and SPs were also determined.

### 3.5 Morphological study

326 samples of E. crassipes, collected from 287 ponds, were studied for a period of 21 months (March,1986 to December, 1987). Each part of the randomly selected samples was thoroughly studied and their measurements were recorded.

After collection, the samples were separated on the basis of their length of petioles. Selected samples, having distinct petiole differences, were grown separately in earthen tubs containing tap water (hereinafter these were designated as cultured plants). Morphological differences of the cultured plants were also recorded. Simple dissection microscope was used for detecting differences in the samples. Plant parts were measured with the help of a meter scale.

Photographs were taken for recording morphological details and the differences between the LPs and SPs.

## 3.6 Pollen grains study

### 3.6.1 Pollen grain stainability

Pollen grains were stained with acetocarmine and glycerine (1:1). The estimates were based on the examination of 1500 pollen grains. Only well inflated, uniformly stained grains were considered to be an estimation of fertile ones.

The freshly opened anthers were placed in a drop of the stain on a clean slide; anthers were gently squeezed with a pair of forceps and the debris was removed. The coverslip was placed and stainability was scored with a microscope. An estimate of the stainability was expressed in percentage.

Photomicrographs of pollen grains were taken of both LPs and SPs.

### 3.6.2 Measurement of pollen grains

The size of the pollen grains were recorded by Ocular micrometer (X 360). For this study only full stained pollen grains were counted for both LPs and SPs.

## 3.7 Growth characteristics

### 3.7.1 Comparative study of the growth of the two forms of water hyacinth in tap water and in 1% Hoagland solution.

Plants of both long petioled and short petioled forms of water hyacinth (LPs and SPs),were planted in earthen tubs containing 40 litre tap water or 1% Hoagland solution (Appendix I). Five plants were planted in large earthen tubs

of 58 cm dia. Each treatment had three replica.Growth record of the experimental plants was maintained for 12 months.

### 3.7.2 Effects of different concentrations of Hoagland solution on the height of short petioled form of water hyacinth (SP)

Five SPs were planted in each of the 12 small earthen tubs of 35.5 cm dia.Each tub contained 10 litre of 1,5 or 10% Hoagland solution (Appendix I). Each treatment had three replica. In the three control tubs only distilled water was added. Two to three litre medium of specific concentrations were added in respective tubs at 10 days intervals. The experiment was conducted for full one year.

### 3.7.3 Propagation of daughter plants from two forms of water hyacinth

To study the propagation of daughter plants from LPs and SPs, three mature plants of each form were placed in six earthen tubs of 58 cm dia, containing 40 litre tap water. Growth characteristics of the two forms were compared on the basis of rate of emergence of daughter plants. Each daughter plant was labeled with a tag.The older plants were discarded when the earthen tubs got crowded with the emergent young plants. On the basis of daily observation, the comparative study of the growth characteristics was conducted for one year.

### 3.7.4 Emergence of daughter plants from rhizome of both LPs and SPs in tap water and in 5% Hoagland solution

#### 3.7.4.1 Rhizomes in tap water

Rhizomes of both forms were planted in earthen tubs of 58 cm dia, containing 40 litre tap water. In each tub there were six rhizomes and there were three replica.

Emergence of daughter plants from the rhizomes was recorded daily. The number of plants was counted.The experiment was conducted for a period of 12 months.

#### 3.7.4.2 Rhizomes in 5% Hoagland solution

The process was the same as described for tap water but in this study smaller earthen tubs of 35.5 cm dia, were used in order to economize the use of chemicals.Six rhizomes were placed in each tub and there were three replica. 10 litre of 5% Hoagland solution was added in each of the six tubs (Appendix I). The experiment was carried out for a period of 12 months.

### 3.7.5 Longevity of two forms of water hyacinth

To study the longevity of two forms (LPs and SPs), three mature plants of each form were placed in six earthen tubs of 58 cm dia, containing 40 litre tap water. Longevity of the two forms were compared on the basis of the emergence of daughter plants and the period of their persistence. Each daughter plant was identified with a labelled tag. On the basis of daily observation, the comparative record of their longevity was maintained for one year.

### 3.8 Development of seed of LPs and SPs

#### 3.8.1 Development of seed by natural pollination

Water hyacinth flowers are attractive and well suited for insect pollination, and yet only 35% of the flowers are pollinated under natural conditions. 100 inflorescences were studied to detect natural pollination. After 18 days, the mature capsules were collected and sun-dried. Number of capsules per inflorescence, number of seed bearing capsules per inflorescence, number of seeds per capsule were recorded. Insects visiting the flowers were also recorded.

#### 3.8.2 Use of bagging method for seed development by self- or cross-pollination

##### 3.8.2.1 Development of seeds by self-pollination

Early in the morning pollen grains from the freshly opened flowers were transferred to the stigma of the same flower with the help of a pair of sterilized forceps. The flowers were then covered with a mosquito net and marked by a labelled tag. After about 18 days the capsules matured. The net was then removed from the inflorescence and the capsules were collected and dried under the sun. The dry capsules were dissected to detect the presence or absence of seed in them. If seeds were present, their number was determined. Record was maintained of the number of capsules per inflorescence, number of seed bearing capsules per inflorescence, number of seeds per capsule. About 50 inflorescences of both LPs and SPs were used in this study.

### 3.8.2.2 Development of seed by cross-pollination

Reciprocal crosses were made between LPs and SPs. The cross-pollinated flowers were covered with a piece of mosquito net material and marked with a labelled tag. After 18 days, the mature capsules were collected and sun-dried. Number of capsules per inflorescence, number of seed-bearing capsules per inflorescence, number of seeds per capsule were recorded as described before. Eighteen inflorescences of both forms were used in this study.

### 3.8.3 Measurement of capsules

Natural pollinated, artificial self-and cross-pollinated freshly collected capsules of both forms (LPs and SPs) were measured with the help of a meter scale.

### 3.8.4 Measurement of seeds

Natural pollinated, artificial self-and cross-pollinated seeds of both LPs and SPs were measured with the help of an Ocular micrometer (X 80).

## 3.9 Germination of self-and cross-pollinated seeds

Water hyacinth seeds remain dormant and retain their viability for several years; the long dormancy may be due to hard seed coat.

Since no clear-cut information is available on the germination of water hyacinth seeds, a part of the present study was devoted towards trying different techniques, for obtain-

ing seed germination.

### 3.9.1 Germination of seed in water on filter paper

One hundred seeds of LPs and SPs, obtained from both self- and cross-pollinations were placed on filter paper, in sterilized Petri dishes (sterilized in drying oven at 100°C for two hours). Fifteen ml tap water or pond water (collected from water hyacinth infested ponds) was added to wet the filter paper as well as soak the seeds. Later on pond or tap water was added to each Petri dish according to necessity (to prevent drying). The seeds were observed every day. The experiment was continued for three months.

### 3.9.2 Alternate wetting and drying

The seeds collected in October, 1989, were used in this study. These were stored in wet condition for 100 days and then stored in dry condition for two weeks and then again stored in wet condition for one week in sterilized Petri dishes.Pond water or Hoagland solution (Appendix I) was used as wetting agents. One hundred seeds of the self-or cross-pollinated plants of both forms were used.

### 3.9.3 Chemical soaking

Chemical soaking was done in 7.5 cm long tube with a dia of 1.7 cm. The volume of the chemical used (conc. $H_2SO_4$) was just enough to cover the seeds. Surface tension was broken by stirring, causing the seeds to sink. After the

pretreatment with conc. $H_2SO_4$ acid for 1 min, the tube was dipped in a beaker containing water to avoid excessive heating. The seeds were then thoroughly washed with distilled water, before being placed in the Petri dishes. Hoagland solution (Appendix I) was used as a germination medium.Petri dishes used were sterilized as mentioned before.

3.9.4 Tissue culture

The following culture media were used for germination of self-or cross-pollinated seeds of LPs and SPs.

(1) MS medium (Murashige and Skoog, 1962)

(2) Hoagland solution (Hoagland and Arnon, 1950).

3.9.4.1 Preparation of culture media

(1) MS medium

Preparation of stock solutions:

(i) Stock solution A (Macronutrients)was made ten times the final strength of the medium in 1000 ml distilled water. Ten times the weight of salts prescribed per litre of the medium were weighed and dissolved one at a time and sequentially in 750 ml of distilled water and final volume was made up to 1000 ml by distilled water.The stock solution was cleared of solid particles by filtering through Whatman No.1 filter paper. It was then poured into a plastic container and stored in a refrigerator at $4^{o}$ C until used.

(ii) Stock solution B (Micronutrients) was also made ten times the final strength of the medium in 100 ml of distilled

water as described above. However, in this case, the two constituents, $FeSO_4$ and $Na_2$-EDTA were separately dissolved in 100 ml distilled water in a 250 ml Erlenmeyer's conical flask by heating until the salts dissolved completely. $CoCl_2$ was also separately dissolved in 100 ml of distilled water. All these stock solutions were filtered and stored in a refrigerator at $4^{o}C$ until used.

(iii) Stock solution C (Vitamins) was also made ten times the final strength of the medium in 100 ml distilled water as described before. This was also filtered and stored in a refrigerator at $4^{o}C$ until used.

Inositol was directly added during preparation of the medium in desired concentrations.

(iv) Hormonal stock solutions: The phytohormones, namely BAP (6-benzyl amino purine) and IAA (Indole-3-acetic acid) were dissolved in appropriate solvents.

| Hormone | Solvent |
|---|---|
| BAP | 0.1N NaOH |
| IAA | 70% ethyl alcohol |

To prepare a stock solution of any of the above hormones, 10 mg of the hormone was placed on a clean watch glass and then dissolved in 1 ml of the particular solvent.The mixture was washed off with distilled water and collected in a 100 ml measuring cylinder and was made up to 100 ml with

distilled water. The solution was then poured into a clean plastic container and stored at $4^{o}C$.

Constituents of MS medium have been shown in Appendix II.

The following steps were taken to prepare 1 litre of the medium.

(a) 30 gm of sucrose was dissolved in 500 ml of distilled water in a 1 litre flask.

(b) 100 ml of stock solution of macronutrients, 10 ml of stock solution of micronutrients and 10 ml of stock solution of vitamins were added to the 500 ml sucrose solution (a) and mixed well. 100 mg of inositol were added to the solution (b) and stirred until dissolved completely.

(c) Hormonal supplements were added to this solution BAP 1 mg/l and IAA 1.5 mg/l were added to this solution and were mixed thoroughly.

(v) The whole mixture was then made up to 1000 ml with distilled water.

(vi) pH of the medium was adjusted to 5.8 with 0.1 N NaOH or 0.1 N HCl.

(vii) 10 gm (1%) of Difco bacto-agar was added to the medium and the whole mixture was gently heated in a water bath while being continuously stirred until agar was completely dissolved.

(viii) The medium was poured into test tubes while still hot and were plugged with nonabsorbent cotton and marked with different codes with the help of a glass marker, to indicate specific hormonal supplement.

(ix) The medium contained in the test tubes was autoclaved at 15 lbs per square inch pressure at 121°C for 20 min. Along with this some Petri dishes were also sterilized.

(x) After sterilization the test tubes were allowed to cool as slants.

### 3.9.4.2 Hoagland solution

Hoagland solution and sucrose were mixed in different proportion to prepare culture medium for seed germination as mentioned below:

Culture medium (a) Hoagland, without sucrose

(b) Hoagland + 1% sucrose

(c) Hoagland + 2% sucrose

(d) Hoagland + 4% sucrose

Before autoclaving, pH of the medium was adjusted to 7. Preparation of Hoagland solution has been presented in Appendix I.

### 3.9.4.3 Culture Technique

a) Seed culture : Self-and cross-pollinated seeds of LPs and SPs were dipped in a 0.1% $HgCl_2$ solution for 20 min. The seeds were subsequently rinsed with sterile distilled

water four times, to remove all traces of $HgCl_2$. Then the seeds were transferred to sterilized Petri dishes containing distilled water,and were left there for six days for softening the hard seed coat. All these operations were performed aseptically inside a laminar flow cabinet.

After six days the sterilized seeds were split longitudinally with the help of a pair of sterilized forceps and the cotyledons were inoculated in the test tubes containing the medium. Seven cotyledons were placed in each of the test tubes.

All the dissection equipments viz. needles, forceps, were dipped in 95% alcohol each time, flamed over a spirit lamp and cooled before use. The floor of the cabinet was sterilized with 70% alcohol before starting the inoculation procedure. Hands were also sterilized with 70% alcohol.

### 3.10 Mitosis and karyotype analysis

Freshly collected 1-1.5 cm long root tips of both LPs and SPs were pretreated with saturated solution of Paradichlorobenzene (PDB) for six hours.

The modified saturated solution of PDB was prepared by dissolving 750 mg of PDB in 50 ml of distilled water and incubated overnight at 60$^{o}$C,as suggested by Palmer and Heer (1973).

The root tips were fixed in 1:3 acetic alcohol for 24 hrs at room temperature, and then transferred to 70% alcohol and stored in a refrigerator, until used.

Root tips were then stained by Haematoxylin method as described by Haque *et al.* (1976) with certain modifications which are as follows:

3.10.1 Haematoxylin method

(a) After washing with distilled water, root tips were transferred to 1N HCl and heated in an oven at 58$^{o}$C for 25 min to dissolve the middle lamella of the cells.

(b) Root tips were washed with distilled water for five min.

(c) After washing mordanting was done by using 2% aqueous solution of iron alum (ferric ammonium sulphate) for 15 min.

(d) Root tips were washed again with frequent changes of distilled water for five min in order to wash away the last traces of mordanting fluid from the tissue.

(e) After washing root tips were stained in 0.5% haematoxylin for 18 hrs and washed with distilled water for five min to remove excess of stain. The time ordinarily recommended for staining of chromosome is about 10 min.

After staining temporary slides were prepared and examined under the microscope for studying mitotic chromosomes. Data were recorded and photomicrographs were taken from the desired preparations.

## 3.11 Chemical analysis of water hyacinth

Estimation of Protein, Potassium and Phosphorus of sun-dried samples of both LPs and SPs.

### 3.11.1 Estimation of Protein

#### 3.11.1.1 Preparation of sun-dried sample

The samples of LPs and SPs growing in the same pond, were collected from three ponds and dried under sun light till a constant dry weight was obtained. Only petioles and leaves were taken for this study.

#### 3.11.1.2 Principle

For protein estimation, total nitrogen was determined by Kjeldahl method (Cocks and Van Rede 1966).Protein content was calculated by multiplying the total nitrogen content by 6.25. The sample was digested with a digestion mixture containing sulphuric acid, phosphoric acid and a catalyst when the nitrogen present, was converted to ammonium sulphate. The solution was then made alkaline, the liberated ammonia distilled off, collected in dilute hydrochloric acid and excess acid titrated.

#### 3.11.1.3 Reagents used

(i) Digestion mixture : The digestion mixture was prepared by mixing together a solution of 1 gm of copper sulphate in 50 ml acid mixture (45 ml conc. $H_2SO_4$ + 5 ml conc. $H_3PO_4$),

with a solution of 1 gm selenium oxide in 50 ml (heating) of the above acid mixture.

(ii) Methyl red indicator solution (0.1% Methyl red in alcohol).

(iii) 50% aquous sodium hydroxide solution.

(iv) 0.1 N hydrochloric acid solution.

(v) 0.1 N sodium hydroxide solution.

3.11.1.4 Procedure

0.2 gm of the sun-dried sample was taken in a 750 ml Kjeldahl flask, and five ml of digestion mixture was added to the flask. The contents then digested in a digestion chamber.Digestion was continued till the sample became clear solution. After cooling, the solution was made alkaline with 50% sodium hydroxide solution using 2-3 drops of phenolphthalein as indicator.The ammonia thus liberated was immediately distilled and the distillate was collected in a standard acid solution (10 ml of 0.1 N Hydrochloric acid) using methyl red as indicator.When about 20 ml of the distillate had been collected,the distillation was completed and the excess acid titrated with standard sodium hydroxide solution.

A blank titration was carried out without sample as described above.

Calculation

$$\% \text{ of Total nitrogen (TN)} = \frac{(x-y) \times N \times M \times 100}{W \times 1000}$$

Where : x is vol of NaOH soln for blank

y is vol of NaOH soln for sample

N is normality of NaOH solution

M is the molecular weight of nitrogen

W is the weight of sample taken.

% of Protein = % TN x 6.25

### 3.11.2 Estimation of phosphorus and potassium

For estimation of phosphorus and potassium, the sun-dried samples of LPs and SPs were burnt to ash and from the ash, stock solution was prepared.

#### 3.11.2.1 Preparation of ash

The sun-dried sample was taken in a previously weighed porcelain crucible and heated very carefully over a bunsen burner for one hr.

It was then heated in a muffle furnace at 550$^{o}$C for four hrs, cooled and weighed. The light gray ash thus obtained was stored for future use.

#### 3.11.2.2 Preparation of stock solution

To the ash obtained from about 2.0 gm of sun-dried water hyacinth samples was added 5 ml of conc. hydrochloric acid

and heated to dryness in a waterbath. To it an additional amount of 2 ml conc. hydrochloric acid was added and heated for a few min in a waterbath. 5 ml deionised water was then added and after cooling, the solution was filtered. The filtrate was collected in a 100 ml volumetric flask and made up to mark with deionised water. For the estimation of phosphorus and potassium, an aliquot portion was used.

3.11.2.3 Estimation of phosphorus

Phosphorus was determined colorimetrically by a UV-Spectrophotometer of the type SP8-100, Pye Unicam, UK.

To 25 ml of the stock solution mentioned above (containing up to 1 mg phosphorus),5 ml of molybdate solution (12.5 gm A.R. sodium molybdate in 500 ml10 N $H_2SO_4$) was added followed by 2 ml hydrazine sulphate solution (1.5 gm hydrazine sulphate in 1 litre deionised water) and diluted to 100 ml in a volumetric flask.The flask was kept immersed in boiling water for 10 min and cooled. The optical density was then measured at 830 nm against a reagent blank.The phosphorus content was determined directly from a calibration curve constructed from standard phosphate solution (A.R. Potassium dihydrogen phosphate in deionised water) treated in the way described above.

3.11.2.4 Estimation of potassium

Potassium was determined in a flame photometer of the model STANDARD II, made in Germany. A.R. Potassium chloride was used for preparation of standard solns.

### 3.11.2.5 Moisture content

For estimation of moisture content of petioles of both LP and SP forms, fresh petioles were used. The petioles were first blotted with blotting papers to remove superficial water and then these were cut into small pieces. 21.93 gms of sample was taken in a previously weighed glass container and heated at 100$^{o}$C for 72 hrs in an oven after which it was cooled and weighed. Heating and cooling was repeated till a constant dry weight was obtained. Percentage of moisture content can be calculated from the weight loss.

## 3.12 Chemical analysis of the water supporting the growth of LPs and SPs

To ascertain whether dissolved foreign materials in water are responsible for the occurrence of the two forms of water hyacinth (LPs & SPs), the water from water hyacinth infested ponds containing either one or both forms of plants, was analysed following the standard methods of American Public Health Association (1965) for: (a) dissolved oxygen, (b) total nitrogen, (c) phosphate and (d) calcium. pH of the water was also recorded.

### 3.12.1 Collection of water

For collection of water samples, clean glass bottles

with plastic stoppers were used. The bottles were immersed into the water and stoppered beneath the water surface to avoid entry of atmospheric oxygen. The dissolved oxygen content was determined within two hrs from the time of collection. The water samples were allowed to decant and the clear supernatant was taken for analysis.

Altogether nine ponds were selected for this study, three with only SPs, three with only LPs and three with both SPs & LPs.

### 3.12.2 Analysis of dissolved oxygen

Winkler method (Azide modification) was followed for estimation of dissolved oxygen.

#### 3.12.2.1 Analysis procedure

For the analysis well stoppered glass bottles of exactly 300 ml capacity were used. The bottle was filled completely with decanted clear sample water and stoppered so that any excess water was drained out without any air bubbles.

To the bottle containing the water sample was added 2 ml manganese sulphate (32.3%) followed by 2 ml alkali-iodide azide reagent (prepared by dissolving in distilled water 50 gm of sodium hydroxide and 15 gm of potassium iodide, making the total volume 100 ml, and then adding to it 1 gm sodium azide dissolved in 4 ml distilled water). Both the reagents were added well below the surface of the liquid.The bottle

was then stoppered carefully to avoid introduction of air bubbles, and mixed by inverting the bottle several times. When the precipitate thus formed settled, leaving a clear supernatant above the manganese hydroxide floc, the bottle was shaken once again and allowed to settle. When about 100 ml clear supernatant is formed, the stopper was removed carefully and immediately 2 ml conc.sulphuric acid was added to run down the neck of the bottle. The bottle was immediately stoppered and mixed by gently inverting until dissolution was complete. Exactly 203 ml supernatant was taken and titrated with 0.025 N sodium thiosulphate solution to the appearance of a pale straw colour. 1-2 ml freshly prepared starch solution was then added. Blue colouration formed. The titration was continued till the blue colour just disappeared.

Calculation

1 ml 0.025 N Sodium thiosulphate is equivalent to 0.2 mg dissolved oxygen. If we take 200 ml of original sample, each ml of sodium thiosulphate titrant is equivalent to 1 mg DO/l.

Since a total of 4 ml (2 ml each) of the manganese sulphate and alkali azide solution was added to 300 ml sample, the vol equivalent to 200 ml original sample is equal to 200 x300/(300-4) ≈203 ml.

### 3.12.3 Analysis of total nitrogen

For analysis of total nitrogen, the Kjeldahl method was followed.

#### 3.12.3.1 Analysis procedure

250 ml of clear water sample was taken in a 750 ml Kjeldahl flask and 10 ml conc.sulphuric acid,6.7 gm potassium sulphate and 1.5 ml mercuric sulphate solution were added to it.The mixture was then digested. Digestion was continued for 30 min, after the solution became clear. The residue was cooled and then 300 ml ammonia-free water was added to it. The solution was then made alkaline by sodium thiosulphate solution (50 gm NaOH + 2.5 gm $Na_2S_2O_3.5H_2O$ in 100 ml water) using phenolphthalein as an indicator. The ammonia liberated was immediately distilled into 50 ml of the indicating boric acid solution contained in a 300 ml conical flask. The indicating boric acid solution was prepared by dissolving 5 gm of boric acid in water, adding 2.5 ml mixed indicator (made by mixing 2 vol of 0.2% methyl red in 95% ethanol with 1 vol of 0.2% methylene blue in 95% ethanol) and diluting to 250 ml with ammonia free water. Distillation was continued until about 200 ml distillate had been collected. The tip of the condenser was extended well below the level of the boric acid solution and the temp. in the condenser was maintained below $29^{o}C$.

The ammonia thus collected was then titrated with 0.25 N

sulphuric acid until the indicator turned a pale lavender.

A blank was run with all the reagents used without sample water and necessary correction was made.

Calculation

$$\text{mg nitrogen/l} = \frac{(A - B) \times 280 \times \text{Normality of sulphuric acid}}{C}$$

Where : A is the vol (ml) of sulphuric acid required for sample

B is the vol (ml) required for blank

and C is the vol (ml) of water taken

### 3.12.4 Analysis of phosphate

Soluble phosphate content in the water sample was determined colorimetrically using a spectrophotometer of the type LKB, ULTROSPEC, model No.4050 at a wave length of 690 nm. It may be stated here that only soluble phosphate content was estimated and the insoluble polyphosphate (if any) was separated by filtration.

The water samples were collected in the usual way and filtered through a Whatman No.1 filter paper to free it from turbidity and suspended materials.Before phosphate analysis, neutrality of the water should be checked. If one drop of phenolphthalein indicator to 100 ml of the filtered water sample produce a pink colour,the water sample is not neutral and should be neutralised first with strong acid.

### 3.12.4.1 Analysis procedure

Four ml of ammonium molybdate reagent (prepared by dissolving 2.5 gm $(NH_4)_6Mo_7O_{24}.4H_2O$ in 17.5 ml water, adding to it 28 ml conc. sulphuric acid dissolved in 40 ml water and making 100 ml with distilled water) and 0.5 ml stannous chloride reagent (prepared by dissolving 2.5 gm $SnCl_2.2H_2O$ in 100 ml glycerine) were added to 100 ml of the filtered water sample with thorough stirring.A blue colour developed. After 10 min of addition,but before 12 min, the intensity of colour was measured.

For calibration, three standard samples were prepared with 1.022 mg, 2.044 mg and 3.066 mg $PO_4$/l from anhydrous $KH_2PO_4$ (analar grade).The same method as described above was followed and colour intensity was measured. The rate of colour development and the intensity of colour depend on temp. of the final solution, each $1^oC$ increase producing about 1% increase in colour. Hence the samples,the standards and the reagents should be within $2^oC$ of one another and at a temp. between $20-30^oC$.

By plotting colour intensity against concentration, a straight line was obtained for the standards. The concentration (mg/l) of phosphate in the water sample was read directly from the curve.

### 3.12.5 Analysis of calcium

Calcium content of the water sample was determined with the help of a Gallenkamp Flame analyser.

### 3.12.6 pH

The pH of the water was read in a pH meter of the type Jenway 3010, UK.

## 3.13 Water purifying capacity of LPs and SPs

To determine if the two forms of water hyacinth (LP & SP) have varying capacity of purifying dirty water, these plants were introduced separately in earthen tubs containing dirty pond water and allowed to grow in it for a specified period of time. The water was analysed before introduction of the plants and after keeping them in the water for the specified period.

### 3.13.1 Procedure

Dirty pond water was collected for the study. Earthen tubs of dia 35.5 cm were used. In each tub, ten litres of dirty pond water was taken. In a set of three tubs, eight young long petioled or eight young short petioled form of water hyacinth plants were placed in two tubs. The third one was kept without plants as control. The water in the tubs was analysed at the beginning and after ten days. Each set was repeated three times.

### 3.13.2 Analysis of water

Analysis of water was carried out according to the method recommended by Theroux *et al.* (1943). The following analysis was conducted before introduction of the plants and after keeping the water for ten days with the plants:

1. Turbidity,
2. Colour,
3. Total solids,
4. Total dissolved solids,
5. Total suspended solids,
6. Chemical Oxygen demand and
7. Biochemical Oxygen demand.

The analysis procedures are described below :

#### 3.13.2.1 Turbidity

500 ml experimental dirty water sample was allowed to settle down for 5 hrs. The supernatant portion was transferred to the HACH tubes, filling them up to the mark and allowed to stand until the air bubbles rose to the surface.

The turbidity was then measured in a turbidimeter (Nephelometric turbidity meter, HACH company, Loveland, Colorado, USA) and expressed in NTU (Nephelometric Turbidity Unit).

#### 3.13.2.2 Colour

To record the colour, the previously in 3.13.2.1 mentioned supernatant was diluted to 1% with distilled

water. 100 ml Nessler tubes were filled with this water sample up to the mark and reading was taken in a HACH spectrophotometer DREL/4,made by HACH company,Loveland,Colorado, USA at a wave length of 455 nm. The results are obtained directly from the apparatus and expressed as mg/l of platinum as chloroplatinate ion.The apparatus was so standardised that a solution of 1 mg/l of platinum as chloroplatinate would show a reading of 1 unit.

3.13.2.3 Total solid

A clean 150 ml glass beaker was dried for one hour at 105$^{o}$C in an oven, then cooled in a desiccator and weighed. The sample of water was thoroughly mixed and 100 ml of it was transferred immediately in the beaker. The liquid was first evaporated over a water bath and then the beaker was heated at 105$^{o}$C in an oven for an hr. Finally it was cooled in desiccator and weighed. From the difference of weights the total solid content can be determined and expressed as mg/l of total solid.

$$\text{mg/l of Total solid} = \frac{\text{Wt of beaker with reside (gm)} - \text{Wt of empty beaker(gm)}}{\text{ml of water taken}} \times 10^{6}$$

3.13.2.4 Total dissolved solid

For determining the total dissolved solid, the same procedure as described in 3.13.2.3 was followed with the exception that the water sample was filtered through a Whatman No. 1 filter paper.

3.13.2.5 Total suspended solid

The total suspended solid was estimated by substructing the total dissolved solid content from the total solid content.

3.13.2.6 Chemical oxygen demand (COD)

(a) Reagents used :

(i) Dilute sulphuric acid (1:3)

(ii) Standard Potassium permanganate solution (0.0125 N)

(iii) Standard ammonium oxalate solution (0.0125 N)

(b) Procedure : 100 ml of dilute sample (1%) as stated in 3.13.2.2 was taken in a 250 ml volumetric flask. To this was added 10 ml dilute sulphuric acid (1:3) and 10 ml of standard potassium permanganate solution (0.0125 N). The solution was then heated in a boiling water bath for 30 min keeping the level of water in the bath above the level of the solution in the flask. The flask was then removed from the water bath and 10 ml of standard ammonium oxalate solution (0.0125 N) was added to it to neutralise excess potassium permanganate. The hot solution was then titrated with standard potassium permanganate solution until the first permanent pink colour appeared. The ml of standard potassium permanganate solution required is equivalent to the ml of standard potassium permanganate solution which reacted with the chemical oxygen present.

Calculation

$$\frac{\text{ml of standard potassium permanganate required} \times 100}{\text{ml of sample water}} = \text{ppm of COD}$$

3.13.2.7 Biochemical oxygen demand (BOD)

(a) Reagents used :

(i) Manganous sulphate solution,

(ii) Alkaline potassium iodide solution,

(iii) Standard sodium thiosulphate solution,

(iv) Starch solution and

v) Conc. sulphuric acid.

(b) Procedure

Dirty water sample (1% as stated in 3.13.2.2) was taken in a well stoppered glass bottle. The bottle was then carefully filled completely to avoid inclusion of air. To the solution were added 1 ml each of manganous sulphate solution (32.3%) and alkaline potassium iodide solution by pipette, dipping the end of the pipette just below the surface of the water. The bottle was then stoppered carefully and the solution was mixed by inverting the bottle several times. The precipitate thus formed was allowed to settle half way and was mixed again. This was repeated twice. 1 ml conc. sulphuric acid was then added to it and the bottle stoppered at once and the content mixed as before. The solution was allowed to stand for 5 min. From the solution, 100 ml was taken in a 250 ml conical flask and immediately titrated

with 0.025 N sodium thiosulphate solution until the yellow colour almost disappeared. 1 ml starch solution was then added and the titration continued until the blue colour just disappeared.

Calculation

ml of 0.025 N sodiumthiosulphate required x 2

= ppm of dissolved oxygen/l.

By this procedure, the dissolved oxygen content of the water sample was estimated. This estimation was continued on the same water sample for five alternate days. Everyday the dissolved oxygen content decreased gradually due to the presence of microorganism in the sample water which consumed the oxygen. The difference of dissolved oxygen content in the water sample represents the biochemical oxygen demand (BOD).

### 3.14 Electrophoretic study of protein bands and isoenzyme patterns of two forms of water hyacinth (LPs and SPs)

Electrophoresis, one of the recent and useful methods for the study of enzyme properties and proteins, was included in the present investigation, involving studies of two enzymes namely alcohol dehydrogenase (ADH), malate dehydrogenase (MDH) and protein bands in the leaves of experimental samples. Extracts from the leaves of LPs and SPs were used to separate protein bands and enzyme electrophoretically.

### 3.14.1 Preparation of extract

Extraction was done by homogenizing 5 gm samples in a chilled mortar with 5 ml 0.05 M Tris-HCL buffer (pH 7.6) containing 10 mM B-mercaptaethanol (BME) and 12.5% sucrose. The extract was centrifuged at 10,000 rpm for 30 min at 4$^{o}$C. The supernatant was used directly for estimating gel electrophoresis of enzymes and proteins. Fresh extract was used.

### 3.14.2 Estimation of soluble proteins

The protein concentration of crude extracts was determined according to the method of Lowry *et al.* (1951) using bovine serum albumin as standard. Absorbance was measured at 750 nm on a LKB digital spectrophotometer (Model, ultrospec, 4050). Protein concentration in the experimental samples was calculated by comparing the data with a standard curve.

### 3.14.3 Electrophoretic procedure

Protein and isoenzyme separation was carried out by polyacrylamide disc electrophoresis according to the method of Bloemendaal (1963). Power supply and electrophoretic set, manufactured by Sargent Welch, USA were used.

### 3.14.4 Preparation of stock Tris-glycine buffer

3 gm of Tris (hydroxymethyl) methylamine (0.247 M) and 14.4 gm of glycine (0.191 M) were dissolved in 100 ml redistilled water.

3.14.5 Working buffer

The stock buffer solution was diluted ten times with redistilled water before use. The pH of this buffer was 8.5. For each electrophoretic run 800 ml of this buffer was needed, 600 ml for the lower and 200 ml for the upper buffer reservoirs respectively.

3.14.6 Preparation of stock gel solutions

(a) 30 gm of acrylamide and 0.8 gm of N-N-methylene bisacrylamide were dissolved in 100 ml redistilled water and filtered through Whatman No.1 filter paper.

(b) 1.6 gm of β-dimethyl aminopropionitrile was added to 40 ml stock Tris-glycine buffer which was then diluted 2.5 times to make the final volume 100 ml (for polymerization).

(c) 30 mg % potassium ferricyanide solution was made in redistilled water (for delaying polymerization).

(d) 480 mg % ammonium persulfate solution was made in redistilled water (for enhancing polymerization).

The above solutions remained stable for at least one month, if stored in a brown bottle at $4^{o}C$.

3.14.7 Polymerization of gels

For polymerization of gels and electrophoresis, 12 glass tubes,8 cm long with an inside dia of 5 mm were placed in a vertical position on a plastic rack with suitable holes and screws.The lower ends of the tubes were closedby rubber

caps. 6 ml of each of the stock solutions, a,b,c,and d (sec. 3.14.6) were taken sequentially in a concial flask and shaken thoroughly. The mixed gel solution was immediately pipetted into gel tubes upto 5 mm from the upper rim. The gels were allowed to polymerize at room temperature. The polymerization occurred within 40-50 min to form a colourless gel. The completion of polymerization was indicated by a sharp boundary line between the polymerized and unpolymerized portions. Unpolymerized solution was discarded and the gel surface was washed with the working buffer.

### 3.14.8 Pre-electrophoresis

The tubes with polymerized gels were removed from the plastic rack and the rubber caps were removed carefully. Immediately the tubes were inserted into the rubber grommet-lined bores of the upper electrode buffer reservoir, so that the rims projected about 2 cm above the bores. Pre-cooled ($4^{o}C$) electrode buffer (Tris-glycine working buffer) was slowly poured into the upper and lower electrode reservoirs and special care was taken to prevent the formation of air bubbles, at the rims of the tube. The electrodes were connected with the power supply and pre-electrophoresis was carried out for 15-20 min at 80V (2-3 mA/gel) to purify the gels from unreacted materials of polymerization process and ionic impurities of the gel components. More over, pre-electrophoresis set up a constant resistance inside the gel.

### 3.14.9 Application of sample

After pre-electrophoresis the upper reservoir was removed, and leaf extracts (0.1 ml), mixed with a few crystals of sucrose,to increase the density of the extracts, were applied on top of the individual gel to form a layer under the buffer layer. For comparative study the same amount of each extract was added. In the control, small amount of 0.001% bromophenol blue mixed with 10 µl 20% bovine serum albumin was added on top of the gel. Bromophenol blue formed complex with albumin and the dye served as a marker of the migrating boundary during electrophoresis.

After application of the samples,the upper reservoir was replaced on the lower reservoir and the apparatus was connected to the power supply. Electrophoresis was performed at room temperature at 80V (2-3 mA/gel) for 15 min and then for 90 min at 160V (3-4 mA/gel) for satisfactory separation of bands. Continuous water circulation was ensured around the buffer reservoirs.

### 3.14.10 Treatment of gels and detection of protein and isoenzymes

Immediately after electrophoresis the gels were removed from the tubes with the help of ejecting water from a hypodermic needle,attached to a syringe, and washed in distilled water.The protein and isoenzyme bands were located by treating the gels in appropriate staining mixture, discribed

below. A control gel was treated in the same way as above. In control gel no band was detected.

### 3.14.11 Staining mixture for protein

The gels were stained with amido black (0.6 gm in 100 ml of 7% acetic acid) for 30 min. Then the stained gels were washed with distilled water and placed in test tubes in 7% acetic acid. The gels were kept immersed in acetic acid until protein bands were clear.

### 3.14.12 Staining mixture for ADH

For each gel, the reaction mixture consisted of 3.7 ml Tris-glycine buffer (pH 8.5), 0.1 ml 99.8% ethanol and 0.5 ml colour reagent. The gels were allowed to stain in a water bath at 37$^{o}$C in the dark for 30-45 min until the bands had reached the desired intensity.

### 3.14.13 Colour reagent

The colour reagent for visualizing dehydrogenase was prepared by dissolving 5 mg nicotinamide adenine dinucleotide (NAD), 0.8 mg phenazine methosulphate (PMS) and 2 mg 3-(4-Iodophenyl) -2- (4-nitrophenyl) -5- phenyl-2H-tetrazolium chloride (INT), in 1 ml Tris-glycine buffer. The reagent was prepared freshly just before use.

### 3.14.14 Staining mixture for MDH

For each gel the staining mixture consisted of 3.7 ml Tris-glycine buffer (pH 8.5),20 mg Na-malate in 0.5 ml Tris-

glycine buffer and 0.3 ml colour reagent.The gels were incubated in a water bath at 37°C in darkness for 45-60 min.

### 3.14.15 Storage of stained gels

The stained gels were washed with distilled water and placed in stoppered test tube in 2% acetic acid. In this solution, the gels remained stable without loss of intensity for several months, if stored in the dark at 4°C.

### 3.14.16 Processing and representation of electrophoretic results

The distance of each zone of protein and enzyme activity on the gel was measured from the starting line (line of application of samples), and on the basis of these measurements,the mobilities of isoenzyme and protein were expressed.

The protein bands and isoenzymes in the investigated samples have been shown schematically. The line of application of samples are always shown on the upper end of the gel. The electrophoretically separated bands have been numbered according to the recommendation of standard committee of enzyme (Webb 1964). In each schematic representation positive and negative poles have been marked by (+) and (-) signs respectively.

## 3.15 Comparative morphological study of white-flowered and lilac-blue flowered cultured water hyacinth

White-flowered water hyacinth was found and collected

only once during this study. It was collected from a pond in Mirpur at the end of the year, 1989.

Comparative study of white-flowered and lilac-blue flowered water hyacinth was carried out in the field of BCSIR Laboratories, Dhaka, and also in Curzon Hall campus in Dhaka University. They were grown in earthen tubs of 58 cm dia, containing only tap water. The measurements of 100 flowers of both were taken with the help of a meter scale. Simple dissection microscope was used for comparing floral parts. Photographs were taken to record the differences.

### 3.15.1 Measurements of pollen grains

Measurements of pollen grains were recorded by Ocular micrometer (X 360).For this study full stained pollen grains were counted.Photomicrographs were taken for recording the differences.

### 3.16 Isolation of total cellular DNA using agarose gel electrophoresis

Principles: With the help of mortar and pestle and using lysis buffer, plant cell wall may be broken, whereby both the nucleic acids (DNA,RNA) and protein are liberated in the solution. The protein is then extracted with phenol, chloroform, isoamyl alcohol, leaving DNA and RNA behind. While precipitating the nucleic acids, chromosomal DNA is spooled out on a glass rod, thus the DNA is separated from RNA.

### 3.16.1 Chemicals used

(i) Lysis buffer: For the preparation of 200 ml lysis buffer the following reagents were used:

| | | |
|---|---|---|
| (a) | Urea | 84 gm |
| (b) | 5 M NaCl | 14 ml |
| (c) | 1 M Tris - Cl (pH 8.0) | 10 ml |
| (d) | 0.5 M EDTA | 8 ml |
| (e) | 20% Lauryl sulphate | 10 ml |
| (f) | Phenol | 10 ml |
| (g) | Distilled water | |
| (ii) | 10% Sodium dodecyl sulphate (SDS) | 20 ml |
| (iii) | TE buffer (10 mM Tris pH 8.0, 1 mM EDTA) | 500 ml |
| (iv) | 3M Potassium acetate | 100 ml |
| (v) | Phenol equilibrated with TE buffer | |
| (vi) | Phenol, chloroform, Isoamyl alcohol mixed together in the ratio of 25:24:1 | |
| (vii) | Ice cold ethanol. | |

### 3.16.2 Preparation of extract

(a) Five gm of young leaves of lilac-blue LPs, SPs and white-flowered form LPs were cut into small pieces and crushed to paste in 20 ml lysis buffer, separately, by mortar and pestle.

(b) Four ml homogenate of each form were poured into 18 separate screw-capped tubes, 20 µl of 10% SDS were added

in each tube, and then all the 18 tubes were thoroughly shaken by hand.

(c) Four ml of phenol, chloroform and isoamyl alcohol mixture were added in each tube,shaken for 10-15 min and then centrifuged at 3500 rpm at room temperature (MSE Angle centrifuge, made in UK).

(d) Phenol, chloroform and isoamyl alcohol with no SDS were added to the aqueous phase and centrifuged. The procedure was repeated twice.

(e) To the final aqueous phase 1/20th vol of 3M potassium acetate and 2 vol of ice cold ethanol were added in the tubes and swirled with a rod, till the chromosomal DNA was obtained. Sometimes DNA was not obtained as threads but as a precipitate. Thread-like DNA was separated out, washed with 80% ethanol, dried and dissolved in TE. If precipitate was obtained, it was centrifuged again for 10 min, pellet dried and then dissolved in 2 ml of 10 mM Tris (pH 8), 1 mM EDTA.

(f) Precipitate was not totally dissolved, hence it was left in a water bath at 37$^{o}$C for about one hr following which the temp.was gradually increased up to 67$^{o}$C until all precipitate got dissolved.

### 3.16.3 Electrophoretic procedure

Separation of chromosomal DNA in the molecular level was carried out by agarose gel electrophoresis,according to

the method recommended by Shure *et al*. (1983). Power supply and electrophoretic set (manufactured by USA) were used for this purpose.

For the polymerization of agarose gel and for the preparation of running buffer solution,TBE buffer (Tris-borate EDTA) was used.

The following were used for the preparation of stock TBE buffer for 100 ml :

| | |
|---|---|
| Tris | 12.1 gm |
| Boric acid | 5.5 gm |
| $Na_2$-EDTA | 0.74 gm |

### 3.16.4 Running buffer

The stock TBE buffer solution was diluted 10 times with distilled water before use.

### 3.16.5 Polymerization of gel

Ninety six gm agarose (0.8%) were taken in a beaker, 120 ml TBE buffer were added to it and then the mixture was heated until dissolved. The solution was cooled to 58$^{o}$C and then poured into the gel chamber. A comb was set up on the upper portion of the gel plate. The gel was then allowed to polymerize at room temp. Within 45 min polymerization was completed. The comb was then taken out of the gel plate, leaving some grooves on the gel.

### 3.16.6 Application of sample

50 µl sample were mixed with 10 µl loading buffer (0.25% bromophenol, 0.25% thylene cyanol, 30% glycerol in water) in an Eppendrof tube. From that, 60 µl extract was applied on top of above mentioned groove. For comparative study, the same amount of sample of lilac —blue flowered LPs, SPs and white-flowered form LPs were applied. In control, same amount of known DNA was used which acted as a marker.

After application of the samples, 900 ml running buffer (TBE buffer) were poured upon the gel, just covering it. The upper reservoir was then replaced on the lower reservoir and the apparatus was connected to the power supply. Electrophoresis was performed at room temp. at 60 volt and 34 mA for eight hr for satisfactory separation of the DNA bands.

### 3.16.7 Pretreatment of gel and detection of DNA

Immediately after electrophoresis, the gel was removed from the gel apparatus and washed with distilled water. The DNA bands were located by treating the gel in appropriate staining mixture.

### 3.16.8 Staining mixture for DNA

The gel was stained with ethidium bromide (10 mg/ml) for 30 min and the DNA bands were detected by direct examination of the gel under UV light, using transilluminator (Chromato-Vue transilluminator,model C-62,made in USA)Photograph was also taken under UV light,using transilluminator.

# EXPERIMENTAL RESULTS

# EXPERIMENTAL RESULTS

The results of the present investigation are described under the following heads.

## 4.1 Ecological study

Both short petioled form (SPs) and long petioled form (LPs) of water hyacinth plants were found in ponds throughout the whole year. Their growth was found maximum for nine months from March to November. Both LPs and SPs were found to grow in shallow water (Figs.1a and 1b) as well as in deep water (Figs.2a and 2b). During the winter months from December to February,when water level falls, their growth is reduced considerably. They also grow in wet muddy land.

### 4.1.1 Survey of water hyacinth in Dhaka city and its suburbs

Results of this study are presented in Table 1. Thirty-one localities were surveyed from March 1986 to February 1987.

In Gulshan area (Table 1) 15 ponds were surveyed : of these,in two there were only SP form,in six only LP form and in the remaining seven a combination of both LPs and SPs were found. In this areas, the percentage of ponds with SP form were 13.33, with LP form 40 and a combination of both LPs + SPs were 46.67. The distribution of forms in the

Fig.1a : Crowded population of long petioled form (LPs) of water hyacinth in shallow water, Mirpur, Dhaka.

Fig.1b : Crowded population of short petioled form (SPs) of water hyacinth in shallow water, Mirpur, Dhaka.

Fig.1a

Fig.1b

Fig.2a : LPs growing in deep water, Gulshan, Dhaka.

Fig.2b : SPs growing in deep water, Bangla College, Dhaka.

Fig.2a

Fig.2b

remaining thirty areas is shown in Table 1.

A total of 277 ponds were surveyed in thirty one areas. Out of these, in 79 there were only SP form, in 123 only LP form and in the remaining 75, a combination of both LPs and SPs. The percentage of ponds with SPs was 34.80, LPs 54.19 and a combination of both LPs and SPs were 33.04. In this study LPs were found to be more abundant and frequency of SPs and the combination of both LP and SP forms were more or less the same.Practically no change in the relative frequency of the two forms and their combination was observed during the year under observation.

Table 1. Survey of availability of water hyacinth in Dhaka City and its suburbs

| Period of survey | Area surveyed | No. of ponds studied | No. of ponds with short petioled form (SPs) | No. of ponds with long petioled form (LPs) | No. of ponds with a combination of both forms(LPs and SPs) | Percentage of ponds with different forms (LPs and SPs) |
|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1986 | | | | | | |
| March - April | Gulshan | 15 | 2 | 6 | 7 | SPs = 13.33<br>LPs = 40<br>LPs + SPs = 46.67 |
| May - June | Shajahanpur | 15 | 3 | 7 | 5 | SPs = 20<br>LPs = 46.67<br>LPs + SPs = 33.33 |
| July - August | Mirpur | 96 | 30 | 45 | 21 | SPs = 31.25<br>LPs = 46.88<br>LPs + SPs = 21.87 |
| August | Tongi | 5 | - | 3 | 2 | SPs = 0<br>LPs = 60<br>LPs + SPs = 40 |
| August - September | Savar | 34 | 5 | 20 | 9 | SPs = 14.71<br>LPs = 58.82<br>LPs + SPs = 26.47 |
| September | Joydebpur | 3 | - | 1 | 2 | SPs = 0<br>LPs = 33<br>LPs + SPs = 67 |

Table 1 (contd.)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| October | Narayangonj | 5 | – | 2 | 3 | SPs = 0<br>LPs = 40<br>LPs + SPs = 60 |
| October | Demra | 5 | – | 2 | 3 | SPs = 0<br>LPs = 40<br>LPs + SPs = 60 |
| October | Keraniganj | 5 | 1 | 1 | 3 | SPs = 20<br>LPs = 20<br>LPs +SPs = 60 |
| October | Zinjira | 5 | – | 2 | 3 | SPs = 0<br>LPs = 40<br>LPs + SPs = 60 |
| October | Kamrangirchar | 5 | – | 2 | 3 | SPs = 0<br>LPs = 40<br>LPs + SPs = 60 |
| October | Nabinagar | 5 | – | 2 | 3 | SPs = 0<br>LPs = 40<br>LPs +SPs = 60 |
| November | Manikganj | 5 | 1 | 2 | 2 | SPs = 20<br>LPs = 40<br>LPs + SPs = 40 |
| November | Dhamrai | 5 | 2 | 1 | 2 | SPs = 40<br>LPs = 20<br>LPs +SPs = 40 |

Table 1 (contd.)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| November | Nayerhat | 5 | 3 | 2 | – | SPs = 60<br>LPs = 40<br>LPs + SPs = 0 |
| November | Utholy | 5 | 3 | 2 | – | SPs = 60<br>LPs = 40<br>LPs + SPs = 0 |
| November | Kalampur | 5 | – | 5 | – | SPs = 0<br>LPs = 100<br>LPs + SPs = 0 |
| November | Aricha | 5 | 2 | 3 | – | SPs = 40<br>LPs = 60<br>LPs + SPs = 0 |
| December | Tejgaon | 5 | 4 | 1 | – | SPs = 80<br>LPs = 20<br>LPs + SPs = 0 |
| December | Kaliakur | 3 | 1 | 1 | 1 | SPs = 33.33<br>LPs = 33.33<br>LPs + SPs = 33.33 |
| December | Dhaka Cantonment Area | 5 | 3 | 2 | – | SPs = 60<br>LPs = 40<br>LPs + SPs = 0 |
| December | Rajendrapur | 3 | 1 | 2 | – | SPs = 33.33<br>LPs = 66.67<br>LPs + SPs = 0 |

Table 1 (contd.)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| December | Sripur | 3 | 1 | 1 | 1 | SPs = 33.33<br>LPs = 33.33<br>LPs +SPs = 33.33 |
| December | Vowal Rajargar | 3 | 2 | 1 | - | SPs = 66.67<br>LPs = 33.33<br>LPs + SPs = 0 |
| 1987 | | | | | | |
| January | Lalbag | 3 | 2 | 1 | - | SPs = 66.67<br>LPs = 33.33<br>LPs +SPs = 0 |
| January | Satkhamair | 3 | 3 | - | - | SPs = 100<br>LPs = 0<br>LPs + SPs = 0 |
| January | Kaoraidth | 3 | 2 | - | 1 | SPs = 66.67<br>LPs = 0<br>LPs + SPs = 33.33 |
| February | Sutrapur thana Area | 5 | 3 | - | 2 | SPs = 60<br>LPs = 0<br>LPs + SPs = 40 |
| February | Pubail | 4 | 3 | 1 | - | SPs = 75<br>LPs = 25<br>LPs + SPs = 0 |
| February | Arikhhola | 4 | 1 | 2 | 1 | SPs = 25<br>LPs = 50<br>LPs + SPs = 25 |
| February | Kapasia | 5 | 1 | 3 | 1 | SPs = 20<br>LPs = 60<br>LPs + SPs = 20 |

### 4.1.2 Kind of water hyacinth (LPs and SPs) in ten selected ponds

The monthly observation record of ten selected ponds containing both forms of water hyacinth throughout the year are presented in Tables 2 and 3. In January, pond numbers 1, 5,6,7,9, and 10 showed a combination of both LP and SP forms while in pond numbers 2,3,4 only LPs were recorded with none in pond no. 8 (Harvest of by public may have been the cause of their absence).The percentage of ponds with LPs were 33.33 and that with a combination of both LPs and SPs were 66.67. In February, in pond nos. 1,2,3 only LPs were found while in pond nos. 4,6,7,9,10 there was a combination of both LPs and SPs, pond no. 5 contained SPs and in pond no. 8 there was no water hyacinth plant. The percentage of ponds with SPs were 11.11, LPs 33.33 and a combination of both LPs and SPs were 55.56. Rest of the data are shown in Tables 2 and 3.

The average monthly percentage of ponds with SPs were 5.27 with LPs 47.41 and a combination of both LPs and SPs were 47.32 (Table 3). The location of the ponds are mentioned in Table 2.

Table 2. Kind of water hyacinth (SPs and LPs) in ten selected ponds (1987).

| Months | Pond no.1 | Pond no.2 | Pond no.3 | Pond no.4 | Pond no.5 | Pond no.6 | Pond no.7 | Pond no.8 | Pond no.9 | Pond no.10 |
|---|---|---|---|---|---|---|---|---|---|---|
| January | LPs+SPs | LPs | LPs | LPs | LPs+SPs | LPs+SPs | LPs+SPs | 0 | LPs+SPs | LPs+SPs |
| February | LPs | LPs | LPs | LPs+SPs | SPs | LPs+SPs | LPs+SPs | 0 | LPs+SPs | LPs+SPs |
| March | LPs | LPs | LPs+SPs | LPs+SPs | LPs+SPs | SPs | LPs+SPs | LPs+SPs | LPs+SPs | LPs+SPs |
| April | LPs+SPs | LPs | LPs+SPs | LPs | LPs+SPs | SPs | LPs+SPs | LPs | LPs | LPs |
| May | LPs | LPs | LPs | LPs | LPs+SPs | LPs+SPs | LPs+SPs | LPs | LPs | LPs |
| June | LPs | LPs | LPs | LPs | LPs+SPs | LPs+SPs | LPs+SPs | LPs | LPs | LPs |
| July | LPs | LPs | LPs | LPs | LPs+SPs | LPs+SPs | LPs+SPs | LPs | LPs | LPs |
| August | LPs | LPs | LPs | LPs | LPs+SPs | LPs+SPs | LPs+SPs | LPs+SPs | LPs+SPs | LPs |
| September | LPs | LPs | LPs | LPs | LPs+SPs | LPs+SPs | LPs+SPs | LPs+SPs | LPs+SPs | LPs |
| October | LPs | LPs | LPs | LPs | LPs+SPs | LPs+SPs | 0 | LPs | LPs+SPs | LPs |
| November | LPs | LPs | LPs+SPs | LPs | LPs+SPs | LPs+SPs | 0 | LPs+SPs | LPs+SPs | LPs+SPs |
| December | LPs+SPs | LPs | LPs+SPs | LPs | SPs | SPs | LPs+SPs | 0 | LPs+SPs | LPs+SPs |

The location of ponds indicated by number (Table 2) was around : (1) Sundarban Hotel, (2) Kathal Bagan, (3) Mohakhali, (4) Mohakhali Pathological Hospital, (5) Budda Jhill, (6) New Airport, (7) Bangla College (8) Road Research, (9) Jikatolla No.15 Staff Quarters and (10) Moghbazar Wireless Area.

Table 3. Percentage of water hyacinth (LPs and SPs) ae recorded in ten selected ponds in 1987.

| Observation period | No.of ponds surveyed | Percentage of ponds with different forms of water hyacinth | | |
|---|---|---|---|---|
| January | 10 | SPs | = | 0 |
| | | LPs | = | 33.33 |
| | | LPs+SPs | = | 66.67 |
| February | 10 | SPs | = | 11.11 |
| | | LPs | = | 33.33 |
| | | LPs+SPs | = | 55.56 |
| March | 10 | SPs | = | 10 |
| | | LPs | = | 20 |
| | | LPs+SPs | = | 70 |
| April | 10 | SPs | = | 10 |
| | | LPs | = | 50 |
| | | LPs+SPs | = | 40 |
| May | 10 | SPs | = | 10 |
| | | LPs | = | 70 |
| | | LPs+SPs | = | 20 |
| June | 10 | SPs | = | 0 |
| | | LPs | = | 70 |
| | | LPs+SPs | = | 30 |
| July | 10 | SPs | = | 0 |
| | | LPs | = | 70 |
| | | LPs+SPs | = | 30 |
| August | 10 | SPs | = | 0 |
| | | LPs | = | 50 |
| | | LPs+SPs | = | 50 |
| September | 10 | SPs | = | 0 |
| | | LPs | = | 50 |
| | | LPs+SPs | = | 50 |
| October | 10 | SPs | = | 0 |
| | | LPs | = | 66.67 |
| | | LPs+SPs | = | 33.33 |
| November | 10 | SPs | = | 0 |
| | | LPs | = | 33.33 |
| | | LPs+SPs | = | 66.67 |
| December | 10 | SPs | = | 22.22 |
| | | LPs | = | 22.22 |
| | | LPs+SPs | = | 55.56 |

The average monthly percentage of different forms water hyacinth in various ponds: SPs = 5.27;
LPs = 47.41;
LPs+SPs = 47.32.

## 4.2 Morphological differences between LPs and SPs

Comparative morphological studies of different forms of water hyacinth (LPs and SPs), collected from various ponds, revealed striking differences in the size of their vegetative parts, number of flowers per inflorescence and also in the shape of their petioles. The LPs were characterised by long slender petioles while SPs by short swollen petioles.

The measurements of different vegetative parts (Figs.3 to 5) have been presented in Table 4 and those of floral parts (Figs.6 to 8) in Table 5.In Table 6 the leaf and flower numbers have been given.

The figures following means of various values represent standard errors in Tables.

Table 4. Comparative values of vegetative parts of LPs and SPs. a : Plant heights

| Form | Plant height (cm) | Mean | 't' value |
|---|---|---|---|
| Natural LPs | 28.0 - 109.0 | 78.20 ± 5.38 | 11.54** |
| Natural SPs | 5.0 - 30.0 | 13.34 ± 1.62 | |
| Cultured LPs | 20.0 - 75.0 | 44.74 ± 3.98 | 6.81** |
| Cultured SPs | 5.0 - 30.0 | 15.41 ± 1.66 | |

Note : ** represents significance at 1% level.

Fig.3a : Comparative plant height and petiole length of LPs (right) and SPs (left), collected from natural ponds. X 0.11

Fig.3b : Comparative plant height and petiole length of LPs (right) and SPs (left), collected from laboratory culture. X 0.11

Fig.3a

Fig.3b

Significant differences at 1% level were observed between LPs and SPs in respect of their average height under both natural and cultured conditions.

Table 4 b. Size of petioles.

| Form | Size of petioles (cm) | | Petiole length | Petiole dia | Obs 't' value for length | Obs 't' value for dia |
|---|---|---|---|---|---|---|
| | Length | Dia | Mean | Mean | | |
| Natural LPs | 11.5-81.5 | 0.5-2.9 | 60.56± 4.70 | 1.43±0.13 | 10.65** | 6.70** |
| Natural SPs | 3.4-22.0 | 1.7-5.0 | 8.51± 1.07 | 2.76±0.13 | | |
| Cultured LPs | 11.0-65.0 | 0.5-2.9 | 38.58± 3.76 | 1.47±0.14 | 7.24** | 3.67** |
| Cultured SPs | 3.4-19.5 | 1.0-4.0 | 10.27± 1.08 | 2.35±0.19 | | |

Significant differences at 1% level were observed between LPs and SPs in respect of their average petiole length and dia under both natural and cultured conditions.

Table 4 c. Size of leaf blades

| Form | Size of leaf blades (cm) | | Leaf length | Leaf breadth | Obs 't' value for length | Obs 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | Mean | Mean | | |
| Natural LPs | 7.0-22.7 | 5.9-20.5 | 16.61± 0.09 | 16.07±0.93 | 9.92** | 7.63** |
| Natural SPs | 3.0-10.5 | 2.0-12.2 | 5.63± 0.52 | 7.66±0.59 | | |
| Cultured LPs | 6.0-18.0 | 5.2-15.0 | 11.86± 0.76 | 10.22±0.74 | 6.64** | 3.39** |
| Cultured SPs | 2.6-9.5 | 2.0-10.5 | 5.98± 0.45 | 6.99±0.61 | | |

Significant differences at 1% level were observed between LPs and SPs in respect of their average leaf blade length and breadth under both natural and cultured conditions.

Fig.4a : Comparative size of leafblade of LPs (left) and SPs (right) collected from natural ponds. X 0.23

Fig.4b : Comparative size of leafblade of LPs (left) and SPs (right) collected from laboratory culture. X 0.23

Fig.4a

Fig.4b

Table 4 d. Size of roots

| Form | Size of root (cm) | | root lengths | root dia | Obs 't' value for length | Obs 't' value for dia |
|---|---|---|---|---|---|---|
| | Length | Dia | Mean | Mean | | |
| Natural LPs | 5.0-53.0 | 0.1- 0.3 | 25.25± 3.51 | 0.17±0.02 | 3.39** | 1.56 |
| Natural SPs | 5.0-30.5 | 0.1- 0.2 | 11.58± 1.98 | 0.14±0.06 | | |
| Cultured LPs | 5.0-53.0 | 0.1-0.3 | 22.55± 3.35 | 0.17±0.02 | 2.37* | 1.56 |
| Cultured SPs | 2.1-35.0 | 0.1-0.2 | 13.44± 1.90 | 0.14±0.06 | | |

Note : * and ** represents significance at 5% level and 1% level respectively.

Significant differences at 1% level were observed between LPs and SPs in respect of their average root length in natural conditions and at 5% level in cultured conditions but there were no differences between two forms in respect of their average root dia.

Table 5. Comparative measurements of inflorescence and floral parts of LPs and SPs. a : Length of inflorescence.

| Form | Length of inf (cm) | Mean | Obs 't' value |
|---|---|---|---|
| Natural LPs | 24.0 - 36.5 | 29.78 ± 0.89 | 7.90** |
| Natural SPs | 11.0 - 25.0 | 19.71 ± 0.91 | |
| Cultured LPs | 23.0 - 36.0 | 29.58 ± 3.81 | 6.79** |
| Cultured SPs | 17.0 - 25.5 | 22.30 ± 0.55 | |

Significant differences at 1% level were observed between LPs and SPs in respect of their average inflorescence length under both natural and cultured conditions.

Fig.5a : Comparative size of roots of LPs (right) and SPs (left) collected from natural ponds. X 0.20

Fig.5b : Comparative size of roots of LPs (left) and SPs (right) collected from Laboratory culture. X 0.20

Fig.5a

Fig.5b

Fig.6a : Comparative length of inflorescence of SPs (left) and LPs (right) collected from natural pond. X 0.30

Fig.6b : Comparative length of inflorescence of SPs (left) and LPs (right) collected from laboratory culture. X 0.30

Fig.6a

Fig.6b

Table 5 b. Size of flowers.

| Form | Size of flower (cm) | | Flower length | Flower breadth | Obs 't' value for length | Obs 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | Mean | Mean | | |
| Natural LPs | 4.5-6.5 | 4.0-5.2 | 5.70± 0.12 | 4.64±0.11 | 4.91** | 4.60** |
| Natural SPs | 4.2-5.7 | 3.5-4.6 | 4.93± 0.09 | 4.01±0.08 | | |
| Cultured LPs | 4.5-6.5 | 4.0-5.2 | 5.58± 0.14 | 4.57±0.10 | 2.69* | 3.83** |
| Cultured SPs | 4.2-5.9 | 3.3-4.6 | 5.1 ± 0.11 | 4.05±0.09 | | |

Length of flowers included the pedicel.

Significant differences at 1% level were observed between LPs and SPs in respect of their average flower breadth under both natural and cultured conditions.Significant differences in respect of their average flower length were also observed in LPs and SPs at 1% level in natural and at 5% level in cultured conditions.

Table 5 c. Size of posterior lobes.

| Form | Size of posterior lobes (cm) | | Length of post.lobes | Breadth of post.lobes | Obs 't' value for length | Obs 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | Mean | Mean | | |
| Natural LPs | 3.0-4.9 | 1.5-2.6 | 4.27± 0.13 | 2.16±0.03 | 2.22* | 1.31 |
| Natural SPs | 2.7-4.6 | 1.4-2.6 | 3.85± 0.14 | 2.02±0.08 | | |
| Cultured LPs | 3.0-4.9 | 1.4-2.7 | 4.08± 0.13 | 2.14±0.08 | 2.15* | 1.37 |
| Cultured SPs | 2.7-4.7 | 1.3-2.6 | 3.67± 0.13 | 1.97±0.09 | | |

Fig.7a : Comparative size of flowers of SPs (left) and LPs (right) collected from natural ponds.

Fig.7b : Comparative size of flowers of SPs (left) and LPs (right) collected from laboratory culture.

Fig.7a

Fig.7b

Significant differences were observed at 5% level in respect of average length of posterior lobes of LPs and SPs, both under natural and cultured conditions, but differences of average breadth of posterior lobe were statistically insignificant under both conditions.

Table 5 d. Size of three outer lobes.

| Form | Size of three outer lobes (cm) | | Length of three outer lobes Mean | Breadth of three outer lobes Mean | Obs 't' value for length | Obs 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| Natural LPs | 2.6-4.6 | 1.2-2.1 | 3.59± 0.14 | 1.62±0.07 | 2.33* | 1.45 |
| Natural SPs | 2.3-4.0 | 1.1-2.0 | 3.16± 0.12 | 1.35±0.06 | | |
| Cultured LPs | 2.4-4.4 | 1.2-2.1 | 3.59± 0.13 | 1.64±0.07 | 3.14** | 1.43 |
| Cultured SPs | 2.3-4.0 | 1.1-2.0 | 3.02± 0.12 | 1.50±0.07 | | |

Significant differences between LPs and SPs were observed at 5% level in respect of average length of three outer lobes under natural conditions and at 1% level under cultured conditions. In average breadth of the three outer lobes the differences were statistically insignificant under both natural and cultured conditions.

Fig.8a : Comparative view of floral parts of LPs (left) and SPs (right) collected from natural ponds.

Fig.8b : Comparative view of floral parts of SPs (left) and LPs (right) collected from laboratory culture.

Fig.8a

Fig.8b

Table 5 e. Size of three inner lobes.

| Form | Size of three inner lobes (cm) | | Length of three inner lobes Mean | Breadth of three inner lobes Mean | Obs 't' value for length | Obs 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| Natural LPs | 3.0-4.9 | 1.5-2.6 | 4.1 ± 0.11 | 2.01±0.08 | 2.37* | 0.38 |
| Natural SPs | 2.5-4.6 | 1.3-2.6 | 3.7 ± 0.15 | 1.96±0.09 | | |
| Cultured LPs | 2.7-4.9 | 1.5-2.6 | 3.99± 0.16 | 2.02±0.09 | 2.15* | 0.66 |
| Cultured SPs | 2.4-4.6 | 1.3-2.6 | 3.53± 0.14 | 1.94±0.09 | | |

Significant differences were observed at 5% level between LPs and SPs in respect of their average length of three inner lobes under both natural and cultured conditions, but average breadth of three inner lobes were statistically insignificant under both natural and cultured conditions.

Table 5 f. Size of the yellow blotch .

| Form | Size of yellow blotch (cm) | | Length of yellow blotch Mean | Breadth of yellow blotch Mean | Obs 't' value for length | Obs 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| Natural LPs | 0.4-1.1 | 0.3-0.9 | 0.85± 0.04 | 0.51±0.04 | 1.96 | 1.85 |
| Natural SPs | 0.4-1.0 | 0.3-0.7 | 0.73± 0.04 | 0.42±0.03 | | |
| Cultured LPs | 0.4-1.3 | 0.3-0.9 | 0.82± 0.05 | 0.53±0.04 | 1.89 | 1.88 |
| Cultured SPs | 0.4-1.0 | 0.2-0.7 | 0.69± 0.04 | 0.43±0.03 | | |

Differences between LPs and SPs in respect of their average length and breadth of yellow blotch were insignificant under both natural and cultured conditions.

Table 5 g. Length of three posterior large stamens.

| Form | Length of 3 posterior large stamens (cm) | Mean | Obs 't' value |
|---|---|---|---|
| Natural LPs | 1.8 - 2.6 | 2.29 ± 0.05 | 1.61 |
| Natural SPs | 1.6 - 2.5 | 2.17 ± 0.06 | |
| Cultured LPs | 1.6 - 2.7 | 2.26 ± 0.06 | 1.39 |
| Cultured SPs | 1.5 - 2.5 | 2.13 ± 0.06 | |

Differences between LPs and SPs in respect of average length of their three posterior large stamens were statistically insignificant.

Table 5 h. Length of three anteterior small stamens.

| Form | Length of three anterior small stamens (cm) | Mean | Obs 't' value |
|---|---|---|---|
| Natural LPs | 0.4 - 1.0 | 0.63 ± 0.04 | 0.19 |
| Natural SPs | 0.4 - 0.8 | 0.64 ± 0.03 | |
| Cultured LPs | 0.4 - 1.0 | 0.72 ± 0.04 | 1.41 |
| Cultured SPs | 0.4 - 0.8 | 0.64 ± 0.04 | |

Differences between LPs and SPs in respect of average length of their three anterior small stamens were statistically insignificant.

Table 5 i. Length of style.

| Form | Length of styles (cm) | Mean | Obs 't' value |
|---|---|---|---|
| Natural LPs | 2.4 - 2.6 | 2.51 ± 0.02 | 4.61** |
| Natural SPs | 2.2 - 2.5 | 2.39 ± 0.02 | |
| Cultured LPs | 2.3 - 2.6 | 2.40 ± 0.02 | 2.56* |
| Cultured SPs | 2.1 - 2.5 | 2.30 ± 0.03 | |

Differences between LPs and SPs in respect of average length of their style were statistically significant at 1% level under natural conditions and at 5% level under cultured conditions.

Table 5 j. Size of Ovary.

| Form | Size of Ovary (cm) | | Length of ovary | Breadth of ovary | Obs 't' value for length | Obs 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | Mean | Mean | | |
| Natural LPs | 0.5-0.9 | 0.2-0.3 | 0.75± 0.03 | 0.25±0.01 | 3.94** | 3.10** |
| Natural SPs | 0.5-0.8 | 0.1-0.3 | 0.61± 0.02 | 0.05±0.01 | | |
| Cultured LPs | 0.5-0.9 | 0.2-0.3 | 0.73± 0.03 | 0.24±0.01 | 2.14* | 2.08* |
| Cultured SPs | 0.5-0.08 | 0.1-0.3 | 0.65± 0.02 | 0.21±0.02 | | |

Significant differences at 1% level were observed between LPs and SPs in respect of average length and breadth of their ovary under natural conditions and at 5% level under cultured conditions.

Table 6. Number of leaves and flowers of LPs and SPs.
a : Number of leaves per plant.

| Form | No. of leaves/plant | Mean | Obs 't' value |
|---|---|---|---|
| Natural LPs | 4 - 15 | 9.95 ± 0.73 | 0.76 |
| Natural SPs | 4 - 12 | 9.25 ± 0.57 | |
| Cultured LPs | 5 - 15 | 9.60 ± 0.69 | 0.66 |
| Cultured SPs | 5 - 13 | 9.00 ± 0.59 | |

The differences between LPs and SPs in respect of average number of leaves were statistically insignificant.

Table 6 b. Number of flowers per inflorescence .

| Form | No. of flowers/inf | Mean | Obs 't' value |
|---|---|---|---|
| Natural LPs | 7 - 40 | 20.40 ± 2.53 | 5.06** |
| Natural SPs | 2 - 14 | 6.95 ± 0.81 | |
| Cultured LPs | 6 - 27 | 15.60 ± 1.41 | 5.07** |
| Cultured SPs | 2 - 14 | 7.76 ± 0.77 | |

Significant differences at 1% level were observed between LPs and SPs in respect of average number of flowers in an inflorescence.

### 4.3 Study of pollen grains of LPs and SPs

The pollen grains were variable in shape, oval and round (Figs.9 a to d) in both LPs and SPs. Round pollen grains were found to be less in number in comparison to the oval ones in both the forms. The stainability was also found to be more or less same, in 1500 pollen grains studied, 91.2% in SPs and 91.3% in LPs (Table 7 a).

#### 4.3.1 Size of oval pollen grains

The average length and breadth of the oval pollen grains were more in SPs than in LPs. The differences were significant at 5% and 1% level respectively (Table 7 b).

#### 4.3.2 Size of round pollen grains

The average dia of round pollen grains was more in SPs in comparison to LPs. The differences were significant at 1% level (Table 7 c).

Table 7. Pollen characteristics of LPs and SPs. a: Pollen stainability of two forms of water hyacinth SPs and LPs.

| Form | Total no. of pollens studied | Total no. of stained pollens | Total no.of non-stained pollens | Percentage of stained pollens | Percentage of non-stained pollens |
|---|---|---|---|---|---|
| SPs | 1500 | 1368 | 132 | 91.2 | 8.8 |
| LPs | 1500 | 1370 | 130 | 91.3 | 8.7 |

Fig.9a : Photomicrographs showing the oval pollen grains of LPs. X 165

Fig.9b : Photomicrographs showing the oval pollen grains of SPs. X 165

Fig.9a

Fig.9b

Fig.9c : Photomicrographs showing the round and oval pollen grains of LPs. X 165

Fig.9d : Photomicrographs showing the round and oval pollen grains of SPs. X 165

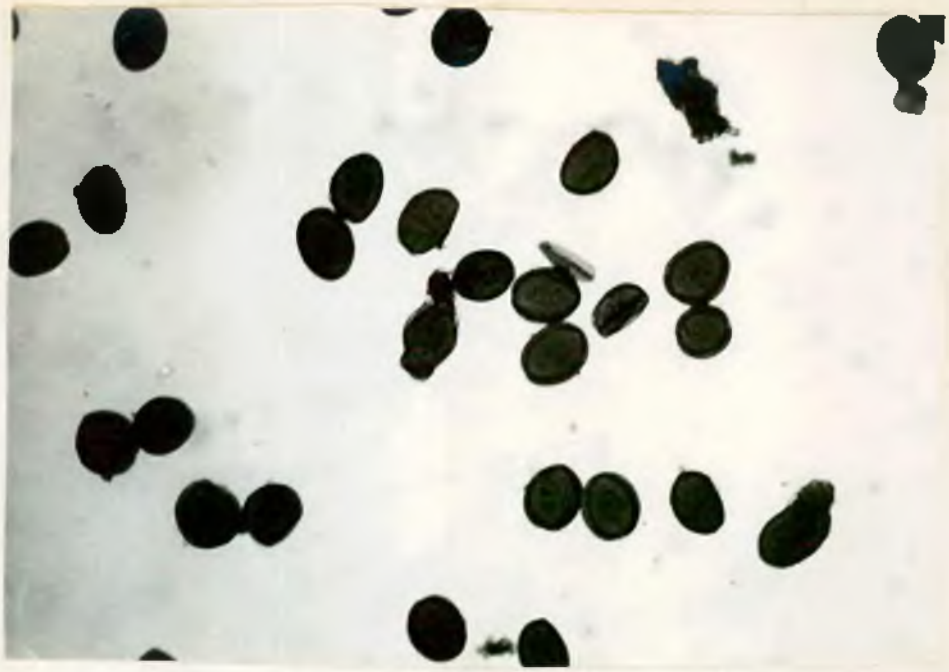


Fig.9c

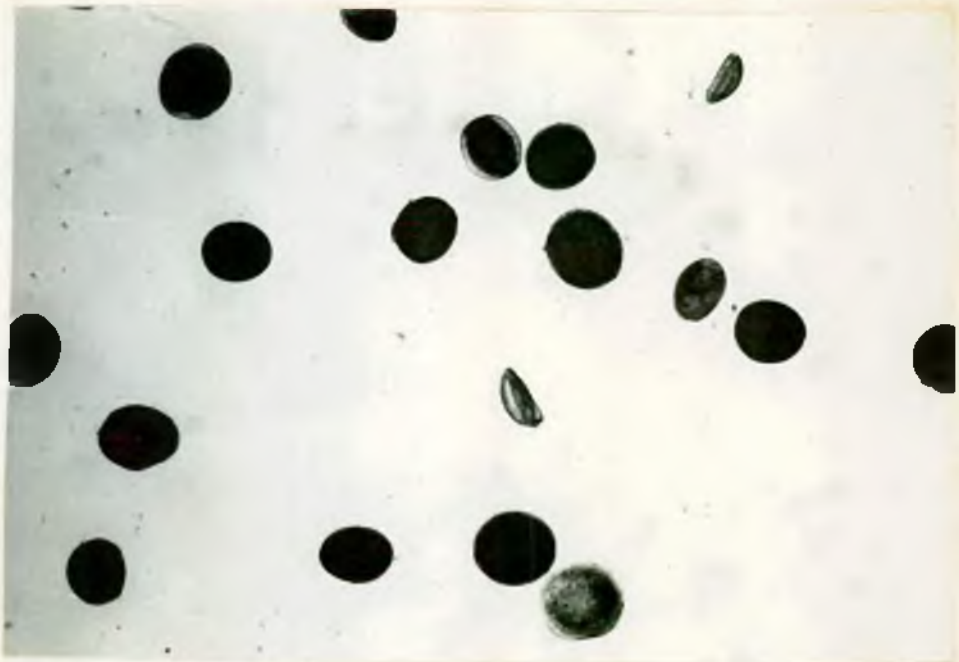


Fig.9d

Table 7 b. Size of Oval pollen grains.

| Form | Size of Oval pollen grains (μm) | | length | breadth | Obs. 't' value for length | Obs 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | Mean | Mean | | |
| LPs | 40.0-85.0 | 37.0 -73.0 | 65.27 ± 2.95 | 50.16 ± 2.34 | 2.43* | 4.55** |
| SPs | 48.0-96.0 | 31.85-88.5 | 75.22 ± 2.84 | 66.78 ± 2.80 | | |

Table 7 c. Size of round pollen grains.

| Form | Diameter of round pollen grains (μm) | Mean | Observed 't' value |
|---|---|---|---|
| LPs | 40.0 - 65.0 | 57.49 ± 1.31 | 4.09** |
| SPs | 52.0 - 77.4 | 66.86 ± 1.88 | |

## 4.4 Growth Characteristics of LPs and SPs.

### 4.4.1 Comparative study of the growth of two forms of water hyacinth, in tap water and in 1% Hoagland solution

Experiments were designed to study the performance and growth characteristics of LPs and SPs in tap water and also in 1% Hoagland solution (Appendix I), in the laboratory. Five plants of LPs and five plants of SPs were grown in large earthen tubs separately, containing tap water or 1% Hoagland solution. Both in the 1% Hoagland solution and tap water the SPs gave rise to SPs only (Fig.10 a) during winter to autumn (December to August) and LPs produced only LPs (Fig.10 b) during late spring to autumn (April to November). In natural

Fig.10a : Showing the young SPs along with the parental SPs. X 0.54

Fig.10b : Showing the young LPs along with the parental LPs. X 0.09

Fig.10a

Fig.10b

ponds also same type of result was observed in both LPs and SPs (Figs.11 a and 11 b).At the end of autumn an interchange of forms was noticed. Both in tap water and 1% Hoagland solution, SPs gave rise to 25% LPs (Fig.12 a) and 75% SPs (Fig.12 b)at the end of September and during the period from October to November the proportion of LPs derived from SPs increased to 75% while that of SPs was reduced to 25%. The height of the LPs, derived from cultured SPs, was considerably reduced i.e., 11 to 30 cm (Fig.12 a), while the height of naturally occuring LPs was 28 to 109 cm (Fig.11 a). The length of the petioles of the former ranged from 6 to 19.5 cm as against 11.5 to 81.5 cm in natural LPs. The height of the 25% SPs, that originated during October - November, in the culture solution,ranged from 6 to 10 cm and length of their petioles ranged from 4 to 6.5 cm (Fig.12 b). During this experiment both SPs and LPs produced flowers (Figs.10 c and 10 d).

In the cultured LPs it was found that LPs gave rise to 80% SPs and 20% LPs (Fig.12 c) from winter through spring (December to March). LPs were again found to produce only LPs (Fig.12 d) from late spring through autumn (April to November).

In other words interchange of forms took place for three months in SPs and four months in LPs while for the remaining period (nine months in SPs and eight months in LPs) SPs and LPs remained true to their forms.

Fig.10c : SPs growing in cultured tub.

Fig.10d : LPs growing in cultured tub.

Fig.10c

Fig.10d

Fig.11a : Young LPs (left) and mature LPs (right) collected from natural ponds. X 0.09

Fig.11b : Young SPs (left) and mature SPs (right) collected from natural ponds. X 0.28

Fig.11a

Fig.11b

Fig.12a : LPs obtained from SPs in cultured condition in late Autumn (Length did not exceed 30 cm.) X 0.20

Fig.12b : SPs obtained from SPs in cultured condition during winter to autumn. X 0.54

Fig.12a

Fig.12b

Fig.12c : SPs and LPs obtained from LPs in cultured condition during winter to spring. X 0.28

Fig.12d : LPs obtained from LPs in cultured condition during late spring to autumn. X 0.11

Fig.12c

Fig.12d

### 4.4.2 Effect of different concentrations of Hoagland solution on the height of SPs

Five SP plants were placed in small earthen tubs containing different concentration of Hoagland solution (1, 5 and 10%).The control tubs had only distilled water. During the six months of observation,the height of the experimental plants was not affected by the concentration of the nutrients (Table 8 a).The height and petiole length of SPs were almost the same regardless of the concentration of Hoagland solution. Even in the control i.e. in distilled water, these two characters of SPs remained unaffected (Table 8 a; Fig.13). The results were analysed statistically for 'F' values (Tables 8 b and 8 c). The observed values were less than tabular values at 5% level, indicating that the differences were insignificant in respect of both the characters (plant height and length of petiole).

Table 8 a. Effect of different concentrations of Hoagland solution on height of six months old SPs (The values represent range of 25 readings).

| Concentration in % | Plant height (cm) | Length of petiole (cm) |
|---|---|---|
| 1 | 11.0 - 24.5 | 7.0 - 15.7 |
| 5 | 9.0 - 26.0 | 5.0 - 16.5 |
| 10 | 11.5 - 30.0 | 6.5 - 19.5 |
| 0 (distilled water) | 11.0 - 29.0 | 6.0 - 18.5 |

Fig.13 : Effect of different conc. of Hoagland solution on cultured SPs showing same response. X 0.11
(i) 1%, (ii) 5%, (iii) 10% and (iv) control.

I II III IV

Fig.13

Table 8 b. AOV for plant height of water hyacinth cultured in different concentration of Hoagland solution.

| Source | df | ss | ms | F |
|---|---|---|---|---|
| Plant height | 3 | 84.366 | 28.122 | 1.297 |
| Error | 96 | 2081.696 | 21.684 | |
| Total | 99 | 2166.062 | | |

Table 8 c. AOV for length of the petiole of water hyacinth cultured in different concentration of Hoagland solution.

| Source | df | ss | ms | F |
|---|---|---|---|---|
| Length of petiole | 3 | 24.222 | 8.074 | 0.770 |
| Error | 96 | 1005.885 | 10.478 | |
| Total | 99 | 1030.107 | | |

The results were analysed statistically for 'F' values. The observed values less than tabular value at 5% level indicating that differences were insignificant in respect of both characters.

### 4.4.3 Propagation of daughter plants from LPs and SPs

Water hyacinth reproduces vegetatively by means of stolons and rhizomes. Experiments were conducted with both rhizomes and stolons. Stolons were used in all the experiments except in no. 3.7.4 where the material consisted of only rhizomes.

#### 4.4.3.1 Propagation of daughter plants from stolons of LPs and SPs

Propagation of daughter plants from LPs was observed within 9.3 ± 0.48 days and in SPs within 14.55 ± 1.28 days (Table 9 a). Significant differences at 1% level were observed between LPs and SPs in respect of their average days of shoot or daughter plants emergence.

#### 4.4.3.2 Emergence of root from daughter plants of LPs and SPs

Roots emerged from the daughter plants of LPs within 5.95 ± 0.35 days and from SPs within 2.90 ± 0.14 days (Table 9 b). The differences were significant at 1% level.

#### 4.4.3.3 Emergence of daughter plants from rhizomes of both LPs and SPs in tap water and in 5% Hoagland solution

When rhizomes of LPs and SPs were planted in tap water/ 5% Hoagland solutions in earthen tubs, 66.67% of SP rhizomes produced SP form in tap water, and in 5% Hoagland solutions (Appendix I) only 38.89% rhizomes gave rise to SP form. The rhizomes from LPs did not produce any plant in tap water but in Hoagland solution LP rhizomes formed 11.11% LP form (Table 9 c; Figs.14 a to d) of plants.

#### 4.4.3.4 Longevity of LPs and SPs

Longevity of LPs and SPs was compared in the laboratory in earthen tubs containing only tap water. The study was conducted for one year. Longevity of LPs was found to be

Fig.14a : SP rhizomes producing SP daughter plants in tap water. X 0.50

Fig.14b : SP rhizome producing SP daughter plants in 5% Hoagland solution. X 0.40

Fig. 14a

Fig. 14b

Fig.14c : LP rhizome producing LP daughter plants in 5% Hoagland solution. X 0.26

Fig.14d : LP rhizome producing both LP and SP daughter plants in 5% Hoagland solution. X 0.57

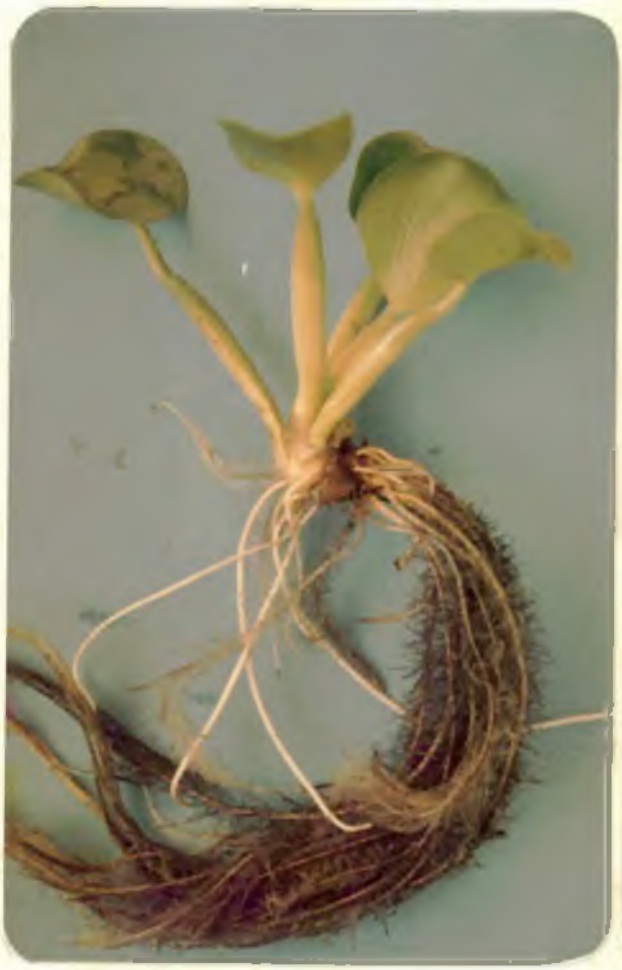


Fig.14c

Fig.14d

restricted within 57.30 ± 4.47 days and SPs within 48.95 ± 3.77 days. The differences are statistically insignificant (Table 9 d).

Table 9. Propagation of LPs and SPs. a : Propagation of daughter plants from stolons of LPs and SPs.

| Form | Propagation (in days) | Mean | Observed 't' value |
|---|---|---|---|
| LPs | 5 - 14 | 9.3 ± 0.48 | 3.84** |
| SPs | 6 - 26 | 14.55 ± 1.28 | |

Table 9 b. Emergence of roots from daughter plants of LPs and SPs.

| Form | Emergence of roots (in days) | Mean | Observed 't' value |
|---|---|---|---|
| LPs | 4 - 9 | 5.95 ± 0.35 | 8.04** |
| SPs | 2 - 4 | 2.90 ± 0.14 | |

Table 9 c. Emergence of daughter plants from rhizomes of LPs and SPs.

| Form | Treatment | Total no. of plants | % of plants |
|---|---|---|---|
| LPs | Tap water | 0 | 0 |
| SPs | | 12 | 66.67 |
| LPs | 5% Hoagland | 2 | 11.11 |
| SPs | | 7 | 38.89 |

Table 9 d. Longevity of LPs and SPs.

| Form | Longevity (in days) | Mean | Observed 't' value |
|---|---|---|---|
| LPs | 28 - 93 | 57.30 ± 4.47 | 1.00 |
| SPs | 27 - 83 | 48.95 ± 3.77 | |

4.5 Development of seed of LPs and SPs

4.5.1 Seed developed by natural pollination

Blooming flowers were visited by different types of insects (Preying mantid, Grassopher, Diptera etc). Over 500 flowers of LPs and SPs have been observed, out of 100 naturally pollinated inflorescences of each forms. The average number of capsules was found to be 15.70 ± 0.77 in LPs and 5.70 ± 0.44 in SPs (Table 11a). The average number of seed bearing capsules was 2.05 ± 0.64 in LPs and 1.50 ± 0.21 in SPs. Some capsules were found to be empty which were also counted (Table 10 a). The average number of seeds per capsules was also found to vary in both the forms. In LPs the average number of seeds was 34.50 ± 5.96 while that in SPs was 12.75 ± 2.19 (Table 11 a).

Significant differences were observed between LPs and SPs at 1% level in respect of their average number of capsules and seeds. The average number of seed-bearing capsules was found to be statistically insignificant (Table 11 a).

4.5.2 Use of bagging method for development of artificial self-and cross-pollinated seeds in LPs and SPs

Development of seeds in artificially self-pollinated flowers

LPs and SPs 648 and 334 flowers were self-pollinated ively by hand (Figs.15 a and 15 b) These flowers were ed from 50 inflorescences each. The average number of les, seed-bearing capsules and number of seeds per cap- were counted. The results are shown in Table 11 b and compared with those, developing under natural conditions (Table 11 a). Number of empty capsules were also counted (Table 10 b). In artificially self-pollinated flowers, the average number of capsules was found to be 13.80 ± 1.10 in LPs and 6.50 ± 0.44 in SPs. The average number of seed-bearing capsules was 11.40 ± 1.44 in LPs and 5.70 ± 1.41 in SPs. The average number of seeds per capsule was found to be 158.72 ± 13.94 in LPs and 86.65 ± 8.15 in SPs.

Significant differences were observed between LPs and SPs at 1% level in respect of their average number of capsules, seed-bearing capsules and also number of seeds per capsule (Table 11 b).

#### 4.5.2.2 Development of seeds in artificially cross-pollinated flowers

Two hundred and twenty five flowers of LPs and 113 flowers of SPs were cross-pollinated by hand (Figs.15 a and 15 b). The results are shown in (Tables 10 c and 11 c), and compared with those, developing under natural conditions (Tables 10 a and 11 a) and also with those, self-pollinated

Fig.15a : Flowers of SPs, covered by mosquito net, supported by stick for artificial self-and cross-pollination.

Fig.15b : Flowers of LPs, covered by mosquito net, supported by stick for artificial self-and cross-pollination.

Fig.15a

Fig.15b

by hand (Tables 10 b and 11 b).

In artificially cross-pollinated flowers, the average number of capsules was found to be 12.44 ± 0.64 in LPs and 6.28 ± 0.40 in SPs; the average number of seed-bearing capsules was 11.67 ± 0.09 in LPs and 5.94 ± 0.50 in SPs; the average number of seeds per capsule was 107.92 ± 10.26 in LPs and 71.52 ± 6.60 in SPs. The differences observed between LPs and SPs in respect of their average number of capsules, seed-bearing capsules and also seeds per capsule were statistically significant at 1% level (Table 11c).

Furthermore, morphologically no differences were observed between the size of the seed-bearing capsules and those without seed (Table 12). No other workers reported that capsules are of two kinds: seed-bearing and empty capsules. The two types were found to be more or less of same size.

The number of seed-bearing and empty capsules is shown in Table 10.

The number of empty capsules was found to be more in naturally pollinated plants in comparison with the artificially self-and cross-pollinated plants (Tables 10 a to c) of both LPs and SPs.It was also observed that number of seed-bearing capsules was found to be less in naturally pollinated plants in comparison with artificially self-and cross-pollinated plants of both LPs and SPs (Tables 10 a to c).

Table 10. Number of seed-bearing and empty capsules in LPs and SPs. a : Naturally pollinated plants.

| Form | Total no. of flowers (100 inf) | Total no. of seed-bearing capsules | Total no. of empty capsules | % of seed bearing capsule or seed set | % of empty capsules |
|---|---|---|---|---|---|
| LPs | 1533 | 43 | 1490 | 2.80 | 97.20 |
| SPs | 588 | 35 | 553 | 5.95 | 94.05 |

Table 10 b. Artificially self-pollinated plants.

| Form | Total no. of flowers (50 inf) | Total no. of seed-bearing capsules | Total no. of empty capsules | % or seed bearing capsule or seed set | % of empty capsules |
|---|---|---|---|---|---|
| LPs | 648 | 567 | 81 | 87.50 | 12.50 |
| SPs | 334 | 293 | 41 | 87.72 | 12.28 |

Table 10 c. Artificially cross-pollinated plants.

| Form | Total no. of flowers (18 inf) | Total no. of seed-bearing capsules | Total no. of empty capsules | % of seed bearing capsule or seed set | % of empty capsules |
|---|---|---|---|---|---|
| LPs | 225 | 211 | 14 | 93.78 | 6.22 |
| SPs | 113 | 107 | 6 | 94.69 | 5.31 |

Table 11. Number of capsules and seeds/capsule in LPs and SPs. a : Naturally pollinated plants.

| Form | Mean of capsules | Mean of seed bearing capsule | Total no. of seeds | Mean of seeds/ capsule | 't' value for capsule | 't' value for seed-bearing capsule | 't' value for seeds/ capsule |
|---|---|---|---|---|---|---|---|
| LPs | 15.70±0.77 | 2.05±0.64 | 885 | 34.50±5.96 | 11.17** | 0.80 | 3.42** |
| SPs | 5.70±0.44 | 1.50±0.21 | 334 | 12.75±2.19 | | | |

Table 11 b. Artificially self-pollinated plants.

| Form | Mean of capsules | Mean of seed bearing capsule | Total no. of seeds | Mean of seeds/ capsule | 't' value for capsule | 't' value for seed-bearing capsule | 't' value for seeds/ capsule |
|---|---|---|---|---|---|---|---|
| LPs | 13.80±1.10 | 11.40 ± 1.44 | 47455 | 158.72±13.94 | 6.16** | 2.81** | 4.46** |
| SPs | 6.50±0.44 | 5.70 ± 1.41 | 15209 | 86.65± 8.15 | | | |

Table 11 c. Artificially cross-pollinated plants.

| Form | Mean of capsules | Mean of seed bearing capsule | Total no. of seeds | Mean of seeds/ capsule | 't' value for capsule | 't' value for seed-bearing capsule | 't' value for seeds/ capsule |
|---|---|---|---|---|---|---|---|
| LPs | 12.44±0.64 | 11.67±0.09 | 18214 | 107.92±10.26 | 8.03** | 5.70** | 2.72** |
| SPs | 6.28±0.40 | 5.94±0.50 | 7297 | 71.52± 6.60 | | | |

### 4.5.3 Measurements of capsules of LPs and SPs

After 18 days of pollination, capsules were found to be mature. At this stage, capsules (Figs.16 to 18) were collected and their measurements were recorded with the help of a meter scale.

Fig.16a : Freshly collected capsules of naturally pollinated LPs.

Fig.16b : Freshly collected capsules of naturally pollinated SPs.


Fig. 16a

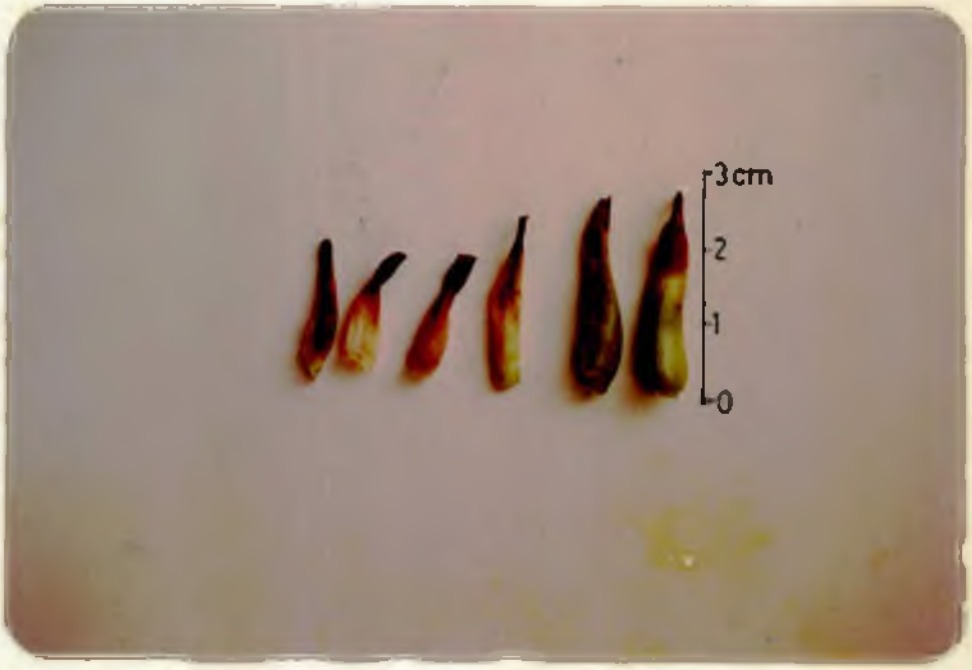



Fig. 16b

Fig.16c : Sun-dried natural pollinated capsules of LPs.

Fig.16d : Sun-dried natural pollinated capsules of SPs.

Fig. 16c

Fig. 16d

Fig.17a : Freshly collected capsules of artificially self-pollinated LPs.

Fig.17b : Freshly collected capsules of artificially self-pollinated SPs.


Fig. 17a


Fig. 17b

Fig.17c : Sun-dried artificially self-pollinated capsules of LPs.

Fig.17d : Sun-dried artificially self-pollinated capsules of SPs.

Fig.17c

Fig.17d

Fig.18a : Freshly collected capsules of artificially cross-pollinated LPs.

Fig.18b : Freshly collected capsules of artificially cross-pollinated SPs.


Fig.18a


Fig.18b

Fig.18c : Sun-dried artificially cross-pollinated capsules of LPs.

Fig.18d : Sun-dried artificially cross-pollinated capsules of SPs.

Fig.18c

Fig.18d

#### 4.5.3.1 Measurements of capsules in naturally pollinated plants

The average length of capsules were found to be 1.89 ± 0.07 cm in LPs and 1.76 ± 0.11 cm in SPs, while average breadth of capsules were found to be 0.55 ± 0.03 cm in LPs and 0.54 ± 0.03 cm in SPs (Table 12 a). The differences between LPs and SPs in respect of their average length and breadth of capsules were found to be statistically insignificant (Table 12 a).

#### 4.5.3.2 Measurements of capsules in artificially self-pollinated plants

The average length of capsules were found to be 1.90 ± 0.08 cm in LPs and 1.83 ± 0.11 cm in SPs, while the average breadth were found to be 0.60 ± 0.05 cm in LPs and 0.57 ± 0.04 cm in SPs (Table 12 b). The differences between LPs and SPs were found to be statistically insignificant in respect of their average length and breadth of capsules (Table 12 b).

#### 4.5.3.3 Measurements of capsules in artificially cross-pollinated plants

The average length of capsules were found to be 2.04 ± 0.10 cm in LPs and 1.76 ± 0.16 cm in SPs, while the average breadth were found to be 0.63 ± 0.04 cm in LPs and 0.56 ± 0.05 cm in SPs (Table 12 c). The differences between LPs and SPs in respect of their average length and breadth of capsules were found to be statistically insignificant (Table 12 c).

Table 12. Comparative measurement of capsules of LPs and SPs. a : In naturally pollinated plants.

| Form | Size of naturally pollinated capsules (cm) | | Mean of capsule length | Mean of capsule breadth | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| LPs | 1.5-2.3 | 0.4-0.7 | 1.89±0.07 | 0.55±0.03 | 0.95 | 0.21 |
| SPs | 1.2-2.3 | 0.4-0.7 | 1.76±0.11 | 0.54±0.03 | | |

Table 12 b. In artificially self-pollinated plants.

| Form | Size of self-pollinated capsules (cm) | | Mean of capsule length | Mean of capsule breadth | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| LPs | 1.5-2.3 | 0.4-0.8 | 1.90±0.08 | 0.60±0.05 | 0.74 | 0.50 |
| SPs | 1.2-2.3 | 0.4-0.7 | 1.83±0.11 | 0.57±0.04 | | |

Table 12 c : In artificially cross-pollinated plants.

| Form | Size of cross-pollinated capsules (cm) | | Mean of capsule length | Mean of capsule breadth | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| LPs | 1.2-2.5 | 0.4-0.8 | 2.04±0.10 | 0.63±0.04 | 1.49 | 1.02 |
| SPs | 1.2-2.5 | 0.3-0.8 | 1.76±0.16 | 0.56±0.05 | | |

### 4.5.4 Measurements of seeds in LPs and SPs

After counting the number of seeds (Figs.19 a to f) per capsule,the size of the seeds (Figs.20 a to f) were measured with the help of an Ocular micrometer. The readings were converted into millimetre and presented in Table 13.

#### 4.5.4.1 Measurements of seeds in naturally pollinated plants

The average length of seeds were found to be 1.24 ± 0.03 mm in LPs and 1.23 ± 0.02 mm in SPs, while the average breadths were found to be 0.55 ± 0.02 mm in LPs and 0.56 ± 0.01 mm in SPs (Table 13 a). The differences between LPs and SPs in respect of their average length and breadth of seeds were found to be statistically insignificant (Table 13 a).

#### 4.5.4.2 Measurements of seeds in artificially self-pollinated plants

The average length of seeds were found to be 1.36 ± 0.04 mm in LPs and 1.27 ± 0.02 mm in SPs, while the average breadth were found to be 0.65 ± 0.01 mm in LPs and 0.60 ± 0.02 mm in SPs (Table 13 b). Significant differences were observed at 5% level between LPs and SPs in respect of their average length and breadth of seeds (Table 13 b).

#### 4.5.4.3 Measurements of seeds in artificially cross-pollinated plants

The average length of seeds were found to be 1.35 ±

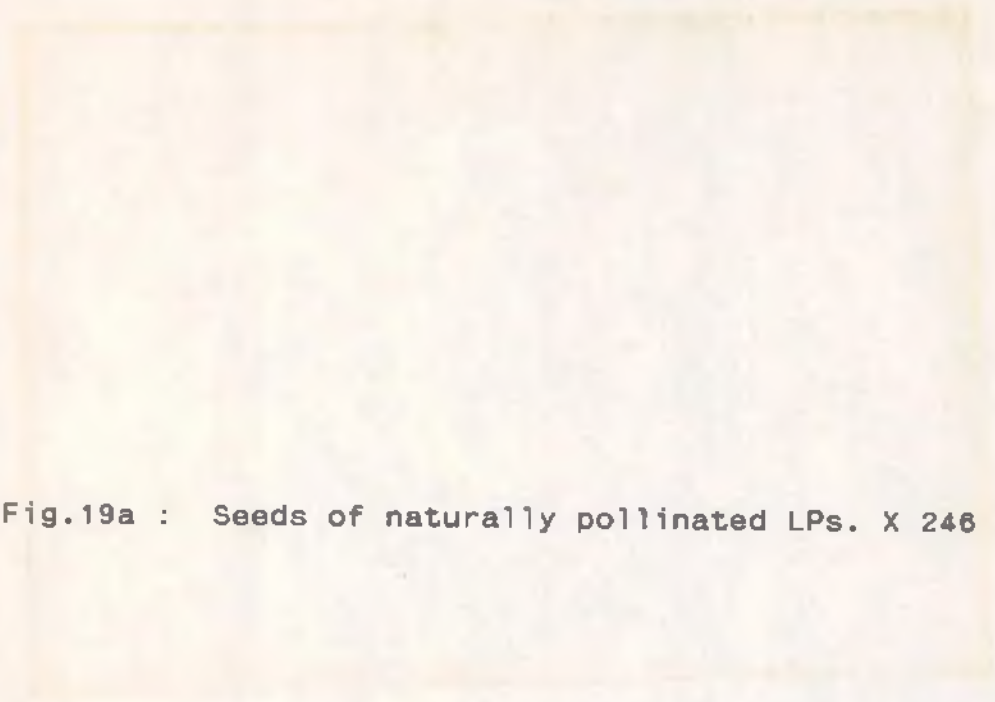


Fig.19a : Seeds of naturally pollinated LPs. X 246

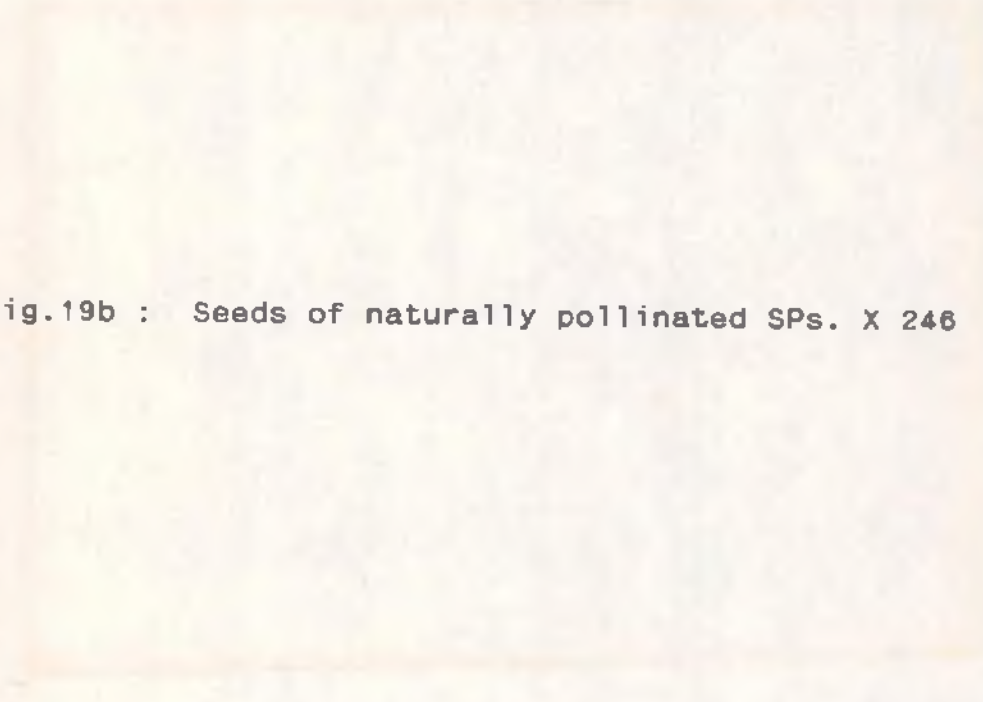


Fig.19b : Seeds of naturally pollinated SPs. X 246

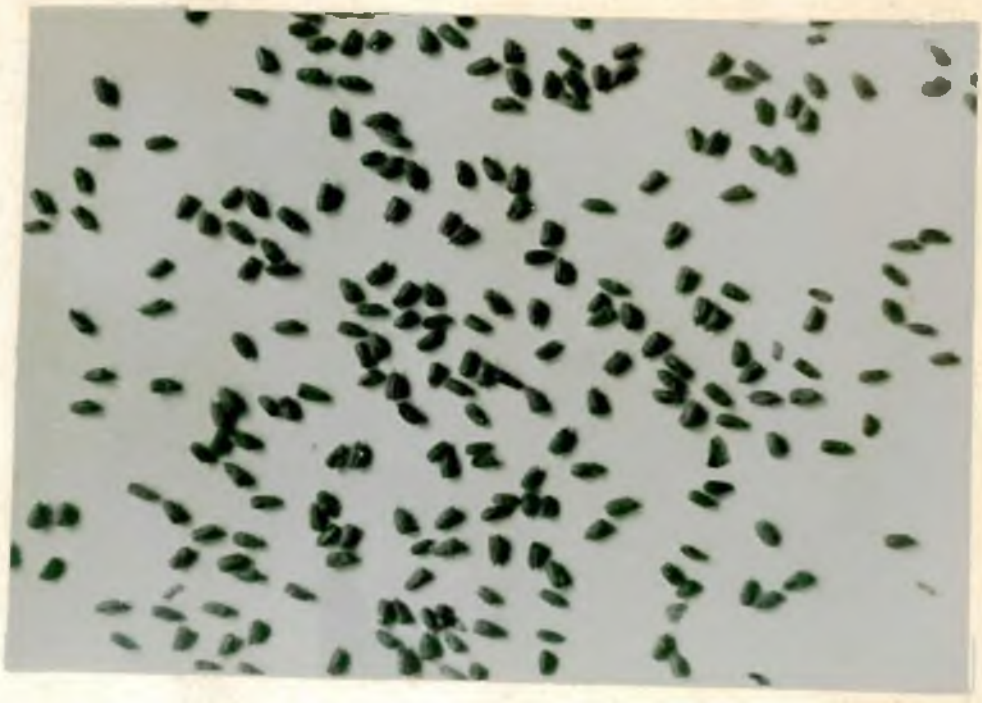


Fig.19a

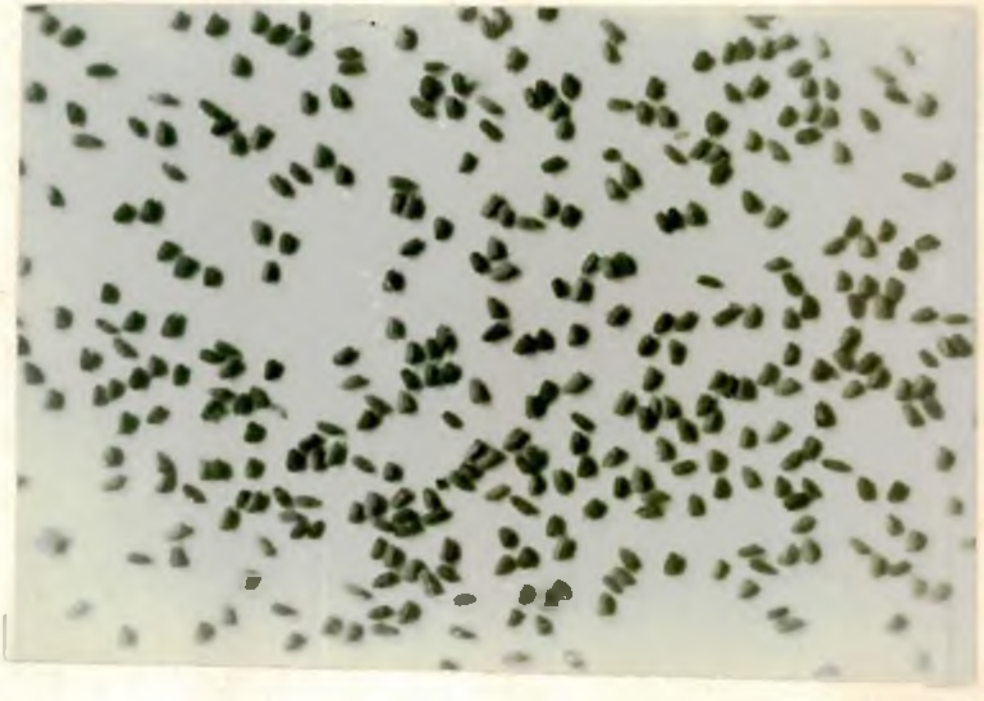


Fig.19b

Fig.19c : Seeds of artificially self-pollinated LPs. X 246

Fig.19d : Seeds of artificially self-pollinated SPs. X 246

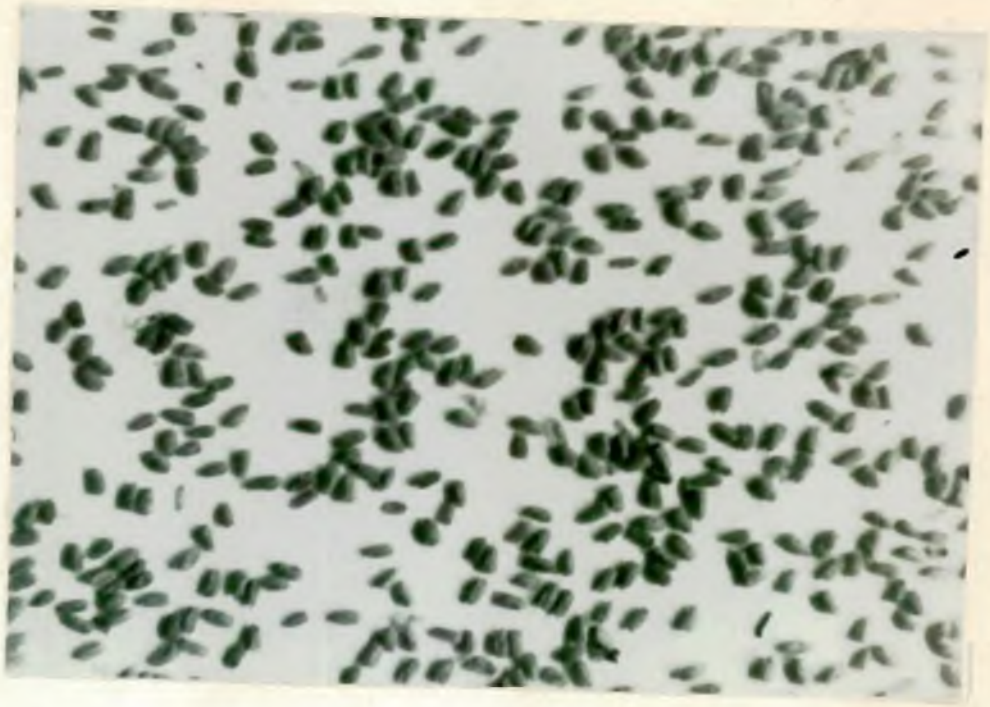


Fig.19c

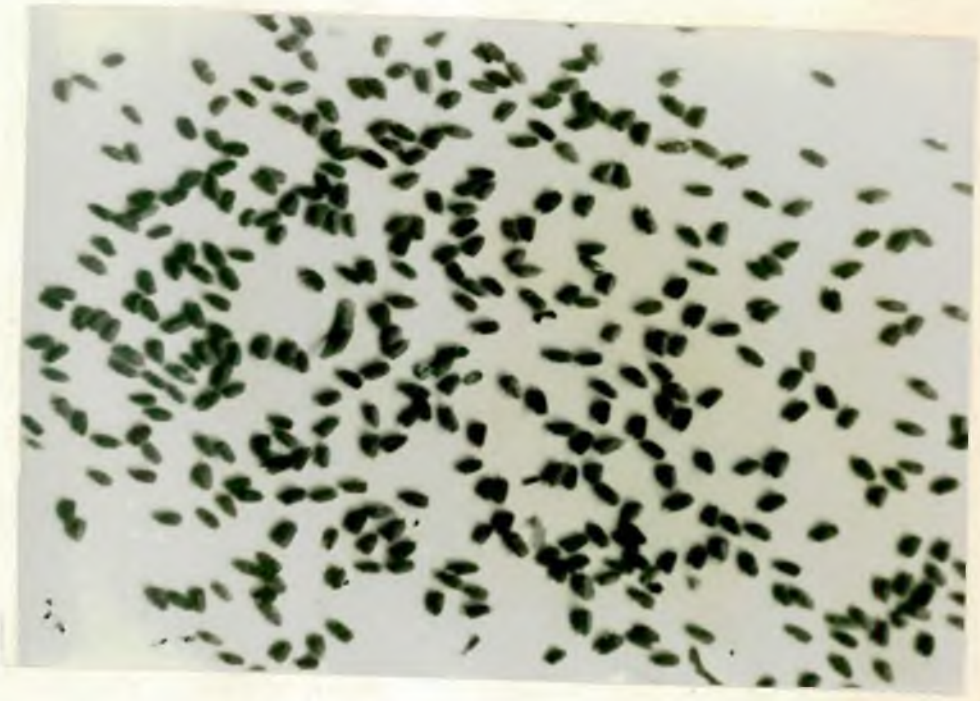


Fig.19d

Fig.19e : Seeds of artificially cross-pollinated LPs. X 246

Fig.19f : Seeds of artificially cross-pollinated SPs. X 246

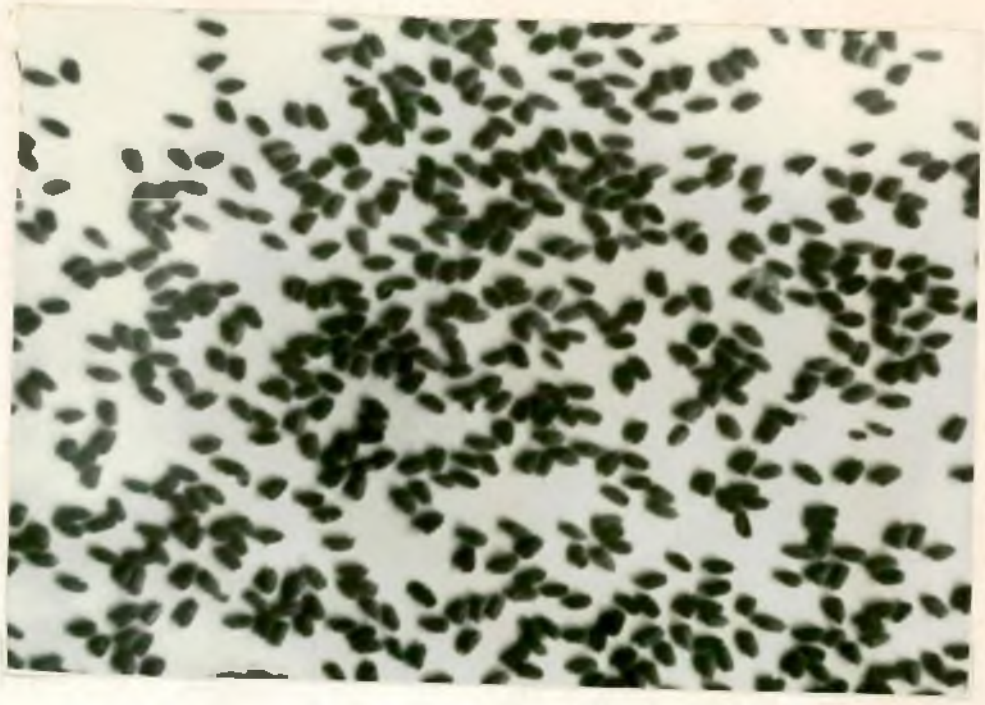


Fig.19e

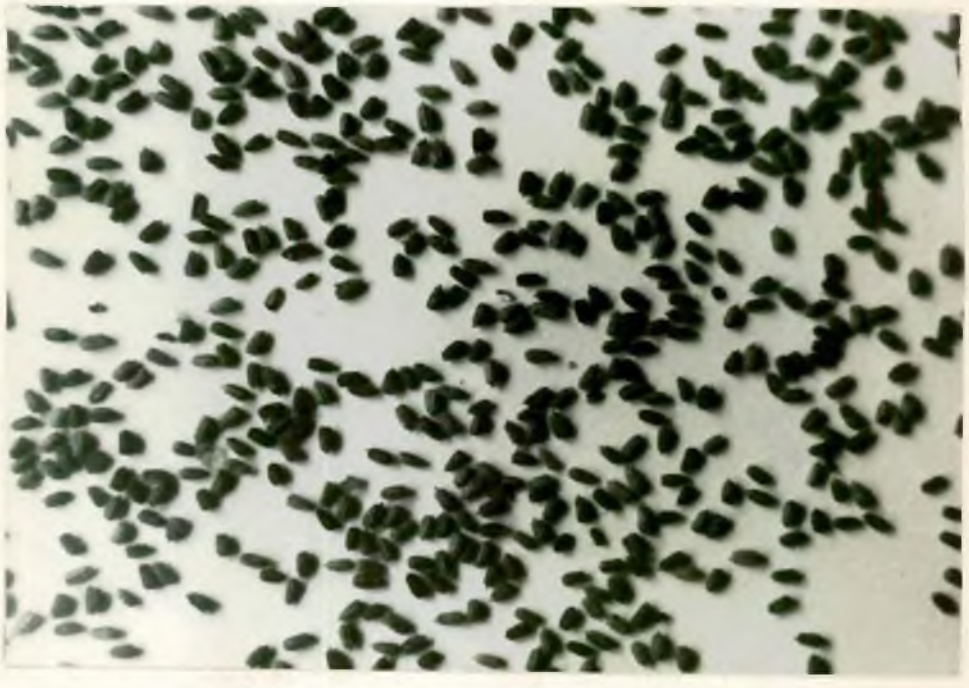


Fig.19f

0.03 mm in LPs and 1.30 ± 0.01 mm in SPs, while the average breadth was found to be 0.64 ± 0.01 mm in LPs and 0.61 ± 0.01 mm in SPs (Table 13 c). The differences between LPs and SPs in respect of their average length of seeds was found to be significant at 1% level while the average breadth of seeds were found to be statistically insignificant (Table 13 c).

Morphological appearance of natural, self-and cross-pollinated seeds was found to be the same. Seeds were minute, brown in colour, with an oval base and tapering apex. The hard seed coat is provided with ten longitudinal ridges (Figs.20 a to f).

Table 13. Comparative measurement of seeds of LPs and SPs.
a : In naturally pollinated plants.

| Form | Size of naturally pollinated seeds (mm) | | Mean of seed length | Mean of seed breadth | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| LPs | 1.08-1.43 | 0.48-0.67 | 1.24±0.03 | 0.55±0.02 | 0.39 | 0.75 |
| SPs | 1.08-1.35 | 0.50-0.63 | 1.23±0.02 | 0.56±0.01 | | |

Table 13 b. In artificially self-pollinated plants.

| Form | Size of self-pollinated seeds (mm) | | Mean of seed length | Mean of seed breadth | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| LPs | 1.15-1.50 | 0.57-0.73 | 1.36±0.04 | 0.65±0.01 | 2.14* | 2.70* |
| SPs | 1.08-1.43 | 0.47-0.72 | 1.27±0.02 | 0.60±0.02 | | |

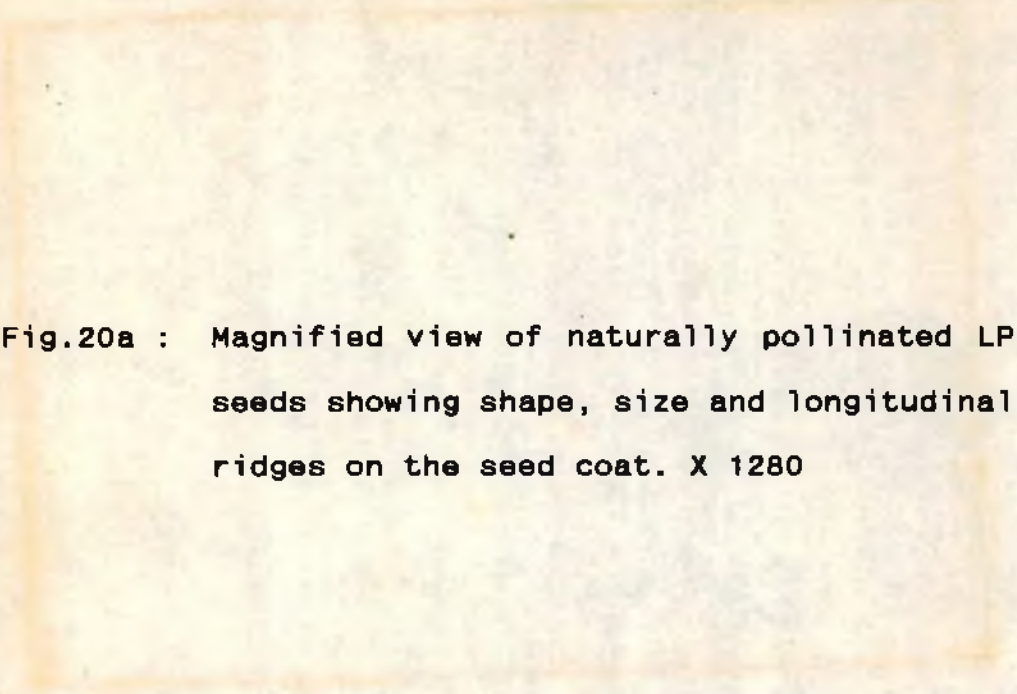


Fig.20a : Magnified view of naturally pollinated LP seeds showing shape, size and longitudinal ridges on the seed coat. X 1280

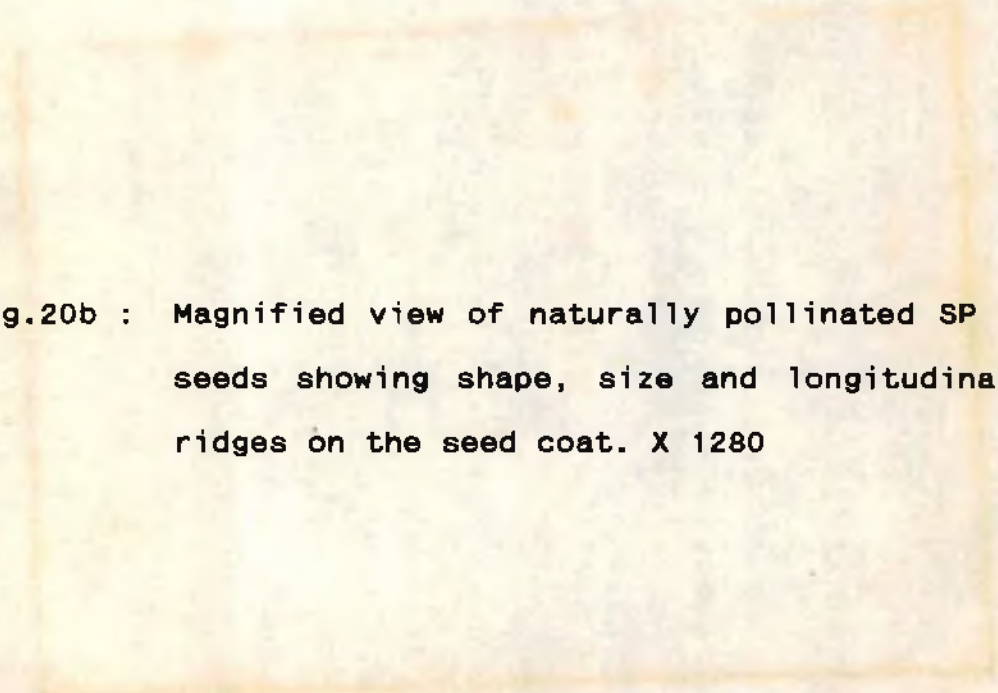


Fig.20b : Magnified view of naturally pollinated SP seeds showing shape, size and longitudinal ridges on the seed coat. X 1280

Fig.20a

Fig.20b

Fig.20c : Magnified view of artificially self-pollinated LP seeds showing shape, size and longitudinal ridges on the seed coat. X 1280

Fig.20d : Magnified view of artificially self-pollinated SP seeds showing shape, size and longitudinal ridges on the seed coat. X 1280

Fig.20c

Fig.20d

Fig.20e : Magnified view of artificially cross-pollinated LP seeds showing shape, size and longitudinal ridges on the seed coat. X 1280

Fig.20f : Magnified view of artificially cross-pollinated SP seeds showing shape, size and longitudinal ridges on the seed coat. X 1280

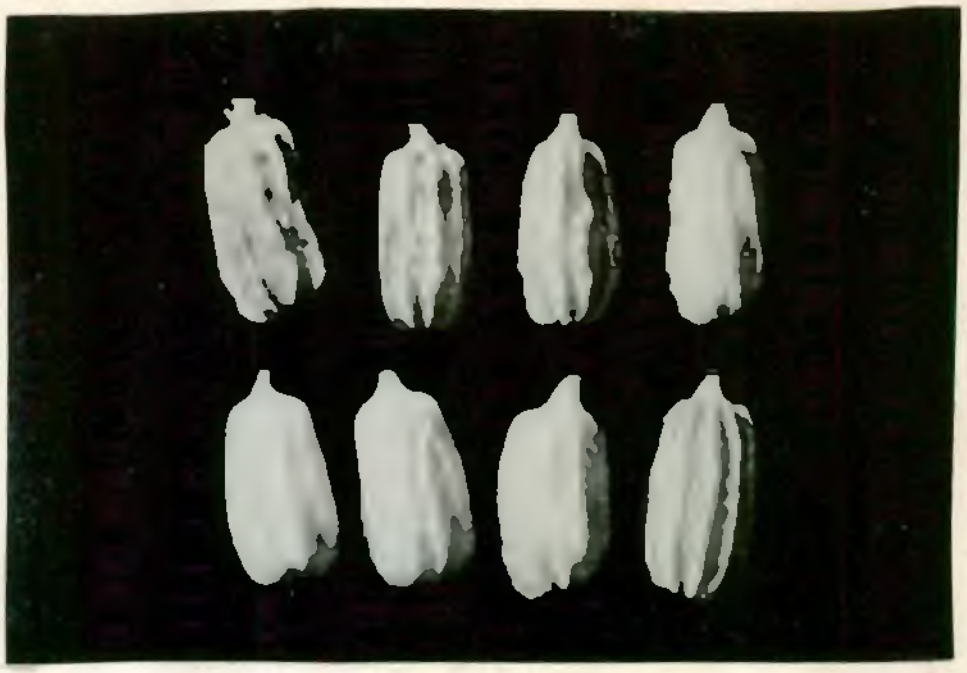


Fig.20e

Fig.20f

Table 13 c. In artificially cross-pollinated plants.

| Form | Size of cross-pollinated seeds (mm) | | Mean of seed length | Mean of seed breadth | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| LPs | 1.17-1.58 | 0.53-0.72 | 1.35±0.03 | 0.64±0.01 | 1.72** | 1.56 |
| SPs | 1.18-1.40 | 0.49-0.73 | 1.30±0.01 | 0.61±0.01 | | |

### 4.6 Germination of artificially self-and cross-pollinated seeds of LPs and SPs

Since no clear-cut information is available on the germination of water hyacinth seeds, a part of the present study was devoted trying different techniques for obtaining germination. The results of this study are given below.

#### 4.6.1 Germination of seeds in tap water/pond water on filter paper

One hundred seeds of LP and SP forms, obtained from both self- and cross-pollinated plants, were used in this study. None of the seeds germinated within three months (Table 14 a).

#### 4.6.2 Alternate wetting and drying

The alternate wetted or soaked and dried seeds of self-and cross-pollinated plants of both forms (LP and SP) were soaked in Hoagland solution (Appendix I), pond water for three months. No germination was observed (Table 14 b). These findings contradict those of Tag El Seed

and Obeid (1976), who were able to germinate the seeds after one day and recorded 100% germination on the next day, following the same treatment.Differences in ecological environments may be the factor responsible for the lack of germination of seeds of LPs and SPs, available here. The varietal differences between the materials used by Tag El Seed and Obeid (1976) in Sudan, and those in the present investigation, may also account for the different behavior of the seeds.

### 4.6.3 Chemical soaking

One hundred seeds of LP and SP forms from both self-and cross-pollinated plants were soaked in Hoagland solution (Appendix I) after treatment of the seeds with conc.sulphuric acid. None of the seeds germinated within three months (Table 14 c).

Table 14. Study of germination of artificially self-and cross-pollinated seeds of LPs and SPs (Observation period, 90 days).

| Table no. | Treatment | Form | Type of seeds* | % of germination |
|---|---|---|---|---|
| 14 a: | (i) Tap water | SP | SPS | 0 |
| | | | SPC | 0 |
| | | LP | LPS | 0 |
| | | | LPC | 0 |
| | (ii) Pond water | SP | SPS | 0 |
| | | | SPC | 0 |
| | | LP | LPS | 0 |
| | | | LPC | 0 |
| 14 b: | Alternate wetting and drying treatment; seeds soaked in: (i) Hoagland solution | SP | SPS | 0 |
| | | | SPC | 0 |
| | | LP | LPS | 0 |
| | | | LPC | 0 |
| | (ii) Pond water | SP | SPS | 0 |
| | | | SPC | 0 |
| | | LP | LPS | 0 |
| | | | LPC | 0 |
| 14 c: | Soaking of conc.$H_2SO_4$ treated seeds in Hoagland solution | SP | SPS | 0 |
| | | | SPC | 0 |
| | | LP | LPS | 0 |
| | | | LPC | 0 |

* Note:

SP self-pollinated = SPS

SP cross-pollinated = SPC

LP self-pollinated = LPS

LP cross-pollinated = LPC

### 4.6.4 Tissue culture

Since none of the previous workers in Bangladesh succeeded in germinating the seeds, and since in the present investigation germination did not occur by means of soaking in water, chemicals and alternate wetting and drying process, attempts were made in this study to germinate seed through tissue culture method.

#### 4.6.4.1 Tissue culture using solid MS medium

In this study, in LPs, 7.14% of self-pollinated seeds sprouted with only shoots emerging from seeds (designated here as germination) and among cross-pollinated seeds 4.76% germinated. In case of SPs, the percentage of germination in both self and cross-pollinated seeds was found to be 7.14 (Table 14 d). In this medium, seeds germinated within six days (Figs.21 a and 21 b). However, the shoots died after sixteen days.

#### 4.6.4.2 Hoagland solution (liquid medium)

In the treatment: (a) in which Hoagland minus sucrose was used,none of the self-and cross-pollinated seeds of the two forms (LPs and SPs) germinated. In the treatment (b) in

Table 14 d. Effect of MS medium and Hoagland solution on the germination of water hyacinth seeds.

| Table no. | Treatment | Form | Type of seeds* | No. of seed | % of germination |
|---|---|---|---|---|---|
| 14 d:(i) | MS Solid medium | SP | SPS | 21 | 7.14 |
| | | | SPC | 21 | 7.14 |
| | | LP | LPS | 21 | 7.14 |
| | | | LPC | 21 | 4.76 |
| 14 d:(ii) | Hoagland soln. with different conc. of sucrose | | | | |
| (a) | Hoagland without sucrose | SP | SPS | 32 | 0 |
| | | | SPC | 32 | 0 |
| | | LP | LPS | 32 | 0 |
| | | | LPC | 32 | 0 |
| (b) | Hoagland+1% sucrose | SP | SPS | 32 | 0 |
| | | | SPC | 32 | 0 |
| | | LP | LPS | 32 | 25 |
| | | | LPC | 32 | 0 |
| (c) | Hoagland+2% sucrose | SP | SPS | 32 | 0 |
| | | | SPC | 32 | 0 |
| | | LP | LPS | 32 | 12.5 |
| | | | LPC | 32 | 0 |
| (d) | Hoagland+4% sucrose | SP | SPS | 32 | 12.5 |
| | | | SPC | 32 | 12.5 |
| | | LP | LPS | 32 | 0 |
| | | | LPC | 32 | 0 |

Fig.21a : Emergence of shoot from cotyledons of artificially self-and cross-pollinated seeds of LPs.

Fig.21b : Emergence of shoot from cotyledons of artificially self-and cross-pollinated seeds of SPs.

Fig.21a

Fig.21b

which 1% sucrose was added to Hoagland,none of the self-and cross-pollinated seeds of SPs germinated, while in self-pollinated seeds of LPs, 25% seeds germinated; however, none of the cross-pollinated seeds of LPs germinated. In the treatment (c) in which 2% sucrose was added to Hoagland, no germination was recorded in self-and cross-pollinated seeds of SPs, and cross-pollinated seeds of LPs, while in self-pollinated seeds of LPs, 12.5% seeds germinated. In the treatment (d) in which 4% sucrose was added to Hoagland, in self-and cross-pollinated seeds of SPs,12.5% seeds germinated in both, while in LPs no germination was recorded in self-and cross-pollinated seeds (Table 14 d ii a to d). In this medium seeds germinated within eleven days (Figs.21 a and 21 b). Shoots survived upto two months but the germinating seeds were not seen to produce any root. However, the shoots grew for two months and became slightly taller than those where shoots degenerated after 16 days (MS medium).

## 4.7 Mitosis and Karyotype analysis of LPs and SPs

For mitosis and karyotype analysis the root tips of both LP and SP were pretreated with a saturated solution of paradichlorobenzene (PDB). In root tip cells metaphase chromosomes were found well separated from one another in both forms.When the roots were mordanted with iron alum (cf. Materials and methods no. 3.10 for details) the chromosomes took brilliant stain against clear background of cytoplasm.

The chromosomes were classified according to the position of the centromere and their arm ratio (short/long arm). The suggestion put forward by Kutarekar and Wanjari (1983) was followed in the classification of the water hyacinth chromosomes. According to this suggestion depending upon their arm ratios the chromosomes were grouped into median, submedian and subterminal. Depending upon the range, general average of chromatin length of chromosomes and the position of their centromeres, they were classed as follows:

| Grouping of chromosomes | Chromatin length (in micrometer) | Chromosome class | Short/long ratio |
|---|---|---|---|
| Long (L) | 3.00 above | Median (m) | 0.76 - 1.00 |
| Medium (M) | 2.25 - 2.99 | Submedian (sm) | 0.51 - 0.75 |
| Relatively short ($S_1$) | 2.24 - 1.50 | Sub terminal (st) | 0.25 - 0.50 |
| Short ($S_2$) | 1.49 below | | |

The metaphase chromosomes of both LPs and SPs have been shown in Fig.22 along with their camera lucida drawing. Karyotype of LPs and SPs have been shown in Figs.23 a and 23 b.

**4.7.1 Karyotype analysis in LPs**

The chromosome length ranged from 1.77 to 3.51 µm. The total chromatin length (TCL) of the compliment was 41.70 µm. The whole complement was made up of 16 pairs of chromosomes

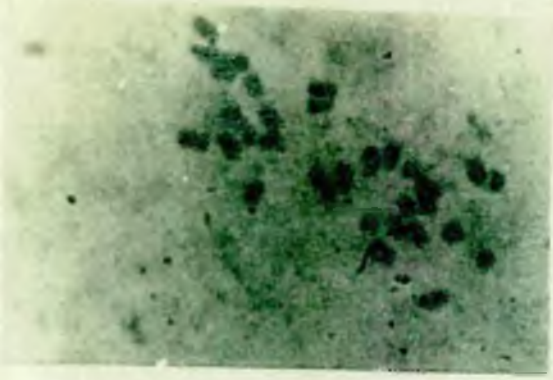
Fig.22 : Photomicrographs and camera lucida drawing of mitotic metaphase chromosomes of LPs and SPs.

(I) Photomicrograph of mitotic metaphase chromosomes of LPs. X 1000

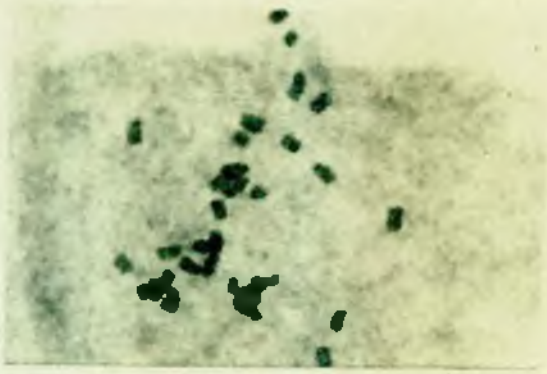
(II) Photomicrograph of mitotic metaphase chromosomes of SPs. X 1000

(III) Camera lucida drawing of mitotic metaphase chromosomes of LPs. X 1200

(IV) Camera lucida drawing of mitotic metaphase chromosomes of SPs. X 1200

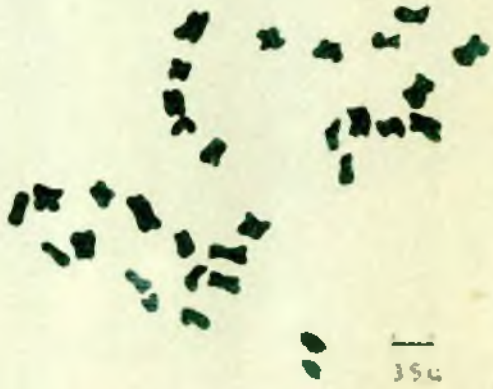


I



II


III




IV

Fig. 22

Fig.23a : Karyotypes of LPs, the longest pair 1 is on the extreme left and the remaining pairs onward in accordance with their graded length.

Fig.23b : Karyotypes of SPs, the longest pair 1 is on the extreme left and the remaining pairs onward in accordance with their graded length.


Fig. 23 a

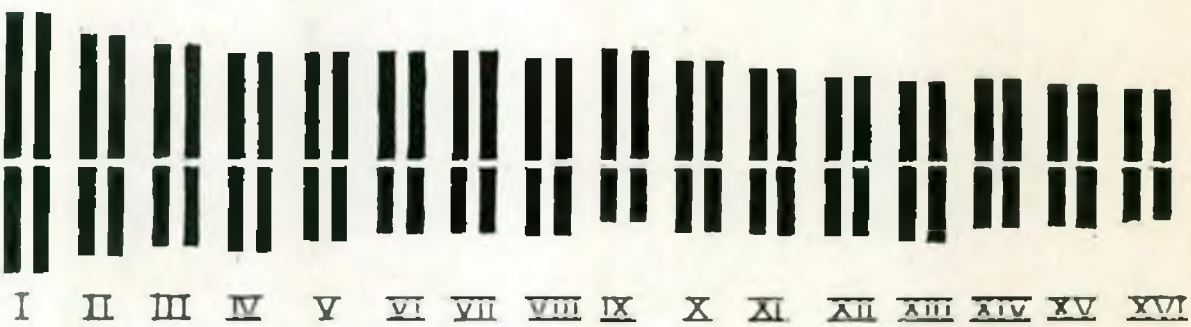



Fig. 23 b

of which four pairs belong to large (I,II,III,IV), eight pairs medium (V, VI,VII,VIII,IX,X,XI,XII) and the remaining four relatively short group (XIII,XIV,XV,XVI). In the large group all the four were submedian (sm).Out of eight pairs in the medium group, two median and six submedian chromosomes were observed.In the relatively short group, one pair median and three pairs of submedian chromosomes were found (Table 15 a). Chromosomal abnormalities, such as bridges at metaphase were also observed (Fig.24).

### 4.7.2 Karyotype analysis in SPs

The chromosome length ranged from 1.64 to 3.32 µm. The total chromatin length (TCL) of the complement was 36.33 µm. The whole complement was made up of 16 pairs of chromosomes: one large (I), seven medium (II,III,IV,V,VI,VII,VIII) and eight relatively short (IX,X,XI,XII,XIII,XIV,XV,XVI). Only one pair of submedian chromosomes was observed in the large group. In the medium group, one pair of median and six pairs of submedian chromosomes were observed. On the otherhand in the relatively short group, there were eight pairs of chromosomes. Of these four were median and the remaining submedian (Table 15 b). No short chromosome was observed in either of the two forms.

Fig.24 : Photomicrographs showing the chromosome bridge of LPs. X 1380

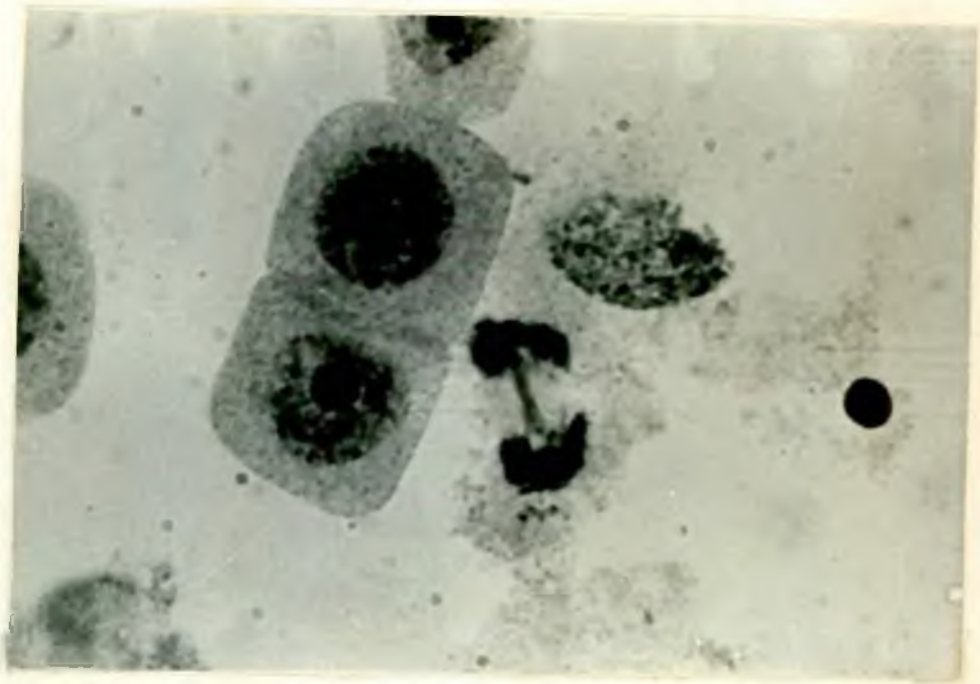


Fig.24

Table 15. Mitosis and Karyotype analysis of LPs and SPs.
a : Measurement of chromosomes and arm length ratio of LPs.

| Pairs | Long arm (μm) | Short arm (μm) | Total length (μm) | Arm ratio short/long | Primary constriction | Grouping |
|---|---|---|---|---|---|---|
| I | 2.05 | 1.46 | 3.51 | 0.71 | sm | L |
| II | 2.06 | 1.26 | 3.32 | 0.61 | sm | L |
| III | 1.95 | 1.18 | 3.13 | 0.61 | sm | L |
| IV | 1.87 | 1.23 | 3.10 | 0.66 | sm | L |
| V | 1.80 | 1.08 | 2.88 | 0.66 | sm | M |
| VI | 1.68 | 1.10 | 2.78 | 0.65 | sm | M |
| VII | 1.45 | 1.14 | 2.59 | 0.79 | m | M |
| VIII | 1.56 | 0.95 | 2.51 | 0.61 | sm | M |
| IX | 1.49 | 1.01 | 2.50 | 0.68 | sm | M |
| X | 1.44 | 1.03 | 2.47 | 0.72 | sm | M |
| XI | 1.48 | 0.91 | 2.39 | 0.61 | sm | M |
| XII | 1.21 | 1.06 | 2.27 | 0.88 | m | M |
| XIII | 1.38 | 0.82 | 2.20 | 0.59 | sm | $S_1$ |
| XIV | 1.33 | 0.84 | 2.17 | 0.63 | sm | $S_1$ |
| XV | 1.15 | 0.96 | 2.11 | 0.83 | m | $S_1$ |
| XVI | 1.08 | 0.69 | 1.77 | 0.64 | sm | $S_1$ |
| Total | 24.98 | 16.72 | 41.70 | | | |

Table 15 b. Measurement of chromosomes and arm length ratio of SPs.

| Pairs | Long arm (μm) | Short arm (μm) | Total length (μm) | Arm ratio short/long | Primary constriction | Grouping |
|---|---|---|---|---|---|---|
| I | 1.91 | 1.41 | 3.32 | 0.74 | sm | L |
| II | 1.62 | 1.16 | 2.78 | 0.72 | sm | M |
| III | 1.50 | 1.06 | 2.56 | 0.71 | sm | M |
| IV | 1.38 | 1.15 | 2.53 | 0.83 | m | M |
| V | 1.45 | 0.98 | 2.43 | 0.68 | sm | M |
| VI | 1.43 | 0.91 | 2.34 | 0.64 | sm | M |
| VII | 1.42 | 0.86 | 2.28 | 0.61 | sm | M |
| VIII | 1.33 | 0.94 | 2.27 | 0.71 | sm | M |
| IX | 1.46 | 0.75 | 2.21 | 0.51 | sm | $S_1$ |
| X | 1.33 | 0.84 | 2.17 | 0.63 | sm | $S_1$ |
| XI | 1.18 | 0.92 | 2.10 | 0.78 | m | $S_1$ |
| XII | 1.08 | 0.90 | 1.98 | 0.83 | m | $S_1$ |
| XIII | 1.01 | 0.97 | 1.98 | 0.96 | m | $S_1$ |
| XIV | 1.09 | 0.82 | 1.91 | 0.75 | sm | $S_1$ |
| XV | 1.03 | 0.80 | 1.83 | 0.78 | m | $S_1$ |
| XVI | 0.94 | 0.70 | 1.64 | 0.74 | sm | $S_1$ |
| Total | 21.16 | 15.17 | 36.33 | | | |

Karyotype formulae of LPs and SPs as determined on the basis of chromosomal length and the position of primary constriction are given in Table 15 c. The karyotype formulae give an idea about similarities and differences of the chromosomes between LP and SP forms of water hyacinth plant.

't' test was carried out to determine whether the difference between chromosome length of LPs and SPs was significant. They were found to be significant at 5% level (Table 15 d).

Furthermore the total chromatin length of chromosomes in LPs was found to 41.70 µm while that in SPs it was 36.33 µm (Tables 15 a and 15 b). This value was determined from the study of 50 cells.

**Table 15 c. Karyotype formulae of LPs and SPs : Karyotype formulae****

| Form | L (3.00µm above) | M (2.25–2.99µm) | $S_1$ (1.50–2.24µm) | $S_2$ (1.49µm below) |
|---|---|---|---|---|
| LP | $4L^{4sm}$ | $8M^{2m+6sm}$ | $4S_1{}^{1m+3sm}$ | - |
| SP | $1L^{sm}$ | $7M^{1m+6sm}$ | $8S_1{}^{4m+4sm}$ | - |

** L = Large, M = Medium, $S_1$ = Relatively short, $S_2$ = short.

Pc = primary constriction.

m = median (0.76–1.00 µm), sm=submedian (0.51–0.75 µm).

st = subterminal (0.25 - 0.50µm).

**Table 15 d. 't' test of chromosome lengths of LPs and SPs.**

| Form | Mean | 't' value |
|---|---|---|
| LPs | 2.61 ± 0.12 | 2.16 * |
| SPs | 2.27 ± 0.10 | |

## 4.8 Chemical composition of water hyacinth (LPs & SPs)

Sun-dried samples of LPs and SPs growing in the same pond were analysed for protein, potassium and phosphorus. The analysis result and location of ponds are given in Table 16 a. The average analysis value is given in Table 16 b.

Table 16 a. Chemical composition of LPs and SPs.

| Pond no. | Water hyacinth form | Moisture (%) | Total solid (%) | % of Nitrogen (on dry basis) | % of Protein (on dry basis) | % of Ash (on dry basis) | % of Phosphrus (on dry basis) | % of Potassium (on dry basis) |
|---|---|---|---|---|---|---|---|---|
| 1 | LPs | 93.84 | 6.16 | 2.50 | 15.63 | 13.80 | 1.51 | 1.05 |
| | SPs | 93.50 | 6.50 | 2.63 | 16.43 | 18.90 | 1.80 | 1.68 |
| 2 | LPs | 93.84 | 6.16 | 2.01 | 12.55 | 9.78 | 0.74 | 0.66 |
| | SPs | 93.24 | 6.76 | 2.22 | 13.88 | 10.80 | 0.68 | 0.32 |
| 3 | LPs | 92.07 | 7.63 | 1.33 | 8.31 | 16.60 | 1.13 | 2.11 |
| | SPs | 93.06 | 6.94 | 0.85 | 5.31 | 14.89 | 0.69 | 1.06 |

Pond 1 : Paikpara, Mirpur, Dhaka.

Pond 2 : Gulshan, Wireless Area, Dhaka.

Pond 3 : Kallyanpur, Mirpur, Dhaka.

Table 16 b. Average chemical composition of LPs and SPs (on dry basis).

| Water hyacinth form | Nitrogen (%) | Protein (%) | Ash (%) | Phosphorus (%) | Potassium (%) |
|---|---|---|---|---|---|
| LPs | 1.95 | 12.16 | 13.39 | 1.13 | 1.27 |
| SPs | 1.90 | 11.87 | 14.86 | 1.06 | 1.02 |

### 4.8.1 Protein content

In pond no. 1, 2 and 3, the percentage of protein was found to be 15.63, 12.55 and 8.31, respectively in LP form while in SP form it was 16.43, 13.88 and 5.31, respectively (Table 16 a).

### 4.8.2 Ash content

In pond no. 1, 2 and 3, the percentage of ash was found to be 13.80, 9.78 and 16.60 in LP form while in SP form it was 18.90, 10.80 and 14.89, respectively (Table 16 a).

### 4.8.3 Phosphorus content

In pond no. 1, 2 and 3, the percentage of phosphorus in LP form was found to be 1.51, 0.74 and 1.13 while in SP form it was 1.80, 0.68 and 0.69, respectively (Table 16 a).

### 4.8.4 Potassium content

In pond no. 1, 2 and 3, the percentage of potassium was found to be 1.05, 0.66 and 2.11 in LP form while in SP form the value was 1.68, 0.32 and 1.06,respectively (Table 16 a).

### 4.8.5 Average chemical composition

The average nitrogen content was found to be 1.95 in LPs and 1.90 in SPs. This corresponds to 12.16% protein in LPs and 11.87% protein in SPs. The average value of ash,phosphorus and potassium content was found to be 13.39, 1.13 and 1.27% in LPs and 14.86, 1.06 and 1.02% in SPs respectively (Table 16 b).

### 4.8.6 Moisture content

The moisture content was found to be more or less similar in both LPs and SPs (Table 16 a).

## 4.9 Chemical composition of water supporting the growth of LPs and SPs

For the study of chemical composition of water supporting the growth of LPs and SPs,nine ponds in and around Dhaka city were selected.Of the nine ponds, three were found with only SPs, three with only LPs and the remaining three ponds were found with both SPs and LPs. The results of the study are shown in Table 17.

### 4.9.1 Dissolved oxygen

Dissolved oxygen in ponds invaded by only SPs ranged from 0.84 to 2.01 mg/l whereas in ponds with only LPs it was 0 - 1.48 mg/l and in ponds with both forms it was 0 - 4.00 mg/l.

### 4.9.2 Total nitorgen

Water in ponds with only SPs was found to contain 1.7 -2.0 mg/l total nitrogen. In ponds with only LPs the value was 1.39 - 9.30 mg/l and in ponds with both forms, the value was 2.80 - 18.31 mg/l (Table 17).

Table 17. Chemical analysis of water having Lps and SPs (either alone or in combination).

| Water hyacinth form in in water | No. | pH of water | Chemical composition of water (mg/l) | | | |
|---|---|---|---|---|---|---|
| | | | $N_2$ | DO | Ca | $PO_4$ |
| SPs | 1 | 6.4 | 1.70 | 0.84 | 0.00 | 0.08 |
| | 2 | 7.4 | 2.00 | 2.01 | 3.00 | 0.01 |
| | 3 | 6.6 | 1.80 | 1.60 | 2.10 | 0.03 |
| LPs | 1 | 7.7 | 9.30 | 0.00 | 11.00 | 3.00 |
| | 2 | 6.5 | 1.39 | 1.48 | 11.50 | 0.22 |
| | 3 | 6.6 | 2.00 | 0.32 | 5.50 | 0.11 |
| LPs + SPs | 1 | 6.6 | 2.80 | 0.00 | 3.80 | 0.08 |
| | 2 | 7.7 | 15.73 | 4.00 | 26.00 | 0.52 |
| | 3 | 7.5 | 18.31 | 2.33 | 22.50 | 2.18 |

Location of the ponds :

SP (1) : Near Zia international airport, Dhaka,

SP (2) : Dhaka national museum pond,

SP (3) : In Kallyanpur, Dhaka,

LP (1) : Jikatola, near No. 15 staff quarter,

LP (2) : In Shamoli, Dhaka,

LP (3) : In Kathal Bagan, Dhaka,

(LP+SP) 1 : Near Bangla college, Dhaka,

(LP+SP) 2 : Near Mogbazar Wireless colony,

(LP+SP) 3 : Near Road Research Laboratory, Mirpur, Dhaka.

### 4.9.3 Phosphate

The phosphate content of water in ponds supporting only SPs ranged from 0.01 - 0.08 mg/l, in ponds supporting only LPs it was 0.11 - 3.00 mg/l and in ponds with a combination of both forms it was found to be 0.08 - 2.18 mg/l(Table 17).

### 4.9.4 Calcium

The calcium content in water of SP ponds ranged from 0 - 3.00 mg/l. The value in LP ponds was 5.50 -11.50 mg/l whereas in ponds with both forms the value was 3.80 - 26.00 mg/l (Table 17).

### 4.9.5 pH

In SP ponds,the pH was found to be 6.4 - 7.4.In Lp ponds it was 6.5 - 7.7 and in mixed LP and SP ponds the value was 6.6 - 7.7 (Table 17).

## 4.10 Water purifying capacity of the two forms of water hyacinth (LPs and SPs)

The result of analysis of water with and without water hyacinth in it is shown in Table 18. Fresh plants of both forms of water hyacinth were included in this study.

As seen in the Table 18, the presence of water hyacinth plants reduced the turbidity of water to a great extent. The turbidity was reduced from 75 NTU to 1.33 NTU by LPs and to 1.52 NTU by SPs in ten days whereas in control it was 72 NTU.

Colour of the water was also found to be reduced by the plants. The colour before treatment was 35 ml/l of Pt. as chloroplatinate ion.The value was reduced to 20 with LPs and to 25 with SPs whereas in control it was 30. Total solid, dissolved solid and suspended solid content was also found to be appreciably reduced by the presence of plants in water.

The COD of water was reduced from 380 mg/l to 160 mg/l with LP and to 120 mg/l with SPs whereas in control it was 200 mg/l.The BOD was found to be 80 mg/l on 2nd day,112 mg/l on 3rd day, 130 mg/l on 4th day and 142 mg on 5th day with LPs.The value was 61, 85, 100 and 113 with SPs and 123, 170, 205 and 225 in control (Fig.25).

Fig.25 : Graph showing biological oxygen demand (BOD) of LPs and SPs.

Curve A : BOD of dirty pond water before treatment

Curve B : BOD of dirty pond water (control)

Curve C : BOD of dirty pond water treated with LPs

Curve D : BOD of dirty pond water treated with SPs

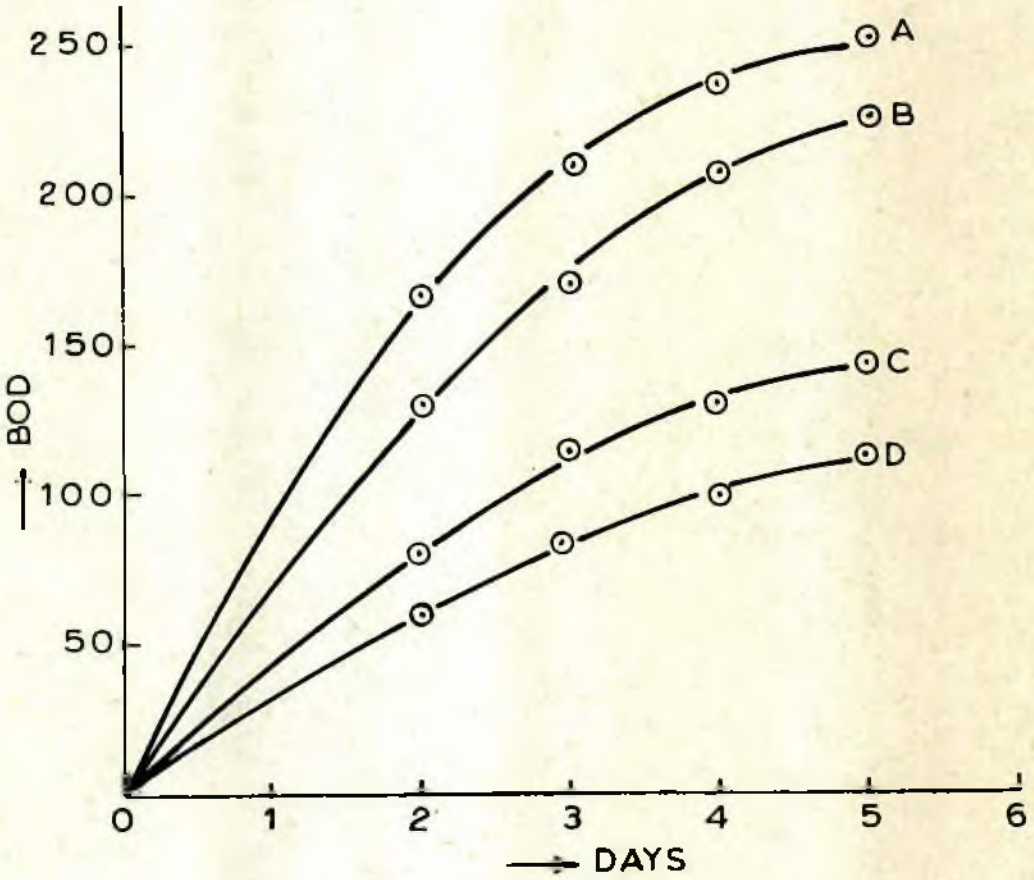



Fig.25

Table 18. Water purifying capacity of the two forms of water hyacinth (LPs & SPs).

| Condition of water | Turbidity (NTU) | Colour (equivalent) to ml/l of Pt. as chloropl. | Total solids (mg/l) | Total dissololved solid (mg/l) | Total suspended solids (mg/l) | COD mg/l | BOD (mg/l) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | at 0 day | at 2nd day | at 3rd day | at 4th day | at 5th day |
| Dirty pond water before treatment | 75 | 35 | 542 | 228 | 314 | 380 | 0 | 164 | 207 | 234 | 252 |
| Dirty pond water as control | 72 | 30 | 524 | 232 | 292 | 200 | 0 | 123 | 170 | 205 | 225 |
| Dirty pond water with LPs | 1.33 | 20 | 223 | 168 | 55 | 160 | 0 | 80 | 112 | 130 | 142 |
| Dirty pond water with SPs | 1.52 | 25 | 248 | 189 | 59 | 120 | 0 | 61 | 85 | 100 | 113 |

## 4.11 Electrophoretic study of protein bands and isoenzyme patterns of LPs and SPs

### 4.11.1 Electrophoretic study of protein bands

Since genetic differences are reflected in shifts of protein patterns, more refined separation method, such as disc electrophoresis are of special interest for research on taxonomy,genetics and cultivation of plants (Ericksson 1968; Davies *et al.* 1973).

The application of disc electrophoresis as a method for solving taxonomic and physiological problems was emphasised by Fox *et al.* (1964) and Vaughan and Waite (1967a) who investigated the seed proteins of legumes and Brassica.

Fox *et al.*(loc.cit.), Vaughan and Waite (loc.cit.) pointed out that morphological classification could be supplemented by biochemical categories.It is even possible to identify different genotypes of one species by their protein patterns.

In the present investigation, 11 protein bands were observed in both LPs and SPs in the same position. The bands showed the same mobilities within the two forms.

Starting from below,they have been serially numbered from 1-11. The colour intensity of the bands have been indicated by solid black (very intense),oblique lines (fairly intense), vertical lines (intense) dot (weak) and completely white (very weak).In both forms band no.10 was very intense, band nos.7, 9, 11 were fairly intense,band nos.1, 2, 4, 6, 8 were

intense, band no. 5 was weak and band no. 3 was very weak (Fig.26).

4.11.2 Isoenzyme studies

Isoenzymes may be defined as multimolecular forms of enzymes which are derived from the same origin or tissue and have atleast one substrate in common.

Applying isoenzyme technique, biochemical, genetical or other problems can be successfully solved now-a-days. Isoenzymes are being used by geneticists because of a number of advantages over the conventional morphological markers. They are being used for estimating degree of genetic polymorphism present in populations, genetic differences between species, differential gene action and expression during development, gene dosage effects on enzyme structure and function, intragenic complementation and heterosis and the evolution of genes and organisms (Gomes 1978).

Isoenzymes are also effectively being employed in studies to ascertain phylogenetic relationship (Lewontin and Hubby 1966; Hart 1969, 70; Allard and Kahler 1971; Whitt *et al.* 1973; Scandalios 1967).

Plant isoenzymes have been proved to be very useful to biologists, systematists, plant breeders and others. It is being used as a marker for selecting and indentifying cultivars of different crops. Plant breeders supplement their morphological, cytological and reproductive data with the

Fig.26 : Schematic diagram of electrophoretogram showing the patterns of protein bands in SPs (left) and LPs (right).

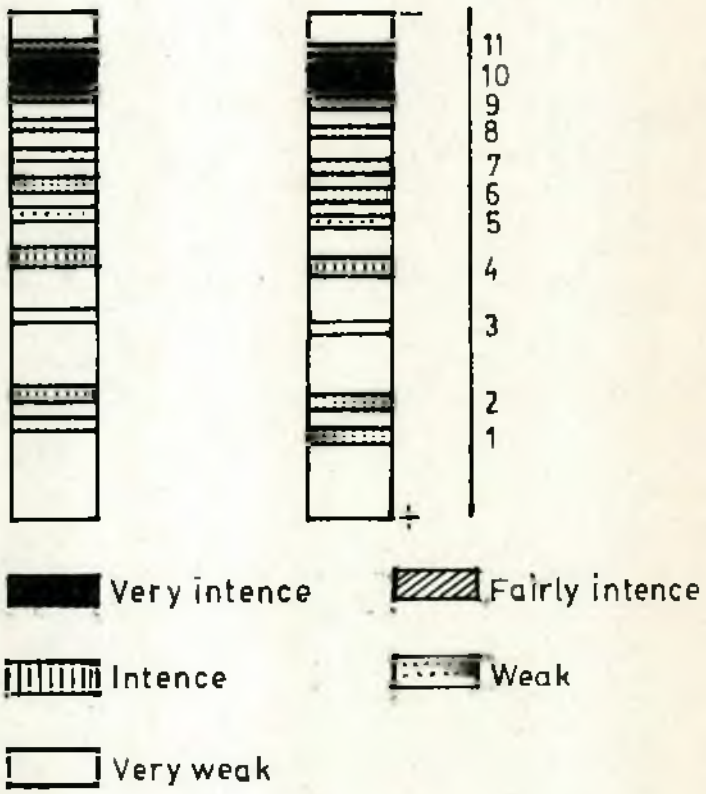



Fig. 26.

help of isoenzymes (Brewer 1970).

Hunter and Markert (1957) developed a technique, known as zymogram technique, for determining enzyme heterogeneity. Using this technique, Markert and Moller (1959) were able to separate lactate dehydrogenase enzyme into five distinct forms. Changes in isoenzyme patterns are indicators of changes in gene expression and/or reflection of genetic and epigenetic events inside and outside of a differentiating cycle (Gomes 1978).

In the present investigation isoenzyme studies have been used to detect whether there is any difference exists between the two forms of water hyacinth (LPs and SPs).

Accordingly a comparative study of alcohol dehydrogenase (ADH) and malate dehydrogenase (MDH) isoenzymes have been undertaken.Results of these observations are discussed below under separate heads.

#### 4.11.2.1 Alcohol dehydrogenase (ADH) isoenzyme of LPs and SPs

ADH isoenzymes have been investigated in a large number of plants namely, maize (Scandalios 1967),wheat (Hart 1970), legumes (Gomes *et al.* 1982; Nilufar *et al.* 1986).

In the present investigation only one ADH isoenzyme band was observed in both LPs and SPs. But the colour intensity and length of the band were higher in SPs in comparison to LPs. In SPs the band was very intense and the length was 8mm while in LPs the band was very weak and the length was 3 mm

(Fig.27). Depending on the mobilities of the bands also they could be distinguished from each other.

#### 4.11.2.2 Malate dehydrogenase (MDH) isoenzyme of LPs and SPs

Vesell and Bearn (1958) reported for the first time that human serum could be separated into three fractions of MDH by starch block electrophoresis. Markert and Moller (1959) fractionated the MDH activity from various animal tissue extracts by starch gel electrophoresis. According to Bailay *et al.* (1969) and Whitt (1970), extensive studies have revealed that the MDH isoenzymes are species specific and also tissue specific.

In the present investigation, in case of MDH isoenzymes, two bands were found in both LPs and SPs (Fig.28). One band was very weak and identical in both the forms. The other band was more intense in SPs than LPs and the length of the band was higher (5 mm) in SPs than in LPs (3 mm). The mobilities of the bands were also different in LPs and SPs.

## 4.12 Comparative study of white and lilac-blue flowered water hyacinth

### 4.12.1 General differences between white and lilac-blue flowered water hyacinth

Some conspicuous general differences were observed between the above forms. The differences are stated below. (1) Lilac-blue flowered water hyacinths are common in the water bodies of this country, while white-flowered water

Fig.27 : Schematic diagram of zymogram showing the ADH isoenzyme band patterns in SPs (left) and LPs (right).

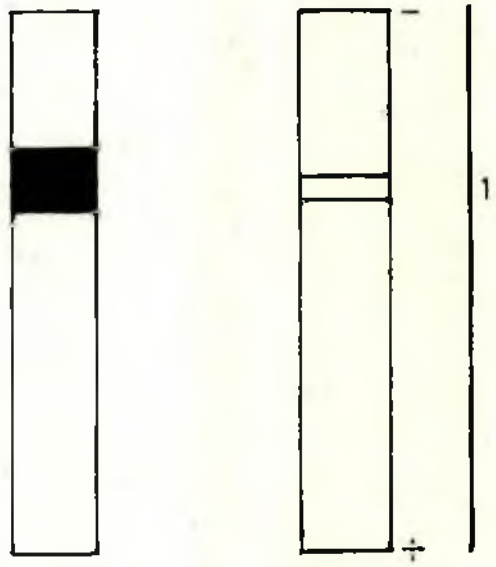



Fig. 27

Fig.28 : Schematic diagram of zymogram showing the MDH isoenzyme band patterns in SPs (left) and LPs (right)

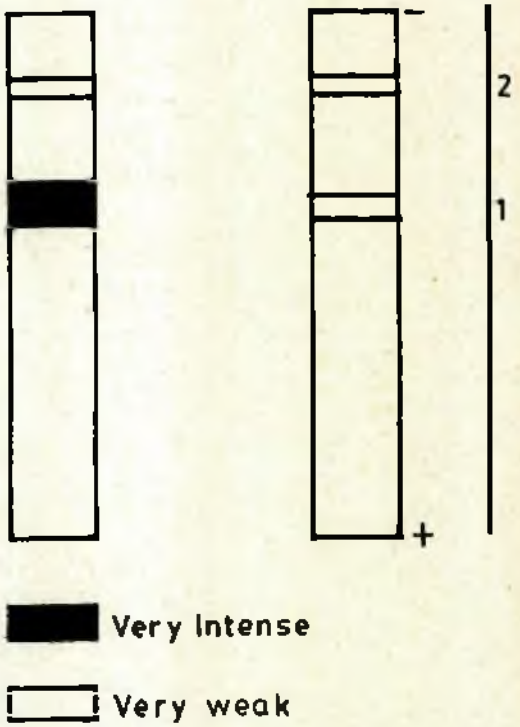



Fig. 28

hyacinths are rare.The latter was collected only once during the present study.

(2) Both LPs and SPs are common in naturally growing lilac-blue water hyacinth. White-flowered water hyacinth collected only once during the present study, were SPs (Fig.29 a). During the laboratory culture for three years, lilac-blue LPs produced both LPs and SPs, and SPs also produced both LPs and SPs. But white-flowered SPs, collected from nature, produced only LPs (Fig.29 b) during the observation period of three years in the laboratory.

### 4.12.2 Comparative morphological study of cultured white flowered LPs and cultured lilac-blue flowered LPs.

#### 4.12.2.1 Similarity in morphological characters

Some morphological characters were found to be similar between white LPs and lilac-blue LPs : (a) length of petiole, (b) size of leaf blades, (c) number of flowers per inflorescence (lilac-blue LPs, 6 to 27 and in white-flowered LPs, 5 to 24), (d) shape of the flower (except the posterior lobe), (e) number of floral parts (six petals, six stamens and one pistil).

#### 4.12.2.2 Differences in morphological characters

(a) There was a striking difference in the cultured white and lilac-blue flowers (Figs.30 a and 30 b) in their colouration and also in the yellow blotch of their posterior lobe (Fig.31). In that the blotch in the lilac-blue flower has

Fig.29a : Short petioled form of white-flowered water hyacinth collected from nature. X 0.40

Fig.29b : Long petioled form of white-flowered water hyacinth collected from laboratory culture. X 0.14

Fig.29a

Fig.29b

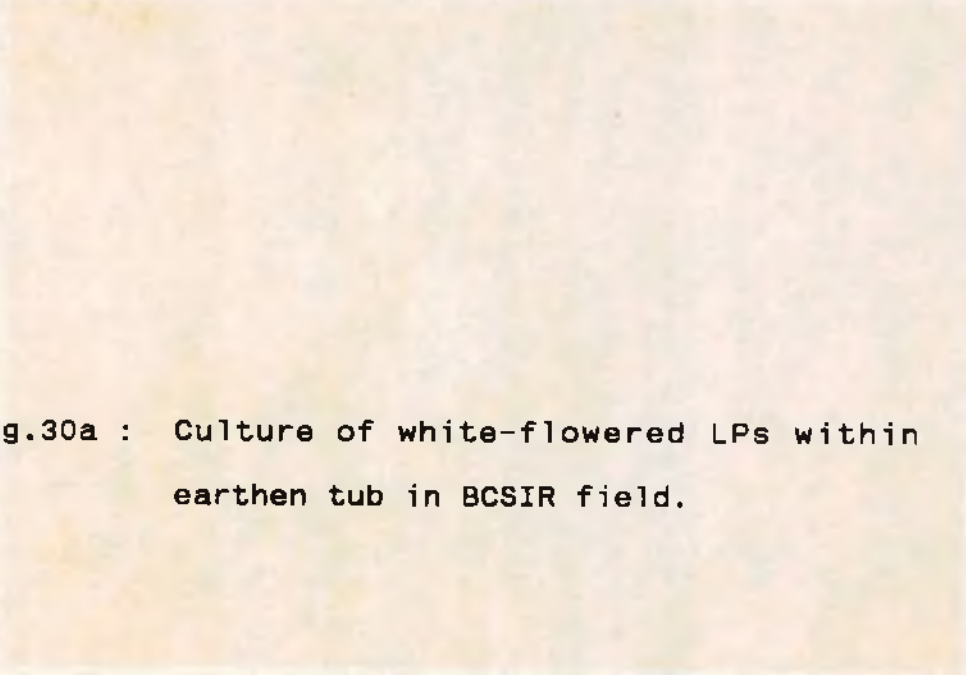


Fig.30a : Culture of white-flowered LPs within earthen tub in BCSIR field.

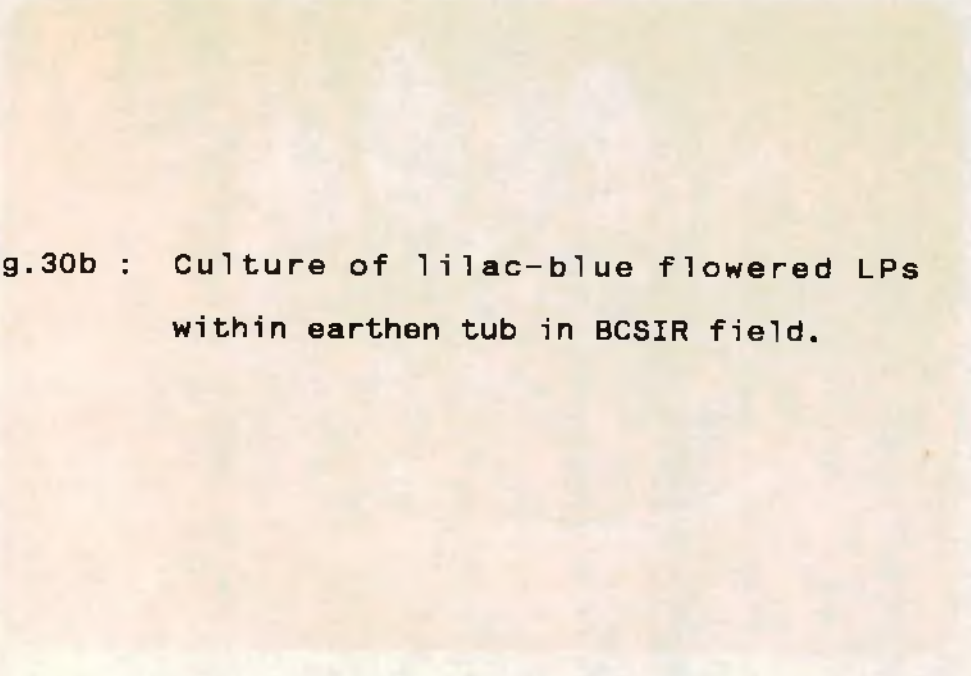


Fig.30b : Culture of lilac-blue flowered LPs within earthen tub in BCSIR field.

Fig.30a

Fig.30b

Fig.31 : White flower (left) and lilac-blue flower (right) showing the colour differentiation.
Note : White flower showing only the yellow blotch in the posterior lobe while lilac-blue flower showing the yellow blotch, surrounded by a conspicuous dark violet border in the posterior lobe.

Fig.32 : White flower (left) with slightly curved posterior lobe; lilac-blue flower (right) with uncurved posterior lobe.

Fig.31

Fig.32

a conspicuous dark violet border around it, while that of the white flower has no such border (Fig.31).

(b) Posterior lobe of the white LPs is slightly curved while that of the lilac-blue LPs has never been found to be curved (Fig.32).

(c) Out of 10,000 flowers, some white flowers had mid-styles while the others had short-styles. Some inflorescences were found to bear flowers of either types or a combination of both (Fig.33). In case of lilac-blue inflorescences, all the flowers were found to bear only mid-styles in the 10,000 flowers studied (Fig.33).

The length of inflorescences of both the forms (Fig.34) have been presented in Table 19 and the measurements of the floral parts (Figs.35 and 36) are presented in Tables 20 a to m.

Table 19. Length of inflorescences of white LPs and lilac-blue LPs.

| Form | Length of inf (cm) | Mean | 't' value |
|---|---|---|---|
| White LPs | 25 - 43 | 32.86 ± 0.94 | 2.49* |
| Lilac-blue LPs | 23 - 36 | 29.58 ± 3.81 | |

Note: * represents significance at 5% level.

Fig.33

Fig.33 : Pistils of white-flowered LPs (left) showing both short and mid-styles; Pistils of lilac-blue flowered LPs (right) showing only mid-styles.

Fig.34 : Comparative view of length of inflorescence of white-flowered LPs (left) and lilac-blue flowered LPs (right). X 0.28

Fig.34

Fig.35 : Comparative view of length of white-flowered LPs (left) and lilac-blue flowered LPs (right).

Fig.36 : Comparative view of floral parts of white flowered LPs (left) and lilac-blue flowered LPs (right).

Fig.35

Fig.36

Table 20. Measurements of floral parts of white LPs and lilac-blue LPs.
a : Length of flowers (including pedicel).

| Form | Length of flower (cm) | Mean | 't' value |
|---|---|---|---|
| White LPs | 3.7 - 5.5 | 4.74 ± 0.07 | 8.93** |
| Lilac-blue LPs | 4.2 - 6.5 | 5.73 ± 0.08 | |

Note: ** represents significance at 1% level.

Table 20 b. Size of posterior lobes (petal no.1).

| Form | Size of posterior lobes (cm) | | Mean (length) | Mean (breadth) | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| White LPs | 2.5-3.7 | 0.8-1.8 | 3.15±0.08 | 1.32±0.04 | 8.54** | 11.97** |
| Lilac-blue LPs | 3.0-4.9 | 1.5-2.6 | 4.34±0.11 | 2.33±0.07 | | |

Note: ** represents significance at 1% level.

Table 20 c. Size of the yellow blotch.

| Form | Size of the yellow bloch (cm) | | Mean (length) | Mean (breadth) | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| White LPs | 0.3-0.7 | 0.2-0.5 | 0.44±0.03 | 0.29±0.02 | 6.17** | 5.30** |
| Lilac-blue LPs | 0.4-1.1 | 0.3-0.9 | 0.76±0.05 | 0.10±0.03 | | |

Note: ** represents significance at 1% level

Table 20 d. Size of petal no. 2.

| Form | Size of petal no. 2 (cm) | | Mean (length) | Mean (breadth) | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| White LPs | 1.8-3.0 | 0.6-1.4 | 2.34±0.04 | 1.01±0.02 | 8.17** | 7.99** |
| Lilac-blue LPs | 2.3-4.4 | 0.8-2.1 | 3.34±0.10 | 1.63±0.07 | | |

Note: ** represents significance at 1% level.

Table 20 e. Size of petal no. 3.

| Form | Size of petal no. 3 (cm) | | Mean (length) | Mean (breadth) | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| White LPs | 1.2-3.1 | 0.6-1.4 | 2.59±0.05 | 1.07±0.04 | 7.45** | 9.29** |
| Lilac-blue LPs | 2.5-4.4 | 1.0-2.2 | 3.49±0.10 | 1.69±0.05 | | |

Note: ** represents significance at 1% level

Table 20 f. Size of petal no. 4.

| Form | Size of petal no. 4 (cm) | | Mean (length) | Mean (breadth) | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| White LPs | 1.9-3.4 | 0.6-1.6 | 2.63±0.06 | 1.09±0.03 | 8.23** | 8.26** |
| Lilac-blue LPs | 2.3-4.7 | 1.0-2.2 | 3.49±0.09 | 1.68±0.07 | | |

Note: ** represents significance at 1% level

Table 20 g. Size of petal no. 5.

| Form | Size of petal no. 5 (cm) | | Mean (length) | Mean (breadth) | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| White LPs | 1.9-3.4 | 0.6-1.5 | 2.61±0.06 | 1.07±0.04 | 7.71** | 8.26** |
| Lilac-blue LPs | 2.4-4.2 | 0.9-2.1 | 3.48±0.09 | 1.69±0.06 | | |

Note: ** represents significance at 1% level

Table 20 h. Size of petal no. 6.

| Form | Size of petal no. 6 (cm) | | Mean (length) | Mean (breadth) | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| White LPs | 2.0-3.3 | 0.7-1.5 | 2.60±0.04 | 1.07±0.03 | 6.74** | 8.96** |
| Lilac-blue LPs | 2.4-4.4 | 0.9-2.1 | 3.51±0.13 | 1.72±0.07 | | |

Note: ** represents significance at 1% level

Table 20 i. Length of posterior large stamens.

| Form | Length of posterior large stamens (cm) | Mean | 't' value |
|---|---|---|---|
| White LPs | 1.5 - 2.2 | 1.80 ± 0.03 | 4.19** |
| Lilac-blue LPs | 1.8 - 2.6 | 2.16 ± 0.08 | |

Note: ** represents significance at 1% level.

Table 20 j. Length of posterior small stamens.

| Form | Length of posterior small stamens (cm) | Mean | 't' value |
|---|---|---|---|
| White LPs | 1.3 - 2.0 | 1.63 ± 0.03 | 4.38** |
| Lilac-blue LPs | 1.5 - 2.3 | 2.01 ± 0.08 | |

Note: ** represents significance at 1% level.

Table 20 k. Length of anterior three small stamens.

| Form | Length of anterior three small stamens (cm) | Mean | 't' value |
|---|---|---|---|
| White LPs | 0.4 - 0.6 | 0.51 ± 0.01 | 4.30** |
| Lilac-blue LPs | 0.4 - 1.0 | 0.69 ± 0.04 | |

Note: ** represents significance at 1% level.

Table 20 l. Length of style.

| Form | Length of style (cm) | Mean | 't' value |
|---|---|---|---|
| White LPs | 1.1 - 2.0 | 1.72 ± 0.02 | 9.21** |
| Lilac-blue LPs | 2.3 - 2.6 | 1.40 ± 0.02 | |

Note: ** represents significance at 1% level.

Table 20 m. Size of ovary.

| Form | Size of ovary (cm) | | Mean (length) | Mean (breadth) | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| White LPs | 0.4-0.6 | 0.1-0.2 | 0.52±0.07 | 0.19±0.002 | 7.17** | 6.53** |
| Lilac-blue LPs | 0.5-0.9 | 0.1-0.3 | 0.68±0.02 | 0.24±0.007 | | |

Note: ** represents significance at 1% level

### 4.12.3 Measurement of pollen grains of white LPs and lilac-blue LPs

Two types of pollen grains (oval and round) were observed in both white LPs and lilac-blue LPs (Figs.37 a and 37 b). Round pollen grains were found to be less in comparison to the oval ones. Measurement of pollen grains have been presented in Tables 21 a and 21 b.

Table 21 Measurement of pollen grains of white LPs and Lilac-blue LPs.
a : Size of oval pollen grain.

| Form | Size of oval pollen grain (µm) | | Mean (length) | Mean (breadth) | 't' value for length | 't' value for breadth |
|---|---|---|---|---|---|---|
| | Length | Breadth | | | | |
| White LPs | 52.0-88.5 | 40.0-77.4 | 70.65±2.44 | 61.94±2.82 | 1.40 | 3.22** |
| Lilac-blue LPs | 48.0-85.0 | 37.0-73.0 | 65.27±2.95 | 50.15±2.34 | | |

Average difference of length of oval pollen grains of white LPs and lilac-blue LPs were found to be insignificant and average breadth of oval grains was found to be significant at 1% level.

Table 21 b. Diameter of round pollen grain.

| Form | Diameter of round pollen grain (µm) | Mean | 't' value |
|---|---|---|---|
| White LPs | 55.00 - 70.00 | 60.90 ± 1.42 | 1.76 |
| Lilac-blue LPs | 54.65 - 65.00 | 57.49 ± 1.30 | |

Difference between white LPs and lilac-blue LPs was found to be insignificant in respect of their average dia of the round pollen grain.

Fig.37a : Photomicrographs showing different types of pollen grains (oval and round) of white-flowered LPs. X 165

Fig.37b : Photomicrographs showing different types of pollen grains (oval and round) of lilac-blue flowered LPs. X 165

Fig.37a

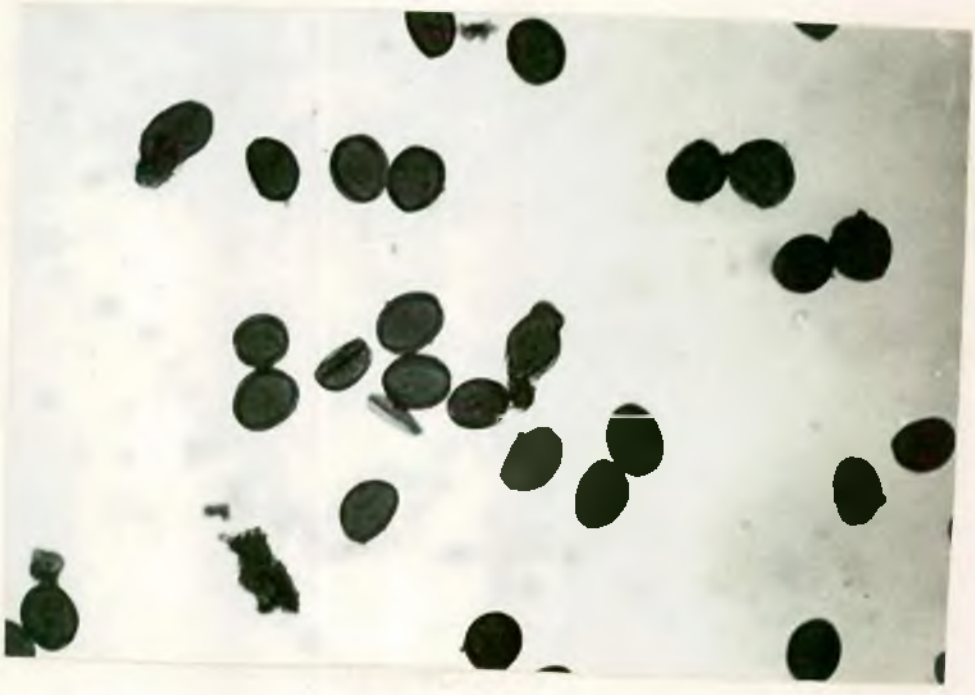


Fig.37b

## 4.13 Mobility of total cellular DNA from LPs and SPs of lilac-blue coloured flower and also LPs of white coloured flower by using agarose gel electrophoresis

The DNA preparation was electrophoresed in .8% agarose gel. A sample of Hind III digested $\lambda$ DNA, which is used as a molecular size marker was also electrophoresed in the same gel. The gel was stained with ethidium bromide (10 mg/ml) for 30 min and photographed under UV light using transilluminator.

Molecules of linear, duplex DNA, which are believed to migrate in an end on position, travel through gel matrices at rates that are inversely proportional to the logarithm of their molecular weights (Maniatis *et al.* 1982).

DNA fragments of a given size migrate at different rates through gels containing different concentrations of agarose. There is a linear relationship between the logarithm of the electrophoretic mobility of DNA and gel concentration.

Using the $\lambda$ DNA/Hind III fragments a standard curve was made by plotting the distance (mm) migrated by $\lambda$ DNA/Hind III fragments against $\log_{10}$ of their base pair length. The range of molecular weight of the last two fragments of $\lambda$ DNA/Hind III (used as a marker) is much lower in comparison with the samples.For the proper separation of high molecular weight DNA, a high percentage of agarose is recommended (Maniatis *et al.* 1982). Therefore % of agarose used for

running the gel was higher (0.8%). The separation of the last two fragments of the marker did not fall in a linear pattern in the 0.8% agarose gel. Therefore the last two points in the graph were disregarded.

By putting the value of the distance migrated by the cellular DNA, the size was calculated to be approximately 33.00 Kb in white-flowered form LPs and 30.80 Kb in SPs, 28.80 Kb in LPs of lilac-blue coloured flower. (Fig.38).

Fig.38 : Graph showing the size of isolated cellular DNA of white-flowered LPs and lilac-blue flowered LPs and SPs

A = White-flowered LPs,

B = Lilac-blue flowered SPs,

C = Lilac-blue flowered LPs.

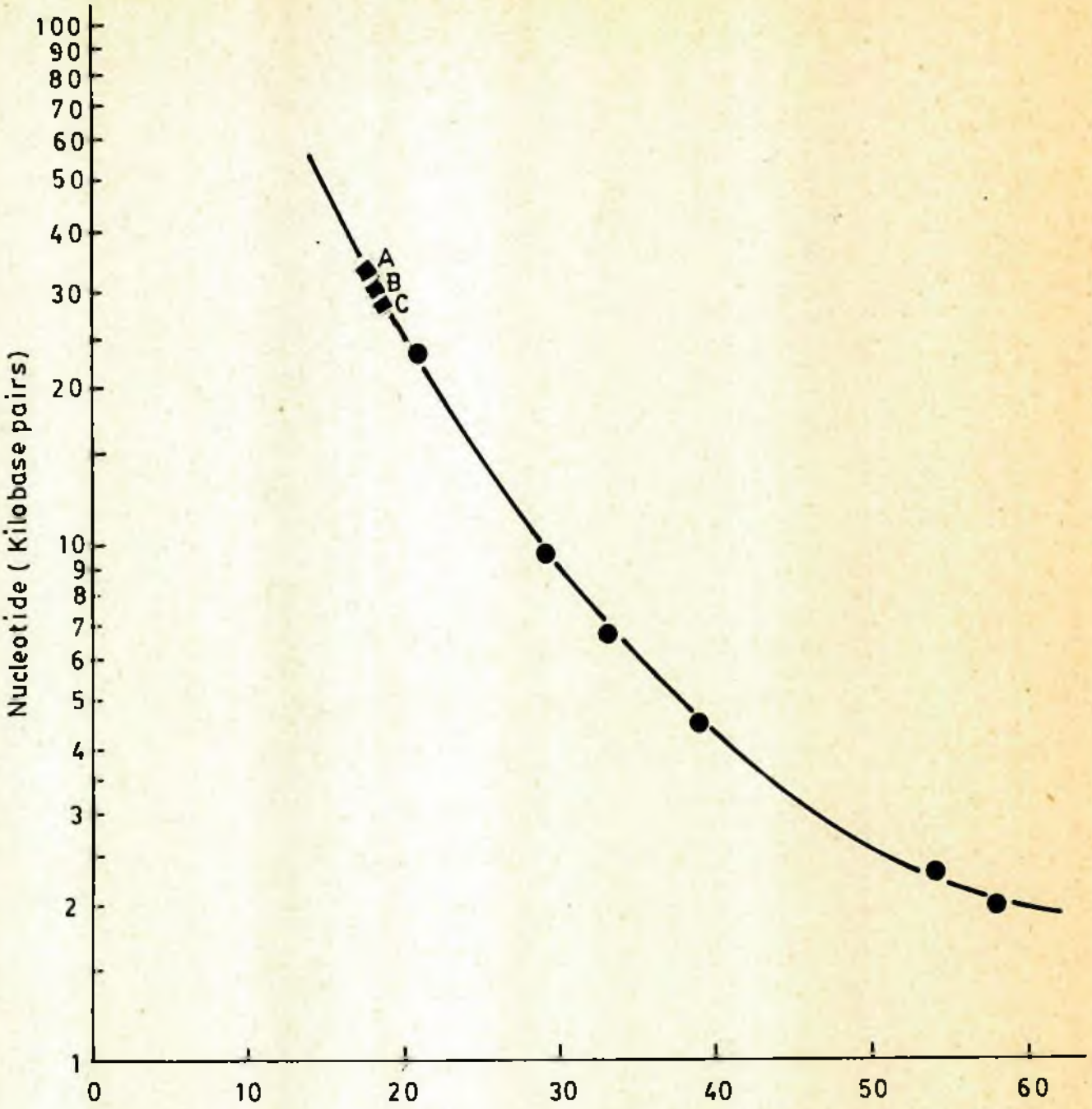



Fig.38

# DISCUSSION

# DISCUSSION

## Ecological study

Both short petioled form (SPs) and long petioled form (LPs) of water hyacinth plants found in ponds throughout the whole year comprised the present study (Table 1). The depth of water in the pond did not seem to affect the presence or absence of different forms.

## Kind of water hyacinth (LPs and SPs) in ten selected ponds

Ten ponds were selected to score the frequency of different forms of water hyacinth during different seasons of a year (Table 2).The selected ponds were surveyed once a month. SPs were exclusively found in 5.27% of the ponds, LPs in 47.41% and a combination of both LPs and SPs in 47.32% (Table 3).According to Avadhani *et al.*(1984) there are two forms of water hyacinth: the floating and the anchored form. Avadhani *et al.* (loc.cit.) also reported that the floating form is associated with deep water and/or crowded conditions. In the present study SPs were generally found to be free floating, but in winter these also survived as anchored form.Generally LPs were anchored to the ground but they were also found in deep water(Fig.2a).Both LPs and SPs were found in deep water and also in crowded condition. This observation contradicts

that of Avadhani (1984).The effect of fluctuating water level on the growth and morphology of water hyacinth has been studied by several workers. According to Gopal and Sharma (1981) the change in water level does not affect the growth. Gopal and Sharma (loc.cit.) also mentioned that water hyacinth is an amphibious plant which can easily produce both emerged and upland type according to circumstances. This observation is in agreement with the present study.

Jamil (1990) reported that water hyacinth plants growing on the shaded side of the pond were tall,those in the partly shaded part were medium sized and those growing under the full illumination of the sun were short with bulbous petioles. The above observations do not agree with the present study as both LP and SP forms were found to grow well in shaded as well as in illuminated areas of the pond. Jamil (loc.cit.) also mentioned that depending on the environmental conditions water hyacinth plants could be classified into three types : (I) tall with elongated petioles, (II) short with bulbous petioles and (III) medium with bottle-necked petioles.In the present study only two forms of water hyacinth were observed which corresponded to type (I) and (II) described by Jamil (1990).Thomas (1984) reported three types of water hyacinth. Variation among them was so pronounced that some of these appeared to him as distinct species.Thomas (loc.cit.) observed that small types had thick bulbous inflated petiolar

base and the tallest type only thick long petioles.The observation of Thomas (1984) on the presence of small bulbous and tall nonbulbous type agrees with the findings of the present study. Wolverton (1979) reported that water hyacinth plants possess spongy swollen petioles which allow it to float. This finding of Wolverton (loc.cit.) is in agreement with the present study. Wolverton also mentioned that in crowded condition the petioles became tall and slender. The findings of the present study differed from those of Wolverton because both LPs and SPs were found to occur in crowded conditions (Figs.1a and 1b).

Morphological differences between LPs and SPs

Only two forms of lilac-blue flowered water hyacinth were recorded in the ponds under observation. These were : LPs and SPs. Some vegetative and floral differences of both natural and cultured LPs and SPs were found to be statistically significant, while some were insignificant.

Plant height

The difference of height between LPs and SPs was statistically significant at 1% level (Table 4 a).

Reza and Khan (1981) reported three types of water hyacinth and their height vary from 20 to 70 cm,20 to 30 cm and less than 20 cm which does not agree with the present findings. However, Ueki's (1978) data on the height (40-100 cm)

of water hyacinth corresponded roughly to those recorded here for LPs (20 - 109 cm)in the present study (Table 4 a; Figs. 3 a and 3 b).

Size of petioles

The differences between LPs and SPs in size of the petioles were found to be significant at 1% level (Table 4 b).

Backer (1951) reported that in young individuals,petioles were short,spongy and very much swollen,while in adults, petioles were much longer (up to 30 cm). The length of the petioles described by Backer (loc.cit.) was comparable to that of SPs ( Table 4 b). The present observation indicates that the swollen form is not the young stage of the plant, as mature SPs with swollen petioles were found to flower as abundantly (Fig.2 b) as LPs.

Size of leaf blade

Leaf blades of LPs were found to be larger in size in comparison to those of SPs, the differences being statistically significant at 1% level (Table 4 c; Figs.4 a and 4 b).

Jamil (1990) reported that leaf area per plant was greater in emerged plants. Jamil's (loc.cit.) observation agrees with the present study (Table 4 c). Thomas (1984) reported three varieties of water hyacinth, the length of leaves of which were as follows: (i) 2 to 3 cm, (ii) 10 to 15 cm and (iii) 40 to 60 cm. Thomas (loc.cit.) also observed the length of the leaves ranging from 15 to 20 cm. In the

present study the length of the leaf blade in LPs was found to be 6.0 to 22.7 cm and that of SPs was 2.6 to 10.5 cm (Table 4 c). In this character the data of Thomas (loc.cit.) on the length of leaves (15 to 20 cm) were closer to those recorded for leaf blade of LPs (6 - 22.7 cm). Reza and Khan (1981) reported three types of leaves : big, medium and small while in the present study only two types, namely, big and small leaves were found.

Length of roots

In LPs roots were found to be longer in comparison with those of SPs, the differences were statistically significant at 1% level under natural condition and 5% level under cultured condition (Table 4 d; Figs.5 a and 5 b).

Reza and Khan (1981) reported the length of the roots as follows : (i) 10 to 90 cm (long type), (ii) 10 to 30 cm (medium type) and (iii) 5 to 15 cm (short type). In the present study, the roots were found to range from 5 to 53 cm in LPs and 2.1 to 35 cm in SPs (Table 4 d). The maximum length was found to be 53 cm (LPs) instead of 90 cm reported by Reza and Khan (1981).

Length of inflorescence

The inflorescences were longer in LPs than in SPs (Figs.6 a and 6 b), the differences being significant at 1% level (Table 5 a).According to Subramanyam (1961) the length

of the inflorescence varied from 15 to 30 cm. The length of inflorescence in LPs was 23.0 to 36.5 cm and in SPs it was 11.0 to 25.5 cm. The findings of Subramanyam (1961) came close to those observed in the present study.

Size of flowers

The size of the flowers was bigger in LPs than in SPs. The differences were significant at 1% level under both natural and cultured conditions (Table 5 b; Figs.7 a and 7 b). However, the difference of length of flower in cultured condition was significant at 5% level.

Posterior lobes

The length of posterior lobes was found to be bigger in LPs than in SPs. The differences were significant at 5% level. The length of posterior lobe was reported to be 3.0 to 3.5 cm by Subramanyam (1961), which corresponded to both LPs (3.0 to 4.9 cm) and SPs (2.7 to 4.7 cm) of the present study (Table 5 c).

Three outer lobes

The average length of the three outer lobes of LPs and SPs were found to differ at 5% level in natural and 1% level in cultured conditions (Table 5 d).

Three inner lobes

The average length of three inner lobes between LPs and SPs differed significantly at 5% level (Table 5 e).

Length of style

In the present study mid-styled form lilac-blue flowers of LPs and SPs were observed. The average difference in the length of style between the two forms was found to be significant at 1% level in natural and 5% level in cultured conditions (Table 5 i; Figs.8 a and 8 b). The average style length reported by Barrett (1979) in mid-styled flowers was 2.44 ± 0.11 cm to 2.61 ± 0.07 cm, which correspond to that of LPs (2.40 ± 0.02 to 2.51 ± 0.02 cm) of the present study (Table 5 i).

Barrett (1977) reported three types of styles, long, medium and short. Barrett (loc.cit.) also mentioned that mid-styled form predominated over long-styled form. Barrett (1979) reported that out of 1272 flowers, 1020 were mid-styles and 252 were long-styles. Barrett (1982) mentioned that form with short-styles was found to be predominant in the Amazon Basin and Mato Grosso of Brazil but was absent from North and central America and the Caribbean. In the present study all the 10,000 lilac-blue flowers examined were mid-styled form with the exception of three, where styles were short. No long-styled form was found. Barrett (1977) also observed that the top of the mid-styles was lavender coloured while short-styles were completely white at the top. In the present study all the styles were completely white (Figs.8 a and 8 b). Haigh (1936) reported that most of the flowers were long-styled. Reddy and Bahadur

(1977) reported that water hyacinth is heterozygous with mid and long-styled forms.Muller (1871),Agharkar and Banerjee (1930),Bock (1966) reported that in Brazil, India, the USA and Jamaica almost all the flowers were mid-styled form, this finding is in conformity with the finding of the present investigation.

Size of ovary

The differences in the average length and breadth of ovary were found to be significant at 1% level in natural and at 5% level in cultured conditions (Table 5 j).

The following morphological characters were found to be statistically insignificant : breadth of the posterior lobes, breadth of the three outer and inner lobes,size of the yellow blotch, length of the stamens and number of leaves per plant (Tables 5 c to h and 6 a). The average length of the large and small stamens of mid-styled flowers, reported by Barrett (1979) was found to be higher (2.86 $\pm$ 0.14 cm to 3.36 $\pm$ 0.11 cm in large stamen and 1.10 $\pm$ 0.23 cm to 1.74 $\pm$ 0.08 cm in small stamen) in comparison with the findings of present study (Tables 5 g and 5 h).Backer (1951) reported three short anterior stamens and three long posterior stamens.The present study is in agreement with Backer's (1951) observation. Subramanyam (1961) reported that stamens are curved, glandular and hairy. The present study is in agreement with the above findings.

Number of flowers per inflorescence

The number of flowers per inflorescence varied greatly between LPs and SPs. The difference is significant at 1% level (Table 6 b).

Khan and Halim (1987) reported 10 to 20 flowers per inflorescence for Bangladeshi specimens; Subramanyam (1961) in India reported 8 to 35 flowers per inflorescence; Backer (1951) in Malaysia reported 3 to 35 flowers per inflorescence; Agharkar and Banarjee (1930) in India reported 10 to 15 flowers per inflorescence. Bock (1966) reported 8 to 18 flowers per inflorescence in California and 4 to 7 flowers per inflorescence in Louisiana. In the present study LPs were found to bear 6 - 40 flowers per inflorescence, while the number of flowers in the inflorescence of SPs were 2 to 14 (Table 6 b).

Barrett (1980) reported that the average number of flowers per inflorescence varied from 5.30 $\pm$ 0.10 to 22.90 $\pm$ 1.80. The present findings in respect of number of flowers per inflorescence were close to those of Barrett (loc.cit.) in overall range i.e. the combined range of SPs and LPs being 7.76 $\pm$ 0.77 to 20.40 $\pm$ 2.53 (Table 6 b).

Pollen characteristics

Pollen stainability was found to be more or less similar in LPs and SPs, but the size of both oval and round pollen grains was greater in SPs in comparison to the LPs, the

differences being statistically significant (Tables 7 b and 7 c).

The average length (88.60 ± 3.80 μ) of pollen grains reported by Barrett (1977) was found to be greater than LPs and SPs but average breadth (30.30 ± 2.10 μ) of pollen grains reported by Barrett (loc.cit.) was found to be smaller than LPs and SPs (Table 7 b).

Comparative study of the growth of two forms of water hyacinth in tap water and in 1% Hoagland solution

During the present study it was found that all LPs gave rise to LPs during late spring to autumn and all SPs gave rise to SPs during winter to autumn. But it was also found that SPs gave rise to 25% LPs and 75% SPs during mid-autumn and 75% LPs and 25% SPs in late autumn. The above observations suggest that SPs gradually produce more LPs in late autumn. However, the height of LPs derived from SPs was much smaller in comparison to those derived from LPs grown under the same condition. SPs were found to produce only SPs again during winter to autumn.

Cultured LPs produce 80% SPs and 20% LPs during winter, while again in spring all LPs propagated true to type. This observation indicates the possibility that seasonal variation somehow influences the genes responsible for conversion of one form to another.

Effect of different concentrations of Hoagland solution on the height of SPs

SP form of plants were grown in different concentrations

of Hoagland (1, 5 and 10%) solution. In this experiment it was found that height of the plant and length of the petiole were not affected by this treatment, they were found to be more or less similar to those observed in control(Table 8a). The values of F indicating the differences among different concentrations of Hoagland solution were found to be insignificant (Tables 8 b and 8 c).

Thomas (1984) reported that plants were found to be smaller in size in the absence of nutrients. The present finding disagrees with the findings of Thomas (loc.cit.), as plant growth was not affected by different concentrations of the nutrients supplied in the medium.

Haider (1984) and Balasooriya *et al.* (1984) reported that plants with swollen petioles turned into long and slender petioles with the increase of nutrients in water. The findings of Haider and Balasooriya *et al.* (loc.cit.) differed with the findings of the present study. Backer (1951) reported that swollen form and long and slender form of water hyacinth belong to the same taxon. The present findings are not in agreement with the findings of Backer (loc.cit.). Backer's (1951) conclusion does not seem to be based on facts. If SPs represent young and immature stage of water hyacinth, they would not have bloomed abundantly like LPs in identical cultured conditions (Figs.10 c and 10 d). In nature also flowering of SPs was common (Fig.2 b).

Avadhani *et al.*(1984) reported that swollen form and plants which are anchored in the soil, are of two different morphological forms.The findings of Avadhani *et al.* (loc.cit.) are in agreement with those recorded in the present study.

Propagation of daughter plants from LPs and SPs

Water hyacinth reproduces vegetatively both by means of stolons and rhizomes.

Propagation of daughter plants from stolons of LPs and SPs

Propagation of daughter plants from stolons of LPs was found to be earlier in comparison to that of SPs. The differences were found to be significant at 1% level (Table 9 a).

Review of literature revealed contradictions regarding the propagation or doubling time of water hyacinth. Freidel (1979) reported that the time of propagation was less than one week; Das (1969) reported it to be within 9 to 15 days; Wolverton (1979) reported that it was after ten days; Penfound and Earle (1948) found it to be 15 days;Balasooriya *et al.*(1984) reported that acording to Bagnall *et al.*(1974), Batanouny and EL-Fiky (1975) and McLean (1922) it to be 9.2 to 22.0 days; Thomas (1984) reported, it was within 12 days; Bakar *et al.* (1984) observed it was 10 to 14 days. In the present study, doubling or propagation time of LPs was found to be 5 to 14 days, whereas this period was 6 to 26 days in SPs (Table 9 a).When the data are compared with those repor-

ted earlier, it becomes obvious that some authors dealt with only LPs, while the others with SPs. Balasooriya *et al.* (1984) seemed to have studied SPs,as the doubling time reported by them was close to that observed in SPs.Others (Freidel 1979; Das 1969; Wolverton 1979; Penfound and Earle 1948; Thomas 1984; Bakar *et al.* 1984) probably studied LPs, as their findings corresponded approximately with those of LPs.

### Emergence of root from daughter plants of LPs and SPs

Emergence of roots from daughter plants of LPs and sPs showed significant variation. It was found to be earlier in SPs in comparison to that of LPs (Table 9 b).

### Emergence of daughter plants from rhizomes of LPs and SPs in tap water or 5% Hoagland solution

Both LPs and SPs produced their own types in 5% Hoagland solution (Appendix I), however, in tap water LPs remained quiescent but SPs produced SPs. It has been observed that the response of LP rhizomes was totally negative in tap water but positive to some extent in 5% Hoagland solution (this might be due to the presence of nutrients in the Hoagland solutions); but in case of SP rhizomes, the response was found to be reverse (more positive response in tap water than in Hoagland solution). In 5% Hoagland solution the response of SP rhizomes was more positive than that of LP rhizomes (Table 9 c).

As the response of the rhizomes did not appear to be

affected by nutrient or pH (pH 7.3 in 5% Hoagland and 6.76 to 6.80 in tap water), it could be due to some genetical factors.

Exceptions were recorded in 0.5% cases, in which rhizomes were found to produce both LP and SP forms of plants (Fig.14 d). If LPs and SPs represent two genetically divergent forms; it becomes difficult to explain as to how the same rhizome could produce both SP and LP forms. It is true that under ordinary conditions each form maintained its morphology, but both forms sometimes developed from the same rhizomes, which points to the possibility that factors other than genetical may be involved in transforming one form to the other. That this factor is not nutritional, has been proved by our experiments, specially designed for this purpose.

### Development of seed by natural and artificial self-and cross-pollination

Ridley (1930) observed that the water hyacinth plant reproduces only vegetatively in its adventive range. Bock (1966) reported the absence of sexual reproduction in California. Penfound and Earle (1948) observed mature fruits from May to December. Mitchell and Thomas (1972) did not find seeds or mature fruits in any areas of South America. Bruhl and Sengupta (1927) obtained seeds by artificial pollination. Agharkar and Banerjee (1930) reported that bagging

the flowers produced fruits indicating that self-pollination occurs to a considerable extent. Agharkar and Banerjee (loc. cit.) also noted that pollination was not always followed by fruit formation because of interference by other factors (temp. and humidity). In general Agharkar and Banerjee (loc. cit.) reported that seed formation took place in submerged flowers. Subramanyam (1961) also mentioned that seed formation took place only in submerged inflorescences. Das (1968) observed that submergence was not necessary for seed formation. The present study agrees with the observation of Das (loc.cit.). The confirmation was obtained when seed-bearing capsules resulted from artificial pollination of flowers, supported above water level, by means of net (Figs.15 a and 15 b). Tag El Seed and Obeid (1975) reported that in water hyacinth in artificially self-pollinated plants, most of the flowers formed capsules containing seeds. The present findings are in confirmity with Tag El Seed and Obeid's (1975) findings, as most of artificially pollinated flowers produced seed-bearing capsules. Agharkar and Banerjee (1930) in India reported that about 35% of the flowers were successfully naturally pollinated. In the present study, only 2.80% of the flowers were naturally pollinated in LPs and 5.95% in SPs. Haigh (1936) in Sri Lanka reported that 29% of the flowers bore capsules and McLean (1922) in Bengal observed 1% of the flowers setting seeds,while according to Backer (1951) in Malaysia and Java fruits were unknown.

Barrett (1979) reported that seed production in artificial and naturally pollinated flowers was found to be higher in mid-styled than in long-styled form. In the present study, long-styled form was totally absent and only mid-styled form was found to produce seeds. The number of seeds were found to be higher in artificially self-and cross-pollinated plants in comparison to the naturally pollinated plants (Tables 11 a to c).

Barrett (1977) reported that out of 2,546 flowers, 94.7% produced capsules with an average of 143.3 seeds per capsule. Barrett (loc.cit.) also reported that no significant differences in seed set had been observed between self-and cross-pollinated plants of Louisiana, Florida, Mexico and Southern Brazil. Haigh (1936) observed an average of 15 seeds per capsule, while Tag El Seed and Obeid (1975) observed an average of 99 $\pm$ 80.3 seeds per capsule.Das (1968) observed an average of 41 seeds per capsule.

In the present study two forms were observed (LPs and SPs).In 100 naturally pollinated plants, in LPs, out of 1533 flowers, 2.80% produced seed-bearing capsules (Table 10 a) with an average of 34.50 $\pm$ 5.96 seeds per capsule(Table 11a) while in SPs out of 588 flowers 5.95% produced seed-bearing capsules (Table 10 a) with an average of 12.75 $\pm$ 2.19 seeds per capsule (Table 11 a). In 50 self-pollinated plants, in LPs out of 648 flowers,87.50% produced seed-bearing capsules

(Table 10 b) with an average of 158.72 ± 13.94 seeds per capsule (Table 11 b) and in SPs, out of 334 flowers, 87.72% produced seed-bearing capsules (Table 10 b) with an average of 86.65 ± 8.15 seeds per capsule (Table 11 b).In 18 cross-pollinated plants,in LPs,out of 225 flowers, 93.78% produced seed-bearing capsules (Table 10 c) with an average of 107.92 ± 10.26 seeds per capsule (Table 11 c); while in SPs, out of 113 flowers, 94.69% produced seed-bearing capsules (Table 10 c), with an average of 71.52 ± 6.60 seeds per capsule (Table 11 c).

Barrett (1980) reported that seed set was significantly higher in cross-pollinated plants in comparison to self-pollinated plants in California, Sudan and Calcutta.

The findings of Barrett (loc.cit.) are in agreement with the present study (Tables 10 a to c).

The number of fruits or capsules per plant also varies greatly, Dekimpe (1957) observed only four capsules out of 139 inflorescences; Das (1968) observed 11 capsules out of five inflorescences. Tag El Seed and Obeid (1975) reported zero to 16 (average 1.5 ± 2.3) capsules per inflorescence; and some workers observed five capsules per inflorescence.

In the present study, out of 100 naturally-pollinated inflorescences, 1 to 14 (average 2.05 ± 0.64) capsules per inflorescence in LPs and 1 to 4 (average 1.50 ± 0.21) capsules per inflorescence in SPs were observed (Table 11 a). In

self-pollinated plants, out of 50 inflorescence, 1 to 25 (average 11.40 ± 1.44) capsules per inflorescence in LPs and 1 to 10 (average 5.70 ± 1.41) capsules per inflorescence in SPs were observed (Table 11 b). In cross-pollinated plants, out of 18 inflorescene 2 to 17 (average 11.67 ± 0.09) capsules per inflorescence in LPs and 1 to 9 (average 5.94 ± 0.50) capsules per inflorescence in SPs were observed (Table 11 c).

It was also observed that the number of seeds per capsule was found to be much more variable. Dekimpe (1957) reported three to 364 seeds per capsule; Gopal and Sharma (1981) reported two to 160 seeds per capsule; Penfound and Earle (1948), Robertson and Thein (1932) reported 50 to 60 seeds per capsule; Muller (1883) observed more than 260 seeds per capsule; Tag El Seed and Obeid (1975) recorded five to 452 seeds per capsule; Francois (1964) obtained upto 390 seeds per capsule, while Reddy and Bahadur (1977) obtained a maximum of 67 seeds per capsule.

In the present study, in naturally pollinated plants one to 108 seeds per capsule in LPs and one to 45 seeds per capsule in SPs were observed. In self-pollinated plants, one to 273 seeds per capsule in LPs and one to 226 seeds per capsule in SPs were observed. In cross-pollinated plants, two to 206 seeds per capsule in LPs and three to 148 seeds per capsule in SPs were observed. The number of seeds per

capsule was found to be higher in self-pollinated plants than in cross-pollinated plants in both forms.

Oki and Ueki (1984) reported that the emerged plants produced more inflorescences and seeds than floating plants. The present study agrees with this observation.

In the present study it was found that some capsules contained seeds while others did not. The percentage of seed-bearing capsules was found to be higher in artificially self-and cross-pollinated plants in comparison with naturally pollinated plants (Tables 10 a to c). On the other hand the number and percentage of empty capsules were found to be higher in naturally pollinated plants in comparison with the artificially self-and cross-pollinated plants (Tables 10 a to c). From the above observation it may be concluded that both artificial self-and cross-pollinations produce a greater number of seed-bearing capsules with a higher number of seeds per capsule in both forms.

Morphological appearance of seeds

Parija (1934) observed that seeds were minute with an oval base and tapering apex and characterized by hard seed coat with 12-15 longitudinal ridges.

In the present study, morphological appearance of seeds was more or less the same as described by Parija (loc.cit.) but the number of longitudinal ridges were found to be 10 in the present investigation instead of 12 to 15 as reported

by Parija (loc.cit.).

Size of the seeds

In artifically self-pollinated plants, the size of the seeds was found to be statistically significant at 5% level (Table 13 b) and in artificially cross-pollinated plants, the average length of the seeds was found to be statistically significant at 1% level (Table 13 c).

Germination of artificially self-and cross-pollinated seeds

Biswas and Calder (1954) found that water hyacinth seeds can retain their viability in dormant condition for five to seven years; Robertson and Thein (1932), Penfound and Earle (1948) found water hyacinth seeds failing to germinate in water. Das (1969) reported that scarification of seeds with dilute sulphuric acid was helpful for seed germination. Wolverton (1979) reported that water hyacinth seeds germinated only when the exact process of drying and rewetting had taken place. Obeid and Tag El Seed (1976) reported that different workers studied germination of seeds of water hyacinth for 90 years; some of their findings were conflicting. Tag El Seed (loc.cit.) also reported the results of Crocker (1907) who found that water hyacinth seeds germinated within seven days when kept constantly in water.Haigh (1936) in Sri Lanka found that seeds germinated after seven days from the date of collection and found neither drying nor

prolonged dry storage necessary for germination. Haigh (loc. cit.) also found that high temp. and intense light induced germination. Hitchcock *et al.* (1949) agreed that high temp. encouraged germination.Muller (1883) believed that in Brazil desiccation was essential for germination.Robertson and Thein (1932) in Burma and Parija (1934) in India concluded that germination was governed by alternate wetting and drying. Hitchcock *et al.* (1949) reported that seeds stored under dry condition took twice as much time for germination as those stored wet. Tag El Seed and Obeid (1975) reported that very few capsules set seed in the Nile. Tag El Seed (loc.cit.) also reported that Parija (1934) found seeds remained viable for several years and after two years of dry storage, 78% of the seeds were still viable. Parija (1934) suggested that the cause of dormancy of seeds was due to hard seed coat, which offers mechanical resistance and prevents the entry of oxygen into the seed. According to Parija (loc.cit.) alternate wetting or chemical action loosened the seed coat facilitating the entry of oxygen, there by causing germination.

Barrett (1980) surveyed 19 populations and found that seed production occurred in all of the 19 populations but only three of them grew into seedlings. Barrett (loc.cit.) also reported that although seeds are produced in most populations, they frequently fail to produce plants because of the absence of suitable environmental conditions for germination and seedling establishment.

Oki and Ueki (1984) reported that submerged condition was favourable for seed germination and growth of seedlings. Barrett (1977) reported that in California, Malaysia and parts of India there was little or no seed set or seedling development.

In the present study different techniques were followed for the germination of seed (Table 14). Very little response was obtained through tissue culture techniques. In MS solid medium, percentage of germination (only rudimentary shoot) in both LPs and SPs was more or less same, but they died after 16 days. In liquid Hoagland medium, sucrose was added in different concentrations. In Hoagland + 1% sucrose only LP seeds responded (25%) and also in 2% sucrose, LP seeds responded. But in 4% sucrose only SP seeds responded. However, they all degenerated after two months. In Hoagland medium shoots were slightly taller in comparison to those observed in MS medium (Table 14 d i and 14 d ii a to d).

Mitosis and karyotype analysis of LPs and SPs

The no. of chromosomes in LPs and SPs was found to be same (2n = 32). Banerjee (1974) also reported the same no. of chromosomes. However, they differed considerably in their chromosome length. Depending on the chromosome lengths LPs and SPs were found to differ at 5% level. Of the 16 pairs, on the basis of chromatin length, three types of chromosomes were observed (long, medium and relatively short) in both the

forms. Out of these, LPs had four pairs of long chromosomes against one pair in SPs. In the medium chromosome length group, LPs were found to have eight, while SPs had seven. There was a marked difference between LPs and SPs in the number of relatively short chromosomes.In LPs the number was found to be four and in SPs it was eight.No short chromosome was found in either of the forms (Tables 15 a and 15 b).

In the large chromosome group, the four pairs in LPs were submetacentric (submedian) while there was only one large chromosome pair in SPs, which was also submetacentric. Of the eight medium chromosomes, in LPs two were metacentric (median) and six submetacentric; the SPs showed the same number of submetacentric and one metacentric chromosome. The differences in chromosome morphology in the relatively short chromosome group were remarkable. In SPs, eight chromosomes were equally divided between metacentric (m) and submetacentric (sm) but in LPs this proportion (m:sm) was 1:3 (Table 15 c).

Three types of chromosomes were also reported by Krishnappa (1971), four pairs of long, eight pairs of medium and four pairs of short chromosomes. This report is more or less similar to those observed in LPs (Table 15 a). Banerjee (1974) reported four types of chromosomes. The observation of Banerjee (loc.cit.) disagrees with the present study.

From the mitotic study it may be concluded that the

difference in karyotype and chromosome length between LPs and SPs is an indication that they represent two different forms of water hyacinth. Since chromosomal abnormalities, such as presence of bridges at anaphase are seen only in LPs (Fig.24), not in SPs, the former may have originated or have been derived from the latter.

Chemical composition of LPs and SPs

Sun-dried samples of LPs and SPs were analysed and their protein, ash, phosphorus and potassium contents were determined in the present study.

According to Reza and Khan (1981),water hyacinth plants are of three types and they all contain high percentage of protein and phosphorus. Reza and Khan (loc.cit.) reported that the percentage of protein was found to be 21.19 in large, 8.21 in medium and 14.90 in small type.

In this study, the percentage of protein was found to be 8.31 to 15.63 with an average of 12.16 in LPs while in SPs it was 5.31 to 16.43 with an average of 11.87 (Tables 16 a and 16 b).The percentage of protein was also estimated by other workers. Taylor and Robins (1968) reported 4.7 - 9.2%; Osman *et al.* (1975) reported 5.6-12.1%; Boyd (1968) observed 12-19.8%; Philipp *et al.*(1983) reported 7.2-19.8%; Meksongsee (1984) reported 16%;Abu Bakr (1984) reported 49.6%;Wolverton and McDonald (1979) reported that the average percentage of protein was 22.30. Wolverton and McDonald (loc.cit.) did not

mention the type of plant studied.

The percentage of ash, reported by Reza and Khan (1981) was 17.60 in large, 14.99 in medium and 17.30 in small type. According to Wolverton and McDonald (1979) the percentage of ash was 15.1. Philipp *et al.*(1983) reported that the percentage of ash was found to be 18.1 to 24.2. In this study the percentage of ash was found to be 9.78 to 16.60 in LPs while in SPs it was 10.80 to 18.90 (Table 16 a).

The percentage of phosphorous reported by Reza and Khan (1981) was 0.53 in large, 0.51 in medium and 0.43 in small type.According to Wolverton and McDonald (1979) it was found to be 0.89 and Philipp *et al.*(1983) reported that it was 0.31 to 0.68.In this study the percentage of phosphorus was found to be 0.74 - 1.51 in LPs while in SPs it was 0.68 -1.80 (Table 16 a). Gosset and Norris (1971), Boyd and Scarsbrook (1975) reported that nitrogen and phosphorus level in the plant correlate with the content of the nutrients in the growth medium. According to Reza and Khan (1981) the percentage of potassium was found to be 0.07 in all of the three types.Philipp *et al.*(1983) reported that it was 2.58 to 5.32. In LPs, the percentage of potassium was found to be 0.66 to 2.11 while in SPs it was 0.32 to 1.68 (Table 16 a).

Chemical composition of the water supporting the growth of LPs and SPs

A review of literatures showed contradictions regarding

the effects of nutrients in water,on the growth of different forms of water hyacinths. Whether a specific form (LP or SP) requires some specific nutrients,is still a matter of controversy. Balasooriya *et al.* (1984) reported that phosphates, nitrates present in water had direct effect on the morphogenic expression of plants. Balasoorya *et al.* also (loc.cit.) observed that with optimum supply of nitrogen, dense mats of waterhyacinths were formed and with reduced quantity of nitrogen, growth was retarded resulting in the development of varying size of plants. Gopal and Sharma (1981) reported that the growth of water hyacinth responds directly to the level of nutrients in water especially nitrogen and phosphorus. Gopal and Sharma (loc.cit.) also reported that plants growing in nutrient rich waters were reported to possess long petioles and roots.

Thomas (1984) reported that chemical contents of the water (especially nitrogen) in which the plant grows, significantly affect the size of the plants.Thomas (loc.cit.) also observed that in the absence of optimum quantity of nitrogen, growth was found to be retarded resulting in the small size of plants.

In the present study, it was observed that both LPs and SPs were found to grow in water having both higher and lower concentrations of nitrogen. It was also observed that ponds where SPs were found to grow alone had lower concentration

of calcium and phosphate in comparison to ponds supporting the growth of LPs. On the other hand , ponds having higher concentrations of nitrogen, calcium, phosphate and dissolved oxygen were also found to support good growth of mixed LPs and SPs. The concentration of dissolved oxygen was found to be slightly higher in ponds with SPs in comparison to ponds with LPs, where LPs and SPs were found to grow alone (Table 17).

Ueki (1978) reported that at pH 7.0, water hyacinth was found to grow well. Chadwick and Obeid (1966) also obtained the same results. The present study is in agreement with the observations of Ueki (1978), Chadwick and Obeid (1966) as far as their growth at pH 7.0 is concerned. However, it was also observed that water hyacinth plants (both LPs and SPs) were also found to grow at pH 6.4 to 7.7.

Water purifying capacity of LPS and SPs

The water purifying capacity of the two forms (LPs and SPs) of water hyacinth was observed in the present study (Table 18). The water purifying capacity of water hyacinth was studied by various workers.Sinha and Sinha (1969) reported that water hyacinth plants reduced turbidity of digested sugar waste from 150 mg/l to 10 mg/l in four days. Sinha and Sinha (loc.cit.) also obtained similar results with septic tank effluent.Ferrara *et al.* (1989) also reported that water hyacinth can purify the waste water from olive oil factories.

Ferrara *et al.*(loc.cit.) observed the encouraging results by using water hyacinth as a purifier.

In the present study it was observed that the turbidity of water was reduced from 75 NTU to 1.33 NTU by LPs and 1.52 by SPs whereas in control it was 72 NTU within ten days (Table 18).

Haider (1989) reported that water hyacinth plants may be used as a clarifier for removal of some undesirable substances from water.

Balakrishnan and Gunale (1979) reported that water hyacinth can remove nutrients from raw sewage. Wolverton and McDonald (1979) reported that water hyacinth plant actsas a natural pollution control agent and it can be used in purifying domestic and industrial waste water. Wolverton and McDonald (loc.cit.) also mentioned that it reduces the biochemical oxygen demand (BOD) and the total suspended solid of domestic waste water to a great extent.

In the present study, the total suspended solid was reduced from 314 mg/l to 55 mg/l by LPs and to 59 mg/l by SPs whereas in control it was 292 mg/l within ten days. BOD is also reduced gradually as there in Table 18 and in Fig.25.

The present study agrees with the findings of Sinha and Sinha (1969), Haider (1989), Wolverton and McDonald (1979) and Balakrishnan and Gunale (1979) that water hyacinth can be used to purify the waste water.

## Protein study

The present electrophoretic study of protein patterns showed almost similar mobilities in both LPs and SPs.In both forms 11 bands were found.The length and colour intensity of the bands were more or less identical.

Wain and Martin (1980) compared the three "groups" of water hyacinth.They used healthy root tips for their electrophoretic study. The above authors observed 14 bands consistently. From their study it was clear that as far as the protein bands are concerned in their electrophoretogram, no discernable differences existed among the three forms,despite the disparity of size and their occurrence in different environments. Wain and Martin (loc.cit.) also reported that character differences which separate the water hyacinth morphs do not seem to be associated with a major genetic divergence in response to different environmental pressure. According to the above authors (loc.cit.) some of "differences may have a genetic basis".

## Isoenzyme study

In the present study,ADH and MDH isoenzymes band patterns showed some differences in their intensity of colour and the length of the band, which was higher in SP form than in LP form (Figs.27 and 28). The length and colour intensities of bands as found in the present study between LPs and SPs indicate that they belong to two different forms.

## Comparative study of white-flowered and lilac-blue flowered water hyacinth

### General differences

The general differences observed during the comparative study of lilac-blue and white-flowered water hyacinth are described below.

The lilac-blue flowered water hyacinths are abundantly available in the water bodies of Bangladesh, while the white- flowered ones are very rare. During the present study it was collected only once (from a pond) under natural condition and it was reported to be collected earlier only once from Bangladesh (Mahabuba Halim, National Herbarium of Banglaldesh, personal communication). Review of literature also revealed only one report (Wolverton 1979) of this form, but this report contained no detailed description. Barrett also confirmed the presence of white-flowered water hyacinth (personal communication).

There exists a great controversy over the number of lilac-blue flowered forms. In the present study this was resolved partially in that lilac-blue LPs and SPs were found to maintain separate identity for over seven months (April to November for LPs and December to August for SPs), while during the winter period LP form changes into the other in certain proportions. As for white-flowered water hyacinth, the single collection from nature was SP but it produced only LPs for three years, when cultured in the laboratory.

Difference of flower colour was found to be a constant feature in both the forms; white-flowered plants produced only white flowers (observation period: three years) and the lilac-blue flowered plants never produced any white flower during the study (observation period: six years). No contradiction was found in the literature regarding this observation.

Presence of a border in the yellow blotch of lilac-blue LPs and its absence in white LPs was also a constant feature (Fig.31).

Posterior lobe of white LPs was found to be slightly curved whereas that of lilac-blue LPs was never found to be curved (Fig.32).

In white-flowered LPs mid-or short-styles were present, while in lilac-blue LPs, the presence of only mid-style was a constant feature (Fig.33).

Barrett (1977a) reported that water hyacinth plants are heterozygous and style lengths are controlled by a few pairs of genes. Barrett (1989) also reported that two pairs of genes determine the length of the style.From the observation of Barrett (loc.cit.) it may be suggested that the differencesin stylar length, observed in white-flowered LPs and lilac-blue flowered LPs are due to genetical factors.

Length of the inflorescences of lilac-blue and white LPs differed from each other at 5% level (Table 19).

Measurements of all the floral parts of white LPs and lilac-blue LPs differed at 1% level (Tables 20 a to m). The breadth of the oval shaped pollen grains of the two forms differed at 1% level (Table 21 a).

Significant differences were observed between white-flowered and lilac-blue flowered LPs.However, white-flowered form was found at a period when the comparative studies of lilac-blue LPs and SPs were nearly over (physiological, biochemical, ecological, seed development and germination, water purifying capacity, chemical analysis etc); hence due to the limitation of time, the above biosystematic features of white LPs could not be compared with lilac-blue LPs.

Mobility of total cellular DNA from LPs and SPs of lilac-blue coloured flower&also Lps of white coloured flower by using agarose gel electrophoresis

Preliminary studies on the isolation of total cellular DNA from young leaves of lilac-blue coloured flower LPs and SPs and also white coloured flower LPs indicated that there is a difference in the molecular weight of DNA extracted from the samples mentioned above. The size of the largest fragment of marker DNA ($\lambda$ DNA/Hind III) was 23.1 Kb. In comparison with the size of marker DNA, the size of DNA of three samples was found to be higher. In white coloured flower LPs,the size was found to be 33.00 Kb while in lilac-blue coloured flower SPs and LPs, the size was found to be

30.80 Kb and 28.80 Kb respectively (Fig.38). However further experimentation is necessary to confirm that real differences exist between the size of DNA of the lilac-blue coloured flower two forms (LPs and SPs) and also white coloured flower LPs. Due to the nonavailability of reagents including marker DNA, repetition of this experiment was not possible.

# SUMMARY

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Biosystematic studies of water hyacinth were undertaken. For this purpose morphological, biological, ecological, cytological, biochemical and physiological characters of the two forms namely, plants with long petiole (LPs) and short petiole (SPs) were studied.

1. The following vegetative and floral parts were greater in LPs than in SPs : Length of the petiole, size of the leaf blade, length of the root, length of the inflorescence, size of the flower, length of the style, size of the ovary and size of the seeds.
2. The size of pollen grains was larger in SPs than in LPs.
3. Number of flowers/inf, number of seeds/capsule was greater in LPs than in SPs.
4. (a) The total length of chromosomes of LPs was more than that of SPs.

   (b) The number of long chromosomes, medium chromosomes and relatively short chromosomes were different in LPs and SPs.

   (c) The number of metacentric and submetacentric chromosomes were different in LPs and SPs.

(d) Chromosome bridges were observed in LPs but not in SPs.

5. (a) Emergence of shoots from stolon in daughter plants was earlier in LPs than in SPs.

(b) Emergence of roots of daughter plants was earlier in SPs than in LPs.

6. (a) In water, emergence of daughter plants from rhizomes was observed only in SPs.

(b) In 5% Hoagland solution, emergence of daughter plants from rhizomes was more in SPs than in LPs.

7. (a) Generally LPs gave rise to LPs and SPs gave rise to SPs vegetatively in summer.

(b) During autumn, it was also observed that 75% SPs gave rise to LPs and the remaining 25% SPs gave rise to SPs.

(c) In winter 80% LPs gave rise to SPs and the remaining 20% gave rise to LPs while all SPs gave rise to SPs.

8. Water purifying capacity was slightly greater in LPs than in SPs.

9. There were hardly any differences observed in germination of seeds and chemical composition of the plants of LPs and SPs.

10. Depth of water and chemical composition of water did not affect the frequency of LPs and SPs.

11. Protein bands showed almost similar mobilities in both

LPs and SPs. In both forms 11 bands were found. The length and intensity of the bands were more or less identical.

12. In ADH only one band was present in both LPs and SPs. However, the length and intensity of the band were different in two forms.

13. In MDH two bands were present. Here also, the length and intensity of the bands were different in two forms.

14. Comparative study of commonly available lilac-blue flowered water hyacinth and the rare white-flowered water hyacinth revealed the following differences :

(a) LPs and SPs of lilac-blue forms produced LPs and SPs vegetatively and also vice versa; while SPs of white form produced only LPs vegetatively.

(b) Length of the inflorescence was greater in white LPs than lilac-blue LPs.

(c) All the floral parts were smaller in white LPs in comparison to lilac-blue flowered LPs.

(d) Short-styler flowers were present in white LPs while only the medium-styler flowers were present in lilac-blue LPs. There were no long-styler flowers either in white or lilac-blue LPs.

(e) Posterior lobe of white-flowered LPs was slightly curved while it was not curved in lilac-blue flowered LPs and SPs.

15. DNA pattern was slightly different in all the three forms studied (lilac-blue LPs and SPs; and white LPs). According to the morphological, cytological variation and also the differences in growth characteristics and development of seeds, water hyacinth of Bangladesh may be divided into lilac-blue flowered LPs and SPs. White-flowered water hyacinth was collected only once but cultured in the laboratory for three years during the study. This form was found to be morphologically different from the lilac-blue flowered forms.

# CONCLUSION

# CONCLUSION

It is obvious that nutritional variation hardly affects the forms of water hyacinth, while seasonal variation seems to have a definite effect.It is possible that certain genes in this water plant act like extra chromosomal elements. Under one set of environmental conditions, these genes may integrate into its chromosomes and cause one form of petioles, and in another set these may disintegrate and transform the petioles into another form. Probably the presence or absence of such an element decides whether the petiole of the particular water hyacinth will be short or long. Therefore the whole question of this interchange of forms from short petioled form (SP) to long petioled form (LP) and vice versa should be looked at the molecular level, for which further study needs to be undertaken (DNA pattern).

There may be another explanation. The dominant gene A conditions the expression of anthocyanin pigment in maize grains while those having the recessive allele aa are colourless. There is another dominant gene called dotted gene (Dt). In its presence 'a' reverts to its dominant state 'A' changing the portion of the grain where such mutation occurs to coloured spot. In other words, colourless grain

appears spotted with colour in areas where such reversion takes place (Islam 1984). It is possible that genes like dotted one may be present in water hyacinth which convert SPs to LPs. This explanation does not however clear up the situation in which LPs change into SPs.

Some genes interact more with environment than others. The fact that both forms interchange during the autumn and winter suggests that the genes that separate the two taxa are partially inoperative during this period,when teperature is comparatively low, removing the barrier of distinction between the two forms in respect of their petiole mophology. The warmer period brings these genes into play creating again distinction between LPs and SPs.

Distinct difference in the karyotype of LPs and SPs and the presence of anaphase bridges in a few somatic cells of LPs (Fig.24) indicates that LPs and SPs represent two different forms which have separated them from each other in recent times.

The original idea was to cross the two forms (LPs and SPs) and study the segregation of few characters such as short vs long to decide the taxonomic status of two forms. Such a study could not be undertaken because of failure of germination of hybrid seeds.

The picture that has emerged out from the present investigation suggests that LPs and SPs should be regarded

as two forms, on the ground that (a) SPs are not juvenile stage of LPs and (b) they bloom like LPs and multiply true to their type. The situation changes with the advent of the winter, when a portion of each form changes into the other. Until the question as to why these two forms interchange during the period of cooler months (LPs during December to March and SPs during September to November) is sorted out beyond any doubt by means of appropriate techniques, the question whether white-flowered LPs and lilac-blue LPs represent two different taxa should remain an open question.

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# APPENDIX

# APPENDIX I

Hoagland solution (Hoagland and Arnon 1950)

Constituents :

| Macronutrients | Concentration mg/l |
|---|---|
| $Ca(NO_3)_2$ | 180 |
| $KNO_3$ | 510 |
| $MgSO_4 . 7H_2O$ | 492 |
| $NH_4H_2PO_4$ | 120 |
| **Micronutrients** | |
| $FeSO_4 . 7H_2O$ | 28 |
| $Na_2$-EDTA | 37 |
| $H_3BO_3$ | 28 |
| $MnSO_4$ | 34 |
| $CuSO_4 . 5H_2O$ | 1 |
| $ZnSO_4 . 4H_2O$ | 2 |
| $(NH_4)_6Mo_7O_{24} . 4H_2O$ | 1 |

# APPENDIX II

MS medium (Murashige and Skoog 1962)

Constituents

| Macronutrients | Concentration mg/l |
|---|---|
| $KNO_3$ | 19,000.00 |
| $NH_4NO_3$ | 1,650.00 |
| $KH_2PO_4$ | 170.00 |
| $CaCl_2.\ 2H_2O$ | 440.00 |
| $MgSO_4.\ 7H_2O$ | 370.00 |
| **Micronutrients** | |
| $FeSO_4.\ 7H_2O$ | 27.80 |
| $Na_2$-EDTA | 37.30 |
| $MnSO_4.\ 4H_2O$ | 22.30 |
| $H_3BO_3$ | 6.20 |
| $ZnSO_4.\ 4H_2O$ | 8.60 |
| KI | 0.83 |
| $Na_2MoO_4.\ 2H_2O$ | 0.25 |
| $CuSO_4.\ 5H_2O$ | 0.025 |
| $CoCl_2.\ 6H_2O$ | 0.025 |
| **Vitamins** | |
| Glycin | 2.00 |
| Nicotinic acid | 0.50 |
| Pyridoxine-HCl | 0.50 |
| Thiamine-HCl | 0.10 |
| Inositol | 100.00 |
| Sucrose | 30,000.00 |