

COMPARATIVE ECOLOGY OF SELECTED HERBS AND SHRUBS IN
RELATION TO SOIL MOISTURE AND SOIL TYPES

BY
SABIHA SULTANA

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DECLARATION

This is to declare that the thesis entitled “ Comparative ecology of selected herbs and shrubs in relation to soil moisture and soil types” submitted by Sabiha Sultana for the award of the degree of DOCTOR OF PHILOSOPHY IN BOTANY is based on the results of experiments carried out by her under my guidance and supervision. The thesis is based on original research work and has not been submitted to a degree of any other university.

Dr. A.K.M. Nazrul-Islam
Professor
Department of Botany
University of Dhaka
Dhaka, Bangladesh

To my beloved Parents, Husband and late mother-in-law and my beloved children

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ABSTRACT

Eco-physiology and nature of adaptation of *Cassia tora*, *C. occidentalis* and *C. sophera* the pioneer of dry derelict land were investigated in relation to environmental conditions. Comparative study with these three *Cassia* spp. was carried out with reference to germination eco-physiology and seedling growth in relation to seed storage, salinity and pH; growth in relation to wet (W) and dry-moist (DM) conditions; sun (100% light) and shade (15% light) treatments and tissue water relation in their ecological significance. The objectives of the present investigation were to know the reasons of abundance of *C. tora* and low population or causes of the absence of *Cassia occidentalis* and *Cassia sophera* in the natural habitat.

Seed morphology of these species showed variation in colour, size and shape and also showed similarities and dissimilarities. Freshly harvested, one week, one month and three months old seeds showed very little germination in *Cassia tora*; in contrast freshly harvested seeds of *Cassia occidentalis* and *Cassia sophera* showed better germination than *Cassia tora*. However, the scarified seeds of *Cassia occidentalis* and *Cassia sophera* showed high percentage of germination. The effect of salinity on germination of three *Cassia* spp. was investigated to know their degree of tolerance at different concentrations of NaCl solution and showed a strong effect on the percentage of seed germination. Germination percentage was decreased in all the three species with the increase of the NaCl concentrations and showed lowest germination at 0.1M NaCl. Lack of germination of normal seeds of *C. occidentalis* and *C. sophera* at 0.2M and 0.5 M NaCl concentrations indicated their sensitivity to salinity.

The seedling length of *C. tora* at 0.05M NaCl was significantly ($p=0.05$) decreased to 5.71cm and further increase of salinity to 0.1M, the seedling length was decreased to less than one third (2.15 cm) from that of control (6.92cm). Seeds of all the species failed to germinate at 0.5M NaCl solution.

Different pH levels did not show any significant ($p=0.05$) difference in seedling growth except *Cassia sophera* which at acidic pH significantly ($p=0.05$) decreased seedling length and dry weight. As far as germination and early establishment are concerned it was established that low germination percentage in *C. occidentalis* and *C. sophera*, and sensitivity to salinity are the factors most likely to exclude the growth, and adaptation in the natural habitat.

The three species grown in wet (W) and dry-moist (DM) conditions and at two light intensities (sun-100% light and shade 15% light) in potted soil to explain the nature of adaptation. Vegetative growth was vigorous in all water regimes in the early stages of growth of three *Cassia* spp. The roots of wet plants were on the top 1-2 cm of the soil surface, stunted and dark brown to black in colour.

In contrast, the roots were penetrated deep into the base of the pot in DM treatment. The individual leaves of *C. tora*, *C. occidentalis* and *C. sophera* at different stages of development were found to be progressively larger with taller petiole

(approximately 70-135 mm) as soil water was decreased i.e. in DM treatment. There was a progressive increase in leaf size, leaf area with the decrease of soil moisture in all three *Cassia* spp. At dry moist treatment *C. tora* started flowering 3 weeks earlier than wet treatment; under DM condition *C. tora* showed flowering 12 weeks earlier than *C. occidentalis* and 17 weeks earlier than *C. sophera*. In wet treatment there is no flower and fruit production at all in *C. occidentalis* and *C. sophera*. However, *C. tora* shows very poor flowers and fruit production under wet condition. Root growth was poor in wet condition. In the dry-moist treatment the largest component of the plant is leaf material in all three *Cassia* spp.

Vegetative growth under sun (100% light) treatment was more vigorous than shade (100% light) condition. At sun (100% light) treatment *C. tora* showed earlier flowering than *C. sophera* and *C. occidentalis*.

The relation between leaf water content and stomatal closure, the rate of transpiration and the relative turgidity under two different soil moisture (wet and dry-moist) and two light (sun and shade) treatments were investigated. The plant showed contrasting behavior with respect to transpiration rate, stomatal behavior and the development of water deficits. Highest total transpiration rate was observed in *C. occidentalis* followed by *C. sophera* and *C. tora*. In all the species, there was a relation between the total water and stomatal water loss. The value of RT was very similar between sun and shade conditions in *Cassia* spp. except *C. sophera* where the value of sun condition is significantly lower ($p=0.05$) than shade condition.

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Cassia tora



Cassia occidentalis



Cassia sophera

CHAPTER 1

GENERAL INTRODUCTION

The study of relations of all organisms to their environments can be divided into

(i) Autecology (Ecological geo-botany or Eco-physiology; Muller-Dumbois and Ellenberg 1974)

(ii) Synecology (Sociologic geo-botany; Muller-Dumbois and Ellenberg 1974).

The first one deals with the ecology of individual species (Clapham 1956) and is also known as physiological ecology i.e. adaptation, distribution and abundance of plants in relation to their function (Bannister 1976) and the later with communities as a whole.

In autecology, attention is sharply focused on a particular organism with the purpose of seeing how it fits into the general ecological picture. In order to understand the ecology of the community, the ecological life histories of plants must be studied. (Baskin and Baskin 1972, 1973, 1988). Therefore, autecology is of necessity, the foundation upon which a sound study of synecology can be built. Salisbury (1927) while stressing the necessity of compiling a “Biological flora of the British Isles” wrote such autecological data are of great value for their own sake and the progress of study of the plant communities is greatly hampered by the lack of information relating to the life history and biotic relations of their constituent species.

Further, until such data are available, it is less valuable to attempt to unroll complex tangle of factors involved in the phenomena of competition. A correct assessment of the ecological niches of any plant can be gained by the study of its autecology (Tansley 1939; Misra 1957).

Olmsted (1941) and Duncan (1952) laid much emphasis on the importance of autecological studies to the applied plant species, especially forestry, range management, soil conservation and weed control. Tansley (1949) even went to the extent of remaining that thorough knowledge of a community cannot be acquired without close study of the autecology of its dominants.

It has frequently been stated or implied that vegetation provides a measure of the environment or may be used as an indication of the total environment. The character of the individual plant and the conditions under which it grows has much to do with the distribution of the species (Clapham 1956).

The “Biological flora of the British Isles” published in the Journal of ecology from 1941 made an important contribution to autecology (Baker 1947; Adams 1952; Beddows 1961, 1967, 1973; Bannister 1965; Bell and Tallis 1973). Autecological studies of selected plants were done by Montasir (1950), Bakshi (1952, 1954), Bakshi and Kapil (1952). They have shown the average seed output per plant and also average reproductive capacity of the species. Substantial amount of work on the autecology of herbaceous plant species was done by Mall (1955).

Weed society of America said that weed is a plant where it is not desired. Professor Beal (1910) defined weed as “a plant out of place”, for example – In a rice field, wheat and jute are weeds and vice versa. So all plants are weeds and all plants are crops. Apart from their practical importance to the gardener and farmer, weeds are of exhaustible interest to Ecologists, Agricultural Scientists and Farmers. (Salisbury and Sir Edward 1961).

In nature, the *Cassia* spp. generally grows in fallow land. *Cassia* belongs to the

family Leguminosae; sub-family Caesalpiniodae. It is a large and predominantly tropical genus of about 580 species of herbs, shrubs and trees with about 20 representatives in India. These three species *Cassia tora*, *Cassia occidentalis* and *Cassia sophera* are widely distributed in Bangladesh and commonly found to grow both in open (Sun) and closed (Shade) habitats. *Cassia tora* (Hindi and Bengali names are Chakunda) is ephemeral (Summer annual), grows profusely also in derelict land and begins to grow in the field just after the first rain i.e. in the middle of March or first week of April and is usually found up to October/November. These plant species are economically important as well as possesses medicinal value. The seeds of *Cassia* spp. are used as a coffee substitute (Burkill 1935) and also reported to be used in indigo dyeing and protein rich feed leaves for livestock.

Cassia tora is an important medicinal plant, belongs to the family of Leguminosae. It is an annual herb grown in tropical parts of India; pinnate leaves and yellow flowers blooming in the month of August to September. Leaves used in ringworm and other skin troubles; seeds used as substitute for coffee and as a mordant in dyeing (Ramachandran *et al.* 1992), it acts as a liver stimulant, mild laxative and heart tonic. The consumption of cooked leaves or use of extract of leaves of this plant helps the body in maintaining the normal level of cholesterol. The alcoholic or vinegar maceration of pounded fresh leaves is used externally to treat eczema and dermatomycosis. The extract of leaves of *C. tora* acts as a nerve tonic. Its powder proves useful in combating indigestion, toning up heart muscles and purifying blood. It is also used as an antidote in case of various poisoning. The plant extract has been reported to possess hyperglycemic actions (Mukherjee, 2003).

Cassia occidentalis is also an ayurvedic plant with huge medicinal importance. Leaves of *C. occidentalis* plant have ethno medicinal importance like paste of leaves is externally applied on healing wounds, sores, itch, cutaneous diseases, bone fracture, fever, ringworm, skin diseases and throat infection (Jain *et al.* 1998) ; (Burkill 1995). Previous pharmacological investigations showed that *C. occidentalis* leaf extracts have antibacterial (Saganuwan and Gulumbe ,2006); antimalarial, (Jafri *et al.* 1999 ; Tona *et al.* 1999) antimutagenic, antiplasmodial (Sharma *et al.* 2000) anticarcinogenic and hepatoprotective activity (Yadav *et al.* 2009).

For more than a third of the world's population, the real energy crisis is a daily scramble to find wood to cook meals. Fire wood scarcity is probably most acute today ` in the under developed countries. About 15% of the world's annual fuel comes from biomass (Anon. 1980). In this aspect herbaceous weeds and shrubs play a vital role by providing biomass for fuel and thus would be a great help to face the firewood shortage to some extent. It may be considered as the cheapest and most accessible form of biomass source in Bangladesh. Because of the immense economic importance and medicinal value these weeds must be protected, which are now a days going to be endangered.

In view of the complexity of the factors involved, it is not surprising that little experimental work has been done to determine the tolerance limits of individual species. Although it is difficult to work out the effect of environment as a whole, conclusion drawn by the study of a single factor at a time are coordinated to have desire results. It is to be understood very clearly that no single factor of the environment affects the plant in "three cardinal point" fashion but it closely follows

the law of “Limiting Factors”.

It is suggested that the differences in growth and distribution of the three species are determined by their general biology. Comparative study of Biology was studied by Myerscough and Whitehead 1966 ; Shamsi and Whitehead (1974) with *Epilobium hirsutum* and *Lythrum salicaria*. Germination ecology in a number of species was done by Caudle and Baskin (1968); Baskin and Baskin (1970, 1972b, 1989) in relation to environmental factors. Seed dormancy type was reported by Thompson and Grime (1979) and causes of dormancy was mentioned by Baskin and Baskin (1989). The influence of salinity on germination response was also studied in halophytes and non-halophytes (glycophytes) Rozema (1975). However, inhibition of germination by salt could play a decisive role in limiting geographical distribution of a species and determining their position in the vegetation (Waisel 1958).

Many seeds do not germinate when placed under condition, which are normally regarded as favorable to germination. Such seeds are said to be dormant or to be in a state of dormancy. Dormancy can be due to various causes. It may be due to immaturity of the embryo, impermeability of the seed coat to water or to gases, prevention of embryo development due to mechanical causes, special requirements for temperature or light, or the presence of substance inhibiting germination.

Seeds, which are surrounded by an impermeable seed coat, will not swell even under favorable conditions. Impermeable seed coats are frequently found in Leguminosae as well as in other groups. The seed coat is usually multilayered membrane containing a number of layers or cell. The seed coats of various species show different permeability to water. He was able to relate these differences to the

composition of the seed coat and especially to lipid components.

The first process that occurs during germination is the uptake of water by the seed in the process of imbibitions, which is determined by the permeability of the seed coat. In case of *Cassia sophera* seed germination even under favorable condition is found to be difficult due to the impermeability of seed coat. Imbibitions of seeds also depend on the pH of the solution. Soil pH was found to determine species distribution by affecting germination.

Jha and Sen (1981) have made detail studies of seed production and germination in purple nut sedge. Nut sedge produces a large number of seeds; but there are conflicting reports about their viability and germination. Most of the work on seed germination and dormancy is a percentage of germination in relation to temperature. Caudle and Baskin (1968) and Baskin and Baskin (1970, 1971 a, b) have found that germination of winter annuals during the summer in the Tennessee Cedar glades is prevented by high temperatures and that germination normally does not occur even if the soil is kept moist for extended periods of time. It was also found that in the field, germination did occur during spring and or summer the seedlings of winter annuals would be killed by dry summer condition.

Eco-physiological study of seed germination is fundamental to the understanding of growth and development of a plant species and is an important aspect of crop interaction. A high correlation exists between the environmental condition of a particular habitat and the successful germination of a particular weed seed. (Sen 1973). A typical winter annual germinates in the autumn, passes the winter in the dormant state, flowers, sets seeds and dies in the following spring. Thus, seeds are

normally liberated in late spring or winter, but they do not germinate until next summer. The reasons for the failure of the *Cassia* spp. (*Cassia tora*, *C. occidentalis* and *C. sophera*) to germinate in the winter are not known. In most species of annuals, there is a change in the ability of the seeds to germinate as they age (Newman 1963). Seeds of annuals are dormant at maturity and must undergo physiological changes (after ripening) before they will germinate. There is evidence that freshly harvested seeds required period of after ripening before they would germinate (Barton 1936). Caudle and Baskin (1968) worked with three winter annuals (*Leavenworthia crassa*, *Arenaria patula* and *Sedum pulchellum*) and have shown that at constant temperatures ranging from 5⁰C to 30⁰C freshly harvested seeds showed high temperature dormancy (20 to 30⁰C). With the increase of age of seeds dormancy disappeared.

Baskin and Baskin (1970) investigated the germination eco-physiology of *Draba verna* (plants of disturbed habitat and road side) in relation to age, temperature and light factors. A high percentage of freshly harvested and one month old seeds were dormant (Baskin and Baskin 1970). Baskin and Baskin (1971b) also investigated with *Leavenworthia* spp. to know the reasons of failure to germinate during summer ; they established that these germination regulating mechanisms are due to adaptation to the seasonally arid glade habitat. Ecological life cycle of *Helinium amarum* and *Holosteum umbellatum* were studied by Baskin and Baskin (1973). They concluded that there are two distinct germination seasons summer-autumn and late winter-early spring. Annual seed dormancy cycles in desert winter annuals (*Eriogonum abertianum* and *Eriastrum diffusum*) were investigated by Baskin *et al.* (1993).

For an annual plant species to persist at a site, even if it has a long lived seed bank, environmental conditions must at least occasionally be favourable for germination and competition of the life cycle. In some habitats this favourable period is unpredictable, while in others it is highly predictable. The primary difference is that seeds of annuals in unpredictable habitats are non-dormant (i.e. are capable of germinating over a wide range of conditions; Baskin *et al.* 1993). There is evidence that pH also affects germination (Singh *et al.* 1975). All these studies indicate that environmental factors including light and dark and temperature may play a part in controlling germination and that these factors need to be investigated in more detail in some species.

The reasons for the failure of these species to germinate during winter are prevented by an internal dormancy mechanism(s). Dormancy in many of the seeds is overcome by a short period of after ripening so that, in nature, a certain percentage of the seeds are nondormant during winter period. In Europe there have been many investigations involving soil plant relationships, especially in connection with the relationships of plant to soil pH and to calcareous and non-calcareous soils. This factor was discussed and reviewed by Braun-Blanquet (1931) and Ellenberg (1958).

There is no other factor but water determines the vegetation of the earth. Life evolved in water that carried out the vital processes of a plant. Relative water content (RWC) of leaves indicates water regime of plants which in turn regulates to greater extent viz. eco-physiological activities such as transpiration, photosynthesis.

There is a need to investigate how soil moisture that differs in physio-chemical properties interacts with other factors to limit the ecological range of a particular

plant species. The chemistry of wetland soils and the changes due to drying and rewetting which affect the growth and distribution of plants will help to explain the nature of adaptation in uncultivated lands, fallow lands and derelict lands. Armstrong and Boatman (1967) have shown an association between the chemical properties of soils particularly redox potential and the distribution types of natural vegetation in wetland soils. In view of the complexity of the environment factors. The effect of drying and rewetting soils may play a major role on the growth, nutrient uptake and distribution of plants. There is evidence that *Erica cinerea* (a species of dry land) is restricted to dry mineral soils, whereas *Erica tetralix* (wetland species) occurs in damp, often waterlogged situations (Bannister 1965, 1966). The work of Bannister (1964c) also indicates that there is a fundamental difference in the response of two species to environmental conditions. Rutter (1955) investigated the comparative dominance of *Erica tetralix* and *Molinia caerulea* which is usually associated with sites which have moving ground water in wet heath vegetation in relation to depth of water table. In Europe there have been many investigations involving soil plant relationships, especially in connection with the relationship of the plants to soil pH and to calcareous and non-calcareous soils. This information has been discussed and reviewed by Braun-Blanquet (1951) and also by Krause (1958) and Ellenberg (1958).

It has frequently been stated or implied that Vegetation provides a measure of the environment or may be used as an indication of the environment. In view of the complexity of the factors involved, it is not surprising that little experimental work has been attempted to determine the tolerance limits of individual species. When

plants are grown in wet conditions their roots are in anaerobic conditions. Previous works suggest that plant species have very different physiological response to such conditions depending on whether they are by nature intolerant or tolerant of flooding (Crawford 1977; Crawford and Taylor 1969; Mcmanmon and Crawford 1971). They have shown that species intolerant of flooding, the oxygen limiting condition causes an acceleration of glycolysis and a subsequent accumulation of acetaldehyde and ethanol which have injurious effects on the plants. Furthermore a malic enzyme decarboxylates the malate present to pyruvate contributing further to ethanol production. In tolerant species there is no acceleration of glycolysis, “malic enzyme” is not found and non-toxic malate is the end product of anaerobic respiration. Gill (1970) has mentioned that there is much observational and experimental evidence of profound differences between species in flooding tolerance and also of significant differences between populations of a single species and he has also given a review of flooding tolerance of woody species.

In the environment, ecological differences in distribution between the species are often related to differences in water availability. Experimental work to investigate the response of growth and internal water relations to water regimes were reported (Etherington and Rutter 1964; Taylor 1960). It was found that growth is reduced before the soil moisture was decreased as far as permanent wilting percentage. Whereas pear, corn and lemon plants have been shown to respond to differences in moisture well above the so called wilting points (Davis1942 ; Furr and Taylor 1959) Progress has also been made in understanding the effect of high moisture and low moisture on growth and nutrient uptake in explaining the adaptation and distribution

of certain plant species. Gore and Urquhart (1966) studied the effect of water-logging on the growth of *Molinia caerulea* and *Eriophorum vaginatum* and reported that *E. vaginatum* grew better in waterlogged conditions and the growth of *Molinia caerulea* was related to soil type. Jones and Etherington (1971) studied dune and dune slack plants under waterlogged and non-waterlogged conditions. The growth (dry weight production) of *Festuca rubra* (dune species) and *Agrostis stolonifera* (dune, slope and slack species) was decreased by water-logging; whereas it was favourable to the growth of *Carex nigra* (wet slack species) and also *Carex flacca* (slack and dune slope species). Growth of these species was related to the uptake of iron and manganese (Jones 1972 b). The growth and nutrient uptake of two closely related species, *Erica cinerea* and *Erica tetralix* was reported by Jones and Etherington (1970). Waterlogged plants of both species took up significantly more iron from soil than plants in non-waterlogged condition.

Since edaphic factors which affect growth and nutrient uptake are both physical and chemical, they must both be considered. Soil moisture directly influences soil water relationship, aeration and permeability through its relationship with inter-particle pore space, and the physical conditions have major influence on the growth and nutrition of plants. Indeed, one of the most complex physiological and ecological problems concerns the need of plants for water and consequence of its shortage. The pattern of soil moisture differs because of variation in texture. The primary effect of water-logging a soil is the virtual exclusion of oxygen (Ponnamperuma 1965; Turner and Patrick 1968). The most important chemical change that takes place when a soil is submerged is the reduction of manganese and iron. Rodrigo and Pollard (1962)

have shown that availability of manganese and iron in soils is dependent on aeration and organic matter. There is evidence that manganese and iron increased in calcareous soil after water-logging (Nazrul Islam 1989) and also in Ganges flood plain soil (Nazrul Islam and Noor Newaz 1991). The reduction and solubilization of iron compounds are much more rapid in the presence of organic matter. To investigate the problem in a comparative way three species of *Cassia* of contrasted ecology were chosen.

There has been a large amount of experimental work to investigate the growth and internal water relations to water regimes (Stanhill 1957). Most of the work has been concerned with herbaceous agricultural plants. Etherington (1962); Slatyer (1958); Stocker (1960) have shown that growth appears to be reduced before the soil moisture was reduced as far as permanent wilting percentage. Seed behavior studies on soil surfaces have shown that the soil micro-topography and water content greatly affect the germination percentage of seeds (Harper and Benton, 1966). Thus germination takes place only if the environment closes to the seed (the microsite *sensu* Harper *et al.*, 1965) and the properties of the seed make possible a net gain in water content. Survival of the seedlings and their dry matter production are related to soil moisture (Jones and Etherington, 1971; Pemadasa and Lovell, 1975; Nazrul Islam and Noor Newaz, 1990) and also in root shoot ratios (Ashenden *et al.* 1975; Akber *et al.* 2012).

From the above discussion it appears that information on growth response on soils with differing moisture regimes is not enough to explain the adaptation and plant

distribution. Hence, it would appear that there is a need to measure the growth response of plant species to moisture supply in soils.

In an effort to resolve some of the problems discussed, comparative aspects of plant response to water stress were investigated in relation to water availability. An approach was therefore, taken by growing three species (*Cassia tora*, *Cassia occidentalis* and *Cassia sophera*) in Wet and Moist conditions (maintained gravimetrically, Read and Bartlett 1972). The effect of moisture on the growth of these species was studied in relation to water availability. There is evidence that (Nazrul Islam 1989) soil water has got strong effect on the chemical changes. The **Chapter 2** includes the germination eco-physiology in relation to age, salinity and pH to explain the adaptability of the three *Cassia* spp. in the natural habitat, and the effect of these factors on the seedling growth is given in **Chapter 3**.

The experiment described in **Chapter 4 and Chapter 6** are the comparative studies of response on the growth of the three species to soil moisture (moist and wet conditions) and light intensities (sun 100% and shade 15% light). The growth was measured from a sequence of weekly harvest. Dry weight, Relative growth rate (RGR), Root: Shoot ratio, number of lateral roots and leaf area were noted to explain the nature of adaptation in relation to soil water and light intensities.

It was found that environmental conditions which favor the growth of these species are the availability of water and light. Low soil moisture and high light intensity are favorable for better growth of these species and the species are very sensitive to wet condition and low light intensity. In the field all these species have been found to grow in dry habitat and occasionally in temporary waterlogged areas during the rainy

season. These species have also been found to grow in open habitat, where they receive full light intensity and occasionally in shade habitat. The distribution of species of wetland and dryland habitats is to a great extent controlled by some aspect of moisture factor and soil type of the environment. Earlier experiment of the effect of moisture conditions (Dry and Wet) on the growth of the three *Cassia* spp. has shown that the species are sensitive to wet conditions.

The epidermis of the aerial parts of vascular plants is perforated by large number of small pores surrounded with guard cells, known as stomata. Stomata are the guardians of gas exchange in plants; they provide paths for CO₂ intake while minimizing the unavoidable efflux of water vapour under continuously changing ambient conditions. The stomatal pore is formed between two guard cells which are specialized cells of the epidermis. The two basic forms of guard cells are: Elliptical (kidney shaped) and Gramineous (Dumbell - Shaped).

Stomata serve two conflicting needs,

- (i) First to conserve water and
- (ii) Second exchange of CO₂ and O₂ for smooth running of metabolic process.

Earlier work (Pandeya *et al.* 1968) suggested that the incidence of light directly controls the epidermal structure and stomatal frequencies in plants of sun and shade habitats. Sen *et al.* (1972) have mentioned that for the opening of the stomata there must be an uptake of water resulting in an increase in the turgidity of the guard cells. The stomatal frequency or density of stomata bearing epidermis varies among species from 20 to 2000 pores mm² with the majority of values falling between 40–350 (Weyers and Meidner 1990). Frequencies of stomata also vary in relation to

water availability. Frequency of stomata was significantly higher ($p = 0.05$) in Jute plant (*Corchorus* spp.) grown at field capacity condition than waterlogged condition (Nazrul Islam and Noor Newaz (1991) and also at different parts of a single leaf (Nazrul Islam and Ahmed 1995) and different regions of a leaf with reference to node distance from ground height. Leaves of a plant growing under different habitats i.e. in sun or shade habitats also show differences in stomatal number (Nazrul-Islam 1988) and size of stomata. Eco-physiological studies of stomatal regulation in some species of Amaranthaceae and compositae were also reported (Nazrul Islam and Sarker 1983).

Stomatal density is the average number of stomata per unit area and stomatal index is the number of stomata expressed as percentage of epidermal cells per unit area. Although the number of stomata per mm^2 of leaf surface may look small, the total number on a plant is usually enormous and commonly runs into many millions. The total number of stomata on a leaf surface can be estimated from stomatal frequency \times leaf area. The frequency of stomata relative to other types of cells on the epidermis may be of greater interest than absolute values. In such cases it may be appropriate to determine the stomatal index (Salisbury 1927). It can be seen that the stomatal index is essentially independent of leaf size once the cells are differentiated. If the frequency and average area of the pores can be estimated, then the total pore area can be calculated as a proportion of the leaf area. Measurement of stomatal aperture is important from the ecological point of view (Slatyer 1967; Barrs 1968; Kramer 1969; Sullivan 1971; Sullivan and Eastin 1975). The stomatal density and stomatal index of *Cassia tora*, *C. occidentalis* and *C. sophera* in isolated epidermal peels of

the plants growing in the natural habitat and also grown in pots were investigated and are given in **Chapter 5**. The main objective of this study was to note general performance in relation to environmental conditions to develop hypothesis to explain the nature of plant adaptation.

These species are resistant to drought and sensitive to high moisture. Adaptation to drought is frequently implied from comparative morphological and anatomical studies. Yanney-Wilson (1963) and Daubenmire (1959), for example, noted that plants of open or dry land habitats are xeromorphic. It was therefore decided to investigate the tissue water relations of these spp. and is included in **Chapter 7**. It was considered that this would provide a useful comparison with the plants of natural habitats. There is evidence that plants of open or sun habitats are comparatively xeromorphic (Daubenmire 1959). In this investigation, attention has been paid to the internal water relations of leaf tissue and the relationship between leaf water content and water potential of leaves (Slatyer, 1958, 1962). These relationships were also used by Weatherley (1950); Weatherley and Slatyer (1957); Jarvis and Jarvis (1963a, b); Bannister (1964a, b); Okali (1971) and Nazrul - Islam (1983) to explain the adaptation of plants to drought.

It is shown that the analysis of tissue water relations of these selected species from the natural environment might show some differences in their physiological behavior that might be useful as a guide for ecological information for a comparative study.

CHAPTER 2

COMPARATIVE ECO-PHYSIOLOGY OF *Cassia tora*, *Cassia occidentalis* AND *Cassia sophera* IN RELATION TO ENVIRONMENTAL FACTORS

2.1. INTRODUCTION

Clapham (1956) has clearly shown that knowledge of the biology of a species is necessary for the understanding of its ecology. This investigation compares the biology of three *Cassia* species, relates them to their limits of habitat and environmental conditions and seeks to give a deeper understanding of their ecology. The three species investigated *Cassia tora*, *Cassia occidentalis* and *Cassia sophera* are typical of derelict land of dry habitat. Information relating to their pattern of growth, physiology, performance, distribution and mutual relationship is not known and is not sufficient to interpret a complex situation. Although the three species studied differ in their habitat requirements they are often successful in occupying newly available territory especially in fallow/derelict land.

All these three species *Cassia tora*, *Cassia Occidentalis* and *Cassia sophera* grow in the wasteland/ derelict land. *C. tora* forms consociation and prefers to occupy flat land, cultivated land and wasteland; in contrast, *C. occidentalis* and *Cassia sophera* grow as solitary plant and do not form consociation and is generally found both on the flat and on slopes. Under natural conditions *C. occidentalis* and *Cassia sophera* are not abundant. In addition, these plants are not found to grow in saline conditions; *C. tora* is found to grow in the Oligohaline zone of the coastal region particularly in the southern region of the country.

Cassia occidentalis has a restricted range and geographical distribution. Usually it is confined to bare- rich wasteland where it is not aggressive after establishment and grows as single individual with other plant species.

Cassia tora forms consociation and found to grow to form large monospecific stands ; these stands persist for 6-8 months and often expand and invade new territory. Occasionally when ground has been bared in the months of winter or early spring *Cassia tora* also forms monospecific stands if the land is irrigated. But the plant species normally tend to be associated with other species of winter season and begin to invade places.

All three species are widely distributed in open places, especially where it is moist. With regard to edaphic factors a requirement of more or less neutral to slightly acid soils is favourable for the growth of these three *Cassia* spp. Field sites showed that soils supporting these species are acidic (pH ranges from 5 to 6.8) in nature.

The process of germination leads eventually to the development of the embryo into seedling. Generally germination is defined as protrusion of some part of the embryo from the seed coat which is the result of growth. Germination naturally is a major factor in the establishment of a plant community as Went (1954) has investigated in some details. The germination process involves “the inception of rapid metabolic activity within the seed, resulting in perceptible the growth of the embryo, first the radical and then aerial parts appearing from inside the testa.” In order to germinate a seed, it must be placed in environmental conditions favourable to this process. Among the conditions required is an adequate supply of water, suitable temperature

and composition of the gases in the atmosphere, as well as light for certain seeds. It appears that there is some correlation between the environmental requirement for germination and the ecological conditions occurring in the habitat of the plant and the seeds. (Mayer and Polijakoff-Mayber 1963).

Baskin and Baskin (1993) have shown that, for an annual species to persist at a site, even if it has a long-lived seed bank, environmental conditions must at least occasionally be favourable for germination and completion of the life cycle. In some habitats, this favourable period is unpredictable, while in others it is highly predictable. Studies on germination responses of seed exhumed after various periods of burial under natural temperature conditions in humid, temperate climates have shown that annual species in unpredictable vs. predictable habitats differ in their germination characteristics.

Baskin and Baskin (1993) also shown that the primary difference is that seeds of annuals in unpredictable habitats are non-dormant (i.e. are capable of germinating over a wide range of conditions) or conditioning dormant (i.e. can germinate over a narrow range of conditions) throughout the year (e.g. Baskin and Baskin 1981,1983a, 1983b, 1983c, 1984a, Roberts and Neilson 1982a,b), while those in predictable habitats exhibit annual dormancy / non-dormancy cycles (e.g. Baskin and Baskin 1983b, 1984b). Dormant seeds do not germinate under any conditions. Thus, in habitats that become favourable for germination and completion of life cycle at unpredictable times, seeds can germinate throughout the growing season for a species, but in predictable habitats germination is most likely to occur at the start of the favourable season-usually in either autumn or spring.

Baskin and Baskin (1971) have discussed that, timing of germination occurs when environmental conditions are favourable for seedling establishment and survival is critical to the success of annuals in a particular environment. In some annuals, germination occurs immediately after seed dispersal because environmental conditions are favourable and seeds are non-dormant.

In other annual species germination is delayed for some time after seed dispersal. Where there is delay in germination, conditions of the environment following seed dispersal usually are such that seedling establishment and survival would not occur for the particular species. Delay in germination by plants growing in a habitat where this occurs, therefore; can be considered to be an adaptation to that type of habitat. The delay in germination following seed dispersal caused by;

A period of true dormancy, i.e.; some internal mechanism (s) within the seed prevents germination.

- i) By Period of quiescence, unfavourable environmental conditions (i.e.; lack of water) cause delay of germination.

Newman (1963) studied the factors controlling the germination of *Aira praecox* and *Teesdalia mudicaulis*. In these two winter annuals he found that the seeds responded to increase temperatures with ageing, but that some germination could take place during the summer if the substrate was continuously moist for several weeks. In a study of the factors controlling germination date of eight winter annuals on the Derbyshire limestone in Great Britain, Ratcliffe (1961) concluded that the seeds

underwent an after ripening period during the summer and germinated in the autumn when moisture conditions was favourable.

Germination is definitely a critical period in the plant life cycle and inhibition of germination by high salt concentration may exclude large segments from its potential distribution area. Thus, the control of germination constitutes a major factor in the zonation and inhabitation of saline habitats (Toole *at al.*1956; Crocker and Barton 1953; Waisel 1958) .

High salt concentration does not have much effect upon seeds unless the later are soaked. At this stage, a salty environment can affect and inhibit germination in two ways;

- a) Preventing uptake of water by the embryo due to the high osmotic potential of the medium; and
- b) Poisoning the embryo, due to toxic effects of certain ions.

Germination of seeds is usually retarded by high concentrations of salt solutions both in halophytes and in glycophytes. The degree of imbibitions, delay or inhibition of germination is proportioned to an increase of the external osmotic potential (Schratz 1934,1939; Ayers and Hayward 1948; Ayers 1952).

The aim of this investigation was to account for: (i) Reasons for failure of the seeds to germinate during winter and (ii) the difference in germination in relation to age of seeds, salinity and pH.

2.2. MATERIALS AND METHODS

2.2.1. Seed collection

For comparative study on the temporal response of seed germination, mature fruits of *Cassia tora*, *Cassia occidentalis* and *Cassia sophera* were collected in the years 2009-2011 from the natural habitats of various locations in and around The Dhaka city. The length of the fruits and number of seeds per fruits were also noted. Fresh weight of the fruit with the seeds and without seeds was also recorded.

Seeds were separated from the fruits, dried in the sun and stored in plastic containers in the laboratory at room temperature until used. The initial experiment was done with fresh seeds just after harvesting fruits from plants.

2.2.2. Germination

Germination tests were performed in petridishes on one sheet of Whatman No.1 filter paper, moistened with distilled water. Four replications of 25 healthy and undamaged seeds were used for each species. All germination tests were carried out at room temperature in the laboratory. Germination was noted at 24 h intervals. Age of the seeds is measured from the time of collection until time of placing them on moist paper. Freshly harvested seeds of species stored for 15 days, 1, 2, 3 and 6 months were used. Final germination counts were made 7 days after placing them on moist filter paper and seeds were considered to be germinated when the radical emerged from the seed coats. (Baskin and Baskin 1998).

Seeds of three species of *Cassia tora*, *Cassia occidentalis* and *Cassia sophera* (with and without scarification) were tested for viability. In addition, the seeds of these species were germinated with different concentrations of NaCl solution (0.05, 0.1, 0.2, 0.5M) and with different pH strength to study their nature of adaptation. Healthy seeds (25 in number) for each species were placed on the moistened filter paper and the seeds in petridishes were moistened daily (approximately 2 ml) with the specific NaCl solution and pH. Germination up to 15 days was recorded and the percentage of germination was calculated. A control with 25 healthy seeds was also done in the same time. In all experiments there were 4 replicates. Maximum and minimum temperatures was recorded during the experimental period are given in Table 2.1.

Table 2.1. Mean daily maximum and minimum temperatures during the experimental period.

Date	Species	Age of the seed	Temperature °C	
			Maximum	Minimum
25.10.2010	<i>C. tora</i>	Freshly harvested seed (0 days)	31	25
2.11.2010		One week	29	21.8
28.11.2010		One month	29	19
30.12.2010		Two months	27	16
1.02.2011		Three months	28	13
10.6.2011		Six months	34	26
4.11.2010	<i>C. occidentalis</i>	Freshly harvested seed (0 days)	29	19
19.11.2010		One week	29	18
08.12.2010		One month	27	16
04.01.2011		Two months	25	12
07.02.2011		Three months	28	13
05.04.2011		Six months	34	25
9.10.11	<i>C. sophera</i>	Freshly harvested seed (0 days)	31	26
26.10.11		One week	31	25
17.11.11		One month	28	21
16.12.11		Two months	23	18
19.1.12		Three months	22	16
15.7.12		Six months	33	27

2.3. RESULTS

2.3.1. *Seed morphology*

The morphological differences among fruits and seeds of the three species are given in Table 2.2. Seeds of the species exhibit variation in colour, size and shape. In addition, there are similarities and dissimilarities between the features of these species. Number of seeds per fruit in *C. occidentalis* and *C. sophera* is approximately three times more than *C. tora*. Seed weight of *C. occidentalis* is significantly lower ($p=0.05$) than the other two species. (Table 2.2)

2.3.2. *Germination and Viability of seeds after storage*

In case of *Cassia tora*, freshly harvested seeds, one week, one month old seeds, showed very little germination i.e. 1 to 6 % only. But in 6 months old seeds, the germination rate increased to 50% with scarified seed and it is 38% without scarification. Low germination in freshly harvested seeds was possibly due to short duration of storage and also possibly for some internal mechanism(s).

The changes in germination capacity with age of the three species are given in Fig. 2.1. (a-f).

Table 2.2. Fruit and seed morphology of three *Cassia* species. 95% confidence limits are also given.

Name of the species.	<i>Cassia tora</i>	<i>Cassia occidentalis</i>	<i>Cassia sophera</i>
Mean length of fruit (cm).	11.65±0.192	10.4±0.089	9.55±0.081
No of seeds /fruit.	18.50±0.603	48.3±1.097	41.50±1.897
Mean Wt. of fruit with seeds. (g)	0.39±0.0239	0.90±0.0176	1.00±0.0168
Mean Wt. of fruit without seeds. (husks) (g)	0.09±0.0048	0.20±0.054	0.28±0.0052
Shape & colour of the seeds.	Seed areole cylindrical. With acute in one side. Dark brown with shining surface.	Ovoid, compressed, hard, smooth and dark olive green with dull appearance.	Broadly ovoid, acute, compressed, light brown to dark olive.
Size of the seeds. (mm)*	5 by 2	4.50 by 4	4 by 4
Weight of 100 seeds.(g)	1.550 ± 0.035	1.383 ± 0.011	1.452 ± 0.032

Each value is the mean of 12 (twelve) replicates measurements and each replicate is the mean of ten fruits. *(length by width).

Although in freshly harvested seeds the germination percentage was very little in the other two species but *Cassia occidentalis* showed highest germination percentage i.e. 37% (without scarification) whereas the seeds showed 94% germination (with scarification). After one week the germination percentage gradually decreased to 93% (with scarification) and 33% (without scarification). As the seeds aged, there was generally an increase in germination after two months; it increased to 94% (with scarification) and 47% (without scarification). After three months, it shows again a tendency to increase germination percentage i.e. 96% (with scarification) and 51% (without scarification). Six months old seeds showed the highest percentage of germination i.e. 98% (with scarification) and 80% (without scarification).

Freshly harvested seeds (October) of *Cassia sophera*, showed germination (99%) with scarification and 38% without scarification (Temperature 31/26⁰C). Then there was a tendency to decrease with the increase of age; i.e. in the month of November (31/25⁰C), December (28/21⁰C) and January (23/18⁰C); it started to increase, i.e. 20%, 29% and 33% without scarification and there was slight decrease 94%, 95% 97% with scarification respectively. After 3 and 6 months storage, germination rate again showed an increasing tendency both with and without scarification. This observation may be related with temperature.

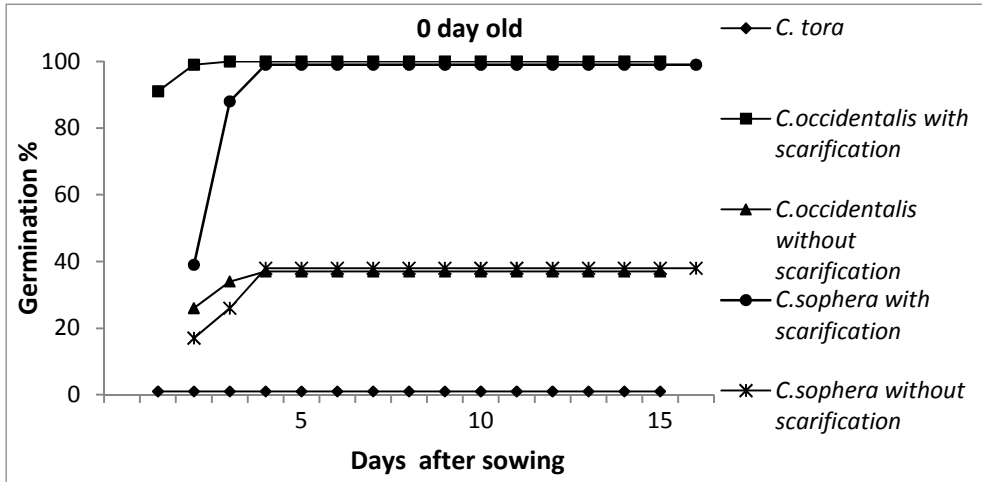


Fig.2.1.a

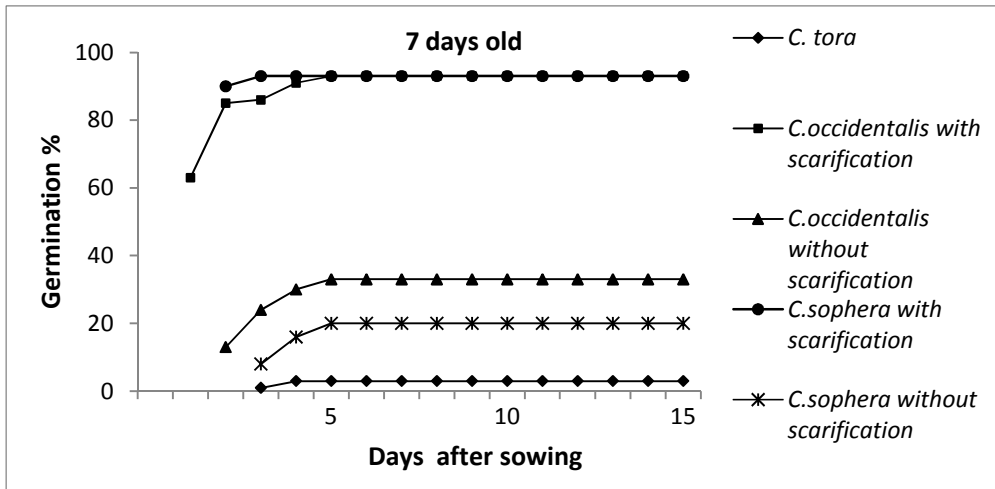


Fig.2.1.b

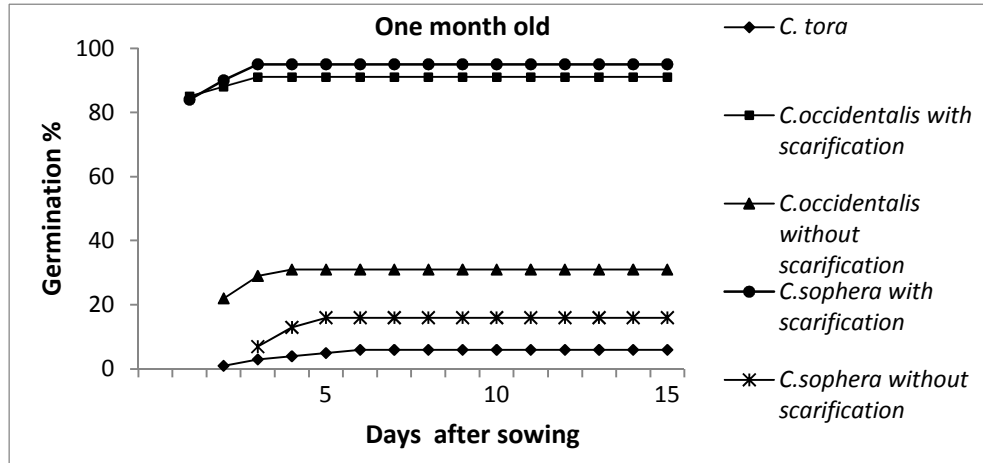


Fig 2.1.c

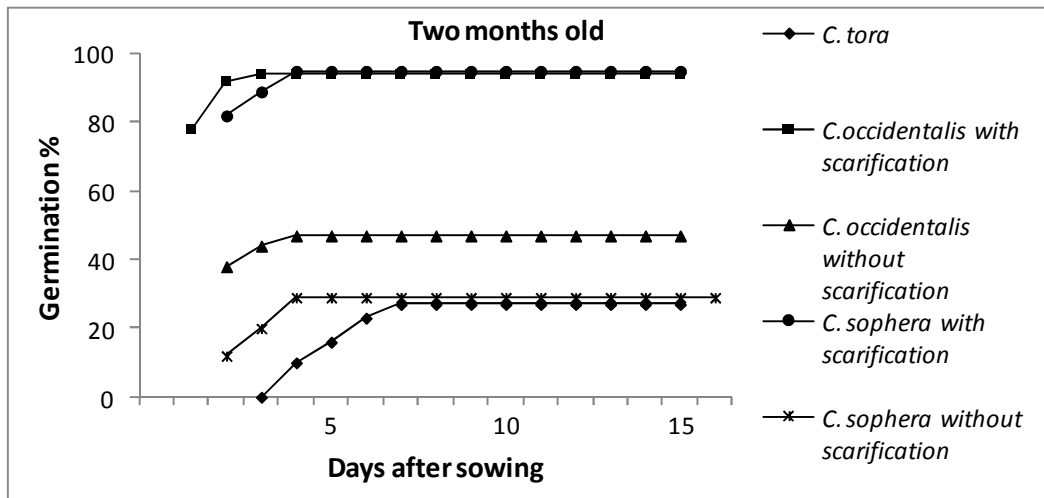


Fig 2.1.d

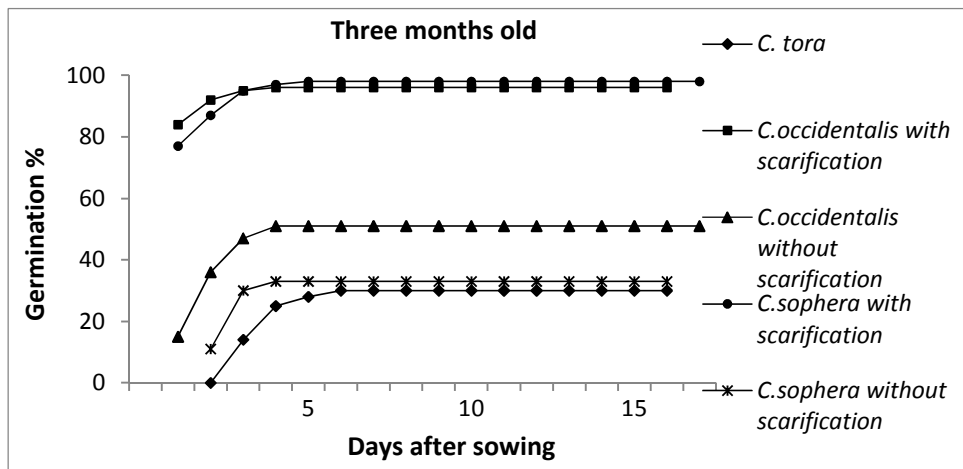


Fig.2.1.e

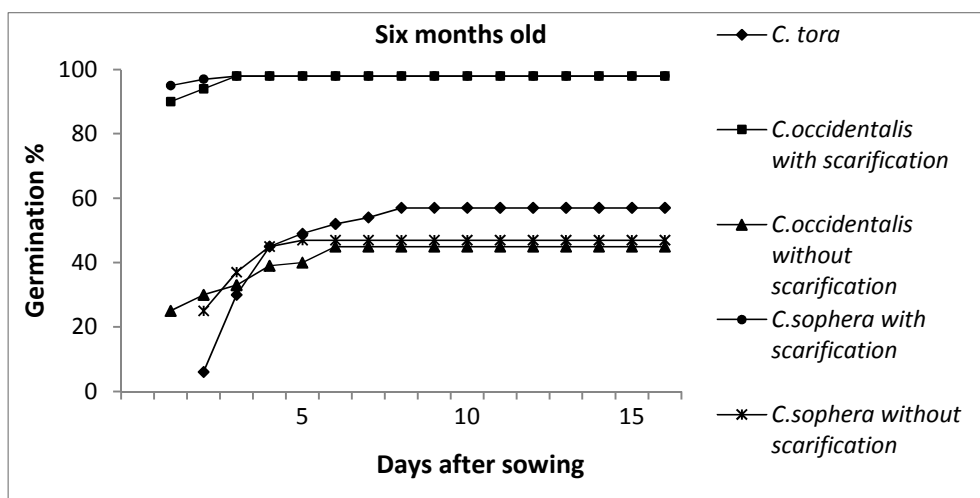


Fig 2.1.f

Fig 2.1. (a-f). Germination of Laboratory stored seeds (0 day to 6 months old) of three *Cassia* spp.

From Fig 2.1 it was observed that, seeds of the *Cassia occidentalis* and *C. sophera* always showed better germination only with scarification than without scarification. This might be one of the reasons that due to hard seed coat and low germination percentage, these two species are not abundant in the field under natural environmental conditions and it is available only when the environmental condition is favourable.

In nature *C. occidentalis* and *C. sophera* do not form consociation possibly because of the low percentage of germination. Since *C. tora* has the high germination percentage it forms consociation. The delay of germination in *C. tora* is caused possibly by a period of true dormancy, i.e. some internal mechanisms within the seed which prevent germination.

2.3.3. Effects of various NaCl concentrations on germination (%)

In the field observation *C. tora* also found to grow in the Oligohaline zone of the coastal habitat where the other two species were not observed to grow.

The Salinity of the medium appears to have a strong effect on the percentage of seed germination in *Cassia* spp. All three species of *Cassia* (*Cassia tora*, *C. occidentalis* and *C. sophera*) responded differently to varying NaCl concentrations during germination (Fig. 2.2).

Germination percentage of *Cassia tora* was highest 51% in control and decreased to 46% at 0.05 M followed by 33% and 30% at 0.1M and 0.2M respectively.

Seeds of both *C. occidentalis* and *C. sophera* showed highest germination percentage in control i.e. 98% (with scarification), 86% (without scarification) and 33% (with scarification), 96% (without scarification) respectively. With the increase of the NaCl concentrations the percentage of germination gradually decreased and showed lowest germination at 0.1 M NaCl. At 0.2 M NaCl unscarified seeds of both *C. occidentalis* and *C. sophera* did not show any germination. In 0.2 M salinity treatment *C. occidentalis* showed 33% germination only with scarification; whereas *C. sophera* showed 58% germination which is significantly higher ($p=0.05$) than that of *C. occidentalis*. Further it was noted that there was no germination in any species at 0.5 M NaCl concentration.

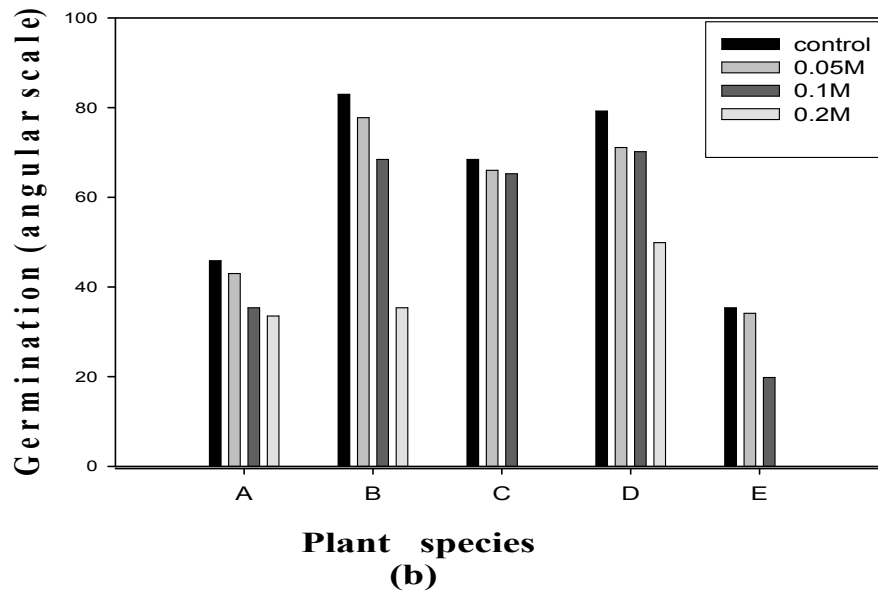
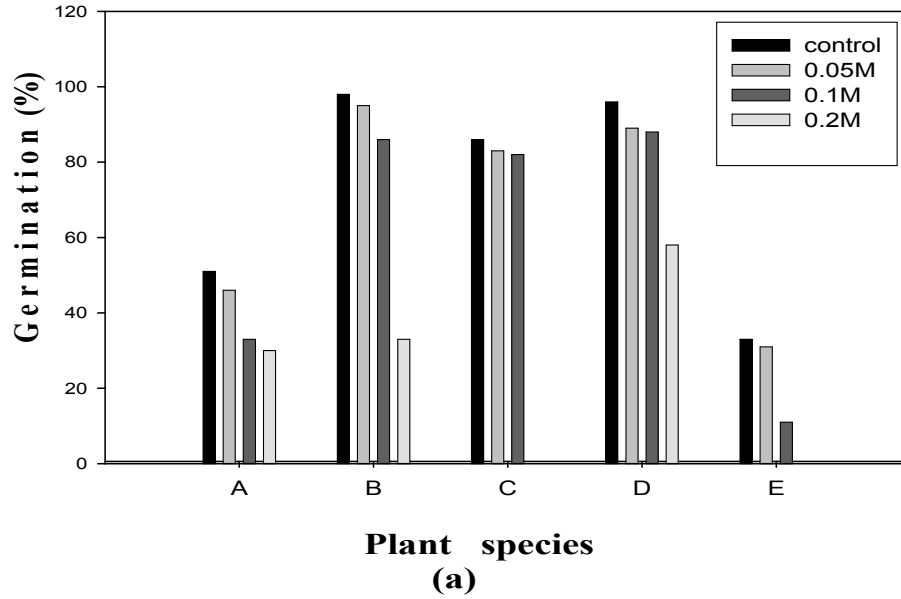
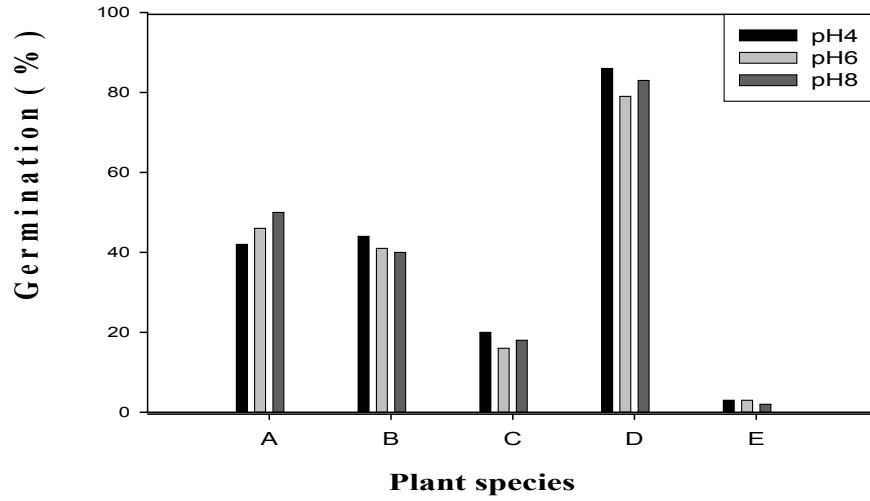


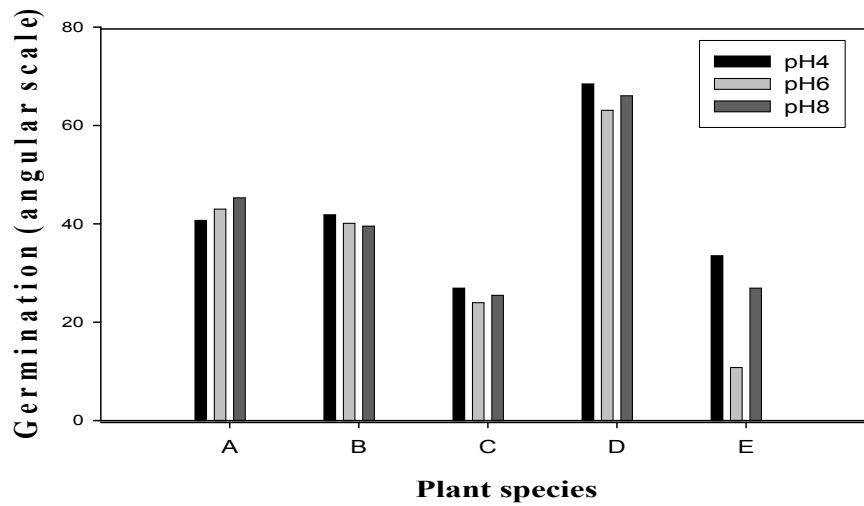
Fig.2.2. (a) Germination rate in percentage (%). (b) Germination rate (angular scale) of three *Cassia* spp. at different salinity treatments. (A=*Cassia tora*, B= *C. occidentalis* with scarification, C= *C. occidentalis* without scarification D= *C. sophera* with scarification E= *C. sophera* without scarification).

2.3.4. Effects of pH on germination

Fig 2.3 shows that all the three *Cassia* spp responded to varying pH levels during germination. Germination rate was very low in *C. sophera* in the unscarified seeds at all pH levels (2 to 3%). However scarified seeds showed high percentage of germination (79 to 86%). *C. tora* (42 to 50%) and *C. occidentalis* (41 to 44% with sacrificed seeds) did not show any significant difference in germination in relation to pH. However the values without scarified seeds in *C. occidentalis* were very low (16 to 20%) *C. tora* showed best germination at alkaline pH. The result suggests that these species can grow in a wide range of pH conditions. In the field it was observed that *C. tora* grows vigorously in the natural habitat where the soil pH ranges from 5 to 7.0.



(a)



(b)

Fig.2.3. (a) Germination rate in percentage (%) (b) Germination rate (angular scale) of three *Cassia* spp. at different pH levels. (A = *Cassia tora*, B= *C. occidentails* with scarification, C= *C. occidentails* without scarification, D = *C. sophera* with scarification, E = *C. sophera* without scarification.)

2.4. DISCUSSION

The morphology of seeds (shape, size and colour) and fruits (fruit length, mean seed and fruit weight) of the three species of *Cassia* showed strong variation in colour, size and shape. Chetan *et al.* (2011) studied the morphology and phytochemistry of *C. sophera*. Their measurement of colour, structure, shape and size of seed is very similar to the present study.

Seeds of the three species of *Cassia* are dispersed from mid to late October and early November. At dispersal they are incapable of germination at any temperature. *Cassia tora* showed lowest percentage of germination in freshly harvested seeds. With the increase of age (storage) the germination increased. *Cassia occidentalis* also showed low percentage of germination but better than *C. tora*. Germination behavior of *Cassia sophera* was very similar to that of *C. occidentalis*. This suggests that some factors are believed to be responsible for dormancy of seeds. Caudle and Baskin (1968) worked with the germination of three winter annuals and found that *Leavenworthia crassa* showed a progressive change of germination with the increase of storage length. In *Arenaria patulalonger*, periods of storage up to 5 months did not result an increase in germination. In contrast, in *Sedum pulchellum* there was very little germination of freshly harvested seeds. In all the three species studied in the present investigation, the fruits are ripen during late October - November. Germination during the winter seems to be prevented by dormancy and water in *Cassia tora*, but in case of *C. occidentalis* and *C. sophera* lack of water is the main factor. Baskin and Baskin (1970) studied germination eco-physiology of *Draba verna*, and noted very little germination in freshly harvested seeds. Baskin and

Baskin (1971b) also worked with germination ecology and adaptation in *Leavenworthia* spp. They have explained that at maturity all the seeds are dormant and germination occurs during late spring and early summer (May and June) apparently is prevented by an internal dormancy mechanism(s). Dormancy in the seeds of many species is overcome by a short period of after ripening so that, in nature, a certain percentage of the seeds are non-dormant (but quiescent) during July and August. In this respect *C. tora* of the present investigation has some similarity with *Leavenworthia* spp. In the field, seeds of *C. tora* began to germinate and grew during the winter months (November – January) if irrigation was done. However, the winter plants of *C. tora* were dwarf in size. This germination regulating mechanisms can be interpreted as an adaptation to the seasonally dry habitat (winter period).

Lack of germination in freshly matured seeds can, therefore, be considered to be due to some internal mechanism(s). The delay of germination in *C. tora* is caused possibly by a period of true dormancy, i.e. some internal mechanisms within the seed which prevent germination. The ability of freshly matured seeds to germinate is the lack of moisture in soil (*C. tora*) and also due to hard seed coat in the remaining two species which inhibits germination. Scarification of seeds clearly showed high percentage of germination in both *C. occidentalis* and *C. sophera*.. Baskin and Baskin (1971b) worked with three species of *Leavenworthia* and have shown that seeds are dispersed in May but germination is prevented by an internal dormancy mechanism (s).

Germination occurs as a result of multiple environmental factors (Bell *et al.* 2009) and in natural environment is often restricted to locations that meet specific environmental conditions. These sites are referred to as 'safe-sites' (Harper *et al.* 1961) or 'regeneration niches' (Grubb, 1977). An insight into eco-physiological characteristics of the species (such as life history and frequency of reproduction, reproductive strategies and seed storage mode) may assist in the prediction of the requirements for seed germination (Bell *et al.* 1993).

The results from these experiments confirm that *Cassia species* seeds take at least 3 days to germinate. Baskin *et al.* (1998) showed that non dormant seeds germinate over a wide range of conditions while dormant ones fail to germinate under any condition.

Conditionally dormant seeds germinate over only a portion of the range of conditions for species (Vegis, 1963). The result of the present study indicates that the seeds of *Cassia* species are not physiologically dormant since they germinated to high percentage without requiring pre-treatment. Therefore, the seeds may either be non-dormant or morphologically dormant. The seeds which are morphologically dormant have undifferentiated or under-developed embryo at the time of dispersal and require a minimum of 5 days for embryo development after which germination can take place (Baskin and Baskin, 1989, 1998). Since seeds of *Cassia* are small, it is unclear whether the embryo of these species is undifferentiated or under-developed at the time of dispersal.

Timing of seed germination is highly sensitive to several aspects of the seed maturation, including water availability, soil nutrients, photoperiod, temperature and light quality (Finch-savage and Leubner Metzger, 2006; Contreras *et al.*, 2008). It was observed that percentage of germination of different species of *Cassia* seeds gradually increased with the increase of age and temperature.

It is interesting to compare the germination of *C. tora*, (a summer annual) with the germination of *Leavenworthia crassa* the freshly-harvested seeds showed very little or no germination at different temperatures; but a few seeds did germinate at the lower temperatures (5 and 10⁰C). As the seeds aged there were an increase in germination at 5, 10, 15 and 20⁰C. Seeds of *Draba verna* are also dispersed in mid to late April. But germination did not occur until September and October and it was evident that germination ability increased with ageing (Baskin and Baskin 1970).

There is evidence that with the increase of temperature, the germination percentage is also increased in *Epilobium hirsutum* (Shamsi and Whitehead 1974). The reason why seeds fail to germinate at low temperature is still obscure. Mayer and Marbach (1982) have suggested the possibility that, membranes themselves are the temperature sensors of seeds.

The entry of water into seed is, however, determined in the first instance by the permeability of the seed coat. Since the seeds of *C. sophera* and *C. occidentalis* are surrounded by an impermeable seed coat and that the imbibitions pressure which develops is not enough to break the hard seed coat, therefore, will not swell even under favourable conditions. However, when these impermeable seed coats are

abraded mechanically at the point of emergence of radical, then the seeds only become permeable to water, which cause more rapid uptake of water followed by rapid emergence of radical than those without scarification. There is evidence that if the suberized layer of the seed coat which prevents the entry of water is fractured or removed, water will enter the seed. In the present investigation it was found that percentage of germination in *Cassia occidentalis* was always significantly higher with scarified seeds in comparison to without scarified seeds.

Salinity (NaCl) showed strong effect on the germination of three *Cassia* spp. and responded differently to varying concentrations of NaCl. There was no sign of germination of *C. occidentalis* and *C. sophera* (without scarification) at 0.2M NaCl; in contrast *C. tora* grew at 0.2M NaCl and indicated its survival in low saline condition. None of the species showed germination at 0.5M NaCl. The tolerance of these species in relation to salinity may be arranged as follows:

C. tora > *C. sophera* > *C. occidentalis*

Increasing salinity always has a decreasing effect on germination for all species (Ayers 1952 ; Ayers and Hayward 1948). *C. tora* showed the highest salt tolerance followed by *C. sophera* and *C. occidentalis*. Greenway (1962 b) with his experiments with *Hordium vulgare* to salinity treatments showed the results of growth of leaf , shoot and roots separately. In general, NaCl treatment decreased the development of leaves. He has also shown that the uptake of sodium by the leaves was the main factor for the decrease of growth. Decreased in germination with increase of concentration of salt has been reported in the rice (Narale *et al* 1969).

Conflicting data exist regarding germination of *Salicornia herbacea* in salt solutions. While some investigators reported highest germination percentage in salt-free media (Feekes 1963); others reported optimal germination in 50% seawater (Walter 1968). Seeds collected from halophytic ecotypes of *Phragmites* (from the saline of Seldon, Israel) tolerated high salinities in germination (up to 0.05 M NaCl) than did seeds of glycophytic ecotypes of *Phragmites* (up to 0.3 M NaCl only).

Effects of pH on germination rate was very low in unscarified seeds of *C. sophera* but scarified seeds showed high percentage of germination. No significant difference in germination was observed in *C. tora* and *C. occidentalis* (scarified seeds) in relation to pH. The results show that the three species can grow in a wide range of pH conditions. It was observed that *C. tora* grew vigorously in the natural habitat where soil pH ranges from 5 to 7. Results of the present investigation with *Cassia* spp. suggest that acidity or salinity at low level has negligible effect on germination. The effect of pH on germination of four common grass species in Ujjain, India was reported by Singh and Billore (1975). No germination was recorded at pH 2.0 in any species. *Iseilema antheboroides* alone was capable of germination at pH 2.5. Seeds of *Apluda mutica*, *Dactyloctenium aegyptium* and *Sehmiia nervosum* showed maximum percentage of germination at pH 5.0.

CHAPTER 3

SEEDLING GROWTH OF *Cassia tora*, *C. occidentalis* AND *C. sophera* IN RELATION TO SALINITY AND pH

3.1. INTRODUCTION

Salinisation of soil is common in arid and semiarid regions where the amount of rainfall is insufficient for substantial leaching. High concentrations of salts have detrimental effect on plant growth (Bernstein 1962, Taiz and Zeiger 2006, Ramoliya *et al.* 2004). Plant species differ in their sensitivity or tolerance to salts (Marschner 1986). There is evidence that organs, tissues and cells at different developmental stages of plants exhibit varying degrees of tolerance to environmental conditions (Munns 1993). It is reported that soil salinity suppresses shoot growth more than the root growth (Maas and Hoffman 1977; Munns 2002, Ramoliya *et al.* 2004). However, fewer studies on the effect of soil salinity on root growth have been conducted (Munns 2002).

The high salt content lowers osmotic potential of soil water and consequently affects the availability of soil water to plants. The salt-induced water deficit is one of the major constraints for plant growth in saline soils. In addition, many nutrient interactions in salt-stressed plants can occur that may have important consequences for growth. The relationship between micro-nutrient concentrations and soil salinity is rather complex and remain poorly understood (Tozlu *et al.* 2000). An understanding of growth and survival of plants under saline conditions is needed for (i) screening the plant species for the afforestation of saline deserts and (ii)

understanding the mechanism that plants use in the avoidance and /or tolerance of salt stress.

In nature the three species of *Cassia* (*C. tora*, *C. occidentalis* and *C. sophera*) appear differently to edaphic factors, climatic factors and their seasonal variation and availability of water. The influence of salinity on germination response was studied in halophytes & non-halophytes (glycophytes) by Rozema (1975). However inhibition of germination by salt could play a decisive role in limiting geographical distribution of a species and determining their position in the vegetation .

Toxic effects of certain ions on seed germination and seedling development had been studied by a number of investigators at the beginning of the century. Stewart (1898) and Harris and Pittman (1918) found that chloride salts were most toxic for germination, sulphate less so, and carbonate the least. Harris (1915) found the relative toxicity of soluble salts to be in the following descending order: NaCl, CaCl₂, KCl, MgCl₂, KNO₃, Mg (NO₃)₂, (Na)SO₄. He also concluded that salt mixtures were not as toxic in soil as in solution culture.

A similar concentration between the ability to germinate under saline conditions and zonation of plants in a salt marsh was also reported by Unger (1965, 1967b). Seeds of *Limonium vulgare* Milll. and *Limonium humile* Mill. slowly germinated in seawater. However, most of the seeds, which did not germinate in such a medium, rapidly germinated after being transferred to fresh water (Boorman 1968). The same was true for *Puccinellia nuttalliana* (Mack and Ungar 1971).

For optimal growth, *Atriplex halimus* and other halophilic species of *Atriplex* require relatively high concentrations of sodium chloride in the soil or culture medium (Black 1956, 1958; Greenway 1968; Brownell 1965). Brownell and Jackman (1966) and Gale and Poljakoff- Mayber (1970) showed that small quantities of sodium are essential for the growth of some *Atriplex* species.

Gale *et al.* (1970) showed that the optimal concentration of sodium chloride in the culture solution for growth of *Atriplex halimus* was a function of environmental conditions.

The present investigation was an attempt to study the seedling growth of the three *Cassia* spp (*Cassia tora*, *C.occidentalis* and *C. sophera*) in relation to salinity and pH.

3.2. MATERIALS AND METHODS

3.2.1. Effects of salinity (NaCl) and P^H on seedling growth

Seeds of three *Cassia* species (*Cassia tora*, *C. occidentalis* and *C. sophera*) were germinated with different concentrations of NaCl solution (0.05, 0.1, 0.2, 0.5M) and with various pH solutions to see the effect on seedling growth. Healthy seeds 25 in number of each species were placed on the moistened filter papers for each NaCl and pH treatments; 4 replications were done and the seeds in petridishes were moistened daily (approximately 2 ml) with the specific NaCl and pH solutions. Seedlings were allowed to grow in the petridishes after germination for 7 days. A control with 25 healthy seeds was also done simultaneously.

After 7 days of growth five seedlings of each species were harvested from each petridish; length of root, shoot and fresh weight of each seedling were taken, then dried in the oven at 80°C for 24 hours and dry weight was then recorded.

3.3. RESULTS

3.3.1. *Seedling growth in relation to salinity*

The growth of seedlings of all three species was best at Control followed by 0.05 M and 0.1 M; and the growth of seedlings was prevented at high levels of NaCl salinity (Plate 1.a; b and c).

The highest seedling length of *Cassia tora* was 6.92 ± 0.32 cm in control and with the increase of salinity to 0.05M, the seedling length was decreased to 5.71 ± 0.90 cm. Further increase of salinity to 0.1 M, the length of the seedling became less than half of 0.05 M and one third to that of control i.e. 2.15 ± 0.40 cm. At 0.2 M the length was lowest (1.66 ± 0.68 cm) and at 0.5 M, seeds did not germinate.

Seeds of *C. occidentalis* (with and without scarification) also showed highest seedling length in control i.e. 7.57 ± 0.94 cm (with scarification) and 4.2 ± 0.82 cm (without scarification) respectively. The length decreased at 0.05 M NaCl treatments i.e. 5.07 ± 0.75 cm (with scarification) and 3.9 ± 0.46 cm (without scarification). At 0.1M it was further decreased to 3.75 ± 0.47 cm (with scarification) and 2.93 ± 0.43 cm (without scarification). In 0.2M it was only 1.95 ± 0.05 cm (with scarification).

Cassia sophera also showed highest length of seedlings in control i.e. 7.74 ± 0.81 cm (with scarification) and 7.28 ± 0.77 cm (without scarification). It was slightly

decreased at 0.05 M NaCl treatments i.e. 7.49 ± 1.28 cm (with scarification) and 6.83 ± 1.12 cm (without scarification). At 0.1M it was further decreased to 6.49 ± 0.28 cm (with scarification) and 3.18 ± 0.11 cm (without scarification). In 0.2M it was only 2.62 ± 0.33 cm (with scarification) but no seedling growth was observed without scarification.

With scarification *C. occidentalis* and *Cassia sophera* germinated in 0.2 M but only rudimentary growth was observed without scarification. At 0.5M NaCl treatment seedling length was prevented in all three species of *Cassia*.

A tentative order of adaptation to salinity in terms of seedling length for the three species may be arranged as follows:

C. tora > *C. sophera* > *C. occidentalis*.

The seedling length of *C. sophera* in different NaCl concentrations is higher than *C. occidentalis*. In spite of this small difference, the results show that both *C. occidentalis* and *C. sophera* are sensitive to salinity. The results suggest that the absence of these two species from the coastal habitat is related to their sensitivity to high salinity.

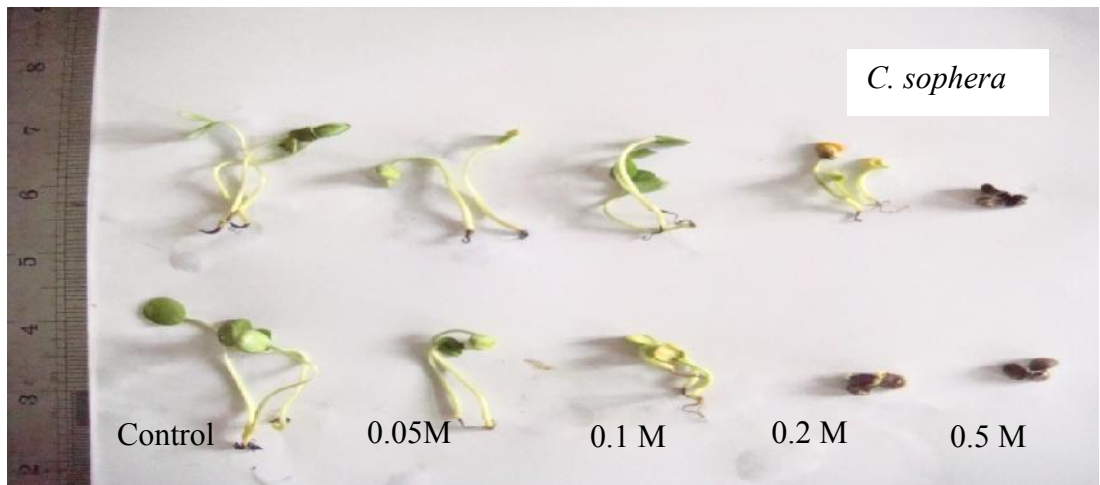
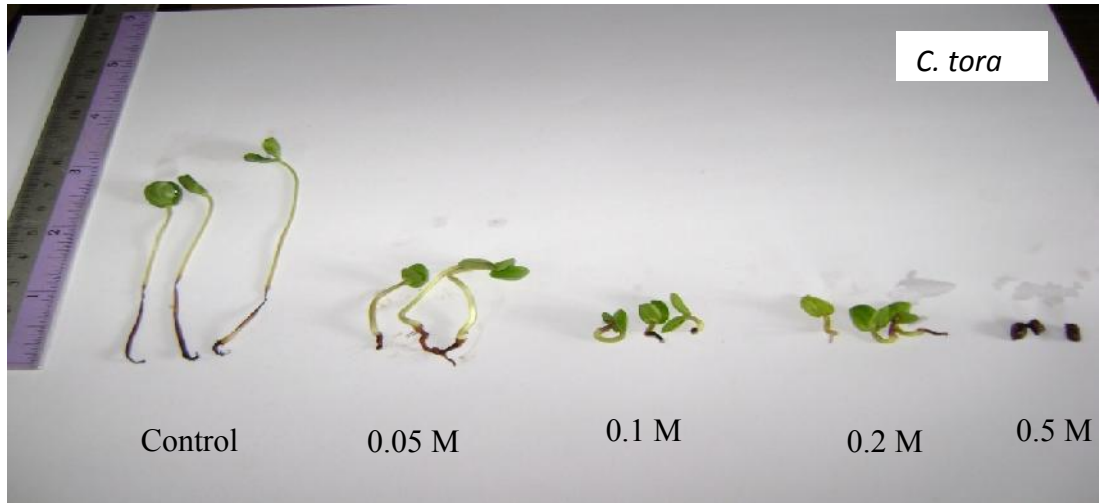
Plate 3.1. Seedlings of *Cassia* spp. at different salinity treatments.

a. *Cassia tora*.

b. *Cassia occidentalis* and

c. *Cassia sophera*.

(In case of *Cassia occidentalis* and *Cassia sophera* the seedlings of upper row are scarified.)



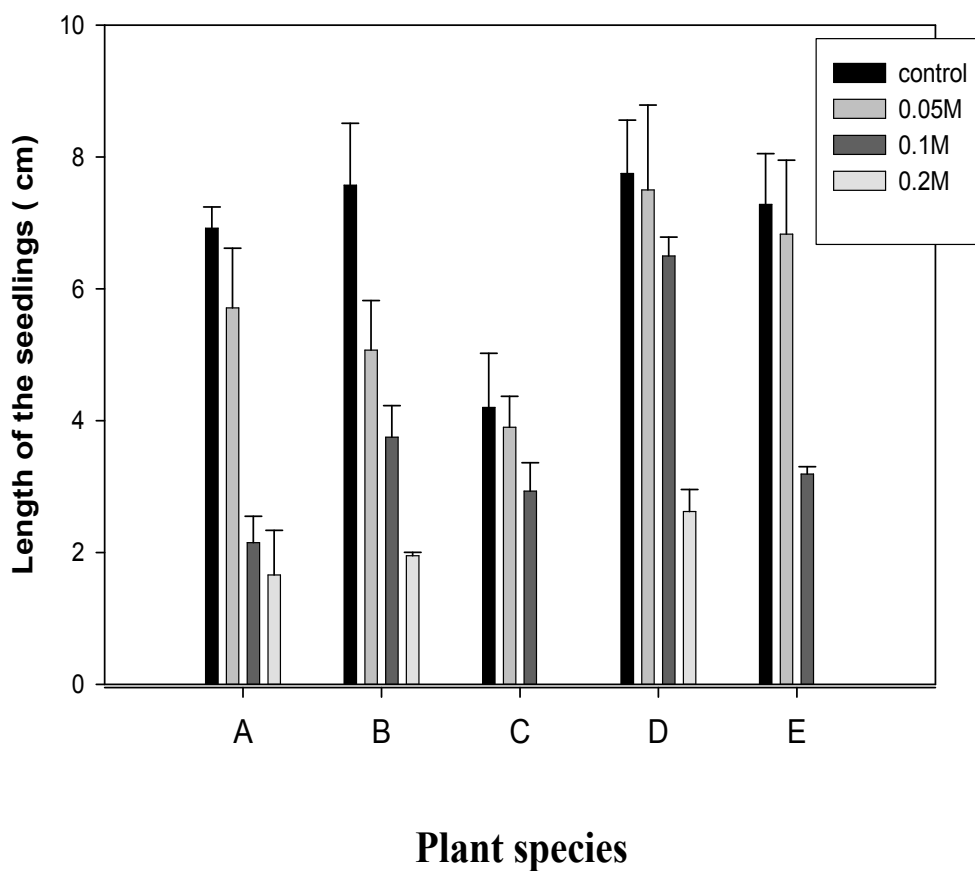


fig. 3.1. Mean seedling length (cm) of three *Cassia* spp. at different salinity treatments. Vertical bars on the graphs show (\pm) 95% confidence limits. (A = *Cassia tora*, B = *C. occidentails* with scarification, C = *C. occidentails* without scarification, D = *C. sophera* with scarification E = *C. sophera* without scarification)

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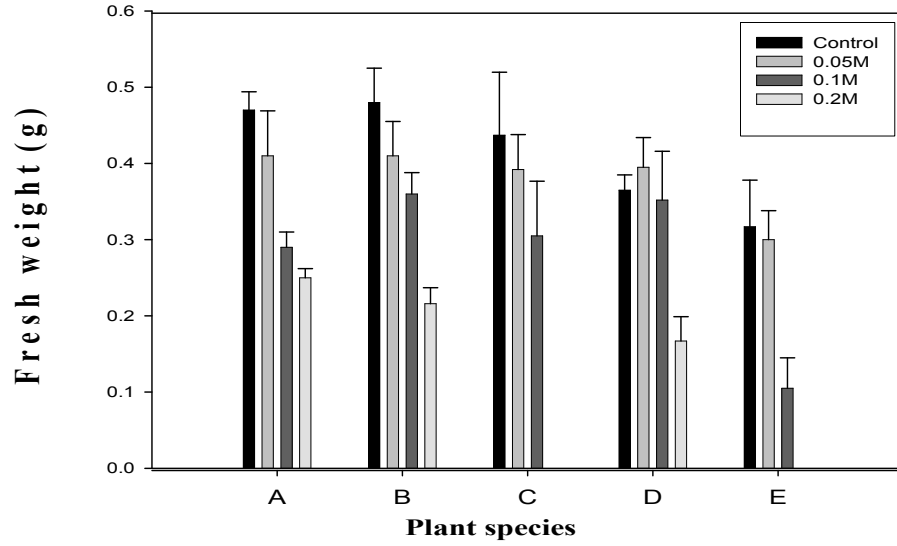
3.3.2. *Fresh and dry weights of the seedlings in relation to salinity*

Fresh and Dry weights due to salt treatment are marked with the increase of salinity levels from 0.1 M onwards in all three species of *Cassia*. (Fig. 3.2)

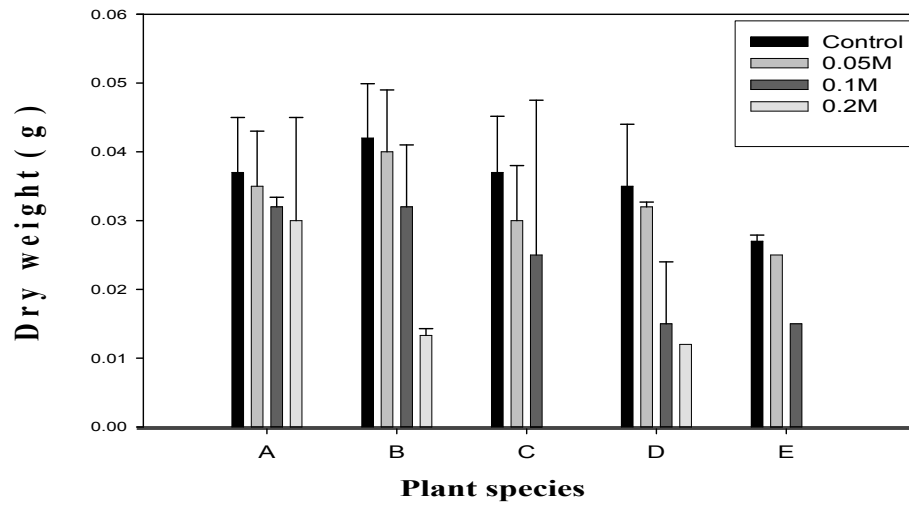
All three species of *Cassia* were sensitive to salinity treatment and showed decrease of fresh weight and dry weight as the salinity levels were increased. There was a significant decrease ($p= 0.05$) of fresh and dry weights (Fig. 3.2) when concentration of NaCl was increased from 0.05 M to 0.1M condition and onward.

Both *C. tora* and *C. occidentalis* exhibited highest fresh and dry weight at control i.e. 0.47 ± 0.024 g (F. Wt.) and 0.037 ± 0.008 g (D. Wt.) and 0.48 ± 0.045 g (F. Wt.) and 0.04 ± 0.008 g (D. Wt.); (with scarification) and 0.44 ± 0.083 g (F. Wt.) and 0.04 ± 0.008 g (D. Wt.) ; (without scarification). With the increase of salinity these values were gradually decreased to 0.41 ± 0.059 g (F. Wt.) and 0.03 ± 0.089 g ((D. Wt.) at 0.05M in *Cassia tora* and in *C. occidentalis* it was 0.39 ± 0.046 g (F. Wt) and 0.03 ± 0.009 g (D. Wt.).

Both species showed drastic reduction of fresh weight and dry weight at 0.2 M salinity treatment. Although the unscarified seeds of *Cassia sophera* exhibited highest fresh and dry weight at control i.e. 0.317 ± 0.061 g and 0.027 ± 0.0009 g respectively; but the scarified seeds of *Cassia sophera* showed highest F. Wt at 0.05 M salinity. i.e. 0.395 ± 0.033 g and lowest at 0.2 M salinity. However, the scarified seeds of *C. occidentalis* and *C. sophera* always showed higher values than the seedlings of non- scarified seeds.



(a)



(b)

Fig. 3.2. (a) Fresh and (b) dry weight (g) of three *Cassia* spp. at different salinity treatments. Vertical bars on the graphs show 95% confidence limits. (A = *Cassia tora*, B = *C. occidentalis* with scarification, C = *C. occidentalis* without scarification, D = *C. sophera* with scarification, E = *C. sophera* without scarification.)

All three species of *Cassia* (*Cassia tora*, *C. occidentalis* and *C. sophera*) exhibited highest fresh and dry weight at control. From the Fig.3.2 it can be said that, the scarified seeds of *Cassia occidentalis* and *C. sophera* always showed higher values than non- scarified seeds. Delay in germination in non-scarified seeds possibly decreased the growth.

3.3.3. Seedling growth in relation to pH

The highest seedling length of *C. tora* was 12.01 ± 0.48 cm in pH 6 and lowest in pH 8 i.e.; 10.25 ± 1.84 cm. Unscarified seeds of *C.occidentalis* showed highest seedling length in pH 8 i.e.; 7.18 ± 1.78 cm and lowest in pH 4 (4.31 ± 0.66) cm. Whereas, scarified seeds showed negligible differences in different levels of pH i.e.; (4.84 ± 1.44) cm, (5.24 ± 0.43) cm and (4.83 ± 1.64) cm in pH 4, pH 6 and pH 8 respectively. (Fig. 3.3)

Cassia sophera showed highest seedling length in pH 6 i.e; (7.42 ± 1.65) cm with unscarified seeds and lowest in pH 4 i.e.; (4.3 ± 0.39) cm. whereas, scarified seeds showed highest length in pH 8 (7.0 ± 0.48) cm and lowest in pH 6 (5.81 ± 0.86) cm.

A tentative order of adaptation to pH in terms of seedling length for three species may be arranged as follows:

C. tora > *C. sophera* > *C. occidentalis*

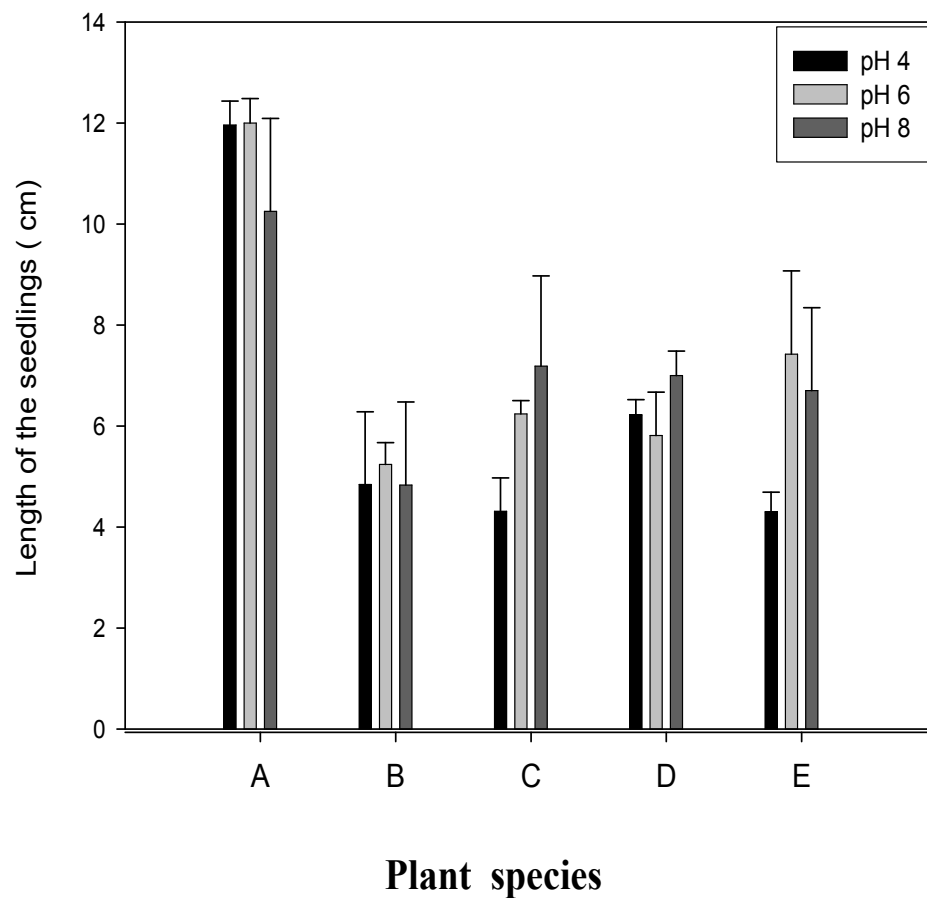


Fig. 3.3: Mean seedling length (cm) of three *Cassia* spp. at different pH level

Vertical bars on the graph show 95% confidence limits. (A = *Cassia tora*, B = *C. occidentalis* with scarification, C = *C. occidentalis* without scarification, D = *C. sophera* with scarification, E = *C. sophera* without scarification).

The seedling length of *C. tora* in different pH levels is higher than *C. occidentalis* and *C. sophera*. The result suggests that these two species may not grow in the field in acid soil.

3.3.2. Fresh and dry weights of the seedlings in relation to pH

Fresh and Dry weights due to pH treatment are marked with the increase of pH levels from 4 to 6 in all three species of *Cassia*. (Fig. 3.4)

The highest fresh weight was found in *Cassia tora* at pH 6 (0.467 ± 0.0590 g) and lowest fresh weight found in *Cassia occidentalis* (scarified seeds) at pH 4 (0.17 ± 0.009 g).

The highest dry weight was noted in *Cassia sophera* (non scarified seeds) at pH 4 (0.03 ± 0.008 g) and the lowest Dry weight was obtained in *Cassia occidentalis* (non scarified seeds) at pH 8 (0.012 ± 0.008 g).

Among the three species *Cassia tora* and *Cassia occidentalis* (with scarification) showed increase of fresh weight at pH 6 but decrease in pH 4 and *Cassia sophera* showed decrease of fresh weight against increasing of pH. Dry weight decreased due to increase of pH except *Cassia tora* and *Cassia occidentalis* (with scarification).

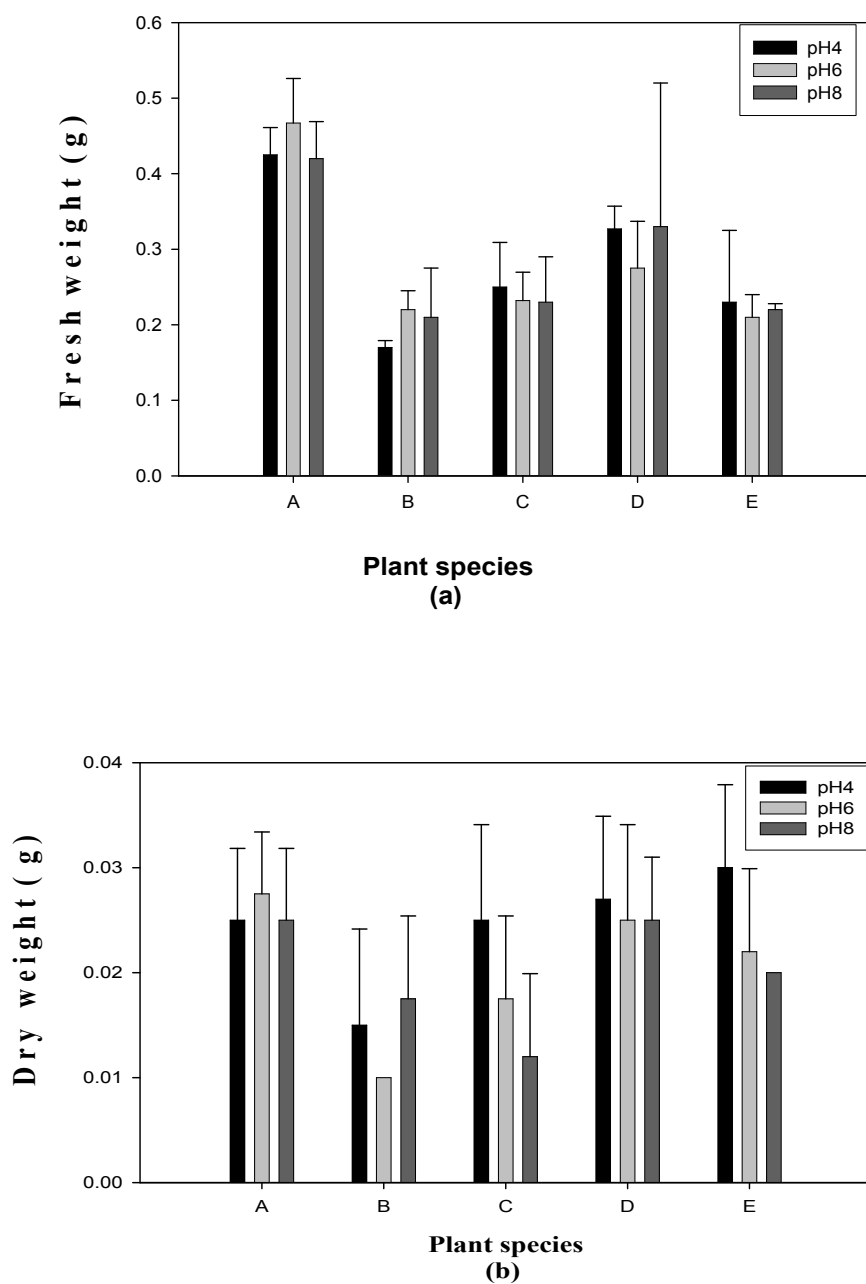


Fig.3.4. (a) Fresh and (b) dry weight (g) of three *Cassia* spp. at different salinity treatments. Vertical bars on the graphs show 95% confidence limits. (A = *Cassia tora*, B = *C. occidentalis* with scarification, C = *C. occidentalis* without scarification, D = *C. sophera* with scarification, E = *C. sophera* without scarification).

From the above Fig it can be said that, both *C. occidentalis* and *C. sophera* are sensitive to acid conditioned. In this respect *C. tora* will be ecologically in advantageous position.

3.4. DISCUSSION

Salinity is a major form of land degradation worldwide (Dudal and Purnell 1986) and is one of the major drawbacks for agriculture in arid and semi-arid regions of the world (Epstein,1985), because salinity decreases the rate of photosynthesis and plant growth to various degrees. Legumes are classified as salt sensitive crop species and their production is particularly affected by salt stress (Lauchli,1984) .

It was found that *C. occidentalis* and *C. sophera* are more sensitive to NaCl treatment than *C. tora*. Throughout the experiment growth of the seedlings of three *Cassia* spp. as a whole was retarded by sodium chloride treatment.

Seedling length of *Cassia* spp. showed significant ($p < 0.05$) reduction at the highest salinity treatment i.e.; 0.1M; but at the lowest salinity treatment i.e. at 0.05M, seedling length increased in all three *Cassia* spp. suggesting that growth of *Cassia* spp. is favored by low NaCl treatment.

In several important grain legumes, including *Phaseolus vulgaris*, have been reported by Delgado *et al.* (1994) a reduction of plant growth and dry-matter accumulation under saline condition. There is a evidence that retardation of germination and growth of seedling at high salinity (Bernstein 1962; Garg and Gupta 1997; Ramoliya and pandey 2003). Reduction in the growth of seedling was also recorded in response to increasing salt stress. (Ramoliya *et al* 2004). In general,

salinity can reduce the plant growth or damage the plant through i) osmotic effect (causing water deficit). ii) toxic effect of ions iii) imbalance of the uptake of essential nutrient.

Greenway (1962b) in his experiment with *Hordium vulgare* to salinity treatments showed the results of growth of leaf, shoot and roots separately. In general, NaCl treatment decreased the development of leaves. He has also shown that the uptake of sodium by the leaves was the main factor for the decrease of growth. Mer *et al* (2000) also worked with *Hordium vulgare* and found that seedling emergence for this species was reduced to 50% in soil with salinity of 4.3 dsm^{-1} .

An Eco-physiological investigation into the salt tolerance of *Glaux maritima L.* (salt tolerance species) was carried out by Rozema (1975). He noted that high salt concentration reduced significantly fresh and dry mass production. Tissue water content of shoot in rice was decreased with the increase of salinity although positive response to growth was observed at 1000ppm NaCl (Nazrul-Islam 1989).

Optimal shoot growth of *Aster tripholium* occurred when plants were in a salt solution, whereas, roots grew best in fresh water (Montfort and Brandrup 1927,1928). An increase in root growth and decrease in shoot growth was obtained under high water stress induced by NaCl, whereas, total growth of the plants was reduced (Stocker 1960; Troughton 1963). Shoot growth of *Aster tripholium* was best in 2% NaCl but roots grew best at only 1% (Bickenbach 1932). In *Atiplex polycarpa*, salinity inhibited shoot growth more than the root growth (Chatterton and Mckell 1969).

Wheat cultivars (*Triticum aestivum* L. cv *Inia-66* and c.v *Sonalika*) were found to grow well in low salinity treatment (Nazrul-Islam 1989). They have shown that c.v *Sonalika* more tolerant to salinity than cv *Inia-66* . The results of the present experiment have similarity with the respond of wheat to salinity (Nazrul-Islam 1989).

The toxic effect of NaCl salinity to crop plants has been known for sometimes (Cramer *et al* 1989, Shourbagy and Wallace 1965. Ram Deo and Kanwar (1968, Das and Mehrotra 1971) but the possible ecological significance has been neglected. Seedlings of all *Cassia* spp. in the present experiment grew well at control and 0.05M NaCl salinity but further increase of salinity showed toxic effect in all spp.

The interaction between salinity, nutrition and crop yield is a major concern in improving crop production (Flores *et al.* 2001). They have found that salt stress inhibits the uptake and transport of nutrients which affect the growth of crop plant. Another study analyzing the effect of roadside salinity showed one-fifth of irrigated agriculture is adversely affected by soil salinity (Chinnusamy, *et al.* 2005). A study conducted by them on improving salt tolerance in plants found salt stress can also cause oxidative damage to membrane lipids, proteins and nucleic acids. Although an experiment by Verdez and Menendez, (2001) showed that tomato plants can tolerate high levels of salt, excessive concentrations were shown to have detrimental effects on the plants.

Tozlu *et al.* (2000) obtained death of fine roots of *Poncirus trifoliata* in response to increasing NaCl and designated this mechanism as “fine root turnover” which was observed in our present investigation with *Cassia* spp.

CHAPTER 4

EFFECTS OF SOIL MOISTURE CONDITIONS ON THE GROWTH OF THREE *CASSIA* SPP.

4.1. INTRODUCTION

Soil moisture directly influences soil- water relationships, aeration and permeability through its relationship with interparticle pore space, and the physical conditions have a major influence on the growth of plants. Soil moisture appears to be an important factor in determining time of germination (Ratcliffe 1961 ; Pemadasa and Lovell 1975; Baskin and Baskin 1979; survival of seedlings and their dry-matter production (Jones and Etherington 1971; Pemadasa, and Lovell 1974a), relative growth rate (RGR) and root-shoot ratios (Ashenden *et al.* 1975) and reproductive performance. including number of seeds and seed weight (Newman 1965, 1967; Ernst 1981; Watkinson 1982).

The effect of drying and rewetting the soils plays a major role on the growth, nutrient uptake and distribution of plants (Bannister 1966 ; Nazrul-Islam 1977). *Erica cinerea* (a dry land species) is restricted to dry mineral soil, whereas *Erica tetralix* (a wetland species) occurs in damp, often waterlogged situation (Bannister 1965, 1966) . The work of Bannister also indicated that there is a fundamental difference in the response of the species of *Erica cinerea* and *Erica tetralix* to soil moisture conditions. When plants grow in waterlogged soil, their roots are in more or less anaerobic condition. Previous work suggests that plant species have very different physiological response to such condition depending on whether they are by nature

intolerant or tolerant of flooding (Crawford and Taylor 1969). Armstrong *et al.* (1991) have discussed the role of root adaptation to soil water logging.

Several authors have suggested that low water availability promotes earlier flowering. However, Koller (1969) points out that there are no critical observations which support this suggestion. Indeed experiments with annual crop plants (Salter and Goode 1967 ; Gates 1968 ; Husain and Aspinall 1970) showed that the reverse may well apply and moisture stress may, if anything ,slightly delay the time of initiation, though a small decrease in time to anthesis has been reported in some cases (e.g. Salter and Goode 1967 ; Slatyer 1973).

In the environment, differences of growth are often related to the differences in water availability. Etherington and Rutter (1964) in an experiment with *Agrostis tenuis* and *Alopecurus pratensis* have shown that a small decrease of soil water around the rooting zone cause a significant reduction of dry matter production during the early part of the growing season. Stanhill (1957) analysed data describing studies of soil water and plant growth; in sixty- six cases growth appeared to be decreased before soil moisture appeared to be reduced to the permanent wilting percentage.

The moisture regimes in different soil types have long been considered as one of the environmental factors limiting plant growth, under natural environment. Stocker (1960), Taylor (1960), Etherington (1962) and Slatyer (1967) have shown that plant growth decreased; but corn, pear and lemon plants have shown to respond to differences in moisture well above the so called wilting point. (Aldrich *et al.*, 1935; Davis, 1942; Furr and Taylor, 1959). There is evidence that dry matter production increased due to waterlogging in Soybean plants (Nazrul-Islam *et al* 1980). In

contrast, low moisture was favourable for the growth of tomato plants. (Nazrul – Islam and Roy, 1978).

The distribution of dry matter between root and shoot systems is a process which is strongly influenced by external environmental conditions such as nutritional regime, water regime, light intensity, and temperature (Brouwer 1966), day length (Cris and Stout 1929; Troughton 1961), root temperature (Davidson 1969) and nature of rooting medium (Hunt and Burnett 1973). More complex conditions such as density of population and volume and nature of rooting medium have strong influences on root/ shoot ratio (Troughton 1956); it was also suggested that a constant functional balance might exist between root and shoot systems despite varying external conditions (Davidson 1969).

Relative growth rate influenced virtually due to the environmental variables (Grime and Hunt 1975; Poorter 1989; Poorter *et al* 1990; Poorter and Remkes 1990). In general any departure from an adequate supply of light, mineral nutrients or water or from a suitable temperature regime, or from external toxins, produces a clearly adverse effect on RGR. It might be added that these factors also interact strongly. The fullest examination yet made of the effect of the environment on the RGR of a single species under comparable conditions and has been summarized by Hughes (1965). It appears that information regarding growth response in soils with different moisture regimes is not enough to explain plant growth and distribution. Hence, there is a need to measure the responses of certain plants over a range of moisture conditions.

The present work was designed to include the growth analysis in three *Cassia* spp. subjected to soil moisture regimes (wet and dry conditions) in potted soil to explain the nature of adaptation and distribution.

4.2. MATERIALS AND METHODS

4.2.1. Preparation of soil and pots

Soil was collected from the Botanical garden of Curzon Hall (D.U.); was broken by hand and passed through a 2 mm sieve to make it free from large particles of stones and bricks. Soil was then dried well in the sun. Earthen pots (height 26 cm; diameter 20 cm. in the top and 18 cm in the bottom) with a drainage hole in the bottom were filled with 4 kg of soil.

4.2.2. Experimental Procedures

4.2.2.1. Plant material

Seeds of the three species of *Cassia tora*, *C. occidentalis* and *C. sophera* were collected in late autumn and stored under laboratory conditions more than six months. Healthy seeds 25 in number were germinated on filter paper in the petridishes in laboratory, and seven days after germination uniformly size seedlings (number 14) were transplanted in the earthen pots.

The experiment was set on 29th March 2012. The experimental design was 3 species 2 treatments X 4 replicates; total 24 (twenty four) pots. The pots containing seedlings were kept in the net house of The Botany Department, Dhaka University to protect the seedlings from the disturbance of rats and birds.

All the seedlings were watered regularly, allowed to grow for two weeks, then two moisture regimes were imposed on 23rd April 2012.

The potted plants were subjected to two treatments:

- a. Wet (W)
- b. Dry-moist (DM).

Soil moisture conditions were maintained with the addition of water from the top. In case of Wet treatment the drainage holes of the pots were sealed with cement at the bottom. The plants of W treatment were flooded by submerging the pots with tap water and water table was kept 5 mm above the soil surface. The pots of DM treatment were watered daily with 300 ml of water in the morning when pot becomes wet and in the next morning it was dry hence it was designated as DM. This approximated near to field capacity (Migahid 1937).

The plants were allowed to grow for two weeks before imposing the treatments and an initial harvest was done. After the initial harvest the plants were subjected to treatments and harvested after every week and continued up to 8th harvest. In each harvest, one plant with whole root system was removed from each pot, carefully washed with tap water followed by distilled water rinse and then the fresh weight of plants was recorded. Plants were dried in an oven at 80°C for 48 hours. The dry weight was then recorded. The mean length of the root, shoot height, number of primary and secondary lateral roots of both treatments was recorded in every harvest. Root shoot ratio was also calculated.

After the treatments, during the vegetative growing period of the plants the appearance of first flower buds, fruiting phenologies of the plants were recorded at

each treatment for the three species. The date of fruit appearance and maturity was also noted.

Relative growth rate (RGR) of the plant of the two treatments was calculated from the following formula.

$$\text{RGR} = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{T_2 - T_1} \quad (\text{Fisher 1921})$$

Where, w_1 and w_2 are the dry weights of the plants of initial (T_1) and next (T_2) harvests.

4.2.3. Environmental conditions

The monthly mean average temperature and humidity (data of meteorological station, Dhaka 2012.) during the experimental period are given in Table 4.1.

Table 4.1. Mean monthly maximum and minimum temperatures and Relative humidity . (Morning and Evening).

Month	Temperature °C		Humidity (%)	
	Maximum	Minimum	Morning	Evening
March	33	19.2	53	44
April	35	21.8	75	60
May	34	22.1	75	68
June	32	23.5	74	67
July	31	23.2	87	90
August	31	24.2	83	67
September	31	25	81	80
October	31	20.7	85	78

The maximum temperature ranged from 31 to 35°C and that of the minimum was in the range of 19 to 25 °C. Relative humidity was highest in July 87% and 90 % in the morning and evening respectively.

4.3. RESULTS AND DISCUSSION

4.3.1. *The effect of soil moisture on the morphology and growth*

The morphology of the plants was recorded at various harvests and the appearance of the plants of the last harvest is given in Plates 4.1. (a - c).

Vegetative growth was vigorous in both two water regimes in the early stages of all the three *Cassia* spp. but the time required to reach each particular phase of development was longest at Wet treatment and shortest at Dry-moist; after the fifth harvest some leaves of the three *Cassia* spp. began to die in the wet condition.

In dry-moist treatment, the leaves were dark green in colour all throughout the growing period but at wet condition yellowing and senescence of leaves was noted (Yellow colour appeared in the margin and then moved towards the centre, and ultimately became brown and then dropped.) after 3rd and 4th harvests. Cannell *et al.* (1984) reported similar type of yellowing and senescence of leaves in wheat and barley grown on a clay and sandy loam soil. Grime and Hodgson (1969) investigated ecological significance of lime chlorosis with grasses and reported similar observation.

Plants of all the three species grown in DM treatment were taller and thicker stem than W treatment plants. It should be mentioned that plants of wet treatment of all the three species were dwarf in nature.

Plate 4. 1. The upper two pots are dry-moist (DM) and lower two pots are wet treatments (W) showing poor growth in wet treatment (four weeks after imposing the treatment).

- a. *Cassia tora*.**
- b. *C. occidentalis*.**
- c. *C. sophera*.**



a. *Cassia tora*



b. *Cassia occidentalis*



c. *Cassia sophera*

The roots of wet treatment plants were on the top 1-2 cm of the soil surface, stunted and dark brown to black in colour. In contrast, in DM treatment the roots were seen to penetrate upto the base of the pot which was light brown in colour. System of strong, contractile natural roots was well developed in the dry moist treatment and their apices soon became buried in the soil where their growth continued with repeated branching. Plates 4.2 (a-c). Number of lateral roots was higher in DM than Wet treatment.

The root development in DM treatment clearly indicates the nature of adaptation of the three species in the natural environment. The plants are not found to grow in wet condition in the field and this is definitely due to poor root development. Under wet condition the coiling of roots at the top 1-2 cm of the soil by all the three species are considered to be the most sensitive to anaerobic conditions. Gill (1970) has discussed the flooding tolerance of woody species and suggested that two possible type's anatomical and metabolic adaptations presence in plants tolerant of flooding. Armstrong *et al.* (1991) have clearly emphasized the structural diversity of roots and adaptation to soil waterlogging and emphasized the importance of aerenchyma development for the adaptation of plants under wet condition.

Plate 4.2. Shows the root growth in dry moist (DM) and wet (W) treatments of the three *Cassia* spp. (eight weeks after imposing the treatment).

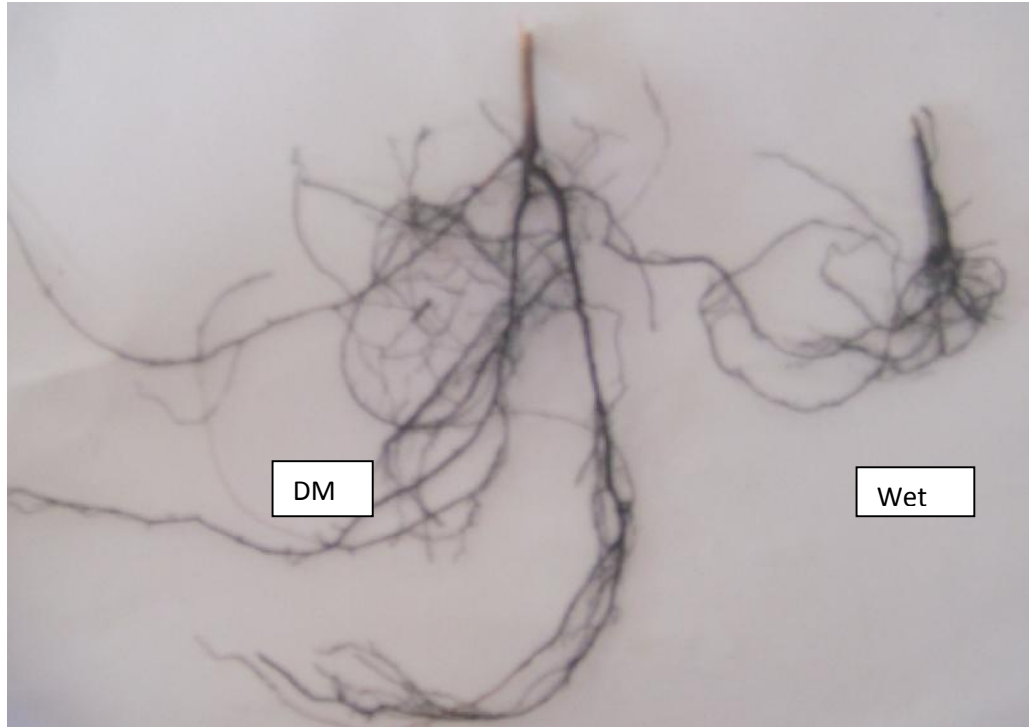
- a. *Cassia tora*
- b. *C. occidentalis*
- c. *C. sophera*



a. *Cassia tora*



b. *Cassia occidentalis*



c. *Cassia sophera*

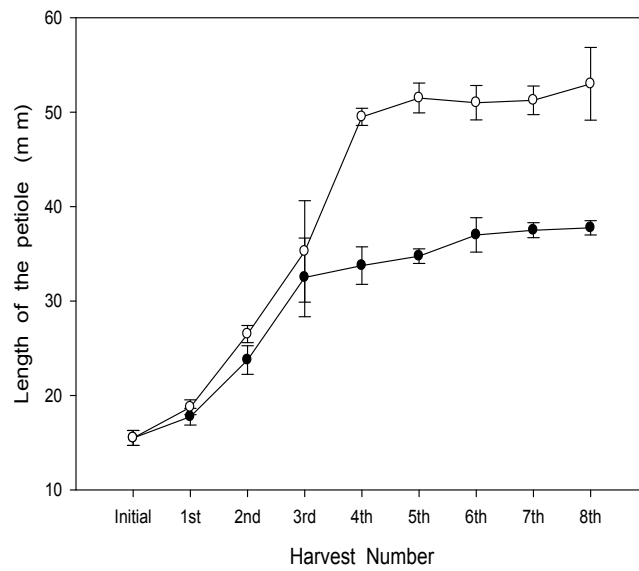
4.3.2. Increase of length of petiole, leaflet area and number of leaflet

The Graphs presented in Fig 4.1 and 4.2 show that all three species are affected at high soil moisture. i.e. wet condition. The area of terminal leaflet showed strong variation in relation to soil water condition and differed markedly in the two water treatments in all three species. The individual leaves of *Cassia tora*, *C. occidentalis* and *C. sophera* at different stages of development were found to be progressively larger with taller petioles as soil water was decreased i.e. in DM treatment (Fig- 4.1).

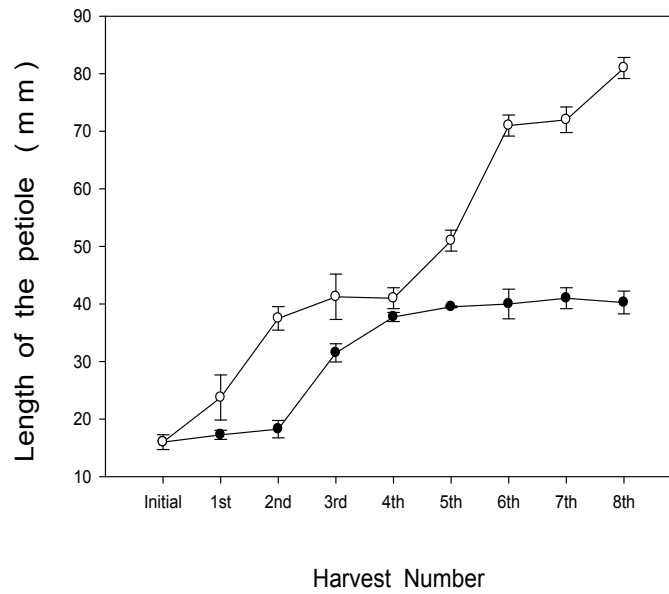
The length of the petiole was short in wet treatment and the value was nearer to the dry-moist treatment up to the 3rd harvest in all the three species. From the 4th harvest onwards the petiole length in dry moist treatment increased and was significantly higher ($p=0.05$) than wet treatment.

In *Cassia tora* the initial value of the petiole length was 15.250 ± 0.790 mm and it reached up to 37.75 ± 0.760 mm in wet treatment and up to 53.0 ± 3.85 mm in DM treatment in 8th harvest (one and half times more than wet treatment). In *Cassia occidentalis* the initial length was 16.00 ± 1.29 mm and was finally 40.25 ± 1.98 mm at wet and it was 81.0 ± 1.82 mm in DM at last harvest (more than double than W treatment). In *Cassia sophera* the length of the petiole of wet treatment was 57.50 ± 1.58 mm whereas it was 91.0 ± 3.16 mm in DM treatment at 8th harvest (one and half times more than Wet treatment.)

(a) *C. tora*



(b) *C. occidentalis*



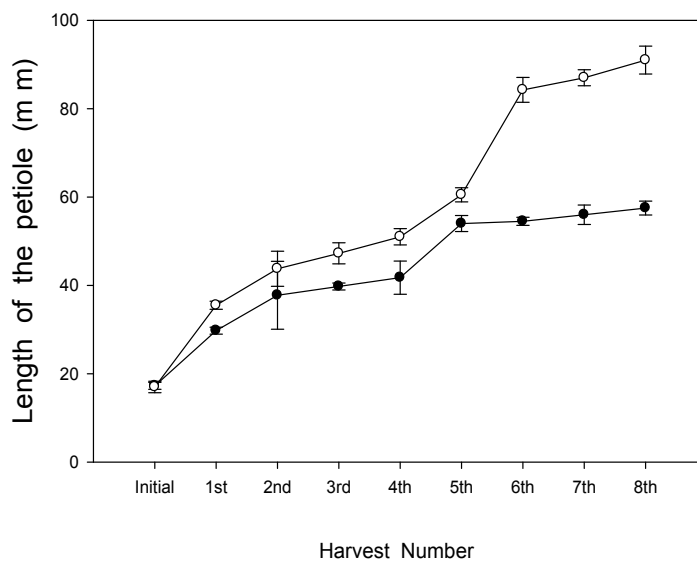
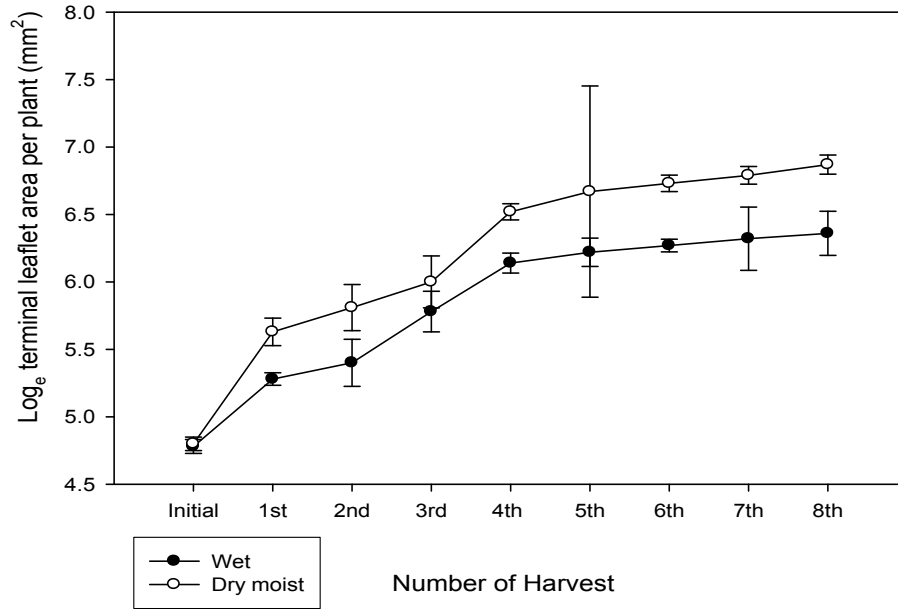
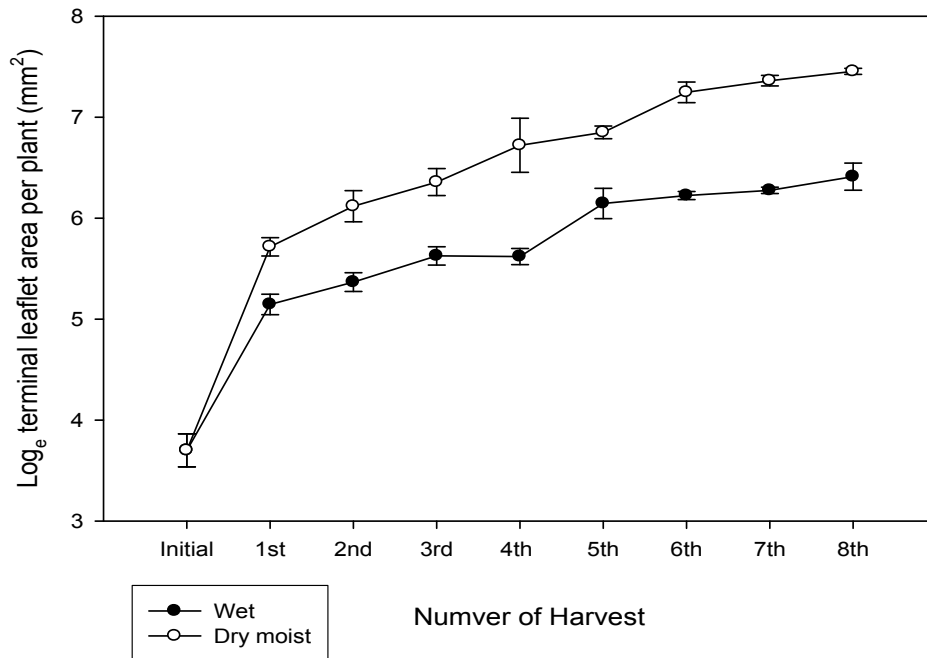
(c) *C. sophera*

Fig 4.1. The effect of soil moisture on the increase of length of the petiole with time. o; dry-moist. ● ; wet. Vertical bars are 95% confidence limits.

Cassia tora



Cassia occiden talis



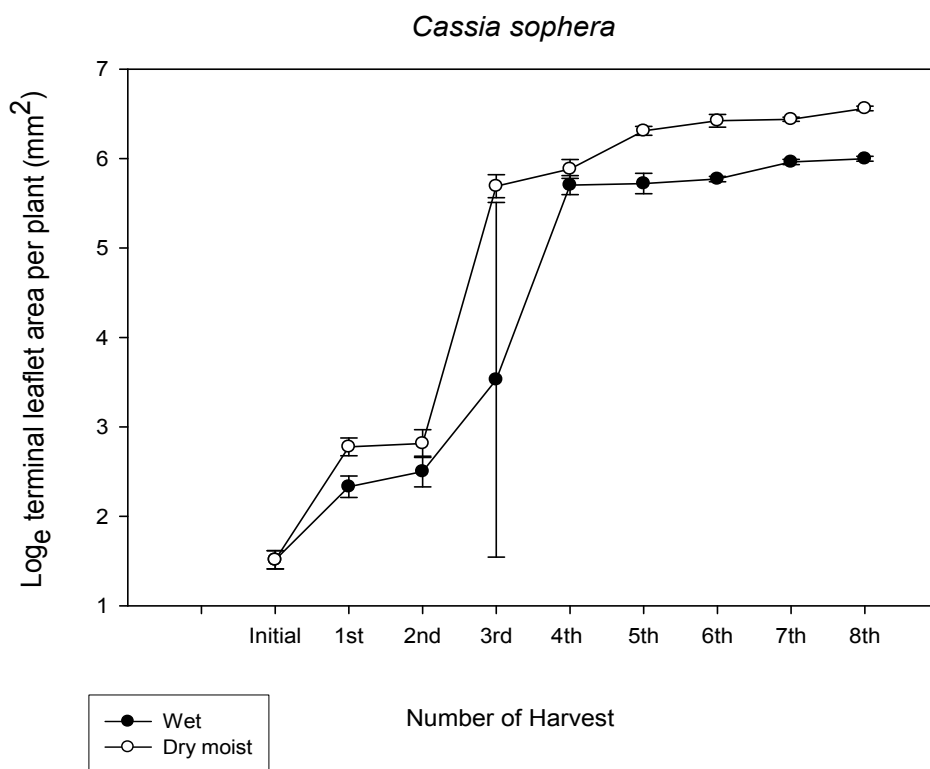
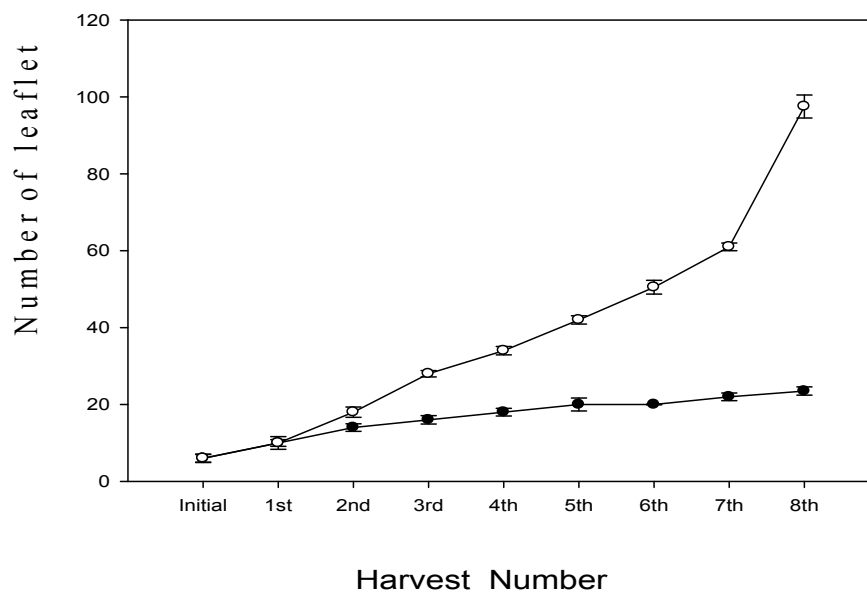


Fig 4.2. The effect of soil moisture on the increase of terminal leaflet area with time. ○; dry-moist ●; wet. Vertical bars are 95% confidence limits.

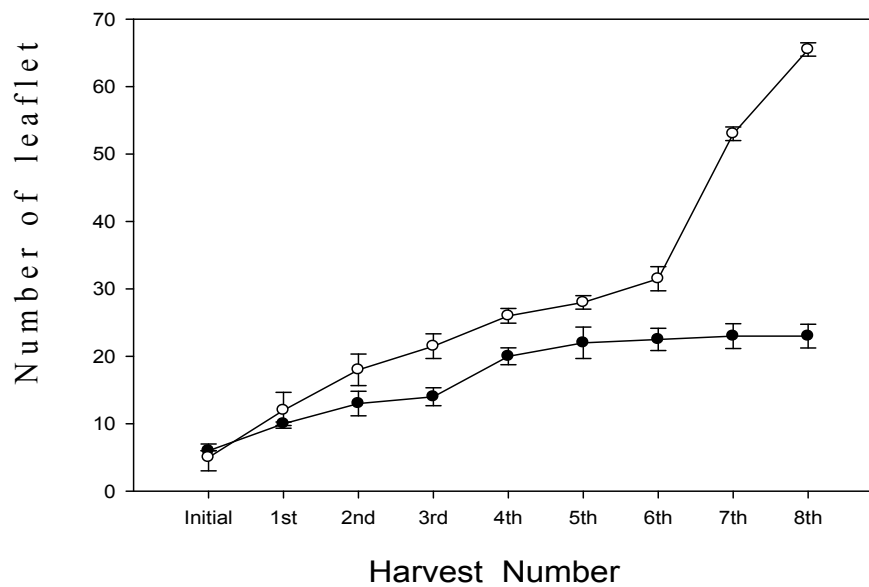
The pattern of increase of terminal leaflet area with time is shown in (Fig- 4.2). There was a progressive increase in leaflet size under DM treatment. Usually the size of the leaflet is bigger in *C. occidentalis* followed by *C. tora* and *C.sophera*. In *Cassia tora* initially the area of the terminal leaflet was $\log_e 4.80 \pm 0.052$ (122.00 mm^2) in both treatments but gradually increased to $\log_e 6.87 \pm 0.048$ (989.5 mm^2) at final harvest in DM, whereas it was only $\log_e 6.36 \pm 0.163$ (549.5 mm^2) in wet treatment. Compare to *Cassia tora* the leaflet area was much higher in *Cassia occidentalis*. Though at initial harvest it was only $\log_e 3.70 \pm 0.164$ (127.5 mm^2) but at final harvest the area of the leaflet was $\log_e 6.560 \pm 0.026$ (1726.0 mm^2) in DM and $\log_e 5.998 \pm 0.028$ (581.00 mm^2) in wet treatment. In *Cassia sophera* at last harvest it was $\log_e 7.454 \pm 0.030$ (675.0 mm^2) in DM when the value was $\log_e 6.411 \pm 0.134$ (392.50 mm^2) in wet treatment.

All the three species are usually considered to be plants of dry habitat. All these species make morphological and other adjustment in response to water stress. *C. tora* is the most successful in wet condition and *C. occidentalis* is the most sensitive to high water (i.e.; wet) in relation to leaflet area, as the leaflet value in the wet condition was less (5.81 mm^2) from that of the dry treatment (6.560 mm^2). Both *C. tora* and *C. sophera* are more resistant to wet condition than *C. occidentalis*.

a) *C. tora*



b) *C. occidentalis*



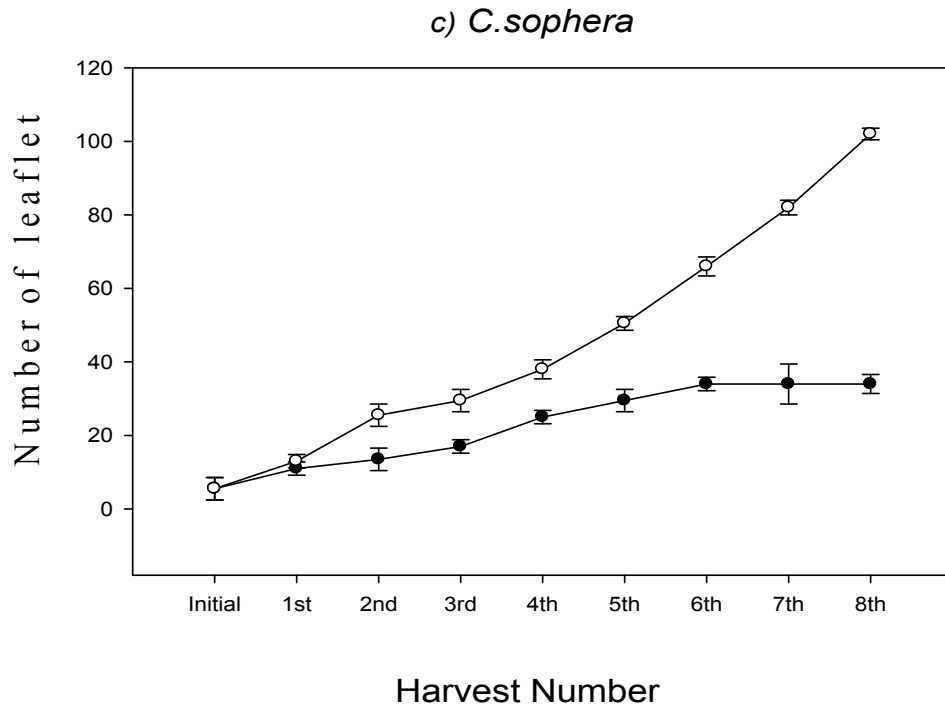


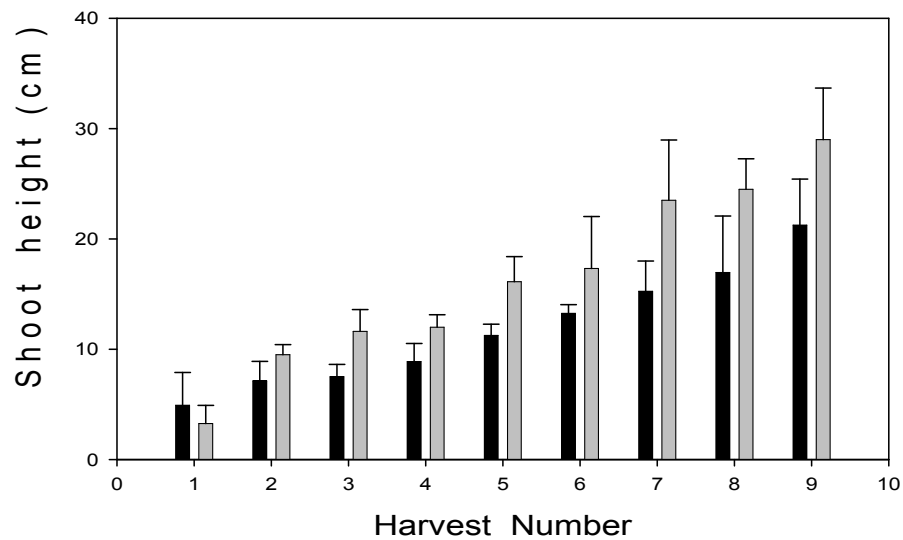
Fig 4.3 . The effect of soil moisture on the number of leaflet with time. o ; dry-
moist .●; wet. Vertical bars are 95% confidence limits.

In all three *Cassia* spp. the number of leaflet was almost half in Wet treatment in comparison to DM treatment (Fig 4.3). Possibly low moisture allowed the plants to grow better and hence the number of the leaflet was greater in DM than Wet condition where the terminal leaflet area (Fig 4.2) is small. Senescence of a few leaflets at the last three harvests in these three species under wet condition was observed. Number of leaflet in the final harvest was less than that of the plants in dry-moist treatment. In *Cassia tora* at wet treatment the number of leaflet did not increase after 4th harvest and remained same up to 8th harvest (20 ± 1.58), whereas it increased to 97.5 ± 0.768 in DM treatment. In *Cassia occidentalis* the number of leaflet was 23.00 ± 1.76 at final harvest in wet treatment and the value was 65.5 ± 1.581 in DM. In *Cassia sophera* the leaflet number in wet treatment was 34.00 ± 2.59 which was less than one third from DM treatment (102.00 ± 1.58).

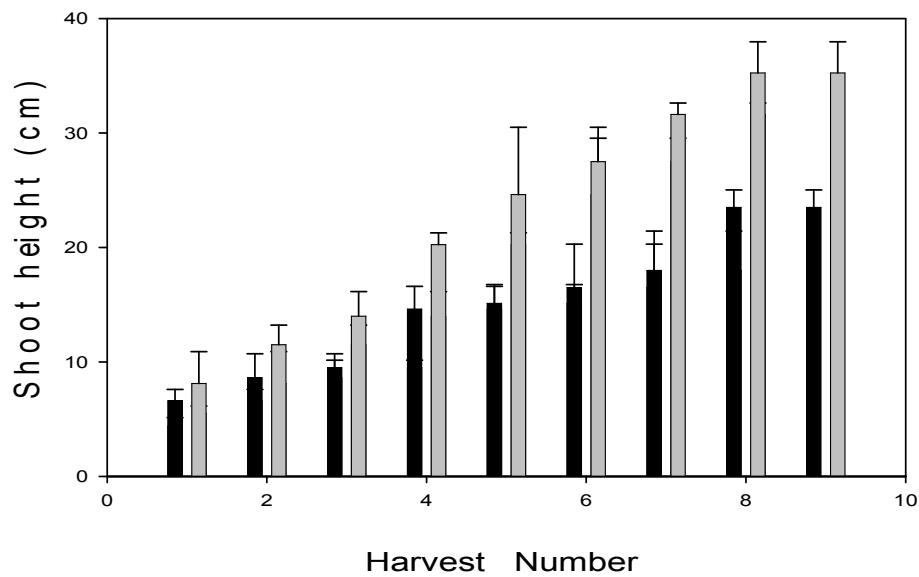
4.3.3. Shoot height, Root length and number of lateral roots

Shoot height at different harvests is given in Figs. 4.4 and 4.5. Shoot height of all the species was significantly higher in DM treatment ($P=0.05$) than that of wet treatment. In wet condition the shoot height was highest in *C. sophera* (24.50 ± 2.75 cm) followed by *C. occidentalis* (23.25 ± 1.53 cm) and was lowest in *C. tora* (21.25 ± 4.17 cm). In DM condition the shoot height was also highest in *C. sophera* (38.50 ± 3.31) followed by *C. occidentalis* (35.25 ± 2.72) cm. In contrast, it was significantly ($p=0.05$) lower in *C. tora* (29.00 ± 4.67 cm). In nature it was observed that *C. occidentalis* reaches height up to approximately 7ft.; *C. sophera* (bushy) 3-4 ft and *C. tora* 2-3 ft.

C. tora



C. occidentalis



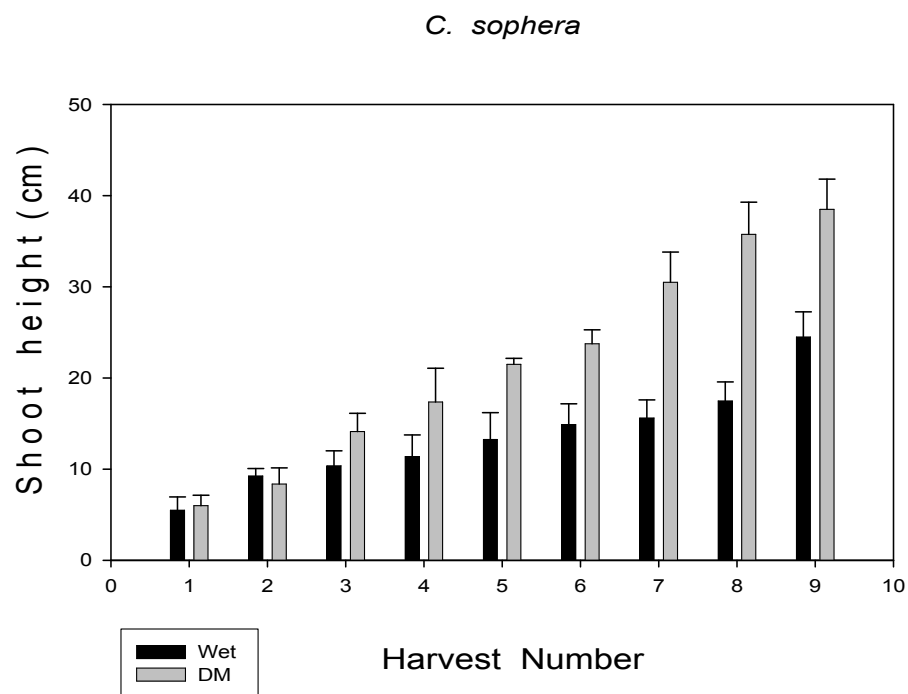


Fig 4.4. Effects of wet and dry-moist treatments on the shoot height per plant at different harvests. Vertical bars are 95% confidence limits.

The role of the increased root development should be correctly evaluated and it is important to explain in more detail the nature of the response. Root response was assessed in the present study to include some measurements which have been shown to be of important from the work of Priestly and Pearsall (1922), Gardner (1961), Cowan (1965) and Newman (1969). The shoot height, root length, number of lateral roots were noted and the results are given in Fig (4.4 - 4.6).

At DM treatment the length of the root of all three species was significantly ($P=0.05$) higher than wet condition. In DM condition the root length of *C. occidentalis* was (30.25 ± 13.96 cm) followed by *C. sophera* (28.50 ± 4.93 cm), and in *C. tora* (24.88 ± 8.83 cm). Among the three species the highest root length in wet treatment at final harvest was in *C. tora* (19.62 ± 2.62 cm) followed by *C. sophera* (17.50 ± 4.58) and *C. occidentalis* (15.12 ± 1.35) cm. At wet treatment the total root length of *C. tora* was higher than the other two species which indicates the efficiency of this species to adapt under wet condition.

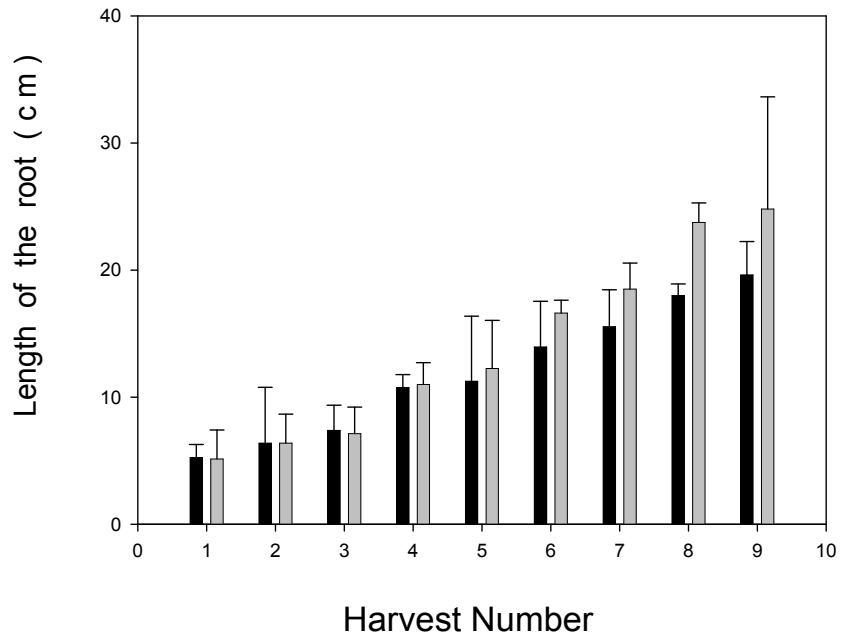
The result suggests that both *C. occidentalis* and *C. sophera* are adapted to less water in the natural condition. In contrast, the adaptation of *C. tora* in relation to root growth and penetration indicating its adaptation not only to dry condition and but also to a lesser extend in wet habitat.

Root adaptation to soil waterlogging was investigated by Armstrong *et al.* (1991). They have proposed that although plants often succumb to soil waterlogging, at least two successful developmental rooting stratagems for survival can be identified and both involve anoxia avoidance. They are (1) superficial rooting and (2) enhanced

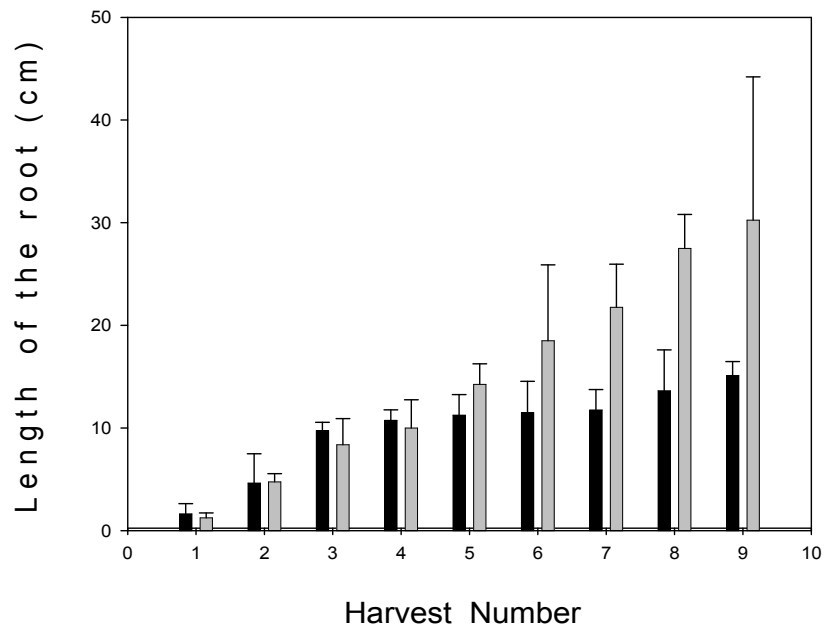
development of internal gas space, which provides a conduit for the transport of O₂ from the shoot system. In the present experiment surface rooting was observed in all the species in wet treatment. It should also be noted that wherever there is internal O₂ transport there will be a potential for radial O₂ loss from root to soil. (Armstrong 1982, Marschner, 1986).

The noticeable morphological response in wet treatment is the restricted root growth. The inability of the roots to penetrate soil under wet condition and their presence on soil surface will exclude all the three species to grow and dominate in wet habitat. Armstrong *et al.*(1991) considered the ways in which the growth and development of roots affect their aeration and thereby influence the plant ability to survive and compete in wetland condition. The major stratagem for survival under wet condition is the enhanced development of gas space in the primary and /or secondary cortex of the root. At low or intermediate soil moisture tension, some response to root growth has been observed by Satoo (1965) and Jarvis and Jarvis (1963a). There is evidence that oxygen diffusion rates are not limiting where there is a fluctuation of water table. (Armstrong and Boatman. 1967; Nazrul-Islam and Rorison.1978). Some measurements of oxidation-reduction potential and oxygen diffusion rates would be more useful to evaluate the results.

C.tora



C. occidentalis



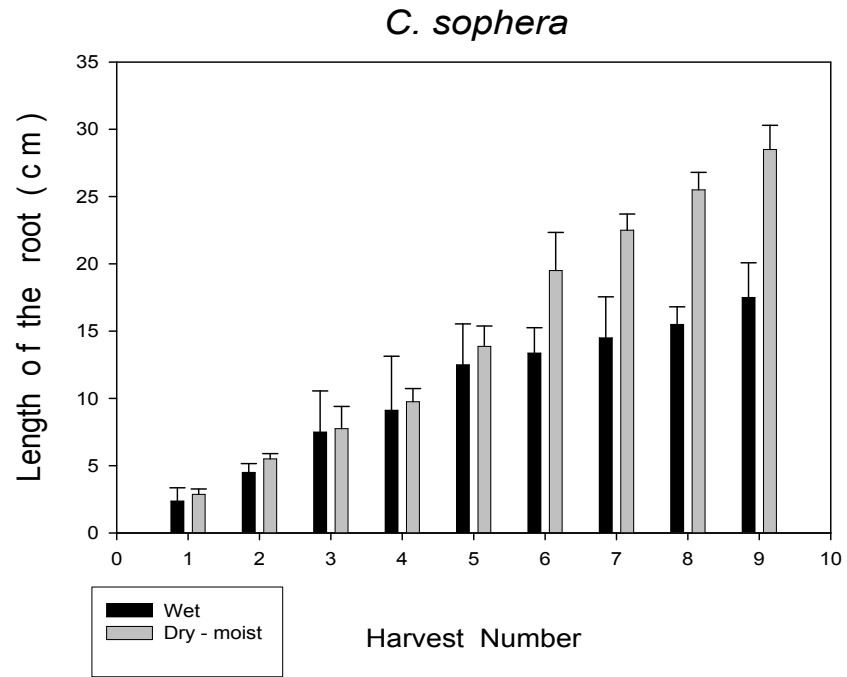


Fig 4.5. Effects of wet and dry-moist treatments on the root length per plant at different harvests. Vertical bars are 95% confidence limits.

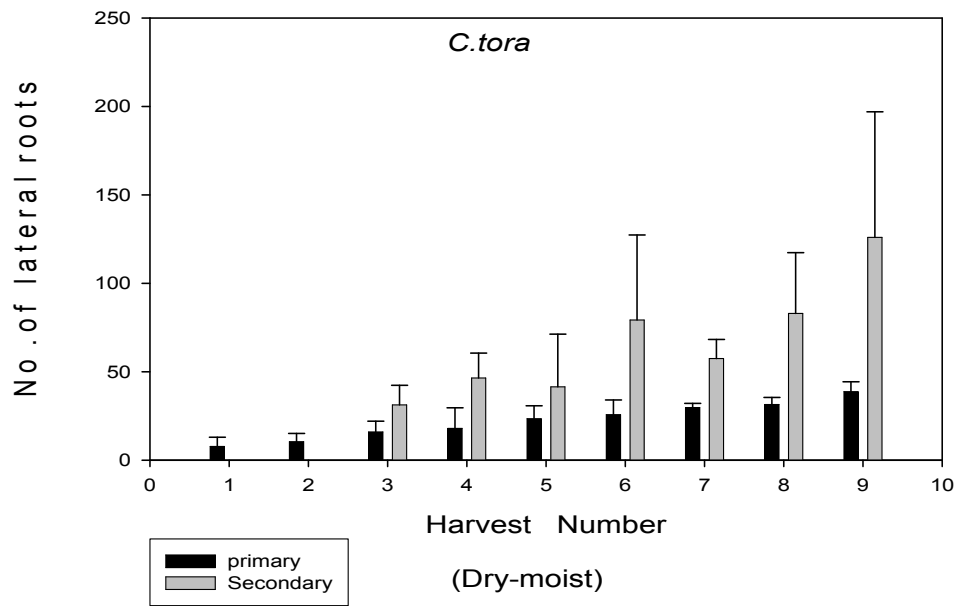
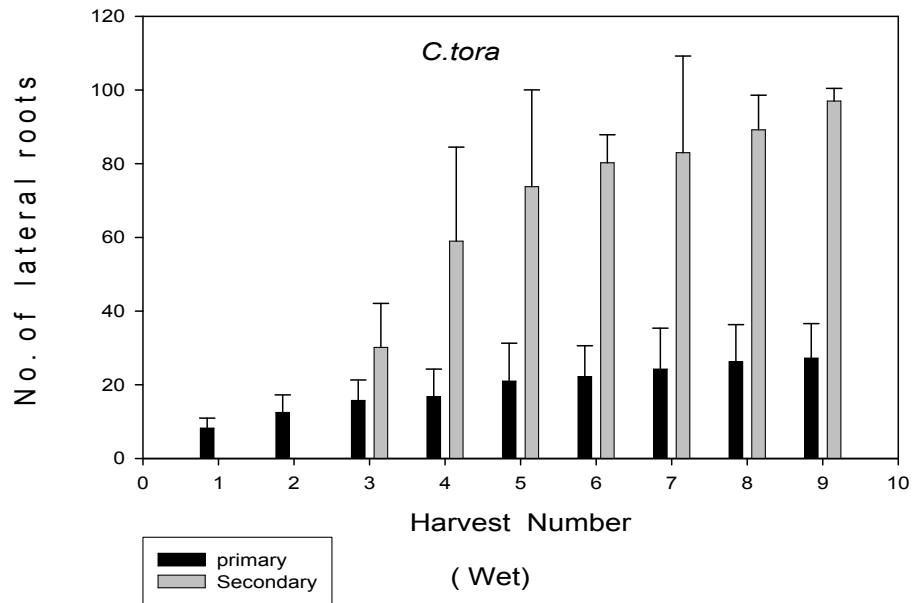
Number of primary lateral roots of *C. tora* in wet treatment at final harvest was 27.25 ± 9.34 and it was 38.75 ± 5.56 in DM. In case of *C. occidentalis* it was 25.75 ± 1.53 in wet and 34.00 ± 6.49 in DM treatment. The number of primary lateral roots of *C. sophera* was only 17.59 ± 10.18 in wet and 24.00 ± 8.71 in DM treatments.

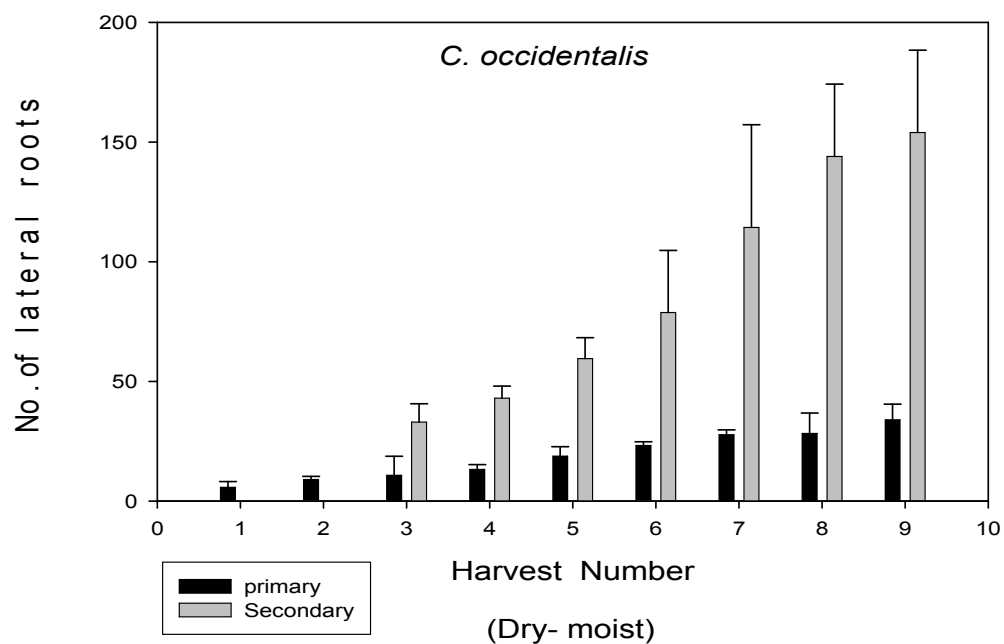
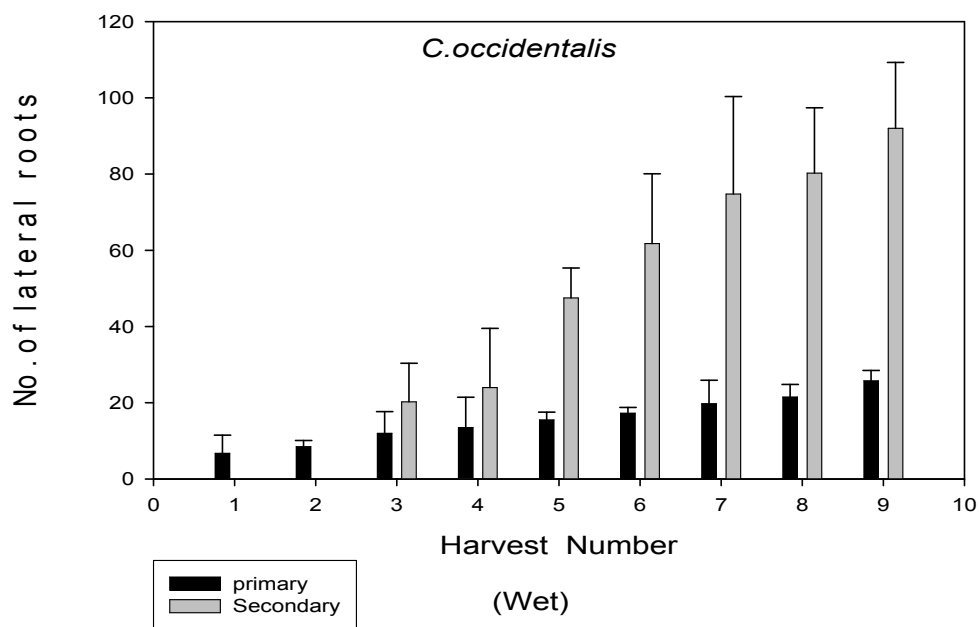
The component part the roots (mean of both primary and secondary lateral roots in number) in *C. tora* (Total mean 124.25) showed luxurious growth in wet treatment in comparison to other two species; in *C. occidentalis* (mean 117.75) and in *C. sophera* (mean 108.50); Whereas, in DM treatment *C. occidentalis* showed highest growth (mean 188.0) followed by *C. sophera* (mean 164.00) and *C. tora* (mean 155.00). This suggests that all the three species can well spread their root system under dry condition. When the results are compared among the species, it is found that the well developed root system of *C. tora* under wet condition is perhaps due to the fact that *C. tora* produces numerous lateral roots and ultimately facilitates anchorage of the plant and therefore helps to tolerate the wet condition up to a certain period.

Development of numerous adventitious roots were reported in maize under flooded condition by Jat *et al.* (1975) and in other field crops by Kozlowski (1984). The species that form adventitious roots at or below the water level was found to survive best (Armstrong 1968). He also reported adventitious roots which perhaps help in absorption of dissolved oxygen present in water. The adventitious roots of flooded plants comprise a supplementary absorbing system in somewhat aerobic zone, while

the original root system does not function normally because of low oxygen tension (Gill, 1970). The three species of *Cassia* did not show any adventitious root development although Kozlowski (1976) concluded that, formation of air space is important in some species and production of adventitious roots appears to be one of the most important features of adaptation.

Under wet condition none of the three species showed deep penetration of root and hence the root development was restricted to soil surface. Similar root development was also observed by Armstrong and Boatman (1967) in calcicole dryland species. However, such root development indicates that wet soil was an unfavorable growth medium and it is known that both the sulphide and ferrous ion in wet soil may have unfavorable effects on plants and deficiency of O₂ inhibits the growth and functioning of roots particularly the mechanism of ion uptake (Ponnamperuma 1972). In addition, production of toxic substances (such as Mn and Fe) as a result of waterlogging also inhibit root growth and even causes death of some species (Bannister 1964 a ; Jones and Etherington 1970 ; Nazrul Islam and Noor Newaz 1991 ; and Nazrul Islam 1989; 2010).





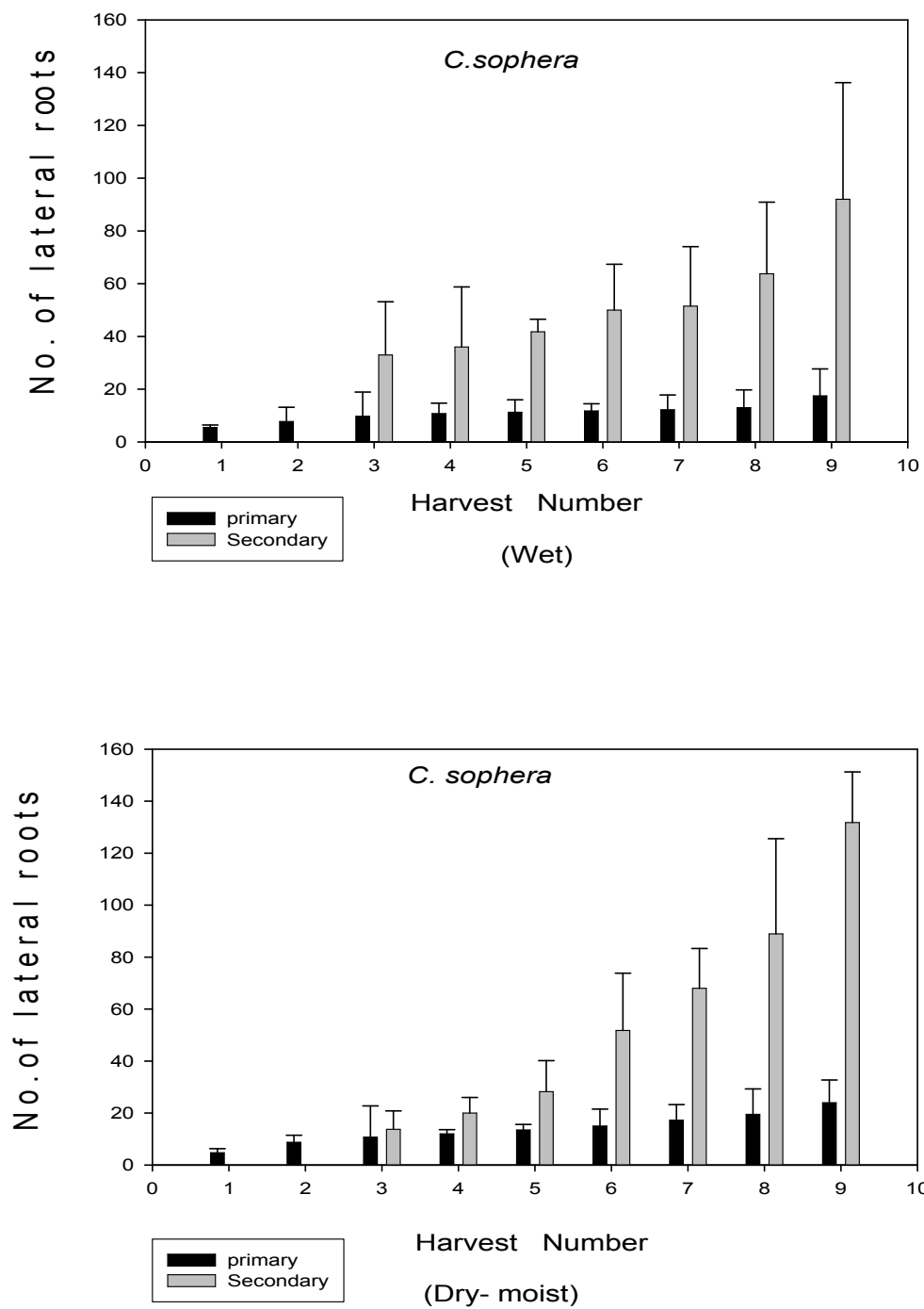


Fig 4.6. Effects of wet and dry-moist treatments on the number of lateral roots per plant at different harvests .Vertical bars are 95% confidence limits.

4.3.4. Flower and fruit production

At dry moist treatment *Cassia tora* started flowering 3 weeks earlier than wet treatment; it is of significant to mention that under DM condition *C.tora* showed flowering 12 weeks earlier than *C. occidentalis* and 17 weeks earlier than *C. sophera*.

Mott and McComb (1975) worked with *Helichrysum cassinianum* and *Helipterum craspedioides* and found that there was a trend for the time to initiation of visible floral primordial to longer in plants under moisture stress than in plants grown on moist soil.

In *C. tora* there was a marked delay and reduction in number of flowers in wet treatment and also reduction in the number of fruits (Fig. 4.7).

The result shows that in wet treatment there is no flower and fruit production at all in *C. occidentalis* and *C. sophera*. However, *C. tora* shows very few flowers and fruits under wet condition. This behavior is possibly due to high amount of water in the soil. In Wet treatment there was also a reduction in the dry weight of fruits and seeds. This reduction was due to the smaller number of seeds per fruit.

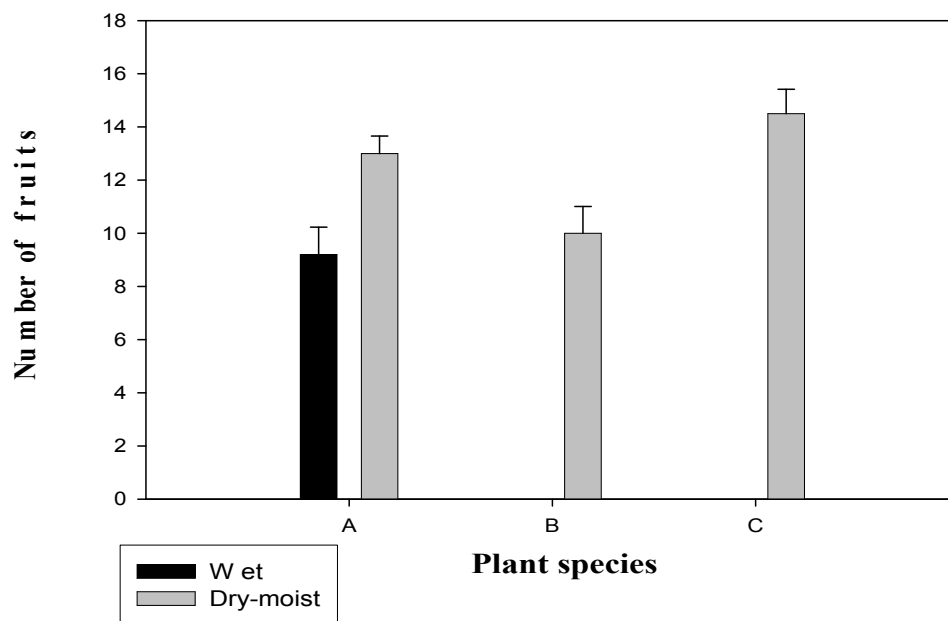
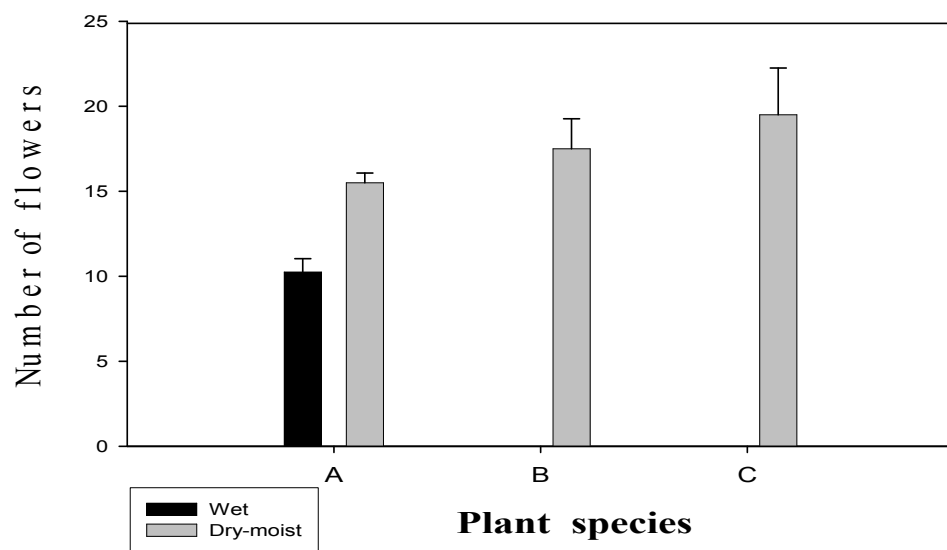


Fig 4.7. Mean number of flowers and fruits in DM and Wet treatments. Vertical bars are 95% confidence limits (A = *Cassia tora*, B = *C. occidentalis*, C = *C. sophera*)

4.3.5. Increase in dry weight with time

It can be seen from Fig 4.8 that the DM treatment had a significant increase ($p=0.05$) in dry-weight. There was also a striking difference in response between three *Cassia* species to the wet treatment. Highest dry matter production was obtained in the plants of dry moist treatment. The dry weight was severely decreased in wet treatment.

In DM treatment the total dry weight of *C. tora* was 1600 ± 64.91 mg/plant and that of the wet treatment was 615.0 ± 37.62 mg which was two and half times less than that of DM.

On other hand, the dry weight of *C. occidentalis* in DM treatment at the final harvest was 1777.5 ± 108.8 mg/plant and in Wet treatment it was 680 ± 34.14 mg/plant (approximately one third of DM treatment).

In *C. sophera* the dry weight in DM was $2505.0 \pm 97.60.0$ mg /plant whereas, in wet treatment it was 807.50 ± 20.01 mg/plant which was less than one third of DM treatment.

The main effect of soil moisture (wet condition) is reducing dry matter production in the three species and this is related to the high amount of water in soil environment, Bannister (1964 a) worked with three species of heath (*Calluna vulgaris*, *Erica cinerea* and *E. tetralix*) plants in relation to soil moisture (wet and moist treatment). *C. vulgaris* showed the best performance in both moisture regims and also showed a good root development, a greater dry matter production and was more branched than the other two species. In the present investigation *Cassia tora* behaved

very similarly to that of *C. vulgaris*. Bannister (1966); Nazrul-Islam (1977) also reported similar type of results in a range of calcicole and calcifuges plant species from U.K. and also in tomato plant. (Nazrul-Islam and Roy 1978).

Dry weight increment in the plant was greater in DM treatment than wet condition. The moisture conditions in soils used in this experiment were chosen to stimulate stress conditions likely to be experienced by the plants growing in the field. The results are valuable since the measured effects of soil moisture made it possible to draw some conclusion about the differences in response of these species.

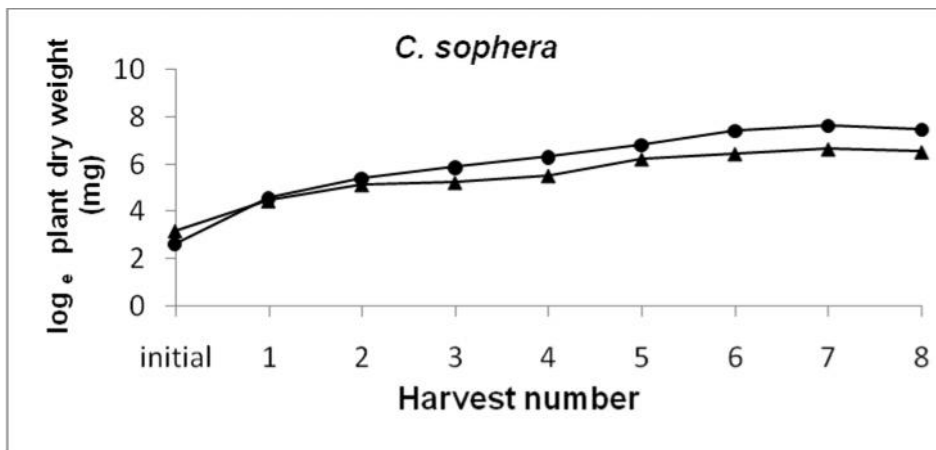
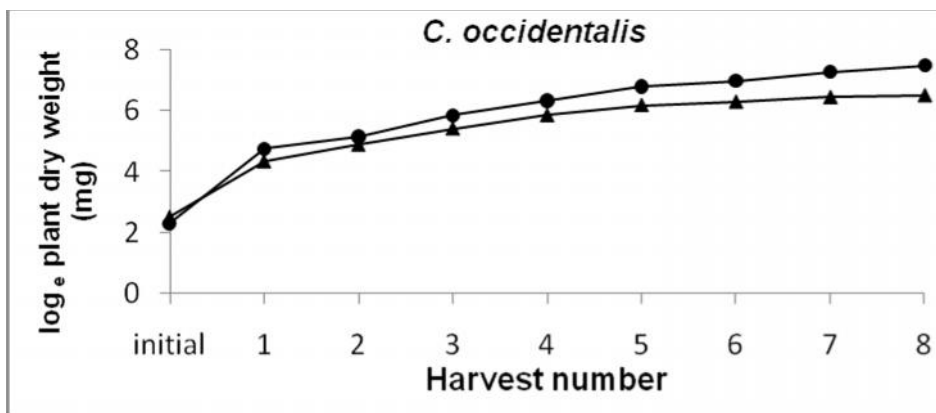
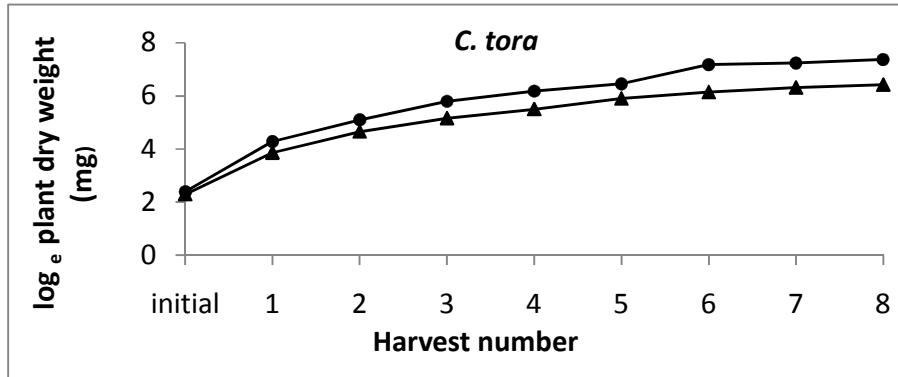


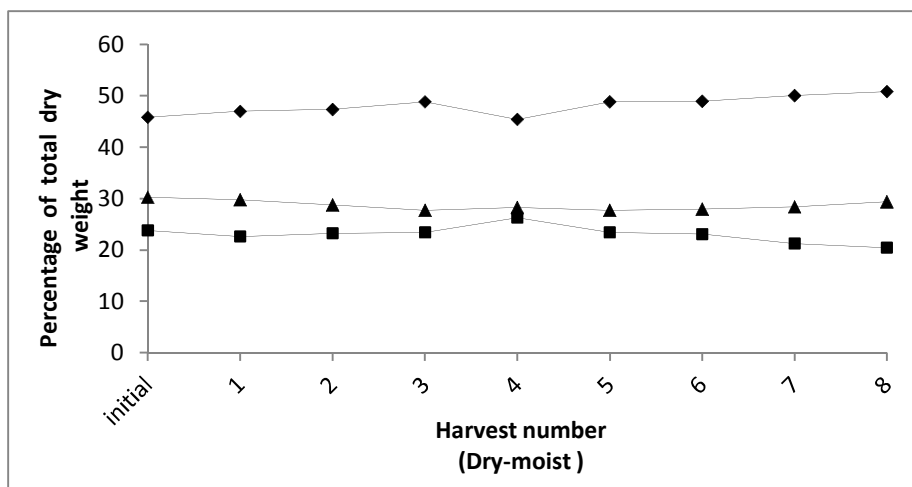
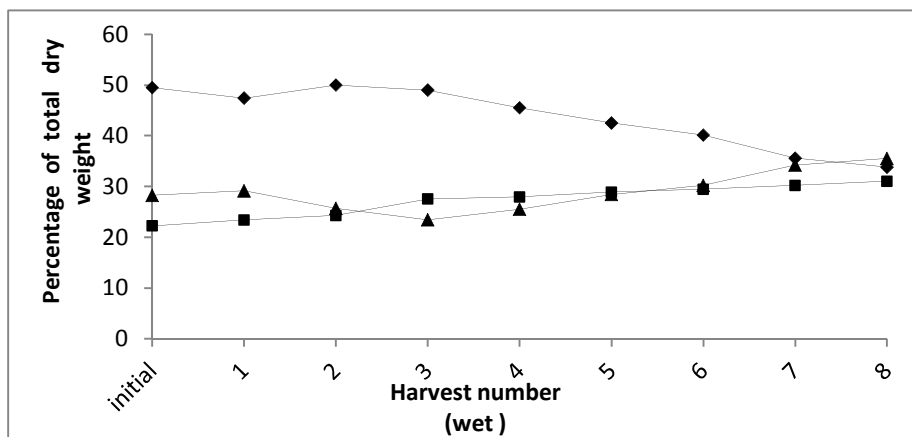
Fig 4.8. The effect of soil moisture on the increase of plant dry weight with time. ●; Dry-moist. ▲; Wet.

4.3.6. *Distribution of dry weight between stem, root and leaf*

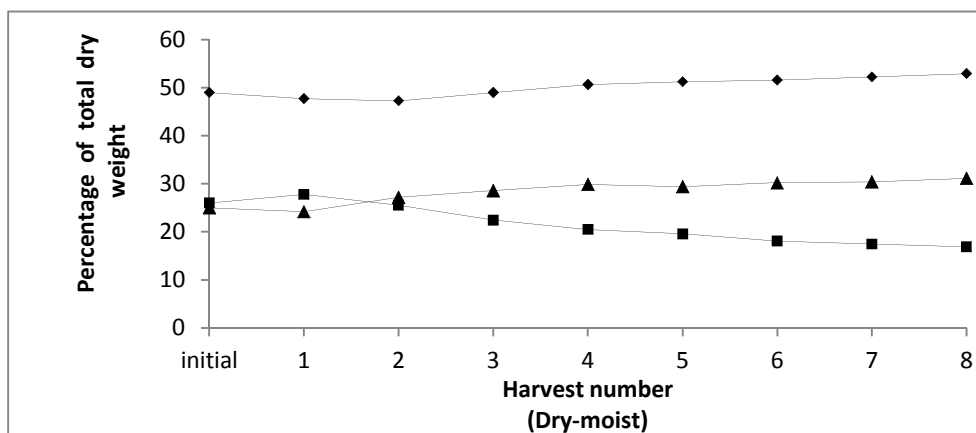
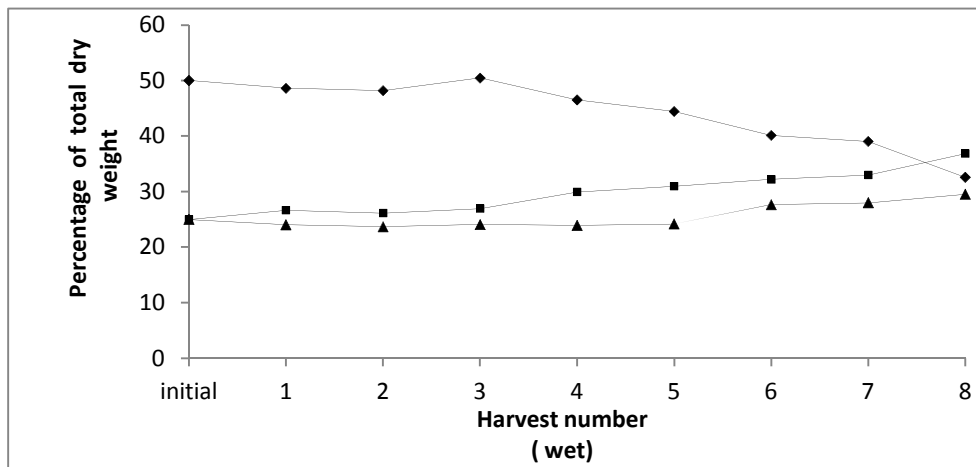
The weight of stem, root and leaf expressed as percentages of the total dry weight, at each harvest are plotted in Fig 4.9 (a-c) to illustrate the ontogenetic changes in three *Cassia* spp.

In *Cassia tora* value of the proportion of stem and root was more or less constant with little decrease in the 3rd and 4th harvests. In wet treatment there was a slight decrease in the proportion of leaf in the second harvest, and shows a continuous decline and ultimately the value fell below 40%. However from the 4th harvest onward there is a tendency for both stem and root to a stable proportion up to the 4th harvest and then shoot and also root increased up to the final harvest. In dry moist treatment the proportion of leaf, shoot and root was constant throughout the harvest.

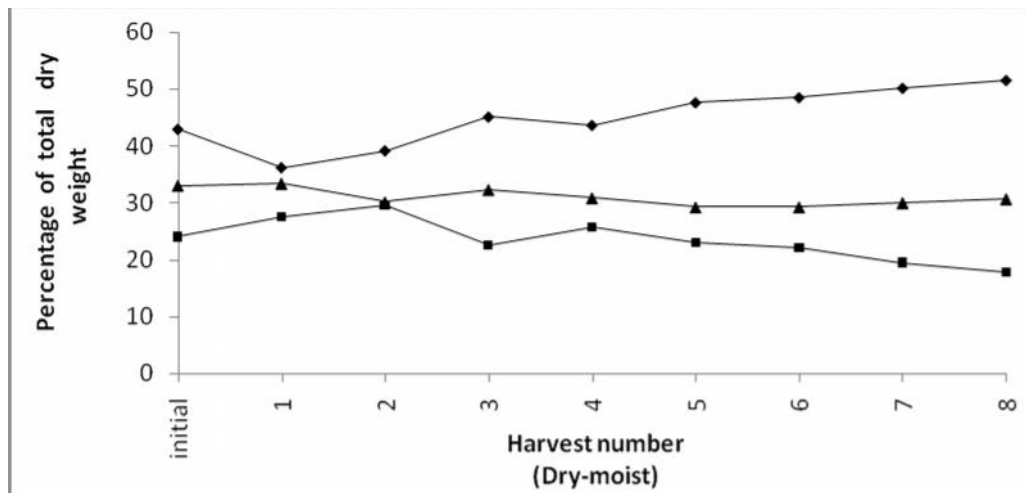
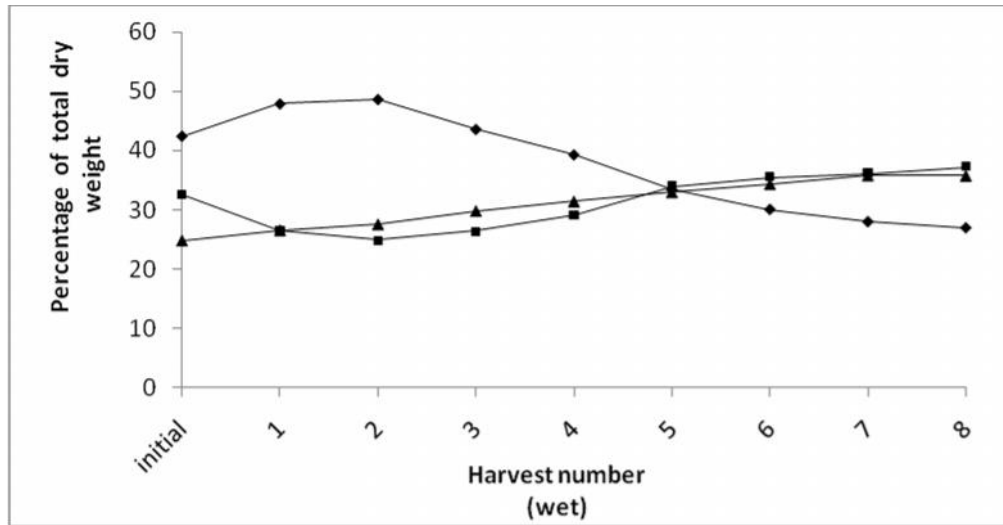
In *Cassia occidentalis*, in the wet treatment the proportion of leaf falls off quite rapidly after the 3rd harvest, the root and stem increase correspondingly from the 4th harvest onwards and the proportion of root in the final harvest was highest. In DM treatment the proportion of leaf was constant in all the harvests. From the 3rd harvest proportion of root began to decline and continued up to final harvest. With *Cassia sophera* the pattern was somewhat different in both wet and dry treatments; in that the proportion of root and shoot is more or less constant from the second harvest but the proportion of leaf showed an increasing tendency in dry moist treatment. In contrast, in wet treatment the proportion of leaf began to decrease from the 3rd harvest onwards with a corresponding increase of stem and root.



a. *Cassia tora*



b. *Cassia occidentalis*.



c. Cassia sophera

Fig 4.9. The effect of soil moisture on the distribution of dry weight between ◆- leaf; ■- root; ▲- shoot at successive harvests.

This changing pattern of growth was also reported in other crops (Kramer 1963). The gradual increase of leaf proportion with the decrease of moisture in the soil (DM) is perhaps due to the fact that for survival in stress condition plants translocate more metabolites into the leaf than root and shoot. It is of interest to mention that in the dry-moist treatment of the three *Cassia* spp. the largest component of the plant is leaf material whereas, root is the largest component in wet treatment plants.

The patterns of ontogenetic changes are the same within each species, irrespective of the moisture treatment, but at any one harvest the values differ with soil moisture in ways which may be ecologically significant, for the proportion of leaf decreases in wet treatment although the proportion of root and stem shows slight increase; In DM treatment the proportion of leaf and shoot increase and the proportion of root decreases. It is clear that the three plant species differ in their ontogenetic development during the early phase of establishment. In all the species the leaf of wet treatment forms a high proportion in the initial stages of growth followed by a decline in the last few harvests whereas the situation was reverse in the plants of DM treatment. Root and shoot are highly affected by soil moisture where it can be seen that the leaf of dry-moist plant forms a high proportion relative to the stem and root. The results clearly indicate the proportion of leaf, root and stem in all the species are strongly affected by high soil moisture. i. e. Wet condition. These differences may be important in the early establishment of seedling in natural condition particularly in the rainy season. Nazrul Islam and Alam (1986) investigated the effect of waterlogging and non- waterlogging in two cultivars of Jute and they have shown the differences in the proportion of dry matter and found that the proportion of leaves

decreases rapidly in both cultivars of Jute with a corresponding rise in stem proportion under waterlogged condition. The highest component of the plant under waterlogged condition is stem material whereas root is the largest component under non waterlogged condition.

4.3.7. Root: Shoot

Fig.4.10 shows changes of root: shoot ratio with time at each harvest together with 95 percent confidence limits. The high value of R/S in all the species of wet treatment means that plants become more Rooty. Initial high value of R/S in all the species is because of the inclusion of remains of the seed in the dry weight of root. Anslow (1962) has discussed this issue under productive green house condition. In the present work the result of final value of R/S attained is entirely due to the influence of wet and Dry-moist treatments.

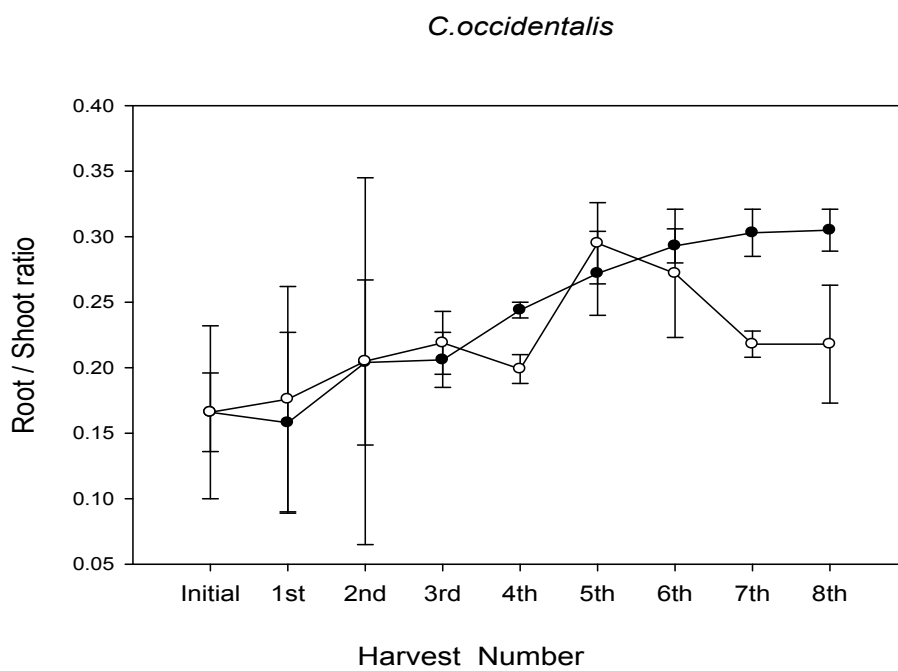
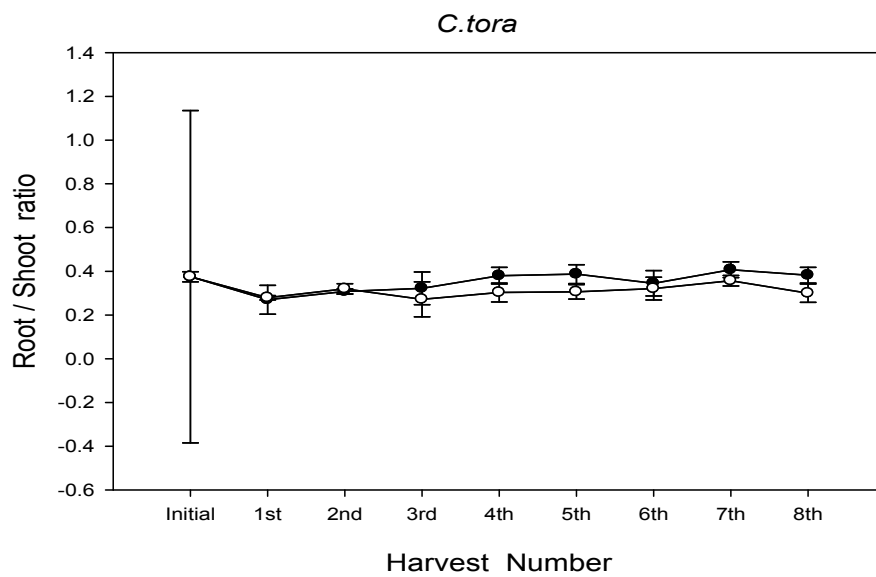
In all the species in wet treatment R/S value was higher than DM treatment and there was a tendency to increase in R/S value. In case of DM treatment there was a tendency to decrease in R/S almost remained constant up to the final harvest .The value of R/S was always low in dry treatment.

The values of R/S in DM in all the species was significantly lower ($p=0.05$) than that of wet treatment in the final harvest. Initial R/S value in *C. tora*; *C. occidentalis* and *C. sophera* was 0.375 ± 0.005 ; 0.166 ± 0.031 and 0.208 ± 0.057 respectively. In *C. tora* the final value was 0.301 ± 0.036 and 0.382 ± 0.023 in dry and wet treatment respectively.

The root: shoot ratio differs significantly ($p=0.05$) at both treatments in *C. occidentalis*. The R/S ratio in final harvest was 0.305 ± 0.016 in wet treatment and in DM treatment the value was 0.218 ± 0.045 . The R/S value did not follow a particular pattern all throughout the harvest. In contrast, in wet treatment the value increased from the 1st harvest onwards and finally reached to 0.305. It is of interest to note that from the 5th harvest onwards the R/S value of wet treatment was significantly ($p=0.05$) higher than dry treatment.

In case of *C. sophera* the curves were different. The R/S value was initially 0.208 ± 0.057 and began to increase and continued up to the final harvest (0.410 ± 0.016). In DM treatment the R/S values were significantly lower ($p=0.05$) in all the harvests. In DM treatment at final harvest the value was 0.397 ± 0.012 .

The reduction of overall growth under wet condition was not, however, reflected in the growth of the root system. The harvest data presented provide clear evidence of a shift in allocation of growth resources in favour of root development in wet treatment. It was noted that plants of wet treatment showed the formation of more lateral roots which altered the root/ shoot ratios. Brouwer (1962) stressed the dynamic nature of assimilate allocation in response to manipulation of root system, whilst Paltridge (1970, 1972) built into his stimulation model of a growing pasture an allocation strategy designed to maximize total growth in response to above and below ground stresses.



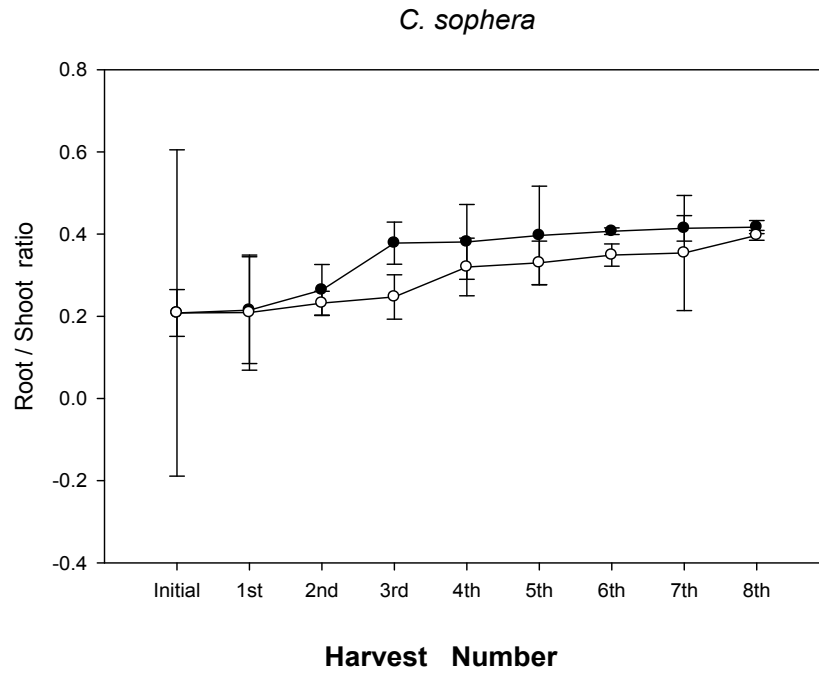


Fig 4.10. Progress curves of root: shoot ratio of the three species. Vertical bars on the graphs show 95% confidence limits. ●; Wet. ○; DM.

Root / shoot ratio in wet condition was higher than DM condition. Gardner (1960) has mentioned the importance of effective root length as a determinant of rate of water uptake from the soil. While it is not yet known exactly what proportion of a herbaceous root system constitutes “effective root length”, it is possible that much of the system laid down in the course of the experiment was capable of uptake since suberization was not well-developed even in the oldest roots. Since the greatest uptake occurs in the zone behind the growing apex (Brouwer 1953) the more profusely branched type of system produced in the non-waterlogged treatment would be expected to provide a more efficient exploitation of soil volume.

4.3.8. Relative Growth Rate (RGR)

It is now well known that relative growth rate in plants is subject to genetic, ontogenetic and environmental control. Relative growth rates (RGR) g/g/week of three *Cassia* spp. in the successive harvests are given in Fig 4.11 (a - c). Any attempt to make meaningful comparisons between species with respect to what is, in fact, a function close to their “economy in working”. (Blackman 1919) would face the considerable difficulty of deciding upon what basis and over what time period to study their growth. Such a study would certainly have to be conducted under controlled condition in which, as far as possible, no environmental factors were limiting, in order that the resulting rates of growth might be internally rather externally determined (Briggs 1928).

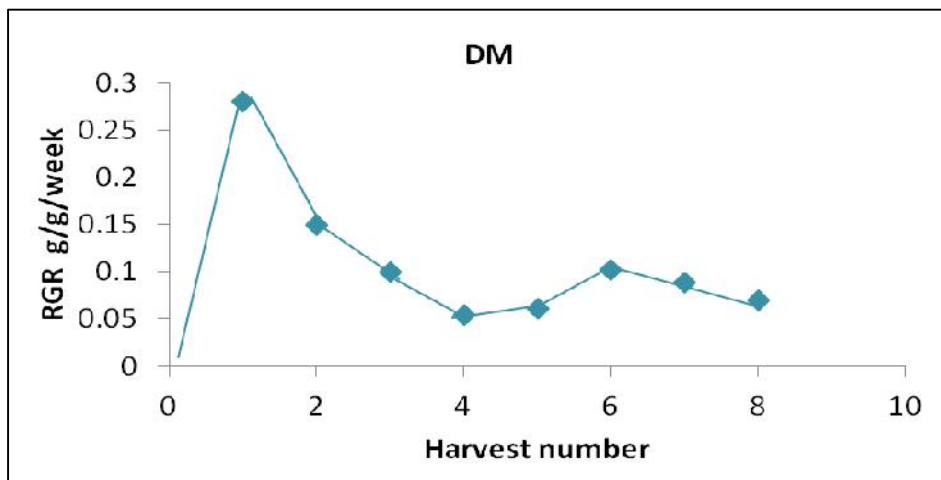
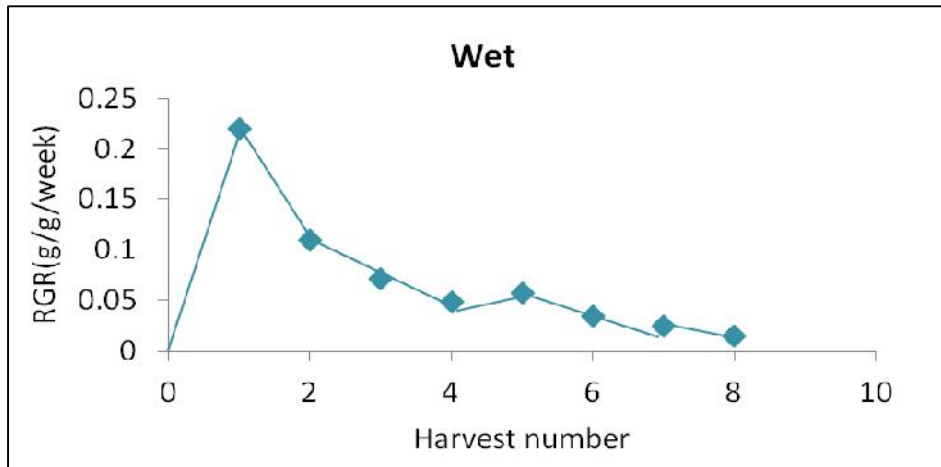
The graphs show dry matter production expressed in terms of relative growth rate; the changes in RGR with the time in relation to moisture. All three species showed

highest RGR initially and began to decrease in the subsequent harvest. RGR decreased drastically in wet treatment and also to a lesser extent in dry treatment in all the three species. The values of RGR of all species showed a continuous decrease up to the final harvest with slight modification in dry treatment in all the three species.

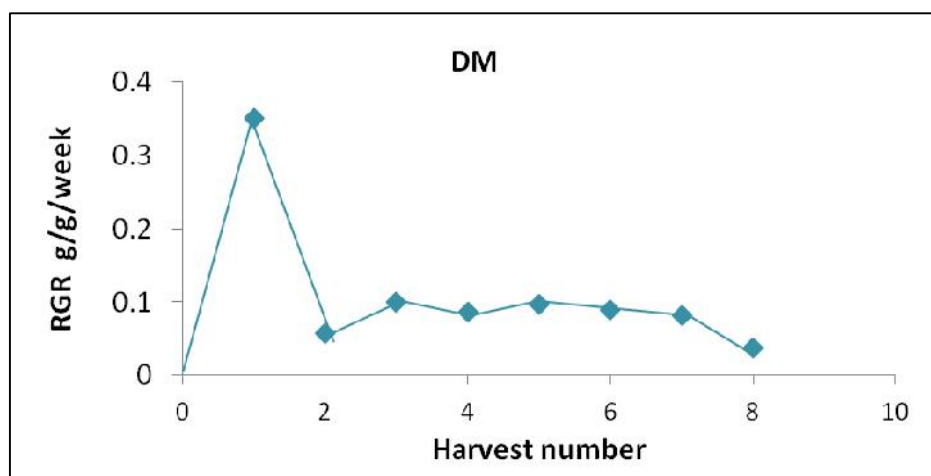
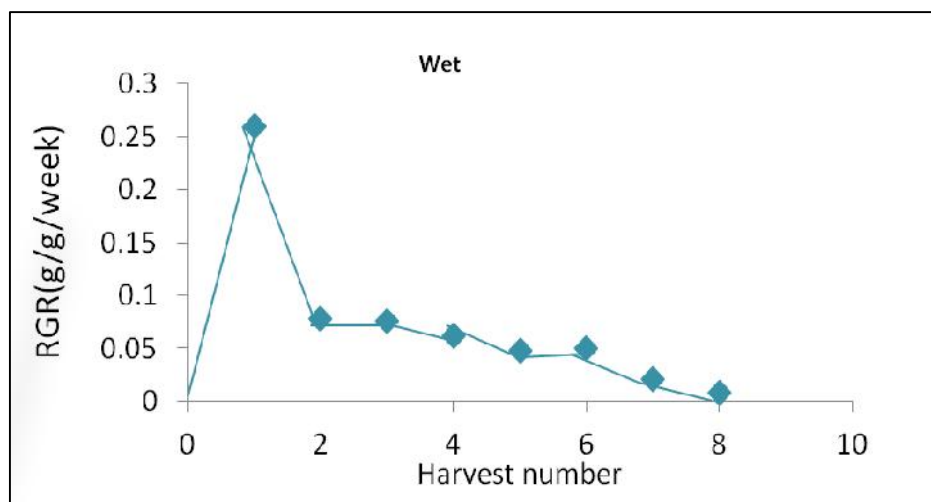
In *Cassia tora* in wet treatment RGR began to decline from the 3rd harvest (0.071) to 0.015 (8th harvest). In Dry moist treatment the decrease of RGR started from 4th harvest (0.041) to 0.0181 (8th harvest).

In *Cassia occidentalis* in wet treatment the RGR although was higher initially (0.078) than dry-moist treatment (0.057) but in the final harvest the RGR value decreased and was lowest in wet (0.008) than dry-moist treatment (0.028). Similar trend was also observed in *C. sophera*.

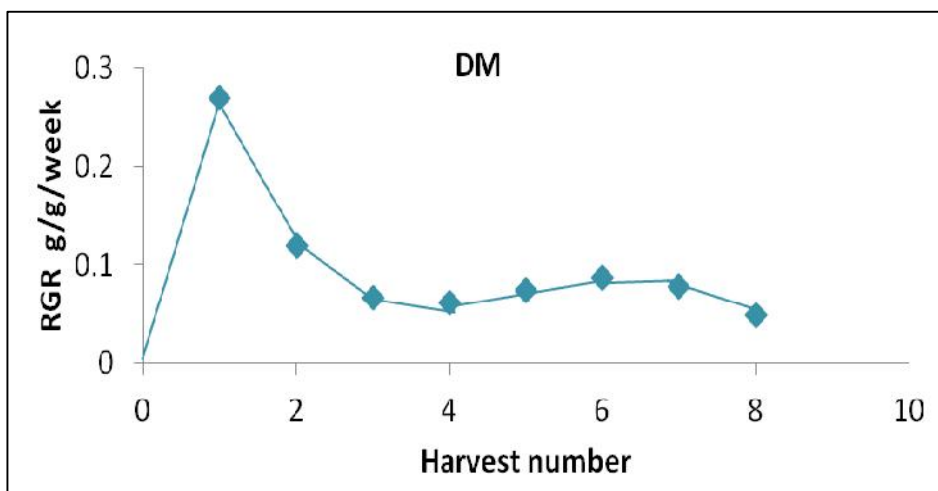
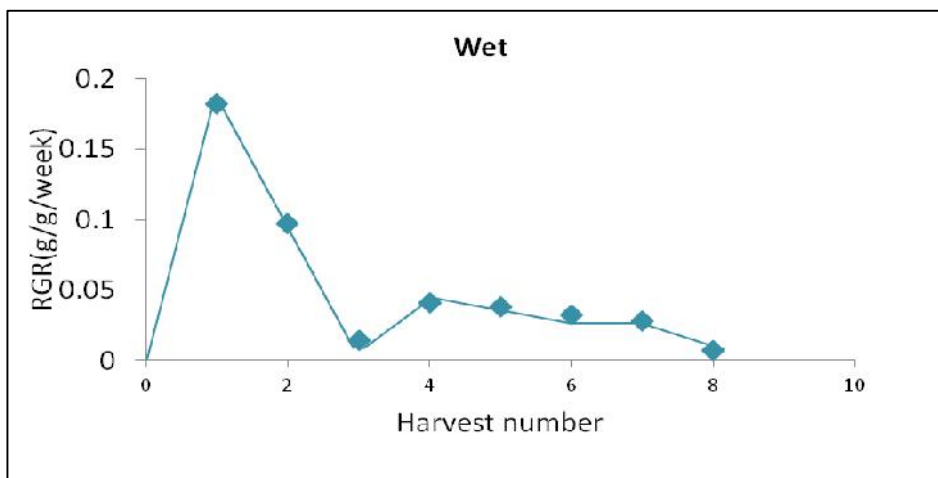
The poor RGR at the wet treatment compare to dry condition in all three species indicate flood susceptibility of these species. Similar type of result was also reported in other crops by Gardner (1970) and Russelle *et al.* (1984).



a. *Cassia tora*



b. *Cassia occidentalis*



C. C. sophera

Fig 4.11. Relative growth rate (RGR g/g/week) a. *Cassia tora*. b. *Cassia occidentalis*. c. *Cassia sophera*

CHAPTER 5

COMPARATIVE STUDY OF STOMATAL DENSITY AND INDEX IN THREE *CASSIA* SPP.

5.1. INTRODUCTION

Greek word “stoma” means mouth and the term “stomata” or “stomates (English) is derived from stoma which being the plural. The epidermis of the aerial parts of vascular plants is perforated by large number of small pores, surrounded with guard cells, known as stomata. Stomata are apertures in the epidermis, each bounded by two guard cells where the existence of stomatal pore is between the two guard cells. In some plants, the guard cells are the elliptical (kidney-shaped or bean- shaped) type, while in other plants they are shaped like a dumbbell (Graminaceous type) with two enlarged ends though there may be other special types of guard cell. By changes in shape, the guard cells bring about the opening and closing of the aperture. The stoma may be surrounded by cells which may or may not differ in shape and content from other epidermal cells. When clearly distinct, such cells are called subsidiary cells or accessory cells. These adjacent cells participate in the osmotic changes involved in the movement of the guard cells. Both types of cells i.e. guard cells and subsidiary cells are organized to form an anatomical unit called stomatal complex or stomatal apparatus. Classification of the many types of stomatal complexes can be made on the basis of the number and position of subsidiary cells and on the ontogeny of the cell types (Baranova 1987). There is a wide variety of configurations for these complexes in plants as described by Dilcher (1974),

Metcalf and Chalk (1979).

Stomata play an important role in the physiological activities of plant viz. first it serves in controlling transpiration; second to control gaseous and third is to prevent leaf surface from evaporative cooling by stomatal closure

(Bannister 1976). Their main function is to allow gases such as carbon dioxide, water vapor and oxygen to move rapidly into and out of the leaf. In leaves they occur either on both surfaces (amphistomatic) or on one only, either the upper (epistomatic) or more commonly the lower i.e.; hypostomatic leaf. On the basis of arrangement of epidermal cell neighboring guard cell, more than 25 main types of stomata in dicot have been recognized, (Metcalf and Chalk 1979). Stace (1980) reported 31 different types of stomata among cotyledonous plants. Metcalf and Chalk (1950) described the 4 types-i.e. Anisocytic, Anomocytic, Diacytic and Paracytic.

Stomatal study is important both from ecological and physiological point of view. The number of stomata per mm^2 of leaf surface, however, look small, the total number on a plant move to infinity. There are differences in the distribution of stomata on different leaf surfaces. Number, distribution, size, shape and mobility of the stomata are species – species characteristics, though they vary according to habitat and even among individuals (Larcher 1973). The stomatal frequency or density of stomata bearing epidermis varies among species from 20 to 2000 pores mm^2 , with the majority of values ranging between 40 to 350 (Weyers and Meidner, 1990).

Incidence of light directly controls the epidermal structure and stomatal frequency in plant of sun and shade habitats (Pandeya *et al.* 1968).

Frequencies and size of stomata also vary at different parts of a single leaf (Nazrul – Islam and Ahmed 1995) and different aged leaf and regions of a leaf with reference to node distances from ground height and also in relation to habitat i.e. in sun or shade (Nazrul – Islam 1988; Nazrul –Islam and Ahmed 1995). Sen *et al.* (1972) have mentioned that for the opening of the stomata there must be an uptake of water resulting in an increase in the turgidity of the guard cells. In general, the number of stomata per unit leaf surface area is higher in dry condition than those grown at high humidity (Lal and Mehrotra 1949; Nazrul –Islam and Alam 1986).

The total number of stomata on a leaf surface can be estimated from stomatal frequency \times Leaf area. The frequency of stomata relative to other types of cells on the epidermis may be of greater interest than absolute values. In such cases it may be appropriate to determine the stomatal index (Salisbury 1927). It can be seen that the stomatal index is essentially independent of leaf size once the cells are differentiated. If the frequency and average area of the pores can be estimated, then the total pore area can be calculated as a proportion of the leaf area. Measurement of stomatal aperture is important from the ecological point of view (Slatyer 1967; Barrs 1968; Kramer 1983; Sullivan 1971; Sullivan and Eastin 1975).

The present observation deals with the stomatal types, stomatal density and stomatal index in the adult plants of *Cassia tora*, *C. occidentalis* and *C. sophera*. The main aim of the present study was to note several performances in relation to environmental conditions to develop a hypothesis to explain the nature of adaptation and distribution.

5.2. MATERIALS AND METHODS

The plants of *Cassia tora*, *C. occidentalis* and *C. sophera* were collected from sun and shade habitats and wet and dry moist treatments. Stomatal density and stomatal index were determined from the both surfaces of the leaves.

5.2.2. Methods

Leaves of the above mentioned species were detached from the plants and the petioles were placed in a Beaker filled with water. These were then brought to laboratory and epidermal peels were taken from adaxial (upper) and abaxial (lower) surfaces.

The peels were mounted in water and then studied by a precalibrated microscope. Number of stomata (S) and epidermal cells (E) were counted from temporary mounts of epidermal peels and then stomatal density and stomatal index were calculated.

5.2.3. Stomatal Index

Stomatal Index (SI) was calculated from the formula using the number of stomata (S) and epidermal cells (E) in a unit area:

$$\text{Stomatal Index (SI)} = \frac{S}{E + S} \times 100 \quad (\text{Pandeya et al. 1968})$$

S = Number of Stomata,

E = Number of epidermal cells.

5.2.4. Stomatal density

Density of stomata was determined as the total number of stomata per unit area and was expressed per mm^2 . The area of the view under the microscope was calculated from the formula πr^2 i.e. area of the circle and is given as below:

Standardization of oculometer at 10 X 40

Serial no.	Oculometer reading in division	Stage micrometer in division	Stage micrometer / Oculometer	Conversion in micron (μ)	Mean
1	19	5	0.26	2.6	2.5
2	39	10	0.25	2.5	
3	59	15	0.25	2.5	

Diameter of oculometer division of a view at 10x40 is 160 divisions.

So the radius (r) is 80 divisions.

1 divisions = 2.5 μ

90 divisions = 2.5 \times 80 μ

= 200 μ

= 0.2 mm

Calculation of area of a circle:

Area of a circle = πr^2

$$= 3.14 \times (0.2 \text{ mm})^2$$

$$= 3.14 \times 0.04 \text{ mm}^2$$

$$= 0.1256 \text{ mm}^2$$

$$\text{Stomatal density} = \text{Mean no. of stomata, } \bar{X} \times \frac{1}{0.1256}$$

$$= \bar{X} \times 7.96$$

$$\text{Therefore, stomatal density} = \bar{X} \times 7.96$$

5.3. RESULTS AND DISCUSSION

5.3.1. Morphology

The leaves of all three species share a number of common features, i.e. shape of epidermal cell, distribution and orientation and Stomatal types.

Epidermal Cell: It was found that the epidermal cell shape varies slightly. They may be squarish, rectangular, variously elongated, with straight anticlinal walls. The leaf epidermal cell shape was pentagonal to polygonal in both surfaces (adaxial and abaxial surface) in all the three *Cassia* spp with the exception in *Cassia sophera* abaxial surface where they have showed irregular shape. This result is in agreement with the work of Reddy and Shah (1981) on the structure, ontogeny and distribution of stomata and trichomes on the pericarps of *Cassia sophera*.

Stomata :

- i) **Distribution and orientation:** The leaves of the three species of *Cassia* investigated were amphistomatic. The stomata are oriented irregularly or in the same direction, scattered or at times closely proximate throughout the

surface of the lamina except the vein regions. Sometimes, they are arranged parallel to the longitudinal axes of the organs. Holm (*cf.* Metcalfe and Chalk, 1950) recorded presence of stomata on both surfaces of Lamina of *Euphorbia corollata* and *Stillingia sylvatica* while Reiche (1923) reported their presence on both the surfaces in *Euphorbia radians*. Inamdar (1969) also found amphistomatic leaves in his work with some Zygophyllaceae and Simarubaceae.

ii) Stomatal types: Two different types (anisocytic and paracytic) of stomata were observed in these species. In anisocytic type the guard cells are surrounded by three unequal sized subsidiary cells. The common wall of which is at right angle to the longitudinal axis of stoma.

The guard cells of paracytic types are accompanied by two subsidiary cells, the longitudinal axis of which is parallel to that of the guard cells and aperture.

A mixture of both types of stomata in the same species was observed in these three *Cassia* spp. They showed paracytic stomata in adaxial surface and in the abaxial surface it showed paracytic stomata along with anisocytic type (Plate 5.1). The present investigation results is supported by the work of Tripath and Mondal (2012). They have worked with the quantitative and qualitative studies of stomata of selected six medicinally viable species of *Cassia* L. and found the similar results. Pandey (1969) described paracytic stomata on the leaflet of *Cassia sophera*. Okpon (1969) also reported paracytic stomata on the leaflets, sepals and pericarp of *C. sophera*. However, according to Shah and Gopal (1971) more than one type of stoma

paracytic , anisocytic, anomocytic and haplocytic occur on the leaflets of *C. sophora*, the paracytic as the most frequent type. Sen (1958) and Palwall (1965) reported different types of stomata on the lower surface of the leaf of *Basella rubra* Linn. The occurrence of diverse types of stomata was also observed on the same surface of the leaf by Pant and Kidwai (1964) ; Pant and Mehra (1964) ; Inamdar (1969 a, b, c).

Plate 5.1. Stomata of *Cassia* spp. at different treatments

a *Cassia tora* (wet) Adaxial 20X

b. *Cassia tora* (wet) Abaxial 20X

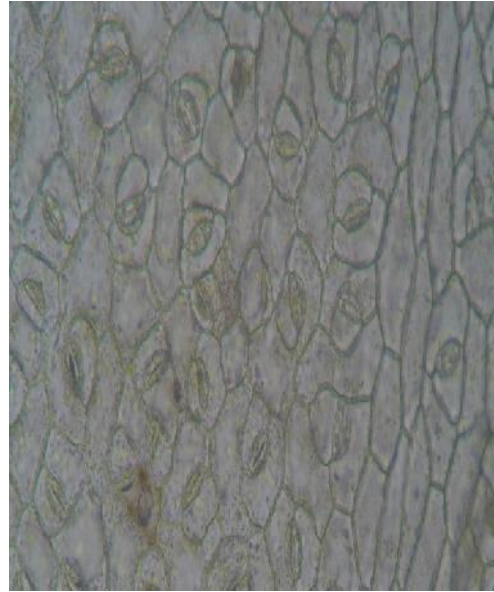
c. *C. occidentalis* (Dry- moist) Adaxial 20X

d. *C. occidentalis* (Dry- moist) Abaxial 20X

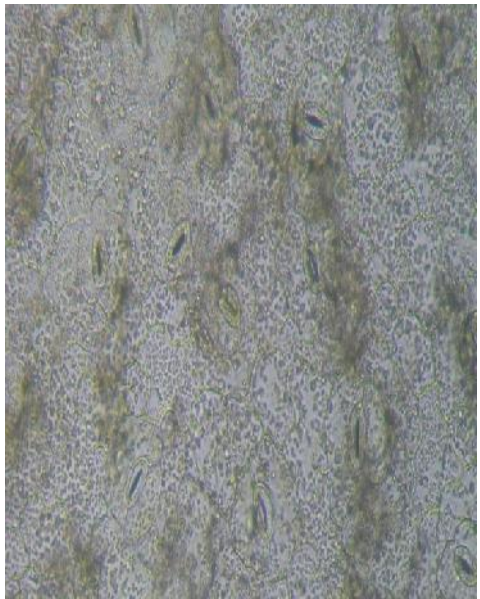
e. *Cassia sophera* (Dry- moist) Abaxial 20X



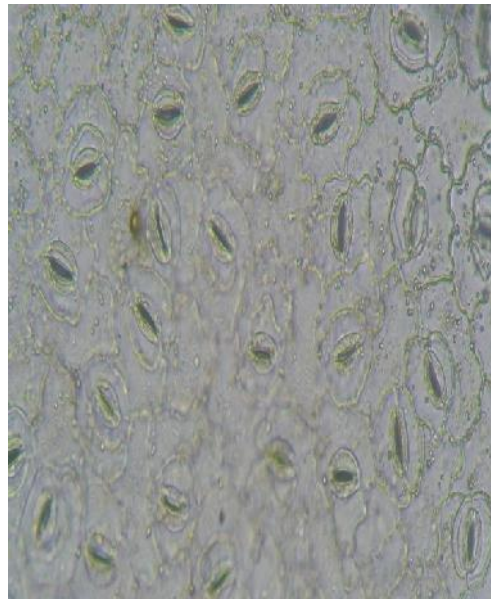
a. *C. tora* (wet) Adaxial (20X)



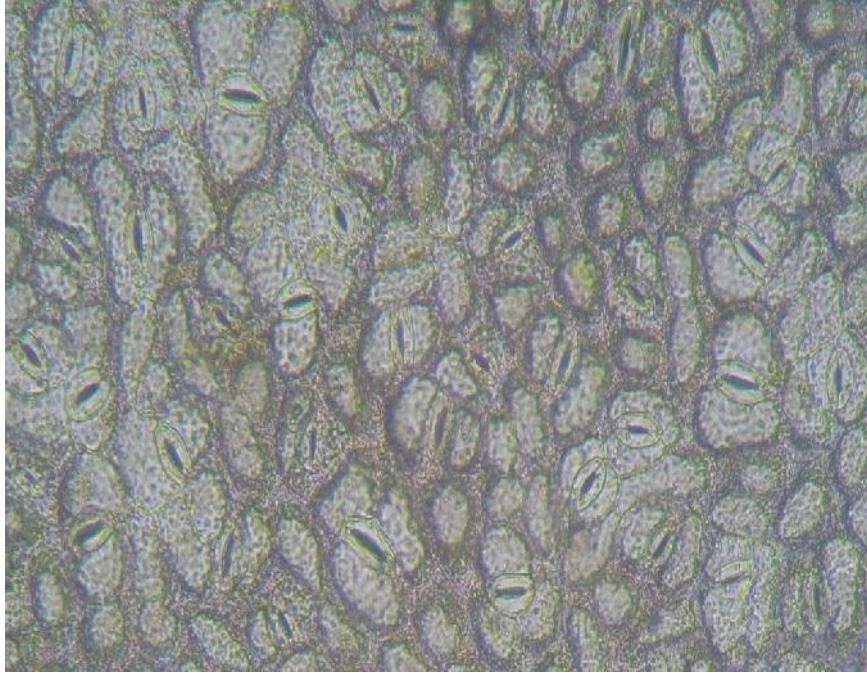
b. *C. tora* (wet) Abaxial (20X)



c. *C. occidentalis* (DM) Adaxial (20X)



d. *C. occidentalis* (DM) Abaxial (20X)



e. *Cassia sophera* (DM) Abaxial (20X)

5.3.2. *Effects of Sun and Shade on Stomatal Density and Stomatal Index*

The value of the stomatal density and stomatal index of plants are given in Table 5.1. stomatal density and the stomatal index, which indicate the proportion of stomata relative to leaf surface, varied from one species to another.

In general, the number of stomata of both adaxial (upper) and abaxial (lower) surfaces were more in the leaves of the plants growing in sun habitat than those of shade habitat in *Cassia tora*, *C. sophera*, *C. occidentalis*. stomatal densities on the abaxial surface in all three species of *Cassia* of sun and shade habitats were significantly ($p=0.05$) higher than those of the adaxial surface. (Table 5.1). Differences in stomatal density and stomatal index in different parts of the same leaf e.g. apex, middle and base were also found. Stomatal density in general was higher in the basal part of the leaf in *C. tora* and *C. occidentalis* and in case of *C. sophera* stomatal density was higher in the apex part. The stomatal index of the three *Cassia* species grown in sun and shade habitats was also higher on the abaxial surface than those of the adaxial surface.

Table 5.1: Stomatal density (mm^2) and index in *Cassia tora*, *C.**occidentalis* and *C. sophera* from different parts of the leaves.

95% confidence limits are also given

a) *Cassia tora*

Habitat	Leaf surface	Parts of the leaf	Stomatal density/ mm^2	Overall mean density/ mm^2	Stomatal index (SI)	Overall mean (SI)
Sun	Adaxial (Upper)	Apex	281.80±12.62	275.34±13.02	27.6±0.72	25.2±1.01
		Middle	239.58±13.54		20.4±1.80	
		Base	305.66±12.92		27.8±0.7	
	Abaxial (Lower)	Apex	385.26±29.67	429.73±18.49	26.8±6.3	30.6±2.6
		Middle	316.0±8.50		28.2±3.3	
		Base	587.94±17.31		36.9±1.2	
Shade	Adaxial (Upper)	Apex	196.59±15.39	216.05±28.50	20.1±0.7	20.0±0.3
		Middle	200.79±40.25		19.8±1.2	
		Base	202.78±29.87		20.1±1.3	
	Abaxial (Lower)	Apex	353.72±15.65	359.09±11.84	29.6± 1.0	29.1±1.9
		Middle	329.04±10.20		30.3±0.39	
		Base	394.52±9.676		27.6± 0.8	

b) *C. occidentalis*

Habitat	Leaf surface	Parts of the leaf	Stomatal density/ mm ²	Overall mean density/mm ²	Stomatal index (SI)	Overall mean (SI)
Sun	Adaxial (Upper)	Apex	145.86±5.87	172.37±8.15	20.5±0.7	21.3±0.6
		Middle	186.67±6.06		23.4±0.3	
		Base	184.6±12.53		20.1±0.9	
	Abaxial (Lower)	Apex	425.86±13.75	442.59±13.79	29.6±1.2	29.9± 0.9
		Middle	494.36±18.55		30.1±0.5	
		Base	407.55±9.06		30.0±1.0	
Shade	Adaxial (Upper)	Apex	153.12±8.82	158.69±9.34	28.8±1.1	26.5±0.9
		Middle	195.81±11.13		26.3±1.0	
		Base	125.26±8.07		24.5± 0.8	
	Abaxial (Lower)	Apex	388.40±6.09	346.60±8.39	29.9±0.3	30.6 ± 0.5
		Middle	332.74±10.13		31.0±0.4	
		Base	318.67±8.95		30.9± 0.9	

c) *C. sophera*

Habitat	Leaf surface	Parts of the leaf	Stomatal density/ mm ²	Overall mean density/mm ²	Stomatal index (SI)	Overall mean (SI)
Sun	Adaxial (Upper)	Apex	187.06±5.51	196.87±8.60	16.7±0.6	17.7±1.02
		Middle	227.65±10.45		19.8±1.78	
		Base	175.91±9.83		16.5±0.91	
	Abaxial (Lower)	Apex	558.79±12.53	524.56±15.43	29.2±0.82	27.1±0.5
		Middle	508.64±19.80		25.3±0.72	
		Base	506.25±13.97		26.9±0.15	
Shade	Adaxial (Upper)	Apex	161.58±8.85	153.89±9.09	13.0±1.9	13.4±2.8
		Middle	148.05±8.98		12.0±0.06	
		Base	152.036±9.46		14.2±0.64	
	Abaxial (Lower)	Apex	530.13±21.82	462.20±15.32	31.7±.11	27.0±0.4
		Middle	440.18±10.74		27.8±.56	
		Base	416.308±13.40		21.5±0.6	

The value of stomatal density in *C. tora* on both surfaces of sun habitat was highest in basal portion of the leaf; 305.66 ± 12.92 /mm² in adaxial surface and 587.94 ± 17.31 /mm² in abaxial surface followed by apex and then middle.

In case of shade habitat, maximum value of stomatal density was found in the middle part of the leaf in adaxial surface and in abaxial surface the highest value was in the basal portion of the leaf (394.52 ± 9.676) /mm² followed by apex (353.72 ± 15.65) /mm² and middle (329.04 ± 10.20) /mm². Highest stomatal index of sun habitat was found in the basal portion (36.9 ± 1.2) /mm²

in abaxial surface and lowest value was found in the middle portion (20.4 ± 1.8) /mm² in adaxial surface. Whereas, in shade habitat the highest value was found in the middle part followed by apex and basal portion of leaf in abaxial surface.

In case of *C. occidentalis* stomatal density of the abaxial and adaxial surfaces in middle part of the both sun and shade habitats were greater than those of apex and basal parts except in the lower surface of shade habitat where the highest value was in apex followed by middle and Base. The overall mean value of stomatal density was significantly higher ($p=0.05$) in the lower surface of the leaf both in sun and shade habitat. In sun habitat the value was (442.59 ± 13.79) /mm² in lower surface whereas, the value was only (172.37 ± 8.15) /mm² in the upper surface, in shade habitat the value was (346.60 ± 8.39) /mm².

Nazrul-Islam and Sarker (1985) have worked with some members of Amaranthaceae and Compositae from sun and shade habitats. They have found the similar results, i.e. the frequency of stomata is usually higher in plants grown in sun than in shade habitat.

Maximum stomatal index was found in middle portion in the sun and shade habitats on both surfaces except adaxial surface in shade condition. In adaxial surface of shade condition maximum stomatal index was found in apex portion (28.8 ± 1.1) followed by middle and base of the leaf.

In *C. sophera*, highest stomatal density was found in the apex portion in both surfaces of the sun and shade habitats except adaxial surface in sun condition. In sun condition of adaxial surface highest density was found in middle portion (227.65 ± 10.45) mm^2 followed by apex and Base (175.91 ± 9.83) mm^2 .

In adaxial surface of the sun, highest value of stomatal index was found in middle portion (19.8 ± 1.78) and apex portion (29.2 ± 0.82) of abaxial surface. In shade habitat, highest stomatal index was in basal portion (14.2 ± 0.64) on adaxial surface and middle portion (27.8 ± 0.56) on abaxial surface.

5.3.3. Effects of wet and dry-moist treatment on stomatal density and stomatal index

Stomatal density and stomatal index was also recorded in the wet and dry-moist treatment of *C. tora* but in *C. occidentalis* and in *C. sophera*; only the value of Dry-moist treatment was noted down as the wet treatment plants died before it reached into maturity (Table 5.2 and Plate 5.1).

In *C.tora* the stomatal density also varied significantly within the adaxial and abaxial surface. In case of wet treatment the highest stomatal density was recorded in the middle portion (624.9 ± 26.69) $/\text{mm}^2$ of the abaxial surface, whereas, the lowest

stomatal density was exhibited in the apex (386.06 ± 15.24) /mm² portion of the adaxial surface.

In case of dry-moist treatment in the abaxial surface the highest stomatal density was exhibited in the apex (626.5 ± 28.50) /mm² part of the leaf followed by base (630.43 ± 12.53) /mm² and middle (568.05 ± 12.33) /mm². In the adaxial surface of the DM treatment apex portion (400.39 ± 7.12) /mm² exhibited the highest value of stomatal density followed by base (386.65 ± 17.39) / mm² and middle portion (268.25 ± 17.62) /mm².

The overall mean density of stomata /mm² was higher in the lower surface of the dry-moist (608.32 ± 17.75) /mm² treatment than wet treatment (593.82 ± 19.54) /mm² but the value of stomatal density in the upper surface differs from where in the wet treatment the overall mean value of stomatal density was higher in upper surface (416.57 ± 12.18) /mm² than in dry-moist treatment (351.76 ± 14.04) /mm². The stomatal index was higher in wet than dry-moist treatment in both surfaces of

C. tora.

Table 5.2. Stomatal density (mm^{-2}) and index in *Cassia tora*, *C. occidentalis* and *C. sophera* from different parts of the leaf. 95% confidence limits are also given (Wet and Dry –moist treatments)

a) *Cassia tora*

Habitat	Leaf surface	Parts of the leaf	Stomatal density/ mm^2	Overall mean density/ mm^2	Stomatal index (SI)	Overall mean (SI)
Wet	Adaxial (Upper)	Apex	386.06±15.24	416.57±12.18	30.07±0.86	29.4±0.7
		Middle	414.72±10.20		28.9±0.49	
		Base	448.94±11.12		29.44±0.8	
	Abaxial (Lower)	Apex	565.16±13.95	593.82±19.54	31.7±1.1	39.9 ± 0.7
		Middle	624.9±26.69		37.8±0.83	
		Base	591.43±18.00		30.2±0.3	
Dry-moist	Adaxial (Upper)	Apex	400.39±7.12	351.76±14.04	28.1±0.9	28.9±1.2
		Middle	268.25±17.62		22.3±1.3	
		Base	386.65±17.39		36.3±1.4	
	Abaxial (Lower)	Apex	626.5±28.50	608.32±17.75	31.3±0.4	30.9±0.5
		Middle	568.05±12.23		31.7±0.68	
		Base	630.43±12.53		29.9±0.42	

b) *C. occidentalis*

Habitat	Leaf surface	Parts of the leaf	Stomatal density/ mm ²	Overall mean density/mm ²	Stomatal index (SI)	Overall mean (SI)
Dry-moist	Adaxial (Upper)	Apex	380.49±5.22	352.24±8.56	21.0±0.42	18.8±1.52
		Middle	348.65±10.31		18.5±0.17	
		Base	327.58±10.15		16.9±0.20	
	Abaxial (Lower)	Apex	403.57±13.16	431.69±13.4	24.4±1.40	25.5±0.80
		Middle	473.62±15.69		26.6±0.40	
		Base	417.90±12.39		25.7±0.80	

c. *C. sophera*

Habitat	Leaf surface	Parts of the leaf	Stomatal density/ mm ²	Overall mean density/mm ²	Stomatal index (SI)	Overall mean (SI)
Dry-moist	Adaxial (Upper)	Apex	382.88±9.47	275.15±7.89	21.1±0.2	17.01±0.3
		Middle	223.68±8.68		12.4±0.51	
		Base	218.90±5.54		12.8±0.34	
	Abaxial (Lower)	Apex	337.50±8.14	362.44±10.32	13.9±0.31	15.0±0.2
		Middle	359.0±7.32		16.4±0.42	
		Base	390.84±15.51		14.9±0.15	

In case of *C. occidentalis* and *C. sophera* only the values of dry-moist treatment were recorded. In *C. occidentalis* the stomatal density was higher in the middle portion (473.62 ± 15.69)/mm², whereas, the lowest value was in the basal portion (327.58 ± 10.15)/mm² of the leaf. Stomatal index was higher (25.5 ± 0.8) in lower surface than in upper surface (18.8 ± 1.52).

In *C. sophera* the value of stomatal density followed the similar pattern of *C. occidentalis*. The value of stomatal index exhibited the opposite pattern where the value was higher in the adaxial surface (17.01 ± 0.3) than abaxial surface (15.0 ± 0.2).

Rugmini (1960) and Nazrul - Islam and Ahmed (1995) have shown that stomatal density was usually lower in the basal portion than the apex and middle portion of the leaf.

Emphasis has been laid down on the stomatal characteristics of selected species in relation to drought including the development and movement of stomata in relation to environment (Inamadar, 1968; Inamdar and Patel, 1969; Kothari and Shah, 1975; Reddy and Shah, 1981; Nazrul - Islam and Sarker, 1983). Franco (1938) noted in coffee leaves that the difference in number of stomata per unit area in the different regions of the same leaf is not significant. In the present investigation, significant difference ($p = 0.05$) in the stomatal density among apex, middle and basal parts of the leaves was observed.

Significant difference ($p = 0.05$) was observed between abaxial surface of the same leaf. Reed (1931) and Rugmini (1960) have studied *Citrus* species and *Ageratum conyzoides* and found that the density of stomata varies in different parts of leaves.

However, the results of the present investigation are comparable to some extent with the work of Rugmini (1960) and Reed (1931).

Stomatal index was always lower in the adaxial portion of the leaf. The lower surface showed high stomatal index. Shidhu (1960) studied the stomatal index in a number of mangrove species. He noted the lowest value 1.2 (*Rhizophora sp.*) to highest 7.0 (*Bruguiera sp.*). Since the results were variable, study of stomatal index at the seedling stage to maturity might provide some valuable information which may be useful to explain adaptation, distribution and abundance of plants in the natural environment.

CHAPTER 6

GROWTH OF THREE *CASSIA* SPECIES (*Cassia tora*, *C. occidentalis* and *C. sophora*) IN RELATION TO SUN (100% LIGHT) AND SHADE (15% LIGHT)

6.1. INTRODUCTION

All living organisms are at various stages in their life history, capable of 'growth' in the sense of change in size, change in forms and in number, given suitable conditions (Hunt 1978a). These three processes together form an important part of the phenomenon of life itself and among natural systems and thus help to distinguish the living from the non living.

Light is an important factor for growth, development, photosynthetic activities and distribution and also affects stomatal movement, photosynthesis, growth, reproduction, flowering, germination and other physiological activities of plants. Percentage of full daylight, which the species requires in the field just to keep itself alive, is known as compensation point. According to Misra *et. al.* (1969), It is the light intensity at which the plant's energy income just balances its expenditure through respiration. Shade plant has not only a lower compensation point than a sun plant, but also it cannot make such good use of high light intensities (Misra *et. al.* 1969; Mahmood *et. al.* 1975). Scientists confirmed the importance of high light intensity on seedling growth and survival. Mayer and Mayber (1982) and Osunkoya *et al.* (1994) found that many trees reduced their growth (e.g. root size and weight) with decreased light intensity.

Plant growth depends on the availability of light and mineral nutrients. In mixed plant communities, the relative amount of intercepted light and the amount of nutrients taken up largely determine whether or not a species can persist in the community (Tilman 1985, 1988; Berendse *et al*, 1987, Aerts *et al* 1990.1992) As a consequence, the allocation of biomass and nutrients to structures involved in the acquisition of light and nutrients is subject to natural selection. In general, the allocation patterns of biomass and mineral nutrients are different (Abrahamson and Caswell 1982 ; Aerts and De Caluwe 1989 ; Ohlson and Malmer 1990.)

Whitehead (1962) showed that plants possess or are capable of advantageous modifications of the phenotype in response to environmental changes, which he called 'Compensating mechanisms '. In later studies, Myerscough and Whitehead (1967), in the course of investigations over a range of environmental conditions, showed that species may react differently in terms of growth and development and that some species have more efficient compensating mechanisms. It was also shown that although such compensating mechanism may aid survival, adverse effects on reproduction both by seed and vegetative means occur.

Comparative studies (poorter 1989, Poorter and Remkes 1990 and Poorter *et al*. 1990) in growth cabinets showed that under optimal conditions of resources supply and in the absence of interference with other plants, high productive plant species are characterized by i) a high biomass allocation to the leaves; ii) a high specific leaf area; iii) a high leaf area ratio (leaf area per unit plant weight); iv) a high nitrogen concentration in the leaves.

In nature, the species *Cassia tora*, *C. occidentalis* and *C. sophera* occur in both open and shade habitats, which indicate their differential tolerance of light intensity.

Hunt (1978a) described the utility of plant growth analysis, the rates of dry weight increase for a variety of plant species under favourable conditions. He has two approaches to plant growth analysis-

1. The 'classical approach, in which the course of event is followed through a series of relatively infrequent, large harvests (with much replication of measurements).
2. The 'functional 'approach in which harvests supplying data for curve fitting are smaller (less replication of measurement) but more frequent.

Every species has a certain range of light requirement under which it shows the best performance, when no other factor limits its growth. On the principle, some species are designated as "Heliophobous" (shade tolerant or shade loving) when they grow under forest canopy or do well under shade conditions. Others have been observed to grow only in open place; with no shade to interfere are regarded as "Heliophilous" (sun loving) plants. Hence there is a need to investigate the effect of light intensity on the distribution of various herbaceous plant species growing in fallow and derelict land. It is really difficult to evaluate the intensity of light and also to know the precise amount of light that plants actually use.

Furthermore, it is desirable to have only light readings at the moment of the determination but continuous records extending over a period of hours a day is necessary. Therefore, the present experiment was designed to examine the effect of

amount of light (sun100%) and (shade 15%) on the growth, development and flowering behavior of three *Cassia* species under sun and shade conditions.

6.2. MATERIALS AND METHODS

6.2.1. Sources of seeds

Seeds of *Cassia tora*, *C. occidentalis* and *C. sophera* were collected (September 2009 and October 2010) from Savar, Agargau and from The Dhaka University campus, dried in the sun and stored in plastic containers in the laboratory at room temperature which were used in the present investigation.

6.2.2. Methods of Preparation of the soil and pots

This procedure is given in Chapter 4.

6.2.3. Experimental Procedure

6.2.3.1. Germination of seeds

Healthy seeds 25 in number of *Cassia tora*, *Cassia occidentalis* and *Cassia sophera* were set for germination in the earthen pots. .

The experiment was carried out on the roof of the Botany Department Building. The experiment was set on 31May 2011 and continued up to 30th November. The experimental design was 3 species x 2 treatments x 4 replicates; total 24 (twenty four) pots. All the potted plants were watered regularly for the first one week and then the following treatments were imposed. Before imposing the treatments the pots

containing seedlings were kept under the cover of a net box to protect them from the disturbance of rats and birds.

6.3.2.2. Treatments

Photosynthetically active radiation (PAR) was measured with the help of a Li-cor Quantum Meter (LI-185B, USA), connected to an aerial sensor (LI-1905B). At first the sensor was leveled with the help of a leveling meter attached to the sensor and then connected with the quantum meter. The meter was put on and the incident radiation was read by adjusting the knob. The value was recorded from the analog scale of the meter in $\mu\text{E m}^{-2}\text{Sec}^{-1}$.

The potted plants were subjected to two treatments

- a) Sun (Full Light ie.100% light)
- b) Shade (15% light); kept by the side of walls.

In case of sun treatment, all the pots were kept to direct sunlight so that the plants could receive full sunlight throughout the day and in direct sun the value of Photosynthetically active radiation (PAR) was $1410 \mu\text{Em}^{-2}\text{Sec}^{-1}$ (100% light), Whereas in case of shade treatment, the pots were placed by the side of the walls in the roof where no direct sunlight could reach throughout the day and the PAR value was $207 \mu\text{Em}^{-2}\text{Sec}^{-1}$ (15% of full light).

6.2.4. Growth analysis

The procedure of harvesting and method of analysis was given in **Chapter 4**

6.3. RESULTS AND DISCUSSION

6.3.1. *The effect of sun (100% light) and shade (15% light) on morphology and growth*

The morphology of the plants was studied in every harvest and the features of the plants at the last harvest are given in Table 6.1 and the appearance of the plants is given in plates 6.1 (a, b, c)

Vegetative growth was decreased from the early stage in shaded treatment (15% light) whereas it was vigorous in sun treatment (100% light). The time required to each particular phase of development was longest at 15% light and shortest at 100% light. The number and stage of development of leaflet was greatly reduced in 15% light. At the same time, system of roots and number of leaflet was well developed in sun condition (100% light) plants and least developed at the 15% light level. Plants of all three species grown in direct sunlight (100% light) were significantly ($p=0.05$) taller with thick stem than plants of shade treatment (15% light) and the plants of shade were dwarf in nature.

The Plate 6.1 shows that the plants of sun treatment have the superiority over the shade plants. There is evidence that all portions of the plants are modified by the amount of light to which they are exposed (Misra *et. al.* 1969).

The leaves differed markedly between light treatments in all three *Cassia* spp. The individual leaflets of all three *Cassia* spp. at similar stage of development were found to be progressively larger and the total leaflet area was higher in the sun treatment (100% light) than the plants of shade treatment. Shamsi and Whitehead (1974) investigated the effect of different light intensities on the growth of *Epilobium hirsutum* and *Lythrum salicaria*. They found that individual leaves of both species at similar stage of development were progressively larger as light intensity was reduced but the total leaf area was higher in full light regimes because of the development of a greater number of leafy shoots.

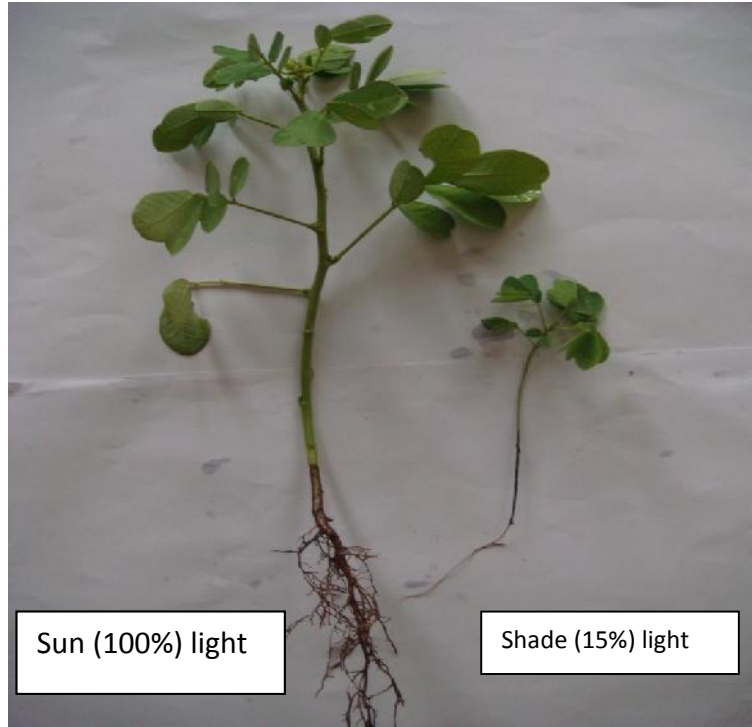
Table 6.1: Morphological features of plants of sun and shade treatments

in *Cassia tora* , *C. occidentalis* and *C. sophera*

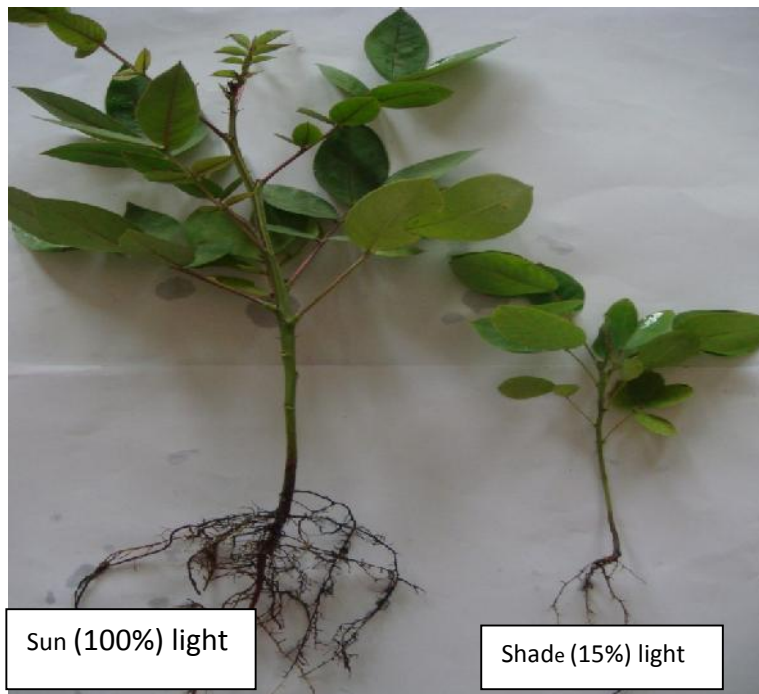
FEATURES	TREATMENTS	
	SUN (100% light)	SHADE (15% light)
Leaflets size	Large	Small
Leaf colour	Light green	Dark green
Branching pattern	High	No branching
Node & internodes length	Large in number but internode length is short.	Internode length is long.

Plate 6. 1. Plants of *Cassia* spp. under sun shade conditions, show very poor growth under shade condition.
(Seven weeks after imposing the treatment).

- a. *Cassia tora*
- b. *C. occidentalis*.
- c. *C. sophera*



C. tora



C. occidentalis



C. sophera

The tendency for a reduction in number of leafy lateral branches was reported by Hughes (1959) for plants grown in low light intensities. A very similar response appears to be common in *Epolibium hirsutum* (Shamsi and Whitehead 1974) and in the Gramineae (Evans *et al.* 1964; Friend 1965) where individual leaves may be larger in low light intensity but tillering is greatly reduced.

The pale green leaf indicates that the photosynthesis did occur poorly. There is evidence that all portions of the plants are modified by the amount of light to which they are exposed (Misra *et al.* 1969). In an experiment, Misra *et al.* (1969) showed that direct light promotes the production of leaves and flowers.

The elongated internode in shade treatment was an agreement with the work of Spence (1976). The internode elongation exhibited by terrestrial plants in the dark or in shade and the reduced elongation found in full sunlight is also shown by submerged aquatic species like *Potamogeton obtusifolium* and *P. crispus* which occur in both shallow and deep water. There is some evidence from plants of *P. crispus* grown experimentally at similar depths that conditions of low or high PAR respectively promote or inhibit internode elongation.

6.3.2. Effects of sun and shade on the root length and shoot height of Plants

The shoot height and root length were noted and the results are presented in Fig 6.1 and 6.2. The root growth of sun and shade plants is shown in plate 6.2. There was a significant difference ($p = 0.05$) in mean root length and shoot height of the plants with the difference of light intensity in almost all the harvests. The roots and their components part (lateral roots) showed luxurious growth in sun treatment. Further, it

was found that in *C. tora* the root length of both the treatments was always lower than that of *C. occidentalis* and *C. sophera*.

Root length was greater in sun plants than those of shade plants ($p=0.05$). Root length of sun condition plants in all three species began to increase significantly ($p=0.05$) from 3rd harvest onward. The root length at the final harvest was highest in *C. occidentalis* (29.84 ± 1.20) cm and (14.260 ± 2.00) cm in sun (100% light) and shade treatments respectively followed by *C. sophera* (25.35 ± 2.005) cm and (13.2500 ± 2.5400) cm, and *C.tora*. (21.85 ± 1.89) cm and (10.35 ± 1.4500) cm. Bakker (1952) ; Myerscough and whitehead (1967) have also shown that lower light intensities result in a reduction in numbers and size of rhizome, poor root development in both *Chaemaeneraion angustifolium* and *Tussilago farfara*.

In all three *Cassia* spp. the shoot height in sun (100% light) treatment was always higher in every harvest than that of shade (15% light) treatment. It is

Plate 6.2. The root growth in Sun (100% light) and Shade (15% light)

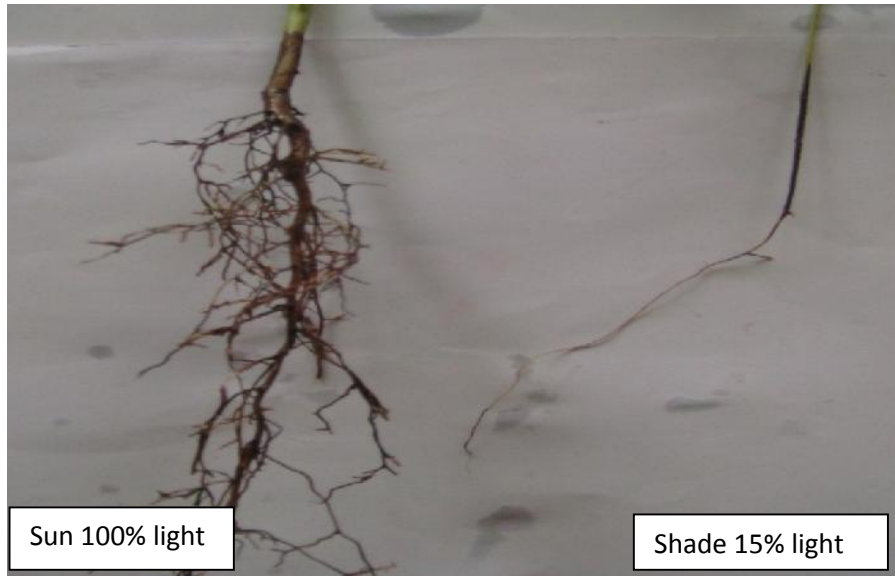
showing poor root development in shade treatments. (Seven weeks

after imposing the treatment).

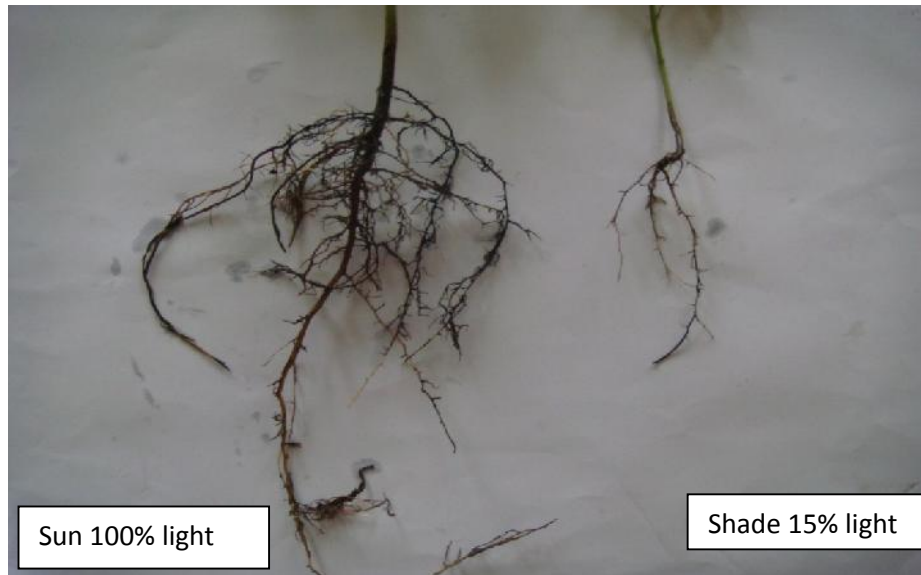
a. *Cassia tora*.

b. *C. occidentalis*.

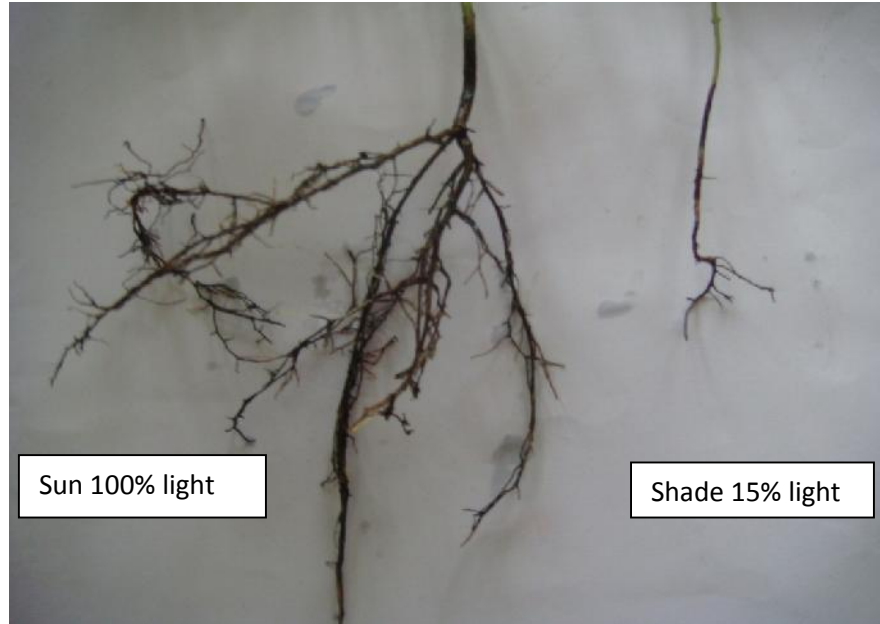
c. *C. sophera*.



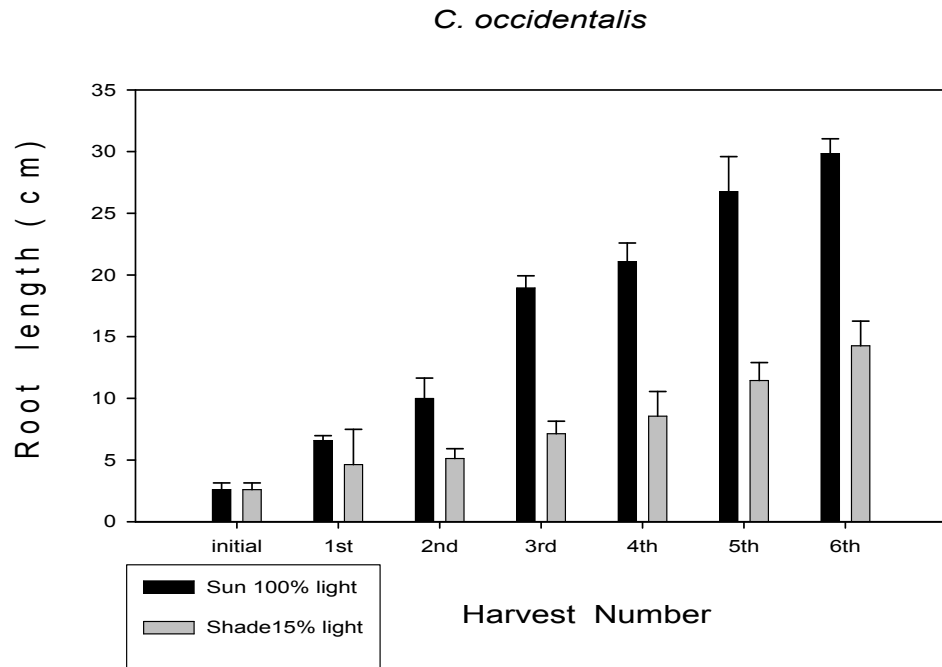
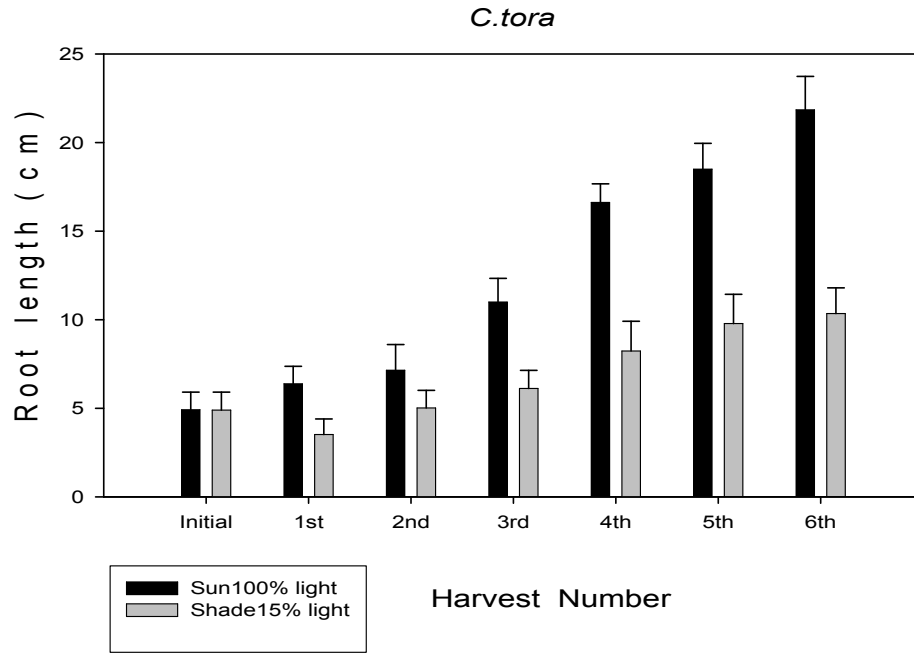
Cassia tora



Cassia occidentalis



Cassia sophera.



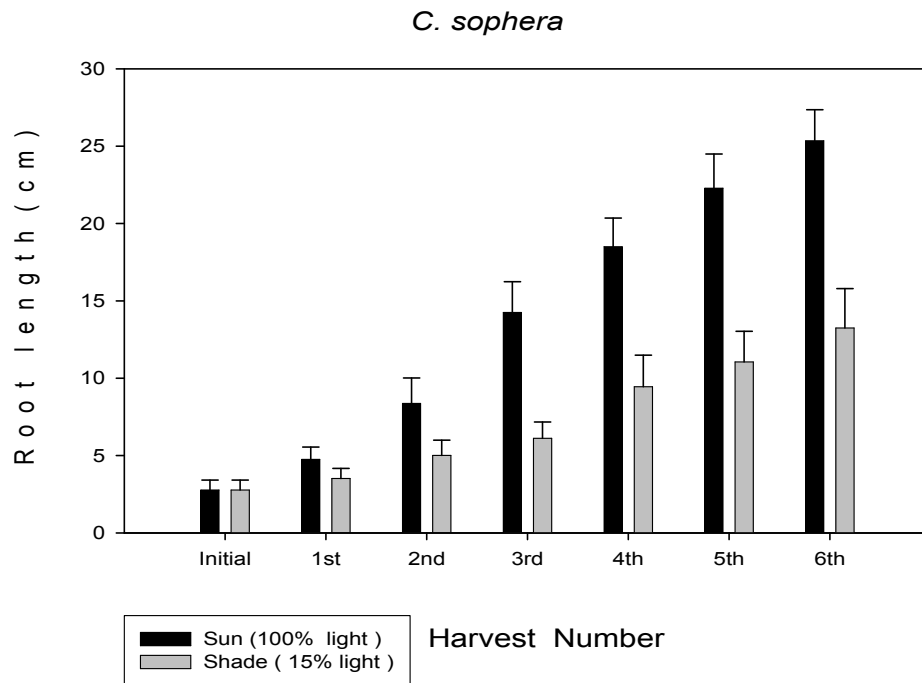
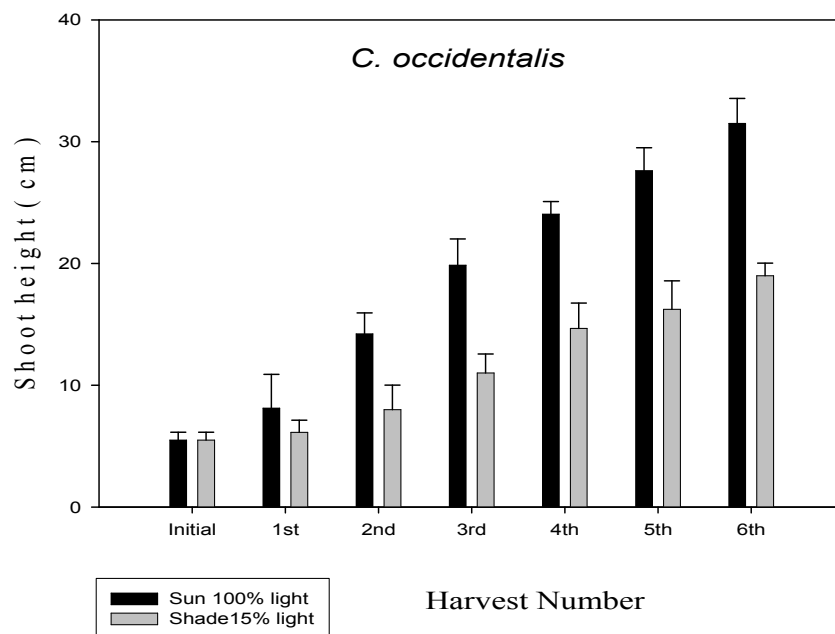
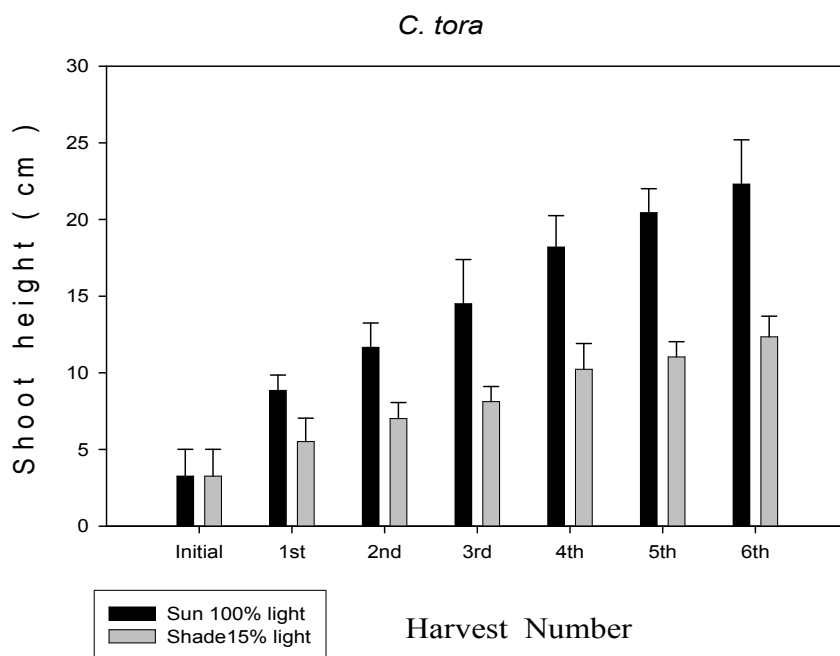


Fig 6.1. The effect of two light intensities (Sun 100% and Shade 15%) on the root length per plant at different harvests. Vertical bars are 95% confidence limit.



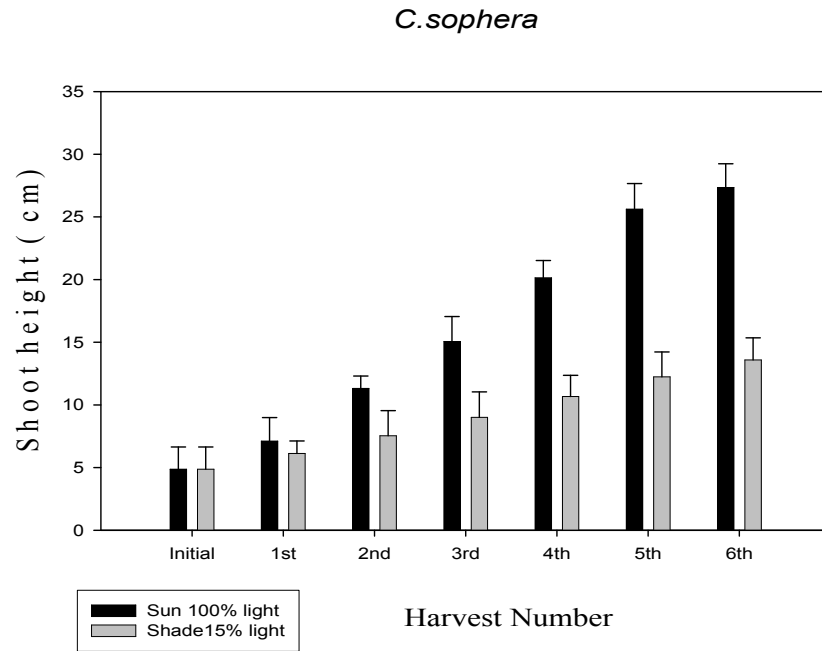


Fig 6.2. The effect of two light intensities (Sun 100% and Shade 15%) on the shoot height per plant at different harvests. Vertical bars are 95% confidence limit.

clearly visible from the plate 6.1 that in all three *Cassia* spp. the plants height are almost half in shade (15% light) treatment than that of sun (100% light) treatment. In *C. tora* the shoot height at final harvest was (22.31 ± 2.89) cm and (12.350 ± 1.340) cm in sun (100% light) and shade (15% light) respectively.

6.3.3. Increase of length of petiole, leaflet area and number of leaf

The individual leaf of *Cassia tora*, *C. occidentalis* and *C. sophera* at different stages of development was found to be progressively larger with tall petioles under the 100% light condition.

In *Cassia tora* the initial value of the length of the petiole was 14.38 ± 0.590 mm and at the final harvest it reached up to 61.25 ± 1.28 mm in sun treatment and up to 29.650 ± 1.11 mm in shade treatment. In *Cassia occidentalis* the initial value of the petiole length was $15.45.00 \pm 2.02$ mm and reached finally in 38.50 ± 0.913 mm at shade treatment and it was 72.350 ± 0.98 mm in sun treatment at last harvest. In *Cassia sophera* the length of the petiole of shade treatment in the final harvest was 36.0 ± 0.21 mm whereas it was 84.250 ± 2.82 mm in sun treatment (more than double than shade treatment) Fig 6.3 and plate 6.1.

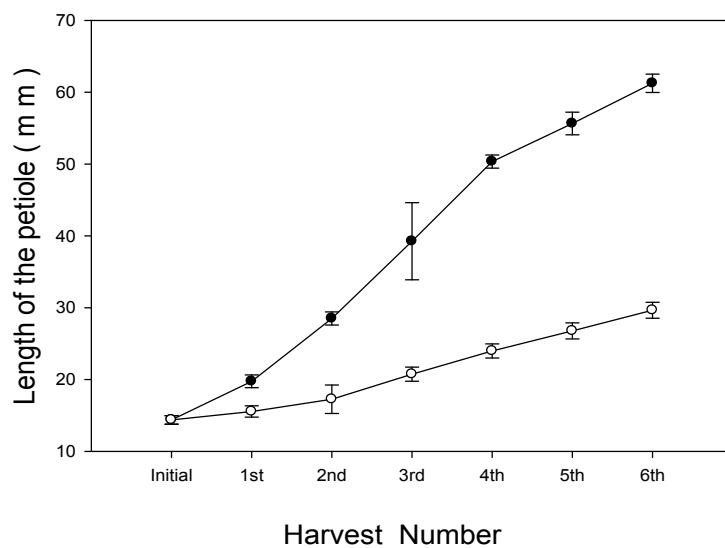
The general pattern of increase in leaflet area with time is given in Fig 6.4. There was a decrease in leaflet area with decreasing light intensity in all three *Cassia* spp. In *Cassia tora* initially the area of the terminal leaflet was $\log_e 4.7$ (122.00 mm^2) but gradually increased to $\log_e 6.73$ (841.5 mm^2) at final harvest in sun treatment and under shade treatment it was $\log_e 6.24$ (515.5 mm^2). In *Cassia occidentalis* leaflet area at initial harvest was $\log_e 2.62$ (129.5 mm^2) but at final harvest the value was

\log_e 7.24 (1404.0 mm²) in sun treatment and \log_e 6.70 (820.00 mm²) in shade treatment. In *Cassia sophera* at last harvest it was \log_e 6.43 (625.5 mm²) in sun and the value was \log_e 6.2 (505.75 mm²) in shade treatment.

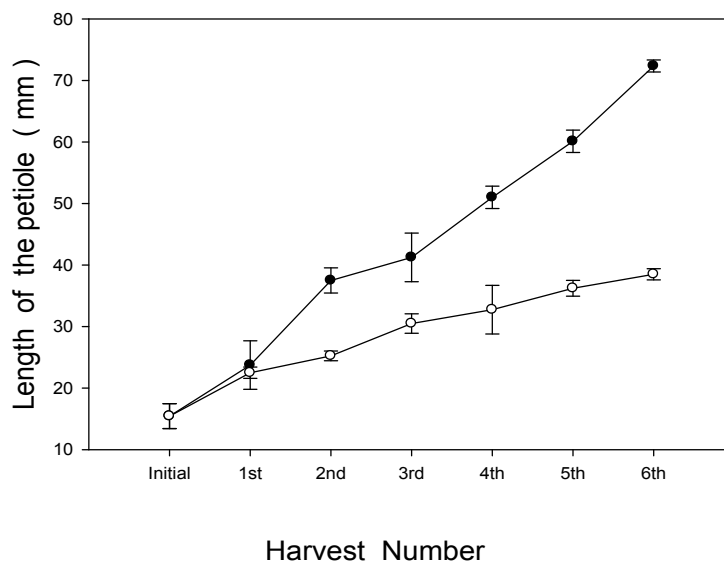
Shamsi and Whitehead (1974) have reported the similar phenomenon on *Epilobium hirsutum* and *Lythrum salicaria*. They have shown that there was a decrease in leaf area with decreasing light intensity. Senescence of a few leaves at the last harvest in the plants at 100% light brought their leaflet area at the final harvest to less than that of the plants in 15% light. The marked increase in leaf area with decrease in light intensities found in *Tussilago farfara* and *Chamaenerion angustifolium* (Myerscough and Whitehead 1967) was not exhibited by either of the species investigated here.

In all three *Cassia* spp. in shade treatment the number of leaflet was always less than sun treatment (Fig 6.3). In *Cassia tora* the number of leaflet at initial harvest was 6.0 ± 0.01 . At the last harvest the number of leaflets in sun treatment (50.50 ± 0.99) was more than the shade treatment (22.75 ± 1.21). In *Cassia occidentalis* the value at final harvest was 26.00 ± 0.99 in and it was 40.50 ± 0.99 in shade and sun treatments respectively. In *Cassia sophera* the number was more than double in sun treatment (66.00 ± 2.58) than shade treatment (31.00 ± 0.25).

a. *C. tora*



b. *C. occidentalis*



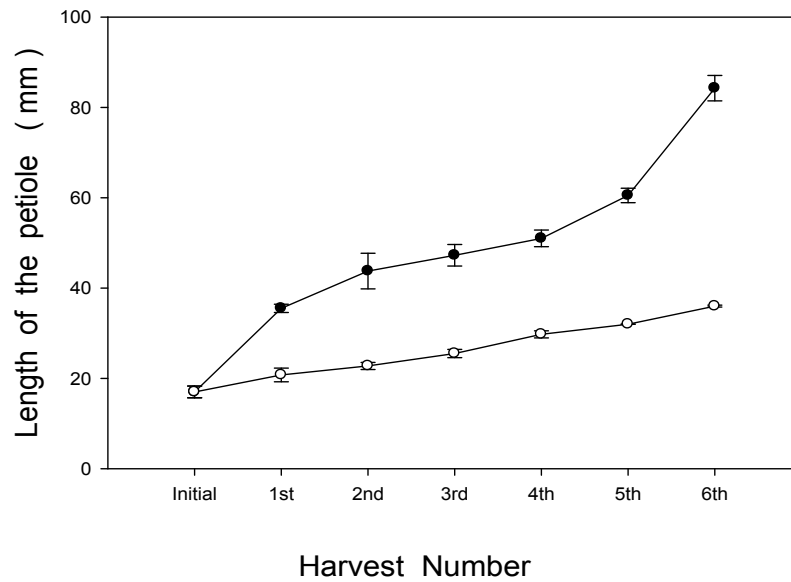
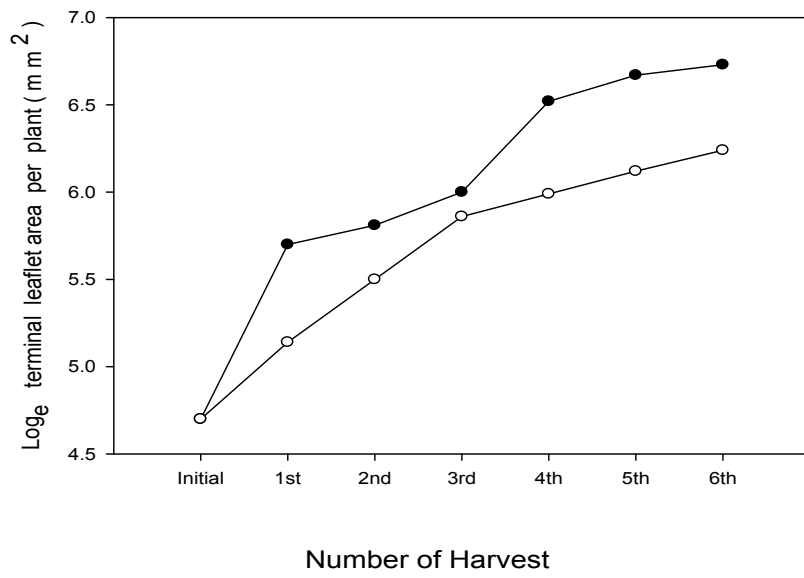
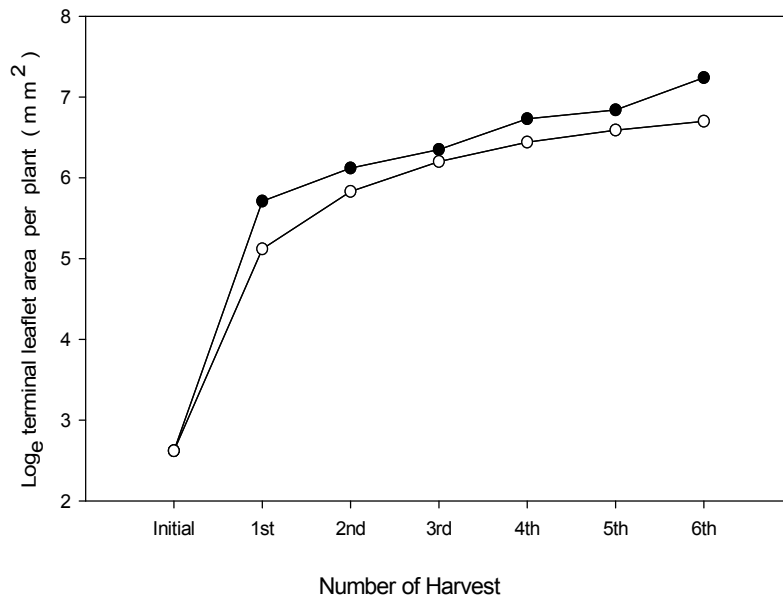
c. C.sophora

Fig 6.3 . The effect of two light intensities (Sun 100% and Shade15%) on the increase of length of the petiole with time. ●; Sun (100% light), ○; Shade (15% light). Vertical bars are 95% confidence limit.

Cassia tora



Cassia occidentalis



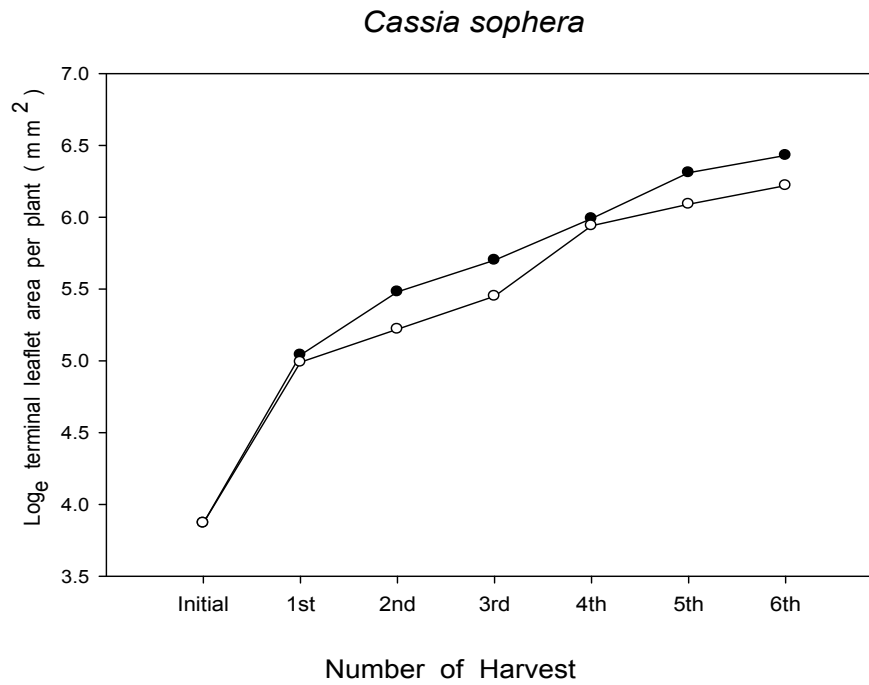
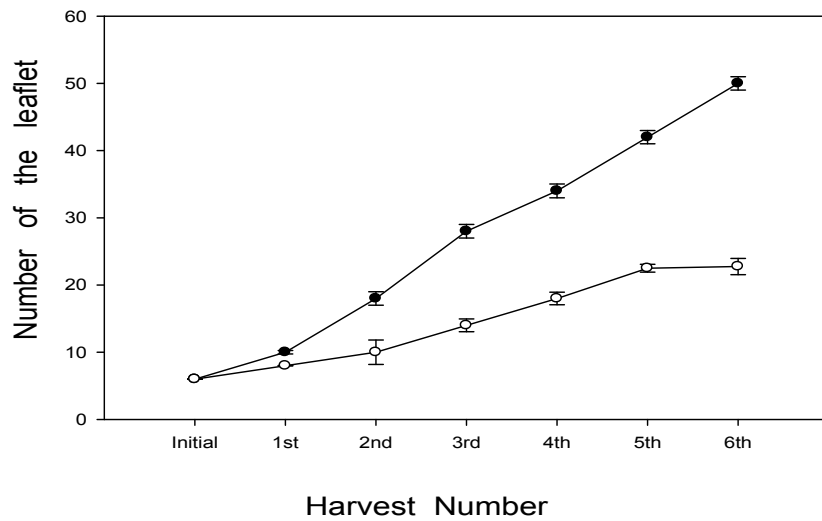


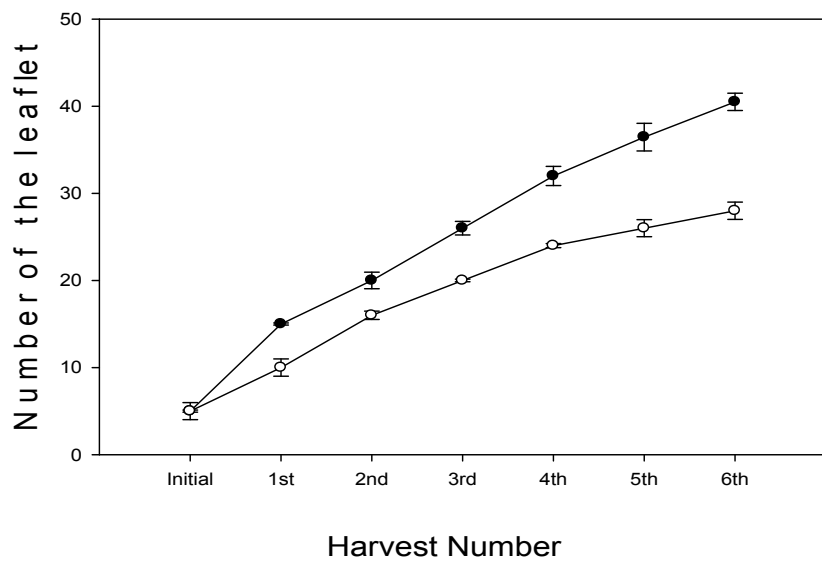
Fig 6.4. The effect of two light intensities (Sun 100% light and Shade 15% light) on the increase of terminal leaflet area with time.

●; Sun (100% light), ○; Shade (15% light).

a. C.tora



b. C.occidentalis



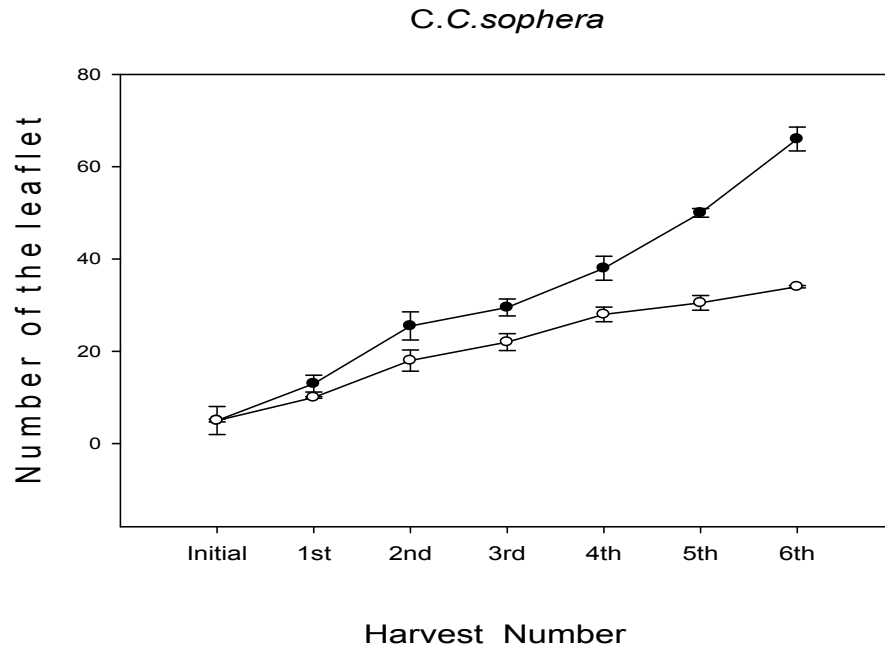


Fig 6.5. The effect of two light intensities (Sun 100% light and Shade 15% light) on the number of leaflet with time. ●; Sun (100% light) ○; Shade (15% light). Vertical bars are 95% confidence limit.

6.3.4 . Flower and seed production

At sun treatment *C. tora* started flowering 11 and 12 days earlier than *C. sophera* and *C. occidentalis* respectively i.e; in case of *C. tora* the bud appeared after 98 days of sowing and in *C. sophera* and *C. occidentalis* after 108 days and 110 days of sowing respectively. In case of shade treatment the bud of *C. tora* appeared after 120 days and in *C. occidentalis* after 128 days of sowing. Shamsi and Whitehead (1974) have reported that at full light intensity *Lythrum salicaria* began flowering two weeks earlier than those of plants grown at low light intensity

The late flowering observed in shade condition in *C. tora* and *C. occidentalis* is in agreement with the work of Fattah and Mallik (1973) who worked with photoperiod in jute plants. Packham and Willis (1981) also reported marked phenotypic plasticity in *Galeobdolon luteum* grown in the open and beneath shade screens.

In *C. tora* mean number of flowers and fruits per plant in sun (100% light) is 16.250 ± 0.57 and 13.00 ± 0.66 respectively and in shade it is 9.500 ± 0.795 and 5.2 ± 1.03 respectively. In *C. occidentalis* mean number of flowers and fruits per plant in sun (100% light) are 18.50 ± 1.77 and 11.00 ± 1.006 respectively and in shade it is 10.0 ± 0.650 . No flower production was observed in *C. sophera* at 15% light. In all three spp. there was a marked delay and reduction in number of flowers in shade treated plants. Again in case of shade condition, flowers were small and weak and were unable to produce

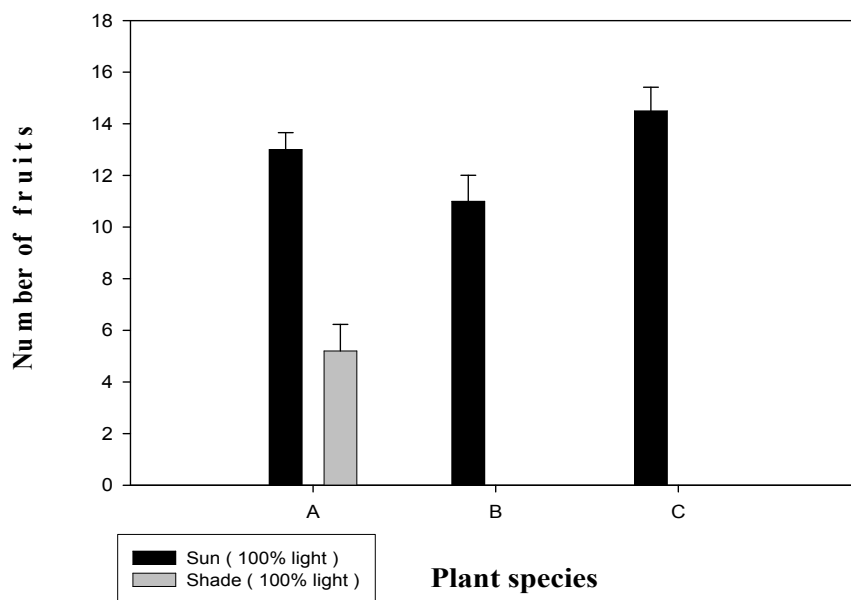
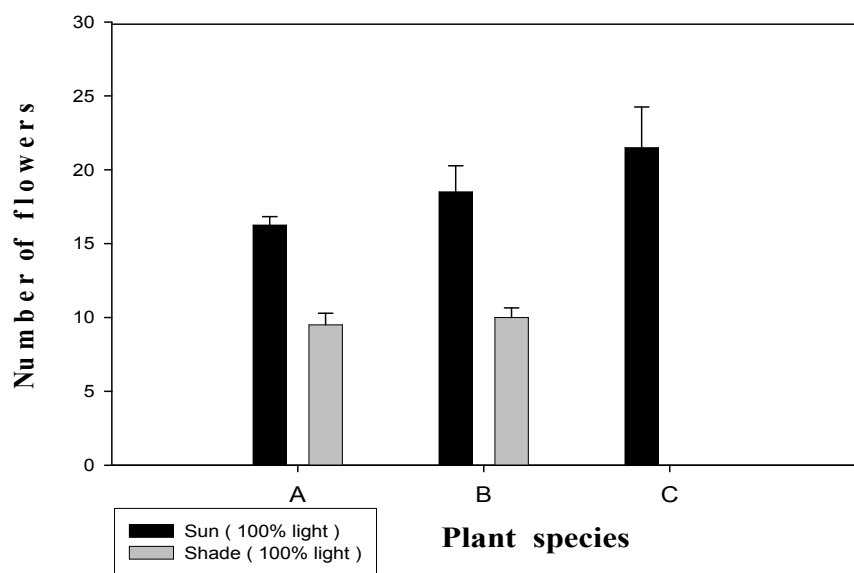


Fig 6.6. Mean number of flowers and fruits in Sun (100% light) and

Shade (15% light) treatments. Vertical bars are 95% confidence limits.

(A = *Cassia tora* B = *C. occidentalis* C = *C. sophera*)

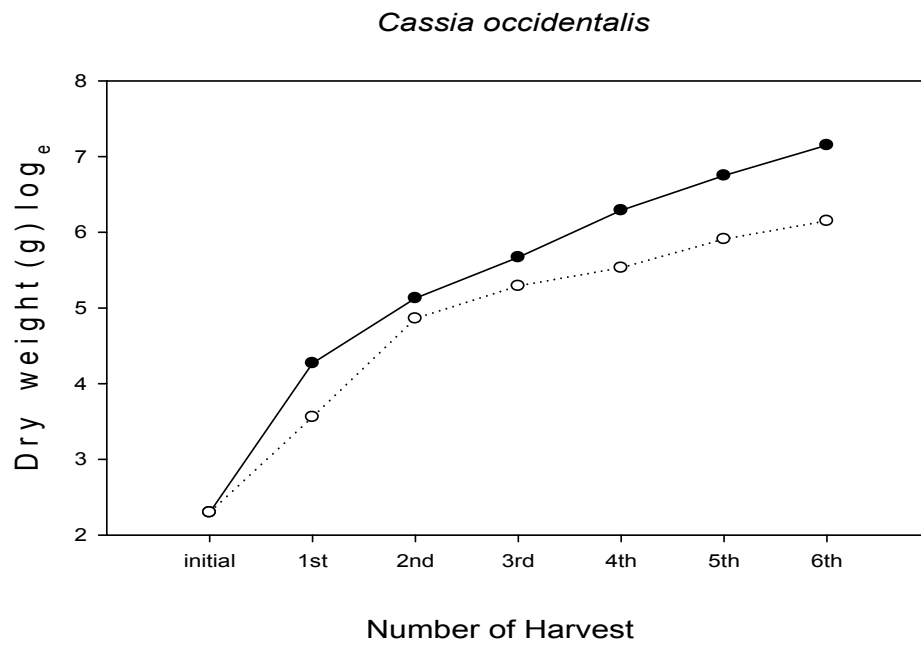
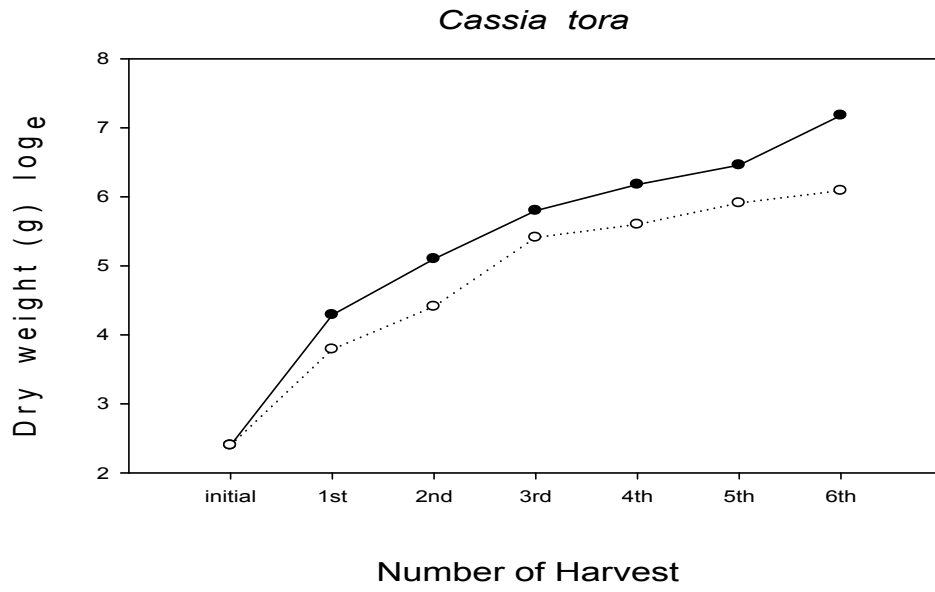
fruit formations and fell before maturity in both species (*Cassia occidentalis* and *Cassia sophera*). Shamsi and Whitehead (1974) also observed this nature in *Epilobium hirsutum* and *Lythrum salicaria* where there was a marked delay and reduction in number of flowers with the decrease of light intensity and corresponding delay and reduction in the number of fruits.

6.3.5 . Increase of dry weight

It can be seen from Fig 6.7 that sun (100% light) and shade (15% light) treatments had a significant effect on the increase in dry-weight of the plants. There was also a striking difference in response between three *Cassia* species to the shade condition. Highest dry matter production was obtained in the plants of sun (100% light). The dry weight was significantly ($p=0.05$) decreased in shade (15% light) treatment.

The total dry weight of *C. tora* per plant initially was $\log_e 2.42$ (11.25 ± 2.65 mg/plant). At final harvest the value was $\log_e 7.18$ (1317 ± 61.41 mg/plant) in sun (100% light) and $\log_e 6.09$ (445.0 ± 20.52 mg/plant) in shade (15%light) condition. Dry weight value of shade condition was less than one third of sun condition. On other hand, in *C. occidentalis* in sun (100% light) the dry weight at the final harvest was $\log_e 7.15$ (1075.0 ± 75.88 mg/plant) and in shade (15% light) treatment it was $\log_e 6.15$ (470.0 ± 31.62 mg/plant) which was two and half times (1/2.5) less than the value of the sun condition.

In *C. sophera* the dry weight in sun condition was $\log_e 7.42$ (1670.0 ± 41.04 mg/plant) whereas, in shade condition it was $\log_e 5.86$ (352.50 ± 20.00 mg/plant) which was nearly 1/5 of full sunlight. With the decrease of light intensity



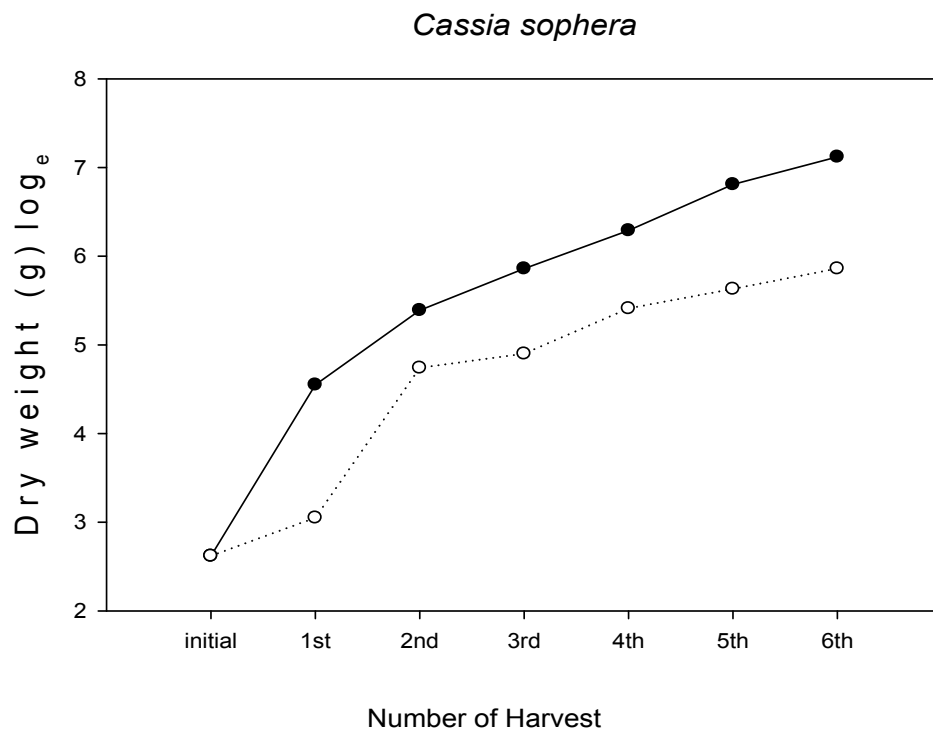


Fig 6.7. The effect of two light intensities (Sun 100% and Shade15%) on the increase of plant dry weight with time. ●; Sun (100% light) ○; Shade (15% light).

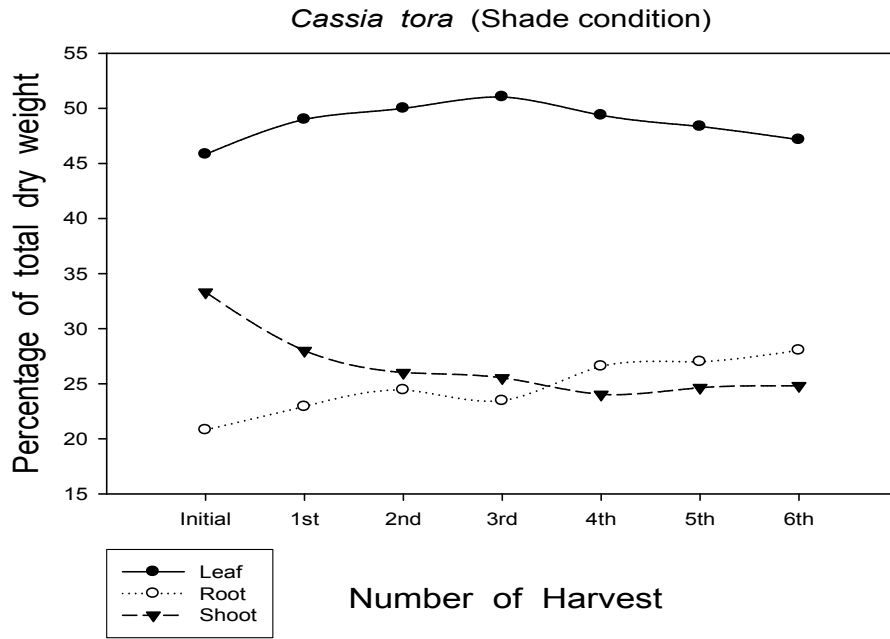
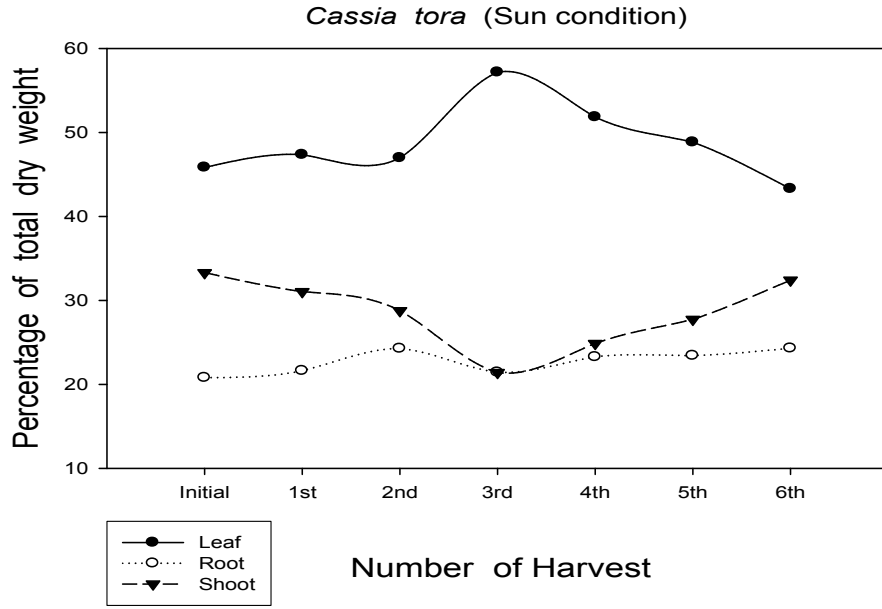
(100%, 70% and 40% the dry weight of *Epilobium hirsutum* and *Lythrum salicaria* was also decreased (Shamsi and Whitehead 1974).

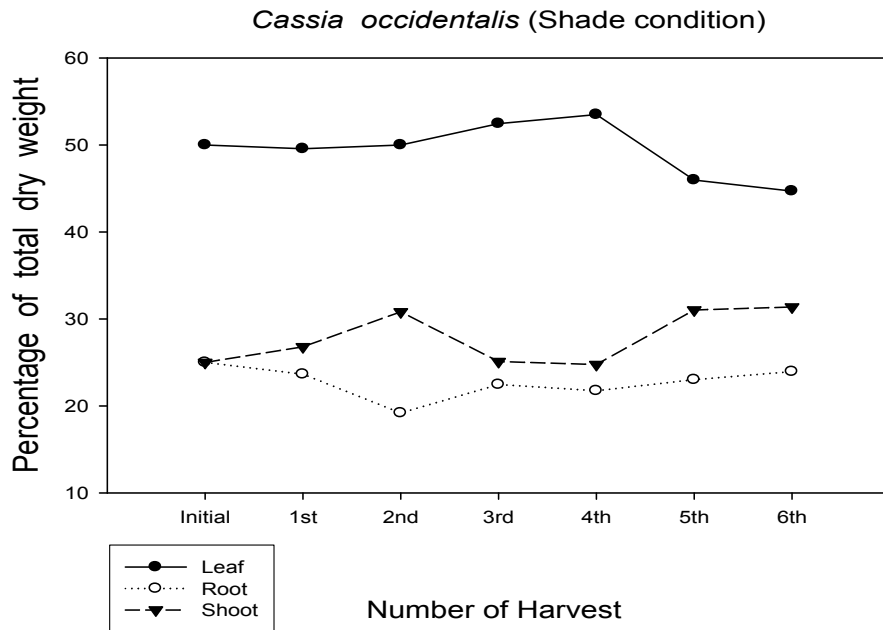
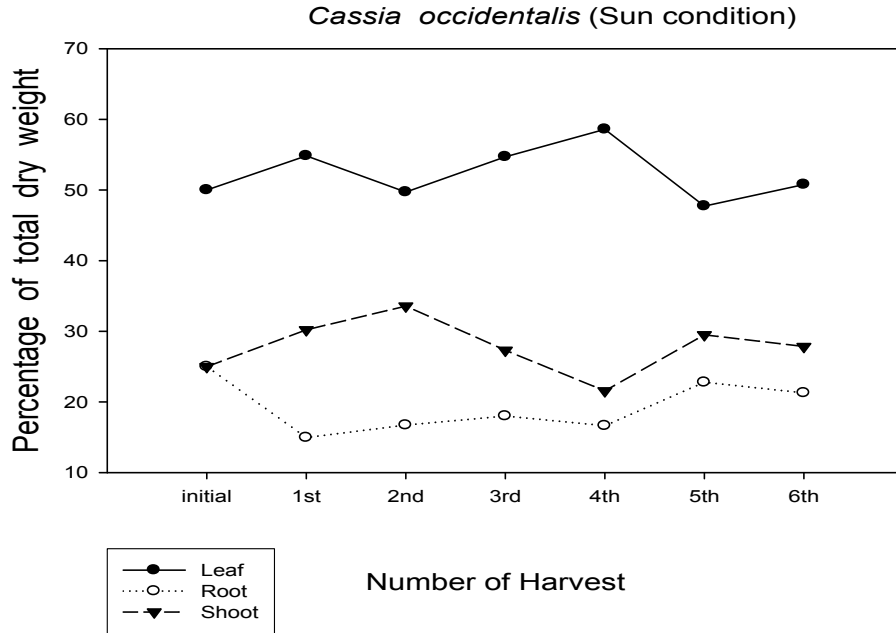
6.3.6. Distribution of dry weight between root, shoot and leaf

The root, stem and leaf dry weights, expressed as percentages of the total dry weight at each harvest are plotted in Fig 6.8 (a - c) to illustrate the ontogenetic changes in three *Cassia* spp.

In *Cassia tora*, the initial value of the distribution of dry weight of leaf, root and shoot was 45.83%, 20.8% and 33.3% respectively. Both in sun and shade treatments the value of leaf was always higher and showed an increasing tendency up to 3rd harvest (57.16%) and (51.04%) respectively and then began to decrease up to the last harvest in sun (43.29%) and in shade (47.16%). The percentage of dry weight of root increased with some fluctuation both in sun and shade conditions. The percentages of dry weight of shoot decreased and the values showed some fluctuation both in sun and shade conditions.

In *Cassia occidentalis*, the initial value of leaf was 50%, root 25% and shoot 25%. In the sun and shade conditions, percentage of shoot and root decreased and in both treatments showed variation. The percentage value of dry weight of leaf did not show any significant variation between sun and shade conditions. In shade condition the percentage value of dry weight of leaf increased up to 4th harvest and then the value decreased. In *Cassia sophera*





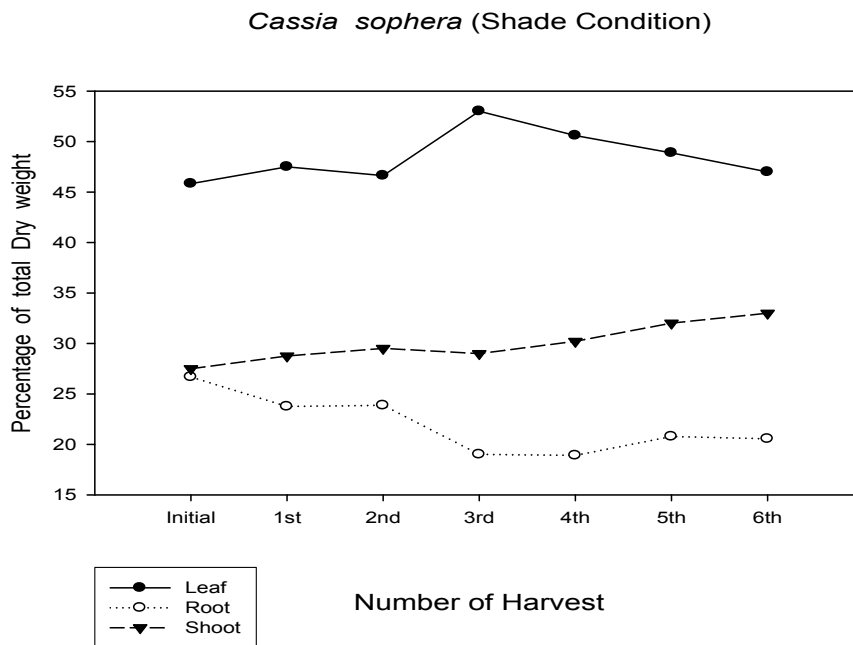
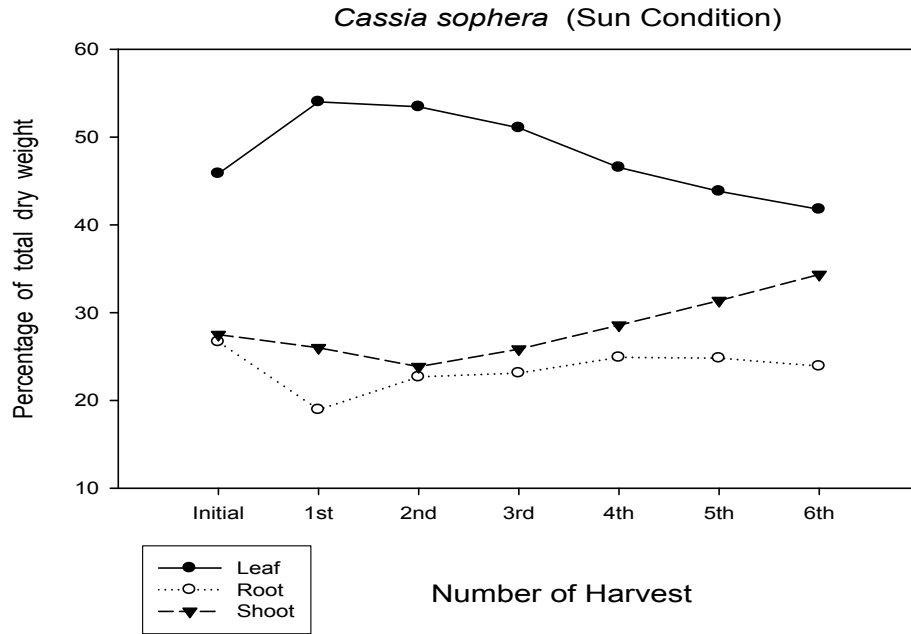


Fig 6.5: The effect of light intensities on the distribution of dry weight between leaf, stem and root at successive harvests. a. *Cassia tora* b. *Cassia occidentalis*. c. *Cassia sophera* —●— leaf;○.....root ; —▼— shoot .

the pattern is somewhat different, over the subsequent six harvests there is a general tendency for the leaf to decrease particularly in sun condition; the proportion of root is slightly affected in shade condition. The shoot is well developed, forms a high proportion relative to root.

It should be mentioned that both in sun and shade conditions in all the three *Cassia* spp. leaf is the largest component part followed by shoot and root.

The patterns of ontogenetic change are the same within each species of *Epilobium hirsatum* and *Lythrum salicaria*, irrespective of light treatment, but any one harvest the values differ with light intensity in way which may be ecologically significant, for the proportion of leaf increases with decrease of light intensity, while the proportion of root decreases (Shamsi and Whitehead 1974).

6.3.7. Root: Shoot

Root /shoot ratio (R/S) is affected by individual factors such as light intensity, and temperature (Brouwer 1966); Troughton (1956). In the present work the final R/S value attained is entirely the influence of sun (100% light) and shade (15% light) treatments.

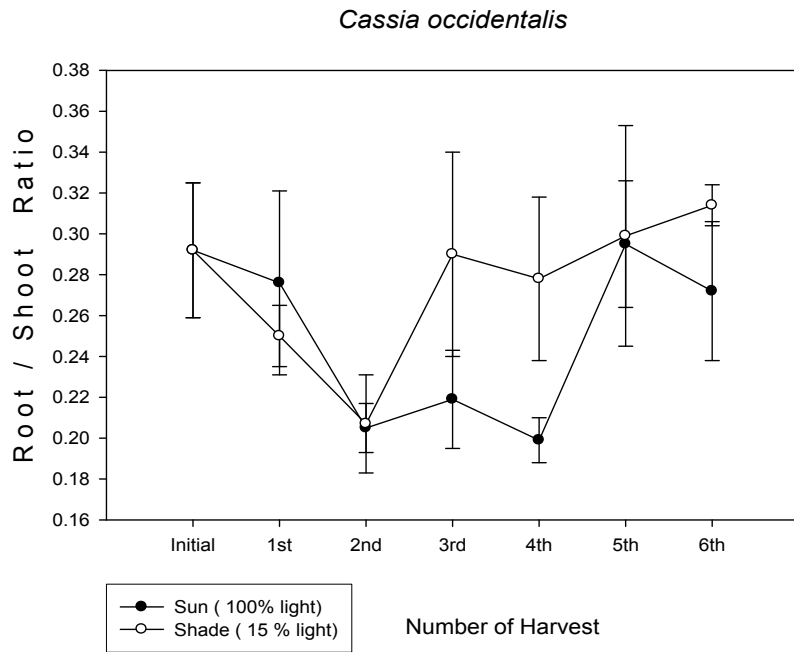
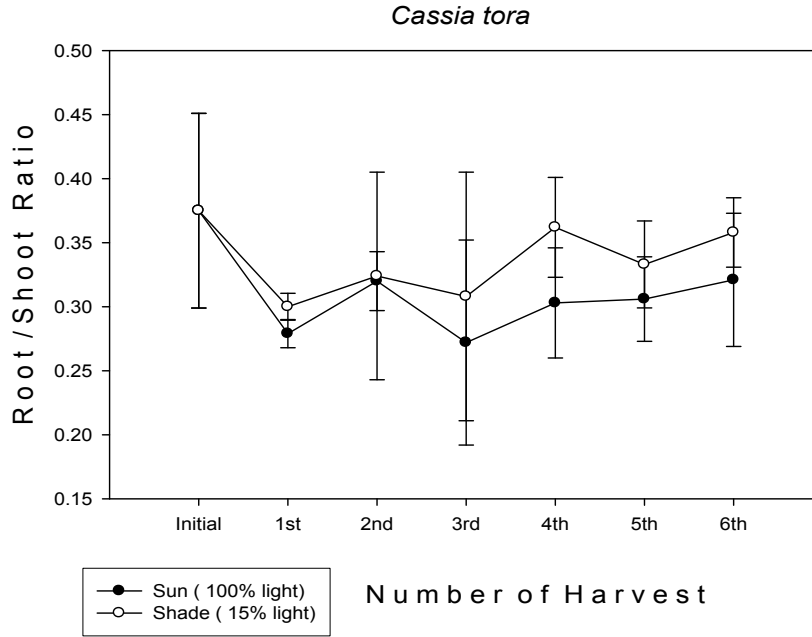
Results are presented in Fig 6.6 in the form of smooth curves plotted against time. A fitted value for the appropriate variable or function is shown at each harvest together with 95 percent confidence limits. These are the limits within which the true value would fall on 95 percent of occasions over an infinite series of identical experiments.

In all the *Cassia* spp the value of R/S was always low in sun treatment than shade condition. In sun and shade conditions of *Cassia tora*, R/S value decreased from 1st harvest onwards with some variation. Initial R/S value was 0.375 ± 0.076 and the final value was 0.321 ± 0.052 and 0.358 ± 0.0271 in sun and shade conditions respectively.

In *Casia occidentalis*, the R/S ratio was 0.292 ± 0.033 at initial harvest and it became 0.272 ± 0.049 at final harvest in sun condition and in shade condition the final value was 0.314 ± 0.011 . In sun condition the value increased from the 2nd harvest onwards and finally reached to 0.272 ± 0.049 . However, the only exception was in *C. sophera* where in sun condition R/S value began to decrease and in shade condition showed an increasing tendency.

In case of *C. sophera* the curves were different. The R/S value was initially 0.250 ± 0.0397 but fell rapidly at 2nd harvest (0.172 ± 0.029) in sun condition followed by a significant recovery particularly from 4th harvest and attained a value of (0.349 ± 0.053) in final harvest. In shade condition at final harvest the value of R/S was 0.258 ± 0.019 .

In all treatments the cause of the initially high value of R/S was the inclusion of remains of the seed in the dry weight of the roots. This phenomenon was discussed by Hunt and Burnett (1973). They did an experiment with different levels of potassium and Light condition with *Lolium perenne* L. where they have found that in low and high light intensities the final level of R/S achieved is almost entirely the result of environmental influence. Several authors have reported marked response to shading in R/S in *L. perenne*. (Throughton 1956;



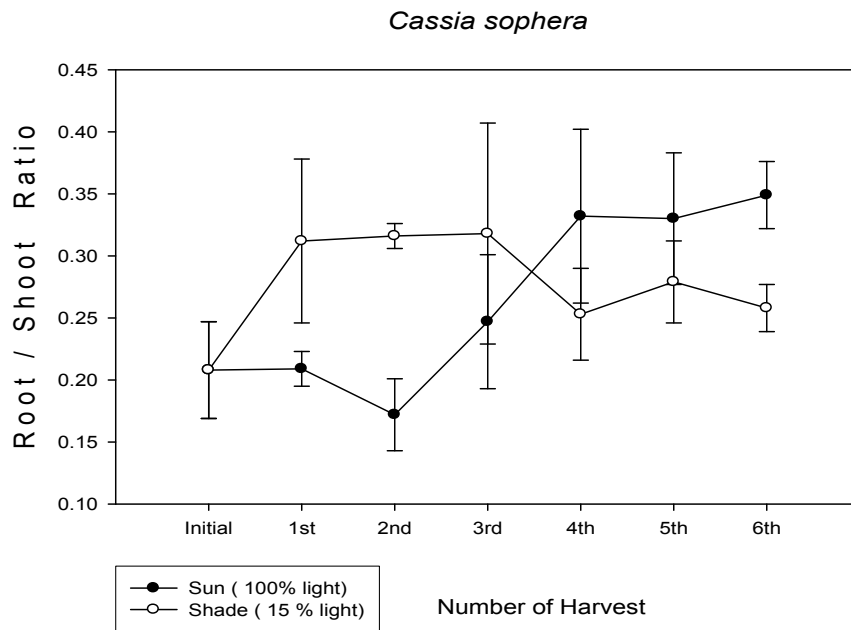


Fig 6.6. Progress curves of Root: Shoot ratio of the three species. ●; Sun (100% light). ○; Shade (15% light).

Davidson 1969). Davidson (1969) showed that in *Lolium perenne* L. and *Trifolium repens* L. carbohydrate is partitioned into root growth in inverse proportion in root activity. Thronlely (1975) has derived an entirely theoretical model in which the total activities of root and shoot are linearly related during exponential vegetative growth at a constant internal composition.

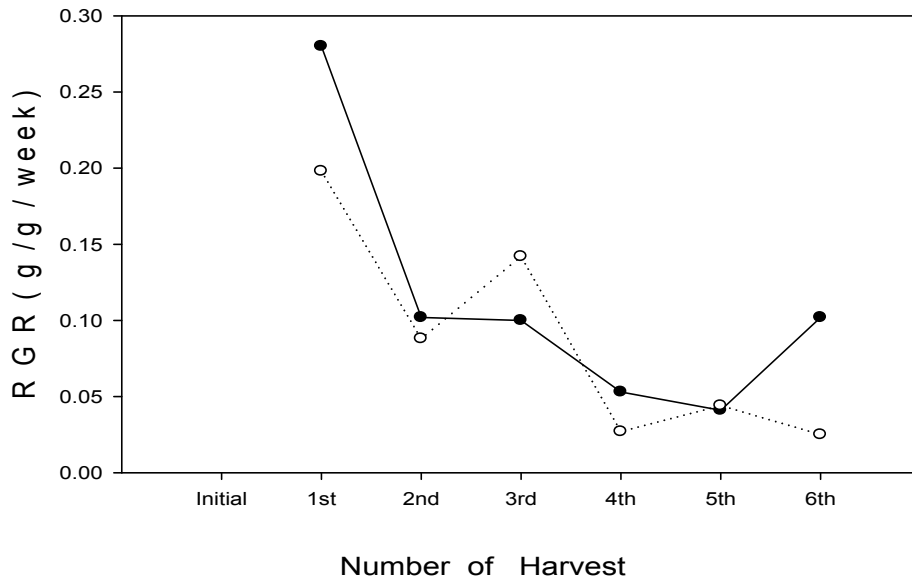
Evans (1971) found a decrease in R/S from 0.344 to 0.313 when plants were shaded to 20 percent of the control condition. Luxmoore and Millington (1971) obtained a decrease from 0.39 at full light to 0.21 at 25 percent light after 60 days.

Monk (1966) studied the ecological importance of root/shoot ratios in a variety of species characteristics of different stages of old field succession. He noted low root/shoot ratio in the annuals and high values in the woody perennials. Monk (1966) also explained the root/shoot ratio decrease with the increase of plant size.

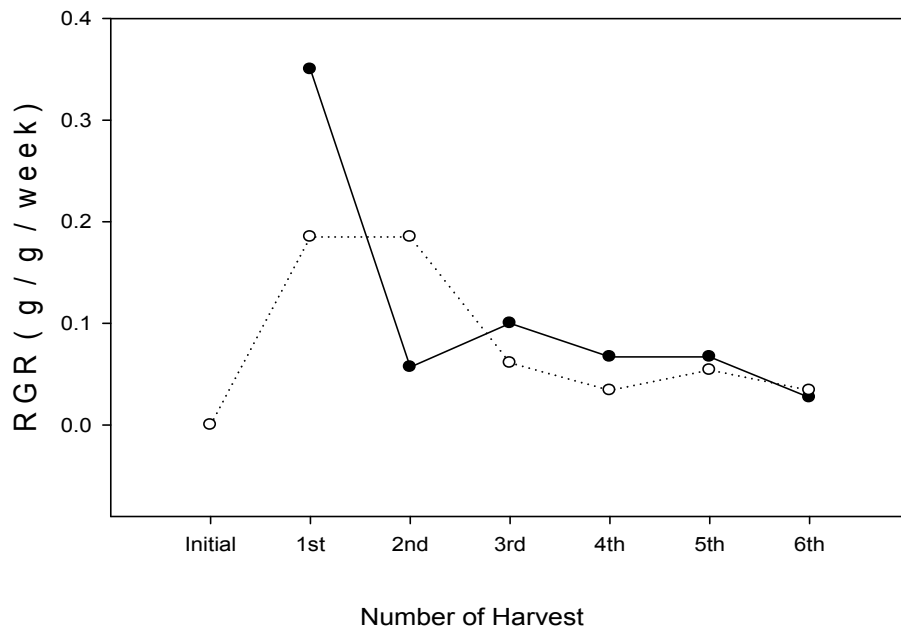
6.3.8. Relative Growth Rate (RGR)

Changes in relative growth rate (RGR) in successive harvests are shown in Fig 6.7. The value of RGR in all the species grown under sun condition in general was higher than shade condition although values of some harvests in shade condition showed a tendency to increase. In *C. tora* the value of RGR of sun treatment declined from 0.28 g/g/week (1st harvest) towards 0.041 g/g/week at 5th harvest without showing any increasing tendency over the central period of experiment (between 2nd and 5th harvest) and at final harvest the value reached to 0.102 g/g/week. In shade treatment the value declined from the 1st harvest

Cassia tora



Cassia occidentalis



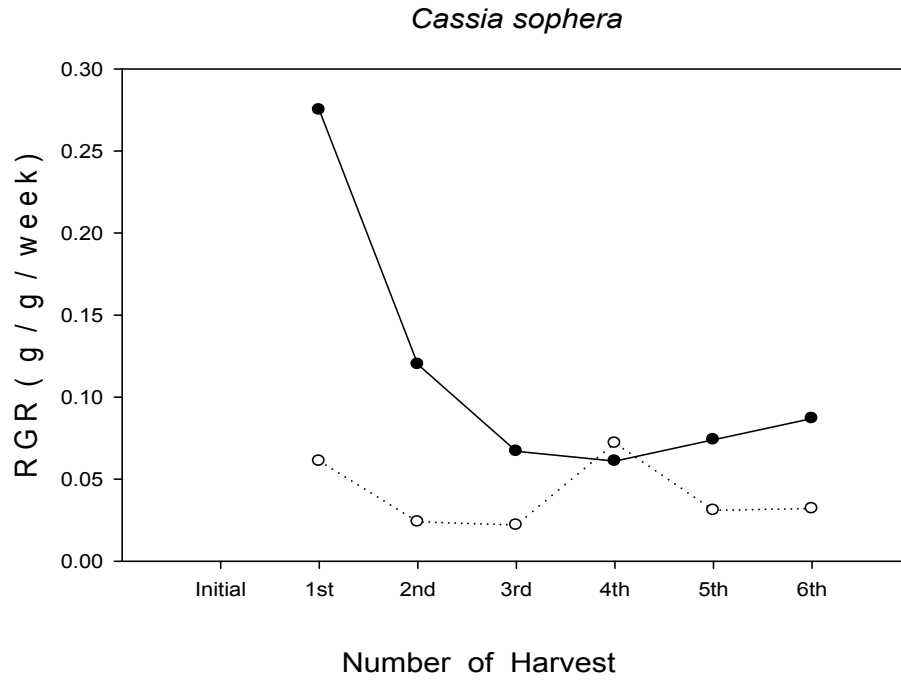


Fig 6.7. Relative growth rate (RGR g /g /week) ●; Sun (100% light). ○;
Shade (15% light.)

(0.198 g/g/week) towards the end of the experiment (0.025 g/g/week) with a fluctuation tendency.

In *Cassia occidentalis* the falling-off in the curves at sun (from 0.35 g/g/week to 0.027 g/g/week) is steadier than shade (0.18 g/g/week to 0.034 g/g/week). There were clearly graded and differences with respect to RGR between the two light treatments were evident over the central part of the experiment. Similar phenomenon was observed by Hunt and Burnett (1973), where they have found that RGR was strongly affected by light intensity in *Lolium perenne*.

In *Cassia sophera* the RGR value in sun treatment was 0.275 g/g/week at 1st harvest and declined to 0.087 g/g/week, whereas in shade treatment the value reached to 0.032 g/g/week at final harvest.

Making similar allowances for differences in environmental conditions the present values of RGR at 100% light and 15% light are broadly comparable to the findings obtained in *L. perenne* by Hunt and Burnett (1973), Hunt (1975), and Grime and Hunt (1975).

Zobel and Antos (1991) have worked with the growth and development of natural seedling of *Abies* and *Tsuga* in old-growth forest and showed that Relative growth rate (RGR) and its components varied with time and between species. RGR declined with plant age for *Abies*. Although these values initially declined for *Tsuga*, for each genus, the RGR values were almost identical.

CHAPTER 7

TISSUE WATER RELATIONS IN *Cassia tora*, *Cassia occidentalis* AND *Cassia sophera* IN RELATION TO SUN AND SHADE , DRY-MOIST AND WET CONDITIONS

7.1. INTRODUCTION

In recent years experimental ecology has developed rapidly and its main aim is often to explain the occurrence of species in particular environment. Transpiration, stomatal aperture and amount of water in leaves are indices which reflect the ability of the plant to take up water under the prevailing atmospheric and soil water conditions.

The water relation study of a plant explains the understanding of adaptability and survival strategies of any species. It is now realized that the water stress causes physiological and morphological changes in plants. Stocker (1960) mentioned that there should be two main objectives of the water relation study. These are

- i) An ecological inquiry into the living condition in the natural habitats.
- ii) A purely eco-physiological inquiry into the changes which cause water deficiency in the structure and function of plants.

These two facts can bring about changes in the protoplasm that may lead into drought resistance capacity.

Tissue takes up water when their water potential is less than that of the environment.

Soil moisture stress usually affects plant growth by influencing the water deficits

produced in plant tissues. Water deficits in plant tissue influence the growth and hence the ecological response of the plant. In many instances the water relations of the plants are analyzed by measuring relative water content (RWC). Relative water content of leaves indicate water regime of plants which in turn regulates to a greater extent the eco-physiological activities, transpiration, stomatal aperture and reflect the ability of the plant to take up water under prevailing atmospheric and soil moisture conditions.

There is evidence (Nazrul-Islam 1983) on the importance of water factor in respect of plant distribution of wetland and dry land habitat species and also in crop plants grown under waterlogged and non- waterlogged conditions (Nazrul-Islam and Yasmin 1982; Nazrul-Islam and Alam 1986; Nazrul-Islam 1987).

Tissue –water relations of *Cassia tora* from sun and shade habitats was also reported by Nazrul –Islam. (1987). Bannister (1964b) has explained that there is a good correlation between the distribution of species with regard to soil moisture and water relations of the species concerned. Data of Lawson and Jenik (1967) suggested that the transpiration rates measured were influenced more by factors related to water supply.

A consideration of the water loss from cut shoot (Bannister 1964d; Nazrul -Islam 1983) showed that the relative water content at the point of stomatal closure varies both between and within species (Nazrul- Islam and Yasmin 1982). Hygen (1951) had attempted an ecological characterization of plants in terms of their rates of stomatal and cuticular transpiration. From a mathematical analysis Hygen was able

to differentiate successfully between the plants, which were considered to be representative of Xeromorphic, Mesomorphic and Hygromorphic types respectively.

Many studies of water relations of plants have been made, only a few of them relate to the behavior of plants under the normal conditions of their habitat. (Weatherly 1950, Willis and Jefferies 1963, Jarvis and Jarvis 1963, Bannister (1964a, 1964b, 1964c, 1964d) Read and Bartlett 1972, Nazrul –Islam 1987).

Plants react to the inevitable loss of water in a variety of ways and their tolerance and avoidance of water stress is the consequence for both physiological and ecological response. The loss of water from a plant will affect both the uptake of water and the water relations of cells and tissues. Water deficits in plant tissues furthermore affect upon cell expansion (Ordin 1958, 1960; Gardner and Nieman 1964), growth (Eaton and Eargle 1948), nitrogen metabolism (Shah and Loomis 1965) production of growth substances (Larson 1964) translocation of materials (Robberts 1964), confirmations of protein molecules (Gaff 1966). Such effects influence the growth and hence the ecological response of the plant.

In sun habitat, conditions tend to be somewhat extreme; where the rate of evaporation are often very high and the water supply is limited during the summer. It was shown that plants of open or sun habitat are comparatively xeromorphic (Daubenmire 1959). Okali (1971) also has shown the water relations of some woody species in relation to their distribution in the natural environment. Okali (1971) in his work has paid particular attention to the internal water relations of leaf tissue and to the behavior of stomata under moisture stress. By use of leaves alone, the necessity

for assuming differential rates of water supply to plant under comparison can be avoided. The measurements examined were the relation between leaf water content and stomatal closure. The relationship between leaf water content and stomatal aperture were studied. These relationships were also used by Weatherley and Slatyer (1957); Jarvis and Jarvis (1963a); Bannister (1964 a); Okali (1971) and Nazrul-Islam (1983) to explain the adaptation of plants to drought. Total, stomatal and cuticular rates of transpiration were also calculated to bring the results to a sharper focus.

Transpiration alone gives little indication of the water balance of the plant. To obtain a complete picture, it was therefore, decided to study the three *Cassia* species in relation to the following measurements:

- i) The relation between leaf water content and stomatal closure and
- ii) The rates of transpiration.
- iii) The relative turgidity (RT)

It was expected that an investigation of tissue water relations of these three *Cassia* spp. might show some differences in their physiological behavior which might provide a useful comparison with previous work and ecological information for a comparative study.

7.2. MATERIALS AND METHODS

7.2.1. Materials

The leaves with petioles of *Cassia tora*, *Cassia occidentalis* and *Cassia sophera* were collected from the natural field growing under sun and shade habitats and from

the Wet and Dry-moist treatments grown in the net house of The Botany Department. Dhaka University 2012.

7.2.2. Methods

7.2.2.1. Relative turgidity

The moisture content of plant material has been expressed as relative turgidity (Weatherley 1950). This is the ratio of the water content after the plant material has been allowed to make up any deficit, expressed as percentage.

The techniques of using leaf water deficit as a measure of water stress has been done by Weatherley (1950); and Bannister (1964a). Weatherley used leaf discs which floated on water to achieve saturation, Bannister (1964a) used shoots of different plant species sampled throughout the year. The method of Bannister (1964a) was adopted in the present investigation to measure deficits of leaves at different times of the day.

In the field, the leaves of more or less uniform size were cut from the plants at 9 A. M. and were collected in saturated polythene bags and brought to the laboratory. The leaves were then rapidly surface dried with filter paper and then weighed (within ten minutes of collection). These weights were taken as the field weight (F). The leaves were then kept to a saturated environment and allowed to stand in water with their cut petioles immersed under water in small specimen bottles (capacity 30 ml) for 24 hours to attain turgidity. At the end of this period the leaves were then taken from the saturated environment and dried with filter paper and reweighed to determine the

turgid weight (T). The leaves were then oven dried at 80°C and finally the dry weight (D) of the leaves was determined.

The relative turgidity (RT) was then calculated from the following formula of Bannister (1964b).

$$RT = \frac{F - D}{T - D} \times 100$$

Water saturation deficit (%) are theoretically independent of changes in dry weight and were determined by the following formula:

$$\text{Water saturation deficit (\%)} = 100 - RT \text{ (Bannister 1964a)}$$

With the help of relative water content and water saturation deficit, individuals of the same and different species from a variety of different habitats can be compared.

7.2.2.2. The relation between leaf water content and stomatal closure

Hygen's (1951) quick weighing method for the analysis of transpiration of detached leaves was used for the comparison of the relations between leaf water content status and stomatal closure. Transpiration rates were estimated from weight losses of detached leaf from the weight at frequent intervals. In this way transpiration decline curves were obtained. The method adopted in this investigation was to cut leaves of convenient size and shape from the plants growing under sun - shade conditions and Wet - Dry-moist treatments.

The leaves used in transpiration were collected from the field at 9.00 A.M. and immediately kept in a sealed saturated polythene bag. The leaves were then

transported to the laboratory, rapidly surface dried with sheets of absorbent filter paper and weighed (within 10 minutes of collection) on an electronic balance. For taking the data of saturation environment, the leaves were kept under a glass jar and allowed to stand in water with their cut petiole immersed under water in small specimen bottles for one hour and was illuminated to tube-lights in a growth room. The water loss was followed for about 3 hours by recording fresh weight (FW) at an interval of 10 minutes (for the first 60 minutes) and then at intervals of 20 minutes. Between weighing the leaves were returned to the natural environment and placed vertically in small specimen bottles (capacity 30 ml). The leaves were then oven dried at 90°C and their dry weights (DW) were determined at the end of the experiment, so that the change of relative turgidity with time could be plotted. The initial weight was taken as the saturated weight (SW).

There were six replicates for each treatment. Leaf water status at any point during a drying cycle was calculated as relative water content (RWC) from the following formula:

$$RWC = \frac{FW - DW}{SW - DW} \times 100 \quad (\text{The relative turgidity of Weatherley 1950})$$

The resulting curve is divisible into three phases: an initial linear phase when stomata are open and the rate of water loss is steady and rapid; a curvilinear phase during the time of stomatal closure and a final linear phase when stomata are closed and water loss is cuticular. The point of intersection of the two linear portions of the curve has been taken as indicating the relative water content at stomatal closure (Jarvis and Jarvis 1963 a, b).

7.2.2.3. *Rates of Transpiration*

Transpiration rates are usually expressed on a weight basis. The most convenient basis of expression in view of the methods used was in mg transpiration per minute, the initial fresh weight at the time of excision was recorded.

The basic data for the determination of points of stomatal closure were used to calculate the rates of transpiration. Total transpiration rates (calculated from transpiration decline curves) in terms of rate of loss of water were calculated in mg/g/minute from the weight loss during the first ten minutes after recording the saturated weight, Cuticular loss of transpiration was determined from the weight loss during the last 40 minutes of transpiration and stomatal loss of transpiration was then obtained by difference. This method of calculation permits the partitioning of total transpiration rate into stomatal and cuticular rates.

7.3. RESULTS

7.3.1. *Relative Turgidity (RT)*

The percentage of water content in the leaves of three species (*Cassia tora*, *Cassia occidentalis* and *Cassia sophera*) grown in sun and shade habitats and Wet and Dry-moist treatments are given in Table 7.1 and 7.2 respectively.

The mean relative turgidity (ratio of water content in the field to the water content after the plant material has been allowed to make up any deficit expressed in percentage) in the leaves of *Cassia tora* of sun habitat was highest (96.09%) among three species followed by *C. occidentalis* (92.99%) and *C. sophera* (85.01). The relative turgidity values of shade habitat were in the range of 91.14% to 93.74 %.

However in *C. sophera* the value of relative turgidity in shade was 91.14% and was significantly ($p = 0.05$) higher than that of sun habitat. Under sun conditions water saturation deficits was highest in *C. sophera* (14.99%) followed by *C. occidentalis* (8.01%) and *C. tora* (3.91%). Water saturation deficit in shade conditions plant was also highest in *C. sophera* (8.86%) followed the same pattern as that of sun conditions. It was expected that the more xeromorphic sun plants would retain open stomata over a wide range of water deficits at stomatal closure than the plants of shaded site. Leaves of *C. sophera* and also to a lesser extent *C. occidentalis* show such a trend from that of *C. tora*. Bannister (1971) measured stomatal closure in *Calluna vulgaris*, *Erica cinerea* and *Vaccinium myrtillus* from sun and shade habitats. Only *E. cinerea* showed such trend.

Similar observation was also done in the leaves of three *Cassia* spp. grown in Dry-moist and Wet treatments. *Cassia sophera* of Dry-moist treatment showed the highest relative turgidity ($98.44 \% \pm 0.801$) among three species followed by *C. tora* and *C. occidentalis*. The relative turgidity values of wet treatment were ($89.73 \pm 0.6.73$) % and 92.463 ± 0.59 % in *C. sophera* and *C. tora* respectively. However in *C. occidentalis* the seedlings could not survive. In *C. sophera* there was a significant difference ($p = 0.05$) in the relative turgidity values between the Dry-moist and Wet treatments. Under Wet treatment water saturation deficits was highest in *C. sophera* (10.27 ± 6.71) % followed by *C. tora* (7.537 ± 0.596) %. Water saturation deficit in Dry-moist treatment plant was highest in *C. occidentalis* (3.884 ± 1.55) % and followed the same pattern as that of Wet treatment.

Table 7.1: The relative turgidity or moisture content in leaves of

Cassia spp. growing naturally in sun and shade conditions. 95% confidence limits are also given.

Species	Habitat	Mean relative turgidity or moisture Content in %	Water saturation deficit (%)
<i>Cassia tora</i>	Sun	86.09 ± 0.64	3.91
	Shade	93.07 ± 4.62	6.93
<i>Cassia occidentalis</i>	Sun	92.99 ± 1.78	8.01
	Shade	93.74 ± 1.86	6.26
<i>Cassia sophera</i>	Sun	85.01 ± 4.70	14.99
	Shade	91.14 ± 3.34	8.86

Table 7.2: The relative turgidity or moisture content in leaves of *Cassia* spp. grown in Dry-moist and Wet treatments. 95% confidence limits are also given.

Species	Treatment	Mean relative turgidity or moisture content in %.	Water saturation deficit (%)
<i>Cassia tora</i>	Wet	92.463 ± 0.59	7.537 ± 0.596
	Dry-moist	97.841 ± 2.500	2.159 ± 0.267
<i>Cassia occidentalis</i>	Wet	Plants did not survive	-
	Dry-moist	96.116 ± 1.55	3.884 ± 1.55
<i>Cassia sophera</i>	Wet	89.73 ± 0.6.73	10.27 ± 6.71
	Dry-moist	98.444 ± 0.801	1.556 ± 0.801

7.3.2. The relation between leaf water content and stomatal closure

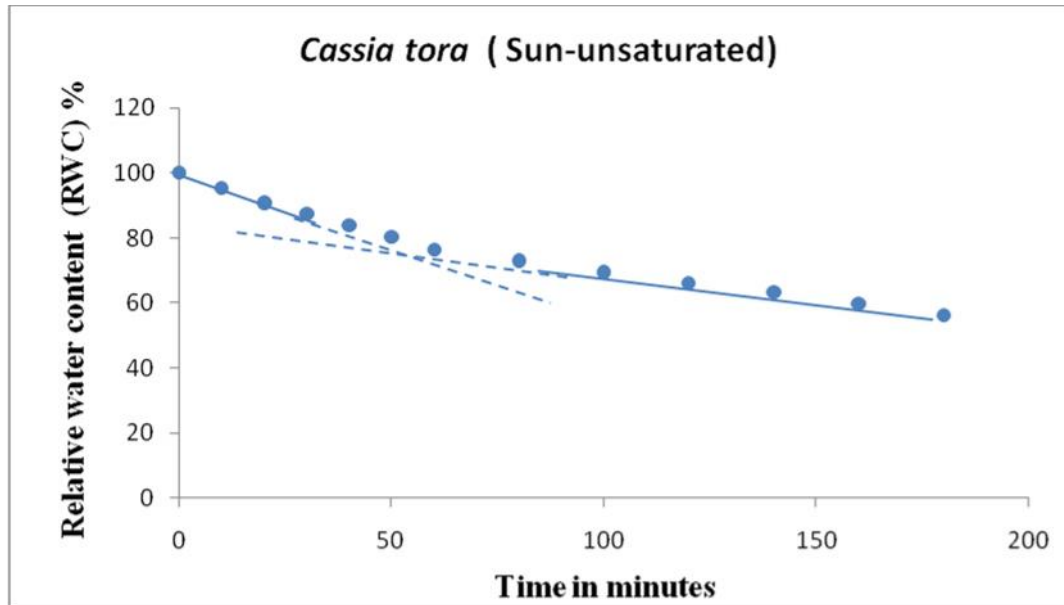
Initially the relative turgidity dropped quickly at a steady rate while the stomata were still widely open. After about half an hour or more (depending on the treatment) the rate of loss of water lessened and eventually settled to a steady low rate which continued for several hours. However, the curves from which relative water content and time taken to stomatal closure were calculated by extrapolation are shown in Fig 7.1. (a - f).

The point of intersection of two linear portions of the curves has been taken as indicating the relative water content at stomatal closure (Jarvis and Jarvis 1963 a; Nazrul-Islam 1983). From such curves the relative water content at any degree of stomatal closure can be determined. The relative water content at the point of intersection of the extrapolated lines of the curves has been taken as a critical level for stomatal closure.

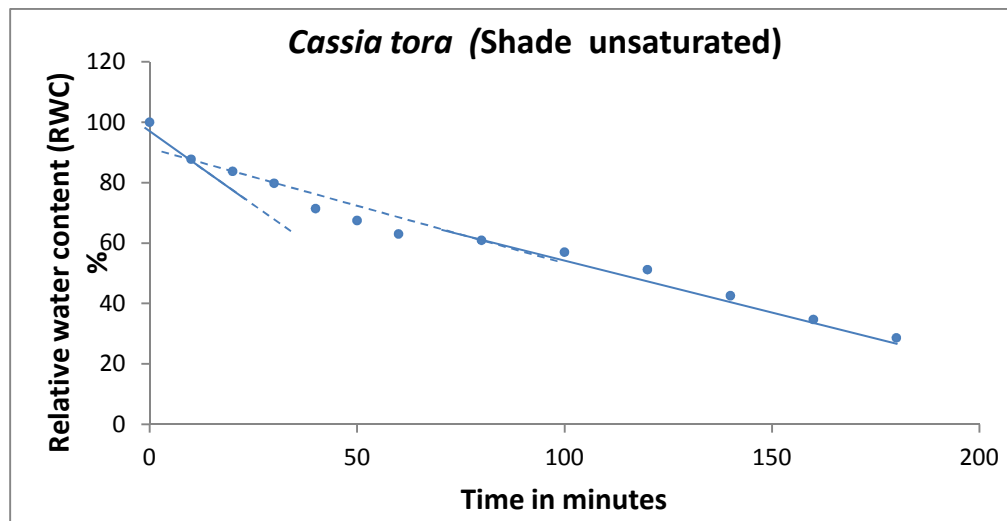
The relative water content of the leaves of *Cassia* spp. of sun and shade treatments and also for the plants grown in Dry-moist and Wet treatments at the points of stomatal closure and also the time taken to stomatal closure are given in Table 7.3 and 7.4 respectively.

Fig-7.1. Examples of curves of the relation between leaf relative water content and stomatal closure showing extrapolations of the straight line parts intersecting at closure in three *Cassia* spp.

<i>Cassia tora</i>	Sun	a.	Unsaturated
	Shade	b.	Unsaturated
<i>Cassia occidentalis</i>	Sun	c.	Unsaturated
	Shade	d.	Saturated for one hour
<i>Cassia sophera</i>	Sun	e.	Unsaturated
	Shade	f.	Saturated for one hour



7.1 .a



7.1 .b

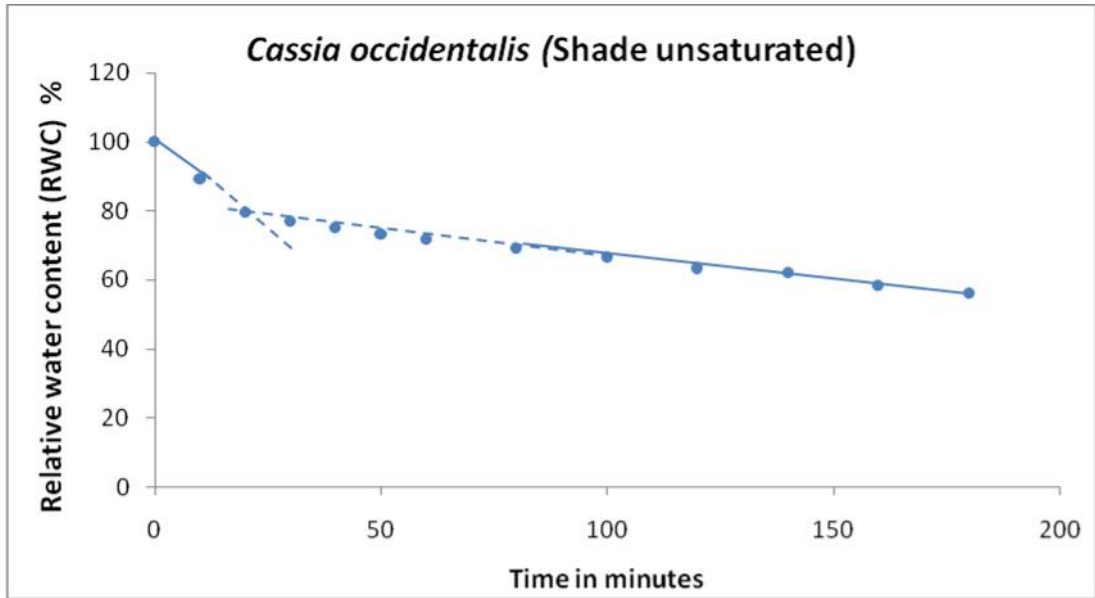


Fig 7.1.c

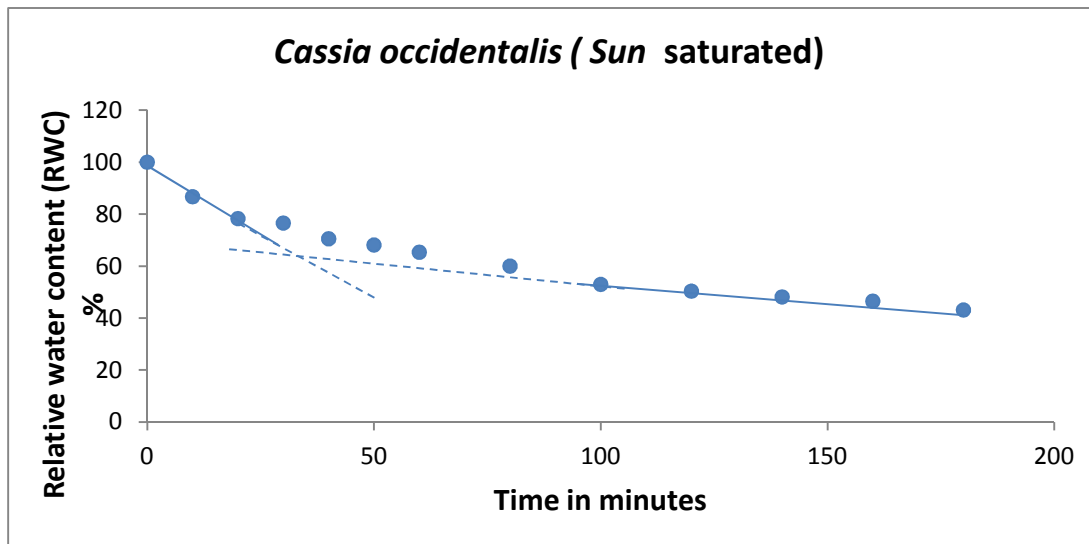


Fig 7.1.d

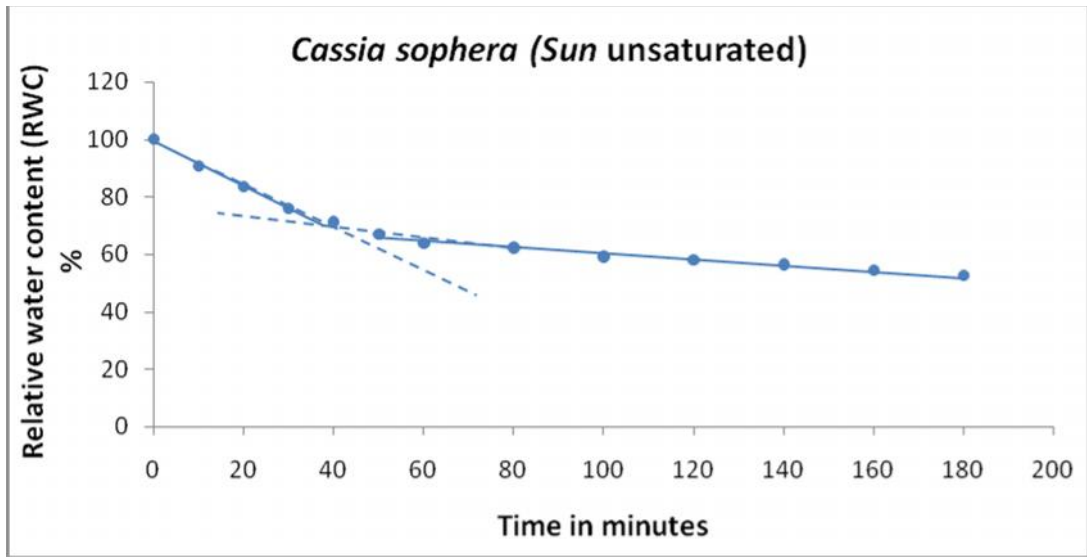


Fig 7.1 .e

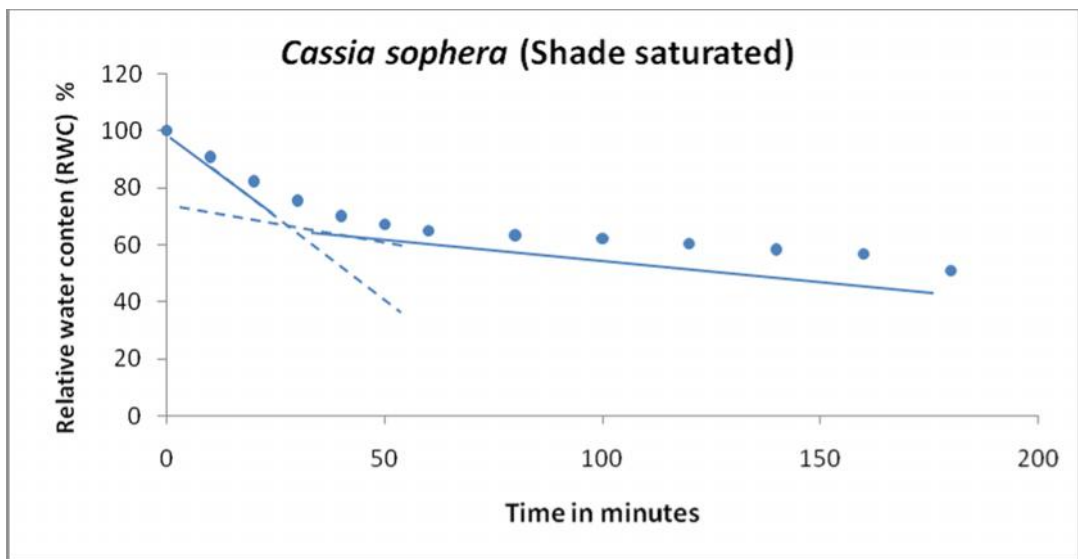


Fig 7.1.f

Fig-7.2. Examples of curves of the relation between leaves relative water content and stomatal closure showing extrapolations of the straight line parts intersecting at closure in three *Cassia* spp.

- a. *Cassia tora* (DM)
- b. *Cassia occidentalis* (DM)
- c. *Cassia sophera* (DM)

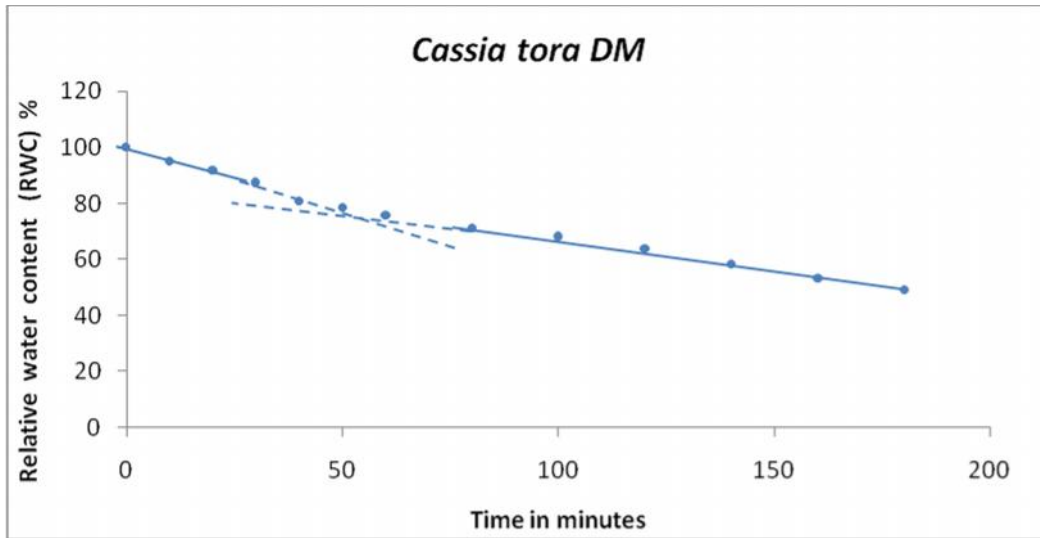


Fig: 7.2 (a)

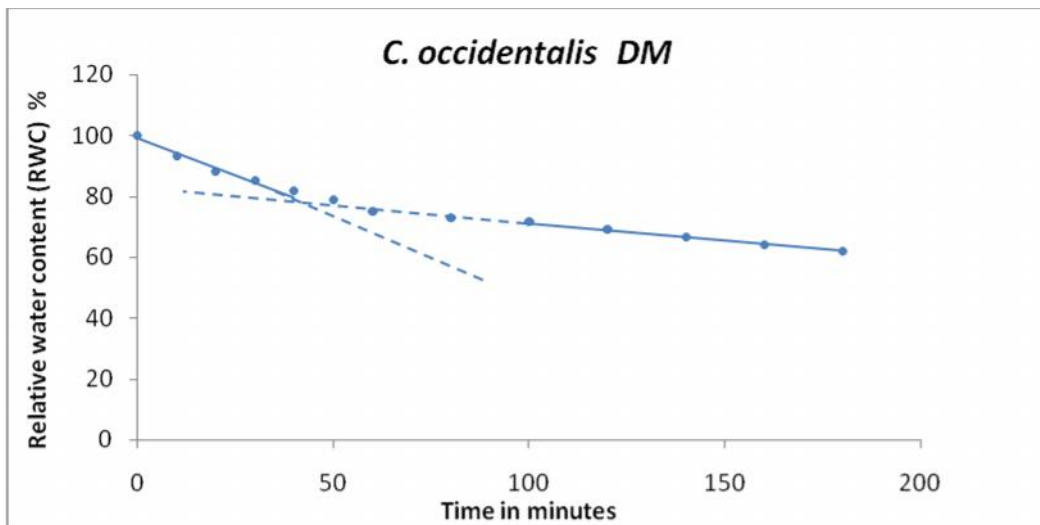


Fig: 7.2 (b)

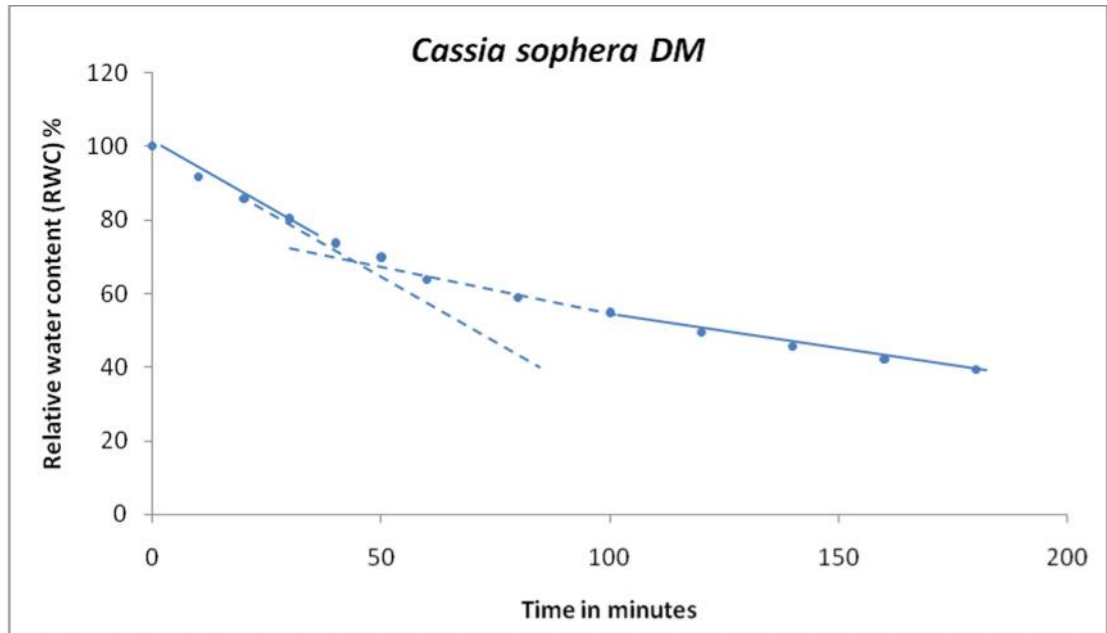


Fig: 7.2 (c)

Table-7.3: Relative water content of leaves at points of stomatal closure and also time taken to stomatal closure. 95% confidence limits are also given. (Collected from natural habitat of Sun and Shade conditions).

Species	Habitat		Relative water content (%) at points of stomatal closure.	Time taken to stomatal closure in minutes.
<i>Cassia tora</i>	Unsaturated	Sun	49.69 ± 7.87	50.24 ± 6.12
		Shade	86.22 ± 4.92	33.80 ± 3.76
<i>C. occidentalis</i>	Unsaturated	Sun	66.33 ± 5.73	41.17 ± 2.69
		Shade	69.33 ± 7.35	21.17 ± 3.00
	Saturated	Sun	68.00 ± 6.11	34.67 ± 4.34
		Shade	67.83 ± 7.40	21.50 ± 2.27
<i>C. sophera</i>	Unsaturated	Sun	62.50 ± 5.78	36.67 ± 7.91
		Shade	70.17 ± 5.65	31.83 ± 7.27
	Saturated	Sun	63.00 ± 8.04	37.50 ± 4.96
		Shade	65.50 ± 4.96	36.33 ± 6.65

In all cases the differences in relative water content at stomatal closure are very small and the differences among treatments are neither consistent nor statistically significant.

Clark and Levitt (1956) opined that drought avoidance was due to increased cuticular resistance, measurements of water loss were made from excised leaves of plants subjected to sun and shade treatments. Two series of estimations (except *C. tora*) were made. In one the leaf weight loss was assessed directly (unsaturated) after removal from the plant. In the second series, the leaves were excised, weighed and placed with their petioles in water for 12 h in a dark room (saturated). Weight loss was determined after this period of saturation. Relative water content at points of stomatal closure and also time taken for stomatal closure are given in Table 7.3. *C. tora* from the shade treatment exhibited highest relative water content (86.22%) at stomatal closure among the three species and suggested that this species is more sensitive to shade habitat than the rest of the species. The value of relative water content at stomatal closure from sun habitat of *C. tora* was 49.69%. In *C. occidentalis* and *C. sophera* the values of relative water content at stomatal closure between sun and shade habitats and also between saturated and unsaturated conditions were in the range of (62.50 to 70.17)%. In *C. sophera* the values of relative water content at stomatal closure were higher in shade condition than those grown under sun condition in both saturated and unsaturated conditions and the leaves of shade condition plants also showed much more rapid stomatal closure than the leaves of sun plants. Bannister (1971) also found that the plants of *Calluna*, *Erica* and *Vaccinium* from shade habitat showed a more rapid stomatal closure than shoots

from open sites (sun habitats). In an investigation of tissue water relations in a range of plant species from dry land and wetland habitats it was noted that *Runex acetosa* L. (dry land habitat species) showed highest relative water content at stomatal closure (Nazrul-Islam 1986) and suggested its sensitivity. However, the time taken to stomatal closure showed significant difference ($p=0.05$) both between the sun and shade treated leaves and also under saturated and unsaturated conditions except *C. sophera* under saturated condition. *C. tora* of sun habitat took longest time to stomatal closure and indicating its adaptation to dry habitat. In the field this species is usually confined to dry areas and occasionally in temporary waterlogged condition which occurs during the monsoon season. In this respect *C. occidentalis* took the shortest time to stomatal closure from shade habitat both under saturated and unsaturated conditions (21 minutes). This suggests that this plant species is unable to adapt in closed habitat. In the field *C. occidentalis* is always found to grow in sun habitat.

Wet condition was in fact most sensitive in terms of relative water content at which stomata closed. The result (Table 7.4) indicates some of the basic differences in the water relations of plants of Wet and Dry-moist treatments. Under the different conditions of water availability, the plants show contrasting behavior with respect to transpiration rates, stomatal change and the development of water deficits.

Ecological differences of the plants of various moisture regimes may well in part have a physiological basis in terms of water relation, characteristically plants of the wet habitat have an efficient means of regulation of transpiration whereas, this is not essential and may be lacking in plants grown in DM treatment.

Table-7.4: Relative water content of leaves at points of stomatal closure and also time taken to stomatal closure of the plants grown in Wet and Dry Moist conditions. 95% confidence limits are also given.

Species	Treatments	Relative water content (%) at points of stomatal closure.	Time taken to stomatal closure in minutes.
<i>Cassia tora</i>	Wet	74.5 ± 3.34	41.3 ± 5.04
	DM	71.9 ± 4.54	53.45 ± 4.95
<i>C. occidentalis</i>	Wet	Plants did not survive	-
	DM	79.9 ± 6.06	45.25 ± 3.33
<i>C. sophera</i>	Wet	70.5 ± 7.55	50.15 ± 7.04
	DM	67.35 ± 4.76	55.05 ± 6.65

This result agrees with the done in tomato plants grown in different soil moisture regimes (Nazrul-Islam and yasmin 1982).

7.3.3. Rates of Transpiration

The pattern of weight loss for both series (saturated and unsaturated) is shown in Table 7.5. If total transpiration rate is partitioned into stomatal and cuticular losses (Table 7.5), it is clear that the differences between the treatments result mainly due to differences in the rate of stomatal transpiration. Among three species, under shade condition *Cassia occidentalis* (unsaturated) exhibited highest total ($16.33 \pm 2.87 \text{ mg g}^{-1} \text{ min}^{-1}$) loss of water and *Cassia tora* from shade condition exhibited lowest total $2.40 \pm 1.03 \text{ mg g}^{-1} \text{ min}^{-1}$ loss of water.

High rate of total and stomatal transpiration is an indication of high uptake of water and ions. On the other hand *Cassia occidentalis* grew in sun (unsaturated) showed the highest cuticular loss of water and *Cassia tora* grew in shade habitat showed the lowest cuticular loss of water. In all the species it was interesting to note that there was a relation between the total water loss and the stomatal loss. It was also noted that highest stomatal loss in *Cassia occidentalis* of unsaturated (shade habitat) and *Cassia tora* (shade habitat) are related to lower water content at stomatal closure. This result agrees with the work done in Jute by Nazrul-Islam and Alam (1986); and also in a range of plant species (Nazrul-Islam 1983).

In dry moist treatment *Cassia sophera* showed highest total loss of water ($3.50 \pm 0.575 \text{ mg g}^{-1} \text{ min}^{-1}$) among the three species followed by *C. occidentalis* and *C. tora*.

C. sophera also showed minimum loss of water ($0.833 \pm 0.429 \text{ mg g}^{-1} \text{ min}^{-1}$) in the Wet treatment.

Stomatal and cuticular water loss of *C. tora* did not show strong difference between DM and Wet treatments. *C. occidentalis* in Wet condition did not survive and hence comparison was not possible. In *C. sophera* stomatal water loss in Wet treatment was less than 1/4th ($0.625 \text{ mg g}^{-1} \text{ min}^{-1}$) from that of DM treatment ($2.833 \text{ mg g}^{-1} \text{ min}^{-1}$). Cuticular transpiration in DM treatment was significantly higher ($p = 0.005$) than that of Wet treatment.

Table 7.5. Rates of transpiration calculated as rate of loss of water

content (stomatal values calculated by difference).95%

confidence limits are also given.

Species	Treatment	Loss of water in $\text{mg g}^{-1} \text{min}^{-1}$			$\frac{\text{Cuticular}}{\text{Total}} \times 100$
		Total	Stomatal	Cuticular	
<i>Cassia tora</i>	Sun	2.40 ± 1.03	1.58 ± 0.99	0.82 ± 0.15	34.16
	Shade	2.34 ± 0.62	1.93 ± 0.62	0.41 ± 0.32	0.17
<i>Cassia occidentalis</i> (Unaturated)	Sun	12.5 ± 2.55	9.5 ± 2.48	3.0 ± 0.66	24
	Shade	16.33 ± 2.87	14.95 ± 2.82	1.37 ± 0.21	8.38
<i>Cassia occidentalis</i> (Saturated)	Sun	15.83 ± 4.31	13.62 ± 4.47	2.20 ± 10.41	13.89
	Shade	13.3 ± 3.23	12.41 ± 3.31	0.91 ± 0.13	6.84
<i>Cassia sophera</i> (Unaturated)	Sun	7.50 ± 1.85	6.75 ± 1.94	0.75 ± 1.65	10
	Shade	7.16 ± 0.78	6.45 ± 0.85	0.70 ± 0.10	9.77
<i>Cassia sophera</i> (Saturated)	Sun	7.51 ± 1.28	6.90 ± 1.22	0.54 ± 0.10	7.2
	Shade	7.80 ± 1.03	7.25 ± 1.02	0.58 ± 0.13	7.4

Table 7.6. Rates of transpiration calculated as rate of loss of water content (stomatal values calculated by difference) of plants 5 weeks after the treatment. 95% confidence limits are also given.

Species	Treatment	Loss of water in $\text{mg g}^{-1} \text{min}^{-1}$			$\frac{\text{Cuticular}}{\text{Total}} \times 100$
		Total	Stomatal	Cuticular	
<i>Cassia tora</i>	Wet	1.333± 0.542	0.875±0.51	0.4583±0.10	37.50 ±14.36
	Dry-moist	1.333±0.542	0.708±0.56	0.6250±0.14	52.08 ±21.02
<i>C. occidentalis</i>	Wet	Plants did not survive-	-	-	-
	Dry-moist	2.66±0.855	2.208±0.83	0.458±0.197	18.06±10.22
<i>C. sophera</i>	Wet	0.833±0.429	0.625±0.36	0.208±0.195	20.83±19.73
	Dry-moist	3.500±0.575	2.833±0.61	0.666±0.213	19.44±7.53

In *Cassia tora* approximately 50% of water loss was through the cuticular in both DM and Wet treatment; loss of water was mainly through the stomata in all the species. During the stomatal phase of transpiration a more rapid weight loss is obtained in the leaves from the DM treatment than from Wet treatment. Read and Bartlett (1972) measured the loss of water in trifoliolate leaves of *Glycine max* at different droughting cycles. Their observation was very similar to that of *Cassia* spp. Thus leaves from DM treatment conserve water more efficiently only after the development of water details sufficient to cause stomatal closure. Further study of stomatal density between the leaves of DM and Wet treatments is warranted in view of the possible relationship between stomatal frequency of DM and Wet treatments.

7.3.4. DISCUSSION

The leaves used in this study were similar in maturity and were taken from plants growing in the same area. The result obtained may thus be expected to reflect real species differences rather than difference due to varying age (Knipling 1967) or varying environmental experience (Jarvis and Jarvis 1963a, b; Gavande and Taylor 1967).

The results therefore obtained bring into sharper focus any interspecific difference in relation to environmental conditions. In the field, *Cassia tora* is usually confined to the dry areas and occasionally in temporary wet places where there is little water stagnation during monsoon season. Nazrul Islam (1983) investigated tissue water relations in a range of dry land and wetland spp. He observed that *R. acetosa* is usually confined to the dry areas and occasionally in waterlogged places where there

is running water. *R. acetosa* closed stomata with high relative water content. In the present investigation all species of shade habitat had high relative water content at stomatal closure. Saturated leaves also showed similar tendency of relative turgidity value at stomatal closure.

The results of the present investigation showed that in general the relative water content values at stomatal closure were higher in shade plants than that of sun plants except *C. tora*. This result is an agreement with work of Bannister (1971) ; Nazrul-Islam and Ahmed (1995). Bannister in a comparative study reported that the relative water content at stomatal closure in *Vaccinium myrtillus* from shade habitat was higher than that of sun habitat. Bannister (1971) has shown that this relationship differs with changing environmental conditions for the same species. The results obtained in the present experiment are important for comparative study among the three species of sun and shade habitats. This results show that the high value of relative turgidity in dry moist treatment and also to some extent in sun condition in all three species of *Cassia* indicating non xeromorphic character. In terms of relatives water content, at which the stomata closed, *Cassia tora* under shade condition was found to be most sensitive (Table 7.3).

Bannister (1964) has also shown that *Calluna vulgaris* from dry site had a lower relative turgidity at a stomatal closure than those from wet sites and that those from intermediate moist sites, lay somewhere between the two extremes, so some precise measurement of soil moisture and also plant moisture (ratio of the water content in the field to the water content after the plant material has been allowed to make up any deficit) from wet and dry sites might show some possible differences.

The sensitivity of the three species (*Cassia tora*, *Cassia occidentalis* and *Cassia sophera*) of both sun and shade habitats in relation to the value of relative water content showed that *Cassia tora* of both habitats is more sensitive than other two species of the same habitat.

Okali (1971) suggested that species with a low degree of tolerance might be expected to possess high stomatal sensitivity as an adaptation against the development of damaging water potentials. The low degree of tolerance with a very low relative turgidity at stomatal closure found in *Cassia sophera* of sun habitat could be related to the views of Williams (1950) who suggested that epidermal modifications might serve to maintain epidermal turgidity and thus enable the stomata to keep open over a wide range of internal water deficits. Bannister (1964) also showed that the hairy variety of *Calluna* possesses lower relative turgidity than the non hairy variety.

Considering the relative water content at stomatal closure, rates of transpiration and plant moisture, it was suggested that drought avoidance of Jute (CVL-1 and 0-4) may be assisted by the capacity to increase cuticular resistance (Nazrul-Islam and Noor Newaz 1991).

(Clark and Levitt 1956) has mentioned that a more complex series of physiological changes are also involved. This change appears to be stimulated by tissue water deficits and they lead to a condition in which a stabilized water balance is achieved by means of both increase of uptake and decrease of loss at given water stress.

There is evidence that the growth of tomato plants is to a great extent controlled by some aspect of moisture factor (Nazrul- Islam and Roy 1978). Data of Lawson and

Jenik (1967) suggested that the transpiration rates measured were influenced more by factors related to water supply than by factor connected with drought avoidance.

The minimum conclusion that can be drawn from this analysis is that the species investigated exhibit different mechanisms of adaptation in water relation of leaf.

CHAPTER 8

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