Characterization of leaf senescence in relation to partitioning and remobilization of dry matter and nitrogen in pigeonpea (*Cajanus cajan* (L.) Millsp.) under two soil moisture regimes

(Charakterisierung der Blattseneszenz in *Cajanus cajan* (L.) Millsp. im Bezug auf die Verteilung und Umlagerung von Trockenmasse und Stickstoff unter zwei Bodenfeuchtebedingungen)

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To everything there is a season,
A time for every purpose under heaven:

A time to be born,
  and a time to die;
A time to plant,
  and a time to pluck what is planted;
...
A time to break down,
  and a time to build up;
A time to weep,
  and a time to laugh;
A time to mourn,
  and a time to dance;
A time to cast away stones,
  and a time to gather stones;
A time to embrace,
  and a time to refrain from embracing;
A time to gain,
  and a time to lose;
A time to keep,
  and a time to throw away;
...
A time to keep silence,
  and a time to speak;
A time to love,
  and a time to hate;
A time of war,
  and a time of peace.

Ecclesiastes 3,1-8
Holy Bible

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ABBREVIATIONS

DOE	Day of emergence
DAE	Days after emergence
DAF	Days after flowering
DAP	Diammonium phosphate
DAS	Days after sowing
DAS_h	Days after sowing at the 1st harvest
DW	Dry weight
DW_{cv}	Dry weight of cultivar
DW_s	Dry weight sub-sample
DW_{sd}	Seed dry weight
DW_t	Total dry weight
ESD	Extra-short duration cultivar
FW	Fresh weight
GA	Ground area
HI	Harvest index
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
JD	Julian day
LAI	Leaf area index
LGS	Length of the growing season
MD	Medium duration cultivar
MSL	Meters above sea level
N	Nitrogen
NUE	Nitrogen use efficiency
PET	Potential evapotranspiration
RGS	Relative growth stage
SAT	Semi-arid tropics
SD	Short duration cultivar
TDM	Total dry matter
TN	Total Nitrogen
W_o	Weight at time zero
W_f	Final weight

1. INTRODUCTION

Pigeonpea, also known as 'red gram', is one of the major grain legume crops of the semi-arid tropics. It is widely grown by small farmers as a subsistence crop which helps to ensure a stable income and which can be used in many and diverse ways. In India, where a high percentage of the population feeds on vegetarian food, pigeonpea is an important source of protein in human nutrition.

Pigeonpea is predominantly grown in marginal areas with unfertile soils and unreliable rainfall but without high inputs such as additional supply of water and nitrogen. Under such conditions, most other crops even fail to complete their life cycle. Pigeonpea plants are able to utilize nutrients and soil moisture more efficiently than other crops while enriching the soil through nitrogen fixation and leaf shedding.

Efficient nitrogen economy is an important crop feature in the SAT where climatic conditions may adversely affect soil properties and therefore crop growth. Research on uptake, partitioning, and remobilization of nitrogen should lead to the improvement in the crop's utilization efficiency of this element for better yield production. The pattern of distribution and mobilization of nitrogen may be genetically determined and may be utilized as a selection criterion.

The senescence of leaves which occurs mainly during the pod filling stage is known to be related to the remobilization of nutrients from leaves to pods. Nitrogen plays a major role as it is an abundant element of protein structures which are broken down in leaves to be resynthesized in developing seeds. The characteristics of leaf senescence in pigeonpea is supposed to be more complicated than in other legumes such as groundnut or soybean but its nature and relation to environmental stress factors have rarely been studied so far.

2. REVIEW OF LITERATURE

2.1. Agroeconomic importance of pigeonpea

2.1.1. Distribution and plasticity

Pigeonpea is widely grown in the tropics and subtropics of Asia, Africa, and America. Compared to other grain legumes such as soybean, field pea, and chickpea, pigeonpea ranks seventh in area and production (Rehm and Espig 1984). Among more than fifty countries where it is grown, the Indian subcontinent has the largest area under pigeonpea cultivation. There, almost 90 % of the world's pigeonpea is produced on approximately 3 Mio. ha amounting to about 2.2 Mio. t (for 1979-84, ICRISAT 1991). Other important countries of pigeonpea production are Myanmar, Nepal, and Bangladesh in Southeast Asia, Kenya, Uganda, and Malawi in eastern Africa and Haiti, the Dominican Republic, and Puerto Rico in Central America (Nene and Sheila 1990). Several of those countries started producing pigeonpea commercially but throughout the tropical region it is commonly grown as a backyard crop for domestic requirements. Pigeonpea grows naturally in a semi-arid to subhumid environment between 30°N to 30°S (Nene and Sheila 1990).

The need for intensive agriculture led to the improvement of the crop through breeding of new varieties and hybrids during the past two decades. The productivity of the traditionally cultivated landraces is low with average yields ranging between 0.4 and 0.8 t/ha (Müller *et al.* 1990). The great plasticity of the pigeonpea taxon facilitates fitting it to diverse agroecological environments and to new growing areas. The recent development of short duration cultivars made commercial and mechanized production systems possible and allows cultivation of the crop even beyond its traditional growing zone up to a latitude of 45°N in the temperate region (Troedson *et al.* 1990). The wide diversity in phenological development and habits existing in pigeonpea results in its adaptation to a wide range of contrasting production systems (Troedson *et al.* 1990). The difference among the genotypes in growth and development is reported to be even greater than between many other crop species. The short duration cultivars are less sensitive to photoperiod and temperature interactions and can be cultivated up to an altitude of 2000m (e.g. in Kenya) (Laxman Singh *et al.* 1990).

2.1.2. Utilization of pigeonpea

Among the many uses of the pigeonpea crop the production of human food is the most important in India. The seeds containing high amounts of protein (up to 29%) are dried, decorticated, and split for preparing a thick soup, called *dhal*. This is commonly mixed with rice for consumption, representing the staple food of India (Nene and Sheila 1990). On other continents, green seeds or pods are cooked as vegetables. As a backyard crop pigeonpea plants provide fodder for cattle from green leaves, husks, and new leaves of harvested plants. Sticks are used as fuel wood or to make fences and baskets. In some countries pigeonpea is used as a green manure crop. Whole plants serve as

windbreak hedges or as field boundaries, and last but not least, there are many folk medicinal uses known from pigeonpea (Morton 1976).

2.1.3. Genotypes and cropping systems

The variety of genotypes available can be grouped according to their duration to maturity. Phenological development has been used as a major crop feature in categorizing production systems (Byth *et al.* 1981) because maturity duration determines the adaptation of varieties to agroclimatic regions and cropping systems (Sharma *et al.* 1981). Short-season, full-season, and long-season crops represent the three classes of production systems defined by Byth *et al.* (1981). Short-season crops comprise typically the extra-short and short duration pigeonpea genotypes (ESD and SD). ESD types mature within 85-100 days and have, for instance, the ability to escape drought in a semi-arid environment because of their short growth cycle (Troedson *et al.* 1990). SD types, like the commonly used ICPL 87, mature within 100-120 days. Full-season crops are referred to as medium duration genotypes (MD) because they occupy the full length of the warm season and mature about 160-200 days after sowing. Long duration genotypes (LD) need more than 200 days for yield formation and can remain in the field for 1-2 years.

Farmers grow pigeonpea mainly in intercropping systems with other legumes (groundnut, soybean, mungbean, cowpea) or with cereals (sorghum, millets, maize, upland rice) by using the MD and LD types. Compared to the companion crop, pigeonpea grows slowly and reaches the stage of broad canopy development only after the companion crop is harvested towards the end of the rainy season. Therefore, both crops do not compete for the same resources, e.g. light, during the same time period (Willey *et al.* 1981). Residual nutrients and soil moisture can be utilized by the continuing pigeonpea crop. The short duration types are more suitable for monocultures, for sequential cropping (e.g. with wheat) and for multiple harvests (ratooning). Because of their shorter size and less branching a higher plant density can be used than in the tall and bushy traditional types.

Intercropping of pigeonpea with sorghum is the most widely used combination in India and Africa and has been proved a stable system of production. The major intercrop of Central and South American systems is maize. Other intercrops than legumes or cereals can be used like cotton, castor, sunflower, etc.

In long-season types yield losses occur mainly because the effective growth period is much shorter than the growth cycle, and the crop has to face drought stress during yield formation (Muchow 1985, Singh and Subba Reddy 1988). Though the growth cycle of ESD and SD types well matches the length of the growing period, they may be sensitive to intermittent drought periods and irregular rainfall distribution in semi-arid environments (Nam *et al.* 1993, Chauhan *et al.* 1993, Nam 1994). Yields obtained from high-yielding ESD and SD types amount to more than 2 t/ha. A maximum yield of 5.2 t/ha per year was obtained in multiple harvests of short duration pigeonpea (Chauhan *et al.* 1984).

2.1.4. Pigeonpea in the semi-arid environment

Pigeonpea is reported to be a crop for dryland agriculture because of its ability to tolerate drought periods, to produce some yield where other crops completely fail and, moreover, because of its improvement of soil fertility. Under rainfed conditions the productivity of the crop over environments and seasons is more stable than that of cereals in such intercropping systems (Singh and Subba Reddy 1988). However, often described as being well adapted to harsh environment (Faris 1982), pigeonpea seems to be rather adapted to survival than to productiveness under water deficient conditions (Muchow 1985, Troedson *et al.* 1990). In fact, the majority of pigeonpea-growing areas lies in sub-humid regions with 500-1000mm rainfall during the growing season (Reddy and Virmani 1981).

Drought tolerance of pigeonpea is due to its deep and widespread root system (main root development in the upper 30-60 cm, Sheldrake and Narayanan 1979a) which provides the plant access to the limited soil nutrients and deep water resources. This is particularly important for cultivars growing into the postrainy and dry season. Water extraction has been reported even up to 180 cm in a Vertisol (Sardar Singh and Russell 1981). Moreover, high dessication tolerance and osmotic adjustment (Flower and Ludlow 1987) enable the plant to expand the root system to deeper soil layers.

Even though pigeonpea is generally grown in a drought prone environment, the crop has to face periods of excess rainfall due to erratic distribution of precipitation (Huda and Virmani 1987). Both drought stress and waterlogging adversely affect the root symbioses of pigeonpea. During moisture stress the water potential of roots and nodules as well as its nitrogenase activity decreases and shedding of nodules may occur (Kumar Rao 1990). Waterlogging also damages the root system and causes the plants to exhibit symptoms of water deficit (Chauhan 1987) as well as symptoms of N deficiency through the inhibition of N_2 fixation (Thompson *et al.* 1981). Pigeonpea plants are very sensitive to temporary waterlogging (Chauhan 1987), and this has been considered to be one of the major contraints to its growth in India. Decreased oxygen content of the soil restricts root and nodule respiration (Okada *et al.* 1991). The problem of soil aeration has been considered more serious in Vertisols than in Alfisols, but Okada *et al.* (1991) found that after heavy rainfalls the soil oxygen concentration can sometimes be even lower in Alfisols.

2.2. Aspects of nitrogen economy in legumes

2.2.1. Assimilation, distribution, and cycling of nitrogen

Nitrogen uptake in legumes is dominated by N_2 fixation of the root nodules which are developed in symbiosis with soil-borne *Rhizobium* bacteria. Atmospheric nitrogen is reduced in the bacteroids by the key enzyme nitrogenase and subsequently translocated to the host cell where it is incorporated into certain amino acids. In the form of organic compounds the fixed nitrogen enters the xylem and is distributed in the plant via the transpiration stream. The compounds characteristic of nitrogen

transport in N_2-fixing plants are species specific. In many temperate legumes, e.g. lupin and field pea, the major transport compounds are amides (asparagine and glutamine), while in certain tropical legumes, e.g. soybean, cowpea and pigeonpea, ureides (allantoin and allantoic acid) are principally carrying the fixed nitrogen from roots to the shoots (Pate 1980, Luthra *et al.* 1981).

Inorganic nitrogen is available in the soil solution in the form of nitrate (NO_3^-) and ammonium (NH_4^+) ions. Plant species vary greatly in their ability to exploit those basic nitrogen sources; nonetheless most species meet their nutritional requirements by using both sources depending on their relative availability in the soil (Bloom 1988). In legumes high soil N concentrations as caused by application of fertilizer are known to have little effect on growth of established plants and rather suppress N_2 fixation by inhibition of nodule formation (Streeter 1988) or nitrogenase activity (Becana and Sprent 1987). N-fertilizer studies have shown such response in pigeonpea also above 25 ppm N (Kumar Rao *et al.* 1981, Quilt and Dalal 1979). In spite of that, pigeonpea cultivars may vary in their response to nitrogen fertilizer in seed yield and dry matter suggesting that nitrogen fixation alone is not able to meet the N requirement of some cultivars (Kumar Rao *et al.* 1981, Kumar Rao 1990). Erratic rains and low organic matter content of soils in the SAT often result in severe leaching of the movable NO_3^- ions into drainage water.

The various organs of a plant receive fixed nitrogen through the vascular bundles, e.g. leaves and stems are supplied directly by the xylem (Pate 1980). In pigeonpea, the N-rich ureides, which are exclusively produced in the nodules, accumulate in the plant parts generally according to the pattern of acetylene reduction (Luthra *et al.* 1983) indicating the close relationship between N_2 fixation and N distribution in the plant.

Developing pods receive about 90 % of their photosynthate and nitrogen supply via the phloem (Pate *et al.* 1977). Photosynthate is either provided by current photosynthesis of green leaves or by the C storage of the leaves. Nitrogen is not directly transported to the reproductive structures after entering the plant via the root system. ^{15}N studies have shown that nitrogen is cycled through various plant parts before being translocated to the pods (Pate and Flinn 1973). Thus, the cycled N may pass several age-groups and types of vegetative structures as well as several generations of proteins before being finally released for seed nutrition. This indicates that apart from current uptake a considerable part of the N fixed during the vegetative stage would eventually be used for seed production (Pate and Flinn 1973). In contrast, a major part of early incorporated C is lost through respiration. Remobilization of N from leaf tissue to developing pods plays an important role in many annual plants resulting in nutrient depletion of the leaves. This process has been extensively discussed in relation to leaf senescence (e.g. Thomas and Stoddart 1980).

2.2.2. Partitioning and remobilization

The pattern of N partitioning changes according to the developmental stage of the plant (Herridge and Pate 1977, Pate and Herridge 1978). The vegetative phase is characterized by expansion of the root and shoot system including leaf area. The C and N assimilates are shared among those organs at seedling establishment, whereas at the later stage, either before flowering (e.g. cowpea) or at the end of flowering as well as during early fruiting, the canopy becomes the major N storage, while the stem accumulates most of the C. The seed filling phase is characterized by predominant N accumulation in the seed which usually rapidly exceeds canopy N. The seed becomes also the major sink for assimilated carbon.

After flowering, when N demand is high, N_2 fixation decreases in many legumes which may be due to decreasing carbohydrate supply to the nodules competing with the developing fruits (Luthra *et al.* 1983a, Herridge and Pate 1977). White Lupin (*Lupinus albus* L.) is reported to be an exception in this regard (Pate and Herridge 1978). Nitrogenase activity in pigeonpea nodules has been shown to decrease after 60 DAS (days after sowing) in several cultivars of different maturity groups (Kumar Rao and Dart 1987). This situation seems to force the plants to translocate N from its own tissues since N uptake rates cannot meet the high demand for seed N as shown by Sinclair and de Wit (1975). Various degrees of self-destruction are visible in legume crops, most severely expressed in monocarpic[1] senescence as for instance in soybean (Lindoo and Noodén 1976).

Partitioning of nitrogen among plant parts is also related to the general growth habit of the plants represented by annual, biennial or perennial types. The majority of crops are grown annually and principle investigations on N partitioning in legumes have been made in plants of this growth habit such as lupin or soybean.

Nitrogen partitioning has often been studied in relation to photosynthate partitioning and utilization in order to obtain whole plant budgets. Therefore, comprehensive studies are available for several legumes like lupin (Pate and Herridge 1978), chickpea (Hooda *et al.* 1986, Hooda 1990), cowpea (Herridge and Pate 1977), and pigeonpea (Rao *et al.* 1984). Many studies were focussed more on the redistribution of N and other minerals during the reproductive stage, as for instance in soybean (Derman *et al.* 1978, Sesay and Shibles 1980, Mauk and Noodén 1992), lupin (Hocking and Pate 1977), and pea (Pate and Flinn 1973, Hocking and Pate 1977). These studies deal mainly with N depletion of senescing leaves and the N accumulation in developing fruits. Remobilization from leaves was also intensely investigated on the biochemical level in relation to the mechanism of leaf senescence (Grover *et al.* 1985). Some studies estimated the remobilization of N from plant parts other than leaves such as stem, podwall or nodulated roots (Pate *et al.* 1977, Herridge and Pate 1977, Rao *et al.* 1984, Hooda 1990).

[1] Monocarpy: single flowering phase followed by whole plant senescence and death

From many of those studies it is evident that N remobilization from plant parts varies among species and cultivars. Understanding of this mechanism can be helpful in evaluating their efficiency in N economy. Pigeonpea is the only legume among the investigated species representing an intrinsically perennial plant type which grows into a woody shrub but is usually cultivated as an annual.

2.2.4. Loss of nitrogen to the soil

The shedding of plant organs during the crop growth cycle results in loss of plant N and usually includes leaves and petioles. Nitrogen which is abscised with the leaves is lost for remobilization and seed protein production. Leaf abscission is enhanced by stress factors such as limited soil moisture availability (Hooda 1990) which may result either in higher remobilization efficiency or in yield reduction. Among pigeonpea cultivars large differences were reported by Kumar Rao and Dart (1987) in the amount of N lost as fallen plant parts (mostly leaves) ranging between 9 to 28 kg N/ha. Accordingly, the residual N varied among cultivars between 16 and 51 kg N ha^{-1} and was well correlated with crop duration (Kumar Rao and Dart 1987). Therefore, residual effects of pigeonpea on a succeeding cereal crop were assumed due to additional soil amelioration through fallen plant parts (Kumar Rao *et al.* 1983). However, the mechanism of the residual effect is not clearly understood. In another study using ^{15}N a mixture of fallen plant parts and soil did not benefit the cereal (ICRISAT 1984).

Flowers and young pods may also be shed to some extent, often depending on environmental conditions. In pigeonpea grown under normal conditions flower shedding has been reported to occur mainly among later-formed flowers indicating that the crop reduces pod setting to a reasonable capacity which, on the other hand, may lie below its capability (Sheldrake and Narayanan 1979b). This is different from chickpea behaviour, for instance, where flower and pod abortion hardly occurs under favourable environmental conditions (Sheldrake and Saxena 1979).

2.3. Leaf senescence

2.3.1. Senescence or aging ?

The general terms *senescence* and *aging* summarize the processes characteristic for the terminal stage of life of organisms, organs or cells, but its often inaccurate use called for more precise definitions, first proposed by Leopold (1980):

Aging may be defined as a passive, gradually proceeding degeneration with time which is driven by mainly exogenous factors and which does not necessarily lead to death (Leopold 1980, Noodén 1988a). Lesions and damages, particularly on molecular and membrane level, caused by external factors accumulate over time decreasing stress resistance while increasing vulnerability and, thereby, the probability of death.

Senescence refers to endogenously induced and controlled processes (Leopold 1980, Noodén 1988a) which drive an organism, organ or cell towards death. Though a whole array of deteriorative steps are involved, senescence appears to be an active process including the synthesis of degradative enzymes (Martin and Thimann 1972). Senescence is not a chaotic breakdown but an orderly loss of normal functions and this seems to be internally programmed. Therefore, senescence is recognized as a fundamental developmental process (Noodén 1988b). However, this process is reversible until late stage, at least in leaves. The ultimate collapse of cells loosing their homeostatic ability would be similar in both aging and senescence.

In many cases it is difficult to distinguish between these two processes because of the difficulty to categorize the metabolic changes and define them in specific biochemical terms many of those pathways being still uncertain (Noodén 1988b). Degeneration may often represent a combination of senescence and aging. Nonetheless, there are clear cases known like monocarpic senescence in soybean or, as an example for aging, the loss of viability in stored seed (Noodén and Leopold 1978, cited in Noodén 1988a). Applied to different growth types of plants, it might be generalized that short-lived plants like annuals and biennials tend to have longevities limited by programmed senescence, whereas perennials tend to die through gradual attrition associated with aging (Leopold 1980).

The time course of leaf senescence is related to the plant's growth type as the general patterns of plant senescence proposed by Leopold (1980) suggest. In annuals and biennials characteristic features are a progressive dieback of leaves in relation to their position as well as a rapid leaf shedding phase towards maturity combined with whole plant death as we know it for soybean (Secor *et al.* 1984). In perennial plants it is usually progressive as well as deciduous leaf senescence (related to winter or dry seasons) which is characteristic. While whole plant death in perennials is mainly a process of aging, the death of their leaves is basically programmed.

2.3.2. Longevity of leaves

During its life time a leaf passes through different developmental phases associated with changes in the major function of the leaf and its metabolic status (reviewed by Woolhouse 1982, Thomas and Smart 1993). The first state, the juvenile or immature phase, covers the development of the leaf from emergence to full expansion and is marked by net import of nutrients like carbon and nitrogen. Leaf maturity follows as a period of active photosynthesis when carbon export becomes the main function of the leaf while nitrogen is still demanded for protein synthesis. The third phase begins with the onset of senescence as a decline in photosynthetic rate and the start of nitrogen export, while physiological integrity is still maintained. Towards the final phase, the breakdown of leaf integrity, leaf yellowing, starts and nitrogen export peaks.

Leaf life spans differ greatly among species and may even be different within plants (Chabot and Hicks 1982). In view of such variability the question arises how leaf life spans are determined. Leaf senescence is commonly interpreted as an integral part of leaf development which is believed to be

under direct genetic control as any other developmental process (Thomas and Stoddart 1980). There is evidence that the nuclear genome is positively involved through cytoplasmatic protein synthesis, for instance (Thomas and Stoddart 1977). Nevertheless, there are several other factors than genetics that may influence the initiation of senescence which can be categorized as being either correlative or environmental (reviewed by Thomas and Stoddart 1980, Woolhouse 1982). Competition within the plant is one of the major reasons for senescence of individual leaves. Various plant organs compete for light, nutrients and growth regulators which may cause induction of senescence in a given leaf as a consequence of its position relative to other organs. Moreover, environmental factors such as temperature, light intensity and photoperiod, water and mineral relations, and pathogen invasion may affect the initiation of the senescence syndrom in certain leaves.

A canopy represents a population of individual leaves of different age and properties borne in different positions of the canopy (Harper 1988). However, canopies are mostly viewed as photosynthetic surface of a plant or a crop expressed in concepts like leaf area index and net assimilation rate which are useful in relation to growth rates of plants. In contrast, investigations of leaf senescence consider the heterogeneity and the changing properties of individual leaves with age within a canopy. The risk of death, being only slight during the juvenile period, may increase more or less rapidly with leaf age. Such changes can be expressed by survivorship curves taken from population dynamics to illustrate the difference of senescence and aging by different slopes (Leopold 1980, Harper 1988).

Reallocation of leaf nitrogen as a part of senescence may have two main objectives. It may take place within the canopy to maximize the plant's carbon gain which could be controlled by leaf age (Field 1983). If the photosynthetic apparatus is broken down in older, shaded leaves and then translocated to more productive positions on the fringe of the canopy, where light levels are high, this will ensure greatest carbon gains. Another objective would be developing the fruit, a storage organ which is built to be utilized only in the next generation. Nevertheless, the carbon provided by a given leaf to the rest of the plant depends much more on the life span of this leaf than on its photosynthetic capacity (Chabot and Hicks 1982).

In crop physiology leaf longevity is important because high leaf area index, prolonged photosynthesis, and extented canopy duration are associated with high productivity (Watson 1952) while senescence would have a negative influence on yield. Certain crop mutants exhibiting delayed senescence have been reported to give high yields and were used in some breeding-programs (Abu-Shakra *et al.* 1978, Thomas and Smart 1993). Nevertheless, there may be circumstances where rapid and efficient remobilization of minerals from leaves is a useful crop feature, for example, when uptake and distribution of nitrogen is restricted under drought conditions or when, even under natural conditions, nitrogen uptake cannot meet fruit N requirement (Sinclair and De Wit 1975).

2.3.3. Metabolic changes during leaf senescence

Leaf senescence is characterized by a number of deteriorative processes leading ultimately to death and abscission of the leaf. The physiological and biochemical changes involved seem to be tightly controlled at the cell and organ level in order to reutilize leaf mineral nutrients most efficiently. In search for the early events indicating the onset of leaf senescence the disappearance of chlorophyll accompanied by yellowing of the leaves has been ruled out. Senescence begins when the leaves are still green.

The earliest detectable symptom for the loss of leaf functioning is typically a decline in photosynthetic rate soon after full expansion (Wittenbach *et al.* 1980, Wittenbach 1983). This decline may proceed slower or more rapid depending on cultivar (Crafts-Brandner and Egli 1987) or on leaf position within a plant as related to reproductive sinks (Secor and Shibles 1984, Jiang *et al.* 1993) but cannot be prevented by fruit removal (Mondal *et al.* 1978, Wittenbach 1983, Crafts-Brandner and Egli 1987).

Declining photosynthesis is closely followed by a decrease in total leaf protein content and, particularly, in activity and amount of Rubisco[2] (Wittenbach 1983). Being the most abundant protein in green leaves Rubisco is located in the chloroplasts where it catalizes the assimilation of carbon. Its breakdown is likely to be genetically determined (Jiang *et al.* 1993) and has been shown to proceed faster than that of most other proteins (Peterson and Huffaker 1975, Wittenbach *et al.* 1980). Rubisco accounts for about 35 % of reduced N in mesophyll cells in wheat (Peoples and Dalling 1988).

Associated with the loss of protein is an increase of proteolytic activity and *de novo* synthesis of proteolytic enzymes (Martin and Thimann 1972, Peterson and Huffaker 1975) which is interpreted as one of the major indications for the controlled nature of the senescence syndrom. Protein is stepwise hydrolized to its constituent amino acids which are released to the cellular soluble N pool (**Fig. 2.1**). Proteins account for about 88 % of reduced N in mesophyll cells and can be divided into soluble and insoluble or membrane-bound, present in proportions of 62 and 38 % of the total protein in wheat (Peoples and Dalling 1988).

The release of amino acids by progressive protein degradation leads to an increase in leaf soluble amino acids unless they are metabolically converted or transported out of the leaf immediately. Atkins *et al.* (1983) noted in a study on lupin that there is a discrepancy between the amino acid composition present in the senescing leaf and those received by the fruit. It was concluded that a considerable amount of amino acids provided by proteolysis as well as those incoming via xylem would have to be converted in the leaf before phloem loading to meet the required composition of the seed.

[2] Rubisco: ribulose-1,5-bisphosphate carboxylase (RuBPCase, Fraction-1-protein)

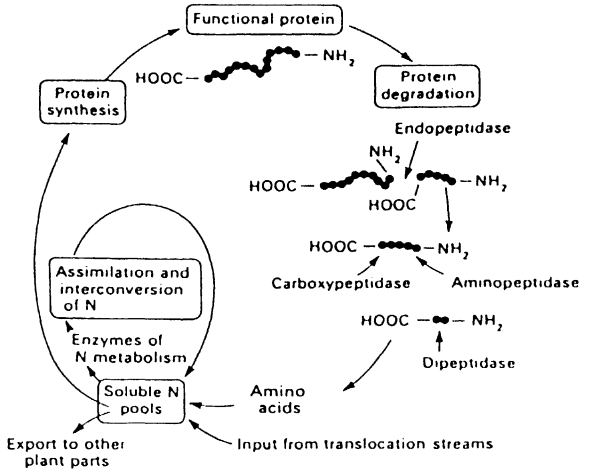


Fig. 2.1: Scheme for the turnover of cellular proteins. Breakdown products are released to the soluble N pool and may undergo metabolism before ultimately being reincorporated into protein or exported to other plant parts (from Peoples and Dalling, 1988).

Ammonium assimilation in the cell may be impaired by proteolytic activity during leaf senescence for it is highly dependent on enzymatic reactions. Since ammonia is toxic for the cell at relatively low concentrations, it is usually re-assimilated immediately by the GS/GOGAT[3] mechanism and/or by the alternative pathway GDH[4]. The amination activity of GDH is reported to be important during leaf senescence in several species, e.g. pigeonpea (Luthra *et al.* 1983, Peoples and Dalling 1988). Ammonium is usually formed in the cell during amino acid metabolism by oxidative deamination, during metabolism of the amides asparagine and glutamine, or by complete degradation of ureides (Thomas and Schrader 1981, Luthra *et al.* 1983). It is also produced by the photorespiratory N cycle which is still functioning during leaf senescence (Berger *et al.* 1985).

Chlorophyll loss occurs along with the degeneration of the chloroplast which also starts early in the course of senescence. The characteristic starch deposits disappear and the thylakoid structures undergo progressive deterioration (Thimann 1980).

Chlorophyll may not be degraded in certain mutants which continue to stay green until maturity, although those plants may lack photosynthetic competence because protein breakdown, inclusive of Rubisco, takes its course (Thomas and Stoddart 1975). The nature of stay-green genotypes is based on gene expression which affects either chlorophyll catabolism or the timing of the initiation of senescence and the regulation of its rate of progress (Thomas and Smart 1993). In the latter, crops might be expected to show a higher yield if carbohydrate is a major component of the harvest because they photosynthesize for longer than normal (Thomas and Smart 1993).

[3] GS: Glutamine-synthetase; GOGAT: Glutamate-synthase
[4] GDH: Glutamate-dehydrogenase

2.4. Aim of the thesis

The present study was conducted to investigate the progress of leaf senescence in pigeonpea as a part of dry matter and N accumulation and partitioning of the crop. The extent of leaf shedding was expected to be different under optimal water availability compared to natural soil moisture conditions (rainfed) at the selected location due to environmental stress. Leaf shedding is related to reutilization of nutrients from older plant parts. Therefore, patterns of leaf abscission within the canopy should be investigated in relation to remobilization of nitrogen to understand differences in the N economy among genotypes. The study was aimed to quantify net remobilization from leaves to reproductive structures and to compare the reutilization efficiency of the distinct genotypes during the period of large nutrient demand. This efficiency should be expressed in the composition of N in the senescing leaves to prove differences in remobilization and to provide information on metabolic changes in pigeonpea leaves during leaf senescence.

The subject was studied in a field experiment including a focussed look at leaves of different age-groups in the canopy.

The following questions were proposed for this work:

(1) *Which patterns of leaf senescence/abscission are present in pigeonpea ?*

(2) *How efficiently does pigeonpea remobilize nitrogen from senescing leaves ? Is this affected by sub-optimal water availability ? What may be the reasons for genotypic differences ?*

(3) *How is leaf senescence as related to dry matter and N partitioning of pigeonpea affected by sub-optimal soil moisture conditions (rainfed) in a semi-arid environment ?*

The thesis pays attention to the two aspects of leaf senescence which are dry matter partitioning and nitrogen economy. In addition, the general performance of the crop including partitioning within the plant is discussed, and the interrelation of N remobilization, leaf senescence and abscission is represented in N flow charts for each genotype and treatment.

3. MATERIAL AND METHODS

3.1. Location of the experiment

3.1.1. Geography

The experiment was conducted at ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), Patancheru, which is located in southern India at 17°35' N, 78°15' E. The Institute is situated near the village Patancheru about 25 km northwest of Hyderabad, the state capital of Andhra Pradesh. The experimental farm extending over 1400 ha includes two major soil types found in the semi-arid tropics: Alfisols (red soils) and Vertisols (black soils). The place is characterized by its semi-arid climatic conditions and by being placed on the so-called Deccan plateau, 545 m above MSL. The major geological formations of the area are granite and basalt. Weathered coarse-grained granite and granite-gneiss are found in the lower pediment on the ICRISAT farm (Murthy and Swindale 1993). Under Vertisols the granite-gneiss pediment is covered by basalt.

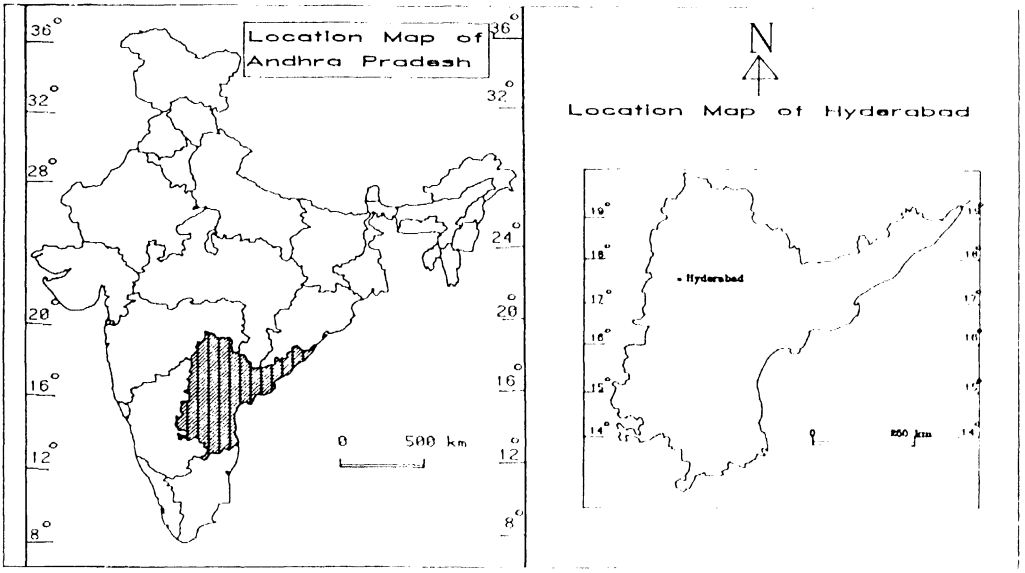


Fig. 3.1: Map of India showing the location of the state Andhra Pradesh and the state capital Hyderabad.

3.1.2. Climate

The climate of Hyderabad is semi-arid tropical. Troll (1965) classified the semi-arid tropics (SAT) as the region within the tropics where mean monthly rainfall exceeds mean potential evapotranspiration (PET) during 2 to 7 months of the year. Within the SAT two sub-zones can be distinguished: the Dry SAT with 2 to 4.5 months rainfall exceeding PET and the Wet-Dry SAT with 4.5 to 7 months rainfall exceeding

PET. ICRISAT lies right at the margin of the Dry and the Wet-Dry SAT with 4.5 months rainfall exceeding PET (Murthy and Swindale 1993).

As in most parts of India there are three seasons in the Hyderabad area. The rainy season (*kharif*), known as monsoon, usually begins in June and extends to October. During those four month more than 80% of the annual rainfall occurs. The average annual rainfall recorded over 30 years is 765 mm (Murthy and Swindale 1993). The pattern of rainfall is bimodal with two peaks, one in July and another in September, but there is high variation in rainfall from year to year.

As between seasons there is also considerable variation within seasons regarding distribution and intensity of rainfall. Rainfall is generally unpredictable which can affect the farmer's situation severely. Nevertheless, estimations of the rainfall probability help in predicting precipitation (Sivakumar *et al.* 1987). The coefficient of variability of rainfall at Hyderabad is 27% (Virmani, pers. communication).

The subsequent postrainy season (*rabi*) or winter season starts in October and continues until January. During this period the climate is dry and cool and the days are short. Crops grown during this time have to live on stored soil moisture. The coldest month is December with mean temperature around 20°C.

From February onwards until the following moonson season the climate is hot and dry. Any crop grown at that time would require irrigation. The hottest month of the year is May with air temperatures of 42° to 43°C. May is followed by stormy pre-monsoon cloud bursts in the early part of June. The regular monsoon rain starts from the second half of June and continues until the first week of October.

The **length of the growing season (LGS)** depends on the availability of water for crop growth which is determined by precipitation, evapotranspiration and water holding capacity of the soil. It is defined as the number of days during a year when rainfall exceeds half the potential evaporation, plus a period required to evaporate an assured 100 mm of water from excess rainfall stored in the soil (Huda and Virmani 1987). The period suitable for crop growth differs widely among different rainfall zones within the SAT (Sivakumar *et al.* 1987). In India the pigeonpea-growing period lasts between 90 to 180 days depending on the location (Huda and Virmani 1987).

For the Hyderabad area the growing period lasts 120 days for Alfisols and 180 days for Vertisols characterized by a water holding capacity of 100 mm and 250 mm, respectively (Virmani, pers. communication). A period of 154 days is reported by Singh and Subba Reddy (1988) for Alfisols and 175 days for Vertisols. Different methods have been used to incorporate soil water balance models for the estimation of LGS leading to deviating values. Moreover, variability of data can be large in the SAT. The beginning of LGS can be assigned to the 15th of June for Vertisols and to the 1st of July for Alfisols (Virmani, pers. communication).

3.1.3. Soil type and characteristics

The experiment was carried out on an Alfisol which is the most abundant soil type in the SAT (El-Swaify *et al.* 1987). Alfisols develop in climates where slight to pronounced seasonal moisture deficits occur but where the soil receives high enough precipitation (above 500 mm) to move clays downwards and form an argillic (clay accumulation, Bt) horizon. The surface horizon is slightly to moderately acid due to leaching. The basic cation saturation (Ca, Mg) of the soil is medium to high exceeding 50%.

In the SAT, diverse production constraints limit crop performance on Alfisols. Most of the South Indian red soils are shallow and gravelly due to erosion (El-Swaify *et al.* 1987). The soil surface tends to form crusts due to rapid drying and low structural stability. This leads to infiltration problems, runoff, and erosion affecting crop establishment adversely under rainfed conditions. Semi-arid tropical Alfisols are well-drained but possess low water-holding and nutrient-retention capacities which limit high and stable yields.

The medium deep Alfisol (maximum depth 1.0 m) used in the experiment belonged to the Patancheru soil series classified by Murthy and Swindale (1993). This mapping unit at the ICRISAT site is described as a sandy clay loam with less than 1% slope and none to slight erosion. Patancheru soils have a soil reaction of pH 6.0 to 6.9 and a base saturation of more than 60%. Extractable bases (CEC) represent 12 meq / 100g soil. The clay fraction consists of nearly 40% Kaolinit and 20% Smectite, while the sand fraction consists of about 40% Quarz and 30% Feldspars.

3.2. Plant material and seed treatment

The experiment was conducted with three distinct pigeonpea cultivars of different maturity dates. These were ICPL 84023 (extra-short duration, ESD), ICPL 87 (short duration, SD) and ICPL 1-6 (medium duration, MD). The growth duration of each maturity group is reported to range between 85-100, 100-120 and 160-200 days, respectively (Troedson *et al.* 1990). The seed was obtained from ICRISAT Breeding unit.

The seed was tested for germination before sowing: 10 seeds per genotype were placed on moist paper in petri-dishes and four replications were tested. Germination was above 90%. Seeds were treated with Captan to prevent fungal infection.

3.3. Experimental design

3.3.1. Layout

The three selected genotypes were grown under irrigated and rainfed conditions as main treatment. Genotypes were assigned to sub-plots. The treatments were allocated in the field in a split-plot design

including 4 replications (**Fig. 3.2**). The fourth replication was arranged to ensure availability of sufficient plant material in case of any severe disease in one of the treatments. The size of the total experimental area was 0.1 ha. Spacing was set at 0.1 m x 0.3 m for all genotypes. The size of the sub-plots was 43.2 m^2 consisting of 18 rows of 8 m length and a plant population of 33.3 plants/m^2.

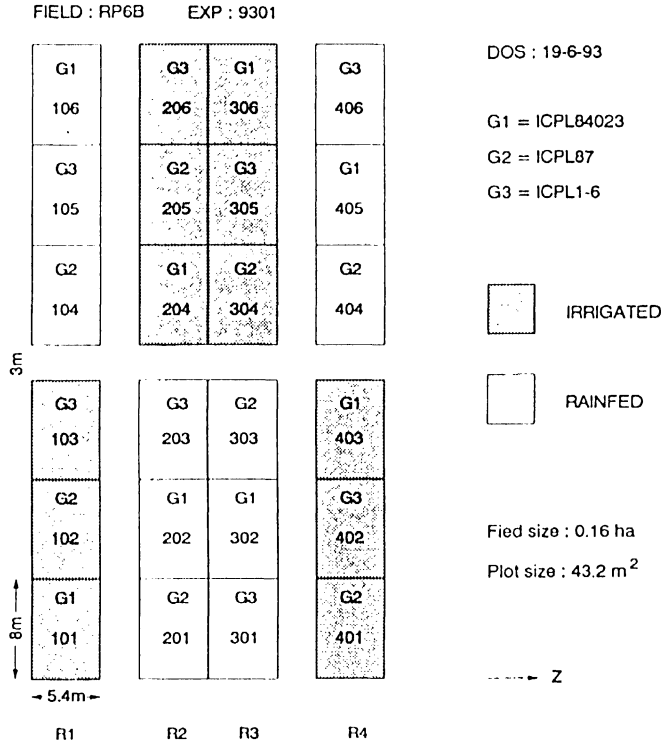


Fig. 3.2: Layout of the field experiment. Randomized split-plot design with 4 replications, water treatment assigned to main factor and genotypes to sub-factor. (DOS: Day of sowing, R: Replication).

3.4. Field management

3.4.1. Sowing and early plant stage

The field was prepared for sowing on the 14th of June 1993. After ploughing by tractor an initial amendment of 100 kg DAP/ha was broadcast as a basal incorporation. Ridges were set at a spacing of 60 cm. The crop was sown by hand on 17th of June and two seeds were placed per hill. About 2-3 kg seed were used per genotype. Pre-emergence herbicide (Basalin 1.65 kg/ha, Prometryn 1.5 kg/ha, Paraquat 4 kg/ha) was applied on the field for weed control.

The experiment started on 19th June (JD 170) with an initial application of irrigation given to the total experimental site for crop establishment. Irrigation was applied into furrows.

Gap filling was done at 5 DAE (days after emergence) of the young plants. Thinning was done at 25 DAS (days after sowing) to obtain the required plant density. Week and small plants were selectively removed. Weeding was done by hand at 8 DAS.

3.4.2. The established crop

Weeding was repeated at four times until mid-August and was generally done by hand. At times weed depression occurred severely in some patches. Especially on the old tracks of the previous year's layout the weeds came up very strongly. One major weed was the common Nut Grass. Chopping and rotavating by tractor was done only once on the main tracks in the beginning of August.

Plant protection concentrated on precautions against diseases and insects. Ridomil 6 g/l was applied to the field during the rainy season at 15 day intervals to prevent Phytophtora Blight, a fungal disease. Endosulfan 0.35 % E.C. at 0.07 % insecticide was sprayed 2-3 times against sucking insects.

The **water regime treatment** started on 22nd of July (33 DAS). Rainfed plots were isolated from irrigated plots by earthen bunds to prevent transgression of water during irrigation. The water applied was measured with a flow meter. At 35 DAS a wet period started for about two weeks and waterlogging effects were observed. Until 1st of October (104 DAS) no irrigation was required because the distribution of rainfall during the main growing season had been well-balanced. Further irrigation was applied during the postrainy season on 5th, 17th, 27th November and 17th December (139, 151, 161 and 180 DAS) to prevent drought stress in the irrigated treatment plots.

3.5. Samplings

3.5.1. Plant samples

Above-ground plant samples were taken in the sub-plots of three replications from defined rows (**Fig. 3.3**). In order to avoid border effects on the collected plant material two rows at a plot edge and 0.5 m in a row were omitted. The samples were taken in 10 day intervals during the vegetative stage and then weekly starting from budding stage onwards. Generally 5 plants and at later stages 10 plants were harvested from 0.5 m or 1.0 m of a ridge to obtain a sufficient amount of sample for subsequent measurement of dry weight and nitrogen.

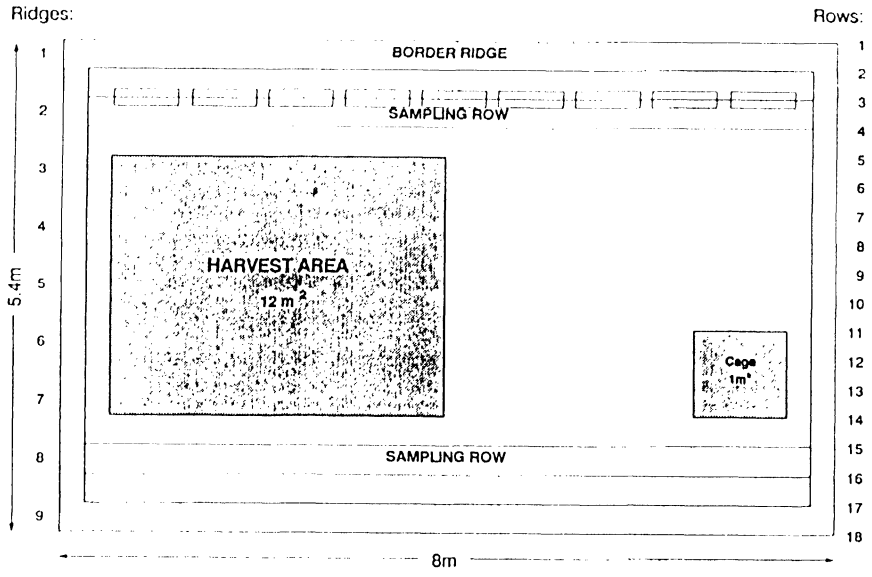


Fig. 3.3: Allocation of sampling areas in an exemplary plot. Sampling rows, harvest area, and cage for collection of fallen plant material were located within the border ridges of the plot.

The plants were separated into various organs like stem, petioles, and leaves at vegetative stage. Thereafter, a more detailed fractionation was used in which leaves and reproductive structures originating from the main stem were collected separatedly (**Fig. 3.4**) representing different age-groups. A fresh sub-sample of about 2g of each main stem fraction was immediately stored in a deep freezer for analysis of soluble nitrogen. All other (sub-) samples were oven-dried at 60°C to constant weight.

Five main stem leaf fractions were obtained as following (**Fig. 3.5**): The main axis was divided into 5 segments according to the total number of nodes counted when the sampling of leaf fractions started (budding stage). The number of nodes determined per fraction was 4, 6, and 6 in ESD, SD, and MD, respectively. In MD a large basal stem segment proportional to the upper stem was excluded from determination because the leaves of this section had already been abscised at budding and the number of leaves per fraction had become too large. The determination of size and location of the leaf fractions L1 (bottom) to L4 were continued throughout the samplings regardless scars of abscised leaves. The top leaf fraction (Top) contained the young and growing apical part of the main axis and had, therefore, an undetermined number of leaves.

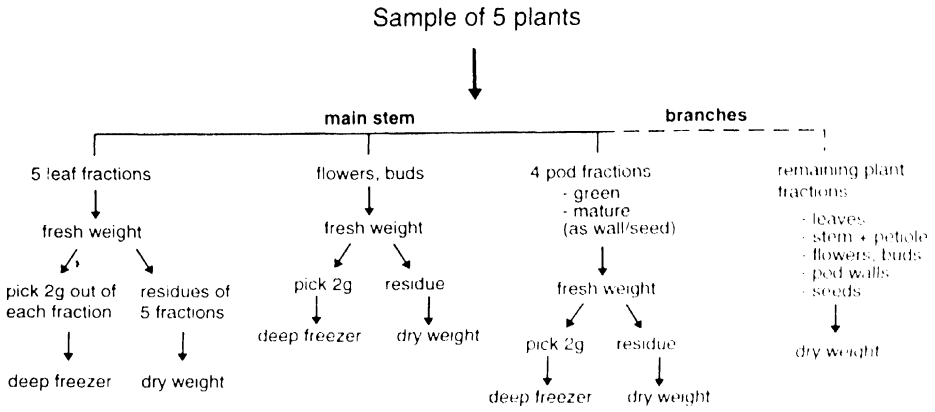


Fig. 3.4: Fractionation of plant samples during the reproductive stage. Five plants were completely separated into plant organs depending on their origin from main stem or branches.

Leaf fraction	Exemplary main stem axis (ESD)	Trifoliate nodes used per genotype and fraction ESD	SD	MD
TOP		>16	>24	>48
L4		13-16	19-24	43-48
L3	Sc	9-12	13-18	37-42
L2	Sc Sc	5-8	7-12	31-36
L1	Sc Sc Sc Sc	1-4	1-6	25-30

Fig. 3.5: Illustration of the sampling method used to obtain five different leaf fractions at the main axis. The fractions L1 to L4 represent leaf layers of defined sizes in ESD, SD, and MD. The number of attached leaflets per fraction was precisely registered. Sc: Scar(s) caused by abscission of 1-2 leaflets or whole leaf (3 leaflets). Each abscised leaflet counted as a scar. Sampling started at late budding stage.

3.5.2. Fallen plant material

Starting from the 20th of August (62 DAS), fallen plant material was collected weekly in cages which were installed in the sub-plots of each treatment in three replicates (**Fig. 3.3**). The biomass was separated into components (leaves, petioles, flowers, pods) before recording dry weight. The cages, made of cotton material which was fixed on sticks, were about 40 cm high and covered an area of 1 m^2. The number of plants per cage was recorded and used for calculation of fallen biomass on plant basis.

3.5.3. Soil samples

Soil samples were taken only in the surface soil layer. The soil moisture measurements were usually carried out with a neutron probe (see chapt. 3.6.2.) except in the surface layer, where the gravimetrical method was used. Volumetric water content (θ_v) was calculated from mass water content (θ_m) of the sample as follows:

$$\theta_v = \theta_m * \rho_B / \rho_w$$

ρ_B stands for bulk density of the layer and ρ_w for density of water.

3.5.4. Harvest

Harvest was carried out from an area of 12 m^2 including 5 ridges of 4 m (**Fig. 3.3**) located in the middle of each plot.

In ICPL 84023 (ESD) and ICPL 87 (SD) the crop remained in the field after harvest of the 1st flush of pods. The pods were harvested by hand. After cleaning from petioles and other plant parts they were dried, threshed and seed weight was taken.

The 2nd flush was also harvested by hand prior to cutting of all plants in the determined area at ground level. The pods were handled as before. The plants were counted, oven-dried and weighed.

In ICPL 1-6 (MD) only one flush of pods was harvested.

3.6. Observations and Measurements

3.6.1. Meteorological observations

Meteorological data were recorded daily at ICRISAT Met Station at 7.17 h in the morning and at 14.17 h in the afternoon including rainfall, maximum and minimum temperature and solar radiation (**Tab. 3.1**). Total annual rainfall was above the long-term average of 765 mm and exceeded 800 mm. Approximately 90% of the year's total precipitation occurred during the rainy season from June to

October. There were less rainy days in September indicating the dry spell which occurred during this time period. Average maximum temperature was 30°C throughout the cropping season but mean monthly minimum temperature decreased continuously towards winter time from 24.6°C in June to 21°C in October and was only 15°C in November.

Table 3.1: Mean monthly weather data of the year 1993.

		---- Mean air temperature ----				
Month	Mean Solar rad. ($MJ/m^2/d$)	Daily max. T. (°C)	Daily min. T. (°C)	Daily avg. T. (°C)	Monthly rain (mm)	No. of rainy days
JAN	17.6	30.2	12.7	21.5	0.0	0
FEB	20.5	30.9	14.0	22.4	0.0	0
MAR	21.6	34.5	19.7	27.1	0.5	1
APR	22.3	37.7	22.6	30.1	17.6	1
MAY	24.0	40.2	25.6	32.9	26.3	2
JUN	21.2	35.9	24.6	30.3	109.9	9
JUL	16.7	30.9	22.9	26.9	182.7	15
AUG	16.5	29.5	22.1	25.8	174.0	17
SEP	18.6	29.9	21.7	25.8	121.0	8
OCT	17.6	30.2	20.7	25.5	170.2	12
NOV	18.0	29.0	15.0	22.0	0.0	0
DEC	15.3	26.2	12.0	19.1	29.0	2
Mean annual	19.2	32.1	19.5	25.8	-	-
Total annual	-	-	-	-	831.2	67

3.6.2. Soil moisture

Soil moisture was measured at 10 day intervals with a neutron probe (Modell 3332 Gauge) which was lowered to various depths in the soil. The aluminium tubes in which the probe was lowered were installed in all plots of three replicates (R1, R2, R4). The neutron count was converted into volurnetric water content by using a calibration curve. Soil moisture content in the 0-15 cm surface layer was determined gravimetrically.

The observed values were used to simulate the soil moisture for each treatment and genotype in seven layers to a depth of 90 cms by using Ritchie's multi-layered water balance sub-routine WATBAL from the CERES-Maize model (Jones and Kiniry 1986). The main input data for running the simulation were the following: initial soil water status, amount of irrigation, weather data on a daily basis for rainfall, temperature and solar radiation, soil profile data, LAI for each genotype and treatment and a light extinction coefficient.

Soil profile data were obtained from an existing data set of a medium deep Alfisol on the ICRISAT site (**Tab. 3.2**) and included thickness of soil layers, lower limit of plant extractable soil water and drained

upper limit of soil water content, saturated water content and moist bulk density of soil in soil layers as well as a weighting factor to determine new root growth .

The output data of the soil moisture simulation included total storage water and plant-available soil water in the profile, volumetric water content of the various layers, cumulative actual plant transpiration and soil evaporation for each genotype and treatment. The simulation output of six layers are presented in chapter 4.1.1.

Table 3.2: Soil profile of a medium deep Alfisol at the ICRISAT site used for soil moisture simulation.

Layer No.	Layer (cm)	Lower Limit (VWC)	Drained upper Limit (VWC)	Saturated water (VWC)	Initial soil water (VWC)	Factor for root growth	Bulk density (g/cm^3)
1	10	0.110	0.200	0.240	0.190	0.50	1.58
2	12	0.100	0.195	0.240	0.195	0.75	1.60
3	8	0.080	0.190	0.255	0.190	0.75	1.60
4	15	0.080	0.180	0.255	0.180	0.75	1.60
5	15	0.090	0.180	0.270	0.180	0.50	1.59
6	15	0.090	0.180	0.270	0.180	0.50	1.59
7	15	0.090	0.190	0.260	0.180	0.35	1.59

VWC: volumetric water content.

3.6.3. Growth analysis

Growth parameters were measured at all sampling dates. Vegetative growth was measured in terms of node number at the main stem (vegetative stage), leaf development (leaf area index), and leaf and stem biomass. The number of nodes was counted from the bottom of the plants starting with the node of the first trifoliate leaf. Reproductive growth was measured in terms of biomass of flowers and buds as well as pod biomass (green and mature as seeds and podwalls separately). Biomass was obtained as dry weight.

Leaf area was measured from fresh leaf samples by using a leaf area meter (LI-3100 Licor). In case of large amounts of leaf sample the dry weight of a sub-sample (DW_t) was used to calculate total leaf area (LA):

$$LA = DW_t * LA_s / DW_s$$

Leaf area index (LAI) was calculated by dividing leaf area (LA) by ground area (GA):

$$LAI = LA / GA$$

Relative growth stage was obtained by dividing the DAS value of a growth stage by the DAS value of the 1st harvest:

$$RGS = DAS / DAS_h$$

Relative growth was compared by dividing the dry weight of each genotype at a given time by the maximum dry weight of the same genotype:

$$RG = DW_{cv} \text{ at given time} / \text{max. } DW_{cv}$$

Growth curves were computed by using total dry matter at sampling dates and were fitted with the following equation:

$$F(t) := W_o * W_f / (W_o + (W_f - W_o) * EXP(-a * t))$$

The function of time representing relative dry weight accumulation includes starting weight (W_o) and final weight (W_f) as well as an exponential term with the factor a which describes the slope of the growth curve.

Harvest index (HI) was calculated by dividing pod dry weight (DW_{sd}) by total dry matter (TDM):

$$HI = DW_{sd} / TDM$$

Values of total dry matter (TDM) were used for calculation of nitrogen use efficiency (NUE):

$$NUE = TDM / TN$$

3.6.4. Phenology

Phenology was recorded in 5 day intervals. Five plants were selected and marked in the field but not harvested. Observations were taken from the same plants throughout the season. Critical stages of whole field plots like budding, flowering, beginning of seed development, and physiological maturity were determined at 50 % of occurrence. Harvest maturity was determined when 75 % of the plants had reached that stage.

3.7. Chemical Analysis

3.7.1. Total Nitrogen

Dry powdered plant sample was digested for total N determination with sulfuric acid. The excess of hydrogen ions was removed by the addition of hydrogen peroxide and the resultant water vaporized. During digestion long chain molecules containing nitrogen are broken down due to the presence of strong acid and high temperature. Nitrogen is reduced to ammonia which can be determined by indophenol colorimetric method (Chaykin 1969).

3.7.2. Fractionation of soluble nitrogen

Approximately 1 g frozen sample was homogenised in a mortar with 10 ml KH_2PO_4 buffer (pH 7). Centrifugation was done at 10,000 rpm for 5 minutes at 5°C. The supernatant was separated and the content of soluble protein, α-amino acids and ammonia was measured. The residue was generally discarded but in case of small amount of sample which could not be separated into dry weight and fresh weight fraction it was dried in an oven at 60°C for total nitrogen analysis.

3.7.2.1. Soluble protein

Soluble protein was analysed immediately after centrifuging by a method based on coomassie brilliant blue according to Bradford (1976). The binding of the dye to protein is a very rapid process (approximately 2 min.), and the protein-dye complex remains stable in solution for approximately 1 hour. The reading of the absorbance was taken during the time interval suggested for very precise determination (10-20 min.).

3.7.2.2. Amino acids

The α-amino acids were analysed by blue color formation with ninhydrin (Snell and Snell 1954). Under heating the α-amino groups bind to ninhydrin. The amino acids are decarboxylated and diaminated in the alkaline solution. The ketone of ninhydrin is reduced to a carbinol group. The amount of amino acid corresponds to the optical absorbance of the color. The ninhydrin test is very sensitive and enables to recover even an amount of 1 µg amino acid.

3.7.2.3. Soluble ammonia

Soluble ammonia was concentrated by Conway microdiffusion technique (Burris 1972). Ammonia molecules present in the liquid sample are forced to volatilize by adding NaOH and then captured by an acidic reagent. The method was modified as described below. Soluble ammonia was analysed by the same colorimetric method as was used for total nitrogen determination (Chaykin 1969).

Modified microdiffusion method:

An amount of 1 ml sample (supernatant) was added into a small glass container. Then 150 µl of 0.5 N HCL was added into a glass tube which had a mouth at a side wall to promote gas exchange. The tube was inserted into the small container and 0.5 ml of 12% (w/v) MgO was added to the sample to alkaline the solution. The glass container was immediately closed with a tight fitting rubber lid as well as a screw lid and kept on a shaker with low speed at maximum 150 r/min. for 24 hours. Ammonia was analysed from HCL in the center tube.

When adding MgO to the sample and removing HCL from the center tube for analysis an extreme care should be taken to avoid contamination between the two compartments. The method had been tested successfully with ammonia standard solution (100% recovery in HCL).

3.8. Statistical analysis

Samples or data collected in the field included three replications for each genotype and treatment and were used to calculate average values (e.g. soil moisture, plant samples).
Plant samples taken to the laboratory for destructive analysis included 5 or, at later stages, 10 plants (from 0.5 or 1.0 m) per replication. Each replication was considered as one sample in terms of biomass.

Statistical analysis was done with the package MSTATC© using the t-test facility for comparison of means. When genotypic differences were large as occurred for several variables, the t-test was sufficient within the genotype data sets to evaluate only the effect of the main factor. The ANOVA facility for factor analysis (randomized complete block design with main and sub factor) could not be used because the sampling dates for the three genotypes were different throughout the experiment.

Regression analysis, confidence limits and equivalence test related to soil moisture simulation was carried out with the statistical software SAS©.

The standard deviation was used to show significant differences occurring, for instance, during the postrainy season.

4. RESULTS

4.1. Water status of soil and crop

4.1.1. Soil moisture pattern during crop growth

The simulated soil moisture distribution of six soil layers reflects the well distributed rainfall during the rainy season in 1993 (**Fig. 4.1**). There was a short drought period at the end of September, when irrigation was given to the irrigated treatment plots as indicated by an arrow at the top of the graph. The postrainy season started at the end of October, and there was very little rainfall which benefitted only the upper 30 cm of the soil profile. Irrigation was applied four times to the irrigated treatment plots during this period.

The simulation model underestimated the soil moisture during the drought periods. The regression analysis showed that the simulated values were not significantly different from the observed values with a confidence of 95% for the upper two layers down to 22 cm depth (**Tab. 4.1**). For the deeper soil layers down to 75 cm depth the simulated values were significantly lower than the observed values (see **Tab. 8.1**, appendix, for precise confidence limits and equivalence test).

Table 4.1: Regression analysis between observed and simulated soil moisture at six soil layers.

Layer	Soil depth (cm)	R^2	b	SE	Confidence limits (95 %)
L1	0-10	0.929	1.043	0.036	0.970 - 1.115
L2	10-22	0.907	1.008	0.041	0.927 - 1.089
L3	22-30	0.975	0.907	0.018	0.871 - 0.944
L4	30-45	0.983	1.110	0.018	1.073 - 1.147
L5	45-60	0.988	1.089	0.016	1.059 - 1.120
L6	60-75	0.987	1.112	0.016	1.080 - 1.144

Three genotypes and two water regimes were pooled together.
R^2: regression coefficient; b: slope; SE: standard error; n = 64.
Confidence limits estimated as: b ±(2 SE).

The observed values of the deeper soil layers were located within a narrow range (**Fig. 4.1**). This may indicate that the actual moisture conditions of those layers had only little fluctuations. It may be also due to unfavourable timing of the measurements that were done at pre-determined intervals (see chapt. 3.6.2.) incidentally not covering larger fluctuation.

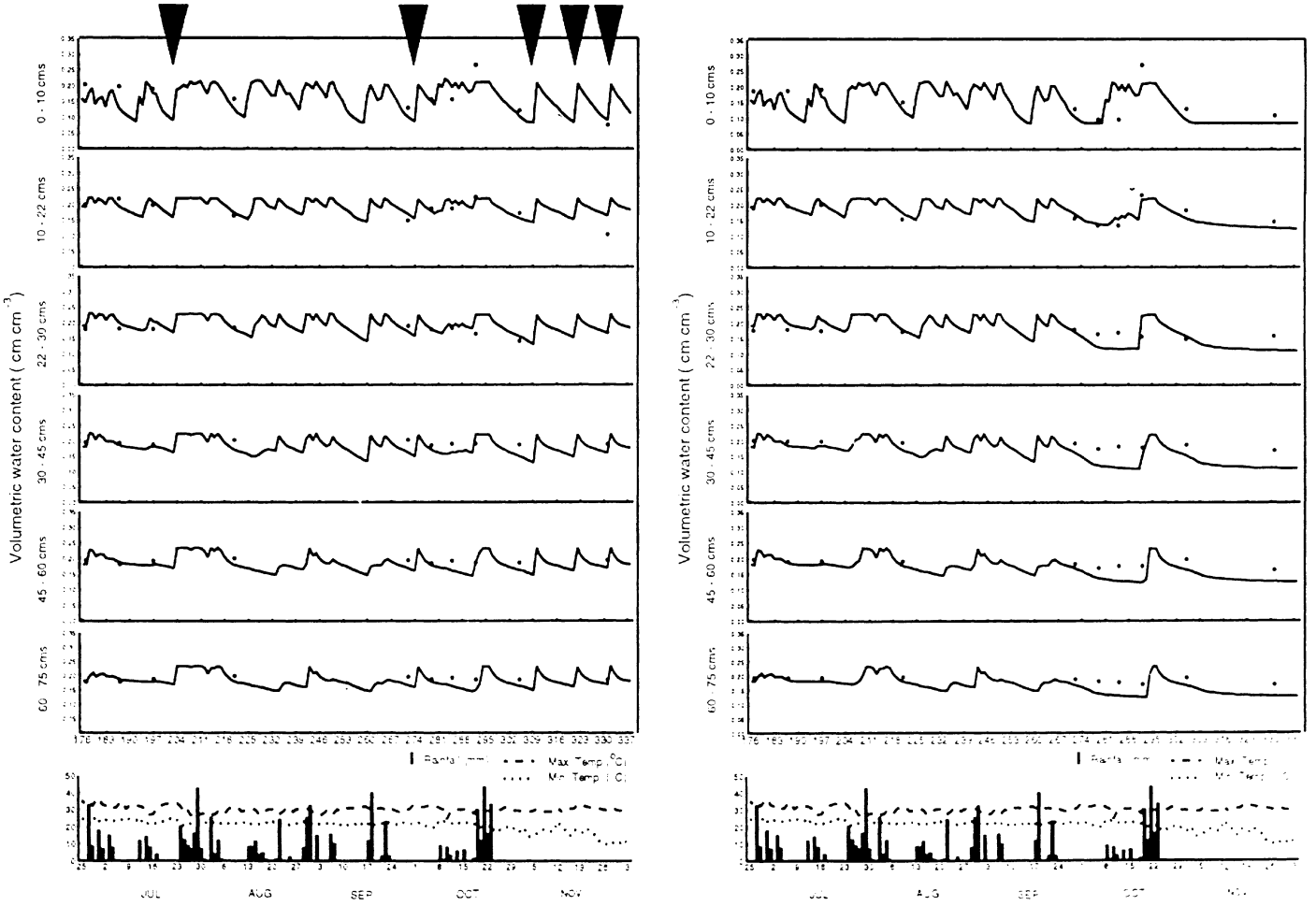


Fig. 4.1a: Rainfall pattern and temperatures during the growing season (bottom) and volumetric water content in soil at six depths measured by gravimetrical method in the top layer and by a neutron probe in the other layers (data points) and those simulated by Ritchie's water balance model (solid line), for ESD genotype in irrigated (left) and rainfed (right) plots.

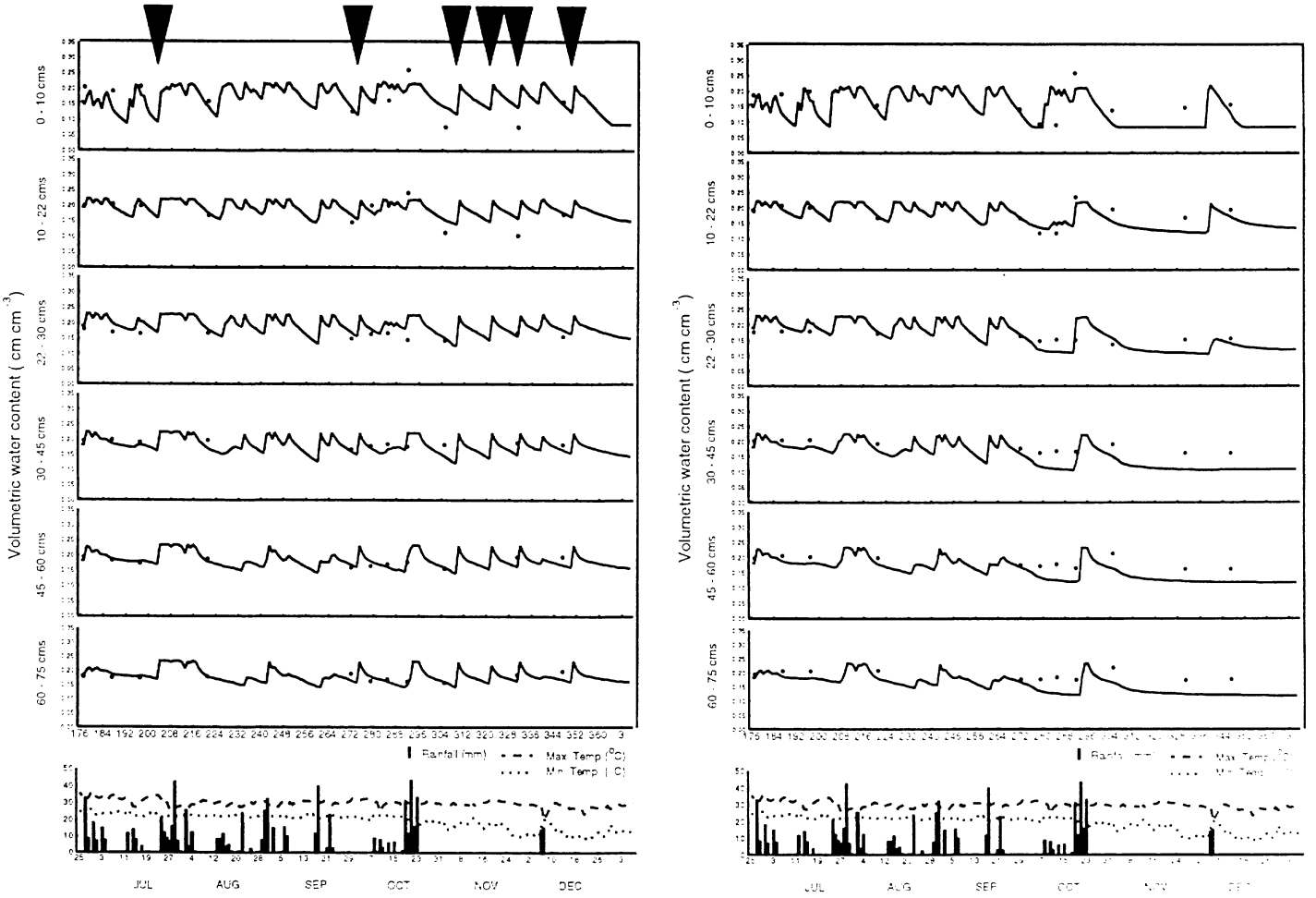


Fig. 4.1b: Rainfall pattern and temperatures during the growing season (bottom) and volumetric water content in soil at six depths measured by gravimetrical method in the top layer and by a neutron probe in the other layers (data points) and those simulated by Ritchie's water balance model (solid line) for SD genotype in irrigated (left) and rainfed (right) plots.

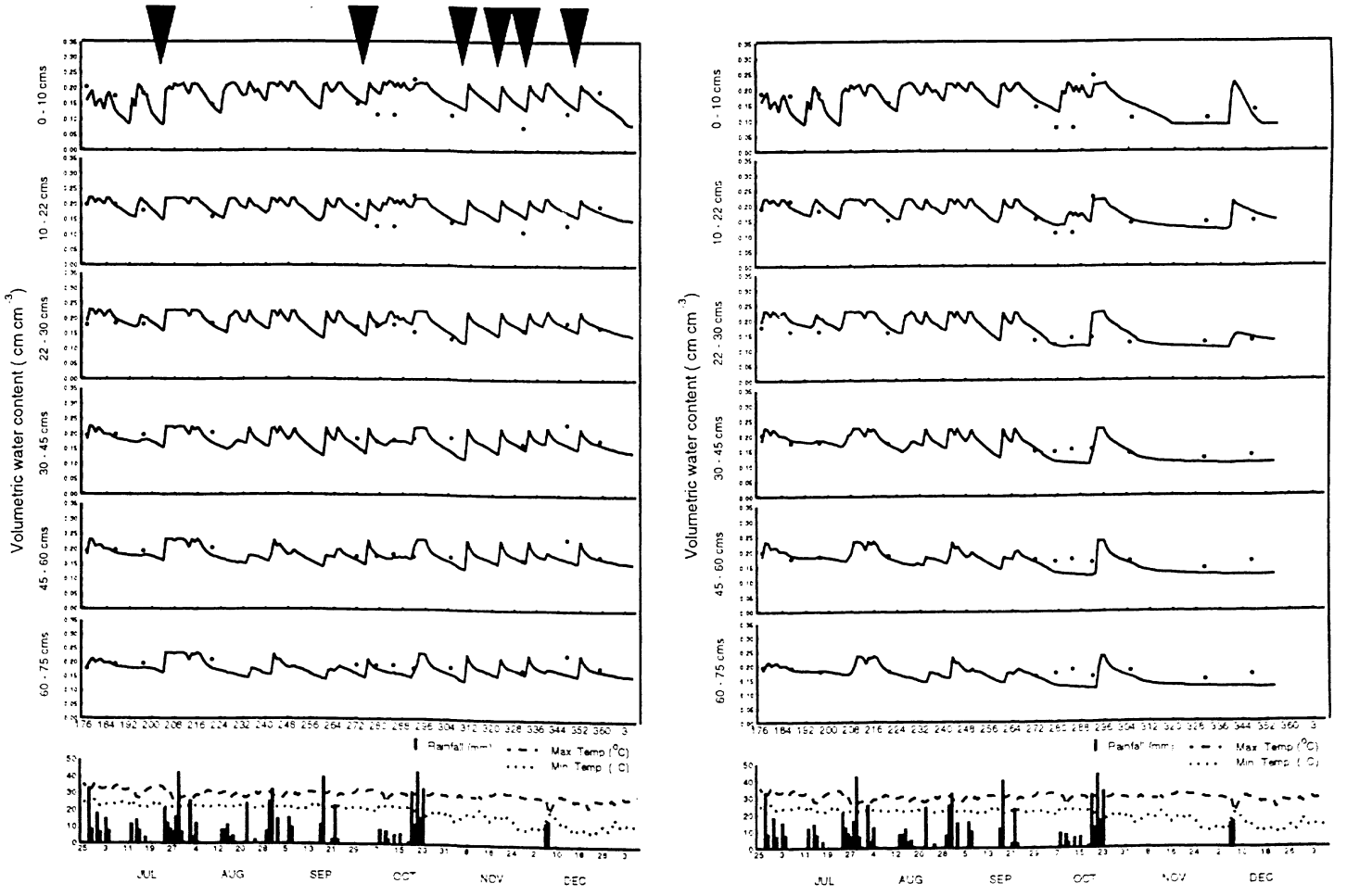


Fig. 4.1c: Rainfall pattern and temperatures during the growing season (bottom) and volumetric water content in soil at six depths measured by gravimetrical method in the top layer and by a neutron probe in the other layers (data points) and those simulated by Ritchie's water balance model (solid line) for MD genotype in irrigated (left) and rainfed (right) plots.

Plant-available soil water in the profile amounted to 9.6 percent by volume representing a water holding capacity of 86 mm (**Fig. 4.2**). Total storage water at field capacity was 168 mm. During the wet spell from 34 to 48 DAS waterlogging occurred in the irrigated plots for a few days. During dry spells plant-available water was reduced to about 23-28 % in the rainfed plots.

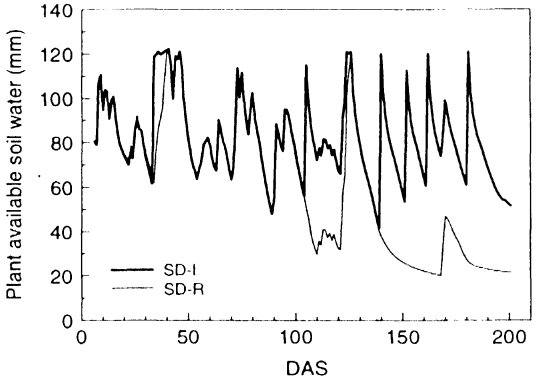


Fig. 4.2: Plant-available soil water in the profile for irrigated and rainfed plots of SD (simulated; data sets of ESD and MD see appendix **Fig. 8.1**).

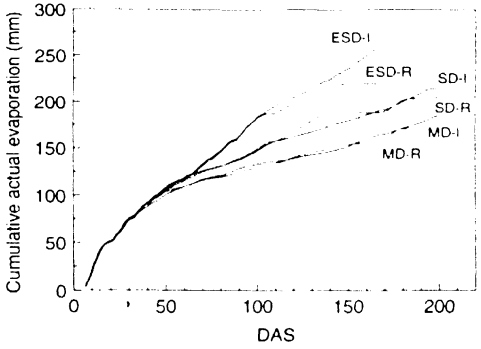
Soil evaporation was highest in ESD and lowest in MD (**Fig. 4.3**). This was probably due to the difference among the three genotypes in leaf area and in the amount of fallen leaves covering the soil surface, which both were largest in MD. Through different canopy structure as represented by branching and plant height, each genotype may create its own microclimate in the crop stand. There was no clear difference in soil evaporation between the two water regimes.

4.1.2. Water status of the crop

During initial plant growth cumulative soil evaporation was generally higher than cumulative transpiration (**Fig. 4.3, 4.4**) because only a part of the soil surface was covered by the plants. Transpiration exceeded evaporation at 65 days after sowing (DAS) in MD, at 72 DAS in SD and at 86 DAS in ESD in the irrigated treatments.

Plant transpiration obtained from simulation output was higher in irrigated plants than in rainfed plants (**Fig. 4.4**). Transpiration was remarkably reduced during the postrainy drought period in the rainfed treatments. During 142 to 168 DAS (after 2 weeks without precipitation) the daily transpiration was reduced to 41% and even down to only 5% of potential transpiration indicating that the plants experienced water stress. After two days of intermittent rain daily transpiration again was reduced to 35% and down to 2% during 172 to 200 DAS.

Among the genotypes, cumulative actual transpiration was highest in MD and lowest in ESD in both treatments throughout the experiment (**Fig. 4.4**).



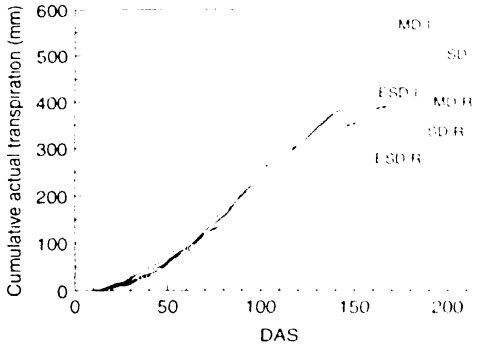


Fig. 4.3 (left): Cumulative actual evaporation in irrigated (I, full line) and rainfed (R, dotted line) plots of ESD, SD, and MD (simulated).
Fig. 4.4 (right): Cumulative actual transpiration in irrigated and rainfed plots of ESD, SD, and MD (simulated)

4.2. Phenology and growth period

4.2.1. Vegetative development

Vegetative development was investigated in terms of leaf appearance at the main axis of the plant. The number of fully expanded leaves on the main stem followed a logistic growth curve (**Fig. 4.5**). Total number of leaves developed at the main stem was significantly reduced during the postrainy season in rainfed MD for the continuously growing top canopy layer (see **Fig 8.3**, appendix, for standard deviation). The appearance of fully expanded leaves slowed down over time in the upper canopy of all genotypes.

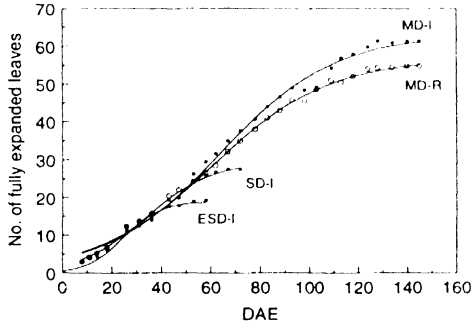


Fig. 4.5: Appearance of fully expanded leaves on the main axis representing vegetative development of irrigated plots of ESD, SD, and MD, and rainfed MD.

4.2.2. Reproductive development

The duration for harvest maturity of the three genotypes was close to the upper end of the range expected for these crops (**Tab. 4.2**). ESD and SD matured at 88 and 108 DAS. MD needed 169 days to reach maturity.

Duration for harvest maturity depends predominantly on the length of vegetative growth prior to reproductive growth. There was a large difference in duration to 50% flowering among the genotypes; 52 and 69 DAS in ESD and SD, but 118 DAS in MD. The time to 50% flowering accounted for 53% and 55% of the whole growth period in ESD and SD, and 59% and 66% in irrigated and rainfed MD, respectively. The proportion of the vegetative growth period to the whole growth period (relative growth stage) was a major difference between the short-duration genotypes and MD, which has a longer vegetative growth stage.

The duration of the reproductive phase was the longest in MD in number of calender days, whereas in growing degree days (°CD) this was relatively close among the genotypes. For setting and filling pods MD needed 51 days (1159°CD), whilst ESD and SD needed 36 days (923°CD) and 39 days (1011°CD), respectively.

Table 4.2: Days after sowing (DAS), relative growth stage (RGS), and growing degree days (°CD) to various reproductive stages on 50% basis, to harvest maturity on 75% basis.

Reproductive stage	ESD-I			SD-I			MD-I			MD-R		
	DAS	°CD	RGS	DAS	°CD	RGS	DAS	°CD	RGS	DAS	°CD	RGS
Budding	34	958	0.35	49	1336	0.39	90	2394	0.45	90	2394	0.51
Flowering	52	1417	0.53	69	1853	0.55	118	3123	0.59	118	3123	0.66
Seed develop. (start)	63	1699	0.64	80	2133	0.64	133	3497	0.67	133	3497	0.75
Physiological maturity	80	2133	0.82	98	2601	0.78	155	4004	0.78	159	4089	0.89
Harvest maturity	88	2340	0.90	108	2864	0.86	169	4282	0.85	169	4282	0.95
1. Harvest	98	2601	1.00	124	3275	1.00	200	4876	1.00	178	4466	1.00
Budding	96	2540	0.98	109	2896	0.88						
Flowering	113	2999	1.15	130	3412	1.05						
Seed develop. (start)	124	3281	1.27	142	3692	1.15						
Physiological maturity	143	3715	1.46	163	4161	1.32						
Harvest maturity	152	3923	1.55	176	4423	1.42						
2. Harvest	164	4184	1.67	198	4835	1.60						

There was no difference between irrigated and rainfed treatments in ESD and SD during the 1st flush. Values of the 2nd flush were estimated from growing degree days of the 1st flush and represent only irrigated treatments.

Since rainfall was well distributed throughout the rainy season (**Fig. 4.1**), there was no effect of drought stress on phenology (**Tab. 4.2**). In case of MD, which continued to grow even after the rainy season, a slight delay in physiological maturity was observed in the rainfed treatments but the crop was ready to be harvested about 3 weeks earlier than the irrigated plots. The 2nd flush in ESD and SD was harvested 66 and 74 days after the first harvest. Phenology observations were not recorded during the second growth period. Values for phenology during the 2nd flush were estimated from thermal time of various reproductive stages from the first growth period (**Tab. 4.2**). Therefore the estimated values have to be considered to represent the irrigated treatment.

4.3. Growth analysis

4.3.1. Leaf area index (LAI)

The maximum LAI in irrigated ESD was 3.8 at 55 DAS (0.56 RGS) during late flowering (**Fig. 4.6A**). Thereafter LAI decreased rapidly to below 2.0 at the harvest of the 1st flush. A similar pattern was observed during the 2nd flush but the peak was lower with a maximum of 3.3 at 121 DAS (1.23 RGS) again during late flowering. Regrowth in the 2nd flush was initiated by irrigation in the plots concerned and stimulated by rain in October. The LAI of irrigated SD reached a maximum of 5.0 at 81-88 DAS (0.64-0.70 RGS) which was the beginning of the seed development period. The following steep decline was quickly restored by irrigation probably due to development of new leaves on the branches. No such restoration of LAI in the 2nd flush was observed in the rainfed SD (**Fig. 4.6B**). The difference was significant for the period of 109-151 DAS (0.87-1.20 RGS) covering the end of a dry spell, a brief wet spell and reaching into the postrainy season (see **Fig. 8.2**, appendix, for standard deviation).

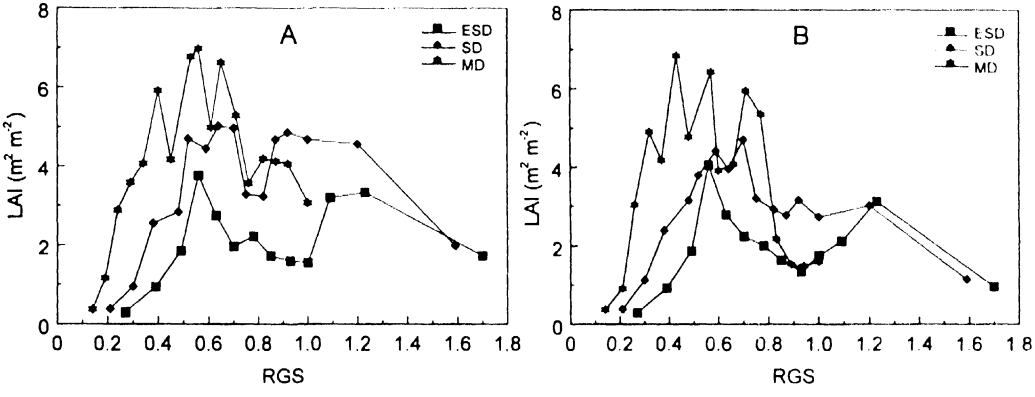


Fig. 4.6: Leaf area index of ESD, SD, and MD under irrigation (A) and under rainfed conditions (B). RGS 1.0 is the growth stage of the 1st harvest.

Results of MD also showed a difference between irrigated and rainfed treatments. Greater fluctuation was observed among the sampling points in MD than in ESD and SD. In the irrigated treatment LAI had a maximum of 7.0 at 111 DAS (0.56 RGS) which was the beginning of flowering stage (**Fig. 4.6A**). Subsequently, LAI declined to around 4.0 and showed an apparent plateau. The LAI of rainfed MD showed a similar tendency as irrigated MD with large variation among the sampling points (**Fig. 4.6.B**). There was a significant difference between irrigated and rainfed MD in LAI for the period of 152-184 DAS (0.76-0.92 RGS) shown by a sharp decline down to 1.5 in the latter (see **Fig. 8.2**, appendix, for standard deviation).

4.3.2. Dry matter partitioning

Dry matter accumulation showed similar patterns in ESD and SD but was entirely different in the MD genotype (**Fig. 4.7**). The major difference came from stem biomass accumulation and leaf shedding. There was no significant difference in dry matter allocation to various plant organs between irrigated and rainfed treatment.

4.3.2.1. Stem and Pods

With increasing length of the growing period, allocation of dry matter to the stem also increased as the stem is the main carbon storage of the plant. During the reproductive stage pods became the major sink for additional carbon in ESD, whilst in SD allocation to stem and pods was almost in balance (**Tab. 4.3**). In contrast, in MD the stem remained the major sink for carbon throughout the growth cycle.

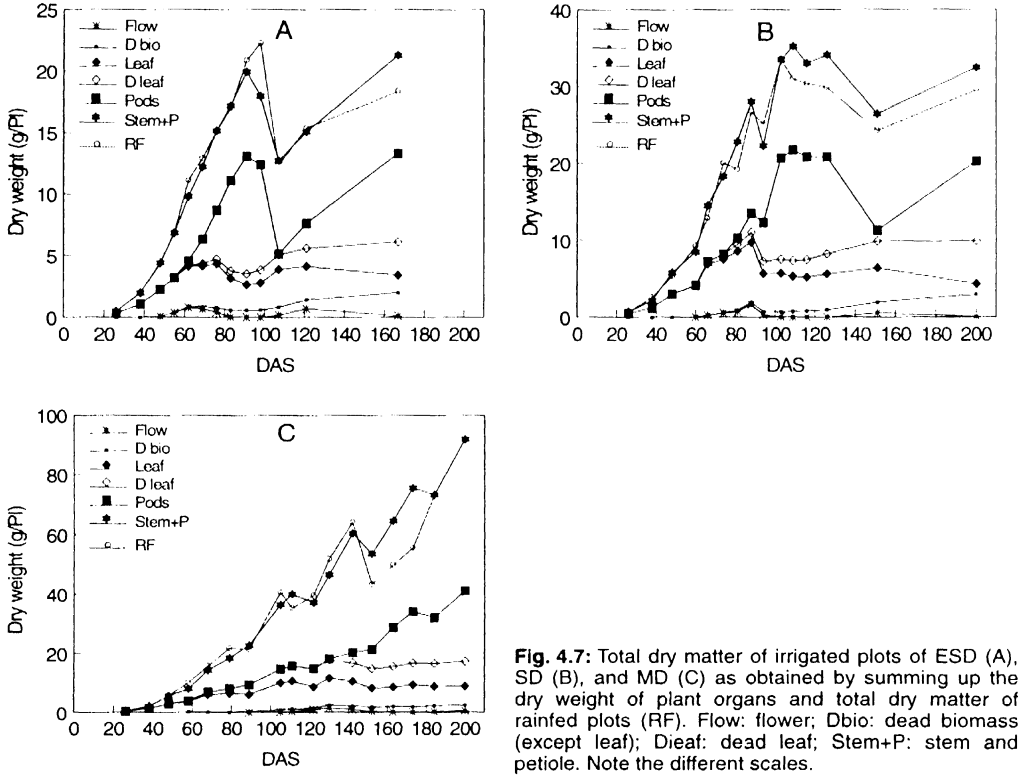


Fig. 4.7: Total dry matter of irrigated plots of ESD (A), SD (B), and MD (C) as obtained by summing up the dry weight of plant organs and total dry matter of rainfed plots (RF). Flow: flower; Dbio: dead biomass (except leaf); Dleaf: dead leaf; Stem+P: stem and petiole. Note the different scales.

In ESD and SD stem biomass remained below 40% of TDM at harvest times but dry matter allocation to pods was considerably higher than in MD. The slight increase in stem dry matter after the harvest of the 1st flush in ESD and SD might have been due to branching. In MD stem biomass increased

continuously, and the majority of TDM was allocated to the stem (**Fig. 4.7**, **Tab. 4.3**). This was due to the indeterminate and bushy growth habit of this genotype. Dry matter accumulated to increase height and diameter of the stem and number of branches. Allocation of dry matter to the pods was the highest in the 1st flush of ESD and the lowest in rainfed MD (**Tab. 4.3**). At the 2nd flush, allocation of dry matter to pods was similar in ESD and SD. There was a tendency in rainfed SD to invest more dry matter in stem than in pods during the 2nd flush which was most probably due to water deficit.

Table 4.3: Total dry matter partitioning (%) to various plant organs at harvest times.

Genotype Treatment	Stem	Petiole	Leaf	Dead Leaf	Flower	Pod	Dead Rem
1st flush							
ESD-I	29.8	1.3	12.1	6.0	0.0	47.5	3.3
ESD-R	30.5	1.1	10.2	5.3	0.0	49.6	3.3
SD-I	37.5	1.4	13.7	7.6	0.1	37.0	2.7
SD-R	38.7	1.3	12.5	9.8	0.2	34.5	3.0
MD-I	54.5	0.8	6.9	9.2	0.6	25.9	2.1
MD-R	63.9	0.4	4.0	12.1	0.3	16.6	2.8
2nd flush							
ESD-I	37.0	0.6	6.8	12.7	0.7	33.7	8.7
ESD-R	35.4	0.6	5.0	16.6	0.2	29.0	13.2
SD-I	37.1	0.4	4.2	17.3	0.3	32.0	8.7
SD-R	42.6	0.2	3.1	17.5	0.2	27.9	8.5

Dead Rem: dead petiole, flower and pod.
I: irrigated; R: rainfed.

4.3.2.2. Leaves

Partitioning of dry matter to leaves increased until early pod development and thereafter decreased in parallel with rapid pod growth (**Fig. 4.7**). At the 1st harvest, the proportion of leaf biomass was similar in ESD and SD but less in MD where dead leaves predominated (**Tab. 4.3**).

The seasonal dry matter allocation to different leaf fractions (**Fig. 4.8**) represents the dynamic structure of the plant canopy. In ESD plants the leaves located at the main stem were the major fraction during the 1st flush. After that, this fraction decreased continuously, while the dead leaf fraction increased steeply (**Fig. 4.8A**). Rapid growth of new leaves on the branches compensated for the loss of photosynthetically active leaf area on the main stem. Thus, the plants were able to restore 86% of the maximum canopy dry weight. In case of SD canopy dry weight was not restored but remained almost constant (**Fig. 4.8B**). This was due to little leaf growth on branches while leaves of the main stem were continuously shed. A second drastic leaf shedding phase occurred towards maturity of the 2nd flush. In MD there was no

steep decline in leaf biomass. Only slight changes occurred in the fraction consisting of leaves from branches whereas the main stem leaf biomass continuously declined similarly to both short-duration cultivars (**Fig. 4.8C**).

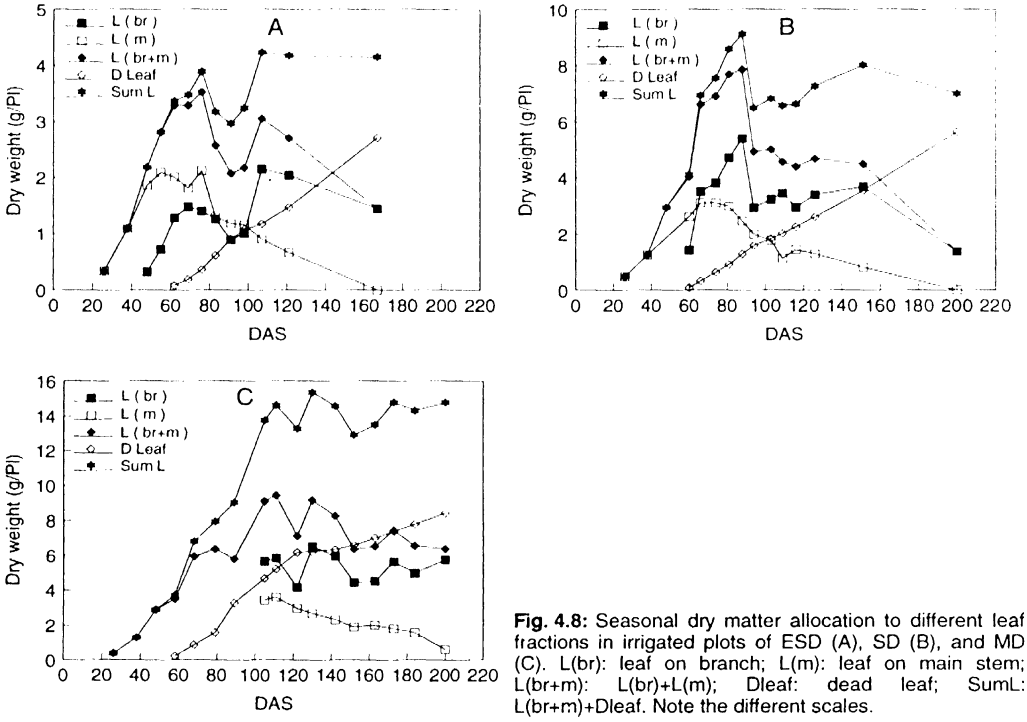


Fig. 4.8: Seasonal dry matter allocation to different leaf fractions in irrigated plots of ESD (A), SD (B), and MD (C). L(br): leaf on branch; L(m): leaf on main stem; L(br+m): L(br)+L(m); Dleaf: dead leaf; SumL: L(br+m)+Dleaf. Note the different scales.

There was a steep increase in the dead leaf fraction of MD until the start of seed development followed by a slow increase, whereas only a linear increase towards the end of the 2nd flush was observed in ESD and SD. Leaf fall started when the canopy was still in an active growing phase, which was initially compensated by new leaf development. In ESD and SD, however, shedding exceeded new leaf growth in late pod fill stage of the 1st flush (76 and 88 DAS), which is shown by the decrease in total leaf biomass (Sum L). The initial leaf fall was most likely due to senescence of the eldest leaves of the plant located at the main axis (**Figs. 4.11, 4.12**).

4.3.2.3. Abscised dry matter

The composition of abscised biomass changed with the phenological stage of the plants. Dead leaves accounted for at least 60% of the fallen biomass in ESD and SD at most of the sampling dates. Abscission of flowers increased from the beginning of seed development and accounted for 53% and 39% of total fallen biomass in ESD and SD, respectively (**Fig. 4.9**), indicating that the flowers formed

later may not be utilized for pod production. Pod abscission in ESD and SD was small during the 1st flush but became larger during development of the 2nd flush. In MD the major part of the fallen biomass consisted of dead leaves. Shedding started at 60 DAS when the plants were still at the vegetative stage. This might have been caused by the dense lower canopy due to the close spacing arrangement.

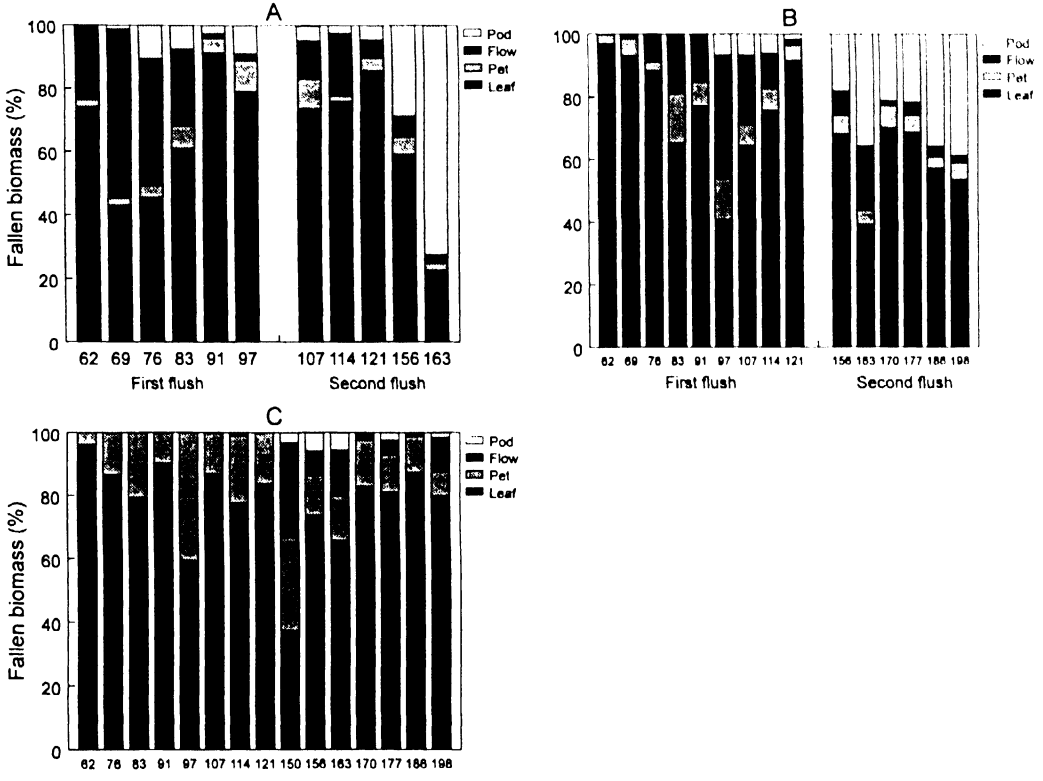


Fig. 4.9: Composition of abscised biomass during the reproductive phase in irrigated plots of ESD (A), SD (B), and MD (C). Flow: flower; Pet: petiole.

In both soil moisture treatments the total leaf biomass abscised accounted for approximately 1 and 2 t/ha in ESD and SD, respectively, which was 60-70% of total fallen biomass (**Tab. 4.4**). In MD approximately 3 t/ha leaves were shed accounting for 80-83% of total fallen biomass. Total fallen biomass was generally higher during the 2nd flush than during the 1st flush. In both periods SD contributed about twice the amount of dead biomass to the soil than ESD. MD contributed three times more than SD in the 1st flush.

Table 4.4: Abscised biomass (dry matter).

Genotype Treatment	Leaves 1st flush (kg/ha)	Leaves 2nd flush (kg/ha)	Leaves Total (t/ha)		Total biomass 1st flush (kg/ha)	Total biomass 2nd flush (kg/ha)	Total biomass Total (t/ha)	
ESD-I	358	542	0.9	± 0.07	557	954	1.5	± 0.19
ESD-R	392	624	1.0	± 0.15	633	1182	1.8	± 0.18
SD-I	866	1010	1.9	± 0.15	1172	1644	2.8	± 0.31
SD-R	974	743	1.7	± 0.04	1271	1283	2.6	± 0.18
MD-I	2807	-	2.8	± 0.07	3455	-	3.5	± 0.22
MD-R	2951	-	3.0	± 0.22	3625	-	3.6	± 0.30

I: irrigated; R: rainfed. ± : Standard deviation of means.

4.3.3. Relative crop growth

Accumulation of TDM over time was compared among the genotypes by using relative growth stage (RGS) and relative growth (dry weight at given time / maximum dry weight) to normalize the differences in crop growth behavior. The growth was simulated by applying a logistic growth equation. ESD and SD plants accumulated dry matter at almost the same rate though growth in SD exceeded that of ESD in relative terms (**Fig. 4.10**). The growth curves of ESD and SD were sigmoidal and reached a plateau at harvest time. In contrast, the growth of MD plants was much slower and almost linear from the beginning of the reproductive phase to harvest. The growth of MD shows a longer initial lag phase and a smaller slope over the linear part of biomass accumulation.

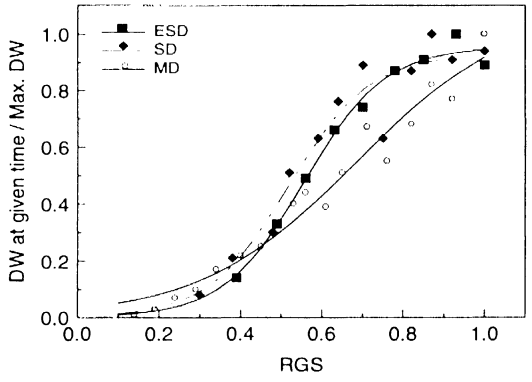


Fig. 4.10: Relative growth of ESD, SD, and MD in irrigated plots.

The short-duration genotypes finished the growth period by slower biomass accumulation towards maturity which enabled them to contribute a higher amount of nutrients and assimilates to pod filling. In MD, the process of biomass accumulation was not influenced by pod development and maturity. The linearity of the growth curve at the later growth stage suggests that pod development would proceed in MD while the plant is still in its active growth phase. This is a typical growth characteristic of perennial

plants. Due to large dry matter fluctuations in rainfed MD relative crop growth was not computed for the rainfed treatments.

4.3.4. Yield and harvest index (HI)

Grain yield obtained from defined harvest areas in the plots varied among the genotypes depending on harvest time and soil moisture treatment. Grain production exceeded 2 t/ha in the 2nd flush of ESD, in the 1st flush of SD and in irrigated MD (**Tab. 4.5**). The lowest grain yield production was obtained in the 2nd flush of rainfed SD and in rainfed MD. Both rainfed SD (2nd flush) and MD had significantly reduced grain yield, harvest index and TDM due to reduced soil moisture availability during the postrainy season (see **Tab. 8.2**, appendix). The increase in yield in the 2nd flush of ESD was most probably related to its active branching.

Yield obtained by sampling of five plants was higher than harvest yield from a greater area in all the plots (**Tab. 4.6**). There were no significant differences between irrigated and rainfed treatment on plant basis (see **Tab. 8.2**, appendix).

Table 4.5: Yield of ESD, SD, and MD in 1st and 2nd flush of irrigated and rainfed treatments.

Genotype Treatment	Grain (t/ha)	Shoot (t/ha)	TDM (t/ha)
1st flush			
ESD-I	1.6	-	-
ESD-R	1.8	-	-
SD-I	2.3	-	-
SD-R	2.5	-	-
MD-I	2.3	10.9	13.2
MD-R	1.3	8.5	9.8
2nd flush			
ESD-I	2.2	4.0	6.3
ESD-R	2.2	3.6	5.8
SD-I	1.8	5.1	6.9
SD-R	1.2	4.6	5.8

TDM: total dry matter.
I: irrigated; R: rainfed.

Table 4.6: Comparison of yield obtained by sampling on plant basis (5 plants) and on area basis (12 m²).

Genotype Treatment	Plant basis TDM (g/Pl)	Plant basis Grain (g/Pl)	Plant basis Grain (kg/12 m²)	Plant basis HI	Area basis TDM (kg/12 m²)	Area basis Grain (kg/12 m²)	Area basis HI
1st flush							
ESD-I	18.0	5.18	2.06	0.29	-	1.92	-
ESD-R	20.6	5.52	2.19	0.26	-	2.11	-
SD-I	34.1	9.18	3.64	0.27	-	2.81	-
SD-R	29.8	7.76	3.07	0.26	-	2.98	-
MD-I	91.8	15.33	6.07	0.17	15.88 *	2.80 *	0.18 *
MD-R	73.1	8.33	3.30	0.12	11.78 *	1.60 *	0.13 *
2nd flush							
ESD-I	21.2	5.06	2.00	0.24	7.50	2.65	0.35
ESD-R	18.3	3.81	1.51	0.21	6.92	2.63	0.38
SD-I	32.5	6.86	2.72	0.21	8.27 *	2.09 *	0.26 *
SD-R	29.5	5.78	2.29	0.20	6.94 *	1.47 *	0.21 *

TDM: total dry matter; HI: harvest index; Pl: plant. I: irrigated; R: rainfed.
* : significant different means between irrigated and rainfed treatment (see Tab. 8.2, appendix).

4.4. Analysis of leaf abscission

4.4.1. Time course of leaf abscission

The progress of leaf shedding was recorded by counting the scars of abscised leaflets (or the number of attached leaflets, respectively) per main stem fraction at each sampling date. Senescence of the main stem leaf started from the bottom of the plants and was predominantly a function of time. Towards the upper leaf positions leaflet abscission began later or proceeded at a slower rate (**Fig. 4.11**).

In ESD, recording of leaflet scars started at about 50% flowering (-4 day after flowering (DAF)). At that time abscission of leaflets in the lower layers L1 and L2 was 17% and 6%, respectively, while the upper layers were still intact (**Fig. 4.11A**). Abscission was most rapid in the bottom layer (L1) which was entirely abscised soon after flowering. The number of leaflets in L2 and L3 decreased continuously towards maturity with a delay in L3. Since the leaflets of L4 and the top layer (Top) had developed later, they remained attached to the petioles almost until physiological maturity of the pods (28 DAF). About 75% of the leaflets in L4 were still attached at harvest time of the 1st flush.

In SD and MD plants the lower layers L1 and L2 had already lost more than 80% and 20% or their leaflets, respectively, before flowering and were entirely abscised long before maturity (**Figs. 4.11B,C**). Differently from ESD the layer L4 was abscised up to 80% in SD and 100% in MD at maturity. This fraction might have been important for redistribution of nutrients during the pod filling stage. Abscission of the leaflets of the growing top layer in MD was also considerable at maturity.

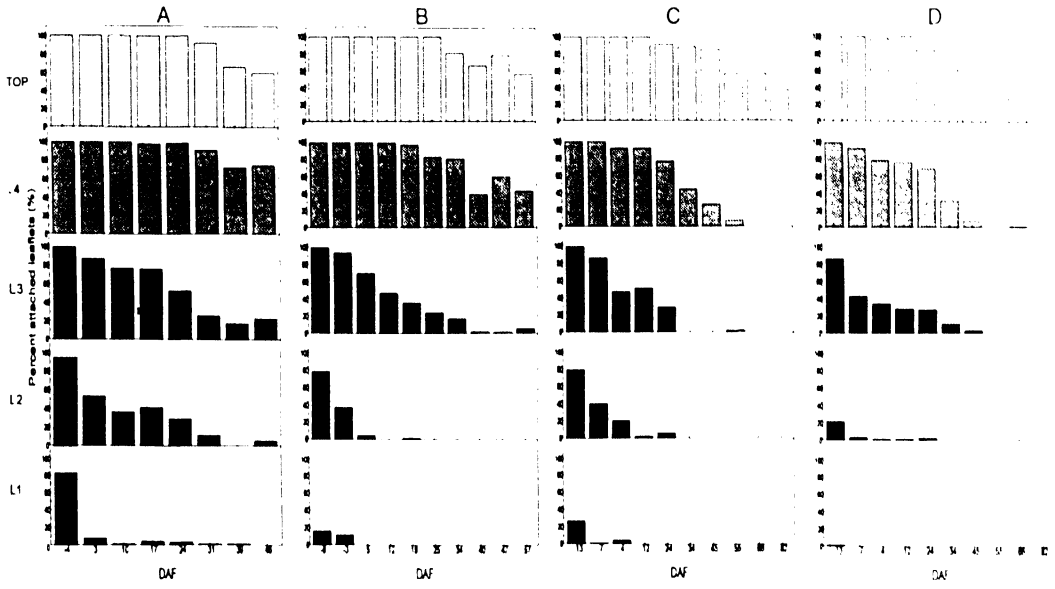


Fig. 4.11: Leaflet abscission as a function of days after flowering (DAF) in irrigated plots of ESD (A) and SD (B) and both irrigated and rainfed MD (C,D). Abscission in upper leaf layers was delayed.

4.4.2. Life span of leaves

The life span of the different leaf layers can be obtained and compared by using the age (days after full leaf expansion) of the last (youngest) developed leaf of each fraction. The proportion of attached leaflets per leaf fraction decreased linearly with time (**Fig. 4.12**) whereby most of the fittings showed statistical significance **(Tab. 4.6)**. The slope of the regression line will be correspondent to the rate of leaf abscission which was the slowest in L4 in ESD and SD. The difference in the rate of leaf abscission among the layers was the smallest in MD. The intercept of the linear regression line with the 100% and the 0% line indicates beginning and end of leaflet abscission **(Tab. 4.6)**. Abscission started at 0 to 20 days after full leaf expansion, which was earliest in L2 for all three genotypes used. Leaflet abscission continued for 50-70 days.

Later developed leaves had generally a longer lifespan than early developed leaves indicating different functions of leaves located at various positions of the main axis. In contrast, the top fraction seemed to senesce along with leaves of the mid-canopy in ESD and SD. This fraction could not be age-determined in MD due to continuous growth during the reproductive stage.

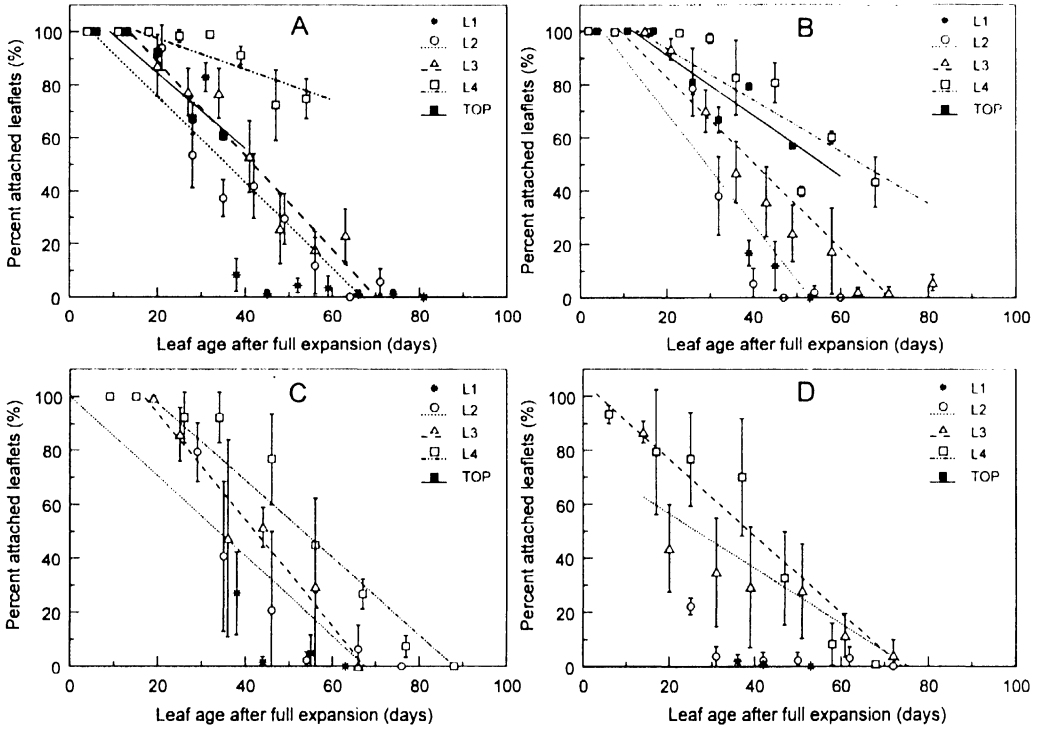


Fig. 4.12: Leaflet abscission as a function of leaf age after full expansion in irrigated plots of ESD (A), SD (B) and MD (C) and rainfed MD (D). Error bars indicate the standard deviation of means. The life span of upper leaves is longer than that of lower leaves.

Table 4.6: Regressions and start and end of leaflet abscission of different leaf fractions at the main stem in ESD, SD, and MD (see Fig. 4.12).

Leaf fraction	r^2	b	a	Leaflet abscission (days)[1] Start	End
ESD-I					
L1	-	-	-	-	-
L2	0.862**	-1.62	108	5	67
L3	0.929**	-1.77	124	14	70
L4	0.732*	-0.58	109	15	-
TOP	0.870*	-1.43	113	9	-
SD-I					
L1	-	-	-	-	-
L2	0.727	-2.09	111	5	53
L3	0.908**	-1.61	115	9	72
L4	0.779**	-0.97	113	13	-
TOP	0.772	-1.14	114	12	-
MD-I					
L1	-	-	-	-	-
L2	0.752	-1.48	100	0	68
L3	0.953**	-1.97	133	17	68
L4	0.941**	-1.43	126	19	89
TOP	-	-	-	-	-
MD-R					
L1	-	-	-	-	-
L2	-	-	-	-	-
L3	0.817**	-1.02	77	(0)	76
L4	0.940**	-1.42	105	3	74
TOP	-	-	-	-	-

[1]: days after full leaf expansion; r^2: regression coefficient; b: slope; a: intercept at Y axis. *: significant at 5%; ** significant at 1%. I: irrigated; R: rainfed.

4.4.3. Dry weight of leaf fractions

The change in dry weight of main stem leaves in different layer fractions showed that the process of senescence started in the lower layers of the plant before 50% flowering (**Fig. 4.13**). In all the genotypes the dry weight of the fractions L1 to L4 declined exponentially. But that of the top fraction followed a linear decrease in ESD and SD. In MD the continuously growing top fraction behaved very differently. Dry matter increased until harvest maturity and declined afterwards.

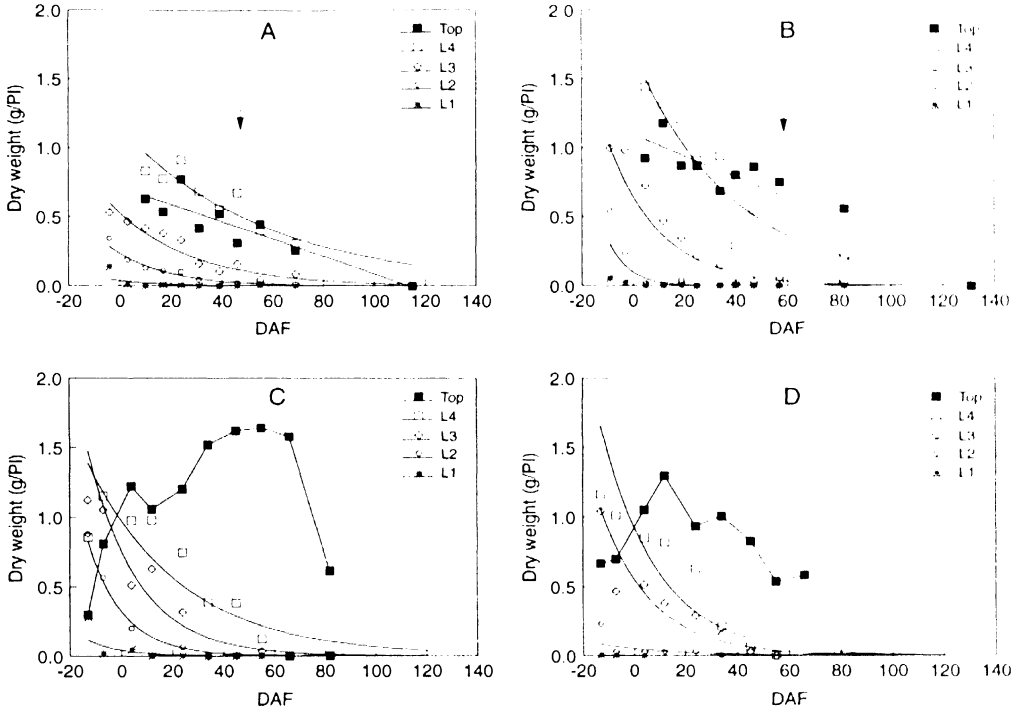


Fig. 4.13: Changes in the dry weight of five main stem leaf fractions in irrigated plots of ESD (A), SD (B), and MD (C) and rainfed MD (D). The arrow indicates the harvest of the first flush.

4.5. Translocation of C from leaves to pods

4.5.1. Translocated carbon

Dry weight loss from the canopy biomass is due to CO_2 respiration, carbohydrate translocation and leaf shedding. The translocation of carbon from leaves to pods can be estimated for time intervals when TDM (including fallen biomass) and pod dry matter increase but leaf dry matter decreases.

Translocation of carbohydrates from leaves to pods was highest in irrigated SD and reached 2.54 g/Pl between 88-109 DAS (**Tab. 4.7, see Figs. 4.7, 4.8**). This was 21.3% of the carbon accumulation in the

pods during that period. Among the short-duration types rainfed ESD translocated the smallest amount, which was 0.77 g/Pl and only 9.2 % of pod dry weight increase at 69-91 DAS. In case of MD it was difficult to obtain a clear picture of the translocation because of the fluctuation of dry matter particularly in rainfed MD. Therefore, translocation was estimated only for a time span of 10 days (130-140 DAS). Irrigated MD plants translocated 0.76 g/Pl, which was 24.6% of pod dry weight accumulation and comparable to that of irrigated SD. Translocation in rainfed MD was the lowest of all the treatments with 0.47 g/Pl or 7.4%.

The translocation rate of carbon was the highest in SD plants. Among the water regime treatments the translocation rate was clearly higher in irrigated plants of ESD and MD, but was the reverse in SD plants.

Table 4.7: Translocation of carbon from canopy leaves to pods during the 1st flush.

Genotype Treatment	Time period (DAS)	Trans-located C (g/Pl)	Translocation rate (mg/Pl*d)	Portion in pod C (%)
ESD-I	76-91	0.93	62	16.7
ESD-R	69-91	0.77	35	9.2
SD-I	88-109	2.54	82	21.3
SD-R	88-109	1.47	98	15.4
MD-I	130-142	0.76	63	24.6
MD-R	130-142	0.47	39	7.4

I: irrigated; R: rainfed.

4.5.2. Loss of leaflet dry weight

Average leaflet dry weight for each main stem fraction was calculated by dividing dry weight by number of leaflets (**Fig. 4.14**). Leaflet dry weight was generally constant in ESD, but there was a clear decline in L4 between 76 and 91 DAS. This indicates a loss of mainly carbohydrates. The leaflet dry weight is also an indication of the leaf size. Bottom leaves are smaller than those of the upper canopy. In SD there was a continuous change in the leaflet dry weight of the layers L3, L4 and Top. The decline pooled in a minimum at harvest maturity. In MD, the top leaf layer had less dry weight than the lower layers L3 and L4. There was a continuous decline in the dry weight of L4 and Top.

The time span of the translocation in irrigated ESD plants matches the decline in dry weight of leaflets in the main stem fraction L4 (see **Tab. 4.7, Fig. 4.14A**). Therefore, the leaf fraction L4 seems to be the major source of carbon translocation to the pods among the investigated main stem leaf fractions in ESD. In contrast, the upper three layers were involved in C translocation in SD plants and the L4 and Top layer in MD.

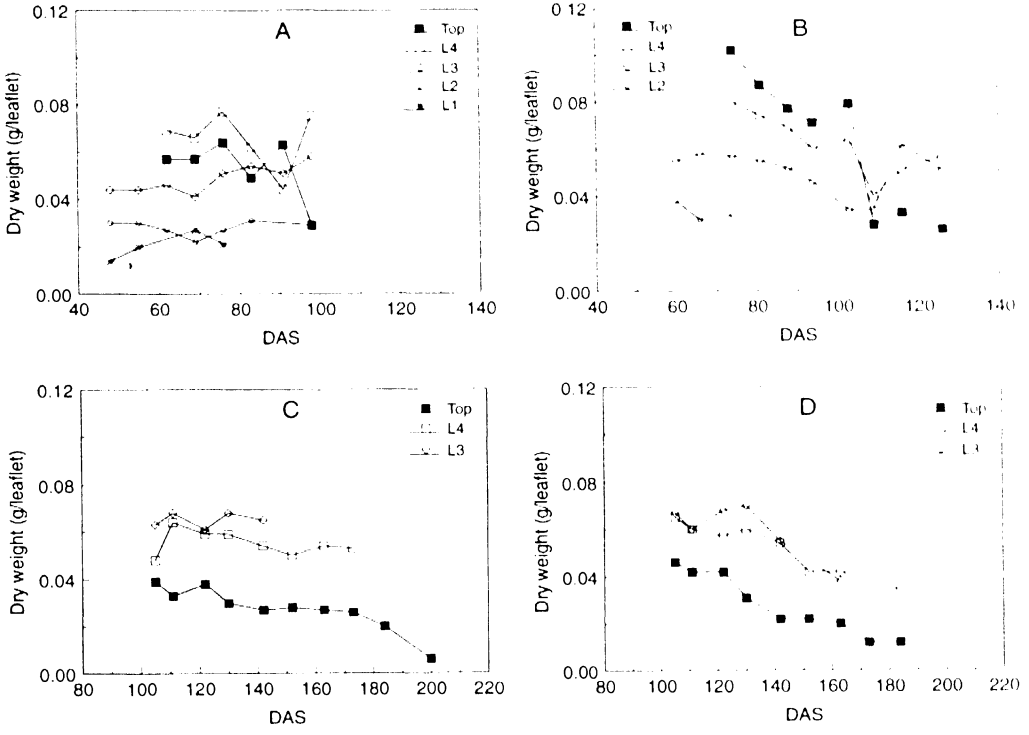


Fig. 4.14: Leaflet dry weight of five leaf fractions of the main stem in irrigated plots of ESD (A), SD (B), and MD (C) and rainfed MD (D).

4.6. Nitrogen partitioning

4.6.1. Nitrogen content

Distribution of nitrogen in the plant was obtained by estimation of the nitrogen content of various plant organs. Though nitrogen accumulation of the organs was closely related to their dry matter accumulation, there was a considerable difference between the two especially in leaves and stem (see **Figs. 4.7, 4.15**).

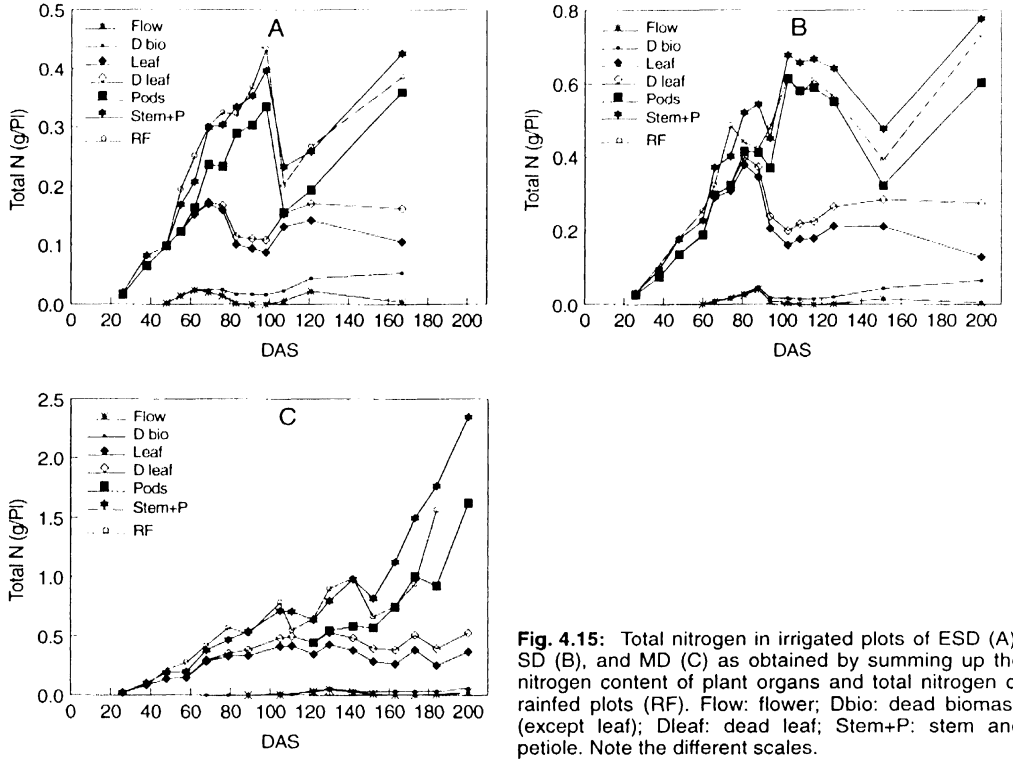


Fig. 4.15: Total nitrogen in irrigated plots of ESD (A), SD (B), and MD (C) as obtained by summing up the nitrogen content of plant organs and total nitrogen of rainfed plots (RF). Flow: flower; Dbio: dead biomass (except leaf); Dleaf: dead leaf; Stem+P: stem and petiole. Note the different scales.

4.6.1.1. Leaves and pods

Nitrogen accumulation in photosynthetically active leaves reached a maximum at early pod filling stage and decreased sharply thereafter in ESD and SD plants (**Fig. 4.16A,B**). In contrast, pod nitrogen increased steeply during that time and reached its maximum when leaf nitrogen was at a minimum. This was at harvest time for ESD and before harvest for SD. Maximum accumulation of nitrogen in leaves and pods was 0.15 g/Pl and 0.23 g/Pl in ESD, and 0.35 g/Pl and 0.42 g/Pl in SD, respectively. Irrigated MD plants accumulated nitrogen to a maximum of 0.4 g/Pl in leaves during flowering stage and 1.15 g/Pl in pods (**Fig. 4.16C**). The amount of leaf nitrogen decreased steadily when pods were accumulating it rapidly.

Allocation of nitrogen to the pods was the highest in ESD with 57 % and the lowest in rainfed MD with only 17.3 % (**Tab. 4.8**). In the other treatments nitrogen allocation to pods varied between 40-48 %. N proportion of active leaves at the 1st flush was relatively high in SD compared to ESD indicating that N partitioning was more focussed on pod support in ESD. In rainfed MD, distribution of nitrogen to various plant organs at harvest was very similar to dry matter allocation (**Tables 4.3, 4.8**). This was different in irrigated MD. A higher percentage of nitrogen was allocated to the pods and a lower to the stem, contrary to dry matter allocation.

Table 4.8: Nitrogen partitioning (%) to various plant organs at harvest times.

Genotype Treatment	Stem	Petiole	Leaf	Dead Leaf	Flower	Pod	Dead Rem
1st flush							
ESD-I	14.9	0.8	18.0	5.2	0.0	57.0	4.1
ESD-R	13.8	0.8	19.0	5.4	0.0	56.3	4.7
SD-I	13.2	1.1	29.7	8.3	0.3	44.5	2.9
SD-R	13.2	0.9	23.5	10.8	0.4	48.4	3.0
MD-I	30.3	0.6	13.1	6.9	1.1	46.6	1.3
MD-R	65.0	0.4	4.5	10.7	0.3	17.3	1.7
2nd flush							
ESD-I	14.9	0.6	12.3	13.4	0.9	46.5	11.5
ESD-R	17.6	0.5	8.1	17.6	0.3	40.5	15.5
SD-I	21.9	0.4	8.2	18.9	0.7	42.4	7.6
SD-R	24.6	0.3	6.4	16.1	0.4	43.8	8.4

Dead Rem: dead petiole, flower and pod.
I: irrigated; R: rainfed.

4.6.1.2. Stem

Stems accumulated generally less nitrogen than leaves except in MD towards harvest maturity (**Figs. 4.15, 4.16**). The proportion of N allocated to the stem was almost equal in ESD and SD at the 1st harvest but much higher in MD particularly in the rainfed treatment (**Tab. 4.8**). In SD, the N proportion clearly increased during the 2nd flush in both moisture regimes. Stems and petioles of ESD and SD seemed to translocate nitrogen during pod filling stage but started to restore the nitrogen level even before harvest (**Fig. 4.16**). These changes probably occurred also during the 2nd flush but were not recorded due to less frequent sampling.

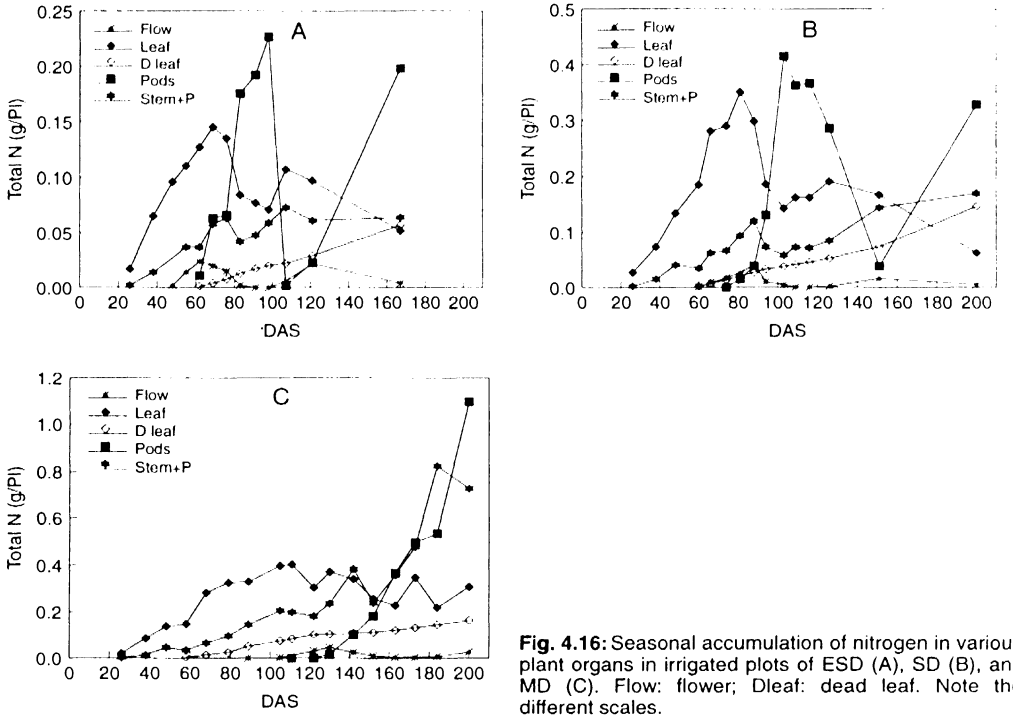


Fig. 4.16: Seasonal accumulation of nitrogen in various plant organs in irrigated plots of ESD (A), SD (B), and MD (C). Flow: flower; Dleaf: dead leaf. Note the different scales.

4.6.2.3. Abscised biomass

The amount of nitrogen in abscised dry matter was obtained separately from two fractions; dead leaf and abscised remaining plant parts (rem) such as petioles, flowers and pods. The percentage of nitrogen in dead leaf of total fallen material was different in the three genotypes (**Fig. 4.17**). This might have been caused mainly by the difference in dry matter accumulated. The percentage was about 56 % in ESD at the beginning of seed development but decreased during the pod filling stage of the 1st flush. During this period the fallen material contained a large amount of abscised flowers (see **Fig. 4.9**). In the case of SD the percentage of nitrogen in dead leaf decreased steadily and remained constant during the 2nd flush at about 70 % of total fallen biomass N. There was a similar pattern in MD but leaf nitrogen accounted for about 80 %.

The total amount of N in abscised material was about double during the 2nd flush compared to the 1st flush except in rainfed SD. Fallen biomass of SD was richer in N content during the 2nd flush than during the 1st flush (**Tab. 4.9,** see **Tab. 4.4**). Irrigated SD and both MD treatments provided more than 60 kg N/ha to the soil; out of that at least 50 kg N/ha came from leaves. In MD there was no difference in nitrogen addition to the soil between the irrigated and the rainfed treatment because rainfed MD lost a higher percentage of its biomass as dead biomass even though it produced less biomass in total.

Table 4.9: Total nitrogen content of abscised biomass

Genotype Treatment	Leaves 1st flush (kg/ha)	Leaves 2nd flush (kg/ha)	Leaves Total (kg/ha)	Total biomass 1st flush (kg/ha)	Total biomass 2nd flush (kg/ha)	Total biomass Total (kg/ha)
ESD-I	6.8	12.2	19.0 ± 2.0	12.0	23.0	35.0 ± 5.3
ESD-R	7.8	14.8	22.6 ± 5.0	14.7	28.0	42.7 ± 5.0
SD-I	17.7	31.2	48.9 ± 5.6	24.0	44.6	68.6 ± 8.6
SD-R	20.3	19.1	39.4 ± 2.0	25.8	34.0	59.8 ± 3.6
MD-I	54.0	-	54.0 ± 1.0	64.3	-	64.3 ± 1.7
MD-R	56.0	-	56.0 ± 6.3	64.5	-	64.5 ± 9.9

I: irrigated; R: rainfed. ±: Standard deviation of means.

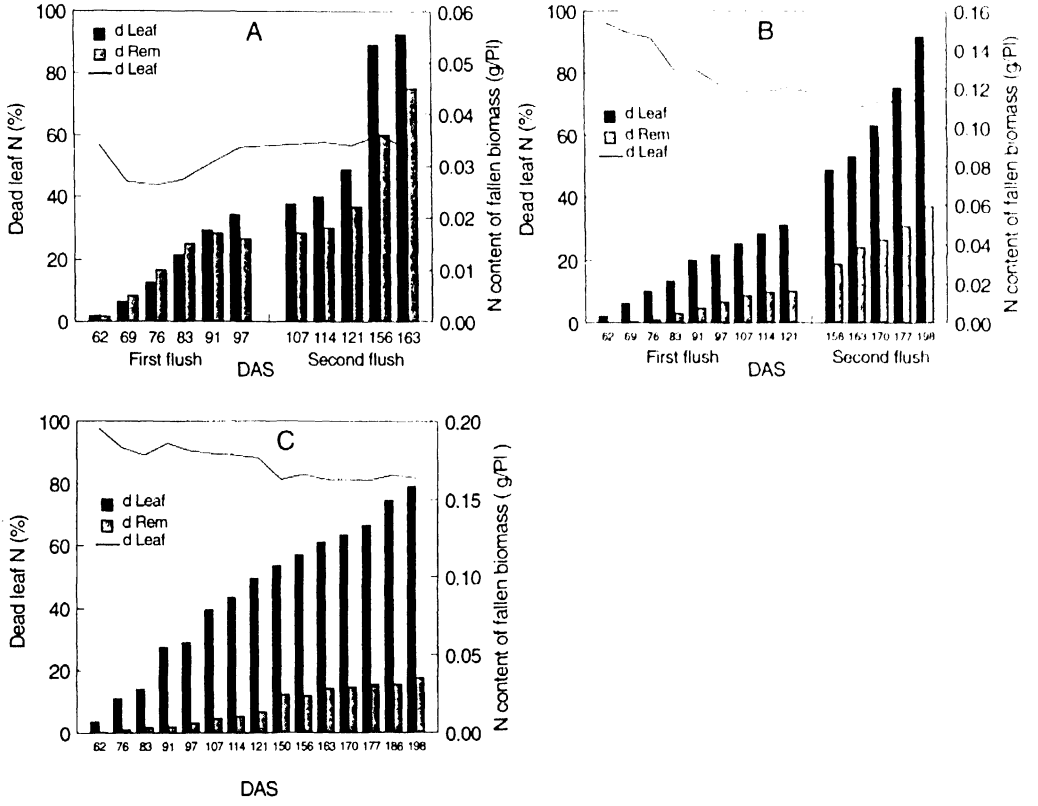


Fig. 4.17: Cumulative N content of fallen biomass components in irrigated plots of ESD (A), SD (B), and MD (C) as g/plant and as percentage dead leaf N in total fallen biomass N. dLeaf: dead leaf, dRem: dead petiole, flower and pod.

4.6.2. Nitrogen concentration of various plant organs

The total nitrogen (TN) concentration of stem, petioles, and leaves of the three genotypes increased until 40 DAS followed by a sharp decline between 40-60 DAS (**Fig. 4.18**). Since dry matter accumulation during initial plant growth is a slow process, especially in pigeonpea, less dilution of nitrogen must keep TN concentration at a higher level. The subsequent decline in TN concentration occurred parallel with an accelerated dry matter accumulation (see **Figs. 4.7, 4.10**) indicating that dry matter accumulation exceeded nitrogen uptake rate.

The TN concentration of the leaves of ESD and SD showed a clear decline during pod filling stage of the 1st flush and was lowest at harvest maturity at about 3.0 % N (**Figs. 4.18,** see **4.19**). During the pod filling stage of the 1st flush there was no increase in leaf dry matter (see **Fig. 4.8A,B**), which means that a certain amount of nitrogen might have been removed from the leaves. During the 2nd flush no decline in the TN concentration was observed. This might be due to less frequent sampling. After harvest maturity of the 1st flush the TN concentration increased again, probably because of the growth of young leaves, and reached a plateau at about 3.5 to 4.0 % N. In MD plants there was a sharp decline in the TN concentration of leaves between budding stage and 50 % flowering (**Fig. 4.18C,D**). This decline could be related to an increase in leaf dry matter on branches and/or main stem (see **Fig. 4.8C**).

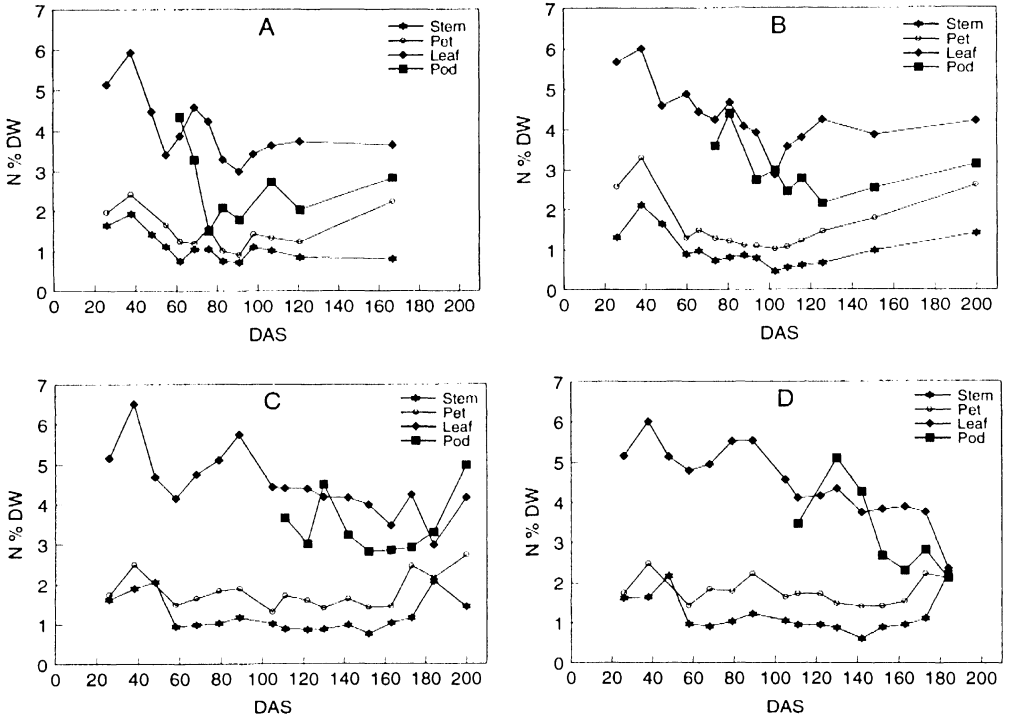


Fig. 4.18: Seasonal changes of total N concentration (dry weight basis) of various plant organs in irrigated plots of ESD (A), SD (B), MD (C), and rainfed MD (D). Pet: petiole.

The TN concentration of stem and petioles was lower than that of leaves. In ESD plants it decreased similarly to that of the leaves (**Fig. 4.18A**) during late pod-fill stage (76-98 DAS). There was also a slight decrease in the TN concentration in SD until harvest maturity (108 DAS) followed by a steady increase during the 2nd flush (**Fig. 4.18B**). This indicates that nitrogen was continuously accumulating in the stem though dry matter did not increase any more (**Fig. 4.7B**). ESD plants increased dry matter during the 2nd flush due to branching so that the TN concentration decreased slightly and the TN accumulation remained almost constant (see **Figs. 4.7A, 4.18A, 4.15A**). There was no apparent pattern of the TN concentration in MD but there was an increase towards harvest (**Figs. 4.18C,D**).

The TN concentration of the pods decreased steeply in the three genotypes due to rapid pod growth.

4.6.3. Nitrogen use efficiency (NUE)

NUE at harvest of the 1st flush was more than 50 g/gN in both SD treatments and in rainfed ESD (**Tab. 4.10**). During the 2nd flush NUE was generally lower except in irrigated ESD. Lowest NUE was obtained in irrigated MD.

Table 4.10: Nitrogen use efficiency (NUE) at harvest times.

	1st flush		2nd flush		1st flush	2nd flush
Genotype Treatment	TDM (g/Pl)	TN (g/Pl)	TDM (g/Pl)	TN (g/Pl)	NUE (gDW/gN)	NUE (gDW/gN)
ESD-I	18.0	0.40	11.9	0.26	45.3	46.3
ESD-R	20.6	0.43	7.1	0.20	47.9	36.2
SD-I	34.1	0.64	11.0	0.42	53.2	26.1
SD-R	29.8	0.56	10.0	0.44	53.2	22.5
MD-I	91.8	2.35	-	-	39.1	-
MD-R	73.1	1.57	-	-	46.6	-

I: irrigated; R: rainfed; TDM: total dry matter; TN: total nitrogen.

4.7. Analysis of leaf fractions for nitrogen

4.7.1. Total nitrogen

The main stem leaves in L4, Top and branches showed similar patterns in the change of TN concentration (**Fig. 4.19A,B**). The leaves in the fractions L1 to L3 lost 1-2 % N during flowering and then either abscised (L1 in ESD, L2 in SD) or maintained TN concentration at 2.5-3.0 % N. This seems to be the lower limit of TN for attached leaves. The TN concentration of fallen leaves generally remained below this range. In MD the TN concentration of leaves on the main stem declined slightly but continuously throughout the reproductive stage (**Fig. 4.19C,D**). There was no clear pattern in the lower leaf fractions.

The TN concentration of fallen leaves remained below that of attached leaves but increased steadily towards harvest maturity. This suggests that nitrogen remobilization from leaves which were abscised at the later stage was much less than from those abscised earlier.

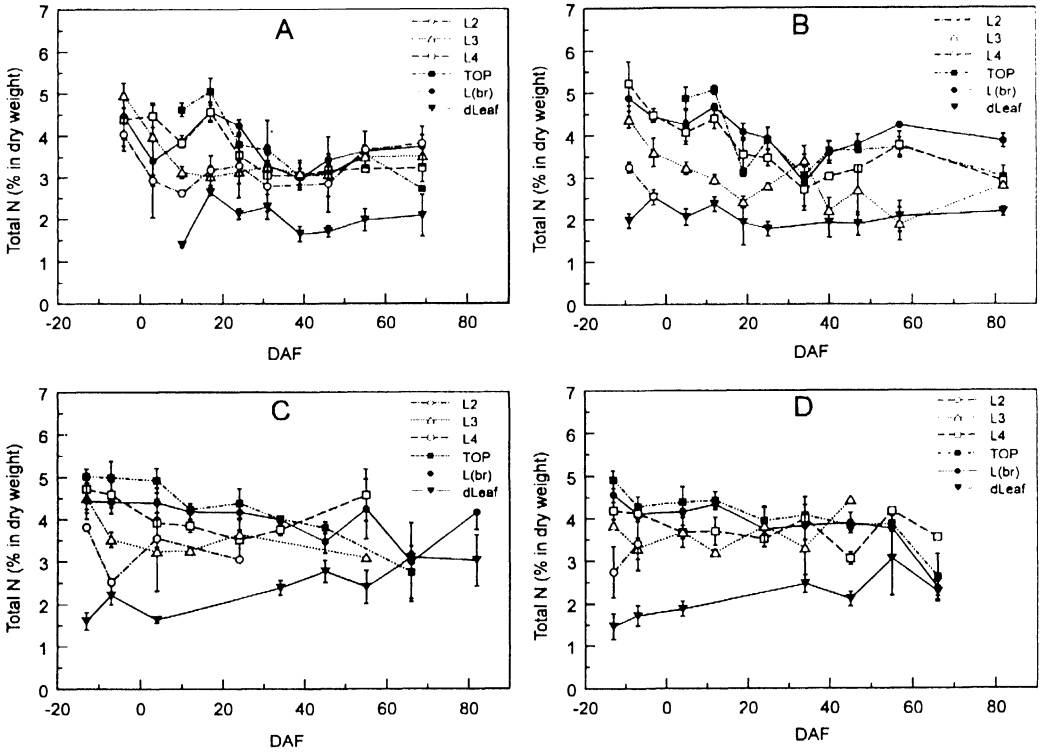


Fig. 4.19: Seasonal changes of total N concentration (dry weight basis) of different leaf fractions in irrigated plots of ESD (A), SD (B), MD (C), and rainfed MD (D). Error bars indicate the standard deviation of means. L (br): leaf on branch; Dleaf: dead leaf.

The amount of total nitrogen decreased in all main stem leaf fractions of the three genotypes. In ESD and SD the amount of nitrogen decreased exponentially in the fractions L1 to L4 but linearly in the top fraction (**Fig. 4.20A,B**). Nitrogen content of the top fraction in MD increased until about 60 DAF when irrigated and until 17 DAF when rainfed (**Fig. 4.20C,D**).

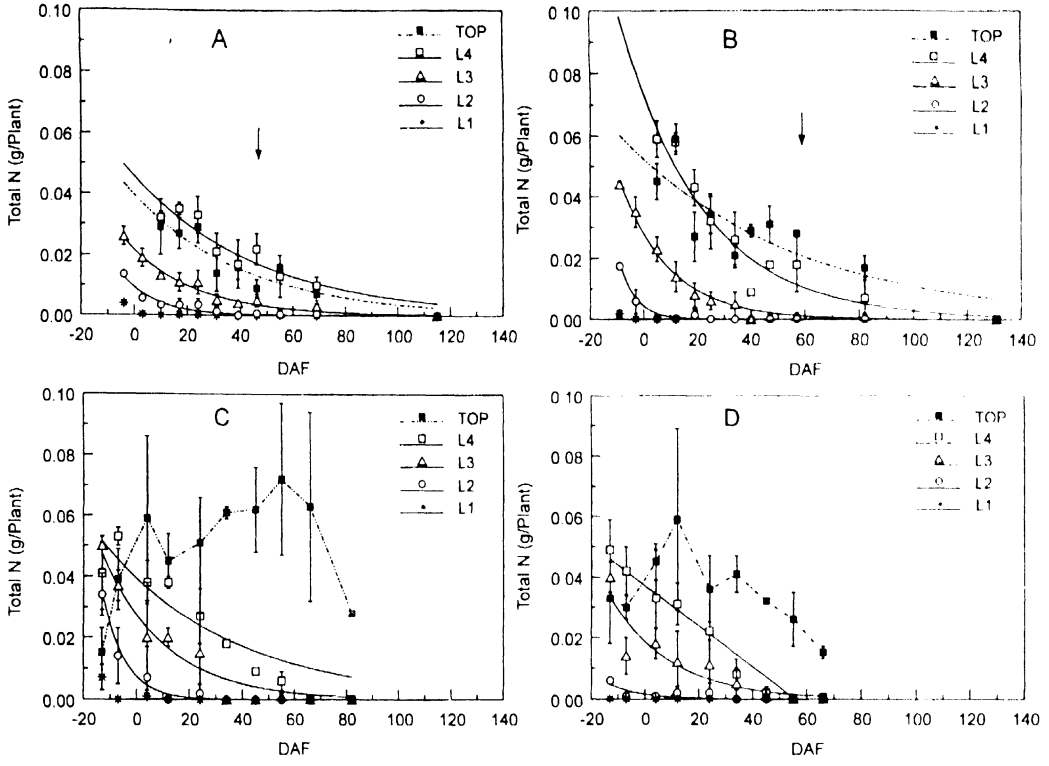


Fig. 4.20: Changes in the nitrogen content of five main stem fractions in irrigated plots of ESD (A), SD (B), MD (C), and rainfed MD (D). The arrow indicates the harvest of the first flush. Error bars indicate the standard deviation of means.

Table 4.11: Regressions for the changes in TN content of leaf fractions at the main stem in ESD, SD, and MD (see Fig. 4.20).

Leaf fraction	r^2	b	a	Leaf fraction	r^2	b	a
ESD-I				**MD-I**			
L1	-	-	-	L1	-	-	-
L2	0.937**	-0.08	0.01	L2	0.980**	-0.12	0.01
L3	0.971**	-0.04	0.02	L3	0.965**	-0.04	0.03
L4	0.893**	-0.02	0.05	L4	0.891**	-0.02	0.04
TOP	0.852**	-0.02	0.04	TOP	-	-	-
SD-I				**MD-R**			
L1	-	-	-	L1	-	-	-
L2	0.984**	-0.16	0.00	L2	0.675	-0.08	0.00
L3	0.990**	-0.05	0.03	L3	0.835**	-0.05	0.02
L4	0.933**	-0.03	0.07	L4	0.951**	-0.00	0.04
TOP	0.722**	0.02	0.05	TOP	-	-	-

r^2: regression coefficient; b: slope; a: intercept at Y axis. *: significant at 5%; ** significant at 1%.

The regression data for almost all fittings were highly significant as shown in **table 4.11** and allowed further calculation of the loss of nitrogen from the leaf fractions in terms of half-life (**Tab. 4.12**). Half-life of leaf nitrogen generally increased in the upper canopy of the three genotypes. Leaf nitrogen of the different fractions decreased with similar half-life values in ESD and MD. In contrast, half-life in the respective SD fractions was clearly less indicating faster remobilization and transport out of the leaves.

Table 4.12: Half-life of total nitrogen in leaf fractions at the main stem in irrigated ESD, SD, and MD during senescence.

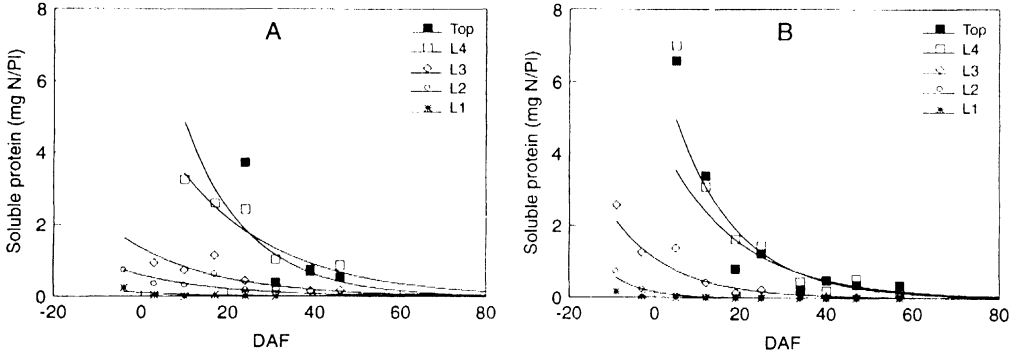
Leaf fraction	ESD (days)	SD (days)	MD (days)
L1	-	-	-
L2	8.8	4.2	5.8
L3	17.6	12.9	16.4
L4	32.8	21.3	33.6
TOP	29.1	44.4	-

Half-life was calculated from the exponential fit shown in Table 4.11 (Fig. 4.20).

4.7.2. Nitrogenous compounds in leaves

4.7.2.1. Soluble protein N

Soluble protein N content of main stem leaf fractions decreased exponentially except in the case of irrigated MD (**Fig. 4.21**). Leaf fractions of the lower canopy contained less soluble protein N than upper fractions. L4 and Top showed similar values in each genotype except rainfed MD. In irrigated MD an increase in soluble protein N was recorded in L4 and the top fraction prior to a rapid decrease. This would be mainly due to the formation of young leaf material.



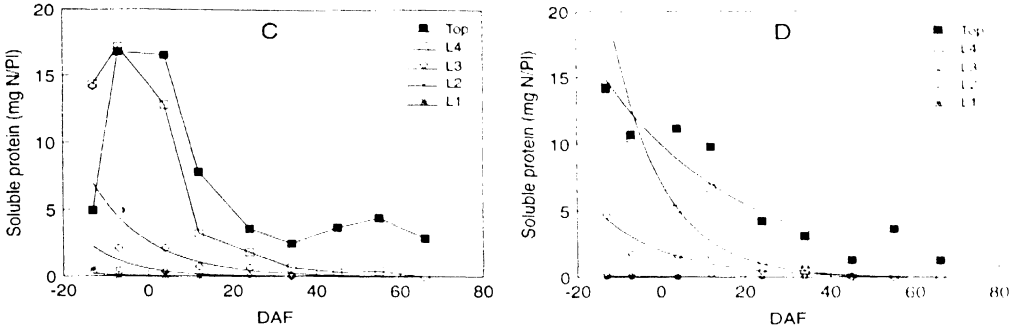


Fig. 4.21: Soluble protein in main stem leaf fractions in irrigated plots of ESD (A), SD (B), and MD (C), and rainfed MD (D). Note the different scales.

4.7.2.2. Amino acid N

Amino acid N content of main stem leaves decreased exponentially in L1 to L3 (**Fig. 4.22**). The fractions L4 and Top showed different patterns. In those fractions amino acid N decreased exponentially with a low slope in ESD and in SD. There was an increase in amino acid N in L4 and the top fraction after physiological maturity of the grain in SD. In irrigated MD the amino acid N content increased steadily in the top fraction. In contrast, there was no increase in rainfed MD.

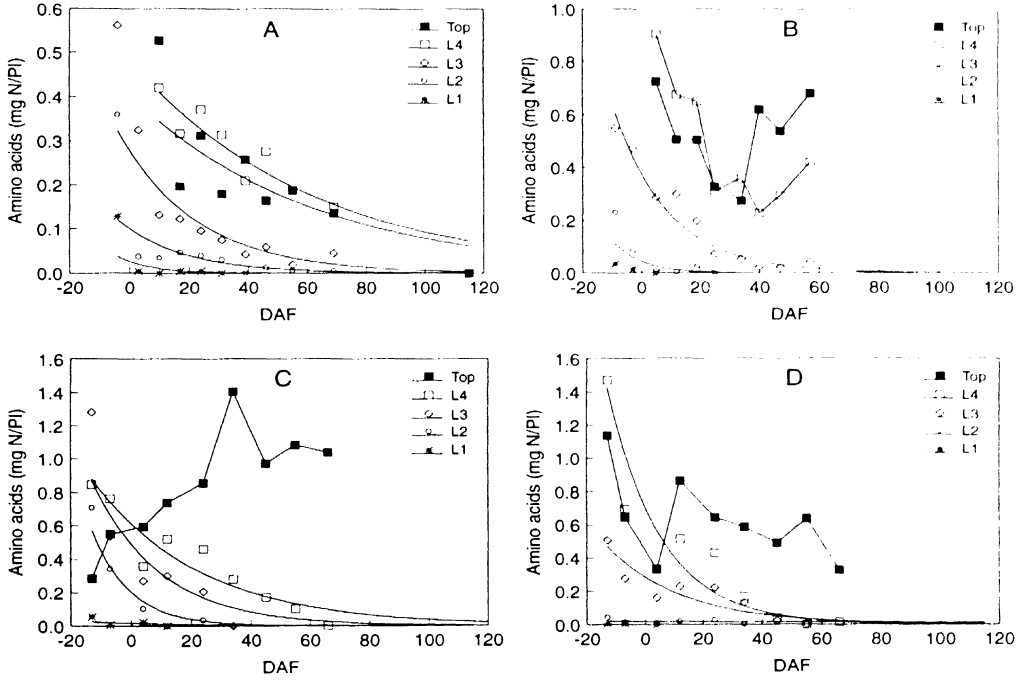


Fig. 4.22: Changes in amino acid N content of five main stem leaf fractions in irrigated plots of ESD (A), SD (B), MD (D), and rainfed MD (D). Note the different scales.

4.7.2.3. Soluble ammonia N

The amount of ammonia N in each leaf fraction declined in the three genotypes after the onset of flowering except in the top fraction of MD (**Fig. 4.23**). ESD tissue contained much higher concentrations until early seed development than SD and MD.

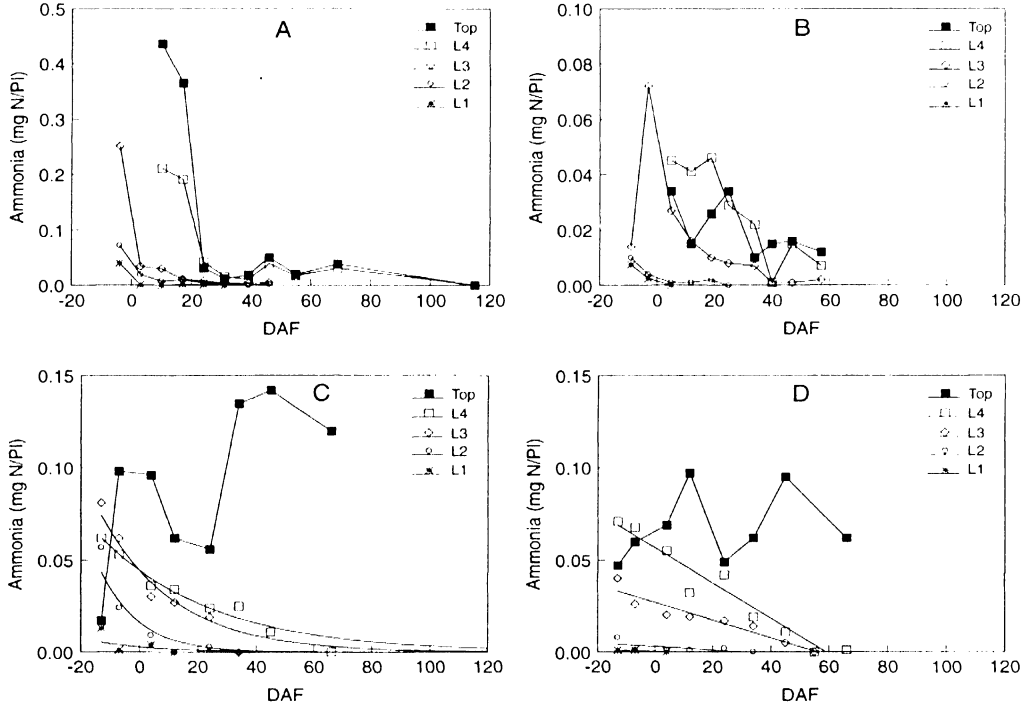


Fig. 4.23: Ammiona N content of five main stem leaf fractions in irrigated plots of ESD (A), SD (B), MD (C), and rainfed MD (D). Note the different scales.

4.8. Remobilization of N from leaves to pods

Mobilization of nitrogen in leaves and stems was expected to occur to supplement the supply of nitrogen for pod development during the reproductive stage. To estimate the amount of remobilized nitrogen it has to be assumed that loss of nitrogen in the concerned organ is due to either remobilization or to abscission. Since total above-ground biomass was determined in this study it was possible to calculate the net remobilization of nitrogen from canopy leaves. Irrigated and rainfed ESD plants remobilized 0.057 gN/Pl and 0.072 gN/Pl, which was about 35 % and 44 % of the nitrogen accumulation in the pods (**Tab. 4.13**). Similar to ESD the SD plants seemed to respond differently depending on soil moisture regime. Irrigated SD translocated 0.189 gN/Pl, which was 47.2 % of pod nitrogen accumulation, but rainfed SD 0.21 gN/Pl corresponding to 56.2 % pod nitrogen accumulation. Remobilization in MD was

less than in SD. Irrigated MD plants remobilized 0.129 gN/Pl, which was 36.8 % of pod nitrogen gain. For rainfed MD it was difficult to obtain reasonable values because of the high fluctuation between sampling points.

Table 4.13: Remobilization of nitrogen from canopy leaves to pods during the 1st flush.

Genotype Treatment	Time period (DAS)	Remobi-zed N (g/Pl)	Translocation rate (mg/Pl*d)	Portion in pod N (%)
ESD-I	69-98	0.057	1.97	34.9
ESD-R	69-91	0.072	3.27	43.9
SD-I	81-103	0.189	8.59	47.2
SD-R	74-116	0.210	5.00	56.2
MD-I	130-163	0.129	3.91	36.8

I: irrigated; R: rainfed.

5. DISCUSSION

5.1. Crop performance and partitioning of dry matter and nitrogen in response to the water regimes

The ESD genotype did not experience any drought during its first growth cycle under rainfed conditions. This was unexpected because rainfall distribution can vary largely in the SAT during the rainy season (Sivakumar *et al.* 1987) affecting crop growth and yield by intermittent droughts. In spite of the seemingly favourable rainfall distribution seed yield of ICPL 84023 was 1.6 and 1.8 t/ha which is below its potential in both irrigated and rainfed treatments. ICPL 84023 is considered to be a high-yielding genotype with a seed production of 2.2 to 2.6 t/ha under optimal conditions and normal sowing on Alfisols (Chauhan 1993, Nam 1994). During the second growth cycle yield performance was better exceeding 2 t/ha.

The low yield produced during the first growth period was most probably due to waterlogging. Short-term waterlogging occurred prior to flowering caused by excess rainfall and additional irrigation in the irrigated plots before the rain started. This may explain the even slightly better performance of rainfed ESD over the respective irrigated treatment expressed in terms of seed yield, TDM and LAI. For a few days, the plants exhibited yellowish symptoms in the top zone. Pigeonpea is known to be very susceptible to waterlogging but genotypic differences seem to exist among ESD pigeonpea (Chauhan 1987). Waterlogging at pre-flowering stage resulted in a yield level similar to that reported by Nam (1994) for drought stress during the same growth stage. Chauhan *et al.* (1993) assumed that fluctuations in the seed yield of ESD genotypes are mainly due to variablility in amount and distribution of rainfall resulting in drought or waterlogging periods, and that this reflects the crop's sensitivity to irregular moisture conditions. The present study confirms this hypothesis.

During the second growth cycle there were two drought periods but the ESD crop yielded similarly well in both treatments. The pre-flowering dry spell reduced plant available soil moisture to at least 50% when the crop was in budding stage. Pigeonpea is known to be remarkably resilient to water deficits prior to flowering (Lawn and Troedson 1990). However, grain yield reductions are reported by Nam (1994) for several ESD genotypes, amongst them for ICPL 84023 by 20% to 30%. Duration and severity of the first dry period was apparently not as large to adversely affect the crop. Monsoon rains coincided to stop at early pod filling stage and the rainfed crop had to grow on residual moisture. At the stage of late pod filling the amount of plant available water started to fall below 40%. It was expected that the crop would suffer from terminal drought stress but seed yield was not affected though there was a tendency of higher TDM and LAI in the irrigated plots. In earlier studies, drought stress at any growth stage resulted in significant yield reduction in various ESD genotypes tested

(Nam 1994). Drought stress during pod-filling stage was found less damaging to seed yield than that of earlier stages.

The SD genotype ICPL 87 performed very well during its first growth cycle with seed yields of 2.3 and 2.5 t/ha in the irrigated and rainfed treatments, respectively. The yield level agrees with that reported by Muchow (1985) and Chauhan *et al.* (1987) for regularly irrigated SD pigeonpea. Nam *et al.* (1993) reported high yielding on Alfisol under rainfed conditions but often yields lie below 2 t/ha without irrigation (Muchow 1985, Chauhan *et al.* 1993, Kumar Rao 1995). In the present study, drought stress coincided with the final phase of pod maturity including predominantly the dehydration of the filled seed and hence did not result in yield reduction. Waterlogging could have been responsible for the slightly better performance of the rainfed crop, however, wetting occurred prior to the reproductive stage and the crop had time to recover. Better yielding of the rainfed crop was not confirmed by the regular plant samplings where rainfed plots produced slightly less seed compared to the irrigated crop.

Seed yield of the second harvest of SD was lower than that of the first harvest. Decreasing seed yield in subsequent harvests of the same crop is also reported by Chauhan (1987a,b). This could partly be related to the relatively small number of new branches developed after harvesting the first flush of pods compared to ESD. ESD developed more branches at the beginning of the second growth cycle though they were shorter according to the plant height. In SD, in spite of new branching total pod production of branches was reduced by 12% compared to the first growth cycle, whilst in ESD it was improved by 30% (though it has to be noted that the main axis contributed much less in ESD to total pod mass of the second harvest). Another reason might be that a considerably higher amount of flowers and pods was shed during the second flush (almost double). Time to maturity was longer than reported elsewhere (Chauhan *et al.* 1987). Since the crop was not ratooned (cut down), which is known to extend the time to maturity, this might have been due to lower temperature. The rainfed crop experienced severe drought stress during seed development and pod filling stage which resulted in yield reduction by 30% as well as in decreased TDM production and low LAI.

The MD genotype ICPL 1-6 faced severe terminal drought stress under rainfed conditions. Seed yield was reduced by 44% but the yield level of the irrigated crop compared with that of one growth cycle of the short-duration types indicating that MD pigeonpea was highly responding to irrigation. The yield level for irrigated MD agrees with that reported by Muchow (1985) and Chauhan *et al.* (1987). Pod development and maturity was hastened in the rainfed crop as a strategy to escape drought and adjust to the harsh environment. Similar observations like seed yield reduction and faster pod development under water shortage were reported by Muchow (1985).

TDM at harvest was different among the genotypes and this was visible by the height of the plants. Planting density was optimal for growing short duration genotypes in peninsular India (Chauhan *et al.* 1987, Chauhan 1990) but adversely affected TDM accumulation per plant in MD which was grown at the same spacing. Plant population in MD was 6.7 times higher than usually practiced (Willey *et al.* 1981) and caused strong inter-plant competition for space resulting in a stalky growth habit and reduced canopy width. Similar observations including reduced yielding ability were made by Singh and Kush (1981) but, in contrast, yield and dry matter partitioning into seed was apparently not affected in the present study. TDM at harvest was less on plant basis but higher on area basis than reported for normal spacing (Willey *et al.* 1981).

Optimal LAI in pigeonpea usually lies between 4-6 (Rachie and Roberts 1974, Lawn and Troedson 1990). Maximum LAI observed during the first flush was 4, 5, and 7 for ESD, SD, and MD, respectively, indicating that leaf area was well developed. TDM at harvest was closely related to maximum LAI attained by the plants during flowering stage (**Fig. 5.1**). Solar radiation intercepted by leaf area is known to be converted into dry matter by a linear relationship (Hughes and Keatinge 1983).

Leaf area reduction took place towards maturity by shedding of leaves. After the first harvest (or even before that in irrigated SD) leaf area was restored by newly developed leaves. Under drought stress as occurred in the second flush of rainfed SD, the transpiring surface was minimized by reduced development of leaf area. Additionally, accelerated leaf shedding occurred towards maturity. Leaf area development in pigeonpea is reduced rather by smaller leaf size and slower leaf expansion rate than by reduced number of leaves developed (Nam 1994).

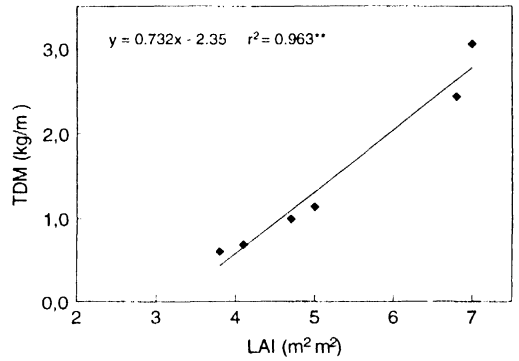


Fig. 5.1: Correlation between TDM at harvest and LAI at flowering over genotypes and treatments for plant samplings.

There was no significant relationship between TDM at harvest and seed yield produced. This was due to genotypic differences in dry matter partitioning to the seed. The primary difference in dry matter

partitioning among the genotypes was the proportion of pod biomass compared to stem biomass. In SD this relation was almost balanced whereas in ESD it was clearly favouring pod biomass. In contrast, even under optimal conditions MD partitioned only one fourth of TDM to the pods. This reveals that the growth of MD mainly concentrates on perennial and bush-like features whereby pod production is supported only secondarily.

TDM accumulation was generally less in the rainfed treatments (except in ESD first flush) although a significant reduction was found only in SD second flush and rainfed MD. Those crops allocated a smaller proportion of TDM to the pods indicating that water shortage adversely affected translocation of mobile carbohydrates from photosynthetically active leaves to the pods. A higher proportion was instead invested in stem biomass and was abscised in dead leaves and other dead biomass. This could be due to reduced transportation and mineral cycling in the plant as a cost of keeping transpiration low under drought conditions. The crop may abscise leaves due to water deficit or to get rid of the high temperature and radiation burden.

Partitioning of above-ground dry matter and nitrogen changed in relation to the developmental stage of the plant. Early vegetative growth was characterized by allocation of major proportions to the leaves regarding particularly nitrogen. Young plants invested primarily in leaf area development to ensure proper assimilate supply for the subsequent reproductive stage. Until about 50-60 DAS the proportion allocated to leaves versus shoot system was similar among the genotypes. Thereafter the diverse genotypic growth habits resulted in different allocation patterns caused predominantly by the length of the vegetative growth phase or the time to flowering, respectively.

Especially the MD genotype behaved differently compared to the short-duration types because of its indeterminate nature and rather perennial growth characteristics like intense branching, late flowering and maturity, and relatively slow crop growth. Accordingly, MD invested a proportionally high amount of dry matter in shoot which was increasing continuously before flowering but maintained at about 55-60% of TDM after flowering. In comparison to shoot growth pod production played a minor role in the first growth cycle of MD, a feature which is typical for perennial shrubs or for cultivars selected primarily as green manure crops (e.g., *Leucaena*, NRC, 1984).

The short-duration types showed a similar allocation of dry matter to the shoot until early pod growth but then reduced shoot allocation to a proportion of 30-40% of TDM and invested mainly in pod dry matter accumulation. The determinate nature of the main axis might have also helped to improve the distribution of DM for pod growth in the short-duration types.

Dry matter partitioning to shoot is reported to range between 40-50% of TDM for groundnut varieties during the growth cycle, while pods received up to 25% and 35% of TDM (Nagaraj and Kumar 1986). The pigeonpea types in the present study accumulated most of their TDM during flowering and pod

development, a situation similar to that of chickpea (Hooda *et al.* 1986) and white lupin (Pate and Herridge 1978). Before flowering only 10%, 17%, and 25% of TDM was acquired in ESD, SD, and MD, respectively. Cowpea accumulates as much as 50% of TDM during the vegetative phase (Herridge and Pate 1977).

Distribution of nitrogen to various plant parts was closely related to DM accumulation but proportioning to leaves and shoot was reversed. Allocation to the shoot was much less accounting for no more than 20% of TN in ESD and SD but around 30% in well watered MD. Yet there was also evidence for reduced allocation of N to the shoot during pod growth in the short-duration types contrary to MD.

Water deficit conditions in MD resulted in proportionally increased TN allocation to the shoot towards maturity indicating that the crop started storing N in shoot tissue before reaching maturity instead of investing in seed protein. Storage of N was also observed in the short-duration types towards the end of the 2nd flush, however, this was mainly due to the early cessation of their shoot growth while N continued to slowly accumulate in the tissue.

Allocation of N to pods was less in rainfed MD because of two reasons: (1) water deficit reduced pod dry matter either through reduction of pod weight or pod number (2) allocation of N on dry weight basis was low towards maturity contrary to the steep increase in non-stressed plants. These conditions increased the sink capacity of the shoot. Water shortage reduced the proportion of N partitioned to the seed in several grain legumes, such as soybean, cowpea, black gram and pigeonpea and increased partitioning to stem and leaves (Chapman and Muchow 1985). In groundnut, but especially in soybean and pigeonpea, water stress limited pod addition and reduced both TN concentration and TN accumulation (DeVries *et al.* 1989).

5.2. Leaf abscission and remobilization of nitrogen

The time course of leaf abscission was a gradual process similar to the decrease of TN in the five leaf fractions. The delay of leaf senescence in upper leaf positions was expected to be predominantly a function of leaf age. Later developed leaves in upper positions would thus be abscised later than earlier developed ones while leaf age would be almost similar among the leaves. Another possibility considered that upper leaves would be having a shorter life span due to destructive drainage of nitrogen from the pods. However, the study showed that later developed leaves of upper positions tended to have longer life spans than those of low positions at the stem axis (except the variable top fraction) (see **Fig. 4.12**). This tendency was most gradually and typically expressed in SD.

Upper canopy positions are more exposed to light and therefore more important for photosynthetic assimilate supply to the pods. Nitrogen is thought to be reallocated from aging leaves to better positions in the canopy to maximize carbon gain (Chabot and Hicks 1982, Field 1983). This was apparently the case in L1 to L3 prior to the phase of N remobilization to the seed. The reallocation process may be expected to be more efficiently in SD due to its pronounced graduality in leaf abscission.

There seem to be also differences in the photosynthetic capacity of leaves at various positions. A higher photosynthetic capacity has been measured in soybean leaves of the mid (pod bearing) canopy compared to those of low canopy position (Jiang *et al.* 1993). It can be assumed that the reason for the slower senescence of the pod supporting leaves was a longer photosynthetic activity in the present study. Those leaves were, at least in ESD and SD, able to survive 40-50 days beyond harvest (see **Fig. 4.13**) despite an increasing risk of death.

Leaves were the main N reserve of the plant utilizable to meet pod N requirement at the stage when nitrogen fixation in pigeonpea usually decreases (Kumar Rao and Dart 1987). The seasonal change in TN concentration of different leaf fractions suggested that a considerable amount of leaf N must have been recycled before the leaves dropped (see **Fig. 4.19**). The results obtained for net remobilization of N during the pod filling phase can be used to develop flow diagrams illustrating roughly the flow of N in the three genotypes (**Fig. 5.2**). The diagrams were drawn for the same time period for which remobilization was estimated. Emphasis was placed on the fate of canopy N and on relative contributions to pod N received from the canopy, other plant parts, and through N uptake by nodulated roots.

Among the genotypes, SD was the most efficient in remobilizing canopy N in both soil moisture treatments. More than 50% was translocated out of the leaves and can be assumed to have entered the pods. Under well-watered conditions only a relatively small proportion of N was abscised in dead leaves of SD. The proportion remobilized from other organs, which may be mainly the stem, was also highest in SD among the irrigated treatments providing 13% of pod N. The ESD genotype remobilized less of its canopy N and lost a relatively high percentage through leaf abscission. However, leaf abscission did not occur at the cost of remobilization. A larger proportion of N remained in the canopy of ESD at maturity compared to SD. Under rainfed conditions remobilization from the canopy was enhanced by 7% in both ESD and SD (no data for MD due to dry matter fluctuations), while the proportion lost to the soil increased only in SD. Moreover, other plant parts contributed a considerably higher amount to pod N in ESD than under well-watered conditions.

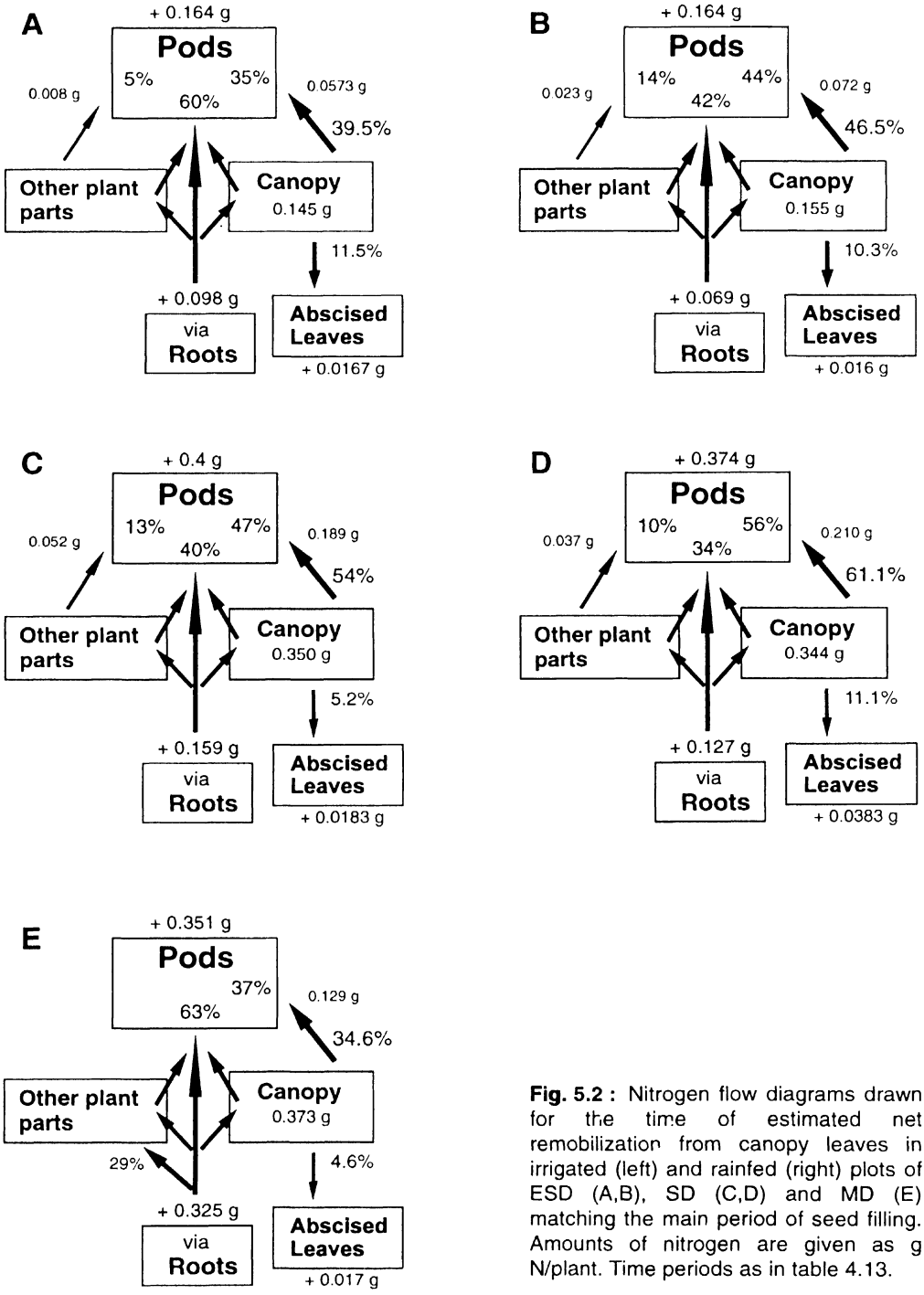


Fig. 5.2 : Nitrogen flow diagrams drawn for the time of estimated net remobilization from canopy leaves in irrigated (left) and rainfed (right) plots of ESD (A,B), SD (C,D) and MD (E) matching the main period of seed filling. Amounts of nitrogen are given as g N/plant. Time periods as in table 4.13.

A large proportion of pod N was received from underground plant parts in well-watered MD and ESD which could be inorganic soil N, N_2 fixation products, and remobilized N from roots and nodules which are usually transported upwards in the plant via transpiration stream (Pate 1980). N_2 fixation is known to decrease after 60 DAS in pigeonpea regardless of the maturity group (Kumar Rao and Dart 1987) which may account for the declining N supply towards maturity, reducing the proportion of biologically fixed N particularly in MD during the reproductive stage. Remobilization from nodulated roots meets about 33% of the seed's requirement for N in chickpea (Hooda *et al.* 1986). Under rainfed conditions the proportion of N contributed by nodulated roots was generally less than under well-watered conditions which could be due to reduced plant transpiration resulting in slower transport rates of nitrogenous compounds and/or to reduced N uptake rates.
Remobilization was least efficient in MD where N came only from leaves. A considerable amount of N taken up by the roots was not used for pod filling but stored in other plant parts, primarily in the stem.

The efficiency of leaves to remobilize N for seed filling was higher than reported by Rao *et al.* (1984) for pigeonpea (23%). In the present study, seed and podwall were not separated, but if so, values for seed N percentage would consequently have even been higher than those for complete pods. For other legumes like chickpea, values were reported around 25% (Hooda *et al.* 1986) or around 40% (Hooda *et al.* 1990). Accordingly, the percentage of seed N met by remobilization from leaves has been relatively small in those studies. Cowpea leaflets remobilized with an efficiency of 31% amounting to 25% of seed N (Herridge and Pate 1977). In this regard, SD pigeonpea showed a very high remobilization efficiency meeting almost 50% of the seed's requirement for N if entirely transferred to the seed. There is hardly any data on remobilization efficiency under water stress conditions available in the literature. Hooda (1990) reported 31% for chickpea which was nearly 10% less than under irrigated conditions, a situation contrary to the present study. The podwall is known to provide only negligible amounts to seed N in pigeonpea (Rao *et al.* 1984).

Remobilization efficiency seems to be closely related to the N uptake rate (inclusive N_2 fixation). Sinclair and de Wit (1975) showed that the high N demand for seed production in legumes cannot be met by N uptake alone. Therefore, the remaining N has to be obtained from vegetative tissue. It was proposed that a low uptake rate should therefore result in an increased translocation from vegetative tissues to the seed, in a reduced period of seed development, and in yield reduction (Sinclair and de Wit 1975). In the present study, the N uptake rate did not seem to be related to net remobilization but simply increased with maturity duration and therefore may not be directly comparable among the genotypes. However, in relative terms, N uptake under well-watered conditions amounted to about 4.6% per day (% of total N taken up during the net remobilization phase) in SD and to 3.5% and 3.0% per day in ESD and MD, respectively. The phase of net remobilization was 7 to 10 days longer in ESD and MD compared with SD. This indicates that the N uptake rate was relatively higher in SD compared to the other genotypes, though SD also provided a relatively high input to seed N by

remobilization. Under rainfed conditions the N uptake rate was drastically reduced in SD to only 2.4% per day supporting the hypothesis that more N has to be drawn from vegetative tissues.

Net remobilization occurred over a period of 3 to 4.5 weeks during seed filling matching the rapid decrease in TN concentration of the upper leaf fractions (L4 and Top) in the short-duration genotypes (see **Tab. 4.13**, **Fig. 4.19**). Those fractions predominantly contained leaves associated with developing pods on the main axis which are believed to constitute functional units in terms of sink and source (Davies 1977, Savithri *et al.* 1978). The major proportion translocated from leaves to pods was derived from those two upper canopy layers. The importance of leaves closely located to reproductive sinks is in agreement with a study conducted by Grover *et al.* (1985). The lower leaf layers redistributed most of their N before seed development which indicates that depletion of leaf N is a gradual process in the plant. This N was most likely to be moved to the upper canopy to support new leaf growth or was finally cycled through those leaves to the growing pods. Nitrogen accumulated during the vegetative stage can also be reutilized for fruit growth with high efficiency (Pate and Flinn 1973).

While N remobilization occurred in all leaf layers along with senescence, net translocation of carbon occurred only in the upper canopy of ESD (L4 and Top) but also in the mid-canopy (L3) of SD and rainfed MD (see **Fig. 4.14**). Early incorporated carbon was hardly used for pod growth indicating the importance of photosynthesis during pod development which requires sufficient leaf area and high photosynthetic activity. SD was not only most efficient in N remobilization but also in net translocation of carbon (see **Tab. 4.7**), and this estimation was supported by the steep decrease in leaflet dry weight. It can be assumed that SD plants have a higher photosynthetic capacity and/or a larger leaflet size in the upper canopy than the other two genotypes.

After remobilization of N the attached leaflets maintained a TN concentration of about 3.0 to 3.5% N in the short-duration genotypes and slightly higher in MD indicating that this was the required amount of N for the leaves to continue metabolic processes and retain physiological integrity (**Fig. 5.3**). A second translocation phase occurred shortly before abscission which can be concluded from the even lower TN concentration of abscised leaves (see **Fig. 4.19**). It is not clear if depletion of individual leaves is more intense in crops with a strong senescent growth habit like soybean. DeVries *et al.* (1989) reported that N loss from soybean leaves (1.0 N % DW) was greater than that from peanut (2.5 N % DW) and pigeonpea leaves (4.0 N % DW) both of them not exhibiting rapid leaf senescence. This phenomenon may be related to a threshold TN concentration preventing or initiating the death of leaves. Species and cultivar differences seem to exist in this regard, e.g. pigeonpea. Nevertheless, the TN concentration of dead leaves was fairly high, an observation that has also been made regarding fallen plant parts of different pigeonpea genotypes by Kumar Rao and Dart (1987).

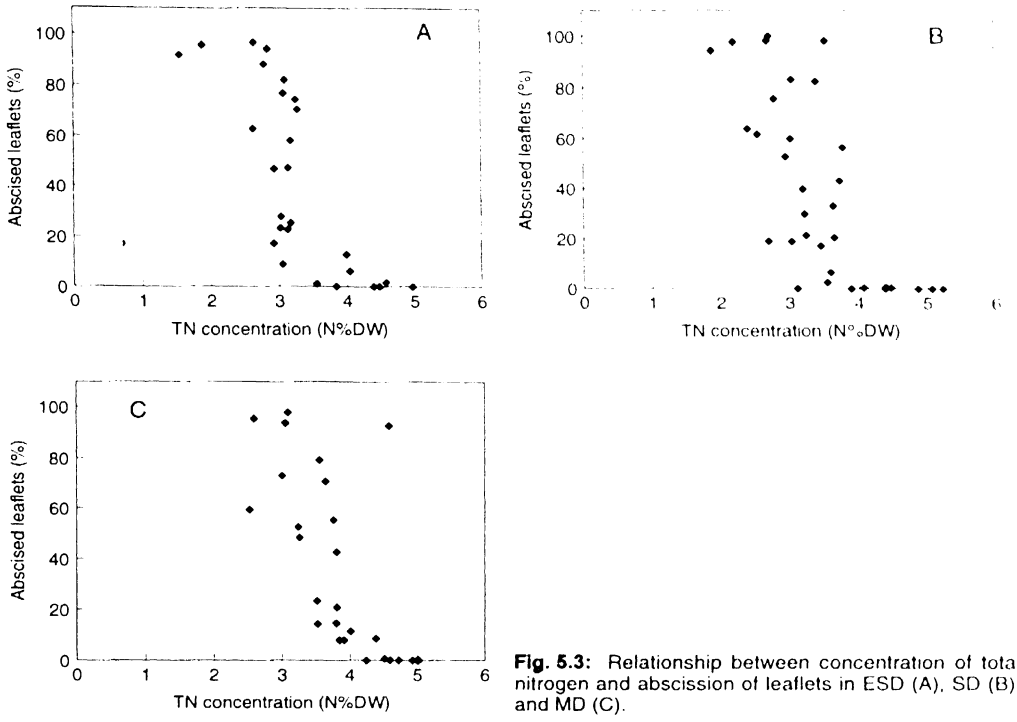


Fig. 5.3: Relationship between concentration of total nitrogen and abscission of leaflets in ESD (A), SD (B), and MD (C).

Accordingly, the amount of N lost to the soil was relatively high and increased with crop growth duration. MD provided the highest input for sustainability of soil fertility. Over a similar growing period N amounts of about 20 kgN/ha in ESD, 40-50 kgN/ha in SD, and about 55 kgN/ha in MD were provided only by abscised leaves. Another 10-20 kgN/ha on average came from other fallen plant parts. For other MD cultivars far smaller values are reported for N in fallen plant parts ranging between 25 to 28 kgN/ha (Kumar Rao and Dart 1987). Approximately 30 kgN/ha was estimated by Sheldrake and Narayanan (1979a) for fallen leaves only. The disagreement of those data with the present study is most probably due to the dense plant stand of the MD genotype causing strong leaf shedding in the lower canopy. The short-duration types finished two growth cycles during this period with two third of the fallen material being abscised during the 2nd growth period. The dead material accumulated below the plant canopies and mixed slowly with the crusty surface layer through rain. Probably progessive decomposition benefitted the late growth phase of the 2nd flush of ESD and SD, and toward maturity of MD.

The fact that ESD lost a relatively high percentage of N in leaf abscission (**Fig. 5.2**) was mainly an effect of the age structure of the canopy. The small plants with only 3-4 branches contained relatively fewer younger leaves in their small canopy at the beginning of seed filling than SD with 6 branches. Later developed leaves are more important for recycling, transitional storage and remobilizing N to

the seed as well as for assimilate supply due to the longer life span. This could be a limitation to the ESD types which, planted as an annual crop, still retain considerable amounts of N in their canopy leaves at maturity. Those could be entirely depleted for protein production. About 70% of the pod supporting leaves were still attached at harvest in ESD, as compared to only 40% in SD, reflecting the difference in remobilization efficiency. Thus, ESD seems to rely mainly on N uptake via roots under non-restrictive availability of water and nitrogen. Studies on the effect of water or N deficit on remobilization and leaf senescence might be of value to improve the N economy in pigeonpea.

Remobilization of nitrogen from senescing leaves denotes the degradation of N-rich structures to constituents with transportable properties. A decline of soluble protein has often been reported as a typical event in leaf senescence which is due to increasing proteolytic activity in the leaf (Wittenbach 1980, Wittenbach 1982). The composition of leaf N, in particular the soluble N pool, changes accordingly and allows conclusions on the progress of degradation and transportation out of the leaf. Changes in the soluble N pool of the upper leaf fractions should support the differences in net remobilization found among the genotypes. Soluble nitrogenous compounds (protein, amino acids, and ammonia) had been determined from fresh sub-samples, while net remobilization had been estimated on the basis of oven-dried samples of the same plants. The soluble N pool of the upper leaf fractions, which have been shown to be important for net remobilization, is composed in **figure 5.4** depicting the two stages before and after remobilization.

Across genotypes and treatments the portion of amino acids in total soluble N was higher at the stage of late remobilization indicating enhanced proteolytic activity (**Fig. 5.4**). The rate of protein degradation was seemingly faster than the export rate of amino acids out of the leaf. Nevertheless, on plant basis amino acid N content did not increase along with protein degradation in the leaf tissues (see **Fig. 4.22**). A similar observation was made by Wittenbach (1980) in senescing leaves of field-grown soybean. The breakdown products must have been translocated to growing regions of the plant immediately after being released from the protein complex. The levels of soluble N and protein N undergo rapid changes in developing seeds of pigeonpea and chickpea (Singh *et al.* 1980, Singh *et al.* 1981). Since the major sinks at the stage of N remobilization are seeds, it is reasonable to assume that most of the amino acids are being transferred to the growing pods for the synthesis of storage proteins.

At the end of the net remobilization phase amino acids accumulated in upper leaf fractions of SD indicating progressive proteolytic activity while translocation drastically slowed down. Differently, in ESD there was no such accumulation which could be due to continued export of amino acids to newly developing leaves or to a slow down of protein degradation. Grover and Sinha (1985) reported an increase of amino acids until 20 DAF in leaves of pigeonpea (cv Prabhat) prior to decline, indicating slow stimulation of amino N translocation in this cultivar.

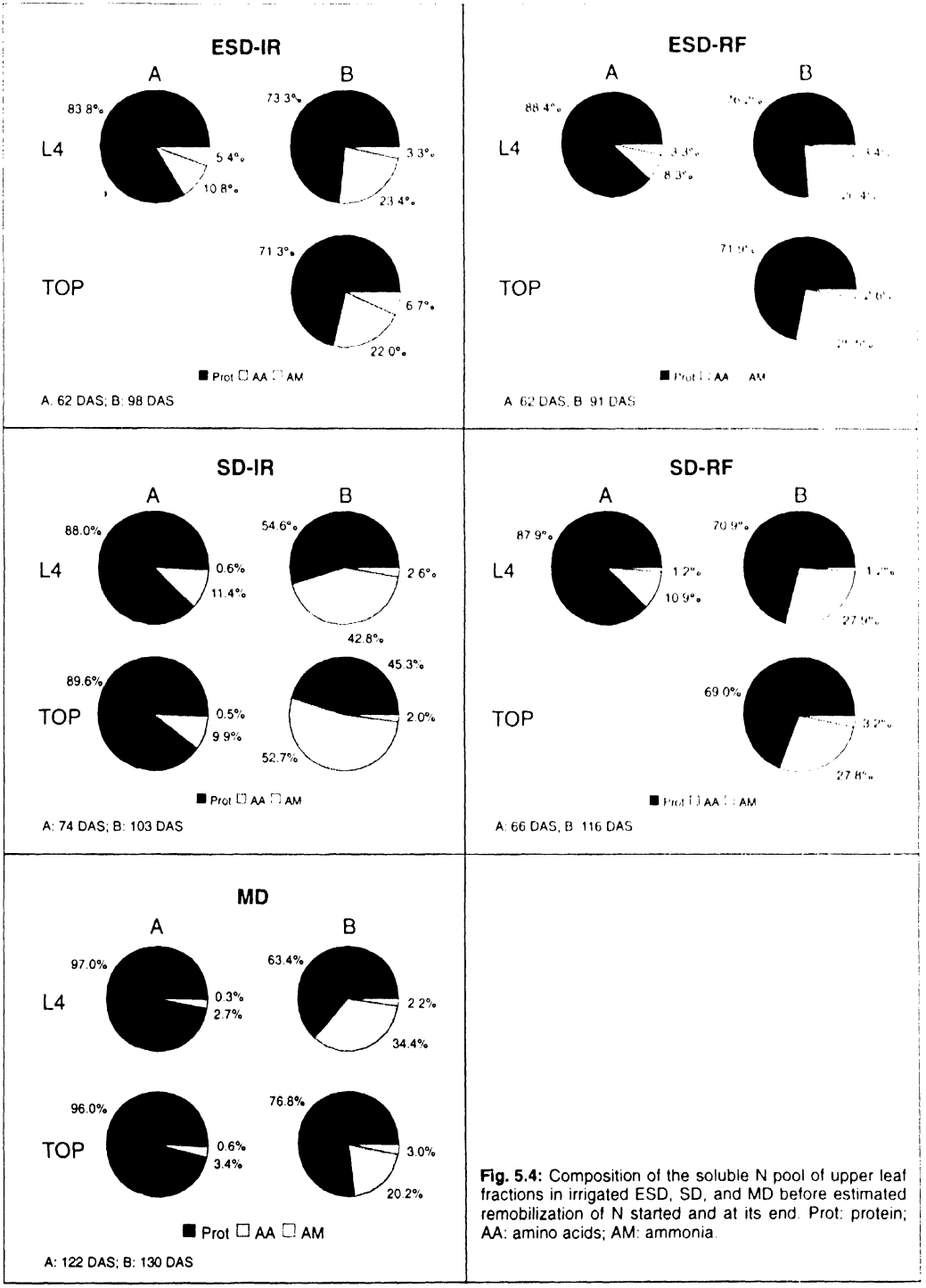


Fig. 5.4: Composition of the soluble N pool of upper leaf fractions in irrigated ESD, SD, and MD before estimated remobilization of N started and at its end. Prot: protein; AA: amino acids; AM: ammonia.

Among the water regime treatments there were only slight differences notable in ESD but large differences in SD at the stage of late remobilization. The larger portion of protein could be due to impaired functioning of certain proteases catalizing the degradation of proteins to amino acids. If translocation had been problematic (due to reduced transpiration) the amino acid portion had increased rather than the protein portion.

Changes in the ratio of soluble N : insoluble N with progressive remobilization showed that the soluble pool decreased in all cases (**Tab. 5.1**). This indicates that soluble N was transported out of the leaf at a faster rate than insoluble structures were degraded and released to the pool of soluble compounds. Among the genotypes, MD had a much higher ratio before remobilization than the short-duration types and its subsequent decrease of leaf soluble N was the most drastic. In comparison to ESD and MD, which both had similar ratios at late remobilization stage, the ratio of SD was very low indicating more efficient depletion of soluble compounds. The flux of N from insoluble structures to the soluble pool and its further export of amino acids seem to take place with different rates among the genotypes.

Table 5.1: Ratio of leaf soluble and insoluble nitrogen at two different stages of remobilization (see Fig. 5.4).

		Irrigated		Rainfed	
	Leaf	Sol : Insol		Sol : Insol	
Genotype	fraction	A	B	A	B
ESD	L4	0.14	0.06	0.12	0.08
	TOP	-	0.09	-	0.07
SD	L4	0.16	0.02	0.14	0.05
	TOP	0.20	0.03	-	0.03
MD	L4	0.53	0.06		
	TOP	0.41	0.09		

A: prior to net remobilization; B: late stage of remobilization (dates see fig. 5.4). Sol: soluble N; Insol: insoluble N.
Insoluble nitrogen was calculated: total nitrogen - soluble nitrogen.

The estimation of the half-life of leaf N has shown the presence of genotypic differences in the rate of N depletion of leaves. A faster loss of N was found in three leaf fractions of SD (L2, L3, and L4), whereas in ESD and MD the values of those fractions were close. Such rates of N depletion are even different among leaf positions at the same plant. They slow down in the upper canopy.

Differences in remobilization efficiency may be related to acitivity levels of protein-degrading enzymes as has been shown for certain genotypes of some cereals with high grain-N (Frith and Dalling 1980, and citation therein). This may also play a role when comparing SD's efficiency with those of ESD and

MD. The diagramic illustration of the soluble N pool of the three genotypes (**Fig. 5.4**) suggests that proteolytic activity was higher in SD than in the other genotypes. The processes responsible for exporting nitrogenous compounds out of the leaf such as phloem loading or the transport itself would also be important in the course of remobilization and may differ in efficiency among genotypes.

It was anticipated that a slight accumulation of ammonia would be observed in the leaves, for ammonia is' produced with rapid protein degradation. Its re-assimilation in the tissue depends on certain enzymes which may also be affected in their functioning by proteolytic activities. The leaf tissues did not show a clear increase in ammonia N, although the composition of the soluble N pool indicated a relative increase of ammonia in soluble leaf N in some cases. Most of the ammonia assimilating enzymes were apparently still functioning during the senescence process. However, enzyme activities of GS and GDH have been estimated to decline in pigeonpea leaves 15 and 30 days before maturity, respectively (cv UPAS-120, Luthra *et al.* 1983).

5.3. Conclusions and outlook

Among the genotypes selected from three different maturity groups SD was the one that performed best in this study. If compared with MD, a more traditional type, it clearly represents an improved plant type with regard to leaf senescence and N remobilization. In contrast, ESD turned out to inherit features that lie more closely to that of MD. If ESD is grown under favorable conditions (rainfall, soil N, etc.) this does not necessarily mean a disadvantage, whereas under adverse environmental conditions, e.g. low soil N, SD may perform better than ESD because it is capable of utilizing its own resources more efficiently. Such efficiency sustains the soil and may reduce farmer's costs for fertilizers. It is not known whether ESD actually gains from the large number of attached leaves kept until harvest, but it is most likely an indication for incomplete adaptation to annuality.

Genotypic differences in N remobilization may be responsible for differences in seed quality which depends on seed composition and protein content. This relationship should be taken into account for futher studies.

The period of available soil water was best matched by the first growth cycle of both short-duration genotypes. Longer crop duration as in SD 2nd growth cycle and rainfed MD resulted in yield losses unless irrigation was provided. The short-duration genotypes clearly outyielded MD by producing around 4 t/ha in two harvests during the same time period when MD produced only 2.3 t/ha or less. The study confirms the sensitivity of ESD pigeonpea to waterlogging. It seems that ideal genotypes should be able to tolerate both drought and waterlogging to avoid yield losses in regions of uncertain rainfall with heavy pourdowns.

It was a basic deficit of this study that the water regime, which was selected as main treatment, could not be sufficiently controlled to ensure more valuable results on leaf senescence and N economy under reduced soil moisture availability.

However, the present study contributes to pigeonpea research by the following findings :

(1) *Which patterns of leaf senescence/abscission are present in pigeonpea ?*
Leaf senescense/abscission starts from the bottom of the plants. The life span of individual leaves increases in the upper canopy (except Top), and there is evidence for genotypic differences in this. There is also evidence that leaf life spans in pigeonpea are shortened under moisture stress. The amount of leaves remaining attached to the plant beyond harvest is genotypically different.

(2) *How efficiently does pigeonpea remobilize nitrogen from senescing leaves ? Is this affected by sub-optimal moisture conditions ? What may be the reasons for genotypic differences ?*
During pod filling pigeonpea remobilizes about 35-54% of canopy N under optimal moisture conditions and this depends on the cultivar. There is evidence that remobilization is enhanced under rainfed conditions. Depletion of leaves of N occurred in two steps: (1) to a TN concentration of 3.0-4.0% N in attached leaves (2) to around 2.0% N before abscission.
The rate of N remobilization and translocation is different among genotypes. It is also different among leaf positions within genotypes. Genotypic differences in remobilization efficiency may be caused by different proteolytic activities and/or proteases in the leaf tissue.

(3) *How is leaf senescence as related to dry matter and N partitioning in pigeonpea affected if grown under sub-optimal soil moisture conditions in a semi-arid environment ?*
Reduced soil moisture availability resulted in reduced grain yield, harvest index and TDM. ESD, SD, and MD lost approximately 1.0, 1.8, and 2.9 t/ha leaf biomass during the same growth period, but no significant difference among the water regime treatments was found. More biomass was abscised during the 2nd flush if TDM was not reduced. ESD, SD, and MD provided about 20, 45, and 55 kg/ha leaf nitrogen to the soil.

There is a whole array of literature on the regulatory impact of fruit development on leaf senescence which mostly involves studies on plant hormones, pod removal effects, and on biochemical and functional changes of leaves. Grover *et al.* (1985) suggested that the senescence of subtending leaves in pigeonpea is regulated by the developing pods. This interesting aspect of leaf senescence was beyond the scope of the present work and could be subject to further research.

6. ZUSAMMENFASSUNG

Unter den drei getesteten Sorten verschiedener Reifegruppen der Straucherbse (*Cajanus cajan* L. Millsp.) war SD (*short duration*) diejenige, die in dieser Studie am positivsten abschnitt. Verglichen mit der traditionelleren Sorte MD (*medium duration*) stellt SD eine deutlich verbesserte Sorte bezüglich Blattseneszenz und N Remobilisierung dar. Im Gegensatz dazu zeigte ESD (*extra-short duration*) Eigenschaften, die näher an MD als an SD lagen.

Blattseneszenz mit Abscission (Blattfall) begann in Bodennähe. Mit zunehmender Höhe der Blattposition an der Pflanze nahm die Lebensspanne einzelner Blätter zu (außer der *top* Fraktion), wobei Hinweise auf sortenabhängige Unterschiede gefunden wurden. Auch gab es Hinweise darauf, daß die Lebensdauer der Blätter unter Wasserstreß verkürzt wurde. Die Anzahl an Blättern, die noch nach der Ernte an der Pflanze hängen blieben, war sortenabhängig und betrug in einer oberen Blattfraktion (L4) nahe den Schoten noch 70% bei ESD, nur 40% bei SD und 0% bei MD.

Während der Schotenreife remobilisierte die Straucherbse abhängig von der jeweiligen Sorte etwa 35-54% ihres gesamten Blattstickstoffs unter optimalen Bodenfeuchtebedingungen. Es fanden sich Hinweise darauf, daß die Umlagerung unter Regenfeldbau gesteigert wurde. Der Entzug von N aus den Blättern erfolgte in 2 Stufen: (1) bis zu einer N Konzentration von 3.0-4.0% N in noch hängenden Blättern (2) bis um die 2% N vor dem Abwurf. Die Rate der N Remobilisierung und Umlagerung war bei den Sorten verschieden. Auch an verschiedenen Blattpositionen innerhalb jeder Sorte war sie unterschiedlich. Die sortenabhängige Effizienz der N Remobilisierung könnte durch unterschiedliche proteolytische Aktivitäten und / oder Proteasen im Blattgewebe verursacht werden.

Reduzierte Bodenfeuchte führte zu reduziertem Kornertrag, Ernteindex und Gesamttrockenmasse. ESD, SD und MD verloren ungefähr 1,0, 1,8 und 2,9 t/ha Blattmasse während der gleichen Wachstumsspanne, wobei jedoch kein signifikanter Unterschied zwischen den Feuchtebehandlungen gefunden. Während dem zweiten Wachstumszyklus wurde mehr Biomasse abgeworfen, wenn die Gesamtbiomasse nicht reduziert war. ESD, SD und MD brachten etwa 20, 45 und 55 kg/ha Blattstickstoff durch Abscission in den Boden zurück.

Die Zeit der ausreichenden Verfügbarkeit von Bodenwasser wurde am günstigsten genutzt durch den ersten Wachstumszyklus von beiden SD Sorten. Längere Zyklen (zweiter Wachtstumszyklus von SD, MD) führten zu Ertragsverlusten wenn nicht bewässert wurde. Beide SD Sorten produzierten während der gleichen Anbauzeit (mit zwei Wachstumszyklen) deutlich mehr Kornertrag als die traditionellere Sorte MD.

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8. APPENDIX

List of sampling dates

Genotype	Date	JD	DAS	DAF	DAE	(IRR) RGS	(RF) RGS
ESD	19/6	170	0			0 00	
	5/7	186	16		11	0 16	
	15/7	196	26		21	0 27	
	27/7	208	38		33	0 39	
	6/8	218	48	-4	43	0 49	
	13/8	225	55	3	50	0 56	
	20/8	232	62	10	57	0 63	
	27/8	239	69	17	64	0 70	
	3/9	246	76	24	71	0 78	
	10/9	253	83	31	78	0 85	
	18/9	261	91	39	86	0 93	
	25/9	268	98	46	93	1 00	
	4/10	277	107	55	102	1 09	
	18/10	291	121	69	116	1 23	
	3/12	337	167	115	162	1 70	
SD	19/6	170	0			0 00	
	5/7	186	16		11	0 13	
	15/7	196	26		21	0 21	
	27/7	208	38		33	0 30	
	6/8	218	48		43	0 38	
	18/8	230	60	-9	55	0 48	
	24/8	236	66	-3	61	0 52	
	1/9	244	74	5	69	0 59	
	8/9	251	81	12	76	0 64	
	14/9	257	88	19	83	0 70	
	21/9	264	94	25	89	0 75	
	30/9	273	103	34	98	0 82	
	6/10	279	109	40	104	0 87	
	13/10	286	116	47	111	0 92	
	23/10	296	126	57	121	1 00	
	17/11	321	151	82	146	1 20	
	5/1	370	200	131	195	1 59	
MD	19/6	170	0			0.00	0.00
	5/7	186	16		10	0.08	0 09
	15/7	196	26		20	0.13	0 14
	27/7	208	38		32	0.19	0 21
	6/8	218	48		42	0.24	0 26
	16/8	228	58		52	0 29	0 32
	26/8	238	68		62	0.34	0 37
	6/9	249	79		73	0.40	0.43
	16/9	259	89		83	0.45	0 48
	2/10	275	105	-13	99	0.53	0.57
	8/10	281	111	-7	105	0.56	0.60
	19/10	292	122	4	116	0.61	0 66
	27/10	300	130	12	124	0.65	0.71
	8/11	312	142	24	136	0.71	0.77
	18/11	322	152	34	146	0.76	0.83
	29/11	333	163	45	157	0.82	0.89
	9/12	343	173	55	167	0.87	0 94
	20/12	354	184	66	178	0.92	1.00
	5/1	370	200	82	194	1.00	

Abbreviations: see page viii.

Julian Day Calender

DAY OF YEAR CALENDAR

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
JAN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
FEB	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60		
MAR	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
APR	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	
MAY	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151
JUN	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	
JUL	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212
AUG	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243
SEP	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	
OCT	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304
NOV	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	
DEC	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365

Add 1 to red values during leap years

Photos

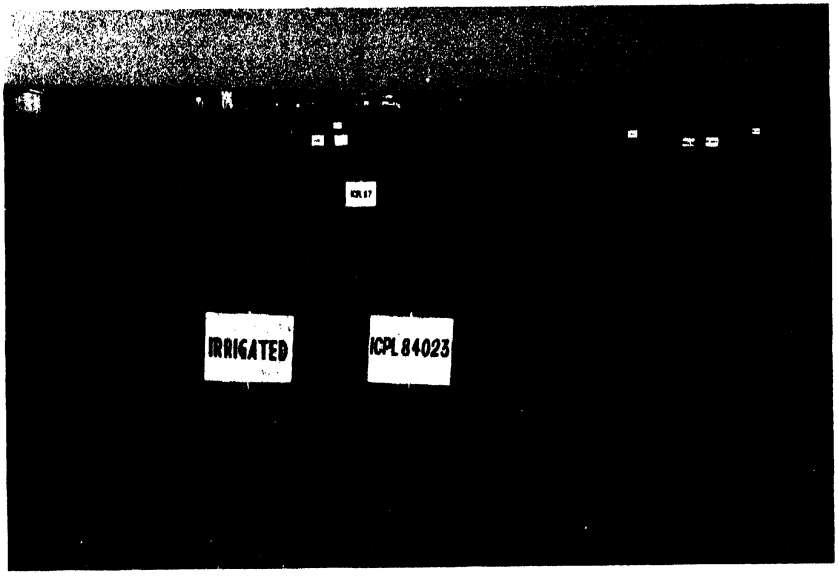

Photo 1: Replication strip R1 on 6th July (17 DAS). Pigeonpea plants were grown on rigdes. The spacing was set at 30 cm between rows and 10 cm within rows (see **chapt. 3.3**, Experimental design). The red lid of a soil moisture tube is seen at the right edge of this picture. (Yellow board for ICPL 1-6 is missing).

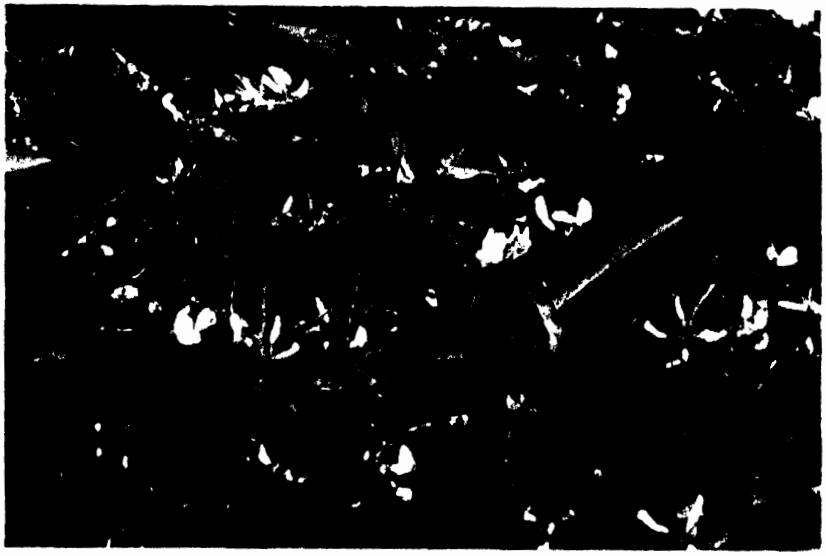

Photo 2: Flowering pigeonpea ICPL 87 (SD) with developing pods around 78 DAS.

Photo 3: Leaf senescence in ICPL 84023 (ESD) towards maturity of the pod. Most of the upper leaves were still green while individual leaflets turned yellow preparing for abscission. Leaves at lower positions were already abscised.

Figures

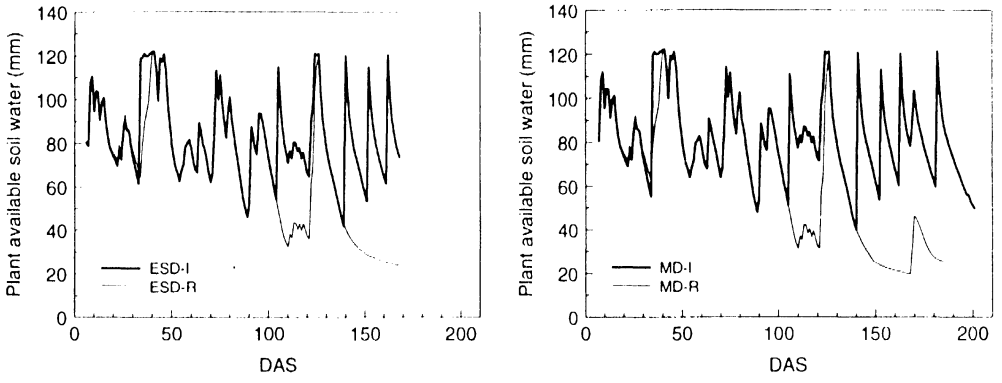

Fig. 8.1: Plant-available soil water in the profile for irrigated and rainfed plots of ESD (left) and MD (right) based on simulation data.

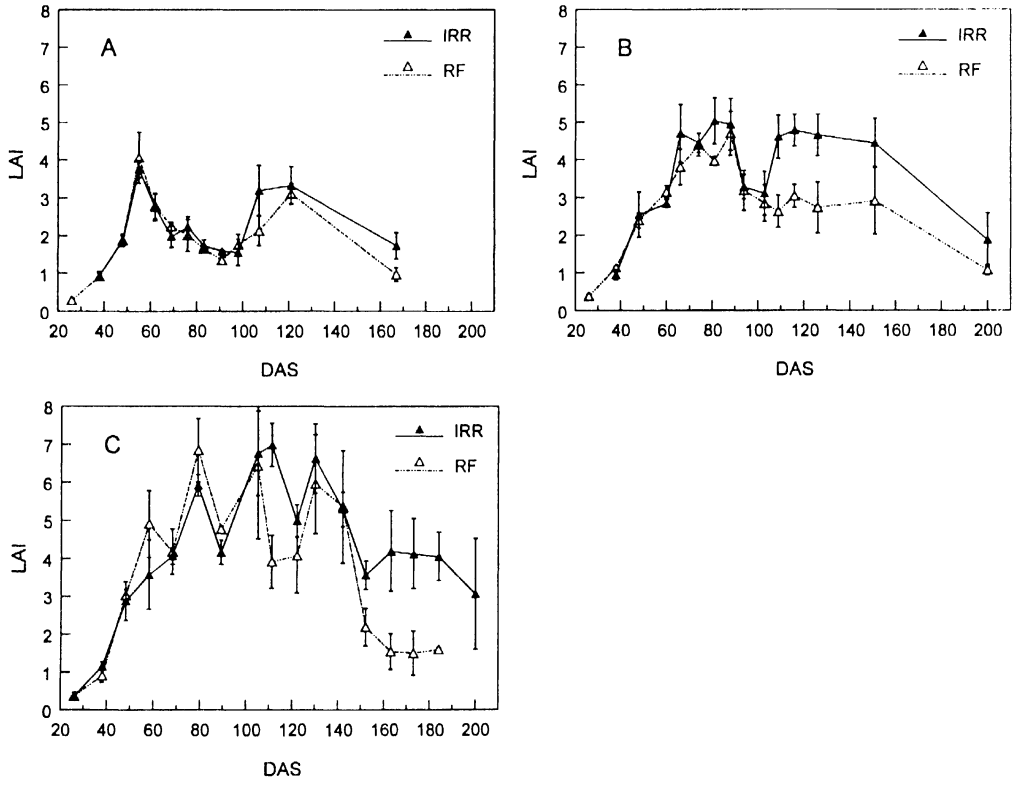

Fig. 8.2: Standard deviation of means of the leaf area index of ESD (A), SD (B), and MD (C) under irrigated and rainfed conditions.

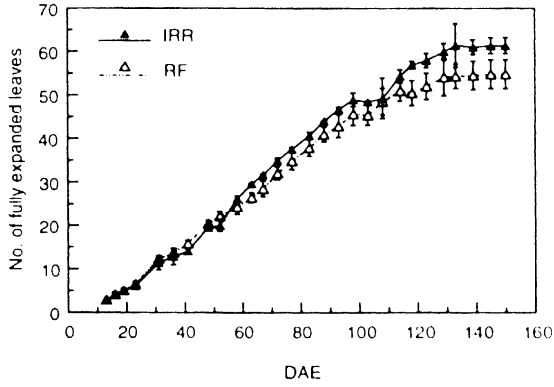

Fig. 8.3: Standard deviation of means of the appearance of fully expanded leaves on the main axis of irrigated (closed symbols) and rainfed MD (open symbols).

Tables

Table 8.1: Confidence limits and equivalence test for observed and simulated soil moisture at six soil layers.

Layer	Soil depth (cm)	$Diff_{MMR - MSim}$	Mean Sim	Mean MR	STD	Confidence limits (95 %) upper	lower
L1	0-10	0.0097	0.148	0.158	0.044	0.021	-0.001
L2	10-22	0.0017	0.179	0.180	0.059	0.016	-0.013
L3	22-30	-0.0125	0.174	0.162	0.028	-0.005	-0.020
L4	30-45	0.0210	0.160	0.181	0.021	0.026	0.016
L5	45-60	0.0171	0.164	0.181	0.019	0.022	0.012
L6	60-75	0.0205	0.162	0.183	0.019	0.025	0.016

Three genotypes and two water regimes were pooled together. MMR: mean of mean of replications (observed values); MR: mean of replications; MSim: mean of simulated values; STD: standard error; n = 64.

Table 8.2: Computation of t-tests for significant differences of three harvest characters of ESD, SD, and MD under irrigated and rainfed conditions.

Genotype Character	$\bar{x}_1$	$\bar{x}_2$	Diff $_{x_1 - x_2}$	t-value	Pr > t	Confidence 95 %
			On plant basis			
1st flush						
ESD						
Seed	5.18	5.52	0.34	-0.30	0.782	-2.88 - 3.57
TDM	17.98	20.62	2.64	-1.16	0.312	-3.44 - 8.35
HI	0.29	0.26	0.02	0.50	0.642	-1.11 - 0.15
SD						
Seed	9.18	7.76	1.42	1.92	0.128	-0.64 - 3.48
TDM	34.09	29.83	4.25	1.84	0.140	-2.17 - 10.68
HI	0.27	0.26	0.01	1.23	0.288	-0.01 - 0.03
MD						
Seed	15.33	8.33	7.00	2.10	0.104	-2.26 - 16.26
TDM	91.82	73.13	18.69	1.13	0.321	-27.17 - 64.54
HI	0.17	0.12	0.05	1.29	0.267	-0.05 - 0.15
2nd flush						
ESD						
Seed	5.06	3.81	1.24	2.14	0.099	-0.37 - 2.86
TDM	21.23	18.31	2.92	1.86	0.136	-1.43 - 7.27
HI	0.24	0.21	0.03	2.60	0.060	-0.00 - 0.06
SD						
Seed	6.87	5.78	1.09	0.97	0.389	-2.04 - 4.22
TDM	32.48	29.51	2.97	0.76	0.491	-7.93 - 13.88
HI	0.21	0.20	0.01	1.11	0.330	-0.02 - 0.05
			On area basis			
1st flush						
MD						
Seed	2.80	1.60	1.20	10.64	0.0004*	0.89 - 1.51
TDM	15.88	11.78	4.10	19.94	0.0000*	3.53 - 4.68
HI	0.18	0.13	0.04	5.81	0.0044*	0.02 - 0.06
2nd flush						
ESD						
Seed	2.65	2.63	0.02	0.08	0.9382	-0.71 - 0.75
TDM	7.50	6.92	0.58	0.83	0.4552	-0.94 - 1.73
HI	0.35	0.38	0.03	-0.81	0.4628	-0.04 - 0.07
SD						
Seed	2.09	1.47	0.62	5.44	0.0056*	0.30 - 0.94
TDM	8.27	6.94	1.33	3.69	0.0211*	0.28 - 2.00
HI	0.26	0.21	0.05	6.71	0.0026*	0.03 - 0.07

TDM: total dry matter; HI: harvest index.
$\bar{x}_1$: mean of irrigated plots; $\bar{x}_2$: mean of rainfed plots; Pr: probability.
*: significant different means.

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Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, daß ich die vorliegende Magisterarbeit selbständig und nur mit den angegebenen Hilfsmitteln angefertigt habe.

Göttingen, den

19. Sept. 1996

Claudia Janetzki