STUDY OF DIVERSITY AMONG SORGHUM (*Sorghum bicolor (L.)Moench.*) GENOTYPES USING MOLECULAR MARKERS

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THESIS SUBMITTED TO
ACHARYA N.G.RANGA AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE
IN THE FACULTY OF AGRICULTURE

DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE, RAJENDRANAGAR
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RAJENDRANAGAR, HYDERABAD - 500 030

1998

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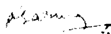

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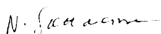

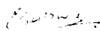

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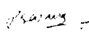

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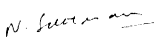

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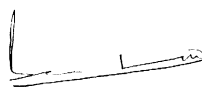

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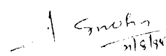
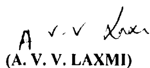


DECLARATION

I, **A. V. V. LAXMI** hereby declare that the thesis entitled **STUDY OF DIVERSITY AMONG SORGHUM (*Sorghum bicolar (L.) Moench.*) GENOTYPES USING MOLECULAR MARKERS** submitted to the Acharya N. G. Ranga Agricultural University for the degree of **MASTER OF SCIENCE IN AGRICULTURE** is a result of the original research done by me. I also declare that the thesis or any part of thereof has not been published earlier elsewhere in any



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ACKNOWLEDGEMENTS

I find no words to express my deep sense of gratitude, sincere and profound thanks to Dr. N. Seetharama, Senior scientist, GREP, ICRISAT for his immensely valuable guidance during the course of investigation, which enabled me to bring out this thesis.

I am greatly indebted to Dr. P. S. Sarma, Professor and Head, department of Plant Physiology, College of Agriculture, Rajendranagar and Chairman of my Advisory committee for, providing me an opportunity to work at ICRISAT, for his constant encouragement and deliberate counsel during the course of investigation.

I also thank Dr B.G.SINGH, Associate Professor, Department of Plant Physiology, Rajendranagar and Dr. S. SUDHEER KUMAR, Assistant Professor Department of Genetics and Plant Breeding, Rajendranagar and members of my advisory committee for their precious suggestions.

I render my special thanks to all the staff of the Department of Plant Physiology for their support throughout the period of my post graduation study. I am also thankful to the TAFP program manager Dr. B. Diwakar, Jagatha Seetharaman, Mr. Prasad and other staff of ICRISAT for supporting my investigation.

I express my gratitude to Mr. Prabhakar (Statistics), Mr. K.D. Prasad (GREP) and Mr. Yousuf for the immense help they have done to me during the course of investigation. I also express my thanks to Shyamala, Beena, Dr. Mythili, Mohita, Soma Raju, Lakminarayana, Santosh madam, Mallareddy, Lakshmi and Shankaramma for the cooperation they extended in the laboratory.

I thank my friends, Padmaja, Vani, Ajitha, Renuka, Prabha, Sailaja, Valli, Pushpa, Gowri, Prachi, Lalitha, Bhavani, Mamta, Rama, Prasuna, Kala, Sonali, Suma, Manju, Sridevi, Vijaya,Rakesh, Shekhar, Manohar, Anand, Rajesh, Hajra, Ajay and Srinivas for their encouragement.

I am greatly indebted to my parents Sri. A. K. Rao and Smt. A.Anuradha for what I am today. I would end by thanking my brother Prasad, Sister in-law-Rohini, Sister Sudha, brother-in-law Sudhakar and Saurabh, Aishwarya and Anusha for their affection and inspiration.

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Faculty : **AGRICULTURE**

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ABSTRACT

Sorghum is an important dual purpose and drought hardy crop of the semi-arid where, drought is the most important constraint limiting crop yields. Drought tolerance is an important agronomic trait but the genetic and physiological mechanisms that condition its expression are poorly understood. Molecular genetics provides a new and powerful approach to understand better expression of this trait. The purpose of this study was to analyze the senescence behavior of sorghum genotypes, to observe the yield stability of these genotypes and quantify their drought tolerance mechanism. Selected sorghum genotypes (48) were also genotyped with 4 microsatellite primers to identify polymorphism. The genotypes showed wide variation for all the senescence parameters under study. The staygreen genotypes had lower rate of senescence when compared to the senescent genotypes. The onset of linear senescence was earlier in the staygreen

genotypes indicating early initiation of grain filling. The offset of senescence was delayed in staygreen genotypes indicating a slow senescence and an extended period of grain filling when compared to the senescent genotypes. Although the staygreen genotypes yielded more than the senescent genotypes, high staygreen resulted in decreased harvest index and an increase in the stalk yields rather than the grain yields. The green leaf number duration during the linear phase of senescence was more in the staygreen genotypes when compared to the senescent genotypes and had a significant influence on the yield of the genotypes. It was observed that grain growth is negatively correlated with senescence. The linear phase of grain growth was longer for the staygreen genotypes than the senescent ones, thus contributing for higher yields in the staygreen genotypes. The staygreen genotypes also showed higher grain growth duration than the senescent genotypes, thus facilitating a longer grain filling period to give higher yields. In other words staygreeness has positive correlation with grain growth and yield. The microsatellite primers V7512, V7518, V7523 and V7525 were used for studying polymorphism among the sorghum genotypes. The microsatellite primers V7512 identified 4 polymorphic bands and primer V7525 identified 3 polymorphic bands between the senescent and staygreen genotypes where as primer V7518 identified only two polymorphic bands and primer V7523 identified 3 polymorphic bands indicating that these primers could be used in selection for staygreen trait. For better understanding of the genetic relationship among the accessions they were clustered into different groups based on their genetic distances. The microsatellite primer V7512 classified the sorghum genotypes into 6 groups, primer V7518 into 3 groups, primer V7523 into 4 groups and primer V7525 classified the sorghum genotypes into 4 groups which helps in classification of sorghum genotypes based on their senescence pattern for their cultivation under drought situation to enhance sorghum yields in semi - arid tracts.

LIST OF ABBREVIATIONS USED

ABA : Absicic acid

AFLP : Amplified fragment length polymorphism

BAP : Benzyl amino purine

bp : Base pairs

DAA : Days after anthesis

DAE : Days after emergence

DAF : Days after flowering

DAF : DNA amplification finger printing

DAS : Days after sowing

DNA : Deoxy ribonucleic acid

d NTPs : di-nucleotide tri-phospates

ET : Evapotranspiration

EW : Epicuticular wax

FCC : Fluorescent chlorophyll catabolite

GGD : Grain growth duration

GLAD : Green leaf area duration

GLND : Green leaf number duration

HCN : Hydrocyanic acid

HI : Harvest index

IAA : Indole acetic acid

LA : Leaf area

JA : Jasmonic acid

M : Molar

M^2 : square meter

MAS : Marker assisted selection

ml : milliliter

μ : micro liter

NCC : Non fluorescent chlorophyll catabolite

ng : nano gram

OA : Osmotic adjustment

PCR : Polymerase chain reaction

PM : Physiological maturity

QTL : Quantitative trait loci

RIL : Recombinant inbred line

RNA : Ribo nucleic acid

RFLP : Restricted fragment length polymorphism

RAPD : Randomly amplified polymorphic DNA

R-GLND : Relative green leaf number duration

RWC : Relative water content

SAM : S-Adenosyl methionine

SSRs : Simple sequence repeats

T1 : Proton spin -lattice relaxation time

TP : Turgor pressure

TDM : Total dry matter

ZR : Zeatin riboside

INTRODUCTION

CHAPTER I

INTRODUCTION

Most of the crop plants are subjected to mild or severe environmental stresses during their life cycle. From the agronomic point of view, stress can be defined as any factor that retards plant growth, resulting in reduced economic yield. Of the 14 billion hectares of land available on the earth, it is estimated that 6.0 billion hectares is under arid or semi-arid and only 0.23 billion hectares is irrigated. So, the most important physiological and breeding objective is to develop plants with tolerance to abiotic and biotic stresses. Among the various abiotic stresses, drought stress is the primary factor contributing to crop yield losses around the world (Boyer and Pherson, 1975). Crop production in areas prone to drought may be enhanced and stabilized by the development and use of crop species and varieties that can tolerate or avoid water deficit. Although many crop species have been shown to possess variation for drought tolerance, selection tolerance while maintaining maximum overall productivity has been a challenge (Rosenow and Clark, 1983).

Sorghum is the fifth most important cereal crop in the world which has a dual purpose both as a grain and fodder . It is vastly grown in the semi-arid tracts (SAT) of the world where moisture stress is the most limiting factor in crop production. The cultivated sorghum have spread throughout the world, and today it is grown on 47.8 million

hectares, with a production of 1.8 metric tons (FAO,1995). Because of its drought tolerance nature it is extensively used to study the mechanisms that condition adaptation to dryland conditions (Blum 1979, Rosenow *et al.*, 1983). Evaluation of sorghum germplasm has identified genotypes that are drought tolerant during one stage of growth but are susceptible at other growth stages (Rosenow and Clark, 1995). Therefore any mechanism which confers tolerance to drought during post-flowering period in sorghum is desirable. Staygreen is one such mechanism or trait that confers post flowering drought tolerance in sorghum by delaying plant and leaf senescence and thereby increasing the duration of grain filling. Staygreen is a delayed leaf and plant death mechanism in *Sorghum bicolor* that circumvents the detrimental effects of reduced soil moisture combined with high temperatures during post-anthesis growth (Henzell *et al.*, 1992). This trait is shown to be heritable and improvement through breeding is possible.

The molecular markers based on DNA sequence variation have significant advantages for genotype identification, genome analysis and mapping. DNA markers are neutral to various environmental factors, highly sensitive and sufficiently reproducible. The DNA based markers are useful in mapping the complex traits where conventional breeding and physiological methods fail. The development of molecular technologies and the use of markers in quantitative trait loci (QTL) analysis has become a powerful approach for studying the genetic and phenotypic basis of complex traits such as staygreen (Williams *et al.*, 1992). If individual genetic components associated with a complex trait can be identified, then research can focus on the function of each locus independently without the confounding effects of other segregating loci (Yang *et al.*, 1995). Construction of genetic linkage map for sorghum will make available a precise but vast information that

can be used by plant breeders and plant physiologists to identify, manipulate and complement traits to their maximum advantage. The purpose of this investigation was to study the diversity among sorghum genotypes. The following are the specific objectives addressed:

1. To quantify the expression of staygreen trait and yield potential of 8 sorghum genotypes.
2. To observe if staygreen has any effect on grain growth.
3. To observe if staygreen and grain growth have any effect on yield of sorghum genotypes.
4. Use of restriction fragment length polymorphism (RFLP) and Simple sequence repeats (SSRs) to identify polymorphism between 48 staygreen genotypes.

REVIEW OF LITERATURE

CHAPTER II

Review of Literature

Sorghum is the most important dual purpose cereals of the semi-arid tropics where drought stress is a major constraint to yield. Staygreen trait is a delayed leaf and plant death resistance mechanism that circumvents the detrimental effects of reduced soil moisture during post-anthesis growth, thus negatively correlated with drought. By identifying DNA-markers associated with staygreen trait and by introducing these markers into non- staygreen genotypes, crop quality and yield can be increased significantly. The literature available on drought tolerance with special reference to sorghum is reviewed below under the following heads.

1. Drought and drought resistance
2. Morphology, anatomy, growth and development of sorghum in relation to drought
3. Drought resistance characters in sorghum
4. Staygreen trait and its relevance in drought tolerance
5. Grain growth pattern in sorghum
6. Molecular markers in relation to sorghum
7. Study of molecular diversity using DNA markers

2.1 Drought and drought resistance :

Drought is the most prevalent environmental stress factor limiting plant survival, growth and productivity. It is the main constraint for agricultural development (Bohnert et al., 1995; Boyer and Pherson,1975). Water stress in early stages of plant growth causes deleterious physiological effects like disruption of cellular organization and metabolism

(Santarius, 1967), impairment of stomatal function (Willmer and Pantoja 1992), membrane disorders due to lipid peroxidation or physicochemical changes and yield reduction (Henson *et al.*,1984). Bakheit (1991) reported that moisture stress during flowering period causes straight reduction in grain yield, panicle weight and plant height, while 1000 grain weight is affected considerably by moisture stress at grain filling period. Under water stress conditions relative height and biomass decrease linearly with transpiration (Donatelli *et al.*, 1992). Ludlow *et al.*(1988) reported that some times the effect of severe water stress on primary head, leads to the production of secondary heads (panicle). The grain yield from these secondary heads compensate for the low yield of primary heads, so that the total DM production and grain yield of water- stressed plants were similar to those of unstressed plants. However it was also found that drought stress decreases CO_2 assimilation rates and electron transport (Masojidek *et al.*, 1991).

2.2 Drought resistance in sorghum

Sorghum is considered as highly drought tolerant species among the cultivated plants. Blum (1979) observed that sorghum genotypes showed wide variations in drought avoidance, drought tolerance and drought escape mechanisms. Early genotypes were drought escaping and show lower evapotranspiration (ET) rates due to smaller leaf area. In drought avoiding types, the root resistance to water uptake was reduced and cultivar rsistance to drought correlated positively to the amount of epicuticular wax on leaves and sheath. Drought tolerant types had a greater ability of leaf cell membranes to function under stress conditions (Blum, 1979). Bennet and Tucker (1986) observed that the epicuticular wax present on the lower side of the leaf and upper leaf sheath aids in moisture stress tolerance. Santamaria *et al.* (1986) found correlation among drought

tolerant traits and they correlated leaf rolling positively with OA. It was found that the extent of leaf rolling in sorghum is a measure of degree of water stress (Bewazir and Idle, 1989). Decreased radiation absorption or light reflection by leaf rolling and reduction in cuticular loss of water helps in drought avoidance. Ross-Leal (1990) showed that intensity of glossiness and waxy bloom, stomatal number contribute to higher water stress resistance compared to lower intensity. The genotypes having higher glossiness showed greater total EW load with characters such as chlorophyll, carbohydrates, wax and hydrocyanic acid (HCN) content which are associated with drought resistance (Garcia saucedo, 1985; Rios-Leal, 1990). Glossy sorghum lines show variability in the contents of EW, chlorophyll a and b and hydrocyanic acid at the seedling stages, that may be related with resistance to drought and insects (Maiti *et al.*, 1991b). Variability in EW among sorghum lines were also reported by Ebercon *et al.*,1977). Sorghum is also well adapted to drought due to a higher root hair density per unit root length (Blum, 1988) and larger rooting depths of up to 2.0 to 2.3 m (Maiti, 1986). Some anatomical characteristics such as intensity of sclerenchyma in the root pericycle and silica particles in endodermis may be correlated with drought resistance and it was observed that more than 80% of the root mass is located in the upper 20-30 cm of the soil profile . Bewazir and Idle (1988) observed that relative conductivity and number of seminal roots were negatively correlated with percent survival and a high relative conductivity indicates drought resistance in lines with less restricted seminal roots.

2.3 Growth and developmental stages in relation to drought stress:

Growth and development of sorghum crop consists of 3 phases: the vegetative (GS1), reproductive (GS2) and grain filling period (GS3) Eastin *et al.*, (1973). These 3 stages influence the growth and yield of crop in different ways:

GS1: Seedling stage, from the day of seedling emergence to the onset of the reproductivephase, panicle initiation phase (PI).

. Establishment of initial root system and shoots producing the panicle (by tillering).

. Termination of GS1 determining the total number of leaves on the panicle.

GS2: Panicle development, from the PI to growth stage 2 terminating with anthesis.

. Expansion of all the upper leaf internodes and all the culms (in case of tillering types).

. Development and growth of panicle and panicle components.

. Potential seed number.

. Continued root growth and nutrient is important as profuse root system is established during GS2.

GS3: Grain- filling, from flowering to physiological maturity of grain.

. Development and filling of grains.

. Seed set and seed size determine the final yield.

. Length of GS3 period influences final yield.

Kreig (1983) suggested that dry-matter production is strongly influenced by leaf area in GS1, which is again directly dependent on period of GS2. Water stress during this stage

inhibits cell expansion, thus reducing leaf area. He has also reported that tillers are more sensitive to water stress than the main stem. Hay and Walker (1989) observed that water stress during GS1 causes reduced yield due to reduction in number of floral initials produced in GS2. Stress during GS2 causes yield reduction through reduction in plant size, leaf area and seeds per head (Kreig, 1983). Fischer and Wilson (1971), observed that only 12 per cent of the grain weight of sorghum is contributed by pre-anthesis assimilates. But in conditions of stress the contribution of preanthesis assimilates to grain weight increases (Kreig 1983). Stout *et al.* (1978) and Lewis *et al.* (1974) observed that water stress at GS2 caused decreased growth rates of leaves, panicles, and reduced seed number per panicle.

The ultimate grain yield however is a function of both the time spent by the sorghum crop in GS3 and the rate of dry matter accumulation by the developing grain (Eastin *et al.* 1973), and about 90 per cent of grain yield is due to photosynthesis in the panicle and the four uppermost leaves. Sorghum starts senescence at milky stage and may have few functional leaves or dried completely by physiological maturity depending on the genotype (Vandelip and Reeves 1974). Moreover entire meristematic activity ceases and no more leaf initiation occurs 25 days after pollination (Wall and Ross 1970). House (1982) observed that as grain begins to dry, the remaining green leaves start senescence, the rate of which is distinct for each variety. Kreig (1983) explained that water stress during GS3 resulted in rapid senescence of lower leaves and consequent reduction in yields due to reduced leaf area, increased stomatal resistance and decreased photosynthesis. The normal activity of the developing panicle is also disturbed. Salam (1995) described dough stage as most critical to drought stress after flowering while

ripening stage is comparatively less sensitive. Tuinstra *et al.* (1992) concluded that in general sorghum genotypes are more drought tolerant at the pre-flowering stage than at the post flowering stage.

According to Salam (1995) resistant species and genotypes showed sufficient decrease in leaf water potentials to maintain leaf turgor during critical stages. Rosenow (1987) observed two distinctly different types of stress response directly related to the stage of growth when stress occurs. One type (pre-flowering) is expressed when plants are stressed prior to flowering during head development, while the other (post-flowering drought resistance) is expressed when moisture stress occurs during grain filling stage. Lines possessing high level of tolerance at one stage tend to be susceptible at the other stage.

2.4 Role of osmotic adjustment and drought stress in sorghum:

Osmotic adjustment (OA) reduces the impact of water stress on the growth and yield of crops. In sorghum, it can confer a yield advantage of up to 30% in water limited conditions. There is considerable variation in (OA) among sorghum lines (Ludlow et al.,1994; Girma and Kreig, 1992) due to net solute accumulation in response to water stress. OA reduces the sensitivity of turgor-dependent processes such as leaf expansion, stomatal conductance and leaf rolling with declining leaf water potentials (Jones and Turner, 1980; Morgan 1984) and allows plant growth at otherwise inhibitory leaf water potentials (Cuttler *et al.* 1980; Meyer and Boyer 1981, Takami *et al.* 1982). Henzell *et al.* (1976) suggested genotypic differences of sorghum leaves to adjust osmotically. OA was considered the main trait responsible for stomatal adjustment to leaf water deficits (Ludlow *et al.*,1985). However stomatal adjustment was closely related to the turgor

pressure(TP) of water-stressed leaves (Jones and Rawson 1979, Hsiao *et al.* 1984). Changes in stomatal conductance were shown to occur independently of leaf water potentials (Bates and Hall 1982; Blackman and Davies 1985). Ludlow *et al.* (1992) reported that when water stress occurs before and after flowering, the genotypes having high levels of osmotic adjustment show a mean yield advantage of 33% and 24% respectively, compared to the genotypes having low OA. Osmotic adjustment was positively associated with green-leaf area retention during grain filling and to root length density at 70 cm depth (Tang premsri *et al.*,1991). The drought resistant genotypes showed higher OA and sufficient decrease in leaf water potential to maintain leaf turgor (Salam,1995). Kannagara and Seetharama (1983) showed high linear correlation between abscissic acid, leaf water potential and plant height. In view of these observations Flower *et al.* (1990) concluded that under drought there is little advantage of selecting for plants with higher capacity for OA.

2.4.1 Relative water content (RWC) and drought resistance:

The highly significant linear correlation between RWC and proton spin-lattice relaxation time (T_1) indicated that T_1 could be used as water stress parameter in cereal leaves (Nagarajan *et al.*,1993). Premachandra *et al.* (1995) reported that drought tolerant grain sorghum cultivar (K886) maintains significantly higher relative water content (RWC), osmotic potential at full turgor and turgor pressure (TP) than did a drought susceptible cutivar (CS3541). The rate of grain growth increases linearly with temperature but the duration of grain filling and the rate of development of black layer are not closely related to mean temperature (Muchow, 1990).

2.5 Influence of drought resistant traits on yield and yield components of sorghum:

Khizzah and Miller (1992) correlated components of drought resistance with yield and found negative correlation between lodging and days to anthesis, panicle exertion and harvest index and positive correlation with plant height, panicle length, green leaf retention, grain size and grain weight. Green leaf retention was negatively correlated with panicle exertion, grain yield, harvest index; while grain yield was positively correlated with height, panicle exertion, lodging, harvest index and grain weight; and negatively correlated with days to anthesis and green leaf retention. He concluded that non-lodging and green leaf retention are useful indices for drought resistance. Dhoble and Kale (1988), showed positive correlation of grain yield with plant height, leaf area index (LAI) and panicle length along with high heritability. Blum *et al.* (1989) showed a reduction in yield but not relative yield under stress, due to decreased HI with increased growth duration of the genotypes. They concluded that genotypes showing traits of early heading, high leaf water potential, lower canopy temperatures and higher stomatal conductance yielded more under drought. Wenzel (1988), reported a positive correlation between characters related to growth rate (TDM and LA) and those related to drought resistance (total and relative moisture loss and moisture loss/leaf area).

2.4 Hormonal regulation of drought stress and senescence in sorghum:

Phytohormones like abscissic acid (ABA), indole acetic acid (IAA), ethylene and methyl esters of jasmonates play an important role under drought stress. Kannagara *et al.* (1982)

showed high levels of indole acetic acid in leaves of drought susceptible genotypes, which showed lesser grain yield stability under drought. In contrast, the free ABA concentration had a positive correlation with per cent relative growth. Kannagara and Seetharama (1983) observed under stress an increase in ABA levels and decrease in phaesic acid levels. High linear correlation was observed between ABA levels, leaf water potentials, leaf solute potentials and plant height. When sorghum cultivars BTx 623 grown with or without irrigation terminated at 50% anthesis were sprayed with benzyl amino purine (BAP) on every 3rd day from 50% anthesis until grain maturity , it was observed that BAP delayed leaf senescence and reduced stalk rot in water stressed plants but did not affect yields (Morgan *et al.*,1993). Zhang and Kirkham (1993), noticed considerable contrast in ethylene production levels of two sorghum genotypes under drought. In normal conditions the resistant genotypes produced more ethylene but under conditions of drought the susceptible genotypes produced more ethylene than the resistant ones. When the xylem sap of non-senescence Tx2817 and senescent Tx7000 cultivars were analysed for cytokinins to determine whether the delayed leaf senescence is associated with transport of greater quantities of cytokinins from roots, it was observed that zeatin + zeatin riboside (ZR) in xylem sap per gram shoot dry weight was 1.51 times higher for the non-senescent sorghum. High xylem sap cytokinin contents for Tx2817 suggests that its delayed senescence and enhanced resistance to adverse conditions may be due to high cytokinin productivity by roots when the carbohydrate supply to the roots is reduced (Amber *et al.*,1992). Conclusive evidence of jasmonic acid (JA) and methyl esters of jasmonate accumulation in plant parts under stress conditions was given by

Gunther and Benno (1993). Methyl jasmonate appears to mimic the activity of ABA and ethylene (Wang and Buta, 1994).

2.7 Grain growth pattern in sorghum:

Grain ripening is characterized by grain growth, which is associated with increase in size, weight change in grain colour and leaf senescence. The grain is the ripened ovary with attached glumes. The process of grain development starts with the formation of watery fluid in the grain, which is gradually converted to milky white soft and finally hard endosperm stage (Wilson and Eastin 1982). They also stated that the developing grains derive mineral nutrients from two sources, namely current photosynthates and reserved carbohydrates formed during pre-anthesis photosynthesis. The relative importance of xylem and phloem transport to the grain is supposed to be strongly influenced by the availability of water and mineral nutrients in the root environment during grain-filling (Roy and Wright, 1974). Kiniry *et al.* (1992) reported that environmental stress during grain filling period causes an increased dependency on stored assimilates. Dahlberg *et al.* (1992) conducted field experiment with two reduced progressive and two senescent sorghums by exposing ^{14}C to the first fully expanded leaf and the penultimate leaf for four minutes, they observed that at grain filling stage, the per cent of labeled carbon in the grains of RTx430, RTx7000 and BTx378 were significantly greater than B35. Conversely the per cent recovery of ^{14}C assimilates in the labeled leaf of B35 was greater than other cultivars.

2.7.1 Development and physiological maturity of grain:

The development of grains follows a sequence of stages comprising milky, soft dough, hard dough to the final physiological maturity. A black layer is formed at the hilar region due to formation of callus tissue as the phloem parenchyma at the hilar region becomes blocked with mucilage and pectic compounds and forms a black layer that completely shuts off translocation of photosynthates from the stem to the grain (Quinby 1972; Giles *et al.* 1975). The development of the black layer is reported to be an indication that maximum kernel dry weight has been achived (Daynard and Duncan, 1969). It takes more than one week for the dark layer to move from tip to base of kernels (Eastin *et al.*,1973). The duration of grain filling period is markedly reduced by temperature and under severe environmental stress (Caddel and Weibel, 1971). Singh and Borikar (1990) observed that days trequired for black layer formation on the aleurone layer, seed moisture content, density, electrical conductivity and germination all exhibited genetic variability. The black spot on the aleurone layer appeared during 30-35 days period coinciding with maximum test weight and optimum seed moisture content. Maximum germination occurred a little earlier than black spot formation suggesting that early maturing genotypes should be harvested before 30-35 days after anthesis (DAA) and late genotypes 35-40 DAA. Parvatikar and Manjunath (1991) reported that when the stalk juice was extracted at 50% flowering and at PM stages, the total sugar content in the juice increased with growth and was highest at physiological maturity. Taneja *et al.* (1992) observed that higher grain yields were associated with higher accumulated heat units during the reproductive and grain filling stages up to physiological maturity. It was

observed that dry weight accumulates linearly in the grain at least two weeks before anthesis. There is no significant increase in the non-productive parts and negligible increase in grain weight for two or three DAA (Dickson, 1976). Maiti *et al.* (1985) reported that the grain weight curve assumes almost a flat shape up to 35 DAA and subsequently there was a decline in fresh weight and there was a continuous drop in moisture content over the period of grain development.

2.8 Staygreen trait and senescence:

Staygreen is an important trait associated with drought resistance in sorghum (*Sorghum bicolor*). Plants possessing this trait retain their leaves in active photosynthetic state when subjected to water stress conditions during the grain filling period (Walulu *et al.*1994). Staygreen is an anti-senescence trait (Thomas and Smart 1991). During senescence chlorophyll disintegrates and the ultimate products of catabolism seem not to be pigmented. As plant ages, the built in processes which defend the plant against auto-destruction begin to decline, there by setting in the senescence syndrome with visible and biochemically measurable symptoms. Plants with high heritable staygreen phenotypes defy or postpone such senescence process. This may be due to the abnormally high level of resistance to photo-damage, due to which plants take longer to reach the threshold below which auto-destruction occurs. Thomas and Smart(1991) however did not agree with such a hypothesis. Thomas and Stoddart (1980) described senescence as a two-stage process. In the first stage after leaf passes through its peak assimilatory capacity, the mesophyll tissue begins to yellow and the photosynthetic apparatus is dismantled and assimilates are exported to young tissues or leaves for reserve deposition. In this stage there is tight metabolic regulation and coordination at tissue and organ level, and

characteristically the cells remain viable. The second stage is marked by rapid tissue deterioration and photo-destruction of viable cells. Young leaves which are net heterotrops, subsequently develop photosynthetic competence contributing to carbon budget of whole plant, which declines as the leaf ages. The transition of leaf from period of active photosynthesis to first phase of senescence in which physiological integrity is maintained is essentially a change rather than loss of function. Guiamet and Gianmibelli (1994) reported that during senescence disintegration of chloroplast, disruption of thylakoid network, deterioration of plasma membrane and formation of osmiophilic globuli occurs. Chloroplast breakdown in senescent leaves proceeds in essentially 3 steps, dephytylation by Mg-dechelatase and oxygenolytic cleavage of the chlorin-macromolecule by dioxygenase (Vicentini *et al.*,1995), as a result accumulation of fluorescent (FCC) and non-fluorescent (NCC) chlorophyll catabolites which are the final products of chlorophyll breakdown (Bachmann *et al.*,1994). Toumadje *et al.*, (1994) described the role of S-Adenosyl methionine (SAM) as the key branch point intermediate in the biochemical pathway leading to polyamine and ethylene biosynthesis, which in turn represents a biochemical cross-roads where polyamine synthesis leads towards non-senescent processess and the ethylene pathway constitutes shift towards senescence processes. Rosenow (1993) stated that the term "staygreen" has been used to describe sorghums which possess post-flowering drought resistance. The understanding of these stress responses, their heritability and the time of stress × stage of growth interaction is extremely important when screening for drought resistance. Rate of senescence is negatively correlated with grain dry mass, suggesting that hybrids containing the staygreen trait have a yield advantage when water is limiting during the grain filling

period. A QL 41/QL12 a high staygreen hybrid, produced the highest yield by combining a low rate of leaf senescence with a high harvest index (HI). The rate of leaf senescence was positively correlated with the amount of stem reserves mobilized probably resulting in the association between staygreen and lodging resistance (Borrel and Douglas, 1996). Thus sorghum breeders have selected for non-senescence (staygreen) under moisture stress to alleviate the widespread lodging problem and observed that there exists a negative correlation between senescence ratings and grain yield (Henzell *et al.*, 1992).

2.8.1 Gene action during senescence:

Thomas and Smart (1993) classified genes into 5 broad categories according to their functions and expressions during leaf development and senescence respectively.

1. Genes which encode growth or carbon assimilation components and which contribute to the progressing of senescence by switching off. Example: nuclear and plastid genes for Calvin cycle.

2. Genes controlling the primary metabolic activities of viable cells like, rRNA synthesis, respiratory enzymes, etc.

3. Genes directing development of latent metabolic machinery in mesophyll cells of leaves which later becomes active. Example : vascular enzymes.

4. Genes specifically turned on at the initiation of senescence, the point of convergence of all the various transduction pathways through which environmental and internal ones involve the syndrome.

5. Genes encoding senescence-related activities. Examples : Catabolic enzymes induced *de novo*.

2.8.2 Classification of staygreen:

Thomas and Smart (1991) classified staygreen into four types. Type A and Type B are functionally staygreen and may arise after alteration of genes in the timing of the initiation of senescence and the regulation of its rate of progress respectively. These staygreen types continue to photosynthesize for longer than normal and show a higher yield in crops for which carbohydrate is a major component of the harvest. In contrast, type C and D look green but lack photosynthetic competence either due to senescence syndrome or premature death.

2.8.3 Impact of staygreeness on drought resistance in sorghum:

Tenkouano *et al.*(1993) described that non-senescence is a delayed leaf and plant death resistance mechanism in *Sorghum bicolor* that circumvents the detrimental effects of reduced soil moisture combined with high temperature during post-anthesis growth.

Rosenow and Clark (1995) described two distinct responses to drought in sorghum. The pre-flowering response is expressed when plants are stressed during panicle differentiation prior to flowering (GS2) and the post-flowering response is expressed when moisture stress occurs during grain filling stage (GS3). Drought during post-flowering period accelerates the senescence, affecting the assimilatory capacity needed to avoid drastic reduction in a grain filing (Nooden, 1988). The yield reduction results from reduced seed size as well as premature plant death, stalk rot and lodging of post-flowering drought susceptible cultivars. Therefore any mechanism that postpones the onset of senescence and keeps the leaves green can benefit the corp.

Rosenow and Clark (1995) used the term 'staygreen' to describe the post-flowering drought resistance response. In sorghum, staygreen genes confer resistance to post-flowering drought stress by preventing the premature death of leaves and stems, plant senescence, stalk lodging and charcoal rot disease when the plants are exposed to moisture stress during the late stages of grain development. Under severe post-flowering drought conditions, the hybrids from non-staygreen parents showed 20-55 per cent lodging compared to less than 10 per cent lodging in hybrids with one staygreen parent (Rosenow 1995). Thus the staygreen trait has a major direct benefit to sorghum by reducing moisture stress type lodging associated with the premature leaf and stalk death. Rosenow (1995) observed a high correlation between good staygreen rating and resistance to lodging. He observed that the staygreen hybrids yielded better than commercial hybrids under stress levels, while at the same time exhibit a good staygreen rating and lodging resistance, indicating that the trait can be manipulated in sorghum and is quite independent of yield or yield potential.

Sorghum improvement based on selection for retention greenness has been described by Gerik and Miller (1984). They observed that the stover dryweight of a hybrid between two tropically adjusted 'non-senescent' (staygreen) sorghums was greater than between temperate senescent-type hybrids. Legget (1990) observed that sorghum resembles oat in that greenness is related to degree of annuality or perenniality. Generally sorghum is annual but staygreen types can survive for years through the generation of fresh tillers from the old plant bases and are thus good for ratooning. The annual or senescent types begin to dry during grain filling commencing with the lower leaves until

finally the whole plant is dead. In non-senescent perennial lines, leaves senesce more slowly and the stem and plant base do not die.

Throwing light on normal influence on senescence, Wittenbach (1977) suggested that cytokinins reduce the rate of loss of both chlorophyll and photosynthesis in senescing wheat seedlings. Amber *et al.* (1987) observed high levels of cytokinins than normal in some stray green lines. So Thomas and Smart (1991) suggested that staygreen lines of sorghum may be of Type B (functional satygreen type).

2.8.4 Inheritance of staygreen trait:

Walulue *et al.* (1994) observed that, the broad sense and narrow sense heritability estimates for the staygreen trait were 0.8 and 0.6 respectively, indicating that the staygreen trait is heritable and progress from selection can be attained. In a diallel study of staygreen trait Van Oosterm *et al.* (1996) observed that, the inheritance of the onset of senescence rate was dominant over a fast rate. Consequently a large relative GLAD (slow senescence) was partially dominant over a small relative GLAD. Because of the dominance of a large leaf area at flowering, the partial dominance in relative GLAD translated into over dominance for a large absolute GLAD indicating the importance of staygreen trait in sorghum for drought tolerance and yield improvement.

Molecular techniques for increased use of genetic resources

1 Monitoring plant material and assisting germplasm collection: One major application of molecular markers is for monitoring plant material and assisting in the management of collections. These markers are suitable for assessing how much allelic

diversity is present in a crop and they have the potential for providing unique fingerprints for each genetically distinct genotype, a useful means of identifying different cultivars.

2 **Organzing genetic diversity:** An assessment of genetic diversity based only on morphoagronomic traits might be biased because distinct morphotypes can result from a few mutations and share a common genetic background. However, isozyme studies (Ollitrault *et al.* 1990), as well as with RFLPs (Deu *et al.* 1994), helps in organizing genetic diversity.

3 **Rapid characterization of germplasm:** Early characterization of germplasm from collecting trips can be conducted using molecular markers. This has been demonstrated so far in the laboratory for *Cocoa* (Lanaud 1987) and *Hevea* spp. (Chevalier 1988).

4 **Assisting in the construction of core collections:** Molecular markers are essential for explaining whether existing genetic variability, which is assessed by morphoagronomic traits, is related to genetic diversity, which is assessed by measuring allelicfrequencies using molecular markers. This information can be used to construct core collections.

5 **Assessing genetic distances to guide the use of genetic resources:** Genetic relationships and distances between individuals are revealed by diversity studies and provides useful information for breeders, this can be exploited to obtain benefit from the potential of each population. For sorghum, crosses between groups *Kafir, South African Guinea* and *Caudatum* demonstrate a relationship between genetic distance and grain product (Chantereau *et al.* 1994). Molecular markers have, therefore,

provided a classification of sorghum cultivars which also allows better use to be made of the genetic resources of this species.

6 **Tracing the origin of cultivated varieties**: Molecular marker techniques are very useful in tracing the origin of most of the cultivated cereals, millets, pulses, oilseeds etc.

7 **Identifying different sources of interesting genes in the genome:** For diploid species, the interest in marker assisted selection (MAS) is growing and genome mapping allows us to understand the genetic control of characters and to identify favorable or unfavorable genetic linkages.

2.9.1 Study of molecular diversity using DNA markers:

A molecular marker is a sequence of DNA or a protein which can be readily detected and whose inheritance can be monitored. It is the variation in, or polymorphism of, molecular markers which can be used in genetic diversity studies. Planning the analysis of a genome in view of mapping favorable genes requires a careful assessment of the comparative diversity for molecular markers and for potentially useful morphoagronomic traits (Deu *et al.*,1992). Molecular marker technologies available to plant breeders include restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR) random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs), amplified fragment length polymorphism (AFLP) and DNA amplification finger printing (DAF), Walton 1993).

Sorghum production environments are characterized by various types of biotic and abiotic stresses. Developing sorghum hybrids with both increased yield potential and improved adaptation to these stresses is critical to increasing productivity, which is

highly dependent on efficient evaluation and utilization of exotic germplasm (Bramel *et al.*, 1994). Since the first introduction of RFLP markers in genetic mapping (Bostein *et al.* 1980), molecular markers have opened a new era for plant genetics and breeding. The genetic markers available now are morphological markers, isozymes, RFLPs, RAPDs, microsatellites sequence-tagged sites and AFLPs. Significant progress has been made towards the molecular mapping of the sorghum genome. Several linkage maps have been published by diverse authors (Hulbert *et al.*, 1990; Binelli *et al.* 1992; Berhan *et al.*, 1993; Pereria *et al.* 1994; Xu *et al.* 1994; Tao *et al.*, 1996). Many of these maps are highly saturated and developed with F_2 populations using sorghum and maize RFLP probes. Tao *et al.* (1996) used 40 maize genomic DNA clones and 80 RAPD primers to screen a backcross progeny segregating for osmotic adjustment and tag the genes for osmotic adjustment.

Periera *et al.* (1994) compared RFLP and QTL mapping in sorghum. An F_2 population derived from crossing *Sorghum bicolor* (CK 60) and *S. bicolor drummondii* was used to construct an RFLP linkage map. The map consisted of 201 loci distributed among 10 linkage Groups covering 1530 CM width an average of 8 CM between loci. Interval mapping was used to detect QTL for plant height, maturity, tillering, stalk diameter, panicle length, seed-branch length, peduncle diameter and seed weight. Xu *et al.* (1996) constcucted RFLP linkage map of *Sorghum bicolor* (L.) Moench, with sorghum low copy number and had 190 loci Grouped into 14 linkage Groups. The 10 largest linkage Groups consist of 10 to 24 markers and 103 to 231 CM. The RFLP frequency detected in this population using PCR-amplifiable low-copy number sorghum clone, and five restriction enzymes was 51 per cent. A minimum estimate of the numbers

of clones that detected duplicate sequences was 11 per cent. Null alleles occurred at 13 per cent of the mapped RFLP loci.

In studying these repetitive regions, Polymerase Chain Reaction (PCR) primers can be developed targeting specifically to the conserved sequences flanking the repetitive region. Southern analysis can also be used by hybridizing clones to the unique region of the locus. Polymorphism in VNTR's may be due to the differences in the number of repeating sequences. More than two alleles are present in a locus. An example is the SAT1 locus found in soyabean (Mortgante *et.al.*,1995) in which 25 alleles were found at this single locus. Polymorphism in Restriction Fragment Length Polymorphism (RFLPs) from either low copy sequence or cDNA clones are often the result of the presence or absence of a restriction site. Thus, in most instances only two alleles exist at a locus. Either a cut occurs which results in a short fragment, or no cut occurs in which a larger fragment is found. Since microsatellites can find more alleles at a locus than RFLP's, former is more informative.

SSRs offer a potentially attractive combination of features that are useful as molecular markers

•SSRs have been reported to be highly polymorphic in plants, and thus highly informative, providing many different alleles for each marker screened, even among closely related individuals.

•SSRs can be analyzed by a rapid, technically simple, and inexpensive PCR-based assay that requires only small quantities of DNA.

•SSRs are co-dominant and simple Mendelian segregation has been observed.

•SSRs are both abundant and uniformly dispersed in both human and plant genomes.

Microsatellite DNA markers are useful in many types of studies. They can be used in pedigree analysis to determine kinship among individuals, fingerprinting, forensics, genetic-mapping, and phylogenetic analysis. Genetic mapping is used particularly in crop species with low polymorphism such as wheat and soyabean. Since microsatellite DNA changes rapidly during the course of evolution, and is not influenced by selection, phylogentic analysis can be conducted and also can be used as an evolutionary timeclock by measuring the gain or loss of repeats in a genera over evolutionary time and can possibly detect when speciation occurs. A recent variation of polymerase chain reaction (PCR) has allowed DNA fingerprints to be obtained independently of prior sequence information. DNA amplification fingerprinting (DAF) is nothing but the enzymatic amplification of arbitrary stretches of DNA that is directed by very short oligonucleotide primers of arbitrary sequence to generate complex but characteristic DNA fingerprints. To determine the contribution of primer sequence and length to the fingerprint pattern and the effect of primer-template mismatches, DNA is amplified from several sources using sequence related primers (Merkin *et al.*,1997).

2.9.2 Study of molecular diversity in sorghum using DNA markers:

Molecular markers can be used in screening for drought tolerance (Tuinstra *et al.*, 1992; Rosenow, 1993), cold tolerance (Seetharama *et al.*,1994), tagging genes for disease resistance like downey-mildew resistance (Gowda *et al.*,1993), charcoal rot and anthracnose resistance (Tenkouano,1993), insects (shootfly, stem borer, midge and mirid bug) resistance (Seetharama *et al.*,1997) and to tag agronomically important traits (Xiang and Nguyen,1991). At least seven RFLP maps of sorghum have been constructed using

both DNA probes previously mapped in maize genome (Hulbert et al. 1990; Binelli *et al.* 1992 Whitkus et al.1994) and specific sorghum DNA probes (Chittenden *et al.* 1994; Pereira *et al.* 1994; Xu *et al.* 1994). Information on the relationship between and within wild and cultivated sorghum has been obtained by means of either nuclear or chloroplastic RFLPs (Aldrich and Doebley 1992; Cui *et al.* 1995) or mitochondrial DNA analysis (Deu et al 1994).

2.9.3 Role of molecular markers in drought resistance:

Selection for drought resistance is difficult due to the timing and intensity of water deficit and interaction between plant (especially growth stage) and other environmental factors. Rapid and precise evaluation of large breeding populations for drought resistant traits like staygreen and OA is the key towards incorporation of these traits by breeding. Bohnert *et al.* (1995) suggested molecular and genetic analysis of stress tolerance principles along with physiological studies. Molecular mapping will provide powerful tools to investigate cause-and effect relationships between physiological mechanisms and drought resistance, and eventually to improve the drought resistance efficiently. Tanksley *et al.* (1995), Martin *et. al* (1993) used isolated genes based on phenotype and map position (referred to as map-based gene cloning) for cloning several genes such as disease resistance gene *Pto* in tomato. Marker assisted selection (MAS) is a rapid and precise means to evaluate large breeding population. The molecular mapping of genes controlling staygreen in sorghum will open a way for cloning such genes and their insertion into drought susceptible lines. Studies were conducted with a view to using molecular markers to identify genetic loci associated with the expression of pre-flowering drought tolerance in sorghum (*Sorghum*

bicolor). Two genotypes with contrasting drought reactions, Tx7078 (pre-flowering tolerant, post-flowering susceptible) and 835 (pre-flowering susceptible, post-flowering tolerant), were selected as parents for a sample of recombinant inbred (RI) lines. Ninety-eight RI lines were evaluated in two different years under conditions of pre-flowering drought and full irrigation. This information was used to quantify the drought tolerance of each line. The population was also genotyped with 150 RAPD and 20 RFLP markers that mapped to 17 linkage groups. By means of these markers, 6 regions of the genome were found to be specially associated with pre-flowering drought tolerance. Eight additional regions were more generally associated with yield or yield components under fully irrigated conditions. Several loci were associated with the expression of drought tolerance under both mild and severe drought stress conditions.

Tuinstra *et al.* (1996) identified QTLs associated with pre-flowering drought tolerance in sorghum to identify genetic loci associated with the expression of pre-flowering drought tolerance in sorghum (*Sorghum bicolor*). Two genotypes with contrasting drought reactions, Tx7078 (pre-flowering tolerant, post-flowering susceptible) and B35 (pre-flowering susceptible, post-flowering tolerant), were selected as parents for a sample of recombinant inbred (RI) lines. Ninety-eight RI lines were evaluated in two different years under conditions of pre-flowering drought and full irrigation. This information was used to quantify the drought tolerance of each line. The population was also genotyped with 150 RAPD and 20 RFLP markers that mapped to 17 linkage groups. By means of these markers, six regions of the genome were found to be specially associated with pre-flowering drought tolerance. Eight additional regions were more generally associated with yield or yield components under fully irrigated

conditions. Several loci were associated with the expression of drought tolerance under both mild and severe drought stress conditions. The phenotypic characters of grain yield, yield stability, seed set stability and height stability related to pre-flowering drought tolerance were tagged to these six genomic regions. Significant genotype x drought treatment interactions existed for yield, seed weight and height. Considerable cross-over interaction for yield, seed set and height was observed indicating segregation for drought tolerance in the RIL Lines.

2.9.4 Tagging QTLs associated with senescence in sorghum:

Sorghum is a diploid cereal (2n=20) with a relatively small genome of 748-772 Mbp (Arumuganathan and Earle 1991).

For traits like staygreen, it is hard to determine whether the desired effect linked with a marker locus is due to one or more genes effecting the trait. Therefore, the term QTL is used to describe a region of the chromosome that has a significant effect on the quantitative trait. Tanksley (1993) described the underlying genetic basis of using molecular marker to tag the QTLs as the linkage disequilibrium between alleles at the marker locus and alleles at the QTL. Tanksley (1995) showed that a single major QTL can account for 10-50 per cent of phenotypic variation in seggregating population. Several statistical methods like one-way ANOVA (Stuber *et al.*, 1992) with SAS (SAS, 1990) and interval mapping with computer program MAPMAKER/QTL (Lauder *et al.*, 1987) can be used for systematically searching for QTLs (Dudley 1993, Tanksley 1993).

Xu *et al.* (1996) mapped QTLs associated with staygreen trait in sorghum using a recombinant inbred line population (RIL) developed from the cross B35 × TX7000 and B35 × TX 430. The RFLP data showed 1:1 segregation of B35 and Tx7000 alleles at

most loci in the F_7 RIL population, and had 110 markers covering a map distance of 1407 CM. Over 70 markers were mapped. Xu *et al.(1996)* located major QTLs associated with staygreen on linkage Group, C, G, H altogether accounting for about 48 percent of phenotypic variation with QTL on Group C alone accounting to 38 percent. The map resolution at the QTL interval varied among approximately 5 cM for QTLs on linkage Group C and over 10 CM on linkage Group G and H.

Context of present study

Although exhaustive work has been done on the study of staygreen trait and grain growth aspects of sorghum, there is still a need to evaluate the sorghum genotypes of the trait under the conditions relevant to post-rainy season sorghum production in India. The behavior of the staygreen trait, its impact on grain growth, yield and yield attributes maturity and duration of the crop needs further study. Also the nature of leaf senescence needs to be further elucidated. Hence the present study was taken up.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The genotypes were evaluated for staygreen trait and influence of this trait on grain growth, relative water content (RWC) at physiological maturity, crop yield at the farm of International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru, Andhra Pradesh.

3.1 Location:

Field trail was conducted at ICRISAT Research Farm at Patancheru. The genotypes were grown in irrigated environment in field RCE 24A. The area of the plot was 28.8 m^2 divided into two equal parts where 8 genotypes were grown in 8 blocks of size 1.6 × 0.75 m each.

3.2 Nature of Soil:

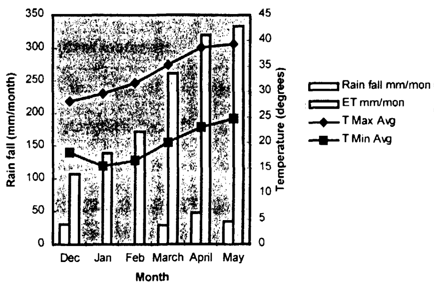
The soil type is red loamy, alfisols, moderate fine to medium sub-angular blocky structure, slightly hard, friable and very slightly sticky. Therefore the soil restores soil moisture for longer period of time. The total plant available water depth is about 8cm.

3.3 Growing Season:

The crop was planted on 9th December 1997, during the post-rainy season of 1997-98. As the stored soil moisture depleted gradually, the crop was irrigated once a week.

Table 1 Weather during post rainy (*Rabi*) season 1997-98 at ICRISAT

Month	Rainfall mm/mon	ET mm/mon	T Max Mean	T Min Mean	RH 07 %mean	RH 14 %mean	Wind speed km/h	Sunshine hr/day
December	31.0	107.4	28.1	18.0	94.2	54.8	8.3	7.0
January	0.0	139.3	29.6	15.4	93.4	41.5	7.2	9.1
February	0.0	171.9	31.8	16.5	80.4	33.1	6.7	9.5
March	29.6	261.0	35.3	20.0	74.7	28.7	8.6	9.4
April	48.8	318.8	38.7	23.0	63.9	27.8	9.2	9.2
May	35.2	332.6	39.4	24.7	63.1	30.2	10.2	8.7



3.4 Agroclimatology and weather during crop period:

In the month of December 1997, the rainfall recorded was 31mm. As most of the rainfall occurred before the sowings, there was enough soil moisture in the soil for seed germination. There was no rainfall during the months of January and February 1998. So irrigation was given at weekly interval to replenish the soil moisture for optimum crop growth. Evapotranspiration (ET) increased steadily from 107.4 mm/month during the post-flowering crop growth stage. So the supplemental irrigation was provided by furrow irrigation once in a week. The rainfall received in March, April and May 1998 were 29.6, 48.8 and 35.2 mm, respectively. Therefore during the flowering and physiological maturity stages crop was irrigated to avoid stress conditions. The average maximum temperatures for the months of Dec 97, Jan 98 and Feb 98 were 29.6 ^{0}C, 31.8 ^{0}C and 35.3 ^{0}C respectively, which were ideal for optimum crop growth and flowering.

3.5 Description of the plant material:

The population used in the study of the staygreen trait consists of eight contrasting genotypes. B 35 is a staygreen, post-flowering drought tolerant and pre-flowering drought susceptible cultivar, which is also resistant to charcoal rot. It is a converted sorghum from IS 125555 (*Zera zera* from Ethiopia). Tx 7078 is a pre-flowering drought resistant, post- flowering drought susceptible and senescent line and also is a charcoal rot susceptible. E 36-1 is a terminal drought sensitive and staygreen cultivar where as R16 is terminal drought susceptible and charcoal rot susceptible cultivar. IS22380 is also a terminal drought susceptible and charcoal rot susceptible cultivar. M35 is a popular *Rabi* sorghum cultivar, generally drought tolerant but susceptible to charcoal rot. CSH6 is a rainy season hybrid, highly susceptible to charcoal rot and moderately susceptible to terminal drought.

3.6 Experimental details:

Crop : *Sorghum bicolor (L.) Moench*

The date of planting: 9th December 1997.

The genotypes were planted on in two row plots which were further divided into blocks of size $1.6 \times 0.75\ m^2$ size in 2 replications.

Design of the experiment: Randomized Block Design (RBD).

Details of Treatments:

Main treatments are 8 genotypes (B 35,IS 12555, TX 7078, E 36-1, R 16, IS 22380, M 35, and CSH 6). From each treatment 8 plants were selected randomly for observations on senescence and grain growth; 6 plants from each treatment were tagged for measuring relative water content (RWC) at physiological maturity stage and for yield attributes.

Number of replications: 2

Number of plots per replication: 2

Number of blocks per plot: 8

Spacing: 75 (between rows) × 10 cm (between plants in a row).

3.7 Crop Management:

Preceding crop: The land was left fallow in the preceding (1997) *Kharif* season.

3.7.1 Field preparation: The field was ridged several weeks before planting. It was then properly divided into 2 row plots, which were further divided into 8 blocks of size 0.75 ×1.6 m.

3.7.2 Method of planting: Seed was sown manually by following line sowing method. The seed rate used was 15 kg/ha.

3.7.3 Emergence: Emergence was recorded on 13th December 1997, 4 days after sowing (DAS).

3.7.4 Fertilization: A basal dose of 150 kg/ha di-ammonium phosphate (DAP) was incorporated into the soil. Urea at the rate of 100 kg/ha was top dressed approximately 20 days after sowing (DAS) i.e. on 29th December 1997.

3.7.5 Irrigation: The field was given a light (15-20 mm) sprinkler irrigation on 10th December (i.e. one day after sowing). Furrow irrigation was given 15 DAS and then repeated at 21 DAS to recharge the soil profile fully. Later furrow irrigations were given once in a week until approximately 30 days after 50% flowering.

3.7.6 Inerculture: Mechanical (interculture) cultivation was taken up twice at 17 and 20 DAS. The initial crop growth period is critical for weed control. When the canopy cover was almost complete and crop was knee- high, no further weeding was needed as at this stage the crop completely smothers the weeds.

3.7.7 Disease control: Seed-borne infections were prevented by seed treatment with *Ridonil*, @ 0.1 ml a.i per kilogram of seed.

3.7.8 Pest control: The major insect pests effecting the young plans at early stages were Sorghum Shoot fly (*Atherigona soccata*), Sorghum aphid (*Rhopalsiphum maidis*) and Earhead bug (*Calocoris angustatus*). The Shoot fly is the most serious problem. It was controlled by application of carbofuron 40 kg/ha with the seed at the time of sowing and with 3 sprays of cypermethrin 25% at 5, 10, and 30 days after emergence (DAE).

Insect damage during GS2 (panicle development stage ending with 50% flowering) causes maximum damage in terms of yield reduction. To control insect pests during this stage, *Carbofuron* 5 % granules were applied within the whorls @ approximately 0.2 g/plant. To control head bugs and aphids *Rogor* and *Sandovit* were sprayed during grain filling stages.

3.7.9 Bird control:

Birds (mainly weavers, sparrows and doves) contribute a serious problem to sorghum especially during maturity and cause severe yield reduction. The bird menace was checked by manning the field and hitting the cans and drums to make noises to scare away the birds.

3.7.10 Harvesting:

The grain was ready to harvest about a fortnight after the physiological maturity is attained. The physiological maturity in sorghum can be judged by black layer formation at the hilum. The mature seed ready for harvest can not be indented by the thumb nail and breaks clear when bitten with the front teeth. The panicles were harvested with sicatures and bagged. Later the culm was also cut to the ground level and bagged separately.

3.7.11 Drying: The grain was sun-dried for 5-7 days to harden the pericarp, to decrease grain moisture content (to prevent fungal attack during storage) and for easy thresing.

3.8 Parameters for observation and data recording:

Data on the expression of staygreen and grain growth was taken to study their consequences on yield and yield attributes. The observations broadly fell into 5 groups.

- Phenological traits i.e days to 50% flowering and physiological maturity.
- Staygreen or senescence traits
- Grain growth
- Relative water content (RWC)
- Yield attributes

3.8.1 Phenological traits:

(a)Time of flowering: The 50% flowering dates for each of the plants tagged in each genotype was recorded. The date on which approximately 50 percent of the spikelets in the majority of plants within the plot started shedding pollen up to half way towards the base of the panicle was recorded. Data was recorded twice in a week.

(b) Physiological maturity: When sorghum grain attains physiological maturity and a black layer is formed at the hilum. The maturity date in the field for each of the line was determined taking into consideration the black layer formation of the grains in the middle of the panicle. The panicle grains were checked at 2 days interval and the date on which majority of plants within a plot showed the back layer at hilum was taken as the date of physiological maturity.

3.8.2.Senescence traits :

All Senescence observations were taken on eight plants tagged in each genotype by counting the number of green leaves. The plants tagged were numbered 1-8, four in each row of the two row plots. The observations (13) were taken at weekly interval from 50 per cent flowering to harvest maturity.

Leaf number count:

The senescence study was taken up using leaf number count. The number of green leaves for each tagged plant at flowering (when the plant is supposed to have developed maximum canopy) was recorded at weekly interval, from the date of 50 % flowering till harvest, the green leaf number was recorded. The average for the eight tagged plants in each plot gave the leaf number for a given tagged plant in each genotype on a particular day after flowering.

Relative green leaf number:

The relative green leaf number was computed at each date of observation in all the eight genotypes in each replication using the following formula:

Relative green leaf number = (absolute leaf number at a given date/ absolute leaf number at flowering)× 100

3.8.3 Grain growth traits: The grain growth observations were taken on 8 plants tagged in each genotype. The plants tagged were numbered 1-8, 4in each row of the 2row plots and from the panicle of each tagged plant 4-5 spike-lets were picked up in all the genotypes and oven-dried for 36 h at 70 ^{0}C. The dry weights of the oven dried samples were recorded. The average of grain weights of the 8 tagged plants in each genotype was taken into consideration for comparing the grain growth traits among the eight genotypes. The observations were taken at 3-4 days interval (i.e. twice in a week) starting from spike-let formation to harvest maturity. Thus observations were taken at 3,7,10, 14,17,21,24,28,31,35 days after flowering (total of 10 observations).

3.8.4 Relative water content:

The relative water content (RWC) at physiological maturity was recorded by tagging six plants in each genotype. The plants tagged were numbered 1-6, three in each row of the two row plots. Twenty leaf discs were taken using a leaf punch in the middle part of the 3rd leaf from the top in each tagged plant. Field fresh weight, turgid weight (by dipping the leaf discs in petridishes containing distilled water for 6h, then using a blotting paper the excess water adhering to leaf discs was removed) and dry weight (after oven-drying the leaf discs at 70 ^{0}C for 3days) were recorded. The RWC at physiological maturity was determined by using the following formula:

RWC = (fresh weight - dry weight/turgid weight - dry weight) × 100

The average of the observations taken in each genotype was taken into consideration to compare the RWC of the eight sorghum genotypes at physiological maturity stage.

3.8.5 Yield attributes:

With regard to the yield attributes, the observations for the variables under study were obtained on 6 plants tagged in each plot. The heads from each plot were bagged separately, tagged and sun-dried. After threshing the grain was separated and the grain weight (g) was determined using an electronic balance.

Head weight: The mature heads from the six tagged plants and their tillers were cut with a sicature leaving about 5 cm below the lowest node of each panicle. These six panicles were individually weighed with a common balance and later averaged.

Stalk weight: The 6 culms were cut to the base and the leaf separated from stem and the weight of the culm (leaf sheath + stem) was recorded as per plant basis and averaged.

Grain weight: The heads from each plot were harvested from the 6 tagged plants and weighed. They were later bagged separately tagged and sun-dried. After threshing, the grain was separated and the grain weight was recorded using an electronic balance.

Standard conversion: To express the head weight, stalk weight and grain weight on standard unit area (m^2) basis, the six panicle weight, grain weight, and six stalk weight were multiplied with a factor 'h' derived based on plot size and spacing.

Plot size = $0.75 \times 1.6 = 12000cm^2 (2.12m^2)$

Spacing = $75 \times 10 = 750\ cm^2$

Approximate number of plants per plot = 12000/750 = 16 plants

$\therefore$ Number of plants /m^2 = 16/1.2= 13.33 plants, approximately.

Weight of a variable per square meter = Six plant weight × 2.22

Head weight /m ^{2} = six heads weight ×2.22

Stalk Weight /m^2 = six stalk weight × 2.22

Grain weight /m^2 = six head grain weight × 2.22

100 seed weight: The dried and threshed grain was separated from husk, chaff and other inert matter. The seed was then taken on a white blotting paper and 100 randomly selected seeds were counted and separated. The 100 seeds were weighted with a sensitive electronic balance and recorded.

Biomass /m^2: It is the total plant biomass /unit area of land. It was calculated by summing up the head weight /m^2 and stalk weight /m^2

Biomass /m^2 = head weight /m^2+ Stalk weight /m^2

Harvest index: it is the ratio of grain weight/m^2 to the total biomass/m^2 expressed on per cent basis.

HI (%) = (grainweight /$m^2 \div$ Biomass /m^2) $\times$100

Threshing percentage: It is the ratio of the grain weight/m^2 to the head weight/m^2 x 100

Threshing percentage = (grain weight /m^2)/(head weight /m^2) $\times$100

Seed density /m^2: It is the number of seeds in a unit area and gives an idea of the panicle compactness yield. It was calculated from 100 seed weight and grain weight/m^2 for all genotypes.

Seed density /m^2 = grain weight /m^2 $\div$ 100 seed weight

Leaf number at harvest : the number of nodes over which the disease has spread in a plant, the length of the spread of the disease and the percent of plants showing soft stalk at maturity and harvest were recorded under charcoal rot traits.

3.9 Statistical analysis :

Statististical analysis was done using GENSTAT 5 statistical software.

The data were analysed for the following:

1. Regression analysis for the staygreen trait.
2. Cluster analysis to group the genotypes based on their senescence pattern.
3. Calculation of relative green leaf number duration under the regression curve.
4. Analysis of variance for agronomic traits.
5. Correlation matrices between all relevant variables.
6. Regression analysis for the grain-growth traits.

3.9.1 Regression Analysis

A regression curve of the relative leaf number for all the Lines and relative leaf area for the 5 selected entries was plotted against DAS. The regression curve fitted was a nonlinear, logistic curve which is typified by the equation.

$$Y = A + C / 1 + e^{-b(x-m)}$$

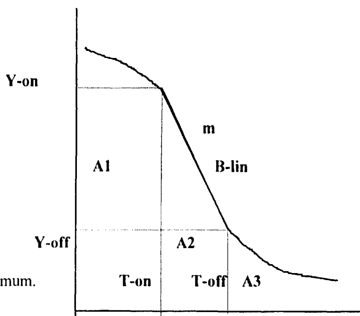
Where ,

A is the lower asymptote

C is the range

b is the slope of the curve

m is the point of inflection where slope is maximum.



The regression curve is continuous and constantly changes unlike the discontinuous curve where the definite point of start of slope and end of slope of the linear phase in the curve can be identified. However the curve presents two points, where the rate of change of slope is maximum. These two points can be considered as the points of onset (T-on), start and offset (T-off), end of linear rate of senescence.

The two points T-on and T-off were obtained using the Newton-Raphson equation and differentiating the 4th differential with respect to the third differential as described by Rajaraman (1990).

$$\textbf{T-on and T-off} = X_0 - [\, f(X_0)^{'''} / f^{''''}(x_0) \,]$$

The slope at 'm' (**b- m**) = **bc/4**

Which is the ratio of first differential to second differential.

The relative leaf number at each given point can be obtained by substituting the 'b', 'm', 'c' and 'x' values obtained from the regression fit for each Line and solving for 'Y'. Finally the parameters given under which define the senescence pattern of a Line were obtained.

TIME PARAMETERS

T-on: Onset of Linear phase of senescence(DAF).

T-off: Offset of Linear phase of senescence(DAF).

T-m: Time to maximum rate of senescence(DAF).

T- lin: The duration of Linear phase of senescence.[(T-off)- (T-on)]

RELATIVE LEAF NUMBER PARAMETERS

Y-on: Relative leaf number at onset.

Y-off: Relative leaf number at offset.

Y- m: Relative leaf number at the point of inflection.

Y-lin: The decrease in relative leaf number from onset to offset of Linear phase.

$$Y = (Y\text{-off}) - (Y\text{-on})$$

SLOPE PARAMETERS

b: The general slope of the regression curve.

b-m: The maximum slope occurring at the point of inflection.

B-lin : The Linear rate of senescence during the Linear phase.

B-lin = Y-lin/ T-lin

Relative green leaf number duration (RGLND) = $\sum\Delta Y$

Relative green leaf number duration is gives an estimate of relative green leaf number over a given period of time. It is obtained by integrating the regression function for 'Y' between any two desired points on the regression curve.

The area under the regression curve was integrated over three sections

A1: RGLND up to onset of Linear phase of senescence (Flowering to T-on).

A2: RGLND during Linear phase of senescence (T-on to T-off).

A3: RGLND from offset on Linear phase to harvest (T-off to harvest).

A: Total RGLND from flowering to harvest **A = A1+A2+A3**

STUDY OF DIVERSITY USING DNA MARKERS

Table: 2 List of genotypes used for studying DNA polymorphism

4 genotypes (M35-1, Btx 623, QL 41, E36-1 were repeated)

Set	Genotype	Comment
1	E36-1	Staygreen, stalk-rot resistant
2	KS19 (Q101)	ex. Short kaura × CK060
3	QL10(Q102)	SCMV resistant version of KS 19
4	QL12(Q103)	SCMV resistant version of KS 19
5	QL27(Q104)	ex. (QL10×TAM2566) × TAM2566
6	QL39	RIL- parent
7	B35-6	RIL- parent
8	R9188	Rio derivative
9	RTx430	Texas
10	BTx623	Senescent
11	P898012	Unknown sudan source
12	P954035	Unknown sudan source
13	PSSD2-19	ex. B35×Tx7078
14	PSSD2-27	ex. B36×Tx7078
15	ICSV 112	
16	M35-1	Drought resistant
17	R16	Moderately staygreen
18	QL41	
19	Tx7078	Senescent
20	P 721	
21	IS 9377	Non-senescent
22	IS22380	Senescent
23	IS296B	Senescent
24	PA62	Drought resistant
25	SP31623-1	Staygreen
26	SP31625-1	Staygreen
27	SP31672-1	Staygreen
28	IS5767	Staygreen
29	ICSV247	
30	DJ1195	Drought resistant
31	IS10699	
32	IS24761	
33	IS9471	Mold resistant
34	IS34219	Mold resistant
35	IS7173	Mold resistant
36	IS23773	Mold resistant
37	IS14384	Mold resistant
38	IS19674	
39	SPV475	Midge resistant
40	IS2313	
41	IS22256	
42	IS2822	
43	IS22238	
44	IS22247	
45	ICSV112	
46	IS9830	
47	QL41	
48	IS12555	Staygreen

3.10.1 Extraction and purification of genomic DNA:

Genomic DNA was extracted from seedlings of sorghum following a modified CTAB DNA isolation procedure described by Saghai-Maroof *et al.*(1991). About 5 grams of fresh and clean leaf material from young actively growing seedlings were collected and frozen in liquid nitrogen. The lyophilized tissue was then ground with dry ice in a coffee grinder and the powdered material was transferred to 30 ml loosely capped polypropylene tubes which were stored overnight at -20 °C so that the CO_2 diffuses out. Pre-warmed (60°C), 10-12 ml isolation buffer was added to each tube. The samples were then incubated in water-bath for 2 hours with occasional gentle mixing. After taking out the samples from water-bath and cooling to room temperature, an equal volume of chloroform and isoamyl alcohol mixture (24:1) was added to the samples and mixed gently to form an emulsion. The samples were then centrifuged for 20 minutes at room temperature in a swing bucket rotor using *Sorvall RC5* preparative centrifuge. The supernatant was reextracted with equal volume of chloroform and isoamyl alcohol mixture (24:1) at 2°C. The supernatant was transferred to corex tubes and the DNA was precipitated by adding 0.6 volumes of ice-cold isopropanol. The DNA was spooled out with the bent ends of pasture pipette, washed with 76 per cent ethanol followed by a 100 per cent ethanol wash. Next DNA was vacuum dried for a few minutes, and dissolved in 2 ml of 1x TE(10 mM Tris. HCl, 1 mM EDTA (pH 8) containing RNase (250μ g /ml). The polysaccharide impurities were removed by treating the sample with 1/10 volume of 5 M NaCl for 20 min at 4^0C, followed by centrifugation at 6000 rpm for 20 min at 4^0C. DNA was further purified by extracting with equal volume of chloroform, and precipitating by the addition of 1/10 volumes of 3 M sodium acetate and 2 volumes of chilled (-20°C) absolute ethanol. The precipitated DNA was spooled, washed, with 70 per cent ethanol, dried under vacuum and dissolved in 200 ul of 1x TE (10 mM Tris.Cl pH

8.0, 1 mM EDTA pH 8.0).The quantity and purity of the DNA samples were determined spectrophotometrically by measuring the absorbance at 260 and 280 nm with a spectrophotometer. DNA quantity was estimated considering that 1.0 OD unit at 260 nm is equivalent to 50 ug of DNA (Sambrook *et al.*,1989).

3.10.2 Ethidium bromide staining:

In this procedure, DNA samples were subjected to agarose gel electrophoresis. An aliquot of genomic DNA was run on 0.8% ethidium bromide stained agarose gel. The dye intercalates into the DNA double helix, and the intensity of floresecence induced by UV light is proportional to the amount of DNA in the lane. Comparison to a standard digest of λ Hind III marker DNA gives an estimate of the amount of DNA in the samples.

This technique also allows (1) DNA quantitation (2) estimation of the extent of contamination by RNA (which usually runs ahead) and (3) evaluation of DNA quality (the extent of degradation)

TABLE B: CTAB Buffer Composition:

STOCK	100 ml	Comment
d H_2O	46 ml	Autoclaved
1.0 M TRIS pH 8	20 ml	Autoclaved
5.0 M NaCl	28 ml	
0.5 M EDTA	4 ml	Autoclaved
$Na_2 SO_3$	250 mg	
C TAB	2 g	
Mercaptoethanol	500 μl	

3.10.3 Microsatellite analysis:

The microsatellite /simple-sequence repeats (SSRs) assay was performed by following the method of Mellersh and Sampson (1993). PCR reaction was performed with a reaction mixture containing a total of 2.5 µl of genomic DNA (concentration of genomic DNA is 100ng/µl), 50mM $MgCl_2$ (*Promega*), 2µl dNTPs (*Sigma* chemicals), 10X PCR buffer (*Promega*), 0.5 µl forward primer, 0.5 µl reverse primer and distilled water to make volume 25 µl per reaction. Amplification was carried out using Perkins Elmer Gene Amp PCR system for 37 cycles described by Mellersh and Sampson (1993) with the following temperature profiles Denaturation was carried out at 94 ^{0}C for 30 sec and extension was carried out at 72 ^{0}C for 1 min. Annealing was carried out between the denaturation and extension steps for 30 sec at 65 ^{0}C for the first 2 cycles, followed by at 63 ^{0}C for 4 cycles, at 59 ^{0}C for 6 cycles, at 57 ^{0}C for 6 cycles and at 55 ^{0}C for 15 cycles. Thus a total of 37 cycles were carried out. A control without template DNA was included in each set of reactions. The reaction products were resolved by overnight electrophoresis on gels containing 3% *Nu-Sieve* Agarose (*FMC*) 3:1 in 1X TBE with ethidium bromide at 25V. For radio-labeled PCR, DNA at a concentration of 20ng/µl was used instead of 100 ng/µl as in case of unlabeled PCR.

Table: 3 List of Microsatellite primers used for studying DNA polymorphism

No.	SSR ID	Primer sequence (5'-3')
1	V7512	GATAGATAGATAGATA
2	V7520	GAGAGAGAGAGAGAGA
3	V7514	CACCACCACCACCAC
4	V7525	ACCCGGGGATCCTCTAGAGTCGAC
5	V7518	GTGTGTGTGTGTYR
6	V7513	GACAGACAGACAGACAGACA
7	V7523	GTGTGTGTGTGTGTGT
8	V7526	CAGGAAACAGCTATGAC

3.10.4 Reactions with γ^{32}P-ATP :

T_4 PNK buffer was diluted ten fold (1:10 dilution, 0.5 µl enzyme + 4.5 µl dilution buffer). Labeling reaction (Forward primer) 10µl reaction volume was done by using 5µl forward primer, 1µl 10X T_4 PNK buffer, 1µl(10µ ci) γ^{32}P-ATP, 1µl diluted T_4 PNK and 2 µl distilled water(total volume = 10ml). Then the labeled forward primer was incubated at 37^{0}C for 1 to 2 hours followed by denaturation of enzyme by heating on a heating block at 70 ^{0}C for 10 min, then it was chilled on ice. PCR reaction was performed with a reaction mixture containing a total of 2.5 µl DNA (20ng/µl), 2 µl dNTPs, 2.5 µl 10 x PCR buffer (*BRL*), 0.5µl reverse primer, 1µl labeled forward primer, 1µl 50mM $MgCl_2$, 0.2 µl *Taq* polymerase(*BRL*) and distilled water to make volume 25 µl per reaction. PCR reaction was carried out using Perkins Elmer Gene Amp PCR system for 37 cycles by following the procedure described above (Mellersh and Sampson, 1993)

3.10.5 Gel Electrophorcsis of radio-labeled PCR Products:

Equal volume (25µl) of bromophenol blue dye was added to the samples and the samples were heated at 85-90 ^{0}C for 5 min, chilled on ice before loading. The then samples were loaded on a denaturing (6% acrylamide, 7.5 M urea, 1X TBE) gel. Samples were electrophorized at 1500 V for 2 hours, then transferred to Whatman 1mm filter paper and after covering with *Saran-Wrap* drying was done under vacuum for 2 hours at 80 ^{0}C.

3.10.6 Data analysis of Microsatellite polymorphism studies

To evaluate the genetic diersity in sorghum genotypes were screeened with 8 diferent microsatellite primers obtained from Univresity of Southampton, U.K. of the 8primers, 4 primers (V7512, V7518, V7523 and V7525) showed goo polymorphism. Cluster analysis was carried out using the data obtained by screening 48 genotypes with 4 printers. Only the most intense and reproducible DNA bands were considered for analysis. These were scored as 1 (for presence) and 0 (for absence). Pairwise genetic distances were calculated by the percentage disagreement method. These data were used to cluster the accessions into the defined groups using GENSTAT software package.

3.11 RFLP analysis in sorghum

Genomic DNA was digested with restriction enzymes *Eco R I, ECO R V, Bam HI* and *Hind III* These restriction enzymes restrict the DNA at different sites depending upon the frequency of the repeating restriction sites.

Protocol for Restriction digestion:

Reaction volume: 40 µl

Concentration of genomic DNA: 10-15 µg

Sorghum DNA was digested with restriction enzymes, *Eco RI, Bam HI and Hind III.*

Reagents for master mix

Restriction buffer	600µl
Restriction enzyme	240µl
Spermidine	240µl
Total reaction volume	1080µl

Mastermix was dispensed into 58 reaction tubes (18µl to each). Next template DNA is added to each tube such that the total amount of DNA was in between 10-15 µg. Reaction tubes were briefly centrifuged and incubated at 37^{0}C for overnight for complete digestion of sorghum genomic DNA. The samples, after digestion, were run on the 0.8% agarose gel at 40 V. If the restriction enzyme is a tetra-cutter, the gel concentration was high because the recognition sites for a tetra cutter 100 times more frequent than that of a hexa-cutter thus producing fragments of smaller length which will migrate up to the end of the gel. Next the run was over the gel was stained with EtBr to examine the restriction pattern. Next the gel was de-stained in distilled water for about 30 min, and the gel was transferred on to the vacuum blot apparatus to transfer the DNA fragments to the nylon membrane.

Southern Blotting

Blotting of digested DNA fragments on to the nylon membrane. The nylon membrane was cut according to the gel size (*Amersham Hybond N*) and it was marked. The gel was carefully transferred on to the membrane and the depurination using 0.25 M HCl, denaturation using 0.4 N NaOH and neutralization reactions were carried out for 20 min each. The transfer was done in 20 XSSC solution for one hour. After the transfer, the DNA was cross-linked to the nylon membrane, followed by baking at 80 ^{0} C for one hour. The blot was wrapped with a *saran wrap*, preserved at 4 ^{0} C. The preserved blots were ready for the hybridization.

Separation of DNA fragments by gel electrophoresis:

The restriction fragments produced are commonly separated by electrophoresis on agarose gels. Since the fragments would be seen as a continuos 'smear' if stained with ethidium bromide, staining alone cannot detect polymorphisms. Therefore, it is necessary to detect specific fragments using hybridization methods.

RESULTS

CHAPTER IV

RESULTS

4.1 LEAF SENESCENCE STUDIES

Data on number of green leaves retained at weekly intervals after flowering was used in leaf senescence studies through regression analysis by plotting leaf number against day after flowering (DAF). Eight genotypes were selected for leaf senescence study (B35, IS12555, Tx7078, E36-1, R16,IS22380, M35-1, CSH6) and the logistic curve plotted for relative leaf number revealed similarity of curves for relative leaf number (Fig. 2).

The regression curve plotted using relative leaf number as a function of time to study the senescence pattern and the genotype differences for the staygreen trait revealed wide variations in the population for leaf senescence.

The R^2(regression coefficient) values for the eight selected genotypes was greater than 0.97 for relative leaf number indicating that the logistic equation gave a good fit. The ratio of the estimated values of the constants 'b'-the slope of the curve, 'm'-the point of inflection and 'c'-the range; to their standard error values were significant (at 5 % level of significance) for the eight genotypes for relative leaf number indicating that the parameters (b, m, c) were effective in defining the logistic equation fitted and the equation is not over parameterized (Table 4). The correlation of days after flowering with both relative leaf number was greater than 0.98 (5 % level of significance) in all cases indicating that green leaf number decreased progressively after flowering. The results indicated that relative number is very effective in senescence studies.

Having inferred that relative leaf number is effective in studying senescence, a detailed regression analysis by fitting the logistic function was done for each of the genotype under wet environment. Using the three primary regression parameters of the logistic curve the parameters which define the senescence, T-on (the onset of senescence), T-off (the offset of senescence), T-lin (the Linear duration of senescence), b-m (maximum rate of senescence), B-lin (the Linear rate of senescence) were found out.

The general slope of the fitted curve (b) ranged from a maximum of 0.07 in the genotype IS22380 to a minimum of 0.01 in genotype B35. The range 'c', varied from 92.25 in genotype E36-1 to 253.1 in genotype B35. The point of inflection (m) occurred earliest at 11 days after flowering in genotype B35 and latest 68 days after flowering in genotype R16. The onset of senescence occurred earliest at 8 DAF in is12555 and latest at 20 DAF in genotype IS22380. The offset of senescence occurred as early as 60 DAF in IS22380 followed by M35-1 and latest in genotype E36-1(78 DAF). The duration of the linear phase was maximum in B35 (65 days) and minimum in IS22380 (43 days). The maximum rate of senescence(observed at the point of inflection) was the highest in E36-1 (-1.1) and the lowest in genotype B35 (-3.99). The maximum relative leaf number at onset of senescence occurred in Lines 57, 65 and 75 while the minimum occurred in CSH6, IS12555 and E36-1 respectively followed by B35, M35-1, Tx7078 and R16 while the minimum occurred in IS22380. At offset, the maximum relative leaf number occurred in genotype E36-1while the minimum occurred in M35-1 and Tx7078. The relative leaf number at 'm' (point of inflection where slope is maximum) was highest in genotype E36-1 followed by M35-1, R16, Tx7078 and lowest in genotypes IS12555, CSH6, M35-1 and B35 respectively.

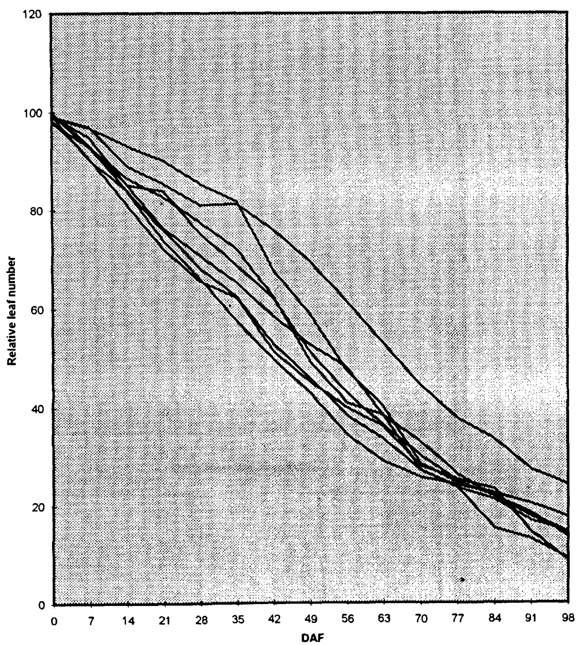


Fig. 2 Regressioncurve of eight sorghum genotypes for relative leaf number
120
100
80
60
40
20
0
Relative leaf number
0
7
14
21
28
35
42
49
56
63
70
77
84
91
98
DAF

Fig. 3 Comparision of regression curves in eight sorghum genotypes for grain growth patern

100grain weight (g)

4.5
4
3.5
3
2.5
2
1.5
1
0.5
0

1 2 3 4 5 6 7 8 9 10 11 12

Days after flowering (DAF)

- B35
- IS22380
- Tx7078
- E36-1
- R16
- IS22380
- M35-1
- CSH6

Table: 4 Comparision of regression parameters for relative leaf number with grain gr

	Relative leaf number				Grain growth			
Entry	constants	estimate	s.e	b/s.e	constants	estimate	s.e	b/s.e
B35	B	-0.01577	0.00873	-1.806415	B	0.04044	0.0162	2.496296
	M	10.72	3.72	2.88172	M	19.957	0.521	38.30518
	C	253.1	15.9	15.91824	C	0.4294	0.0376	11.42021
IS12555	B	-0.04702	0.00691	-6.804631	B	0.1126	0.0247	4.558704
	M	23.98	4.74	5.059072	M	13.07	2.77	4.718412
	C	112.4	14.7	7.646259	C	0.15	0.0286	5.244755
TX7078	B	-0.04403	0.00736	-5.982337	B	0.1902	0.0153	12.43137
	M	42.28	2.85	14.83509	M	18.647	0.431	43.2645
	C	106.7	12.2	8.745902	C	0.12856	0.00493	26.07708
E36-1	B	-0.0477	0.00635	-7.511811	B	0.1056	0.0157	6.726115
	M	59.1	2.44	24.22131	M	13.066	1.12	11.66607
	C	92.25	8.27	11.15478	C	0.1832	0.00753	24.32935
R16	B	-0.05629	0.00613	-9.182708	B	0.404	0.14	2.885714
	M	67.67	2.11	32.07109	M	10.52	0.935	11.25134
	C	96.14	19.17	5.015128	C	0.09225	0.00876	10.53082
IS22380	B	-0.07718	0.0099	-7.79596	B	0.2843	0.0324	8.774691
	M	49.81	1.58	31.52532	M	15.89	0.892	17.8139
	C	85.23	4.86	17.53704	C	0.1321	0.0093	14.2043
M35-1	B	-0.0603	0.0103	-5.854369	B	0.2228	0.0325	6.855385
	M	44.87	2.42	18.54132	M	23.983	0.671	35.74218
	C	89.41	8.31	10.75933	C	0.13504	0.00795	16.98616
CSH6	B	-0.02478	0.00803	-3.085928	B	0.2228	0.0255	8.737255
	M	22.5	15.5	1.451613	M	20.369	0.525	38.7981
	C	178.3	58.5	3.047863	C	0.13738	0.00632	21.73734

B: Slope of the curve

M: Point of inflection where slope is maximum

C: Range

Table: 5 Regression summary for relative leaf number

Genotype	B	M	C	T-on	T-off	T-lin	Y-on	Y-off	Y-lin	Y-m	B-m	B-lin
B35	-0.0158	10.72	253.1	8.46	74.13	65.67	92.5	29.16	63.34	58.33	-1.091	0.9645
IS12555	-0.047	23.98	112.4	8.24	71.97	63.62	95.815	25.823	69.992	61.27	-1.321	1.2559
TX7078	-0.054	42.28	106.7	16.7	68.16	51.43	90.9	24.99	65.91	62.5	-1.475	1.2815
E36-1	-0.0477	59.1	92.25	19.8	78.23	58.41	95.613	35.817	59.796	73.32	-1.1	1.2555
R16	-0.0563	67.6	96.14	9.97	70.18	60.21	90.817	29.988	60.819	66.66	-1.35	1.01
IS22380	-0.0772	49.81	85.23	20.1	63.16	43.61	90.316	32.65	57.666	65.24	-1.645	1.345
M35-1	-0.0603	44.87	89.41	13.6	63.97	50.35	91.65	25.825	63.585	61.06	-1.348	1.26
CSH6	-0.0248	22.5	178.3	8.73	68.26	61.53	97.481	23.32	74.161	60.83	-1.105	1.2052

Table: 7 Regression summary for grain growth of sorghum genotypes

Genotype	B	M	C	T-on	T-off	T-lin	Y-on	Y-off	Y-lin	Y-m	B-m	B-lin
B35	0.04044	19.55	0.129	8.81	29.63	20.82	0.0237	0.0938	0.0672	0.079	0.097	0.00328
IS12555	0.1126	13.7	0.15	7.33	30	20.67	0.029	0.0969	0.0679	0.059	0.003	0.00312
TX7078	0.1902	18.647	0.129	8.9	28.4	20	0.0209	0.095	0.074	0.074	0.004	0.0012
E36-1	0.1056	14.3066	0.183	4.64	24.8	20.16	0.0172	0.08	0.0628	0.047	0.003	0.00168
R16	0.404	10.52	0.092	10.4	23.18	12.78	0.029	0.11	0.081	0.049	0.006	0.0019
IS22380	0.2843	15.89	0.132	11.1	22.4	11.28	0.0448	0.1322	0.0884	0.064	0.005	0.00174
M35-1	0.2214	23.983	0.135	14.3	30.4	16.08	0.0399	0.136	0.0961	0.078	0.006	0.0039
CSH6	0.2228	20.369	0.137	10.8	26.4	15.6	0.0372	0.1279	0.0906	0.067	0.006	0.00275

4.2 COMPONENTS OF REGRESSION

The regression plot of relative leaf number against days after flowering has three phases. The plotted curve is a continuous one, but a sequential change in leaf number as crop growth progresses towards maturity can be envisaged. At the first stage the curve represents slower senescence rate, then gets into the linear phase signaling accelerated senescence due to water stress and again the rate slows down at about physiological maturity. The differences in senescence pattern of the genotypes under study, especially during the linear phase can be studied based on the time parameters, rate parameters and the relative leaf number parameters in each of the sorghum genotypes.

4.2.1 TIME PARAMETERS

4.2.1.1 Onset of Linear phase of senescence (T-on)

The onset of senescence was early in the staygreen genotypes compared to the senescent genotypes. It was earliest in IS12555 (8DAF) and B35 (8DAF) followed by R16 . The onset was latest in genotype Tx7078(17DAF) and IS22380 (20DAF). In the other genotypes E36-1, M35-1 and CSH-6 the onset of senescence occurred at 20, 14 and 9 days after flowering (DAF) respectively (Fig. 4).

4.2.1.2 Offset of Linear phase of senescence (T-off)

The offset of senescence was earlier in the senescent genotypes, as expected which belonged to IS22380 (63DAF), Tx7078 (68DAF) and CSH6 (68DAF). The offset was relatively late in the genotypes R16 (70DAF), B35 (74DAF), E36-1 (78DAF) which were

FIG 4: Onset of senescence in the sorghum genotypes

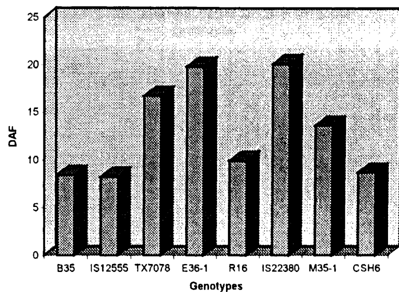
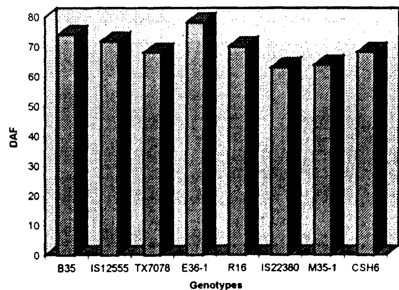


FIG 5: Offset of senescence in the sorghum genotypes



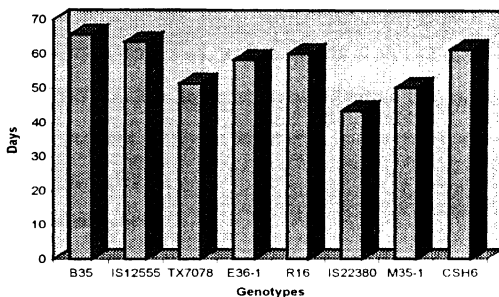


FIG 6: Linear phase of senescence in sorghum genotypes
70
60
50
40
30
20
10
0
Days
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

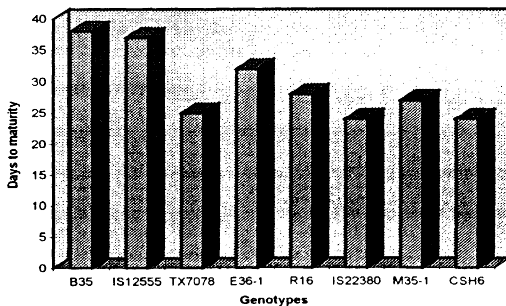


FIG 7: Inflection values in sorghum genotypes
40
35
30
25
20
15
10
5
0
Days to maturity
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

the staygreen types. In IS22380 (63DAF) the offset was the earliest which indicates rapid rate of senescence (Fig 5).

4.2.3.3 Duration of linear phase of senescence

The linear phase of senescence gives a direct indication of the staygreen trait of a genotype. It was observed that the duration of the Linear phase is more in the staygreen genotypes B35, IS12555, E36-1, R16 and CSH6 compared to the senescent genotypes IS22380 and Tx7078. The linear phase was longest in genotype B35 followed by IS12555. The duration of linear senescence was the shortest in genotype IS22380 followed by Tx7078 (Fig. 6).

4.2.3.4 Point of inflection

The point of inflection where senescence rate is maximum occurred earlier in the senescent genotypes than the staygreen genotypes. In genotype IS22380 it occurred as early as 24 days after flowering (DAF) followed by Tx7078 (25DAF). In genotype B35 and IS12555 it occurred late at 38 and 37 days after flowering (DAF). M35-1 and R16 although moderately staygreen genotypes showed maximum senescence rate earlier than the other staygreen genotypes (Fig. 7).

4.2.4 RATE PARAMETERS

The rate of senescence is another important senescence parameter. The general rate of senescence describes the senescence of the plant during the entire period. The maximum rate of senescence occurs at point of inflection. The important parameter is the linear rate of senescence which occurs during the linear phase of senescence.

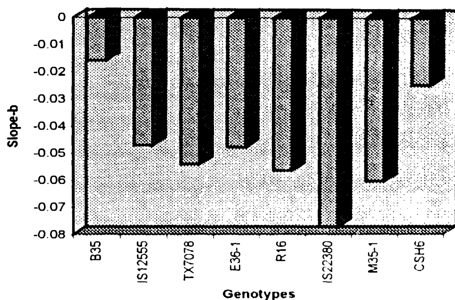


FIG 8: General slope (B) of sorghum genotypes under senescence study
0
-0.01
-0.02
-0.03
-0.04
-0.05
-0.06
-0.07
-0.08
Slope-b
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

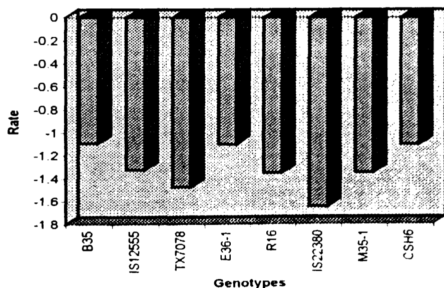


FIG 9: Max. rate of senescence in the sorghum genotypes
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
-1.6
-1.8
Rate
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

FIG 10: Linear rate of senescence in the sorghum genotypes

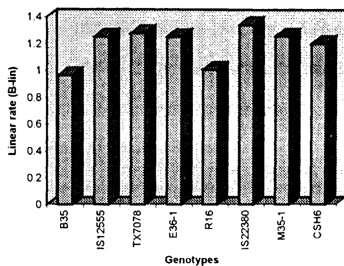
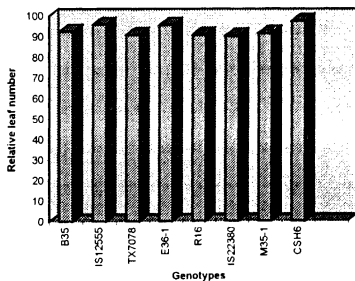


FIG 11: Relative leaf number in the sorghum genotypes at onset of senescence



4.2.4.1 General rate of senescence (B)

The rate of senescence was higher in the senescent genotypes than in the staygreen genotypes. IS22380 had the highest rate of senescence (0.07 leaves/day) followed by M35-1 (0.06), R16 (0.06) and Tx7078 (0.05), leaves/day respectively. Out of the staygreen genotypes B35 (0.01) had the lowest rate of senescence followed by E36-1(0.04) and IS12555 (0.046). There was a marked increase in senescence rates of the senescent genotypes compared to the staygreen genotypes (Fig. 8).

4.2.4.2 Maximum rate of senescence (Bm):

The maximum rate of senescence was higher in the senescent genotypes and lower in the staygreen genotypes.The Bm was highest in genotype IS22380, followed by Tx7078, M35-1 and it was lowest in genotypes B35, E36-1 followed by CSH6 and ISS12555 (Fig. 9).

4.2.4.3 Linear rate of senescence (B-lin)

The Linear rate of senescence which is another good measure of senescence showed that staygreen genotypes had the least Linear rate. The lowest rate was observed in genotype B35(1.04%/day) followed by R16 (1.1%/day). There was a rapid increase in Linear rate of senescence from the genotypes IS12555 (1.3%/day) and E36-1 (1.31%/day) to Tx7078 (1.4%/day). The highest rate of senescence was observed in genotype IS22380 (1.48%/day). Though CSH6 is a moderately senescent genotype it showed lower (1.23%/day) senescence rate (Fig. 10).

FIG 12: Relative leaf number in the sorghum genotypes at offset of senescence

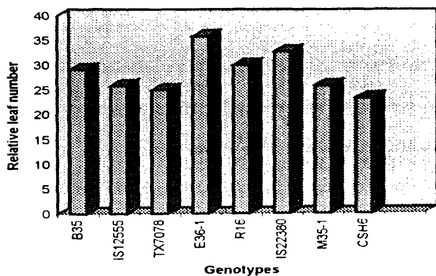
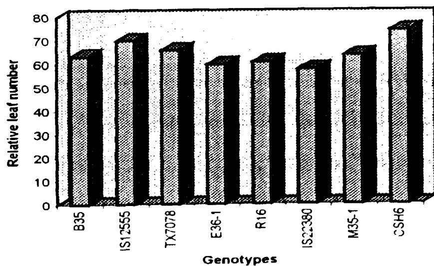


FIG 13: Relative leaf number at linear phase of senescence



4.2.5 Relative leaf number:

4.2.5.1 Relative leaf number at onset of rapid senescence (Y- on)

The relative leaf number at the onset of senescence was higher in the staygreen genotypes compared to the senescent genotypes. While the staygreen genotypes retained more than 85% of their functional green leaves at flowering the senescent genotypes lost more than 80% of their green leaves. The relative leaf number of the senescent genotypes (IS22380, Tx7078) leaf number was lowest at onset. The lower relative leaf number was observed in R16, M35-1 followed by B35. The highest relative leaf number at onset was in the genotype E36-1. Though CSH6 is a moderately staygreen genotype it retained relatively higher relative leaf number at onset of senescence (Fig. 11).

4.2.5.2 Relative leaf number at offset (Y-off)

The highest relative leaf number was recorded in genotype E36-1 followed by R16 and B35 while the lowest was in genotype Tx7078 followed by CSH6. The genotypes IS12555 and M35-1 maintained relative leaf number in the intermediate range at offset of senescence (Fig. 12).

4.2.5.3 Change in relative leaf number from onset to offset (Y-lin)

The decrease in the relative leaf number between onset and offset was more in the staygreen genotypes compared to the senescent genotypes. The decrease was highest in genotypes IS12555 while it was lowest in IS22380 (Fig. 13)

4.2.5.4 Relative leaf number at point of inflection (Y-m)

The relative leaf number at the point of inflection decreased across genotypes from the staygreen genotypes to the senescent genotypes. At 'm' the staygreen genotypes

Table: 6 Relative leaf number duration of the sorghum genotypes

Genotype	A1	A2	A3	Total A
B35	814.275	3994.706	512.69	5321.671
IS12555	806.7578	3389.443	461.909	4658.109
TX7078	1296.879	2980.111	493.35	4770.34
E36-1	1938.525	3838.413	593.83	6370.768
R16	951.2227	3636.835	531.47	5119.527
IS22380	1023.682	3061.298	718.553	4803.533
M35-1	1305.137	2558.424	507.247	4370.808
CSH6	664.5235	3863.126	652.4972	5180.147

Table: 8 Grain growth duration of sorghum genotypes

Genotype	A1	A2	A3	Total A
B35	0.2155	1.2523	1.1615	2.6293
IS12555	0.4754	1.3011	1.6632	3 4397
TX7078	0.111832	1 1592	1 65	2 921032
E36-1	0.0451	0.9793	1 5	2.5244
R16	0.1764	0.88821	2.1183	3.18291
IS22380	0.2311	1.00392	2.9028	4.13782
M35-1	0.38542	1.4142	1.7168	3.51642
CSH6	0.2141	1.2879	2.305	3.807

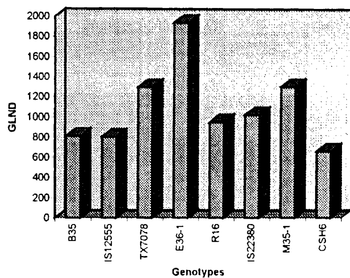


FIG 14: R-GLND in the sorghum genotypes from flowering to onset of senescence (A1)
GLND
2000
1800
1600
1400
1200
1000
800
600
400
200
0
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

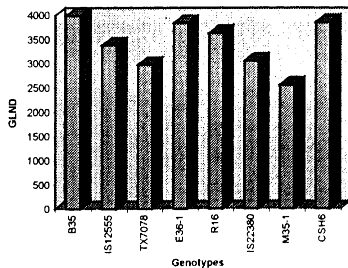


FIG 15: R-GLND in sorghum genotypes from onset to offset of senescence (A2)
GLND
4000
3500
3000
2500
2000
1500
1000
500
0
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

FIG 16: R-GLND in the sorghum genotypes from offset to maturity (A3)

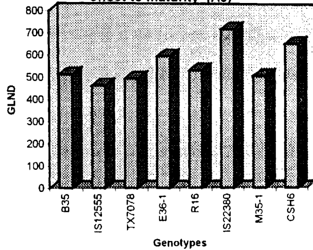
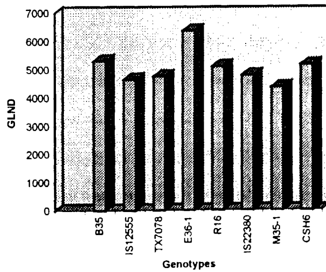


FIG 17: R-GLND in the sorghum genotypes from flowering to harvest (A)



maintained greater than 58% relative leaf number while the senescent genotypes lost greater than 50% of their leaves.

4.3 GREEN LEAF NUMBER DURATION STUDIES

Green leaf number duration studies over the plant growth period can account for differences in performance of the selected genotypes. A relatively high green leaf number duration in the critical period of grain filling can give a distinct yield advantage to a crop. By integrating the function of the regression curve between flowering to onset(A1), onset to offset (A2), offset to harvest maturity (A3) the area under each part and the total relative green leaf number duration- A (from flowering to harvest) for all the eight genotypes the green leaf duration was calculated (Table 6).

4.3.1 Total relative green leaf number duration(A)

The selected genotypes showed wide variation in the Relative GLND from flowering to harvest. The total relative GLND was higher for staygreen genotypes compared to the senescent genotypes. On the whole E36-1 showed highest total green leaf number duration while M35-1 showed the lowest relative green leaf area duration. The the senescent genotypes had a marginally less total green leaf number duration when compared with the staygreen genotypes (Fig 17).

4.3.2 Relative green leaf number duration from flowering to onset (A1)

The relative green leaf number duration (GLND) from flowering to onset (A1) was more in senescent genotypes compared to the staygreen genotypes. The highest A1 values were recorded in genotype Tx7078 and CSH6 had the lowest A1 value followed by IS12555,

B35 and R16. The other genotypes E36-1, M35-1 and CSH6 showed intermediate range of A1 values (Fig.14).

4.4.3 Relative green leaf number duration from onset to offset (A2):

The period from onset to offset is the most important phase of senescence which coincides with the grain filling period. The A2 values for all the selected genotypes showed much variation. The relative GLND values of the staygreen genotypes was higher than the senescent genotypes. While the lowest A2 ,

values were recorded in Tx7078, the highest value was recorded in B35 followed by E36-1 (Fig.15).

4.3.4 Relative green leaf number duration from offset of Linear phase to harvest (A3)

In general the staygreen genotypes retained higher number of green leaves and showed greater relative GLND than the senescent genotypes. A3 values were highest for genotype E36-1, followed by B35 where as it was lowest for Tx7078 followed by IS22380 (Fig. 16)

4.4 GRAIN GROWTH STUDIES

Grain ripening is characterized by grain growth, which is associated with increase in size, weight, change in grain colour and leaf senescence. The process of grain development starts with the formation of watery fluid in the grain, which is gradually converted into milky white soft and finally hard endosperm stage as reported by Wilson and Eastin (1982). Data on grain growth retained twice in a week after flowering was used in grain growth studies through regression analysis by plotting grain weight against days after

flowering (DAF). Eight genotypes were selected for grain growth study (B35, IS12555, Tx7078, E36-1, R16, IS22380, M35-1, CSH6) and the logistic curve plotted for weight of 4-5 grains revealed similarity of curves for grain growth pattern (Fig. 3).

The regression curve plotted using grain weight as a function of time to study the grain growth pattern and the genotype differences for the grain growth trait revealed wide variations among the genotypes under study.

The R^2(regression coefficient) values for the eight selected genotypes was greater than 0.98 for relative grain weights indicating that the logistic equation gave a good fit. The ratio of the estimated values of the constants 'b'-the slope of the curve, 'm'-the point of inflection and 'c'-the range; to their standard error values were significant (at 5 % level of significance) for the eight genotypes for relative leaf number indicating that the parameters (b, m, c) were effective in defining the logistic equation fitted and the equation is not over parameterized (Table 4). The correlation of DAF with both relative leaf number was greater than 0.984 (5 % level of significance) in all cases indicating that grain weight increased progressively after flowering. It was also found that the study of grain growth pattern is very effective in senescence studies.

Having inferred that grain growth pattern is effective in studying senescence, a detailed regression analysis using by fitting the logistic function was done for each of the genotype under wet environment. Using the three primary regression parameters of the logistic curve the parameters which define the senescence, T-on (the onset of senescence), T-off (the offset of senescence), T-lin (the Linear duration of senescence), b-m (maximum rate of senescence), B-lin (the Linear rate of senescence) were found out.

The general slope of the fitted curve (b) ranged from a maximum of 0.4 in the genotype R16 to a minimum of 0.04 in genotype B35. The range 'c', varied from 0.09 in genotype R16 to 0.4 in genotype B35. The point of inflection (m) occurred earliest at 13 days after flowering in genotypes IS12555 and E36-1 and latest 23 days after flowering in genotype M35. The onset of grain growth occurred earliest at 7 DAF in IS12555 and latest at 14DAF in genotype M35-1. The offset of grain growth occurred as early as 22 DAF in IS22380 and latest in genotypes IS12555 (30 DAF) and M35-1 (30 DAF) followed by B35. The duration of the Linear phase was maximum in B35 (21 days) and minimum in IS22380 (12days). The maximum rate of grain growth (observed at the point of inflection) was the highest in B35 (0.099) and the lowest in genotype IS12555 (0.003)and E36-1 (0.003). Where as the rest of the genotypes did not vary much in the linear phase of grain growth. The maximum grain weight at onset of grain growth occurred in IS22380 followed by M35-1 and CSH6 while the minimum occurred in E36-1 followed by IS12555 and B35. At offset, the maximum grain weight occurred in genotype M35-1while the minimum occurred in E36-1 followed by B35, Tx7078 and IS12555. The grain weight at 'c' (point of inflection where slope is maximum) was highest in genotype B35.

4.5.1 COMPONENTS OF REGRESSION

The regression plot of grain growth pattern against days after flowering has three phases. The plotted curve is a continuous one, but a sequential change in grain weight as crop growth progresses towards maturity can be envisaged. At the first stage the curve represents slower grain growth rate, then gets in to the linear phase signaling accelerated

grain growth due to increased rate of photosynthesis because better canopy development and again the rate slows down at about physiological maturity. The differences in grain growth pattern of the genotypes under study, especially during the linear phase can be studied based on the time parameters, rate parameters and the relative leaf number parameters in each of the eight sorghum genotypes.

4.5.1.1 TIME PARAMETERS

4.5.1.1.1 Onset of Linear phase of grain growth (T-on)

There is no significant increase in the non-reproductive parts and negligible increase in grain weight for 2 to 3 days which is supporting the report by Dickson (1976). The onset of grain growth was early in the staygreen genotypes compared to the senescent genotypes. It was earliest in E36-1 (5DAF) followed by IS12555 (7DAF) and B35 (9DAF). The onset was latest in genotype M35-1 (14.32DAF) and IS22380 (11DAF) followed by R16 and CSH6. In rest of the genotypes R16, B35, Tx7078 and E36-1 the onset of grain growth occurred at 10, 9 and 5 days after flowering (DAF) respectively (Fig. 18).

4.5.1.1.2 Offset of Linear phase of grain growth (T-off)

The offset of grain growth was earliest in the senescent genotype, as expected which is IS22380 (22DAF). The offset was relatively late in the genotypes R16 (23DAF), E36-1 (25DAF), CSH6 (11DAF). The offset was very late in IS1255 (30DAF) and M35-1 (30DAF) which indicates rapid rate of grain growth (Fig. 19).

4.5.1.1.3 Duration of Linear phase of grain growth

The Linear phase of grain growth gives the actual duration of grain filling of a genotype. It was observed that the duration of the Linear phase is more in the staygreen genotypes

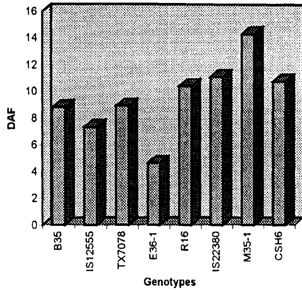


FIG: Onset of grain growth in sorghum genotypes
16
14
12
10
8
6
4
2
0
DAF
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

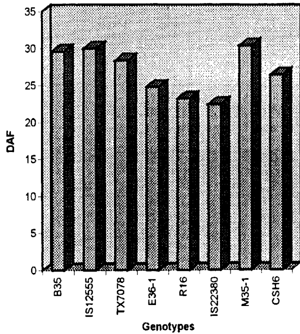


FIG: Offset of grain growth in sorghum genotypes
35
30
25
20
15
10
5
0
DAF
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

FIG : Linear phase of grain growth in sorghum genotypes

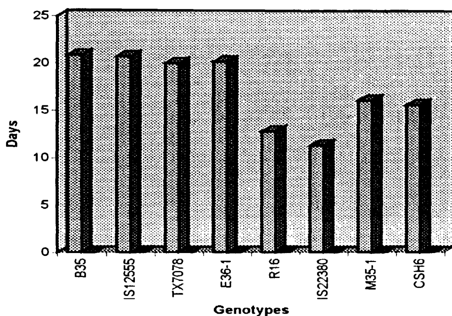
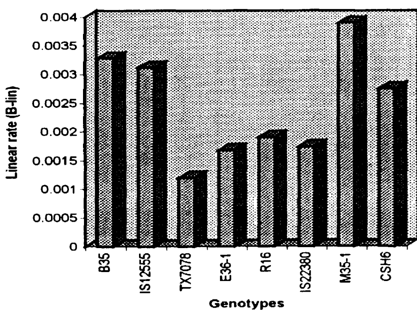


FIG : Linear rate of grain growth in the sorghum genotypes



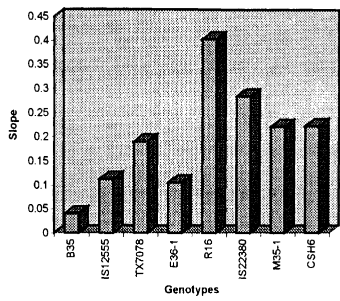


FIG : General slope (B) of sorgum genotypes under grain growth study
Slope
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

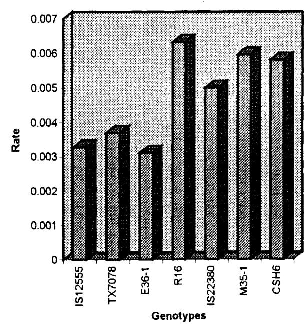


FIG : Max. rate of grain growth in sorghum genotypes
Rate
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

B35, IS12555 and E36-1 compared to the senescent genotypes IS22380 and Tx7078. The Linear phase was longest in genotype B35 followed by IS12555. The duration of Linear senescence was the shortest in genotype IS22380 followed by CSH6 and M35 (Fig. 20).

4.5.1.1.4 Point of inflection

The point of inflection where grain growth rate is maximum occurred earlier in the senescent genotypes than the staygreen genotypes. In genotype IS22380 it occurred as early as 15 DAF. In genotype B35 and M35-1 it occurred lately at 22 and 19 DAF. E36-1 and IS12555 although staygreen genotypes showed relatively lesser grain growth rate than the other staygreen genotypes (Fig 21).

4.5.1.2 RATE PARAMETERS

The rate of grain growth is another important parameter to be considered. The general rate of grain growth describes the grain filling pattern of the plant during the entire period. The maximum rate of grain growth occurs at point of inflection. The important parameter is the Linear rate of grain growth which occurs during the Linear phase of grain filling .

4.5.1.2.1 General rate of grain growth (B)

The rate of grain growth was higher in the senescent genotypes was comparatively than in the staygreen genotypes. R16 had the highest rate of grain growth (0.4lgrains/day) followed by IS22380 (0.3), M35-1 (0.2), CSH6(0.2), grains/day respectively. Out of the staygreen genotypes B35 (0.04) had the lowest rate of grain growth followed by E36-1(0.1) and IS12555 (0.1) grains/day respectively (Fig.22).

FIG : Linear rate of grain growth in the sorghum genotypes

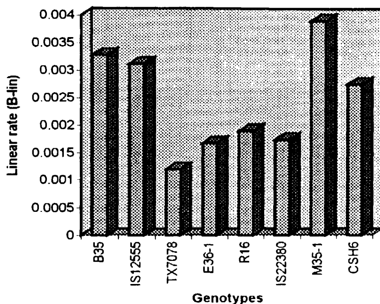
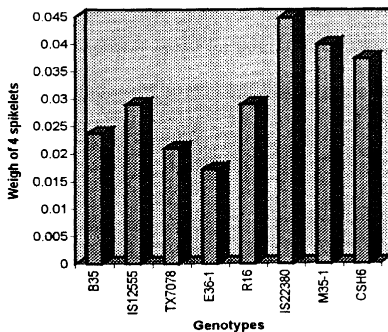


FIG [illegible] : Weight of spikelets at the onset of grain growth



4.5.1.2.2 Maximum rate of grain growth (Bm):

The maximum rate of grain growth was higher in the staygreen genotypes and lower in the senescent genotypes. The Bm was highest in genotype B35 and it was lowest in genotype Tx7078 followed by E36-1, IS12555, IS22380 and M35-1 respectively (Fig.23).

4.5.1.2.3 Linear rate of grain growth (B-lin)

The Linear rate of grain growth which is another important measure of grain growth showed that senescent genotypes had the least Linear rate. The highest rate was observed in genotype ISI2555 (14%/day) followed by B35 (13%/day), E36-1 (10%/day). There was a rapid increase in Linear rate of grain growth from the genotypes IS22380 (3%/day) and Tx7078 (6%/day) to M35-1 (9.7%/day), IS22380 being the lowest .

4.5.1.3 Grain weight

4.5.1.3.1 Grain weight at onset of rapid senescence (Y- on)

The grain weight (4-5 spike-lets) at the onset of grain growth was higher in the senescent genotypes compared to the staygreen genotypes. In the staygreen genotypes grain weight was lesser at the onset of grain growth may be due to utilization of current photosynthates for canopy development rather for grain filling. The grain weight was highest for IS22380 followed by M35-1 and CSH6. The lowest grain weight at onset was for genotype E36-1, followed by IS1255 (Fig.24).

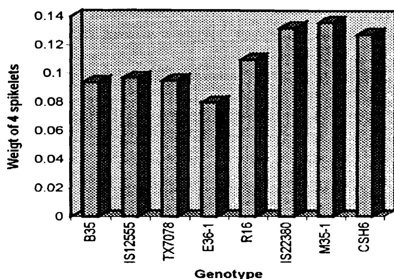


Fig 25 .: Weight of spikelets at the offset of senescence
Weigt of 4 spikelets
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotype

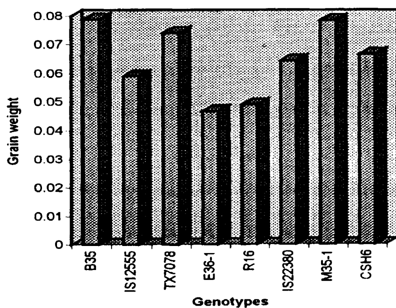


FIG 26 : Grain weight at the point of inflection in the sorghum genotypes
Grain weight
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

4.5.1.2.2 Grain weight at offset (Y-off)

The highest grain weight at offset of grain growth was recorded in genotype E36-1 followed by B35 while the lowest was in genotype IS2380 followed by CSH6, M35, Tx7078 R16 and IS12555 respectively (Fig. 25).

4.2.5.3 Change in grain weight from onset to offset (Y-lin)

The increase in the grain weight between onset and offset was more in the staygreen genotypes compared to the senescent genotypes. The increase was highest in genotypes E36-1, followed by B35, while it was lowest in IS22380 (Fig.26).

4.5.2.5 Grain weight at point of inflection (Y-m)

The grain weight at the point of inflection decreased across genotypes from the staygreen genotypes to the senescent genotypes. At 'm' the staygreen genotypes maintained greater than 79% relative leaf number while in the senescent genotypes it was lesser than 60% of their grain weights (Fig. 26).

4.5.3 GRAIN GROWTH DURATION STUDIES

Grain growth studies over the plant growth period can account for differences in performance of the selected genotypes. A relatively high grain growth duration in the critical period of grain filling can give a distinct yield advantage to a crop. By integrating the function of the regression curve between flowering to onset(A1), onset to offset(A2), offset to harvest maturity (A3) the area under each part and the total grain growth duration- A (from flowering to harvest) for all the eight genotypes was calculated (Table 8).

FIG 27: GGD in the sorghum genotypes from flowering to onset (A1)

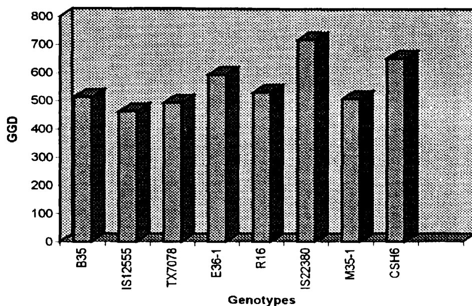
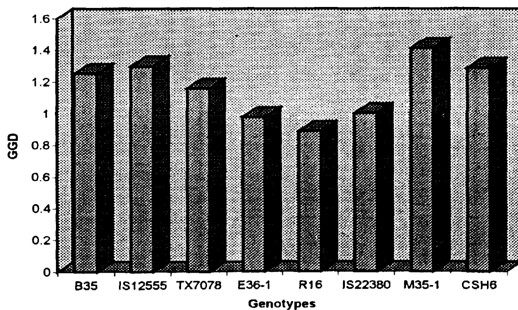


FIG 28: GGD from onset to offset (A2)



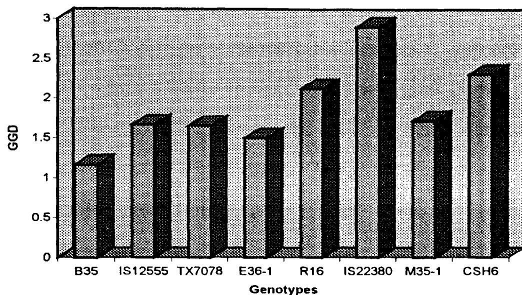


FIG 29: GGD of the sorghum genotypes from offset to maturity (A3)
GGD
3
2.5
2
1.5
1
0.5
0
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

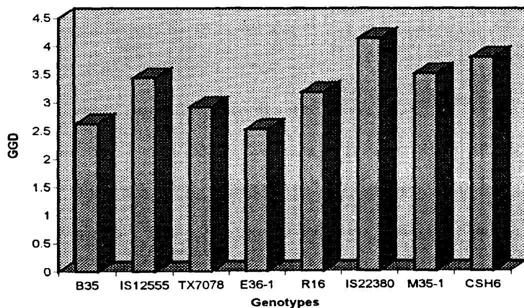


FIG 30: GGD in the sorghum genotypes from flowering to harvest (A)
GGD
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

4.5.3.1 Total grain growth duration (A)

The selected genotypes showed wide variation in the GGD from flowering to harvest. The total GGD was higher for senescent genotypes, when compared to the staygreen genotypes. On the whole IS22380 showed highest total grain growth duration followed by CSH6, M35-1 and IS22380 respectively, where as E36-1 showed the lowest grain growth duration followed by B35, Tx7078, R16 and IS1255 respectively. The senescent genotypes had a marginally greater grain growth duration when compared with the staygreen genotypes (Fig. 30).

4.5.3.2 Grain growth duration from flowering to onset (A1)

The grain growth duration duration from flowering to onset (A1) was more in senescent genotypes compared to the staygreen genotypes. The highest A1 values were recorded in genotype M35-1, followed by IS22380 and E36-1 had the lowest A1 value followed by IS12555, B35 R16 and CSH6 (Fig. 27).

4.5.3.3 Grain growth duration from onset to offset (A2):

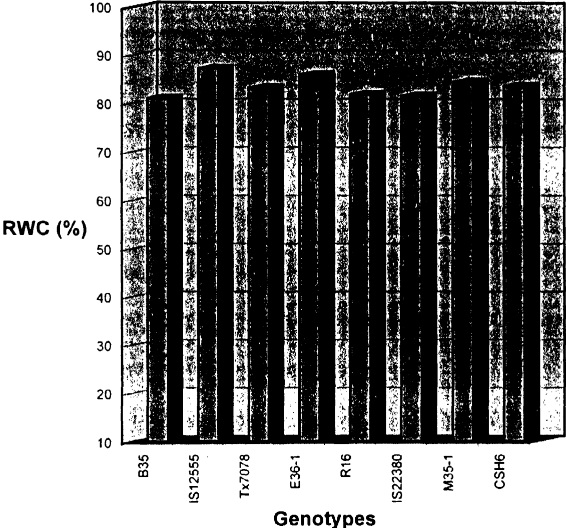
The period from onset to offset is the most important phase of grain growth which coincides with the grain filling period. The A2 values for all the selected genotypes showed much variation. The A2 values of the staygreen genotypes was higher than the senescent genotypes. While the lowest A2 values were recorded in IS22380, the highest values were recorded in B35, IS12555, M35-1 and Tx7078 respectively (Fig. 28).

4.5.3.4 Grain growth duration from offset of Linear phase to harvest (A3)

Table: 9 Analysis of variance (ANOVA) of sorghum genotypes for agronomic traits

	(Phenology)			(Yield traits)							
Genotyp	Days to Fl. DAYS	ays t Ma. DAYS	GS3 DAYS	HDWT/M2 (g)	STWT/M2 (g)	BIO/M2 (g)	GRWT/M (g)	THR (%0	100Sd. Wt.(g)	Sds/M2	HI (%)
B35	61.75	113.4	51.67	841.08	480.8	1321.9	726.04	86.3	3.6	20169	54.926
IS12555	81.083	121.8	40.75	1292.04	1492.3	2784.4	1063.14	82.5	2.345	46336	38.22
TX7078	64.25	110.7	46.42	623.48	382.8	1006.3	542.06	87	3.815	14209	53.872
E36-1	81.083	118.8	46.5	921.64	409.9	1331.5	810.49	88	3.835	17724	60.876
R16	70.167	117.3	47.17	964.12	520.3	1484.5	824.39	85.5	5.329	15472	55.538
IS22380	64.083	90.25	26.17	540.06	279.2	819.2	463.08	85.8	2.819	16429	56.53
M35-1	75	118.9	43.92	881.35	519.7	1401	763.21	86.6	3.977	19197	54.476
CSH6	64.583	116.1	51.5	544.39	294.7	839.1	473.13	86.9	3.412	13870	56.388
Mean	69.16	113.1	44.26	826	547.5	1373.5	708.2	86.1	3.461	20426	53.853
Min.	61	89	24	528.9	270.7	807	458.7	75.4	2.08	13519	35.26
Max.	83	124	54	1423.9	1621.8	3046	1088.8	89.8	5.4	51530	62.2
SE	0.1571	0.258	0.31	1.579	3.35	4.26	1.609	0.34	0.069	1572.6	0.2406
CV(%)	0.3	0.3	1	0.3	0.9	0.4	0.3	0.6	2.7	10.9	0.6
SED	0.2222	0.366	0.438	2.233	4.74	6.02	2.275	0.48	0.098	2224.1	0.3406
LSD	0.5253	0.865	1.037	5.279	11.2	14.23	5.38	1.14	0.232	5259	0.8046

Fig. 31: Relative water content (RWC) of soghum genotypes at physiological maturity



In general the senescent genotypes maintained higher grain weight and showed greater relative green leaf number duration than the staygreen genotypes. A3 values were highest for genotype IS22380 where as it was lowest for B35 followed by E36-1. The A3 values for rest of the genotypes were of intermediate range (Fig. 29)

4.6 ANALYSIS OF PHENOLOGICAL AND YIELD TRAITS:

Having studied the basic senescence pattern, a study of how the differential senescence pattern will influence the phenological and yield parameters was undertaken. Analysis of variance was done for the yield parameters to see if the genotypes differed significantly from each other in their performance (Table 9).

4.6.1 Days to 50% flowering

The eight genotypes differed significantly from each other in days to 50% flowering. The genotype B35 attained days to 50% flowering as early as (62DAS), where as E36-1 (82DAS) and IS12555 (82DAS) and attained days to 50% flowering very lately. The genotypes Tx7078, R16, IS22380, M35-1 and CSH6 differed significantly from each other in the time taken to 50% flowering attained days to 50% flowering. It was attained at 64,70,64,75 and 65 days after sowing respectively (DAS).

4.6.2 Days to Physiological maturity

The eight genotypes differed significantly from each other at the time of physiological maturity. The genotype IS12555 attained physiological maturity lately (121DAS) where as genotype IS22380 attained physiological maturity as early as 90 days after sowing (DAS). The senescent genotypes attained physiological maturity comparatively earlier than the staygreen genotypes. The relative water content (RWC) was measured at

Fig. 32 Comparision of head weight/m2 of sorghum genotypes

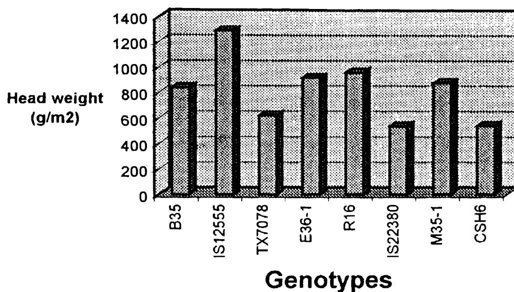
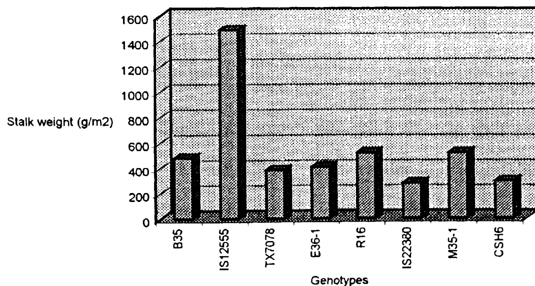


Fig. 33: Comparision of stalk weight/m2 of sorghum genotypes



physiological maturity stages in the sorghum genotypes and it was observed that highest RWC was found in the staygreen genotype IS12555 (87.36%), followed by B35 (86.13%) and E36-1 where as the senescent genotype IS22380 (81.67%) possessed least RWC at physiological maturity stage, followed by R16 (82.02%) and Tx7078 (83.36%) respectively (Fig31).

4.6.3 GS 3 (Grain filling period)

The genotypes differed significantly from each other during the period of grain filling. Staygreen genotypes possessed a longer duration of GS3 than the senescent genotypes, thus contributed for higher rate of dry matter accumulation by the developing grains, which is in agreement with the report of Eastin *et al.* (1973). GS3 was highest for B35 (52days) and shortest for IS22380 (26 days). The duration of GS3 did not differ significantly among the genotypes Tx7078 (46days), E36-1 (46days) and R16 (47days) where as it was 44 days and 44 days for genotypes IS12555 and M35-1.

4.6.4 Head weight per square meter

The performance of the genotypes varied significantly with respect to head weight.. The genotypes also differed significantly from each other in their mean head weights. The genotype IS12555 showed highest head weight/ sq. meter (1292.04 g) and genotype IS22380 (540 g) showed the lowest. The head weight of the staygreen genotypes was comparatively higher than that of senescent genotypes. The head weight per sq. meter for the genotypes B35, Tx7078, E36-1, R16, M35-1, and CSH6 were 841, 623, 921, 964, 881 and 544 g respectively (Fig. 32).

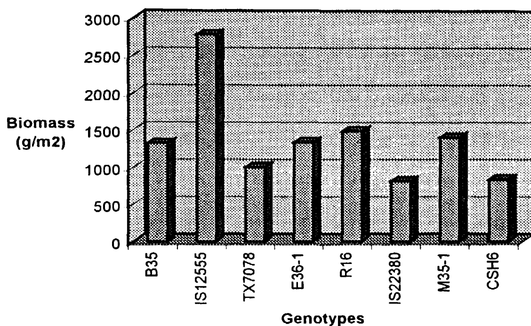


Fig. 34: Comparision of Biomass/m2 of sorghum genotypes
Biomass (g/m2)
3000
2500
2000
1500
1000
500
0
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

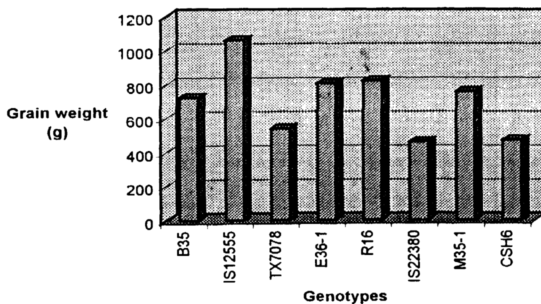


Fig. 35: Comparision of grain weight of sorghum genotypes
Grain weight (g)
1200
1000
800
600
400
200
0
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

4.6.5 Stalk weight per square meter

The stalk weights and the mean stalk weights varied significantly among the genotypes. The senescent genotypes possessed lesser stalk weights than the staygreen genotypes which in agreement to the report given by Gerik and Miller (1984). The stalk weight was found to be highest for IS12555 (1492 g/m^2) and it was lowest for IS22380 (272.9 g/m^2) followed by Tx7078 (382.8 g/m^2). Though CSH6 is a moderately staygreen cultivar the stalk weight was found to be lower (294.7 g/m^2). In general the staygreen Lines recorded higher stalk weight/M2 than the senescent Lines. The genotype IS12555 (highest staygreen) showed highest post harvest stalk weight than other staygreen and senescent genotypes (Fig. 33).

4.6.6 Biomass per square meter

The genotypic difference was significant while considering biomass per sqare meter. The genotypes also differed significantly from each other in the mean values. The biomassIn per sqare meter was found to be higher for the staygreen genotypes than that of senescent genotypes. Genotype IS1255 showed highest biomass per sqare meter (2784g) and it was lowest in IS22380 (819.2g). The staygreen genotypes had a distinct advantage over the senescent senescent genotypes in terms of total biomass accumulation by the plants (recording up to up to 40% higher biomass). Genotype is12555 showed high biomass due to higher head weight as well as stalk weight but CSH6 showed less biomass due lesser stalk weight though it is not a senescent genotype (Fig. 34).

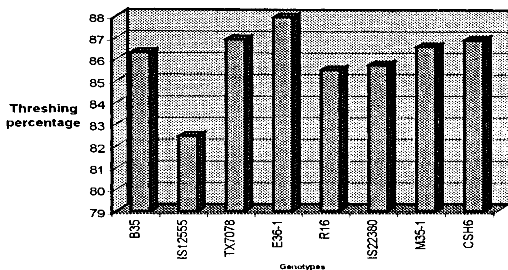


Fig. 36: Comparision of threshing percentage of sorghum genotypes
88
87
86
85
84
83
82
81
80
79
Threshing percentage
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

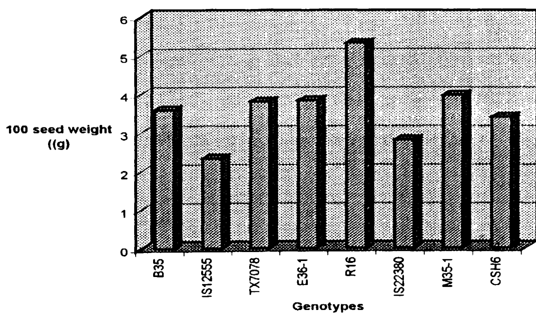


Fig. 37: Comparision of 100 seed weight of sorghum genotypes
6
5
4
3
2
1
0
100 seed weight ((g)
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

4.6.7 Grain weight per square meter

The eight genotypes differed significantly from each other in realization of grain weight. The overall means for the grain weights also showed variation. The highest grain weight per square meter was recorded in genotype IS12555 (1063.14g) while the lowest was recorded in genotype IS22380 (463.08g). The grain weight per square meter values were higher for the staygreen genotypes than that of senescent genotypes (Fig. 34).

4.6.8 Threshing percentage

The threshing percentage of the genotypes did not differ significantly. This indicates that staygreenness did not have any effect on the threshing percentage. However the threshing percentage was the least in genotype IS12555 (82.5%) and highest in genotype E36-1 (88%) (Fig.36).

4.6.9 100 seed weight

Staygreeness had a significant effect on the seed weight of the evaluated genotypes. The genotypes also differed significantly from each other in their 100 seed weights. Genotype E36-1recorded the highest seed weight (5.3g) while genotype IS22380 recorded the lowest(2.81g). Though IS12555 is a staygreen genotype the 100 seed weight was found to be less (2.34g) (Fig.37).

4.6.10 Seeds per square meter

The genotypes also significantly differed from each other in their seed densities. The highest number of seeds per unit area was recorded in genotype IS12555(46,333seeds/

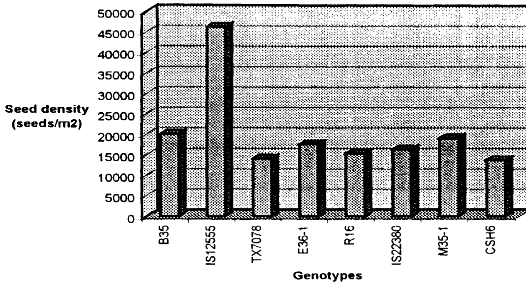


Fig. 38: Comparision of seed density of sorghum genotypes
Seed density (seeds/m2)
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

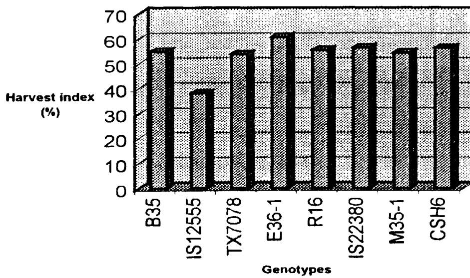


Fig. 39: Comparision of harvest index (HI) of sorghum genotypes
Harvest index (%)
70
60
50
40
30
20
10
0
B35
IS12555
TX7078
E36-1
R16
IS22380
M35-1
CSH6
Genotypes

m^2) while the lowest was recorded in genotype Tx7078 (14,209 seeds / m^2).The seed density in general was lower for senescent genotypes than the staygren genotypes (Fig. 38)

4.6.11 Harvest index (HI)

The genotypes differed significantly from each other in their HI (%). The HI was lowest in genotype IS1255 (38.22%) and highest in genotype E36-1 (60.876%). The HI values of the staygreen genotypes was comparatively lesser than that of senescent genotypes.

The staygreen genotypes had a lower HI compared to the senescent genotypes (Fig.39).

4.7 CORRELATIONS

4.7.1 Correlation between phenological and yield traits

Days to 50% flowering has a significant positive correlation with maturity, head weight/ m^2, grain weight/ m^2, stalk weight/ m^2, biomass/ m^2, seeds/ m^2 and leaf number at harvest while having a negative correlation with GS3, 100seed weight, maturity. The correlation was not significant for threshing %, HI. Days to physiological maturity had significant positive correlation with headweight/m^2, grainweight/m^2, stalk weight/m^2, seeds/ m^2 while having a negative correlation with GS3, 100 seed weight, plant height, HI.

4.7.2 Correlation between regression parameters

The rate parameters had a positive correlation with T-off, T-lin, and relative leaf number at onset and offset while having a significant negative correlation with T-on. T-on had a significant positive correlation with T-off, while having a negative correlation with linear rate of senescence. Relative leaf number at onset and the point of inflection have a

Table:10 CORRELATION BETWEEN REGRESSION PARAMETERS FOR RELATIVE LEAF NUMBER AND AGRONOMIC TRAITS

	a1	a2	a3	atot	b	m	c	ton	toff	tlin	yon	yoff	ylin	ym	bm	blin	dfl	dmat	gs3	hdw	stw	biomass	grwt	th%	sdwt100	seedsm2	hi
a1	1.000																										
a2	-0.182	1.000																									
a3	-0.006	0.135	1.000																								
atot	0.519	0.734	0.255	1.000																							
b	-0.350	0.728	-0.231	0.341	1.000																						
m	0.596	-0.270	0.194	0.202	-0.727	1.000																					
c	-0.515	0.620	-0.092	0.160	0.899	-0.805	1.000																				
on	0.758	-0.379	0.460	0.259	-0.659	0.591	-0.598	1.000																			
toff	0.333	0.750	-0.296	0.809	0.551	-0.088	0.317	0.133	1.000																		
tlin	-0.327	0.776	-0.443	0.365	0.834	-0.480	0.635	-0.774	0.725	1.000																	
yon	-0.077	0.539	0.058	0.408	0.574	-0.417	0.270	-0.344	0.488	0.599	1.000																
yoff	0.592	0.258	0.376	0.668	-0.306	0.541	-0.253	0.607	0.407	-0.170	-0.186	1.000															
ylin	-0.528	0.169	-0.228	-0.247	0.564	-0.644	0.369	-0.649	-0.003	0.484	0.680	-0.835	1.000														
ym	0.760	0.157	0.319	0.688	-0.417	0.794	-0.593	0.654	0.393	-0.195	0.040	0.786	-0.559	1.000													
bm	0.033	0.737	-0.179	0.613	0.860	-0.397	0.620	-0.465	0.724	0.810	0.706	-0.020	0.384	-0.008	1.000												
blin	0.362	-0.604	0.331	-0.214	-0.606	0.195	-0.646	0.643	-0.409	-0.688	0.084	-0.014	0.026	0.235	-0.508	1.000											
dfl	0.491	-0.087	-0.337	0.208	-0.216	0.304	-0.519	0.078	0.395	0.171	0.398	0.241	-0.022	0.480	0.183	0.280	1.000										
dmat	0.094	0.248	-0.694	0.170	0.486	-0.129	0.127	-0.556	0.533	0.708	0.529	-0.309	0.474	-0.034	0.680	-0.339	0.580	1.000									
gs3	-0.045	0.515	-0.463	0.334	0.804	-0.304	0.567	-0.558	0.528	0.733	0.400	-0.347	0.473	-0.192	0.680	-0.339	0.580	1.000									
hdw	0.048	0.092	-0.687	0.009	0.062	-0.010	-0.123	-0.398	0.478	0.525	0.227	0.066	0.049	0.070	0.232	-0.226	0.769	0.660	0.122	1.000							
stw	-0.246	-0.036	-0.604	-0.283	0.042	-0.284	-0.086	-0.465	0.230	0.427	0.312	-0.237	0.345	-0.213	0.024	0.029	0.611	0.471	-0.068	0.863	1.000						
biomass	-0.136	0.015	-0.659	-0.174	0.052	-0.183	-0.104	-0.454	0.339	0.482	0.288	-0.122	0.237	-0.106	0.109	-0.074	0.696	0.564	0.007	0.948	0.979	1.000					
grwt	0.100	0.105	-0.691	0.055	0.071	0.020	-0.124	-0.375	0.509	0.529	0.223	0.101	0.018	0.107	0.265	-0.244	0.783	0.682	0.154	0.997	0.827	0.925	1.000				
th%	0.553	0.099	0.357	0.506	0.119	0.259	0.080	0.485	0.092	-0.241	-0.063	0.259	-0.252	0.345	0.277	0.007	-0.289	-0.104	0.329	-0.641	-0.877	-0.811	-0.585	1.000			
sdwt100	0.226	0.093	-0.171	0.204	-0.025	0.583	-0.106	-0.046	0.092	0.075	-0.379	0.139	-0.321	0.294	0.135	-0.548	-0.099	0.270	0.459	-0.045	-0.435	-0.291	0.000	0.445	1.000		
seedsm2	-0.241	-0.022	-0.489	-0.251	0.051	-0.369	-0.036	-0.393	0.224	0.379	0.353	-0.170	0.316	-0.223	0.023	0.109	0.587	0.359	-0.166	0.805	0.978	0.941	0.767	-0.865	-0.593	1.000	
hi	0.458	0.169	0.556	0.530	-0.021	0.421	0.022	0.508	0.022	-0.302	-0.207	0.466	-0.472	0.455	0.152	-0.097	-0.354	-0.297	0.140	-0.659	-0.939	-0.858	-0.607	0.922	0.531	-0.922	1.000

TABLE: 11 CORRELATION BETWEEN REGRESSION PARAMETERS FOR GRAIN GROWTH AND AGRONOMIC TRAITS

	a1	a2	a3	atot	b	m	c	ton	toff	tlin	yon	yoff	ylin	ym	bm	blin	dfl	dmat	gs3	hdw	stw	biomass	grwt	th%	dwt100	seeds/m2	hi
a1	1.000																										
a2	0.657	1.000																									
a3	0.021	-0.340	1.000																								
atot	0.483	0.163	0.858	1.000																							
b	-0.075	-0.473	0.717	0.518	1.000																						
m	0.198	0.785	-0.174	0.139	-0.308	1.000																					
c	-0.087	0.161	-0.294	-0.252	-0.682	0.109	1.000																				
ton	0.403	0.377	0.421	0.629	0.530	0.553	-0.591	1.000																			
toff	0.543	0.878	-0.701	-0.253	-0.629	0.592	0.163	0.099	1.000																		
tlin	0.001	0.392	-0.884	-0.723	-0.874	0.144	0.537	-0.621	0.698	1.000																	
yon	0.506	0.239	0.761	0.937	0.492	0.307	-0.306	0.794	-0.181	-0.762	1.000																
yoff	0.400	0.278	0.698	0.863	0.570	0.438	-0.399	0.907	-0.135	-0.769	0.957	1.000															
ylin	0.282	0.256	0.647	0.777	0.632	0.476	-0.426	0.923	-0.131	-0.751	0.871	0.974	1.000														
ym	0.295	0.731	-0.205	0.115	-0.305	0.859	-0.189	0.556	0.651	0.173	0.265	0.347	0.338	1.000													
bm	0.035	0.186	-0.182	-0.106	-0.057	0.324	-0.503	0.396	0.159	-0.108	0.143	0.204	0.161	0.491	1.000												
blin	0.748	0.796	-0.313	0.145	-0.316	0.538	0.016	0.431	0.678	0.163	0.339	0.339	0.267	0.478	0.471	1.000											
dfl	0.301	0.025	-0.283	-0.190	-0.177	-0.315	0.559	0.387	0.149	0.300	0.237	-0.308	-0.306	-0.545	-0.598	0.171	1.000										
dmat	0.201	0.348	-0.679	-0.490	-0.274	0.003	0.177	-0.235	0.534	0.556	-0.481	-0.381	-0.292	-0.167	-0.055	0.422	0.580	1.000									
gs3	-0.249	0.251	-0.663	-0.619	-0.288	0.208	-0.038	-0.175	0.407	0.511	-0.566	-0.382	-0.257	0.102	0.351	0.227	-0.047	0.746	1.000								
hdw	0.508	0.107	-0.507	-0.328	-0.252	-0.421	0.158	-0.354	0.371	0.426	-0.338	-0.436	-0.486	-0.357	-0.165	0.372	0.769	0.660	0.122	1.000							
stw	0.727	0.318	-0.301	-0.006	-0.292	-0.317	0.149	-0.274	0.473	0.415	-0.124	-0.278	-0.373	-0.167	-0.290	0.375	0.611	0.471	-0.068	0.863	1.000						
biomass	0.663	0.243	-0.395	-0.137	-0.286	-0.370	0.157	-0.316	0.448	0.434	-0.215	-0.352	-0.432	-0.250	-0.250	0.387	0.696	0.564	0.007	0.948	0.979	1.000					
grwt	0.465	0.090	-0.539	-0.376	-0.261	-0.412	0.176	-0.316	0.448	0.434	-0.215	-0.352	-0.432	-0.250	-0.155	0.367	0.783	0.682	0.154	0.997	0.827	0.925	1.000				
th%	-0.778	-0.182	-0.081	-0.330	-0.016	0.390	0.196	0.017	-0.227	-0.027	-0.207	-0.021	0.120	0.109	0.126	-0.295	-0.289	-0.104	0.329	-0.641	-0.877	-0.811	-0.585	1.000			
sdwt100	-0.476	-0.434	-0.117	-0.373	0.533	-0.169	-0.522	0.187	-0.285	-0.265	-0.246	-0.034	0.127	-0.224	0.285	-0.204	-0.099	0.270	0.459	-0.045	-0.435	-0.291	0.000	0.445	1.000		
seedsm2	0.744	0.356	-0.266	0.045	-0.400	-0.257	0.268	-0.301	0.469	0.427	-0.065	-0.256	-0.384	-0.118	-0.281	0.405	0.587	0.359	-0.166	0.805	0.978	0.941	0.767	-0.865	-0.593	1.000	
hi	-0.797	-0.436	0.160	-0.186	0.219	0.171	0.020	0.084	-0.525	-0.326	-0.042	0.101	0.202	-0.063	0.232	-0.365	-0.354	-0.297	0.140	-0.659	-0.939	-0.858	-0.607	0.922	0.531	0.922	1.000

Table: 12 CORELATION BETWEEN GRAINGROWTH AND SENESCENCE

	a1	a2	a3	b	m	c	ton	toff	tlin	yon	yoff	ylin	ym	bm	blin
a1	1.000														
a2	0.657	1.000													
a3	0.021	-0.340	1.000												
b	-0.075	-0.473	0.717	1.000											
m	0.198	0.785	-0.174	-0.308	1.000										
c	-0.087	0.161	-0.294	-0.682	0.109	1.000									
ton	0.403	0.377	0.421	0.530	0.553	-0.591	1.000								
toff	0.543	0.878	-0.701	-0.629	0.592	0.163	0.099	1.000							
tlin	0.001	0.392	-0.884	-0.874	0.144	0.537	-0.621	0.698	1.000						
yon	0.506	0.239	0.761	0.492	0.307	-0.306	0.794	-0.181	-0.762	1.000					
yoff	0.400	0.278	0.698	0.570	0.438	-0.399	0.907	-0.135	-0.769	0.957	1.000				
ylin	0.282	0.256	0.647	0.632	0.476	-0.426	0.923	-0.131	-0.751	0.871	0.974	1.000			
ym	0.295	0.731	-0.205	-0.305	0.859	-0.189	0.556	0.651	0.173	0.265	0.347	0.338	1.000		
bm	0.035	0.186	-0.182	-0.057	0.324	-0.503	0.396	0.159	-0.108	0.143	0.204	0.161	0.491	1.000	
blin	0.748	0.796	-0.313	-0.316	0.538	0.016	0.431	0.678	0.163	0.339	0.339	0.267	0.478	0.471	1.000

significant negative correlation with linear rate. Relative leaf number at offset has no significant correlation with linear rate.

4.7.3 Correlation between regression parameters for relative leaf number and agronomic traits

Grain yield has a significant negative correlation with T-on and Y-off. Stalk and bio-mass yield have significant negative correlation with T-on and positive correlation with linear rate of senescence(T-lin). HI has a negative correlation with T-lin and Y-lin. Seed density had a negative correlation with yield parameters. The total relative green leaf number duration from flowering to harvest has a significant positive correlation with T-on, Linear duration of senescence, relative leaf number at onset and stalk weight of the plants while having a negative correlation with Linear rate of senescence. Grain filling duration (GS3) was found to be negatively correlated with head weight and seed density and stalk weight is negatively correlated with grain weight, threshing percentage and harvest index where as100 seed weight was negatively correlated with seed density. Biomass, grain weight, threshing percentage, 100 seed weight and seed density were negatively correlated with T-on and positively correlated with T-off (Table 11).

Correlation between relative leaf number and grain growth

The relative leaf number at onset (T-on) and offset (T-off) of senescence were negatively correlated with offset and linear phase of grain growth. The grain growth duration from physiological maturity to harvest was also found to be negatively correlated with relative leaf number at the linear phase of senescence. Total relative grain growth duration (GGD) was found to be positively correlated with the relative leaf number duration (RLND). The

Table: 8 Data showing DNA polymorphism using microsatellite markers (V7512, V7518, V7523, V7525)

Primer	Mol. Wt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	of marker																				
V7512	600bp	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1
	500bp	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	1
	400bp	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
	300bp	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
		1	1	1	1	1	1	1	0	1	1	1	0	1	1	0	1	1	0	1	1
		0	1	1	0	1	1	1	1	0	0	1	0	1	1	0	1	1	1	0	1
		1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1
		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		41	42	43	44	45	46	47	48	49	50	51	52	53							
		1	1	1	1	1	0	1	1	1	1	1	1	1							
		1	0	0	1	1	1	0	1	1	1	0	0	0							
		1	1	1	1	1	1	0	1	1	1	1	1	1							
		1	1	1	1	1	1	1	1	1	1	1	1	1							
Primer	Mol. Wt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
V7525	of marker																				
	400bp	1	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0	1	1	1
	300bp	1	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	1
	200bp	0	1	1	1	1	1	1	0	1	1	1	0	0	1	0	1	1	0	1	0
		21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
		0	1	0	0	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	0
		0	0	0	0	0	1	1	0	1	1	0	1	1	0	0	0	0	0	1	0
		1	0	1	1	1	0	0	1	0	0	1	0	0	1	1	1	1	1	0	1
		41	42	43	44	45	46	47	48	49	50	51	52	53							
		1	1	1	1	1	1	1	1	1	0	1	1	1							
		1	0	0	0	0	1	0	0	1	0	1	0	0							
		0	1	1	1	1	0	1	1	0	1	0	1	1							

Primer	Mol. Wt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
V7518	of marker																				
	400bp	0	1	0	1	0	1	1	0	1	1	1	0	0	0	1	0	1	1	0	0
	200bp	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		21	22	23	24	25	26	27	28	29	30	31	32	33	33	35	36	37	38	39	40
		1	0	1	1	1	0	1	0	1	0	1	1	0	1	1	1	0	1	1	1
		1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
		41	42	43	44	45	46	47	48	49	50	51	52	53							
		1	1	1	0	1	1	1	0	1	1	1	1	1							
		1	1	1	1	1	1	1	1	1	1	1	1	1							
Primer	Mol. Wt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
V7523	of marker																				
	400bp	1	0	0	0	1	1	1	1	0	0	1	0	1	1	0	1	1	0	1	0
	300bp	0	1	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	1	0	1
		1	1	0	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
		21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
		0	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	0	1	0	1
		1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	1	0	1	0
		1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1
		41	42	43	44	45	46	47	48	49	50	51	52	53							
		0	0	0	0	0	1	1	0	0	0	1	0	1							
		1	1	1	1	1	0	0	1	1	1	0	1	0							
		0	1	1	1	1	0	1	1	1	1	1	1	1							

Scoring: 1 Band present
0 Band absent

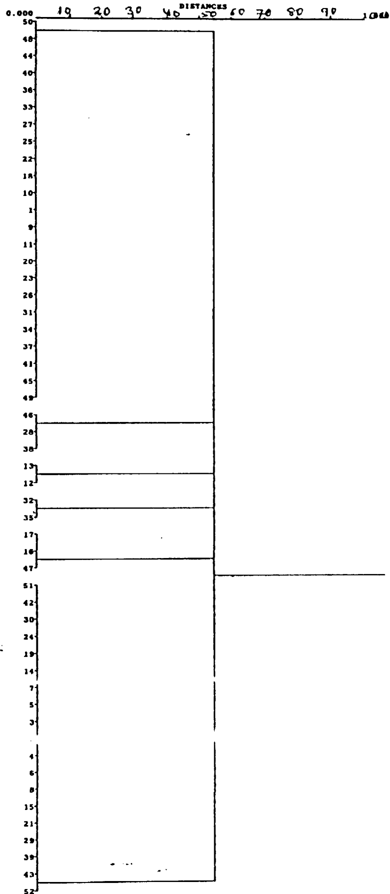


Fig. 40: Dendrogram showing diversity among sorghum genotypes using molecular marker (V7512)

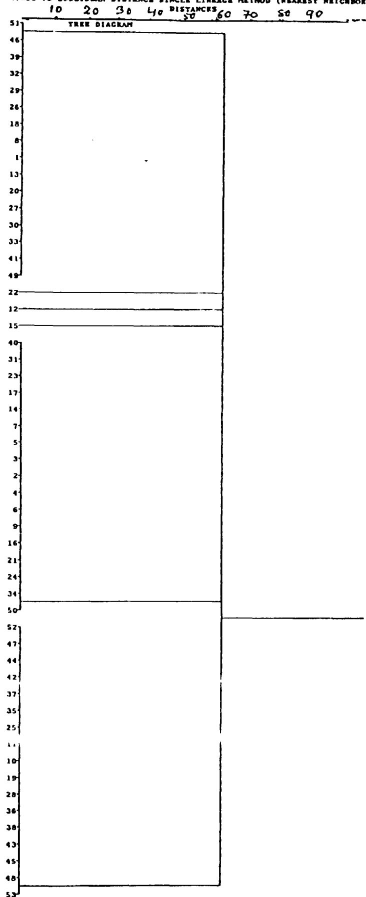


Fig.4I. Dendrogram showing diversity among sorghum genotypes using molecular marker (V7518)

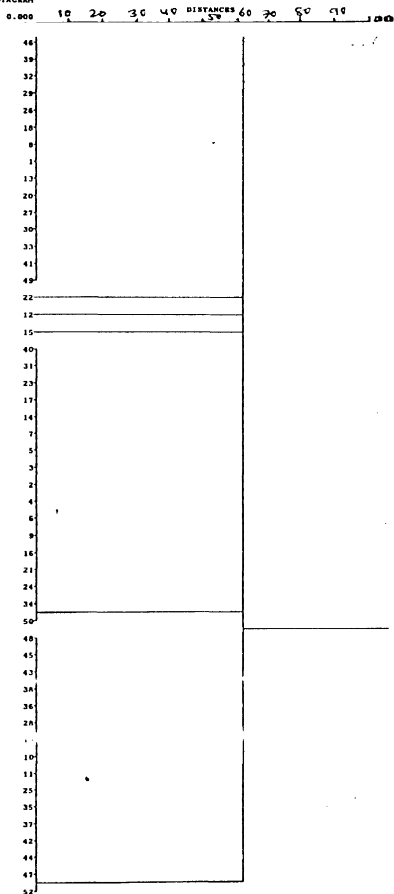


Fig 42: Dendrogram showing diversity among sorghum genotypes using molecular marker (V7523)

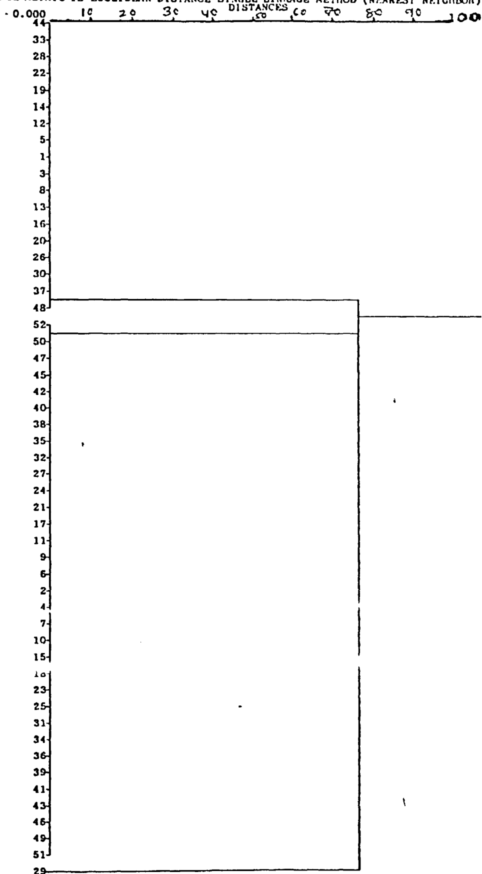


Fig.43: Dendrogram showing diversity among sorghum genotypes using molecular marker (V7525)

relative leaf number duration upto onset and from onset to offset were positively correlated with grain growth duration during these stages(Table 12).

Correlation between grain growth and agronomic traits

The grain filling duration (GS3), head weight, grain weight, biomass and seed density were negatively correlated with grain growth duration from onset to offset (A2), with onset of grain growth (T-on) and with Y-on, Y-off and Y-lin (Table 11).

4.8 MOLECULAR ANALYSIS

4.8.1 Detection of variability among sorghum genotypes using microsatellites

To evaluate the genetic diversity in sorghum, 48 genotypes were screened with 8 different microsatellite primers, obtained from University of Southampton, U.K. Of the 8primers 4 primers (V7512, V7518, V7523 and V7525) showed good polymorphism. Cluster analysis was carried out using the data obtained by screening 48 genotypes with 4 primers. Only the most intense and reproducible DNA bands were considered for analysis. These were scored as 1 (for presence) and 0 (for absence). Pairwise genetic distances were calculated by the percentage disagreement method. These data were used to cluster the accessions into the defined groups using GENSTAT software package.

From the total number of amplification products, they were classified as per polymorphism for atleast one accession tested (Table 13). To better understand the genetic relationships among the genotypes, they were clustered into defined groups based on their genetic distances. The microsatellite primer V7512 classified the 48 sorghum genotypes into 6 divergent groups. The first group composed of, numbers 50, 48, 4, 40, 36, 33, 27, 25, 2, 18, 10, 1, 9, 11, 20, 23, 26, 31, 34, 37, 41, 45and 49. The second group

composed of numbers 46, 28,and 38, where as the third group composed of numbers 13 and 12, fourth group composed of numbers 17,16 and 47 and the fourth group composed of numbers 51, 42, 30, 24, 19, 14, 7, 5, 3, 2, 4, 6, 8, 15, 21, 29, 39, 43,and 52. All the 6 groups were similar to each other at a distance of 0.538 (53.8%) (Plate 1, Fig. 44).

The microsatellite primer V7518 classified the 48 sorgum genotypes into 3 groups. The first group composed of (numbers 44, 33, 28, 22, 19, 14, 12, 5, 1, 3, 8, 13, 16, 20, 26, 30, 37 and 48). The second group composed of (numbers 52, 50, 47, 45, 42, 40, 38, 35, 32, 27, 24, 21, 17, 11, 9, 6, 2, 4, 7, 10, 15, 18, 23, 25, 31, 34, 36, 39, 41, 43, 46, 49 and 51) and the genotype numbered 29 was found to be most divergent from these two groups and formed a separate group. These 3 were similar to each other at a distance of 0.803(80.3%) (Plate 2, Fig. 45).

The microsatellite primer V7523 classified sorgum genotypes into 4 divergent groups. The first group composed of numbers 51, 46, 39, 32, 29, 26, 18, 8, 1, 13, 20, 27, 30, 33, 41 and 49). The second group composed of numbers 22,12 and 1). where as the third group composed of numbers 40, 31, 23, 17, 14, 7, 5, 3, 2, 4, 6, 9, 16, 21, 24, 34 and 51. Fourth group composed of numbers 48, 45, 43, 38, 36, 28, 19, 10, 11, 25, 35, 37, 42, 44, 47 and 52). All the 4 groups were similar to each other at a distance of 0.625 (62.5%) (Plate 3, Fig. 46).

The microsatellite primer V7525 classified sorghum genotypes into 4 divergent groups. The first group composed of numbers 51, 46, 39, 32, 29, 26, 18, 8, 1, 13, 20, 27, 30, 33, 41 and 49. The second group composed of numbers 22,12 and 15. where as the third group composed of numbers 40, 31, 23, 17, 14, 7, 5, 3, 2, 4, 6, 9, 16, 21, 24, 34 and 50). Fourth group composed of numbers 52, 47, 44, 42, 37, 35, 25, 11, 10, 19, 28, 36, 38,

43, 45, 48 and 53. These 4 groups were similar to each other at a distance of 0.625 (62.5%) (Plate 4, Fig. 47).

As microsatellite markers are locus specific and are very useful for the identification of polymorphism, for that particular locus. So these microsatellite primers can be used to screen a large no of genotypes within a short span of time. They are codominant markers allowing us to distinguish between homozygosity and heterozygosity for the staygreen trait.

PLATE 1 Polymorphism of sorghum staygreen genotypes using Microsatellites (SSR)
Primer used V7512

PLATE 2 Polymorphism of sorghum staygreen genotypes using Microsatellites (SSR)
Primer used V7518

PLATE 3 Polymorphism of sorghum staygreen genotypes using Microsatellites (SSR)
Primer used V7523

PLATE 4 Polymorphism of sorghum staygreen genotypes using Microsatellites (SSR)
Primer used V7525

PLATE 5 Field view of sorghum genotype B35 at physiological maturity

PLATE 6 Field view of sorghum genotype IS12555 at physiological maturity

B 35

PLATE 7 Field view of sorghum genotype Tx7078 at physiological maturity

PLATE 8 Field view of sorghum genotype E36-1 at physiological maturity.

TX7078

E36-1

PLATE 9 Field view of sorghum genotype R16 at physiological maturity

PLATE 10 Field view of sorghum genotype IS22380 at physiological maturity

R16

IS22380

PLATE 11 Field view of sorghum genotype M35-1 at physiological maturity

PLATE 12 Field view of sorghum genotype CSH6 at physiological maturity

CSH6

DISCUSSION

CHAPTER V

DISCUSSION

Sorghum is considered as a drought hardy plant adapted to harsh climatic conditions of the semi-arid tropics through the process of evolution. Various cultivars show different morphological and physiological modifications to overcome the various environmental stresses it encounteres during the crop growth. One such mechanism is the staygreen or non senescence. Nonsenescence is a delayed leaf and plant death resistance mechanism in sorghum plants that circumvents the detrimental effects of reduced soil moisture during post-anthesis growth . To study this trait eight sorghum genotypes (B35, IS12555,Tx7078, E36-1, R16, IS22380, M35-1, CSH6) were used. Out of the eight genotypes, B35 is a staygreen, post-flowering drought susceptible cultivar, which is also resistant to charcoal rot and it is a converted sorghum from IS12555 (*Zera zera* from Ethiopia). Tx7078 is a pre-flowering drought resistant, post-flowering drought susceptible and senescent cultivar which is also susceptible to charcoal rot. E36-1 is a terminal drought susceptible and charcoal rot susceptible cultivar. M35-1 is a popular *Rabi* sorghum cultivar, generally drought tolerant but susceptible to charcoal rot. CSH6 is a rainy season hybrid, highly susceptible to charcoal rot and moderately susceptible to terminal drought.

Among the developmental processes in plants, senescence is a relatively gross change or sequence of changes leading to the death of the plant in some cases. In plants these changes are recognized as a decrease in the growth rates and vigor, which in turn

results in increase in susceptibility to environmental challenges (abiotic stresses) or disease susceptibility (biotic stresses). In general, senescence may be categorized into 4 different types (Nooden, 1988)

1. Overall senescence, where the complete plant dies.
2. Top senescence, where the above ground part organ die off seasonally.
3. Deciduous senescence, where there is a seasonal, summer or winter foliage senescence depending on local 'stress' seasons.
4. Progressive senescence, which is encountered on most perennial woody species.

Leaf senescence may be characterized by involvement of all the leaves at the same time (synchronous senescence) or may pass up the stem in a wave in which the older leaves at the basal end of the stem senesce and die first (Sequential senescence) and additionally formed leaves continue to die as the plant reaches physiological maturity. The senescence type observed in the population was of the sequential type with the lower leaves senescing first, as reported by Duncan (1983). In the late reproductive stages, the panicle starteS senescing from tip downwards.

5.1 Climate

The population was evaluated during the post-flowering stage of crop growth for senescence. The crop was grown on preceding soil moisture in the post-rainy (*Rabi*) season and irrigated at ten days interval as and when required. The meteorological data indicates a high evapotranspiration rate and very scanty rainfall in the months of January 98 and February 98 which coincided with the flowering and initial grain filling stage of the crop. But the rapid depletion in soil moisture which was overcome by replenishing soil moisture by irrigating the terminal stages of crop growth.

5.2 Evaluation of Stay-green trait:

5.2.1 Leaf senescence studies

Data on leaf number was fitted against days after flowering to obtain the senescence pattern of the lines under study. Green leaf number gave similar results in all the genotypes suggesting that the parameter can be used in senescence studies especially if large populations are involved. Relative leaf number was used in the senescence studies as it has an advantage over absolute leaf number in that it does not take in to account the genotypic potential, hence represents staygreen per se. Senescence parameters obtained through the regression fit also facilitated study of staygreen trait in a better way. The genotypes showed wide variation for all the senescence parameters under study.

5.2.2 Rate Parameters :

The staygreen genotypes showed lower rate of senescence than the senescent genotypes. The regression curve can be distinguished into three distinct regions. The initial phase of senescence is the phase where the senescence occurs as a normal process in the life cycle of the crop. This phase involves gradual death of the lower leaves and loss of photosynthetic capacity. But in the reproductive stage ,i.e., when grain filling starts the rate of senescence was accelerated. This is the linear phase of senescence . After grain filling the senescence rate of the remaining leaves (if any) is again lowered as no further translocation of assimilates to the grain occurs. Considering that the rate of senescence during linear phase of senescence a normal phenomenon in the life of the plant any increase in rate of senescence in varied climatic conditions may be due to moisture stress.

5.2.2.1 Linear rate of senescence :

The staygreen genotypes had at least a 40 percent lower linear rate of senescence compared to the senescent genotypes. The high linear rate of senescence caused a decrease of 55 to 60 percent in the green leaf number in a period of 13 to 18 days in the senescent genotypes. In contrast the lower rates of linear senescence caused a 60 percent decrease in the effective leaf number in almost double the period ,i.e., 23 to 37 days in the staygreen genotypes. This basic difference in the linear rate of senescence could have a marked effect on the yield .The sudden increase in senescence rate with initiation of grain filling is natural and involves tight metabolic regulation of the tissues involved. The observations were in agreement with Thomas and Smart (1993) who were of the opinion that the accelerated senescence in absence of stress is more or less a change rather than loss of physiological efficiency. It was observed that the linear rate of senescence on an average increased by less than 25 percent in the staygreen genotypes when compared to the senescent cultivars. The increase in senescence rate in the staygreen genotypes may be considered as a normal response to the increased water stress. But the abrupt and rapid increase the senescence rate in the senescent genotypes at the same time implies lack of adaptive mechanism to overcome abiotic and biotic stresses as in stay-green genotypes. This can be inferred that the senescent genotypes enter the second phase of senescence characterized by rapid tissue deterioration and photodestruction of carotenoids which are the main protective pigments present in the leaf tissue protecting chlorophyll from photo-oxidation. This result is again in agreement with Thomas and Smart (1993).

One possibility is that in the senescent genotypes factors like impaired chloroplast function and partial stomatal closure result in decreased current photosynthesis. The failure of current photosynthesis is followed by rapid translocation of stored assimilates to the developing grain thus increasing the rate of senescence of the leaves due to less partitioning of assimilates. In the absence of stress (As replenishment of soil moiture was done by irrigating the crop frequently) the contribution of stored carbohydrates to the grain weight is estimated as only 10 to 15 per cent which is in confirmation with the reports of Kreig (1983). The staygreen genotypes retained more number of functional green leaves and thus were able to photosynthesize even under moisture stress. Genotypic differences in senescence rate was the largest at the point of inflection where the senescence rate was the highest for all the lines ,i.e., when about 55 to 60 per cent of the leaves have senesced in staygreen genotypes and 80 percent of the leaves senesced in the senescent genotypes. The results were in confirmation of those obtained by van Ooesterom *et. al.*(1996).

5.2.3 Onset of linear phase of senescence

The onset of linear phase of senescence was earlier in the staygreen genotypes when compared to the senescent genotypes. The probable reason for this may be the early initiation of grain filling in the staygreen genotypes when compared to the senescent genotypes. The rapid loss of green leaves in the senescent genotypes in half the duration taken by the staygreen types left insufficient green leaves at the end of the linear phase for efficient photosynthesis. The rate of linear senescence has a significant negative correlation with grain weight/m^2 and biomass/m^2. The onset of senescence is also having significant negative correlation with grain weight and biomass. This implies that in

genotypes with early onset of senescence the yields are reduced. At the same time duration of linear phase of senescence has a positive correlation with grain and stalk yield indicating that a longer duration of linear phase of senescence resulted in high grain and stalk yield. It was observed that although in the staygreen genotypes the onset of linear phase of senescence was early compared to the senescent genotypes the duration of linear senescence was longer which contributed to the higher yields in those genotypes as it increases the grain filling duration.

5.2.4 Green leaf number duration (GLND):

The number of physiologically active green leaves from flowering to maturity is very important for synthesis of current photosynthates which contribute the major bulk of the grain weight. The staygreen genotypes have a higher overall GLND when compared to the senescent genotypes In senescent genotypes the duration of A3 phase was more but it did not increase grain weight as during this stage no further partitioning of assimilates to grain growth occurs. But the decline in the green leaf number duration was most prominent (40 to 60 per cent) in the initial phase of senescence. This was due to the shorter duration before the onset of linear senescence. The staygreen genotypes showed 80 per cent more leaf number duration compared to the senescent genotypes during the linear phase of senescence. A high relative green leaf number during the linear phase had a positive influence on the grain weight and stalk weight ($r = 0.71$ and 0.56 respectively) as the duration of grain filling is more. Hence higher yields were observed in the staygreen genotypes when compared to the senescent genotypes as was observed by Gerik and Miller (1984). Grain growth duration during the linear phase of senescence has a significant negative correlation with stalk weight ($r = -0.318$) and no significant

correlation with grain weight (r = 0.09). So grain weight is primarily dependent on the leaf number during the linear phase of senescence while stalk weight depends both on the green leaf number duration during the linear phase and the overall post flowering period also.

5.3 Grain growth traits

Data on grain weight was fitted against days after flowering to obtain the grain growth pattern of the genotypes under study. Grain growth pattern was fitted well to the logistic equation in all the genotypes suggesting that the parameter can be used in senescence studies especially if large populations are involved. parameters obtained through the regression fit also facilitated study of staygreen trait in a better way. The genotypes showed wide variation for all the senescence parameters under study.

5.3.1 Rate Parameters :

The staygreen genotypes showed lower rate of senescence than the senescent genotypes. The regression curve can be distinguished in to three distinct regions. The initial phase of grain growth is the slower grain filling phase as the partitioning of current photosynthates senescence occurs for vegetative growth to a greater extent than for grain filling. But in the reproductive stage ,i.e., when grain filling starts the rate assimilate partitioning to the developing grains was accelerated, which is the linear phase of grain growth . After grain filling no further translocation of assimilates to the grain occurs as more than 80% of leaves showed senescence. Considering that the rate of senescence during linear phase of senescence is a normal phenomenon in the life of the plant the grain filling occurs both from current photosynthates and remobilization of stored

photosynthates which was in confirmation with the reports of Quinby1972; Giles *et al.*, 1975).

5.3.1.1 Linear rate of grain growth:

The staygreen genotypes had at least a 45 percent higher linear rate of grain growth compared to the senescent genotypes. The high linear rate of grain growth caused a increase of 55 to 60 percent in 100 seed weight in staygreen genotypes. This basic difference in the linear rate of grain growth could have a marked effect on the yield. The sudden increase in grain growth rate in the initiation of grain filling is natural and involves tight physiological and metabolic regulation of the tissues. One possibility is that in the senescent genotypes factors like impaired chloroplast function and partial stomatal closure result in decreased current photosynthesis which was in confirmation with the reports of Guiamet (1994). Genotypic differences in grain growth rate was the largest at the point of inflection where the grain growth rate was the highest for all the genotypes, when about 65 to 70 per cent of the grain filling is over in staygreen genotypes and 50 percent of the leaves senesced in the senescent genotypes. The results were in confirmation of those obtained by van Ooesterom *et al.*(1996).

5.3.2 Grain growth duration (GGD):

The grain growth duration from flowering to maturity is very important for synthesis of current photosynthates, that contribute the major bulk of the grain weight. The staygreen genotypes, thus contributing for more leaf area for assimilate synthesis resulting in higher yields in staygreen genotypes.

5.4 Agronomic traits

5.4.1 Days to 50 percent flowering :

The genotypes B35 and IS12555 differed significantly from each other in their flowering dates. However the days to 50 percent flowering is influenced by the genotype as well as the environment (G×E). But the rest of the genotypes does not show a marked difference in days to 50% flowering, the probable reason for uniformity in the flowering dates may be absence of moisture stress up to flowering due to the rains and irrigation received during the pre-flowering stage.

5.4.2 GS 3 :

The genotypes differed significantly from each other in their grain filling duration (GS3).The staygreen genotypes had a longer duration of GS3. Genotype B35 which had the longest grain filling period recorded higher average grain yields than the senescent genotypes, this may be due to greater availability of photosynthates for a longer duration in stay green genotypes. Blum (1985) reported that early maturityi.e., shorter duration of GS3 may be a potential benefit in situations where growth is achieved solely on stored water. Shorter grain filling duration under stress indicates rapid grain filling.

5.4.3 Relative water content (RWC): The relative water content (RWC) was measured at physiological maturity stages in the sorghum genotypes and it was observed that highest RWC was found in the staygreen genotype IS12555 (87.36%), followed by B35 (86.13%) and E36-1(86.1) where as the senescent genotype IS22380 possessed least RWC (81.67%) at physiological maturity stage, followed by R16 (82.02%) and Tx7078

(83.36%) respectively. This indicates that high RWC content of the staygreen genotypes helps in maintaining green leaf number, as well as to tolerate moisture stress to some extent.

5.4.5 Threshing percentage and Harvest index :

The threshing percentage remained more or less constant among indicating that staygreen ness did not have significant effect on the threshing percentage. The harvest index of the genotypes decreased significantly in senescent genotypes in comparision to the senescent genotypes.

5.4.6 100 Seed weight and Seed number/m^2

The 100 seed weight was more for staygreen genotypes than the senescent genotypes. The decrease in seed weight in senescent genotypes was due to reduced duration of linear phase of grain filling when compared to that of stay green genotypes. senescent decreased by 23 It was observed that the 100 seed weight of staygreen genotypes was approximately 56-60% higher than that of senescent genotypes which thus contributed for higher yields in staygreen genotypes. Though IS12555 had a lower 100 seed density the high seed number per unit area (seed density) may be the reason for high grain yields . In contrast CSH6 had the less number of grains per unit area but relatively higher 100 seed weight coupled with greater HI compensated the yields.

5.4.7 Stalk weight :

The stalk weight recorded was higher for the staygreen genotypes when compared to the senescent genotypes. The higher stalk weight of staygreen genotypes can be attributed to lower rate of leaf senescence and harvest index when compared to the senescent genotypes. The correlation of linear senescence rate with stalk weight was negative (-

0.074) indicating that a lower senescence rate contributed to higher stalk weight and linear senescence rate can be used for selecting genotyes with higher stalk weight.

5.4.8 Grain weight :

The grain yields were significantly more in the stay-green genotypes compared to the senescent genotypes indicating a higher genetic potential as well as a high resistance to terminal moisture stress in the staygreen genotypes. The yield of a grain crop like sorghum is a function of carbohydrates that are ultimately stored in the grain. Hence productivity ultimately depends on leaf area development and maintenance along with distribution of assimilates between grain and stover. Turner and Begg (1982) reported that water stress had a greater effect on leaf area than on photosynthtic rate per unit leaf area. Fischer and Turner (1980) suggested that TDM produced is largely a function of water that passes through the plant in transpiration. A high senescence rate in the senescent genotypes causes a rapid decrease in the number of functional leaves which causes significant yield reduction. The results obtained in the present investigation were in confirmation of their reports. The grain yield was highest in ISI2555 staygreen genotype. But B35 and E36 -1 which are also staygreen genotypes the grain weight was relatively less, this may be due to the reason that the leaf which is primarily the source may partially act as a sink in order to maintain its functional integrity thus depriving some of the carbohydrates to the developing grain due to which grain yields are decreased.

5.5 Molecular analysis

Out of the 8 microsatellite (SSR) primers (V7512, V7513,V7514, V7518, V7520, V7523, V7525, V7526) 4 primers were efficient in detecting the polymorphism among the sorghum genotypes. The SSR primer V7512 detected 4 polymorphic bands and classified the sorghum genotypes into 6 groups which were similar by a genetic distance of 0.538, primer V7518 detected 3 polymorphic bands and classified the sorghum genotypes into 3 groups which were similar by a genetic distance of 0.803. primer V7523 detected only 2 polymorphic bands and classified the sorghum genotypes into 4 groups which were similar by a genetic distance of 0.625, where as the SSR primer V7525 detected only 3 polymorphic bands and classified the sorghum genotypes into 4 groups which were similar by a genetic distance of 0.623 for the staygreen and senescence traits. The polymorphism obtained primers can be used in mapping the staygreen trait.

All these results indicated that staygreen is an important trait associated with drought tolerance and to increase yields in sorghum. Breeding for this trait is possible and studies by Van Oosterom *et al.*, (1996) indicated that this trait is heritable. The staygreen helps in selection of genotypes to be grown under drought conditions. The relative water content (RWC) measured at physiological maturity stage showed that the values were higher for staygreen genotypes than the senescent genotypes. This clearly shows that the staygreen genotypes can tolerate moisture stress to a greater extent than the senescent genotypes. The comparision of regression parameters for relative leaf number indicated that the staygreen genotypes have higher grain weight at physiological maturity than the senescent genotypes, thus giving higher yields.

SUMMARY

CHAPTER VI

SUMMARY

Staygreen is an important trait associated with post-flowering drought tolerance in sorghum. The present study for leaf senescence using eight sorghum genotypes (B35, IS12555, Tx7078, E36-1, R16,IS22380, M35-1, CSH6) contrasting in their senescence behavior was taken up at International Crops Research Institute (ICRISAT)- Patancheru, Andhra Pradesh in the post-rainy (*Rabi* season 1997-98 with the following objectives.

(I) Quantifying the expression of staygreen trait and yield potential in 8 sorghum genotypes.

(II) Observe if staygreen has any effect on grain growth pattern.

(III) Observe if staygreeness and grain growth pattern have any effect on yield of sorghum genotypes

(IV) Use Restricted length polymorphism (RFLP) and microsatellites or simple sequence repeats (SSR) primers to identify polymorphism between staygreen and senescent genotypes.

Under senescence study, the relative leaf number was plotted against days after flowering using a logistic nonlinear regression function. The senescence type observed in the population was of the sequential type with the lower leaves senescing first followed by successively formed leaves. Green leaf number gave reliable results justifying its use

in senescence studies. The genotypes were examined based on the senescence parameters of linear rate of senescence, onset and offset of senescence, linear duration of senescence and maximum rate of senescence which were derived by differentiating the fitted equation.

The genotypes belonging to the stay-green groups showed at least 40 per cent lower rate of senescence than the senescent genotypes. The rapid increase in the rate of senescence with growth was more marked in moderate senescence group and the high senescence genotypes. The high linear rate of senescence caused a decrease of 55 to 60 percent in the green leaf number in a period of 13 to 18 days in the senescent genotypes. In contrast the lower rates of linear senescence caused a 60 per cent decrease in the effective leaf number in almost double the period ,i.e., 23 to 37 days in the staygreen genotypes. Genotypic differences in senescence rate was the largest at the point of inflection where the senescence rate was the highest for all the genotypes. The onset of linear phase of senescence was earlier in the staygreen genotypes when compared to the senescent genotypes. The probable reason for this may be the early initiation of grain filling in the staygreen genotypes when compared to the senescent genotypes. The rate of linear senescence has a significant negative correlation with grain weight and biomass. Duration of linear phase of senescence has a positive correlation with grain and stalk yield indicating that a longer duration of linear phase of senescence higher is the grain and stalk yield. It was observed that although in the staygreen genotypes the onset of linear phase of senescence was early compared to the senescent genotypes the duration of linear senescence was longer and the linear rate of senescence lower which contributed to the higher yields of those genotypes.

The staygreen genotypes had higher overall green leaf number duration when compared to the senescent genotypes. The decline in the green leaf number duration was most prominent (40 to 60 percent) in the initial phase of senescence. This was due to the shorter duration before the onset of linear senescence. Among the cultivars the staygreen genotypes showed 40 per cent more leaf number duration compared to the senescent genotypes during the linear phase of senescence. A high relative green leaf number during the linear phase had a positive influence on the grain weight and stalk weight (r = 0.71 and 0.56 respectively). Total relative green leaf number duration had a significant correlation with stalk weight(r = 0.604) and no significant correlation with grain weight(r = 0.058) under agronomic traits. The dependence of grain growth on leaf senescence was studied by taking the weight of 4-5 spike-lets twice in a week and the grain weight was plotted against days after flowering using nonlinear logistic function. It was observe that grain weight is primarily dependent on the leaf number during the linear phase of senescence while stalk weight depends both on the green leaf number duration during the linear phase and the overall post flowering period also. The grain growth study revealed that grain growth was slow in the intial phase of grain filling although the rate of senescence was slow, this may be due the partitioning of photosynthates more for vegetative growth of the plants. The genotypes differed significantly from each other in their grain filling duration (GS3) and the nonsenescent genotypes had a longer duration of GS3.

The harvest index of the genotypes decreased significantly in the staygreen genotypes in comparision to the senescent genotypes. The senescent genotypes in general had a higher HI the decrease in HI was lesser compared to the stay-green genotypes. The stalk weight

recorded was higher for the staygreen genotypes when compared to the senescent genotypes. Lower senescence rate contributed to higher stalk weight and linear senescence rate can be used for selecting genotypes with higher stalk weight. The grain yields were significantly more in the staygreen genotypes compared to the senescent genotypes indicating a higher genetic potential as well as a high resistance to moisture stress and in getting higher yields in the staygreen genotypes. A high senescence rate in the senescent genotypes causes a rapid decrease in the number of functional leaves which causes significant yield reduction. In the high staygreen genotypes the leaf which is primarily the source may partially act as a sink in order to maintain its functional integrity thus depriving some of the carbohydrates to the developing grain due to which grain yields are decreased. Thus it appears that for grain purpose the moderate staygreen genotypes are better suited. Under post-flowering drought stress conditions. The RWC measured at physiological maturity stage was found to be higher in staygreen genotypes than the senescent genotypes, indicating its role in overcoming the moisture stress situations without causing any yield reduction in staygreen genotypes.

The molecular analysis using the 4 microsatellite primers revealed polymorphism between the sorghum genotypes for staygreeness . The SSR primer V7512 showed 4 polymorphic bands, while the primer V7518 showed 3 polymorphic bands and the primer V7523 identified 2 polymorphic bands but primer V7525 showed 3 polymorphic bands. Hence these primers can be used in marker assisted selection of the staygreen trait in sorghum genotypes.

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LITERATURE CITED

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