USE OF MOLECULAR MARKERS TO IDENTIFY PLANT TRAITS RELATED TO TERMINAL DROUGHT TOLERANCE IN SORGHUM

(*Sorghum bicolar (L.) Moench.*)

By

P. SATCHIDANAND
B. Sc. (Ag.)

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

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DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE, RAJENDRANAGAR
ACHARYA N.G.RANGA AGRICULTURAL UNIVERSITY
RAJENDRANAGAR, HYDERABAD - 500 030

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1997

CERTIFICATE

Mr. P. SATCHIDANAND has satisfactorily prosecuted the course of research and that the thesis entitled, **USE OF MOLECULAR MARKERS TO IDENTIFY PLANT TRAITS RELATED TO POST-FLOWERING DROUGHT TOLERANCE IN SORGHUM** (***Sorghum bicolar (L.) Moench.***) submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

Date : 31-10-97

(Dr. P. S. SARMA)

Chairman

CERTIFICATE

Mr. P. SATCHIDANAND has satisfactorily prosecuted the course of research and that the thesis entitled, **USE OF MOLECULAR MARKERS TO IDENTIFY PLANT TRAITS RELATED TO POST-FLOWERING DROUGHT TOLERANCE IN SORGHUM** (***Sorghum bicolar (L.) Moench.***) submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

Date : 29/6/97

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This is to certify that the thesis entitled, **USE OF MOLECULAR MARKERS TO IDENTIFY PLANT TRAITS RELATED TO TERMINAL DROUGHT TOLERANCE IN SORGHUM (*Sorghum bicolar (L.) Moench.*)** submitted in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE of the** Acharya N.G.Ranga Agricultural University, Hyderabad, is a record of bonafide research work carried out by **Mr. P. SATCHIDANAND** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. The published part has been fully acknowledged. All the assistance and help received during the course of investigations has been duly acknowledged by the author of the thesis.

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DECLARATION

I, **P.SATCHIDANAND** hereby declare that the thesis entitled **USE OF MOLECULAR MARKERS TO IDENTIFY PLANT TRAITS RELATED TO TERMINAL DROUGHT TOLERANCE IN SORGHUM (*Sorghum bicolar* (L.) *Moench*)** 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 work done by me. I also declare that the thesis or any part thereof has not been published earlier elsewhere in any manner.

Anand

Date: 31/10/97. **(P.SATCHIDANAND)**

Place: Hyderabad.

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ABSTRACT

Sorghum is an important dual purpose 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 a recombinant inbred population derived from two genotypes with contrasting drought reactions TX 7078 and B35 (pre-flowering tolerant, post-flowering tolerant), observe the yield stability of these lines under post-flowering drought stress and quantify their drought tolerance. The parents and a few selected lines were also genotyped with 2 RAPD primer to identify polymorphism.

The lines showed wide variation for all the senescence parameters under study. The staygreen lines had lower rate of senescence when compared to the senescent lines. The onset of linear senescence was earlier in the stay green lines indicating early initiation of grain filling. The offset of senescence was delayed in staygreen lines indicating a slow senescence and an extended period of grain filling when compared to the senescent lines. Although the staygreen lines yielded more than the senescent lines, 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 lines when compared to the senescent lines and had a significant influence on the yield of the lines. On an average the flowering and maturity were earlier under stress by one day. The height of the senescent lines was more than the staygreen lines and all lines showed a decrease in their height with stress. The incidence of charcoal rot disease was more in the senescent lines when compared to the staygreen lines and plant height had a positive correlation with soft stalk related lodging at maturity.

The RAPD primer *UMC176* identified two polymorphic bands while *OPB8* identified one polymorphic band between the senescent and staygreen types indicating that these primers could be used for selection for staygreen trait.

LIST OF ABBREVIATIONS USED

ABA	: Absicic acid
AFLP	: Amplified fragment length polymorphism
bp	: Base pairs
DAE	: Days after emergence
DAF	: Days after flowering
DAS	: Days after sowing
DNA	: Deoxy ribonucleic acid
d NTPs	: di-nucleotide tri-phospates
GLAD	: Green leaf area duration
GLND	: Green leaf number duration
IAA	: Indole acetic acid
LA	: Leaf area
M	: Molar
M^2	: square meter
MAS	: Marker assisted selection
ml	: milliliter
µ	: micro liter
ng	: nano gram
OA	: Osmotic adjustment

PCR : Polymerase chain reaction

QTL : Quantitative trait loci

RNA : Ribo nucleic acid

RFLP : Restricted fragment length polymorphism

RAPD : Randomly amplified polymorphic DNA

R-GLND : Relative green leaf number duration

TDM : Total dry matter

INTRODUCTION

CHAPTER I

INTRODUCTION

Drought is the primary factor contributing to crop yield losses around the world (Boyer 1982). 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 genetic variation for drought tolerance, selection for tolerance while maintaining maximum overall productivity has been a challenge (Rosenow *et al.*, 1983). There are several explanations for this problem. First, drought tolerance has been defined in several ways and the lack of a simple screening procedure has slowed down the selection of improved genotypes. Some researchers use grain yield *per se* to quantify drought tolerance, but selecting for grain yield under drought conditions is not efficient (Clarke *et al.*, 1992). Grain yield integrates the plant response to the environment over the entire crop season and may not efficiently discriminate between drought tolerant and susceptible genotypes. An alternate measure of drought tolerance is based on the stability of yield or some other trait across drought and non-drought environments (Fischer and Maurer, 1978). The problem with stability measurements is that selection for stability can lead to stable but poor yielding Lines under optimal conditions (Clarke *et al.*, 1992). Selection for drought tolerance should ideally integrate high yield potential with stability of agronomic performance across drought-prone environments. The second difficulty in selecting for drought tolerance is that genotypes must be screened for tolerance in controlled environments where drought can be routinely imposed. Testing under dry-land conditions is difficult because specific

drought conditions cannot be easily and reproducibly imposed. Finally, drought tolerance is subject to strong environmental variation and genotype x environment interaction (Clark *et al.*, 1992). Genotypes selected for adaptation to drought in one environment may show poor adaptation in other dry environments unless the two environments are very similar. Genotypes selected solely for adaptation to drought often display poor grain yield potential under optimal conditions (Blum, 1979; Rosenow and Clark, 1981).

The difficulties of quantifying drought tolerance have led physiologists and plant breeders to search for specific mechanisms that condition tolerance or susceptibility. It has been argued that if components of drought tolerance can be identified and selected independently of yield, then progress toward high-yielding, well-adapted genotypes could be more rapid (Blum 1983, Rosenow and Clark 1981). The problem with this approach is that many traits have been proposed as indicators of drought tolerance, but there has been little evidence supporting their agronomic merit (Ludlow and Muchow 1990).

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. It is one of the most drought tolerant grain crops and has been extensively used to study 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 growth stage but are susceptible at other times (Rosenow and Clark, 1981). Stress during the post-flowering stage causes a rapid decrease in grain and stalk yield in sorghum and increases its susceptibility to pests and diseases. Therefore any mechanism which confers tolerance to drought during the post-flowering period in sorghum is

beneficial. Staygreen is one such mechanisms or trait that confers post-flowering drought tolerance in sorghum by delaying plant and leaf senescence under terminal moisture stress. The trait is shown to be heritable and improvement through breeding is possible.

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). The complex expression of drought tolerance makes this trait difficult to study using traditional genetic and physiological methods. Use of molecular markers and QTL analysis of drought tolerance in Lines grown in replicated and carefully induced drought environments may lead to a better understanding of this trait. The markers can also be used in marker assisted selection for the trait in other populations.

The purpose of this investigation was to study the sorghum recombinant inbred Lines for post-flowering drought tolerance. The following were the specific objectives addressed:

(I) Quantifying the expression of staygreen trait and yield potential in a set of RIL Lines and their parents.

(II) Observe if staygreen has any effect on charcoal rot resistance and lodging.

(III) Use Randomly amplified polymorphic DNA to identify polymorphism between staygreen and senescent Lines.

REVIEW OF LITERATURE

CHAPTER II

Review of Literature

Drought is one of the most important abiotic constraints limiting production in the semi-arid tracks of the world. Sorghum is an important crop of such areas showing many adaptive traits. The literature available on drought tolerance with special reference to sorghum is reviewed below under the following heads.

1. Drought and drought resistance in general
2. Anatomy, morphology, 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. Molecular markers and their importance in screening for drought resistant traits like staygreen

2.1 Drought and Drought Resistance

Water stress in mesophytic cultivated species is the most common type of plant stress in most regions of the world, and is the main bottleneck of agricultural development . Drought is the most prevalent environmental stress factor limiting plant growth, survival and productivity (Bohnert and Jensen, 1995; Boyer, 1982). Water stress causes deliterious physiological effects like disruption of membrane structure and concomitant organelle disarray (Kuiper, 1977), impairment of stomatal function (Willmer and Pantoja 1992), reduction in root growth (Blum and Jhonson, 1992) and reduction in yield (Henson,

2.2 Drought resistance in sorghum

Among all cultivated plants, sorghum is considered as highly drought tolerant species next only to date palm (Horritz, 1983). Blum (1979) observed that sorghum genotypes showed wide variations in drought escape, drought avoidance and drought tolerance mechanisms. Early genotypes were drought escaping, and had lower evapotranspiration due to smaller leaf area. In drought avoiding types, the root resistance to water uptake was reduced and cultivar resistance 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 after stress (Blum, 1979). Santamaria *et al.* (1986) found correlation among drought tolerant traits but not drought avoidance traits; they correlated leaf rolling positively with OA. Bennett and Lucker (1986) reviewed that the epicuticular wax present on the underside of the leaf and upper leaf sheath aids in moisture stress tolerance. Bewazir and Idle (1989) indicated that the extent of leaf rolling in sorghum is a measure of degree of water stress. However decreased radiation absorption or light reflection by leaf rolling and reduction in cuticular loss of water saved insignificant amounts of water and so did not benefit much in drought avoidance. Sorghum is also well adapted to drought due to a higher root hair density per unit length (Blum, 1988), and larger rooting depths of up to 2.0 to 2.3 m (Maity, 1986). Dogget (1988) showed silica deposits in the endodermis of the root of sorghum thus enabling it to withstand high pressures during drought stress. Bawazin and Idle (1988) observed that relative conductivity and number of seminal roots were negatively correlated with per cent survival

and a high relative conductivity indicates drought resistance in Lines with less restricted seminal roots.

2.2.1Growth stages and drought stress

The period of development sorghum has 3 phases, the vegetative (GS1), reproductive (GS 2) and grain filling period (GS 3) (Eastin *et al.*, 1973). Krieg (1983) suggested that drymatter production is strongly influnced 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 also said that tillers are more sensitive to water stress than the main stems. Lira *et al.* (1989) observed that the most resistant genotypes were those characterized by slow vegetative development. 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 preanthesis assimilates. But in conditions of stress the contribution of preanthesis assimilates to grain weight increases (Krieg, 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 drymatter accumilation 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

1984). O'Toole and Chang (1978) and Gaff (1980) observed that crop plants, unlike xerophytes, use more than one mechanism to resist moisture stress. Levitt (1972) grouped drought resistance mechanisms into three types. Drought escape, drought avoidance and drought tolerance.

Turner (1979), observed that the mechanisms that enable crop plants to escape drought are - early maturity, developmental plasticity and remobilisation to grain of stem reserves stored before anthesis. With regards to developmental plasticity, Ludlow and Muchow (1990) pointed out that adaptation of annual crop genotypes to the expected length of the growing season is the single most important aspect to enhance both survival and production in arid environment. Drought resistance is a phenotypic expression of a number of morphological and physiological mechanisms. Ludlow and Muchow (1990) called these characteristics and mechanisms as traits. They further stated that drought resistance is not due to a single trait, but is the combination of mechanically linked traits called strategies. Plants with the avoidance strategy show enhanced water uptake through deep roots and reduced water loss by stomatal closure, leaf movement or rolling and leaf area reduction (Ludlow, 1980). The important strategy in crop plants is drought tolerance. Gaff (1989) observed that plants tolerate dehydration through high desiccation tolerance, which enables them to survive low tissue water status. Osmotic adjustment (OA) enhances dehydration tolerance by lowering the leaf water potential at which critical relative water content (or cell volume) is reached (Flower and Ludlow, 1986).

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 (1985) observed that as grain begins to dry, the remaining green leaves start to senescence, the rate of which is distinct for each variety. Krieg (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. Bradget *et al.* (1994) concluded that in general sorghum genotypes are more drought tolerant at the preflowering stage than at the post-flowering stage.

According to Salam *et al.* (1992) resistant 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 (preflowering) 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 possesing high level of tolerance at one stage tend to be susceptible at the other stage.

2.2.2 Osmotic adjustment in relation to drought stress in sorghum

OA reduces the sensitivity of turgor-dependent processes such as leaf expansion, stomatal conductance and leaf rolling to 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, Takani *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 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). Al-hamdani *et al.* (1988) observed that smaller decrease in water potential, somatal conductance and CO_2 assimilation at preanthesis than postanthesis stage in majority of the drought tolerant genotypes.was observed in most drought tolerant genotypes. The drought resistant genotypes showed higher OA and sufficient decrease in leaf water potential to maintain leaf turgor (Salman, 1995). Santamaria *et al.* (1986) noticed a decrease in OA towards the end of drying cycle in early genotypes and increase in OA in the late genotypes. 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. Kannagara and Seetharama (1983) showed high linear correlation between absicic acid, leaf water potential and plant height.

2.3 Influence of drought resistant traits on yield and yield components of sorghum

Dhoble and Kale (1988) showed positive correlation of grain yield with plant height, leaf area index 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 harvest index 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 (total dry matter-TDM and leaf area-LA) and those related to drought resistance (total and relative moisture loss and moisture loss / unit leaf area).

Khizzak 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, HI; while grain yield was positively correlated with height, panicle exertion, lodging, HI 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.

2.4 Harmonal changes in sorghum under drought stress

Most, if not all of the known phytoharmones have been implicated in water stress. Conclusive evidence of absicic acid (ABA) control or response to drought stress was given by Davies and Jhang (1991). Kannagara *et al.* (1982) showed high levels of indole acetic acid (IAA) 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. Jhang 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.

2.5 Selection for drought tolerance

Yield is not an effective selection criteria for drought tolerance as naturally occurring environments are variable and unrepeatable and the precision of measurement of genotypic differences in yield is often poor with low heritiability (Blum, 1985). Further yield variation under stress may be due to genotypic differences in yield potential and the drought escaping nature (Fischer and Maurer,1978; Bidinger *et al.*, 1987). Accordingly Blum (1983) suggested that selection for drought tolerance must combine selection for yield potential (and presumably an appropriate phenology) in favorable conditions; with selection under stress for the expression of traits thought to be associated with drought tolerance. Bularos and Edneades (1988) advocated that whole plant or crop response to stress (called 'integrated traits') are more effective as selection criteria for drought. Sinha (1987) argued that traits representing phenological and morphological adaptations represent greater integrating effect than physiological and biochemical adaptations for drought resistance. Staygreen is one such trait.

2.6 Staygreen trait and senescence

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, thereby 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 occur. 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.

2.6.1 Senescence related genes

Thomas and Smart (1991) recognized 5 broad categories of genes with functions in senescence according to their patterns of expression doing leaf development.

1. Genes controlling the primary metabolic activities of viable cells like, rRNA synthesis, respiratory enzymes etc.
2. Genes directing development of latent metabolic machinery in mesophyll cells of leaves which later becomes active. Example : vascular enzymes.

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 an 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.

Zartman and Woyedwojic (1979) observed that senescent types had a greater root system than the non-senescent types up to 100 days after sowing(DAS), after which the root density of senescent types declines but the non-senescent types exhibit only a minimal decrease. 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).

2.6.4 Inheritance of staygreen trait :

Studying the heritability of staygreen trait in sorghum, 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 Oosterom *et al.* (1996) at ICRISAT observed that the inheritance of onset of senescence in sorghum was additive, but a slow senescence rate was dominant over fast rate. Consequently a large relative green leaf area duration (GLAD) , i.e., slow senescence was partially dominant over a

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 dry weight of a hybrid between two tropically adjusted 'non-senescent' (staygreen) sorghums was greater

3. 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.
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*.

Alterations within each class of senescence related genes, such as the timing of a genes or extension in the life cycle may cause a charge in the greenness of the phenotype.

2.6.2 Different types 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. Genes involved in generation of type A staygreen Lines come from Group 4 of senescence related genes while genes effecting type B come from Group 5.

2.6.3 Staygreen and its influence on drought resistance in sorghum

Rosenow and Clark (1995) described two distinct responses to drought in sorghum. The preflowering response is expressed when plants are stressed during panicle differentiation prior to flowering (GS2) and the post-flowering response is expressed when moisture

small relative GLAD. Further, because of a larger leaf area at flowering, the partial dominance in relative GLAD translated into over dominance for a large absolute GLAD, suggesting the usefulness of staygreen trait in sorghum for improving drought tolerance in environments with post-flowering drought stress. Xu *et al.* (1995) identified two genotypes B35 and Tx 7000 showing differential response to post-floweirng drought stress. B35 is a staygreen Line retaining much more of the chlorophyll and losing much less in grain yield compared to the non-staygreen Line Tx7000.

2.7 Molecular markers and progress in sorghum genome mapping

Since the first introduction of restricted fragment length polymorohism (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, randomly amplified polymorphic DNA (RAPDs), microsatellites sequence-tagged sites (SSRs) and amplified fragment lenghth polymorphism (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 quantitative trait loci (QTL) mapping in sorghum. An F_2 population derived from crossing *Sorghum bicolor* (CK 60) and *Sorghum*

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.* (1994) 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 11per cent. Null alleles occurred at 13 per cent of the mapped RFLP loci.

2.7.1 Molecular markers and their role in drought resistance breeding

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 in breeding objectives. 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 way for cloning such genes and their insertion into drought susceptible Lines.

2.7.2 Tagging QTLs associated with drought resistance in sorghum

Sorghum is a diploid cereal (2n=20) with a relatively small genome of 748-772 Mbp (Arumuganathan and Earle, 1991). It is well known for drought resistance and successful mappng for drought resistance in this species could serve as a cereal crop model, and as a source of genes for other crops such as maize in which improved drought tolerance is of prime importance.

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 10-50per 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 per cent of phenotypic variation with QTL on Group C alone accounting to 38 per cent. The map resolution at the QTL interval varied between approximately 5 CM for QTLs on linkage Group C and over 10 CM on linkage Group G and H.

Tuinstra *et al.* (1996) identified QTLs associated with preflowering drought tolerance in sorghum by mapping a RIL progeny of two genotypes (TX7078 and B35) contrasting for their drought tolerance. Using 150 RAPD & 20 RFLP markers that mapped to 17 linkage Groups, the established six regions of the genome to be specifically associated with preflowering drought tolerance. The phenotypic characters of grain yield, yield stability, seed set stability and height stability related to preflowering 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.

Context of present study

Although exhaustive work has been done on the study of staygreen trait using RIL derived by crossing B35 with Tx7000, there is still a need to evaluate the RIL for seggregation of the trait under the conditions relevant to post-rainy season sorghum production in India. The behavior of the trait under terminal drought, its impact on yield and yield attributes, maturity and duration of the crop and resistance to charcoal rot needs further study. Also the nature of leaf senescence needs to be further elucidated.

CHAPTER III

Materials and Methods

The genotypes (parents and recombinant inbred lines) were evaluated for staygreen trait and influence of this trait on crop yield and lodging at International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru, Andhra Pradesh.

3.1.1 Locations

Field trail was conducted at ICRISAT Research farm at Patancheru. The lines were grown in both dry (terminal stress with no further irrigation after crop establishment) and irrigated environments in field BL 2B. The area of the plot was 2600 m^2 divided into two equal parts for the Dry and Irrigated treatments with a 20 m bufffer zone.

3.1.2 Nature of soil

The soil type is black loamy. The plot is a shallow Vertisol inceptisol field which does not contain enough stored water for complete grain filling in a dryland crop (and so is ideal for drought stress related field experiments) based on experience during several previous years.

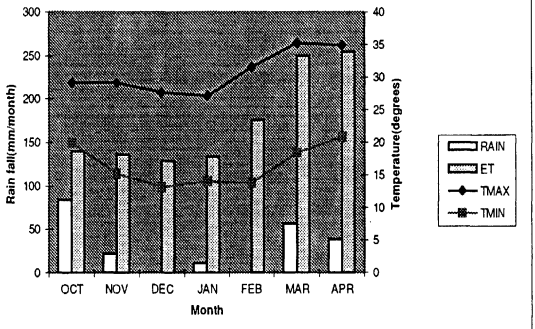
3.1.3 Growing season

The crop was planted on 15 October 1996 during the post rainy season of 1996-1997. So the crop was subjected to continually decreasing residual soil moisture as season progresses.

Table 1 Weather during post rainy (rabi) season 1996-97 at ICRISAT

Month	Rainfall mm/mon	ET mm/mon	T Max Avg	T Min Avg	RH07 %mean	RH14 %mean	ind speed kmph
October	83.7	139.5	29.1	20.0	89.3	59.7	6.4
November	22.4	136.3	29.17	15.3	86.9	43.4	4.3
December	0	129	27.7	13.2	86.6	38.2	4.2
January	11.4	133.7	27.2	14.0	90.0	41.0	6.7
February	0	175.9	31.6	13.7	84.1	24.1	4.7
March	56.8	249.4	35.2	18.4	77.4	23.8	6.7
April	38.4	254.1	34.9	20.9	78.1	28.1	8.2

FIG : Rainfall, evaporation and temperature during crop growth



3.1.4 Agroclimatology and weather during crop period

In the month of October 1996 the rainfall recorded was 83.7 mm. Most of the rainfall occurred before the sowings and so there was enough soil moisture in the soil for seed germination. In November 1996, a little over 22.4 mm of rainfall was received. The rainfall during the months of October and November 1996 coincided with the pre-flowering growth stages of the crop. There was no rainfall during the months December 1996 and February 1997. Only 11.4 mm of rainfall was received in January which is minimal and not enough to relieve the moisture stress which has already set in during that period. Evaporation increased steadily from 129 mm/month during December 1996 to 254.1 mm/month during April 1997. The increased evapotranspiration (ET) levels coupled with lack of rainfall during the post-flowering crop growth stage created ideal moisture stress conditions. The rainfall received in March and April 1997 was 56.8 and 38.4 mm respectively. Therefore, during the flowering and maturity stages the crop received minimal rainfall and the conditions were ideal for experiment to be carried out under water stress. The rainfall during March 1997 fell after postphysiological maturity stage in the crop in Dry environment and physiological maturity in Wet environment and hence does not have any effect on the results of the experiment. The average maximum temperatures for the months of December '96, Jan'97 and Feb '97 were 27.7°C, 27.2°C & 31.6°C, respectively due to which the secondary stress of high temperatures can be considered marginal and the crop performance may be attributed mainly to water stress.

3.1.5 Description of the plant material :

The population used in the study of the staygreen trait was developed in Purdue, USA by Dr. G. Ejeta. It consists of 97 random lines selected from a cross between two genotypes contrasting for the staygreen trait. The parent B35 is a staygreen, postflowering drought tolerant and preflowering drought susceptible cultivar, which is also resistant to charcoal rot. It is a converted sorghum from IS 12555 (*Zera zera* from Ethiopia). The other parent TX7078 is a preflowering drought resistant, post-flowering drought susceptible and senescent line and also is charcoal rot susceptible. The contrasting drought response of these two genotypes was confirmed by Premchandra *et al.* (1994). RI Lines were developed by randomly selecting 97 F2 individuals and selfing them to the F_5 generation by the single seed descent of plant breeding. Selfed seeds from the F_5 generation were grown by head to row method and several panicles from each row were selfed and bulked to represent each Line. In the succeeding generations the RI Lines were planted in rows, the representative plants selfed and bulked to represent the next generation. F8 population was evaluated in this trail for drought tolerance and yield stability.

3.1.6 Experimental details

Crop is *Sorghum bicolor* L. Moench

The date of planting was 15th October 1996.

The Lines in Wet and Dry environments were planted as a two rows plots of 0.75 x 4.0

Design of the experiment was 10×10 triple lattice

tagged plants. The average of leaf area of the six tagged plants gave the absolute leaf area for a particular Line, at the given time after flowering.

Relative green leaf area

The relative leaf green area (RGLA) was computed at each dates for the 5 entries in all replications using the formula:

Relative green leaf area = (Absolute leaf area on the given date/absolute leaf area at flowering) 100

Leaf number count

In view of a large number of plots under study (600), the senescence study was taken up using leaf number count instead of the leaf . The number of green leaves for each Line at flowering (when the plant is supposed to have developed maximum canopy) was recorded. At an interval of about 7 days, from the date of 50 per cent flowering till harvest the green leaf number was recorded. The full green leaf was counted as a unit. Each leaf was visually scored for greenness, and a score of 1 to 0 (i.e. 50per cent green leaf was counted as 0. 5 and 35 per cent green leaf was counted as 0. 35; and so on) was given. The approximation was ± 10 per cent of the exact leaf number when the same partially senescenced leaf was scored by different individuals. The scored values for each leaf were summed up to give the green leaf number per plant. The average for the 6 tagged plants in each plot gave the leaf number for a given Line 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 100 Lines in each replication using the formula:

Date of sowing : Both the Wet and Dry plots were sown on 15 October 1996.

Method of planting : The seed was machine planted using a tractor with calibrated seed drills. The seed rate used was 15 kg /ha.

Emergence: Emergence was recorded on 19 October 1996, 4DAS in both the Dry and Wet environments.

Fertilization: At the time of field preparation a basal dose of 200 kg/ha of NPK 28-20-0 was incorporated in to the soil. Urea at the rate of 100 kg /ha was side dressed approximately 14 days after planting i.e. on 29 October.

Irrigation: The field was given a light (15-20 mm) sprinkler irrigation in both the Wet and Dry plots after planting to ensure full emergence. The Dry plot received no further irrigation. The irrigated plot was furrow irrigated, on 3 DAS and later on 15 DAS to recharge the soil profile fully. Furrow irrigations were later given four times at 15 days interval until approximately 30 days after 50per cent flowering in the Wet environment.

Interculture: Mechanical (interculture) cultivation was taken up twice at 10 and 21 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 crop completely smothers the weeds.

Disease control: Seed borne infections were checked by seed treatment with *Ridonil* at the rate of 0.1 ml a.i per kilogram of seed.

Insect control: The major insect pests effecting the young plant at early stages were sorghum shoofly *(Atherigona soccata)*, Sorghum aphid *(Rhopalsiphum maidis)* and thrips (*Thrips spps*). The shoot fly is the most serious problem. It was controlled by application

of 40 kg /ha *Furadan* with the seed at the time of sowing and 3 sprays of *Cypermethrin* (125 ml /ha mixed in water) at 5,10,& 15 days after emergence (DAE).

Insect damage during GS2(panicle development phase ending with 50% flowering) causes maximum damage in terms of yield reduction. To control insect pests during this stage *Carboforan* 5 % granules were applied with in the whorls at a rate of 0.2 g /plant. The granules were applied twice, the first application being 30 DAS and the second just before panicle initiation. During grain filling stage one more spray of *Cypermithrin* @ 125 ml a.i ha^{-1} was taken up.

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.

Harvesting

The grain became ready to harvest about a fortnight after the physiological maturity is attained. The crop in the Dry environment was harvested approximately 15 days after physiological maturity, and the crop in the Wet environment was harvested approximately 30 days after physiological maturity. 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.

Drying: The grain was sun-dried for 5-7 days to harden pericarp and for easy threshing.

3.3 Parameters for observation and data recording

Data on the expression of staygreen and charcoal rot infection in the lines was gathered to study their consequences on yield and yield component expression. The observations broadly fell into 4 Groups :

i) Phenological traits.

ii) Staygreen or senescence traits.

iii) Yield attributes.

iv) Charcoal rot related traits.

3.3.1 Phenological traits:

a)**Time of flowering**: The 50 per cent flowering dates for each of the Lines was recorded in both the Dry and Wet environments. The date on which approximately 50 per cent of the spikelets in the majority of the plants within the plot started shedding pollen up to half way towards the base of the panicle was recorded. Data was recorded at 2 day interval.

b)**Physiological maturity**: Sorghum grain attains physiological maturity when 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.

c) **Plant height**: Height of each of the six tagged plants per plot was measured using a meter scale and averaged to obtain the average plant height for each plot. The height was measured from the base of the stem to the tip of the panicle and recorded in centimeters.

3.3.2 Senescence traits:

All senescence observation were taken on 6 competitive plants tagged in each plot. The plants tagged were number 1-6, three in each row of the two row plot. The observations were taken approximately 7 days interval starting from 50 per cent flowering to harvest maturity. Observations were taken at 46, 76, 83, 90, 104 and 111 DAS (7 observations) in Dry environment and 74, 81, 88, 95, 102, 109, 116, 123 and 130 DAS in Wet environment (9 observations).

Absolute Leaf area: The absolute leaf area at flowering (when the plant is supposed to attain maximum leaf area) was computed in 5 selected entries - the staygreen parent B35, the two entries for TX7078 (the non staygreen parent), the high charcoal rot resistant selection Line -66, and the low charcoal rot resistant selection Line-91. The length and breadth of each leaf was measured. The area of each leaf was computed using the equation Area = l x b x c where 'c' is a predetermined constant. The value of 'c' for sorghum is 0.75. Then the area of all leaves was summed up to get the whole plant leaf area. The six plant leaf area was thus computed and averaged to get the representative absolute leaf area of the each Line at flowering.

At approximately 7 days intervals, a visual approximation of the green leaf area was made on percentage basis (to an accurately ± ten per cent of actual) and multiplied by the original leaf area of the same leaf obtained during flowering to get the individual leaf area. Then the leaf area of all the leaves were summed up to get the leaf areas of individual

Relative leaf number = (absolute leaf number at a given date/absolute leaf no at flowering) x 100

3.3.3 Yield attributes :

With regard to the yield attributes, the observations for the variables under study were obtained on six competitive 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 was weighted using a electronic balance (in grams).

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 six culm were cut to the base and the leaf separated from stem and the weight of the culm (leaf + stem) was recorded on per plant basis and averaged.

In the Wet environment the six leaf weight were recorded separately since the many leaves still remained green (and leaf weight could contribute substantially to stem weight).

Grain weight : The heads from each plot were harvested from the six 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 x 4000 = 3000 cm^2(3 m^2)

Spacing = 50 x 20 = 100 Cm^2

Seed density /m^2 = grain weight /m^2 ÷ 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 per cent of plants showing soft stalk at maturity and harvest were recorded under charcoal rot traits.

3.3.4 Charcoal rot traits:

Nodes spread: The stems were bisected and the number of nodes over which the charcoal rot was spread was noted and averaged in each plot to get the average nodes spread of the disease in each plot for all Lines.

Length spread: The six tagged stems were bisected and the length spread of the disease was measured with a scale. Stem with charcoal rot show threads with a sooty and dried up appearance of the vascular tissues in the pith of the stem. All the six plant data were averaged to get mean length of spread of the disease for each Line.

Soft stalk per cent at maturity: The number of plants showing soft stalk were counted out of the six tagged plants. Soft stalk per cent at maturity was obtained as the ratio of number of plants showing soft stalk to total number of plants (6) on percentage basis

Soft stalk % (at maturity) = No. of plants with soft stalks / 6 x 100

Soft stalk per cent at harvest: It was obtained as a ratio of number of plants showing soft stalk at harvest to the total number of plants on per cent basis for each Line.

Soft stalk % at harvest = No. of plants with soft stalk at harvest /6 x 100

The soft stalk per cent data was computed in the Dry environment only.

Approximate number of plants per plot = 3000/100 = 30 plants

∴ Number of plants /m^2 = 30/3 = 10 plants

Weight of a variable per square meter = Six plant weight × 1.66

Head weight /m^2 = six heads weight ×1.66

Stalk Weight /m^2 = six stalk weight x 1.66

Grain weight /m^2 = six head grain weight x 1.66

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 was 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 biomax/m^2 expressed on per cent basis.

HI(%) = (grainweight /m^2 ÷ Biomass /m^2) x 100

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

Threshing per centage = (grain weight /m^2)/(head weight /m^2) x 100

Threshing per centage for each Line over three replications was calculated

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 Lines.

3.4 STATISTICAL ANALYSIS :

Statistical analysis was done using *GENSTAT5* statistical software.

The data was analyzed for the following :

1) Regression analysis for senescence traits

2) Cluster analysis to Group the Lines based on their senescence pattern

3) Calculation of relative green leaf number duration under the regression curve

4) Analysis of variance for agronomic and charcoal rot traits

5) Correlation matrices between all relevant variables

3.4.1 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.

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

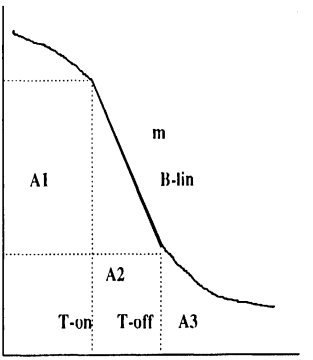
Where ,

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.



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

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**

Group averages for Regression parameters and Leaf number duration:

The average value for all the regression parameters and the RGLND for each of the Groups formed by cluster analysis was obtained by averaging the Lines present in each Group. The averages for each Group in the Wet environment were obtained for all the regression parameters and RGLND parameters by considering the same Grouping as in Dry environment.

3.7 Analysis of variance for agronomic and charcoal rot traits:

A combined analysis of variance for each of the data variables on which observations were taken was initially carried out

1) To know if the Dry environment differed significantly from the Wet environment.
2) To analyze if water stress had any significant effect on the mean performance of the Lines for the variables under study.

A separate analysis of variance done for each of the agronomic and charcoal rot variables to know:

1) If the Lines differed significantly from each other in their mean performance in the Dry environment.

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) and offset (T-off) 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' ($\mathbf{b-m}$) = $\mathbf{bc/4}$

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 .

3.5 CLUSTER ANALYSIS:

To Group Lines based on their senescence pattern cluster analysis is an ideal tool. Using the five parameters T-on, T-off, b-m, T-lin and B-lin the Lines were Grouped by cluster analysis in to six Groups at 94.5 per cent similarity for the senescence parameters under consideration . The regression parameters for the Lines under the Dry environment were used to make the dendogram. The cluster analysis and production of dendograms was done using *GENSTAT5* statistical soft ware.

3.6 RELATIVE GREEN LEAF NUMBER DURATION:

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.

Group averages for agronomic and charcoal rot traits :

The average value for all the agronomic and charcoal rot variables for each of the Groups formed by cluster analysis was obtained by averaging the Lines present in each Group. The averages for each Group in the Wet environment were obtained for all the agronomic and charcoal rot traits by considering the same Grouping as in Dry environment.

Analysis of variance was done using Genstat5 statistical software.

3.8 CORRELATIONS:

Influence of agronomic variables, charcoal rot variables, regression parameters and relative leaf number duration on each other over all the Lines was found out by correlating the desired variable means over all the Lines using *GENSTAT5* statistical package.

3.9 LABORATORY EVALUATION:

Two RAPD primers were used the screen the genotypes under study for polymorphism.

3.9.1 SAMPLE PREPARATION

About 3 grams of seed was sown in moist paper towels. The seeds were incubated in dark at 32° C for 4 to 5 days. The towels were moistened frequently to maintain the needed humidity for germination.

3.9.2 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 five day old 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 capped polypropylene tubes which were stored overnight at -20 °C so that the CO_2 diffuses out. Ten to 12 ml of pre warmed (60°C) isolation buffer was added to each tube and clumps were suspended by gentle shaking with a rotary shaker. 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 (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 - isoamyl alcohol (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 and vacuum dried for a few minutes. Later the DNA was dissolved in 2ml 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 *SHIMADZU UV 160A* spectrophotometer. DNA was quantified considering that one OD unit at 260 nm is equivalent to 50 ug of DNA (Sambrook *et al.* 1989).

TABLE B : CTAB Buffer Composition:

STOCK	100 ml	comment
d H_2O	46 ml	autoclaved
1 M TRIS pH 8	20 ml	autoclaved
5 M NaCl	28 ml	
0.5 M EDTA	4 ml	autoclaved
$Na_2 SO_3$	250 mg	
C TAB	2 g	
Mercaptoethanol	500 µl	

2.5 RAPD ANALYSIS

The RAPD assay was performed following the method of Williams *et al.* (1992). PCR reaction was performed with 25 ul of a reaction mixture containing a total of 20 ng of genomic DNA, 10 uM of arbitary decamers (*Operon Primers* Inc.), 25 mM $MgCl_2$ (*Promega*), 2.5mM dNTPs (*Sigma* chemicals), 10x PCR buffer (*Promega*) and 1unit of *Taq* polymerase *(promega)* and sterile distilled water to make volume 25 ul per reaction.

PCR reaction was carried out using *Perkin Elmer Gene Amp PCR system 9600* for 45 cycles with the following temperature profile:

First cycle	Denaturation at 94°C for 1 min. Primer annealing at 37°C for 45 sec Primer extension at 72°C for 1 min.
Next 44 cycles :	Denaturation at 94°c for30 sec Primer annealing at 37°C for 45 sec Primer extension at 72°C for 1 min
Final cycle	72 ° C for nine minutes.

A control without template DNA was included in each set of reactions with a single primer. Reaction products were resolved by electrophoresis on gels consisting of 1.5 per cent *FMC Nu-Sieve* agarose.

Primer Id.	**Sequence (5'→3')**	**(G+C)per cent**
OPB 08	GTC CAC ACG G	80
UMC 176	CAA GGG AGG T	60

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 or leaf area against day after flowering (DAF). Initial study of five selected Lines(B35, TX7078, TX7078-2, Line66(low charcoal rot), Line91(high charcoal rot), by plotting a logistic curve for both relative leaf area and relative leaf number revealed similarity of curves for both relative leaf area and relative leaf number (Fig. ure 1,2).

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 five selected Lines was greater than 0.97 for both the relative leaf area and 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 five selected Lines for both relative leaf area and 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 2).

The correlation of days after flowering with both relative leaf number and relative leaf area was greater than 0.98 (5 % level of significance) in all cases indicating that both green leaf area and green leaf number decreased progressively after flowering. The result indicated that relative leaf number can be equally effective as relative leaf area in senescence studies.

Having inferred that relative leaf number is effective in studying senescence, a detailed regression analysis using by fitting the logistic function was done for each of the Lines under both the Dry and Wet environments. 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.

Figure 1 Regression curve of B35, TX7078 (in duplicate), Line 66, Line 91 for relative green leaf number under Dry environment

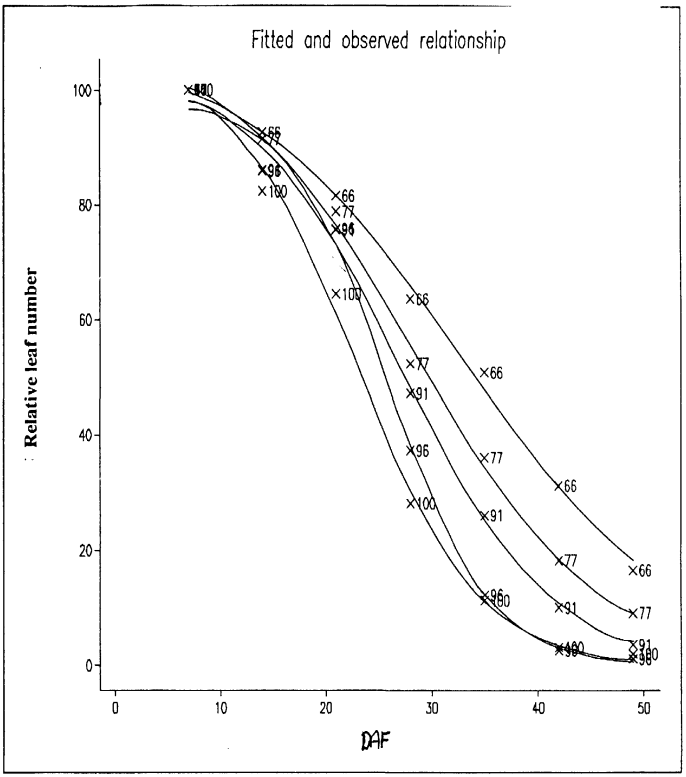


Figure 2 Regression curve of B35, TX7078 (in duplicate), Line 66, Line 91 for relative green leaf area under Dry environment

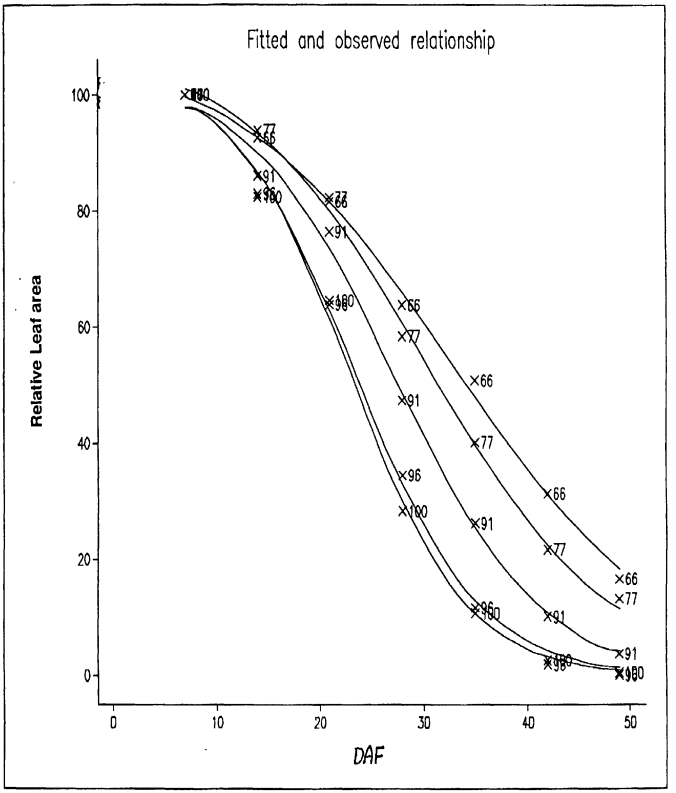


Table 2 Comparison of regression parameters for relative leaf area with relative leaf number

	Relative leaf area				Relative leaf number			
Entry	constants	estimate	s.e.	b/s.e	constants	estimate	s.e.	b/s.e
77	B	-0.11	0.01	-11.829	B	-0.12	0.009	-14.118
(B35)	M	30.15	1.03	29.272	M	28.42	0.91	31.231
	C	108.32	4.17	25.976	C	108.64	3.83	28.366
96	B	-0.17	0.016	-11	B	-0.22	0.027	-8.24
(TX7078-1)	M	23.49	0.755	31.114	M	25.93	0.72	36.014
	C	103.7	4.02	25.796	C	98.28	3.67	26.779
100	B	-0.18	0.017	-10.899	B	-0.18	0.02	-9.231
(TX7078-2)	M	23.1	0.694	33.288	M	23.01	0.866	26.57
	C	102.96	3.83	26.883	C	103.79	4.77	21.759
66	B	-0.1	0.009	-10.728	B	-0.1	0.008	-13.015
(L.Ch.Rot)	M	32.71	1.25	26.168	M	32.75	1.64	19.97
	C	107.71	4.57	23.569	C	107.64	3.78	28.476
91	B	-0.15	0.012	-11.75	B	-0.14	0.013	-11.024
(H.Ch.Rot)	M	27.24	0.808	33.712	M	27.11	0.853	31.782
	C	103.09	3.64	28.321	C	103.43	3.84	26.935

In the Dry environment, the general slope of the fitted curve (b) ranged from a maximum of O.23 in Line 92 to a minimum of 0.07 in Line 41. The range 'c', varied from 98.4 in Line 92 to 137.4 in Line 75. The point of inflection(m) occurred earliest at 21 DAF in Line 58 and latest 34 DAF in Line 89. The onset of senescence occurred earliest at 6 DAF in Line65 and latest at 22 DAF in Line7 and Line 89. The offset of senescence occurred as early as 30 DAF in Line 92 and TX7078 and latest in Line 84 (48 DAF). The duration of the Linear phase was maximum in Line 41(36 days) and minimum in Line 92 (12 days). The maximum rate of senescence(observed at the point of inflection) was the highest in Line 92 (-4.8) and the lowest in Line 53 (1.4). The maximum relative leaf number at onset of senescence occurred in Lines 57, 65 and 75 while the minimum occurred in TX7078 and Line 92 (77%). At offset, the maximum relative leaf number occurred in the Line 75 (29%) while the minimum occurred in TX7078 and Line 92 (20%). The relative leaf number in the Dry environment at 'c' (point of inflection where slope is maximum) was highest in Line 65 (65%) and lowest in Lines 36, 92 and 96 (49%).

In the Wet environment the maximum and minimum values of 'b' were 0.16 in Line 98 and 0.06 in Lines 19, 55, 59 and 79 respectively. The inflection point occurred earliest in Line 92 (35 DAF) and latest in Line19 (55 DAF). The slope at inflection point was maximum in Line 98 (-4.0) and minimum in Line 19 (-1.61). The onset of senescence occurred earliest in Line 74 (24 DAF) and latest in Line 36 (52 DAF) while the offset was earliest in Line 92 (45 DAF) and latest in Line 53 (86 DAF). The Linear duration of senescence was maximum in the Line 53 (52 days) and minimum in the Line 98 (16 days). The relative leaf area recorded at onset was maximum in Line 53 (88%) and minimum in

Line 81 (73%) while that at offset was maximum in Line 73 (23.6%) and minimum in Line 70 (9%). At the inflection point the relative leaf area recorded was maximum in Line79 (55.3%) and minimum in Line 81 (46.5%).

4.2 CLUSTER ANALYSIS

Cluster analysis is a powerful tool using which genotypes can be Grouped based on the similarity for the parameters under consideration.

The inbred Lines were Grouped in to senescence Groups by cluster analysis (Table 3) at 94.5 % similarity for the senescence parameters under consideration ,i.e., T-on , T-off, b-m, T-lin and B-lin. The genotypes clustered in to six Groups (Fig. 3). The Group showing highest senescence (Group 6) has only one Line (Line 92) in it. The Group showing high senescence(Group-5) had ten entries in it including both the entries of the non-staygreen parent TX7078. The Group representing moderate senescence (Group-4) has three Lines. The highest number of Lines (63) occurred in Group-3, the moderate staygreen Group. The Group representing low senescence (high staygreen), (Group-2) has nine Lines in it while the Group which showed highest staygreen(lowest senescence),(Group-5) has 15 entries in it. Out of the five selected entries for which both relative leaf area and relative leaf number were plotted against time, B35 , Line 66 and Line 91 fell in to Group 3 while the TX7078 entries (96 and 100) fell in to Group 5. To further study the senescence pattern and staygreen behavior of the above six identified Groups and the effect of variation in senescence among the Groups on yield attributes and charcoal rot, the Group wise means for all the regression parameters were found out(Table 4).

Figure 3 Dendogram : Cluster analysis and grouping of Lines based on similarity in senescence and senescence parameters

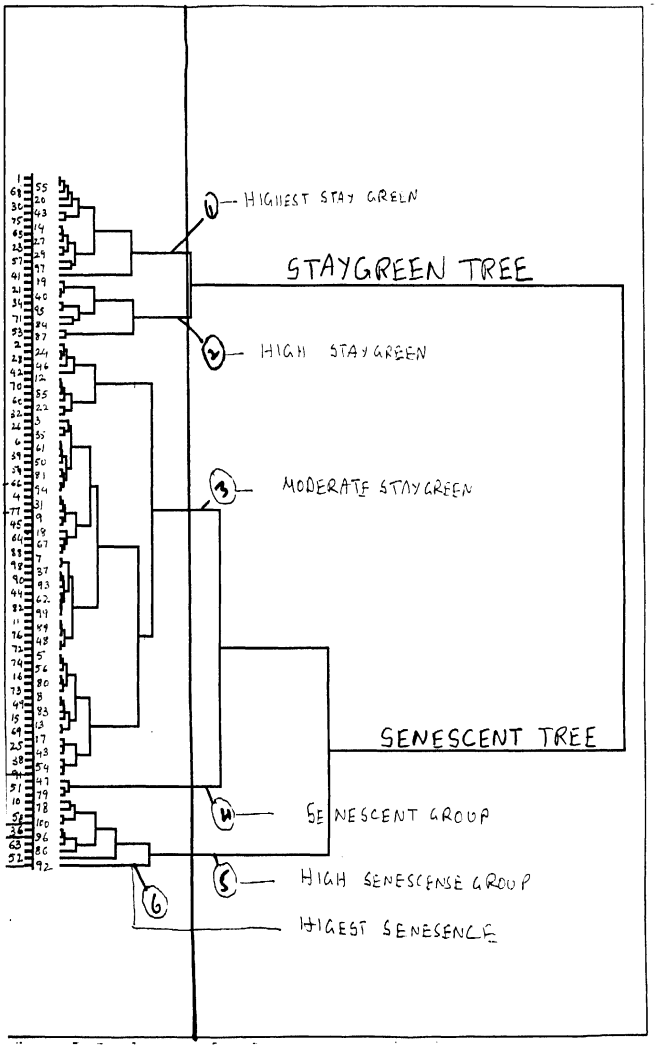


Table 3 Grouping of Sorghum Lines based on cluster analysis

Group	Line	Group	Line		
1	1	4	51	GROUP	RATING
3	2	5	52	1	Highest Staygreen
3	3	2	53	2	High Staygreen
3	4	3	54	3	Moderate Staygreen
3	5	1	55	4	Senescent
3	6	3	56	5	High Senescence
3	7	1	57	6	Highest Senescence
3	8	5	58		
3	9	3	59		
5	10	3	60		
3	11	3	61		
3	12	3	62		
3	13	5	63		
1	14	3	64		
3	15	1	65		
3	16	3	66 L C Rot		
3	17	3	67		
3	18	1	68		
2	19	3	69		
1	20	3	70		
2	21	2	71		
3	22	3	72		
1	23	3	73		
3	24	3	74		
3	25	1	75		
3	26	3	76		
1	27	3	77 B35		
3	28	5	78		
1	29	4	79		
1	30	3	80		
3	31	3	81		
3	32	3	82		
1	33	3	83		
2	34	2	84		
3	35	3	85		
5	36	5	86		
3	37	2	87		
3	38	3	88		
3	39	3	89		
2	40	3	90		
1	41	3	91 H C Rot		
3	42	6	92		
3	43	3	93		
3	44	3	94		
3	45	2	95		
3	46	5	96 TX7078		
4	47	1	97		
3	48	3	98		
3	49	3	99		
3	50	5	100 TX7078		

Table 4 Regression summary for RILs in the Dry and Wet environments

A : Dry environment													
Commer	Group	B	m	c	T-on	T-off	T-lin	Y-on	Y-off	Y-lin	Y-m	b-m	B-lin
	Group 1	-0.09	25.80	121.70	9.44	41.55	32.11	96.84	25.72	71.13	60.85	-2.60	2.22
	Group 2	-0.10	29.19	112.44	15.68	51.90	36.22	88.68	17.42	71.26	56.22	-2.84	2.07
B35,LC,I	Group 3	-0.12	27.50	109.70	16.14	38.87	22.73	86.52	23.18	63.34	54.85	-3.25	2.83
	Group 4	-0.18	27.42	101.83	20.03	34.81	14.78	80.31	21.52	58.80	50.91	-4.58	3.98
TX7078	Group 5	-0.19	25.50	101.30	18.51	32.48	13.96	79.89	21.41	58.48	50.65	-4.84	4.22
	Group 6	-0.23	23.79	98.14	17.96	29.62	11.65	77.40	20.74	56.66	49.07	-5.64	4.86
B : Wet Environment													
Commer	Group 1	-0.09	46.47	100.51	35.40	66.14	30.74	79.27	20.56	58.71	50.25	-2.25	1.96
	Group 2	-0.09	47.85	98.58	40.72	74.40	33.67	77.75	17.72	60.02	49.29	-2.28	1.88
B35,LC,I	Group 3	-0.10	45.98	99.38	37.37	66.76	29.39	78.65	20.65	58.01	49.69	-2.35	2.05
	Group 4	-0.09	44.52	102.72	41.99	72.60	30.61	81.01	21.70	59.31	51.36	-2.29	2.01
TX7078	Group 5	-0.11	44.18	98.78	38.97	63.39	24.42	77.91	20.88	57.03	49.39	-2.78	2.43
	Group 6	-0.15	34.7	96.58	27.08	45	17.9	76.17	20.41	55.76	48.29	-3.55	3.11

4.3 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 Lines 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 six senescence Groups.

4.3.1 TIME PARAMETERS

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

In the Dry environment the onset of senescence was early in the staygreen Groups compared to the senescent Groups. It was earliest in Group-1 (9DAF) followed by Group-2 and Group-3. The onset was latest in Group-4 (20DAF). In the Wet plot earliest onset was seen in Group-6 and onset was similar in all other Groups (Fig. 4).

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

The offset of senescence was earlier in the senescent Lines, as expected which belonged to Groups 4, 5 and 6. The offset was relatively late in the Groups1, 2 and 3 which were the types. In Group-6 the offset was the earliest which indicating shows rapid senescence (Fig. 5).

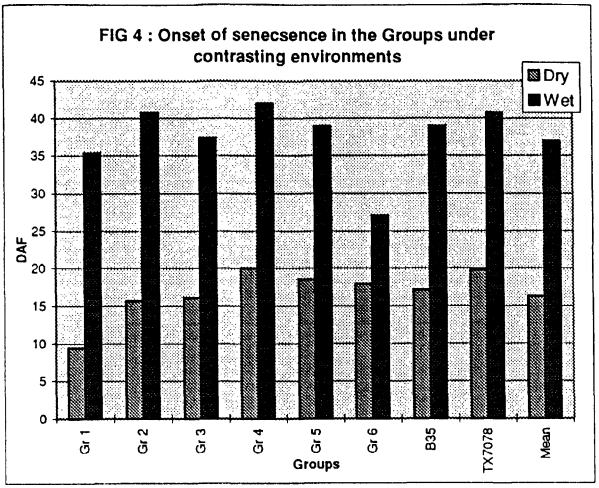


FIG 4 : Onset of senecsence in the Groups under contrasting environments
Dry
Wet
DAF
45
40
35
30
25
20
15
10
5
0
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX7078
Mean
Groups

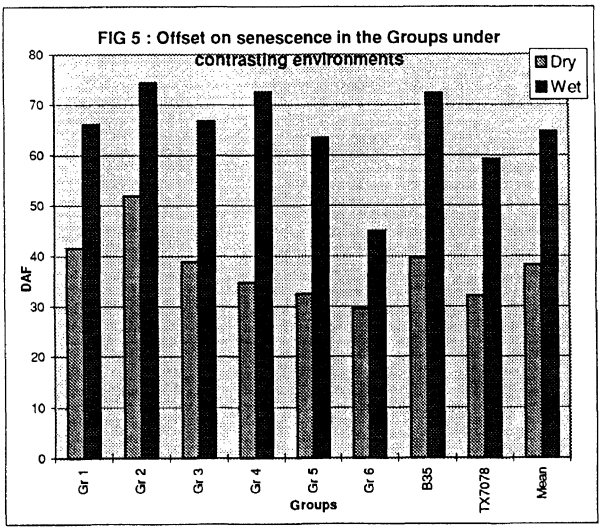


FIG 5 : Offset on senescence in the Groups under contrasting environments
Dry
Wet
DAF
80
70
60
50
40
30
20
10
0
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX7078
Mean
Groups

FIG 6 : Linear phase of senescence in the Groups under contrasting environments

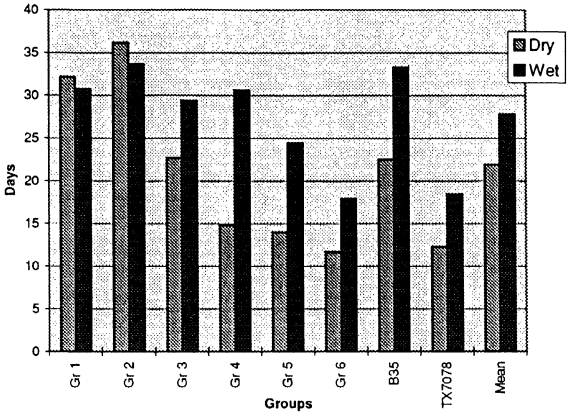
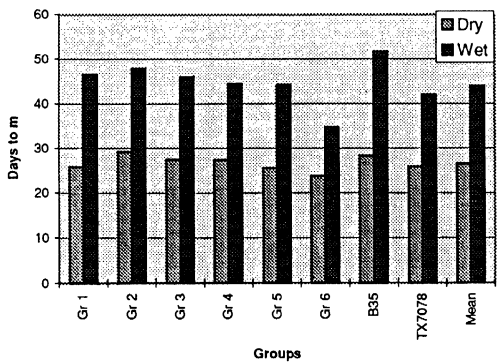


FIG 7 : Inflection values in Groups under contrasting environments



4.3.3.3 Duration of Linear phase of senescence

The Linear phase of senescence gives a direct indicator of the staygreen trait of a Line. In the Dry environment the duration of the Linear phase was more in the staygreen Groups belonging to Groups-1, 2 and 3 compared to the senescent Groups 4, 5 and 6. The Linear phase was longest in (Group-2) followed by Group-1 and Group-3. The duration of Linear senescence was the shortest in Line92 followed by Group-5 and 4. In the Wet environment also the Linear phase of senescence was extended in the staygreen Group while it was the shortest in the senescent Groups (Fig. 6).

4.3.3.4 Point of inflection

The point of inflection where senescence rate was maximum occurred earlier in the senescent Lines than the staygreen Lines. In Group-6 it occurred as early as (24DAF) followed by Group-5 (26DAF). In Group 2 it occurred late (29DAF). Group1 although a staygreen Line showed maximum senescence rate earlier than the other staygreen lines (Fig. 7).

4.3.4 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 occured at point of inflection. The important parameter is the Linear rate of senescence which occurs during the Linear phase of senescence.

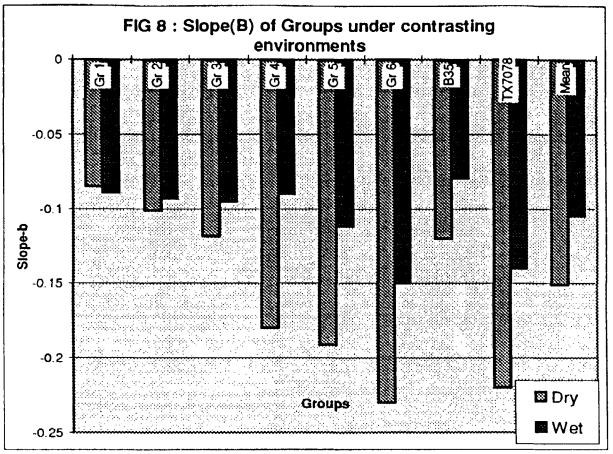


FIG 8 : Slope(B) of Groups under contrasting environments
Slope-b
0
-0.05
-0.1
-0.15
-0.2
-0.25
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX7078
Mean
Groups
Dry
Wet

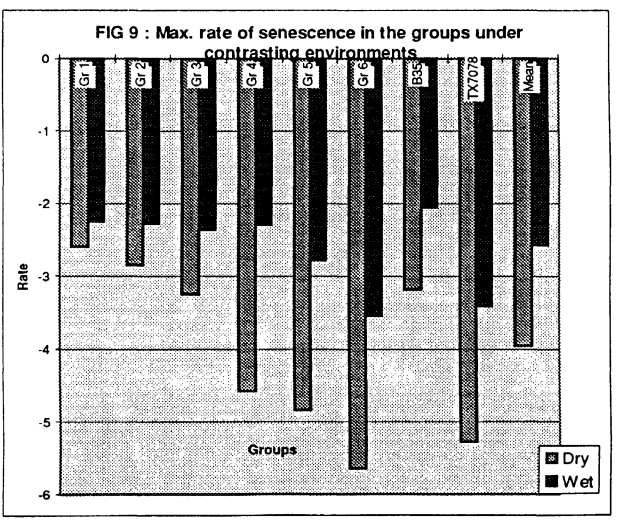


FIG 9 : Max. rate of senescence in the groups under contrasting environments
Rate
0
-1
-2
-3
-4
-5
-6
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX7078
Mean
Groups
Dry
Wet

4.3.4.1 General rate of senescence(B)

In both the Wet and Dry environments the rate of senescence was higher in the senescent Group than in the staygreen Lines. Group-6 (PRIL92) had the highest rate of senescence (0.23 leaves/day) followed by Group-5 and 6 (0.19 and 0.18 leaves/day respectively). Out of the three staygreen Lines Group-1 and the lowest rate of senescence (0.09 leaves/day) followed by Group-2 and Group-3. There was a marked increase in senescence rates of the senescent Lines compared to the staygreen Lines. In the Wet environment however the Group 4 deviated from the other senescent Groups and was comparable to the staygreen genotypes in its rate of senescence (Fig. 8).

4.3.4.2 Maximum rate of senescence (Bm):

In both the Wet and Dry environments the maximum rate of senescence was higher in the senescent Groups and lower in the staygreen Lines. The Bm was highest in Group-6 followed by the Group-5 and Group-4. It was lowest in Group-1 and 2. In the Wet environment however the Group-4 deviated from the other staygreen Groups and was comparable to the staygreen genotypes in its maximum rate of senescence (Fig. 9).

4.3.4.3 Linear rate of senescence

The Linear rate of senescence which is another good measure of senescence showed that Lines belonging to the staygreen Groups had the least Linear rate. The lowest rate was observed in Group-2 (2.07%/day). Group1 (2.2%day) had marginally higher rate than Group-2. There was a rapid increase in Linear rate of senescence from the Group-3 (2.8%/day) to Group-4 (3.98%/day). The highest rate of senescence was observed in Group-6 followed by Group-5 which consists of the senescent parent. In the Wet

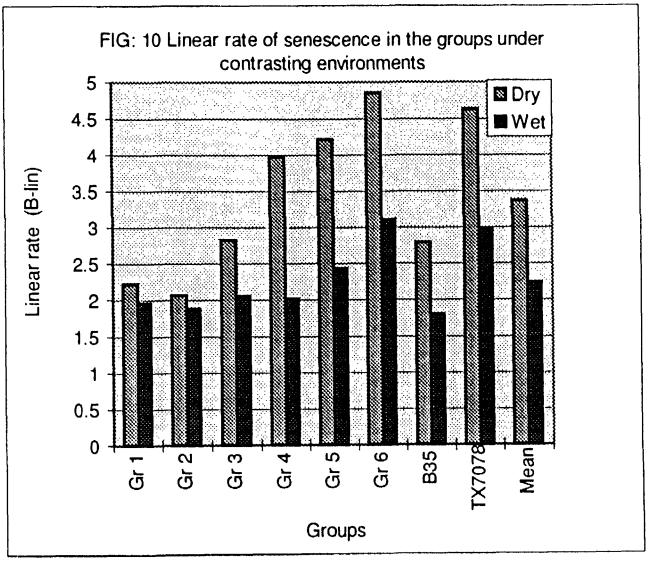


FIG: 10 Linear rate of senescence in the groups under contrasting environments
Linear rate (B-lin)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Dry
Wet
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX7078
Mean
Groups

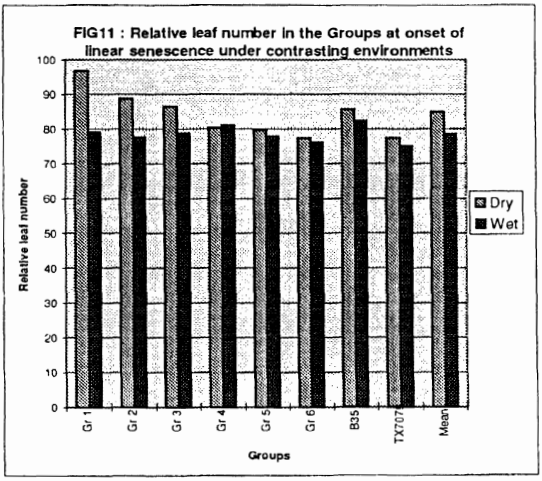


FIG11 : Relative leaf number in the Groups at onset of linear senescence under contrasting environments
Relative leaf number
100
90
80
70
60
50
40
30
20
10
0
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX707
Mean
Groups
Dry
Wet

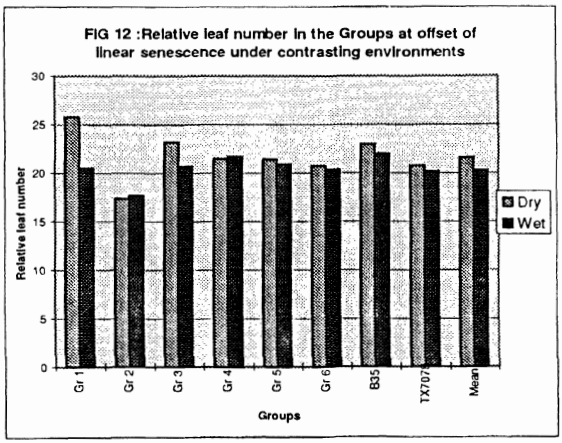


FIG 12 :Relative leaf number in the Groups at offset of linear senescence under contrasting environments
Relative leaf number
30
25
20
15
10
5
0
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX707
Mean
Groups
Dry
Wet

environment the decrease in the Linear rate of senescence was marked in the senescent Groups compared to the staygreen Lines (Fig. 10).

4.3.5 Relative leaf number:

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

The relative leaf number at the onset of senescence was higher in the staygreen Lines belonging to Groups 1, 2 and 3 compared to the senescent Lines. While the staygreen Groups retained more than 85% of their functional green leaves at flowering the senescent Groups lost more than 80% of their green leaves. The relative leaf number of the senescent Lines(Groups 1, 2 and 6) was comparatively lower at onset. The lowest was observed in Line92 followed by Group-5. In the Wet environment relative leaf number was the similar between groups at onset of linear senescence (Fig. 11).

4.3.5.2 Relative leaf number at offset (Y-off)

There was not much difference in the relative leaf number of the selected Lines at offset. The highest relative leaf number was recorded in Group-1 followed by Group-3 and Group-4, while the lowest was in Group-2 followed by Group-6. In the Wet environment the relative leaf number was the highest in Group-4 and lowest in Group-2. In both the environments Group two recorded the lowest relative leaf number (Fig. 12).

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

In the Dry environment the decrease in the relative leaf number between onset and offset was more in the staygreen Lines compared to the senescent Lines. The decrease was

FIG 13 : Decrease in realtive leaf number between onset and offset in the groups under contrasting environments

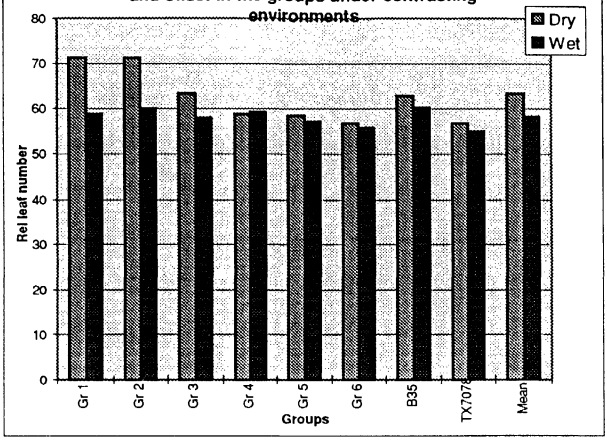
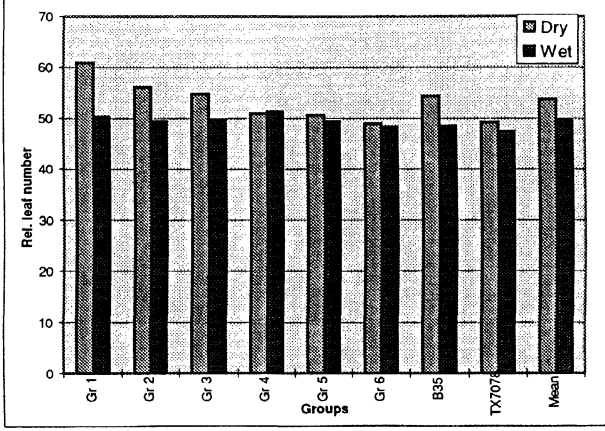


FIG 14 : Relative leaf number at 'm' in the Groups under contrasting environments



highest in Group-2 and Group-1 while it was lowest in Group-6. In the Wet environment the decrease was uniform over all Groups (Fig. 13). It was because of slow but longer duration of linear senescence.

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

In the Dry environment the relative leaf number at the point of inflection decreased across Groups from the staygreen Groups to the senescent Groups. At 'm' the staygreen Lines maintained greater than 55% relative leaf number while the senescent Lines lost greater than 50% of their leaves. In the Wet environment the difference in the relative leaf number at point of inflection was marginal across the Groups (Fig. 14).

Table 5 Relative leaf number duration of the groups in the Dry and Wet environments

DRY ENVIONMENT					
Group	Entry	A1	A2	A3	Total A
Group 1		969.9	1977.8	257.8	3205.5
Group 2		1550	1582.8	148.7	3281.5
Group 3	B35,LC,HC	1577.4	1252.1	199.1	3028.5
Group 4		1911.4	752.5	131.7	2795.6
Group 5	TX7078	1751.2	708.6	125.7	2585.5
Group 6	Line 92	1665.2	572.2	99.1	2336.4
B35		1675.1	1257.2	57.5	3117.3
TX7078		1864	597.1	87.8	2569.2
Mean		1699.9	1049	32.4	2902.4
WET ENVIRONENT					
Group	Entry	A1	A2	A3	Total A
Group1		3186.7	1303.3	116.2	4606.2
Group2		3556.8	1050.8	220.9	4828.5
Group3	B35,HC,LC	3301.2	1127.8	120.6	4549.6
Group4		3596.3	894.2	181.9	4672.5
Group5	TX7078	3420.4	840.9	48.6	4309.9
Group6	Line 92	2440.9	790.2	110.3	3341.4
B35		3689.5	1501.8	176.9	5368.2
TX7078		3488.6	467.4	60.8	4016.8
Mean		3068.6	1240.2	149.8	4458.6

4.4 GREEN LEAF NUMBER DURATION STUDIES

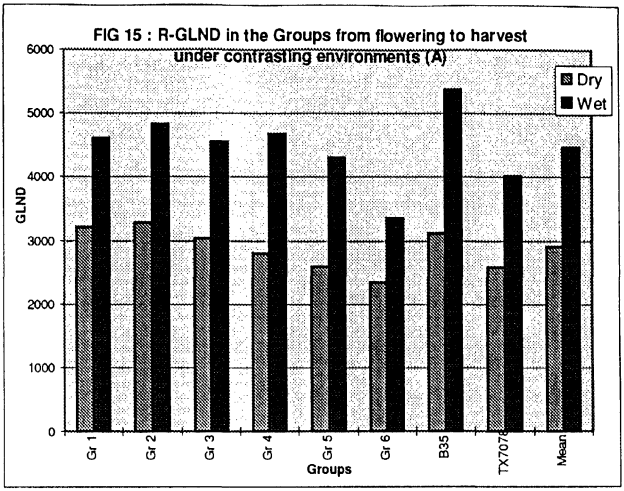
Green leaf number duration studies over the plant growth period can account for differences in performance of the selected Lines. A relatively high green leaf number duration in the critical period of grain filling can give a distinct yield advantage to a crop. By integrating the function of the regression 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 Lines and the representative six senescence Groups was found out in both the Wet and Dry environments (Table 5).

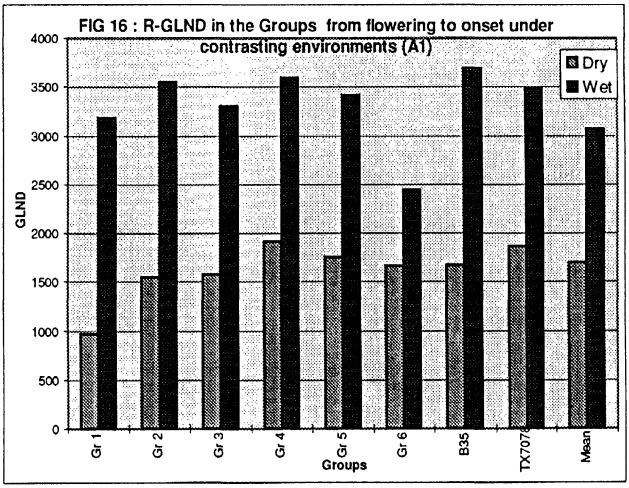
4.4.1 Total relative green leaf number duration(A)

The selected Lines showed wide variation in the Relative GLND from flowering to harvest. The total relative GLND was higher for staygreen Lines compared to the senescent Lines in the Dry environment. On the whole Group-2 showed highest total green leaf number duration while Group-5 showed the lowest relative green leaf area duration. In the Wet environment the senescent Lines belonging to Group-4 and 5 had a marginally less total green leaf area duration and compared well with the staygreen Lines (Fig. 15).

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

The relative green leaf number duration from flowering to onset (A1) was more in senescent Groups compared to the staygreen Groups. The highest A1 values were recorded in Lines belonging to Group-4. Groups-1 had the lowest A1 values. The A1





values for the other two senescent Groups were almost the same. The Wet environment showed a 80% increase in relative green leaf number across all Groups over the Dry environment. Group5 had the lowest A1 values while the highest were recorded in Group-2 and 4 (Fig. 16).

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 Lines showed much variation in both the environments. The relative GLND values of the staygreen Lines were higher than the Lines belonging to senescent Groups in both the environments. While the lowest A2 values in the both the environments were recorded in Group-6, the highest values were recorded in Group-1 (Fig. 17).

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

In general the staygreen Lines retained higher number of green leaves and showed greater relative green leaf area duration than the senescent Lines. In the Dry environment the Lines belonging to Group-1 showed the highest relative leaf number in the post senescence phase while the Lines belonging to Group-6 showed the least. In the Wet environment however Group-1 showed decrease in relative leaf number over Dry environment by more than 100%. Group 4 showed the highest A3 values while Group-5 showed the lowest (Fig. 18).

FIG 17 : RGLND in the Groups from onset to offset under contrasting environments (A2)

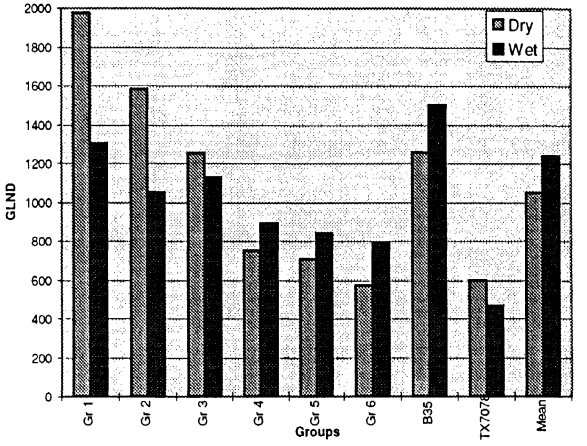
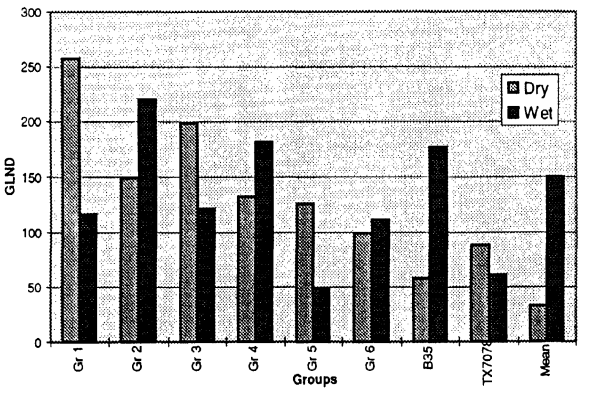


FIG 18 : R-GLND in the Groups from offset to maturity under contrasting environments (A3)



4.5 ANALYSIS OF PHENOLOGICAL, YIELD and CHARCOAL ROT TRAITS:

Having studied the basic senescence pattern , a study of how the differential senescence pattern will influence the phenological, yield and charcoal rot parameters was undertaken. Analysis of variance was done to identify environmental effect on the yield and charcoal parameters and also to see if the Lines differed significantly from each other in their performance (appendix 2).

Effect of environment on performance of the population

A combined analysis of variance over Wet and Dry environments (Table 6) was carried out to determine

⇒ If the two environments differed significantly from each other

⇒ Whether environment had any significant effect on the performance of the RILs. The phenological, yield and charcoal traits were analyzed for significant difference, between the Wet and Dry environments between the Groups

⇒ If the Lines differed significantly from each other in their mean performance.

Table 6 Combined analysis of variance over the two environments for agronomic and charcoal rot traits

	Days to Fl	Days to Mat.	GS3 Hd Wt/m2	St Wt/m2	Biom/m2	Gr Wt/m2	Thr %
Dry	67	109	44	528.4	292.9	819.8	407	77.75
Wet	68	115	49	655.6	356.3	1011.9	549.7	83.91
Mean	68	112	47	592	324.6	915.85	478.35	80.83
SE	0.271	0.777	0.741	16.23	21.74	37.17	16.48	0.965
Environment	NS	*	*	*	*	*	*	*
Genotype	*	*	*	*	*	*	*	NS
G*E	NS	NS	NS	*	*	*	*	NS
SED	0.38	1.10	1.05	22.95	30.74	52.56	23.30	1.36
CD(5%)	0.45	1.28	1.22	26.76	35.85	61.29	27.18	1.59
LSD	0.63	1.81	1.73	37.84	50.69	86.67	38.43	2.25

	100 Sd wt	Sd Den/m2	HI Lf No. Har	Pl Ht	Nd Sprd	Cm Sprd
Dry	2.976	14031	50.26	0.86	95.60	1.18	3.97
Wet	3.58	9569	55.06	1.55	105.50	0.05	0.17
Mean	3.278	11800	52.66	1.20	100.55	0.61	2.07
SE	0.061	251.8	0.5	0.106	1.15	0.17	0.46
Environment	*	*	*	*	*	*	*
Genotype	*	*	*	*	*	*	*
G*E	*	NS	*	*	NS	*	*
SED	0.09	356.05	0.71	0.15	1.63	0.24	0.42
CD(5%)	0.10	415.22	0.82	0.17	1.90	0.28	0.76
LSD	0.14	587.12	1.17	0.25	2.68	0.39	0.69

Table 7 Group means and analysis of variance for agronomic and charcoal rot traits

in the Dry environment

	(Phenology)		(Yield traits)						
Group	Days to Fl.	Days to M:	GS3	HDWT/M2	STWT/M2	BIO/M2	GRNWT/M	THR%	100Sd.Wt.
	DAYS	DAYS	DAYS	(g)	(g)	(g)	(g)		(g)
Group 1	69	112	43	516.9	371.2	884.8	411.7	78.9	3.1
Group 2	66	111	45	488.6	345.2	833.9	376.0	77.3	3.2
Group 3	68	111	43	547.1	285.1	831.0	420.5	77.6	3.0
Group 4	61	108	47	476.2	207.3	683.4	334.4	71.7	3.3
Group 5	66	110	44	464.6	198.4	660.5	355.3	79.7	2.7
Group 6	70	113	43	611.9	244.7	854.8	453.8	72.6	2.7
B 35	**70**	**113**	**42**	**644.3**	**357.3**	**998.4**	**469.8**	**71.3**	**2.9**
TX7078-1	**64.38**	**109.65**	**45.28**	**449.5**	**164.9**	**616.5**	**366.5**	**83.0**	**2.8**
Mean	67	111	43.7	528.4	292.8	819.8	407.0	77.8	3.0
SE	0.214	0.174	0.184	6.1	6.2	10.1	5.2	0.7	0.0
CD(5%)	0.42	0.34	0.36	12.0	12.2	20.0	10.2	1.4	0.1
CV	16.519	72.25	7.3	19.9	36.6	21.4	22.1	16.0	16.9
SED	1.45	1.99	2.14	65.2	55.7	94.7	58.6	9.7	0.3
LSD	2.86	3.93	4.22	128.7	110.0	187.0	115.6	19.1	0.6
Min.	12	0	33	278.0	93.0	411.2	170.3	0.4	1.7
Max.	31	12	49	926.3	833.3	1373.7	756.5	0.9	4.2
Range	19	12	16	648.2	740.4	962.5	586.2	0.6	2.5
P(.01)	**	**	**	**	**	**	**	NS	**

	Charcoal rot traits							
Group	Seeds/m2	Hl% Lf No-Har		Pl. Ht.	Nd Sprd (Cm)	Cm Sprd oft	St. Mat (Cm)	oft St. har
Group 1	13788.2	46.82	1.35	97.47	0.5	1.4	1.23	15.38
Group 2	12134.56	45.25	1.25	94.62	0.63	2.34	6.25	16.69
Group 3	14598.68	51.06	0.81	95.22	1.19	3.9	6.1	36.3
Group 4	10601.67	49.01	0.41	102.38	1.05	2.99	18.32	42.33
Group 5	13260	55.67	0.17	91.89	2.83	10.28	27.5	77.27
Group 6	16193	52.16	0	110.12	1.47	7.86	72.01	100
B 35	**15896**	**45.34**	**0.85**	**79.28**	**1.49**	**5.16**	**1.05**	**41.21**
TX7078-1	**12838**	**60.46**	**0.1243**	**93.82**	**1.51**	**5.293**	**39.44**	**79.64**
Mean	14030.87	50.26	0.86	95.56	1.18	3.97	8.33	35.94
SE	216.56	0.46	0.04	0.95	0.08	0.31	1.08	1.9
CD(5%)	427.49	0.91	0.08	1.88	0.16	0.61	2.13	3.75
CV	26.735	15.93	80.52	17.24	120.1	134.66	225.16	91.39
SED	2123	5.62	0.42	9.4	3.5	3.6	12.98	19.2
LSD	4190.8	11.09	0.83	18.56	6.91	7.11	25.62	37.9
Min.	5873	24.54%	0	56.5	0	0	0	0
Max.	29268	68.79%	2.85	237.83	6.7	29.83	100	100
Range	23395	44.25%	2.85	181.33	6.7	29.83	100	100
P(.01)	**	**	**	**	**	**	**	**

Table 8 Group means and analysis of variance for agronomic and charcoal rot traits in the Wet environment

Entry	DaystoFl.	Dayst Mat	GS3 DAYS	HDWT/M2 (g)	STWT/M2 (g)	BIO/M2 (g)	GRWT/M (g)	THR%
Group 1	70	118	48	668.47	433.56	1103	555.24	83.7
Group 2	67	117	50	592.18	381.59	972.9	489.6	82.137
Group 3	68	117	49	666.86	349.31	1016.16	561.18	84.22
Group 4	61	115	54	566.2	216.3	783.67	454.23	81.43
Group 5	67	117	51	621.82	297.51	919.11	533.19	85.31
Group 6	73	117	44	975.1	406.7	1381	782.4	79.61
B35	70	115	45	834.1	513.5	1347	711.3	84.98
TX7078	66	116	49	461	258.3	720	404.3	87.75
Mean	68	117	49	656.6	356.3	1012.9	550.65	83.93
SE	0.217	0.17	0.24	8.5	7.87	14.09	7.4	0.34
CD(5	0.43	0.34	0.47	16.78	15.54	27.81	14.61	0.67
CV	16.39	28.6	8.38	22.42	38.27	24.09	23.28	6.97
SED	1.7	2.49	2.732	103.3	87.78	162.5	89.77	5.38
LSD	3.36	4.92	5.39	203.91	173.28	320.78	177.21	10.62
Min.	14	6	39	308.8	97.9	458	252.3	51
Max.	30	17	58	1129	874	1840	929.6	98
Rang	16	11	19	820	780	1383	677.3	47
P(.01)	**	**	**	**	**	**	**	NS

Entry	100Sd.Wt. (g)	Seeds/m2	HI	HI Leaf	Lf No. Har	Pl. Ht. (Cm	Nd Sprd (Cm)	Cm Sprd
Group 1	3.54	9692.29	51.02	7.66	1.79	113.3	0	0
Group 2	3.59	8169.6	50.391	7.6	1.76	103.28	0.06	0.14
Group 3	3.57	9887.56	56.08	6.73	1.57	103.77	0.04	0.14
Group 4	3.7	8145	58.67	4.37	1.06	115.23	0.08	0.39
Group 5	3.67	8925.11	57.95	6.68	1.11	100.5	0.14	0.54
Group 6	4.09	11792	56.82	5.44	0.65	139.6	0.17	0.84
B35	3.51	11775	53.63	11.68	2.13	100	0	0
TX7078	3.99	5984	55.49	9.84	0.16	96.8	0.72	2.33
Mean	3.58	9586.12	55.1	6.86	1.56	105.45	0.05	0.17
SE	0.22	161.51	0.44	0.16	0.05	0.96	0.01	0.03
CD(5	0.43	318.82	0.87	0.32	0.1	1.9	0.02	0.06
CV	12.44	29.18	13.68	40.62	55.91	15.68	384.18	394.36
SED	0.24	1784	5.4	2.29	0.65	5.58	0.15	0.5
LSD	0.47	3521.62	10.66	4.52	1.28	11.01	0.3	0.99
Min.	2.4	4042.6	31.5	1.5	0	71.2	0	0
Max.	4.9	25511	74.7	23.2	4.15	184	1.7	4.5
Rang	2.5	21468	43.1	21.7	4.15	112.8	1.7	4.5
P(.01)	**	**	**	**	**	**	**	**

4.5.1 Days to 50% flowering

The two environments(Wet and dry) did not differ significantly from each other up to days to 50% flowering. The mean flowering date in Dry and Wet environments were 22'Dec 96 and 23'Dec 96 respectively. Stress did not have a significant effect on days to 50% flowering and the flowering date is stable for the Lines across environments. The Lines differed significantly from each other in the time taken to 50% flowering

In the Dry environment flowering occurred earliest in Line42 (59DAS) followed by Line79 (60DAS) and latest in Line66 (74DAS) followed by 52 (73DAS). In the Wet environment flowering occurred earliest in Line79 (60DAS) followed by Line42 (61DAS) while it occurred latest in the Lines 52, 66, 33 and 17 (74DAS).

The Groups showed wide variation in their mean flowering dates. The Lines belonging to Group-4 showed the earliest flowering while Group-6 flowered the last in both the Dry and the Wet environments (Fig. 19).

4.5.2 Days to physiological maturity

The two environments(Wet and dry) differed significantly from each at the time of physiological maturity. Stress did not have significant effect on the days to physiological maturity across environments. The Lines differed significantly from each other in their physiological maturity. In the Dry environment the earliest maturing Line was Line42 (106DAS) while Line50 (124DAS) was the last to mature. The mean maturity was 109 DAS in the Dry environment and 115 DAS in the Wet environment .In the Wet

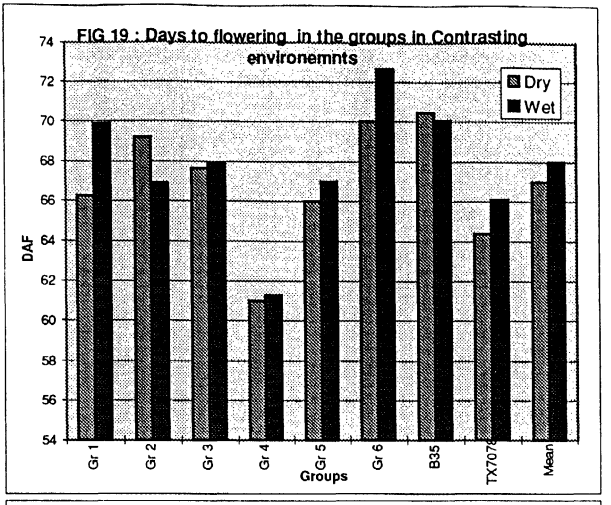


FIG 19 : Days to flowering in the groups in Contrasting environemnts
Dry
Wet
DAF
74
72
70
68
66
64
62
60
58
56
54
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX7078
Mean
Groups

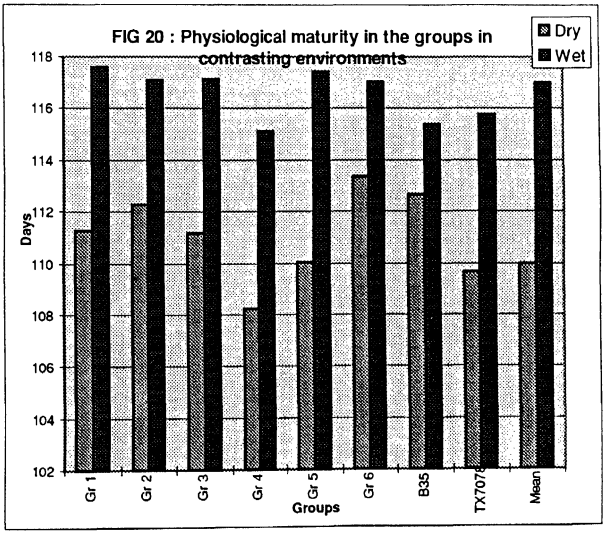


FIG 20 : Physiological maturity in the groups in contrasting environments
Dry
Wet
Days
118
116
114
112
110
108
106
104
102
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX7078
Mean
Groups

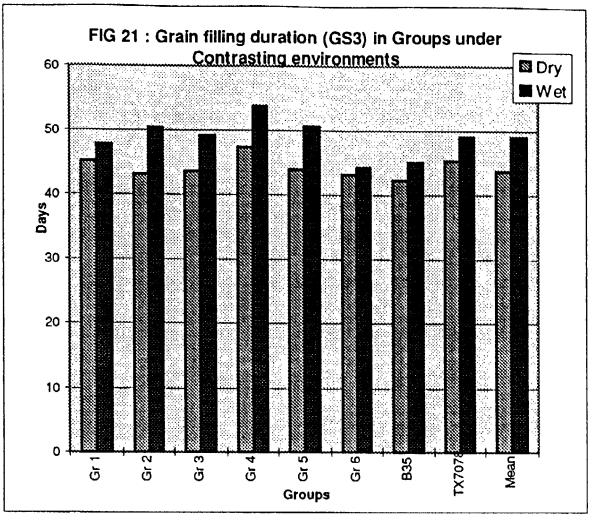


FIG 21 : Grain filling duration (GS3) in Groups under Contrasting environments
Dry
Wet
Days
60
50
40
30
20
10
0
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX707
Mean
Groups

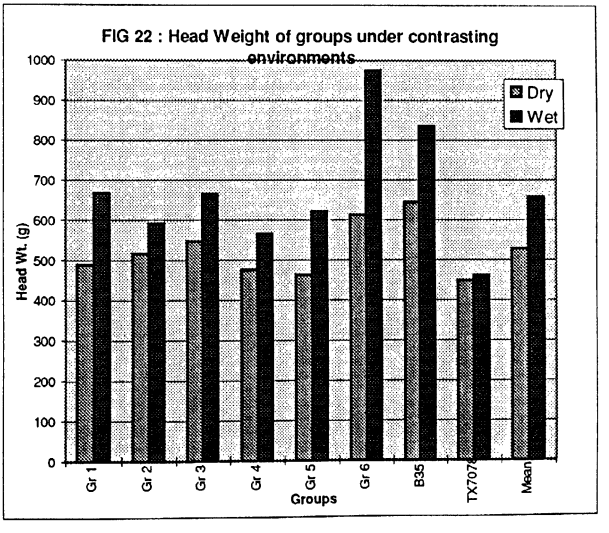


FIG 22 : Head Weight of groups under contrasting environments
Dry
Wet
Head Wt. (g)
1000
900
800
700
600
500
400
300
200
100
0
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX707
Mean
Groups

environment maturity was earliest in Line60 (113DAS) while it occurred latest in the Line56 (120DAS).

Group4 matured slightly earlier than the remaining Groups otherwise the senescent Lines were having similar maturity duration as the staygreen Groups (Fig. 20).

4.5.3 GS 3 (Grain filling period)

The Dry and the Wet environments differed significantly from each other during the period of grain filling. Water stress had no significant effect on duration of GS3 and the grain filling period did not differ significantly between the two environments. In the Dry environment the duration of GS3 was the shortest in Line91 (38days) followed by Line66 (39days) while it was the highest in Line60 (48 days). In the Wet environment Line6 had the least growth duration of 43 days while Line79 had the highest GS3 duration of 55 days. The mean duration of GS3 was 44 days in the Wet environment and 49 days in the Dry environment.

In both the environments the duration of GS3 was longer in Group-4. The senescent Groups with the exception of Line92 (Group-6) had a relatively longer duration of GS3 than the staygreen Groups. Group-6 had the least duration of grain filling amongst the Groups (Fig. 21).

4.5.4 Head weight per square meter

The performance of the treatments varied significantly between both the environments i.e. water stress effected the performance of the genotype significantly. The genotypes also differed significantly from each other in their mean head weights. In the Dry environment, Line40 showed the lowest head weight/ sq. meter (343.2 g) and Line89 (789 g) showed the highest. In the Wet environment, head weight was the least in Line34 (381 g) and

maximum in Line92 (975 g) followed by Line89. The mean head weight per square meter was 528.4g in Dry and 655.6g in the Wet environment.

In the Dry environment in general the Lines belonging to the staygreen Groups showed higher head weight than the rest. Group 6 showed the highest head weight Followed by Group-3 and Group-1. The lowest head weight was recorded in the Lines belonging to Group-5 followed by Group-4. In the Wet environment the Group-6 recorded the highest head weight followed by Group-1. The lowest head weight was recorded in Lines belonging to Group-4 in the Wet environment. The head weight recorded for Group-5 was higher and comparable to the staygreen Lines in the Wet environment. (Fig. 22)

4.5.5 Stalk weight per square meter

The stalk weights varied significantly between the two environments. Significant reduction in stalk weight was observed in Dry environment over all the Lines. The mean stalk weight values for the Dry and the Wet environments were 292g and 363g respectively. The Lines also differed significantly from each other in their stalk weights. In the both the environments the lowest stalk weight was recorded in Line42 (150.5g in Wet and 129g in dry). The highest stalk weights recorded were 641g (Line41) and 764g (Line99) in the Dry and the Wet environments respectively.

In general the staygreen Lines recorded higher stalk weight/M^2 than the senescent Lines. Lines belonging to Group-1(highest staygreen) showed highest post harvest stalk weights in both the environments. In the Dry environment Group-5 showed the least stalk weight recording a 66% decrease in stalk weight over the Wet environment. The mean stalk

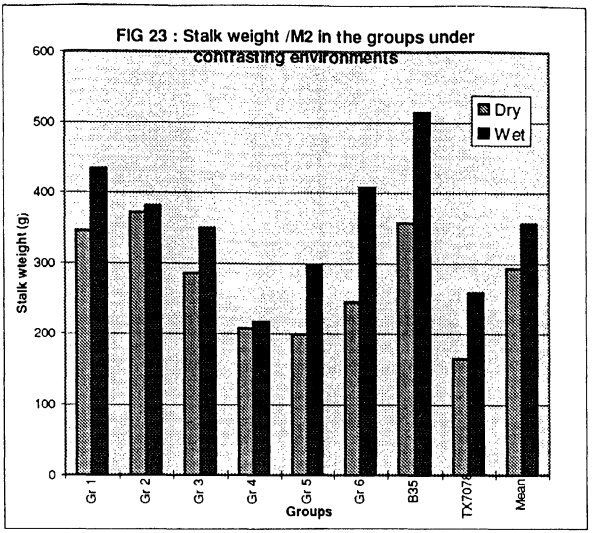


FIG 23 : Stalk weight /M2 in the groups under contrasting environments
Stalk wieight (g)
600
500
400
300
200
100
0
Dry
Wet
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX707
Mean
Groups

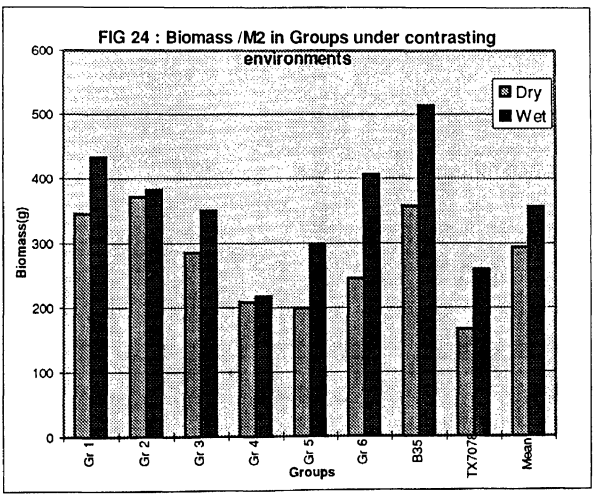


FIG 24 : Biomass /M2 in Groups under contrasting environments
Biomass(g)
600
500
400
300
200
100
0
Dry
Wet
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX707
Mean
Groups

weight of Line92 was higher and comparable to the staygreen Lines in the Wet environment (Fig. 23).

4,5.6 Biomass per square meter

The effect of the environment on the total biomass realized was significant. The genotypes also differed significantly from each other. In the Dry environment Line63 showed the least biomass(539g) and Line41 showed the highest biomass(1172.7g). In the Wet environment the Lines 42 and 33 showed the lowest and the highest biomass of 535g and 1489 g respectively. The mean of the Dry environment was 820g an that of the Wet was 1012g.

The staygreen Groups had a distinct advantage over the senescent Lines in terms of total biomass accumulation by the plants especially in the Dry environment (recording up to 40% higher biomass). Line92(Group-6) recorded high biomass due to higher head weight even though it was a senescent Line. Within the Groups, Group-1 followed by Group-3 recorded the maximum biomass while Group-5 showed the least biomass. In the Wet environment Group-6 recorded the highest biomass followed by Group-3, while Group-5 recorded the least biomass (Fig. 24).

4.5.7 Grain weight per square meter

Drought stress caused a significant reduction in the mean grain yield of the population when compared to the Wet environment. Lines differed significantly from each other in realization of grain weight. The overall means for the Dry and Wet environments are 407g and 550g respectively. In the Dry environment the highest grain weight was recorded in Line89 (565g) while the lowest was recorded in Line40 (263.6g). In the Wet environment

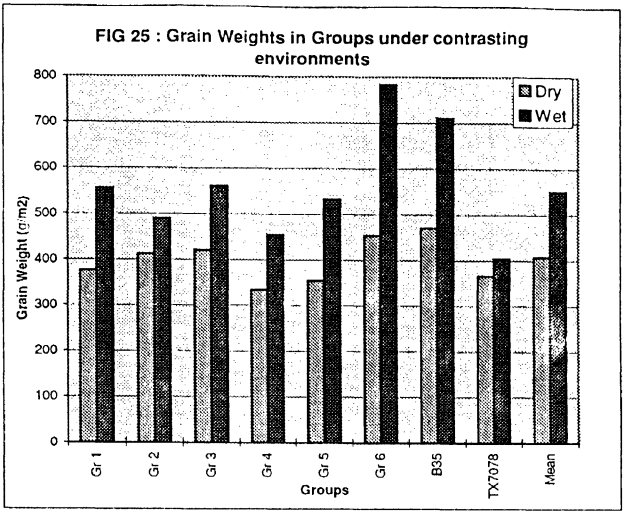


FIG 25 : Grain Weights in Groups under contrasting environments
Dry
Wet
Grain Weight (g/m2)
800
700
600
500
400
300
200
100
0
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX7078
Mean
Groups

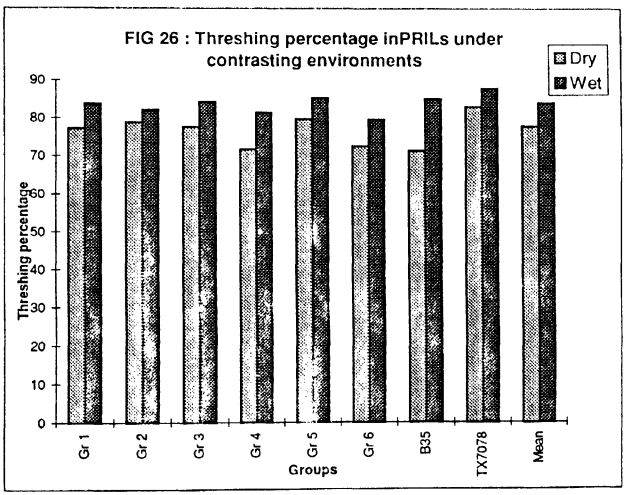


FIG 26 : Threshing percentage inPRILs under contrasting environments
Dry
Wet
Threshing percentage
90
80
70
60
50
40
30
20
10
0
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX7078
Mean
Groups

the lowest and the highest grain weights recorded were 297g (Line34) and 821g (Line89) respectively.

In general, the staygreen Lines recorded the higher grain yields compared to the senescent Lines (Fig. 25). Within the Groups Group-6 consisting of Line92, recorded the highest grain weight. The staygreen Lines of Groups-3 showed higher grain weight than the other staygreen groups in the Dry environment. The lowest grain yields in both the environments was recorded in Lines belonging to Group-4.

4.5.8 Threshing per centage

The threshing percentage of the genotypes did not differ significantly over the two environments which means that drought stress did not have any effect on the threshing percentage. The groups did not differed significantly from each other in their threshing percentages. In the Dry environment the threshing percentage was the least in Group-4 (71.7%) and highest in Group-5 (79.7%). In the Wet plot the threshing percentage was maximum in Group-5 (85.3%) and minimum in Group-6 (79.6%). The means of the two environments are 77% and 84% respectively (Fig. 26).

4.5.9 100 seed weight

Drought stress had a significant effect on the seed weight of the evaluated Lines. The Lines also differed significantly from each other in their 100 seed weights. In the Dry environment Line17 recorded the lowest seed weight(2.06g) while Line51 recorded the highest(3.94g). In the Wet environment Line21 showed the highest seed weight(4.57g) and Line89 showed the lowest(2.8g). The Dry and the Wet environment means are 3 g and 3.6g respectively.

FIG 27 : 100 Seed Weight in the Groups under contrasting environments.

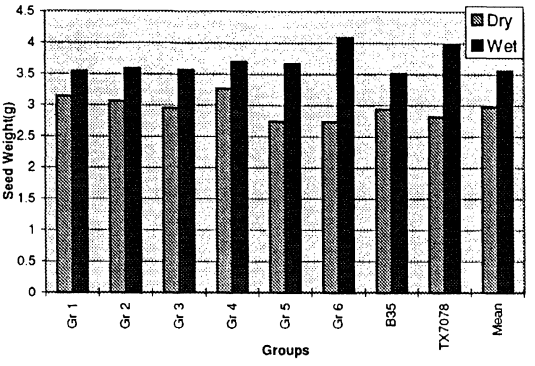
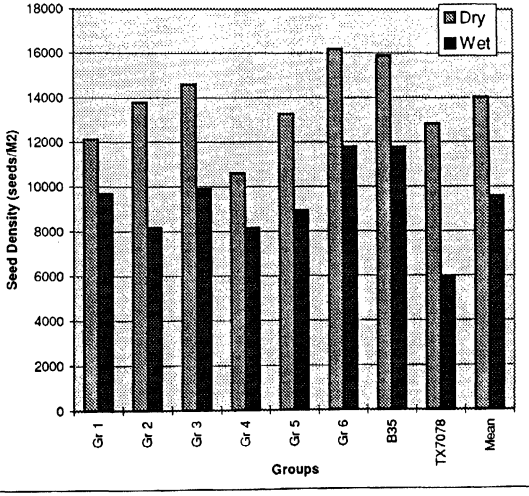


FIG 28 : Seeds/M2 in groups under contrasting environments



Group-4 recorded the highest 100 seed weights in the Dry environments and Group-6 under Wet environment respectively. In the Dry environment the staygreen Groups recorded a higher seed weight than the senescent Groups, but in the Wet environment the senescent Lines had higher seed weights than the staygreen Lines (Fig. 27).

4.5.10 Seed per square meter

The Lines also significantly differed from each other in their seed densities. The highest number of seeds per unit area was recorded in Line57 (23,586 seeds /M ^{2}) in the Dry environment while the lowest was recorded in Line51 (8,512 seeds /M ^{2}). In the Wet plot the highest number of seeds per unit area was recorded in Line34 (5344 seeds /M ^{2}) and the lowest values were recorded in Line89 (17874 seeds /M ^{2}). The mean number of seeds per unit area for the Dry and the Wet environments are 14031 seeds /M ^{2} and 9569 seeds /M ^{2} respectively.

The seed density in general was lower in the Wet environment compared to the Dry environment. In the Dry environment highest seed density was recorded in Group-6 followed by Group-3 while the lowest was recorded in Group-4 (Fig. 28).

4.5.11 Harvest index (HI)

The genotypes differed significantly from each other in their HI. The environment means for the Dry and Wet environments are 50.2% and 56% respectively. Water stress caused a significant reduction in HI across environments. The environment had a significant effect on the performance of the genotypes. In the Dry environment the HI was

lowest in Line1 (34.9%) and highest in Line 8 (62.15%) while in the Wet environment the HI lowest in Line 99 (39.57%) and highest in Line 80 (67.29%).

The harvest index for the Dry environment over all the Groups is less than the Wet environment. The staygreen Groups had a lower HI compared to the senescent Groups in both the Wet and Dry environments (Fig. 29).

4.5.12 Leaf number per plant at harvest

The Lines differed significantly from each other in their green leaf number at harvest. The environmental means for the Dry and Wet are 0.85 leaves and 1.55 leaves respectively. The environment had significant effect on the leaf number at harvest. In the Dry environment the highest leaf number was recorded in Line 95 (1.98 leaves) and the lowest in the Lines 52 and 92. In the Wet treatment the highest leaf number was recorded in Line19 (3.41) while the lowest leaf number was recorded in TX7078.

In general, the staygreen Groups retained greater number of green leaves compared to the senescent Groups. Out of the six Groups Group-1 showed the highest leaf number in the Dry environment followed by Group-2 at harvest while the Groups 5 and 6 showed complete senescence at harvest. In the Wet environment the staygreen Lines retained 50% more green leaves than the senescent Lines at harvest (Fig. 30).

4.5.13 Plant height:

Water stress did not have a significant effect on the plant height. The means were 95.6 cm and 105.5 cm for the Dry and Wet environments respectively. The Lines however differed significantly in their heights. In both the environments the Line 7 (67cm in Dry and 73cm

FIG 29 : HI in groups under contrasting environments

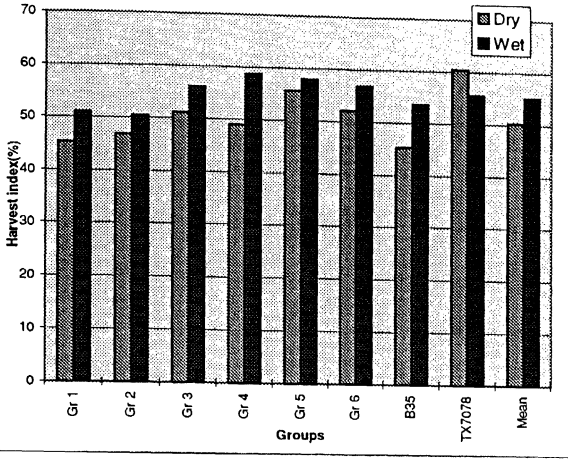
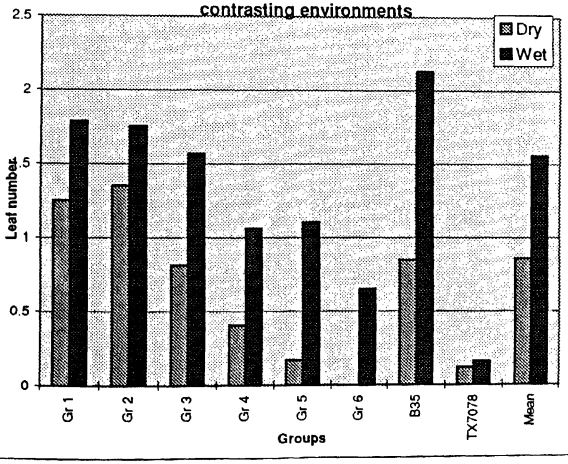


FIG 30 : Leaf number at Harvest in Groups under contrasting environments



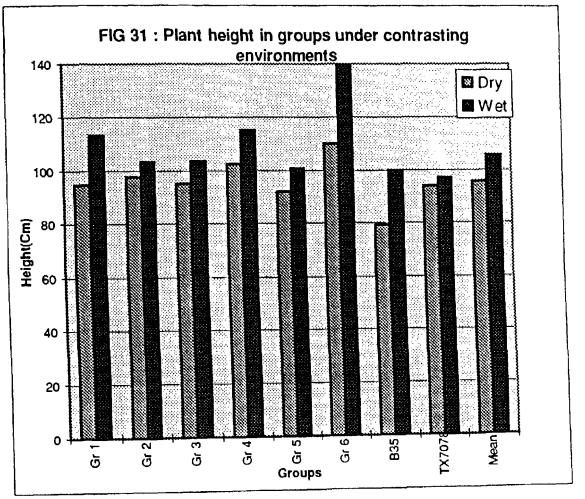


FIG 31 : Plant height in groups under contrasting environments
Dry
Wet
Height(Cm)
140
120
100
80
60
40
20
0
Gr 1
Gr 2
Gr 3
Gr 4
Gr 5
Gr 6
B35
TX7078
Mean
Groups

in Wet) was the shortest and the Line41 was the tallest (156cm in Dry and 173 cm in Wet).

The plants were higher in the Wet environment compared to the Dry environment. Within the Groups Lines belonging to Group-6 and 4 were the tallest (Fig. 31).

4.6 INCIDENCE OF CHARCOAL ROT AND LODGING:

4.6.1 Soft stalk percentage

The soft stalk data was taken in the Dry environment at crop maturity and again at harvest.

The genotypes differed significantly the number of plants effected both at maturity and at harvest. At maturity the Lines belonging Group-5 showed soft stalk incidence in greater than 60% of the plants while 22 Lines recorded no soft stalk at all. At harvest fifteen Lines had less than 10% of the plants infected. Within the Lines 92 and 52 showed 100 % soft stalk infection . GROUP-5 showed infection in 65% of the plants. Over the whole population soft stalk occurred in 8% of the plants at maturity and 36% of the plants at harvest.

4.6.2 Nodes spread

The environment had significant influence on the number of nodes the charcoal rot disease was spread over. The genotypes differed significantly in the number of nodes on which the disease is spread. The mean number of nodes infected were 1.18 and 0.049 in the Dry and Wet environments respectively. In the Dry environment Line52 showed the highest infection with 5 nodes being infected while no disease was seen in the Lines 85, 41, 34,

30, 22, 99, 1, 50 and 71 (belonging to Groups 1 and 2). In the Wet environment the disease was very marginal with 68 Lines showing no symptoms at all.

All the Groups had Lines showing infected nodes. The number of nodes infected was less (< 0.5) in the Lines belonging to Groups1 and2, medium(<1.2) in Groups3 and 4 and high in Groups 5 and 6 (<2.0). In the Wet environment the infection was minimal (Fig. 32).

FIG 32 : Nodes infected by the fungus In the Groups under contrasting environments

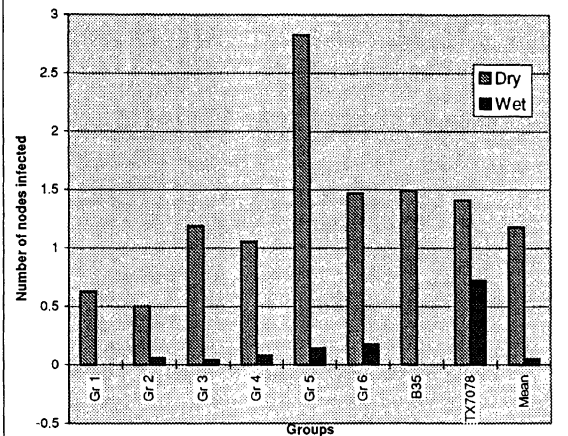
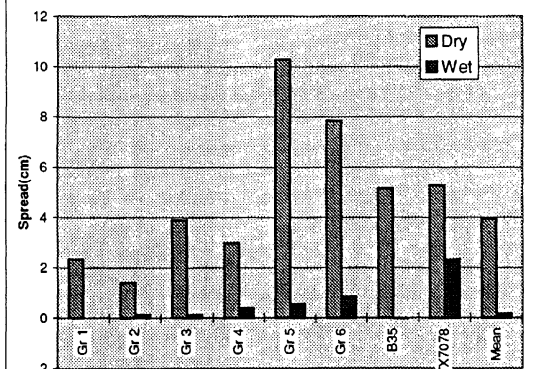


FIG 33 : Spread of fungus in the groups under contrasting environments



4.6.3 Spread of the disease

The environment had significant influence on the spread of the charcoal rot disease. The spread of the disease was significantly different between the Lines.. The mean spread recorded was 3.97 cm and 0.168 cm in the Dry and Wet environments respectively. In the Dry environment Line91 showed the highest infection with a 17cm spread followed by Line63 and Line52 while no disease was seen in the Lines Line85, 41, 34, 30, 22, 99, 1, 50 and 71 (belonging to Groups 1 and 2). In the Wet environment the disease was very marginal with 60 Lines showing no disease spread.

The spread of the disease in the vascular bundles of the stem was minimal in the staygreen Lines compared to the senescent Lines. In Group-5 and 6 the spread is 110% more than the remaining Groups. Amongst the staygreen Groups the Lines belonging to Group-3 showed the maximum spread of the disease(Fig. 33).

4.7 CORRELATIONS

4.7.1 Correlation between phenological, yield and charcoal rot 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, plant height and soft stalk at maturity. The correlation was not significant for threshing %, HI and other charcoal rot parameters. Days to physiological maturity had significant positive correlation with headweight/M^2, grainweight/M^2, stalkweight/M^2, seeds/M^2 while having a negative correlation with GS3, 100 seed weight, plant height, HI, soft stalk at maturity and soft

stalk at harvest. The correlation was not significant with GS3, threshing percentage, 100seed weight, plant height and spread of charcoal rot disease in the stem. GS3 had significant negative correlation with head weight /M ^{2}, grain weight/ M ^{2}, stalk weight /M ^{2}, seeds /M ^{2} while having a positive correlation with 100 seed weight, plant height and soft stalk at maturity. No significant correlation existed with stem weight/M ^{2}, threshing %, HI and leaf number at harvest.

Grain weight /M ^{2} had a positive correlation with biomass/M ^{2}, head weight/M ^{2}, stalk weight /M ^{2}, seeds /M ^{2}, threshing %, HI, leaf number at harvest and plant height while it had a negative correlation with 100seed weight and soft stalk at maturity. No correlation was observed with the other soft stalk parameters. Stalk weight/M ^{2} had a significant positive correlation with all the yield and phenological parameters except threshing %; and showed a significant negative correlation with HI and the charcoal rot parameters.

100 seed weight had a significant negative correlation with seeds /M ^{2} and all the charcoal rot parameters.

Leaf number at harvest had a significant correlation with biomass /M ^{2}, grain weight /M ^{2}, stalk weight/M ^{2} and significant negative correlation with all the charcoal rot parameters. No significant correlation was observed with plant height.

Charcoal rot :

The charcoal rot parameters were significantly positively correlated with each other and negatively correlated with stem weight /M ^{2}, biomass /M ^{2}, 100 seed weight and leaf number at harvest. The correlation with grain yield was not significant (Table 9).

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, Linear rate while having a negative correlation with Linear rate of senescence. Relative leaf number at onset and the point of inflection have a significant negative correlation with Linear rate. Relative leaf number at offset has no significant correlation with Linear rate (Table 9).

4.7.3 Correlation between regression parameters and agronomic traits

Grain yield has a significant negative correlation with T-on and T-off. Stalk and bio-mass yield have significant negative correlation with T-on and Linear rate of senescence. Harvest index has a negative correlation with T-on and T-off. Seed density had a negative correlation with yield parameters.

The charcoal rot parameters had a significant negative correlation with the linear rate of senescence (Table 10).

4.7.4 Correlation between regression parameters, agronomic traits and relative green leaf number duration

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 and both soft stalk at maturity and soft stalk at harvest.

TABLE 9 : CORRELATIONS FOR PHENOLOGICAL, YIELD, CHARCOALROT TRAITS IUNDER DRY EN' UNDER DRY ENVIRONMENT

Flowering	1																			
Maturity	0.76	1																		
GS3	-0.82	-0.25	1																	
Head wt	0.45	0.36	-0.36	1																
Stalk Wt	0.32	0.45	-0.07	0.30	1															
Bio-mass	0.46	0.50	-0.25	0.78	0.83	1														
Grain Wt	0.47	0.38	-0.37	0.77	0.42	0.73	1													
THR %	0.02	0.00	-0.02	-0.32	0.10	-0.10	0.34	1												
100 Sd Wt	-0.34	-0.08	0.43	-0.20	0.25	0.05	-0.19	-0.01	1											
Seed Den	0.52	0.31	-0.50	0.65	0.14	0.47	0.78	0.22	-0.717	1										
HI	-0.08	-0.21	-0.07	-0.16	-0.59	-0.47	0.23	0.64	-0.287	0.301	1									
Lf No -Har	0.15	0.25	0.02	0.02	0.68	0.46	0.16	0.13	0.433	-0.182	-0.437	1								
PL Ht	-0.21	-0.04	0.27	0.08	0.41	0.32	0.17	0.11	0.260	-0.022	-0.170	0.12	1							
Nd Sprd	0.14	-0.05	-0.27	0.13	-0.39	-0.19	0.02	-0.10	-0.496	0.345	0.303	-0.62	-0.13	1						
Cm Sprd	0.06	-0.11	-0.20	0.08	-0.40	-0.22	-0.03	-0.11	-0.429	0.242	0.292	-0.58	-0.06	0.94	1					
Soft % Ma	-0.28	-0.22	0.22	-0.19	-0.42	-0.38	-0.24	-0.05	-0.186	-0.086	0.267	-0.50	0.00	0.24	0.36	1				
Soft % Har	-0.01	-0.17	-0.16	0.07	-0.58	-0.34	-0.04	-0.09	-0.560	0.320	0.445	-0.83	-0.10	0.78	0.78	0.62	1			

Flowering Maturity GS3 Head wt Stalk Wt Bio-mass Grain Wt THR % 00 Sd Wt Seed Den HI Lf No -Har PL Ht Nd Sprd Cm Sprd oft % Mat Soft % Har

TABLE 10 CORRELATIONS BETWEEN REGRESSION PARAMETERS AND YIELD PARAMETERS IN DRY ENVIRONMENT

B	1																					
M	0 13	1																				
T-on	-0 64	0 24	1																			
T-off	0 53	0 38	0 19	1																		
T-lin	0 92	0 09	-0 69	0 57	1																	
Y-on	0 79	-0 36	-0 7	0 35	0 84	1																
Y-off	0 37	-0 16	-0 33	-0 07	0 22	0 47	1															
Y-lin	0 62	-0 29	-0 55	0 44	0 78	0 76	-0 19	1														
Y-m	0 79	-0 41	-0 67	0 35	0 81	0 98	0 48	0 75	1													
b-m	0 99	0 25	-0 61	0 54	0 91	0 72	0 35	0 56	0 72	1												
B-lin	-0 92	-0 24	0 57	-0 6	-0 92	-0 67	-0 01	-0 74	-0 65	-0 93	1											
Gr Yld	0 49	0 26	-0 46	0 09	0 45	0 3	0 11	0 26	0 26	0 5	-0 49	1										
Bio Yld	0 25	0 17	-0 34	-0 17	0 16	0 11	0 09	0 05	0 08	0 25	-0 23	0 73	1									
HI	0 03	0 01	-0 34	-0 46	-0 05	-0 04	0 05	-0 08	-0 05	0 03	0 0	0 49	0 8	1								
Sd Den	-0 42	-0 15	0 24	-0 35	-0 45	-0 3	-0 02	-0 32	-0 29	-0 42	0 44	-0 49	0 22	0 29	1							
Pl Ht	0 08	-0 13	-0 02	0 1	0 09	0 19	0 16	0 09	0 17	0 04	-0 02	0 32	0 18	-0 02	-0 17	1						
Nd Spr	-0 53	-0 23	0 11	-0 63	-0 56	-0 42	-0 18	-0 34	-0 4	-0 55	0 54	-0 18	0 01	0 33	0 29	-0 12	1					
Cm Spr	-0 55	-0 27	0 16	-0 58	-0 56	-0 42	-0 2	-0 33	-0 4	-0 59	0 56	-0 22	-0 04	0 24	0 29	-0 05	0 94	1				
Sol% Mat	-0 52	-0 31	0 4	-0 19	-0 47	-0 33	-0 18	-0 24	-0 32	-0 55	0 52	-0 49	-0 34	-0 16	0 31	-0 04	0 22	0 31	1			
Sol % Har	-0 61	-0 3	0 3	-0 56	-0 66	-0 5	-0 18	-0 43	-0 49	-0 63	0 62	-0 41	-0 07	0 22	0 52	-0 09	0 55	0 66	0 62	1		
	B	M	T-on	T-off	T-lin	Y-on	Y-off	Y-lin	Y-m	b-m	B-lin	Gr Yld	Bio Yld	HI	Sd Den	Pl Ht	Nd Spr	Cm Spr	Sol% Mat	Sol % Har		

TABLE 11 : Correlation of relative green leaf number with regression and agronomic traits

	A1	A2	A3	Total A	T-on	T-off	Y-lin	B-lin	Y-on	Y-off	St Wt	Biomass	Gr Wt	Soft mat	soft har	PL ht
A1	1															
A2	-0 86	1														
A3	-0 85	0 87	1													
Total A	-0 57	0 91	0 74	1												
T-on	1	-0 9	-0 88	-0 64	1											
T-off	-0 41	0 79	0 44	0 91	-0 48	1										
Y-lin	-0 68	0 94	0 66	0 94	-0 73	0 95	1									
B-lin	0 66	-0 95	-0 77	-0 99	0 72	-0 91	-0 97	1								
Y-on	-0 89	0 99	0 93	0 87	-0 93	0 7	0 88	-0 91	1							
Y-off	-0 57	0 31	0 72	0 09	-0 55	-0 31	-0 04	-0 1	0 44	1						
St Wt	-0 73	0 89	0 6	0 82	-0 76	0 87	0 95	-0 88	0 83	-0 05	1					
Biomass	-0 53	0 54	0 3	0 4	-0 54	0 54	0 61	-0 5	0 47	-0 13	0 82	1				
Gr Wt	0 15	0 75	0 12	0 13	-0 12	0 06	0 09	0 01	-0 05	-0 24	0 37	0 82	1			
Soft mat	0 34	-0 72	-0 66	-0 91	0 42	-0 76	-0 74	0 86	-0 71	-0 13	-0 52	-0 03	0 42	1		
soft har	0 49	-0 85	-0 72	-0 98	0 57	-0 85	-0 86	0 94	-0 83	-0 13	-0 71	-0 27	0 29	0 93	1	
PL ht	0 33	-0 5	-0 56	-0 56	0 38	-0 39	-0 44	0 57	-0 53	-0 28	-0 22	0 25	0 46	0 76	0 54	1

The relative green leaf number duration up to onset of Linear phase of senescence(A1) has a significant positive correlation with T-on while having a negative correlation with A2, A3 and relative leaf number at offset of Linear phase.

The relative green leaf number duration from onset to offset has a significant positive correlation with A3, A, T-off, Linear rate of senescence stalk weight and biomass while having a negative correlation with both soft stalk at maturity and soft stalk at harvest. The relative green leaf number duration from offset of senescence to harvest maturity had a positive correlation with relative leaf number at both onset and offset of senescence. The correlation was significant and negative with soft stalk % at harvest and the time of onset of senescence.

Plant height had a significant correlation with soft stalk % at maturity and no significant correlation with other parameters. Biomass had a significant negative correlation with soft stalk at harvest. T-on, Linear rate of senescence and Linear duration of senescence had a significant negative correlation with soft stalk at both maturity and harvest.(Table11)

MOLECULAR ANALYSIS

RAPD results :

RAPD analysis is simple and fast involving PCR amplification followed by gel electrophoresis of genomic DNA. It requires very minute amounts of genomic DNA (25 ng per reaction) and analysis is free from the radioactive materials. As the primers used are of 10 bp length the condition for PCR amplification such as annealing temperature,

PLATE 1 RAPD profile of the two contrasting parents using probes UBC 176 an

OPB 08

PLATE 2 RAPD profile of the six Lines from six groups using the probes UBC 176

and OPB 08

$MgCl_2$ concentration dNTP's concentration and G+C content of primers are very crucial to get reproducible results.

The two parents which showed contrasting senescence response were screened with 2, ten base pair long primers. The senescence groups were also screened by selecting one nine from each group in testing with the two rapid primers. As shown in Plates 1&2 a total of 15 amplified fragments were identified when PCR amplified products were run on 1.5 % agarose gels using the RAPD primers UBC 176 and OPB 08. The RAPD primer UBC 176 showed 5 amplified fragments where as the primer OPB 08 showed 4 amplified fragments. Out of the 5 bands amplified by UBC176 2 were polymorphic. Out of the 4 bands shown by OPB 08 one was polymorphic. With the primers UBC 176 , band 2 was absent in Groups 1 and 2, which are staygreen, was faint in Group 3 which was moderate staygreen and prominent in the Groups 4, 5 and 6 which were senescent lines

PLATE 3 Field view of B35 at physiological maturity

PLATE 4 Field view of TX7078 at physiological maturity

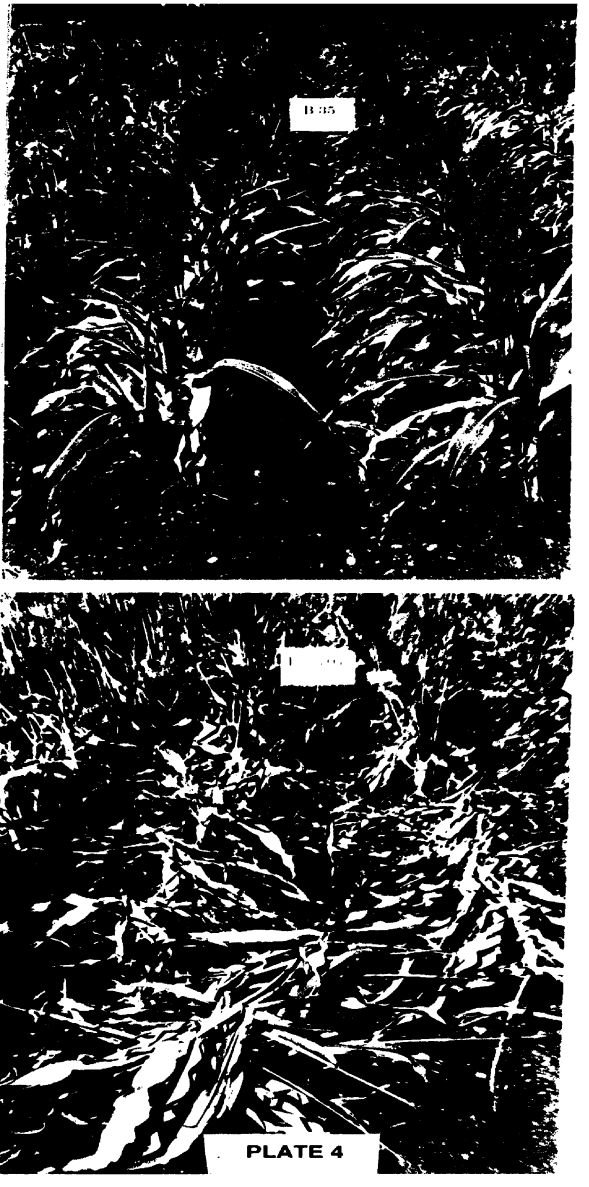


PLATE 4

PLATE 5 Field view of Line 92 at physiological maturity

PLATE 6 Comparison of single plants of B35 and TX7078

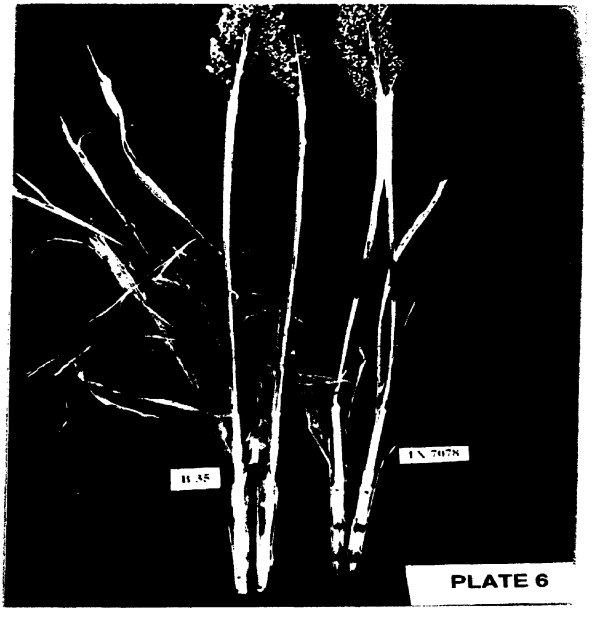


PLATE 6

PLATE 7 Comparison of heads of B35 and TX7078 under stress

PLATE 8 Spread of charcoal rot in the whole stem of B35 and TX7078

PLATE 7

PLATE 8

PALTE 9 Spread of charcoal rot in the basal nodes of B35 and TX7078

'LATE 10 Infected TX7078 plant

PLATE 9

PLATE 10

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. Different cultivars show different morphological and physiological modifications to overcome the various environmental stresses encountered during the crop growth . One such mechanism is the staygreen or non senescence. Staygreen 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 a population of 97 Recombinant inbred Lines ((RILs) were used. The RILs offer an advantage in that they have attained a high level of homozygosity (>99per cent) and show little segregation for the trait under study and hence represent an ideal plant material to study the staygreen trait showing least within the line variability.

In the development of plants, senescence is a relatively gross change or sequence of changes ultimately leading to the death of the plant. In plants, these changes are recognized as a decrease in the growth rates and vigor and increase in susceptibility to environmental challenges or disease susceptibility. 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 and as reported by Duncan (1983). In the late reproductive stages the panicle started senescence from tip downwards.

5.2 Evaluation of Staygreen trait

5.2.1 Leaf senescence studies

Data of leaf number was fitted against days after flowering to obtain the senescence pattern of the Lines under study. Green leaf number gave similar variance as green leaf area 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 to produce large leaf area, hence represents staygreen *per se*. The categorization of Lines into Groups by cluster analysis basing on the various senescence parameters obtained through the regression fit also facilitated study of staygreen trait and comparison of Groups. The Lines showed wide variation for all the senescence parameters studied.

5.2.2 Rate Parameters

The Lines belonging to the staygreen Groups showed at least 25 % lower rate of senescence than the senescent Lines under water stress. But in the Wet environment (absence of stress) the Groups did not differ much in their senescence rates. The rapid increase in the rate of senescence with stress was more marked in moderate senescence Group and the high senescence Group (4 and 5).

The regression curve can be distinguished into three distinct regions. The initial phase of senescence where the senescence occurs as a normal process in the life cycle of the crop. This phase involves gradual loss of photosynthetic capacity and death of the lower leaves.

But during the crucial stage 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 in the Wet environment a normal phenomenon in the life of the plant any increase in rate of senescence in the Dry environment over the Wet environment may be due to moisture stress. Hence Dry environment is more suitable for screening for senescence and drought resistance.

5.2.2.1 Linear rate of senescence

The dependence of leaf senescence on soil water as illustrated by accelerated senescence under Dry environment resulted in G×E interaction for staygreen amongst the Lines. The staygreen Lines belonging to the Groups 1, 2 and 3 had at least a 40 per cent lower Linear rate of senescence compared to the senescent Lines under terminal moisture stress.. The high Linear rate of senescence caused a decrease of 55 to 60 per cent of the total green leaf area during a period of 11 to 15 days in the senescent Lines. 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 Lines. 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 even in the absence of stress is natural and involves tight metabolic regulation of the tissues involved. The observations were in agreement with Thomas and Smart (1991) who opined that the accelerated senescence in the absence of stress is more a change rather than loss of function and physiological integrity. Under moisture stress it was observed that the Linear rate of

senescence on an average increased by less than 20 per cent in the staygreen Lines when compared to the Wet environment. In contrast the Linear rate of senescence increased by over 70 per cent in the senescent Lines in the Dry environment when compared to the Wet environment. The increase in senescence rate in the staygreen Lines can be considered a normal response to the increased water stress. But the abrupt and rapid increase in the senescence rate in the senescent Lines at the same time implies lack of adaptive mechanism as in staygreen Lines. The inference is that the senescent Lines enter the second phase of senescence characterized by rapid tissue deterioration and photodestruction. This result is again in agreement with Thomas and Smart (1991).

One possibility is that in the senescent Lines 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. In the absence of stress the contribution of stored carbohydrates to the grain weight is estimated at only 10 to 12 per cent. But under stress significant increase in contribution of stored carbohydrates to the grain weight especially in the senescent Lines was observed. This is in confirmation with the reports of Kreig (1983). The staygreen Lines 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 Lines and 50 per cent of the leaves senesced in the senescent Lines. The results were in confirmation of those obtained by vanOoesterom *et. al.* (1996).

5.2.3 ONSET OF LINEAR SENESCENCE

The early onset of Linear senescence under moisture stress when compared to Wet environment implies GxE interaction for onset of senescence. The onset of Linear phase of senescence was earlier in the staygreen Lines when compared to the senescent Lines in the both the environments. The probable reason for this may be the early initiation of grain filling in the stay- green Lines when compared to the senescent Lines. In the Dry environment the rapid loss of green leaves in the senescent Lines 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 and biomass. The onset of senescence is also having significant negative correlation with grain weight and biomass. This implies that in Lines 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 higher is the grain and stalk yield. It was observed that although in the staygreen Lines the onset of Linear phase of senescence was early compared to the senescent Lines the duration of Linear senescence was longer which contributed to the higher yields of those Lines.

5.2.4 GREEN LEAF NUMBER DURATION

The number of physiologically active green leaves from flowering to maturity is very important for production of current photosynthates which contribute the major bulk of the grain weight. The staygreen Lines belonging to Groups 1, 2 and 3 have a higher overall green leaf number duration when compared to the senescent Lines. Across environments stress caused a 25 to 30 per cent decrease in the green leaf number duration in the Lines

after flowering indicating varying GxE interaction. But the decline in the green leaf number duration was most prominent (40 to 60 %) in the initial phase of senescence. This was due to the shorter duration before the onset of Linear senescence under moisture stress. In between the Groups the staygreen Groups showed 90 per cent more leaf number duration compared to the senescent Lines during the Linear phase of senescence. A high relative green leaf number during the Linear phase had a positive influence on the grain weight (r=0.75) and stalk weight (r=0.54) as is indicated by the highly significant correlation values. Hence higher yields were observed in the staygreen Lines when compared to the senescent Lines as was observed by Gerik and Miller (1984). High relative green leaf number duration during the Linear phase of senescence has a negative correlation with charcoal rot parameters (> -0.75). Thus the staygreen Lines which have high relative green leaf number duration during the Linear phase of senescence and showed lower incidence of charcoal rot disease when compared to the senescent Lines. Total relative green leaf number duration had a significant correlation with stalk weight (r = 0.82) and no significant correlation with grain weight (r = 0.15) under moisture stress. 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 AGRONOMIC TRAITS

5.3.1 Phenology

5.3.1.1 Days to 50 per cent flowering

The Lines differed significantly from each other in their flowering dates. However it did not vary significantly between the Wet and Dry environments. The flowering date on an

average over all the Lines was one day earlier in the Dry environment which is not significant. The probable reason for uniformity across environments in the flowering dates may be absence of moisture stress until up to flowering due to the rains and irrigation received during the pre-flowering stage.

5.3.1.2 Flowering to physiological maturity (GS3)

The Lines differed significantly from each other in their grain filling period (GS3). Due to water stress the mean maturity of the Lines was earlier in the Dry environment when compared to the Wet environment.. Within the Groups the senescent Lines had a longer duration of GS3. Group4 which had the longest grain filling period recorded the lowest average grain yields. The results were in confirmation with those obtained by Blum (1985) who reported that early maturity , i.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. Group 5 also had a longer duration of GS3. Besides a rapid rate of senescence the longer duration of grain filling under stress resulted in its lower yields. Amongst the staygreen Lines those belonging to Group-2 had a longer duration of GS3 compared to the other two Groups which may be the possible reason for the comparatively lower yields of the Group. It was observed that in the highly senescent Line 92 belonging to Group-6 the duration of grain filling was shorter unlike other senescent Group. A longer duration of GS3 may also cause a decrease in the harvest index under stress which also brings about reduction in yields.

5.3.2 Yield attributes The Lines showed significant differences between each other for all the yield attributes under consideration.

5.3.2.1 Threshing percentage and Harvest index

The threshing percentage remained more or less constant across the environments indicating that moisture stress did not have significant effect on the threshing percentage. The harvest index of the Lines decreased significantly due to moisture stress which is not in confirmation with the reports of Jordan and Sullivan (1982) who suggested that HI is maintained although the grain yields decreased under moisture stress. The senescent Groups in general had a higher HI in both the environments and the decrease in HI with moisture stress was lower compared to the staygreen Lines. The observations Jordan and Sullivan look more valid in the senescent Lines than in the staygreen Lines. The decrease in HI was the most for Group-4 which can be another contributing factor for the lower yields of that Group. Amongst the staygreen Lines Group-3 showed the highest HI in both the environments and the least decrease in HI with stress which may be one of the reasons for the high yields of that Group.

5.3.2.2 Seed size and Seed number /M^2 :

The 100 seed weight decreased under moisture stress indicating significant GxE effect. The decrease in seed weight was due to reduced grain filling under stress. The seed weight of the Lines belonging to the senescent Groups decreased by 23 per cent of the Wet environment. In contrast the staygreen Lines showed less than 10 per cent decrease in their seed weights with stress which is one more contributing factor to the higher yields of the staygreen Lines. However all the Groups showed higher seed number under moisture stress when compared to the Wet environment. The senescent Lines had lower number of seeds /M^2 than the staygreen Lines in both the environments with the exception of Group-

6 (Line 92) which had 30 per cent more number of seeds compared to all other Groups. The high seed number per unit area coupled with a high harvest index may be the reason for high grain yields of Line 92. In contrast Group-4 had the lowest number of grains per unit area (and also lowest harvest index) due to which it recorded the lowest yields in both the environments. With in the staygreen Lines Group-2 had lowest seed number /M^2 and Groupp3 the highest. There was no significant difference in the 100 seed weights. So grain yield of Lines belonging to Group-3 was more than Group-1 while Group-2 recorded lower yields.

5.3.2.3 Stalk weight :

Moisture stress had a significant effect on the stalk weight of the Lines under study. The stalk weight recorded was higher for the staygreen Lines when compared to the senescent Lines. The higher stalk weight of staygreen Lines can be attributed to lower rate of leaf senescence and harvest index when compared to the senescent Lines. The correlation of Linear senescence rate with stalk weight was negative(- 0.5) indicating that a lower senescence rate contributed to higher stalk weight and Linear senescence rate can be used for selecting Lines with higher stalk weight.

5.3.2.4 Grain weight :

The grain weight /M^2 was significantly more in the staygreen Lines compared to the senescent Lines across both the environments indicating a higher genetic potential as well as a high resistance to terminal moisture stress in the staygreen Lines. 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 photosynthetic 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 Lines will cause a rapid decrease in the number and area of functional leaves which causes significant yield reduction. The results were in confirmation of their reports. Of all the staygreen Lines the grain yield was the highest in Lines belonging to Group-3 which represents the moderate staygreen Group. In the high staygreen Lines 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 Lines are better suited.

5.3.2.5 Plant height :

Water stress did not have a significant effect on plant height although the Lines showed a marginal decrease in their heights. The correlation of green leaf number or rate of senescence with plant height was not significant. But plant height had a significant negative correlation with soft stalk and lodging at maturity indicating that taller plants tend to lodge more quickly.

5.3.3 CHARCOAL ROT and LODGING :

Lines with a higher rate of Linear senescence showed greater incidence of charcoal rot than the staygreen Lines. Under post-flowering drought stress conditions, senescent Lines showed 20 to 25 per cent lodging compared to less than 10 per cent lodging in the staygreen Lines. Thus the staygreen trait has a direct benefit to sorghum by reducing

moisture stress related lodging associated with premature leaf and stalk death. The results were in agreement with those obtained by Rosenow *et al.* (1995). A significant negative correlation was observed between plant height and soft stalk(r = 0.75) indicating that Lines which were taller were more susceptible to soft stalk and its associated lodging.

Molecular analysis

The two primers used (UBC 176 and OPB 08) were efficient in detecting polymorphism between the Lines contrasting in their senescence behavior. The RAPD primer, UBC176 detected two polymorphic bands while OPB 08 detected one polymorphic band between the two parents contrasting for their post-flowering drought tolerance. The primers can hence be used in marker assisted selection for the staygreen trait. Testing with a group of related primers can produce more data and polymorphism which can be ultimately used in mapping the trait.

All these results indicate that staygreen is an important trait associated with post-flowering drought tolerance in sorghum. Breeding for the trait was shown to be possible and studies by vanOosterom *et al.* (1996) indicated that the trait is heritable. The trait helps in selection for drought tolerance while maintaining the overall productivity and yield stability under terminal moisture stress. Although the trait is advantageous, very high staygreen rating may not lead to high yields as, in such lines the source (which is the leaf) may act as a sink thus decreasing the availability of current photosynthates to the developing panicles. However it was observed that staygreen lines were less effected by charcoal rot disease and showed lesser lodging compared to the senescent lines. The taller lines tended to lodge earlier than the shorter lines and the staygreen lines in general were shorter than the senescent lines.

SUMMARY

CHAPTER VI

SUMMARY

Staygreen is an important trait associated with post-flowering drought tolerance in sorghum. The present study using a RIL population derived from two lines contrasting in their drought response (B35 is post-flowering tolerant and TX7078 is pre-flowering tolerant) was taken up at ICRISAT- Patancheru, Andhra Pradesh in the post-rabi season 1996-97 with the following objectives.

(I) Quantifying the expression of staygreen trait and yield potential in a set of RIL Lines and their parents.

(II) Observe if staygreen has any effect on charcoal rot resistance and lodging.

(III) Use Randomly amplified polymorphic DNA to identify polymorphism between staygreen and senescent Lines.

Both relative leaf number and relative leaf area were 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 similar variance as green leaf area justifying its use in senescence studies. The lines were clustered in to six groups based on the senescence parameters - 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 lines belonging to the stay-green groups showed at least 25 per cent lower rate of senescence than the senescent lines under water stress. The rapid increase in the rate of senescence with stress was more marked in moderate senescence group and the high senescence group. The dependence of leaf senescence on soil water as illustrated by accelerated senescence under dry environment resulted in G×E interaction for stay-green amongst the lines. The stay-green lines belonging to the groups 1, 2 and 3 had at least a 40 per cent lower linear rate of senescence compared to the senescent lines under terminal moisture stress.. The high linear rate of senescence caused a decrease of 55 to 60 per cent in the green leaf number in a period of 11 to 15 days in the senescent lines. 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 stay-green lines. Under moisture stress it was observed that the linear rate of senescence on an average increased by less than 20 per cent in the stay-green lines when compared to the wet environment. In contrast the linear rate of senescence increased by over 70 per cent in the senescent lines in the dry environment when compared to the wet environment. Genotypic differences in senescence rate was the largest at the point of inflection where the senescence rate was the highest for all the lines. The onset of linear phase of senescence was earlier in the stay-green lines when compared to the senescent lines in the both the environments. The probable reason for this may be the early initiation of grain filling in the stay- green lines when compared to the senescent lines. 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 stay-green lines the onset of linear phase of senescence was early compared to the senescent lines the duration of linear senescence was longer and the linear rate of senescence lower which contributed to the higher yields of those lines.

The stay-green lines belonging to groups 1, 2 and 3 have a higher overall green leaf number duration when compared to the senescent lines. Across environments stress caused a 25 to 30 per cent decrease in the green leaf number duration in the lines after flowering indicating varying G×E interaction. 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 under moisture stress. In between the groups the stay-green groups showed 90 per cent more leaf number duration compared to the senescent lines 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 ($r = 0.75$ and 0.54 respectively). High relative green leaf number duration during the linear phase of senescence has a negative correlation with charcoal rot parameters(> -0.75) due to which the stay-green lines which have high relative green leaf number duration during the linear phase of senescence showed lower incidence of charcoal rot disease when compared to the senescent lines. Total relative green leaf number duration had a significant correlation with stalk weight($r = 0.82$) and no significant correlation with grain weight($r = 0.15$) under moisture stress. 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.

The flowering date on an average over all the lines was one day earlier in the dry environment which is not significant. The lines differed significantly from each other in their grain filling duration (GS3). Due to water stress the mean maturity of the lines was earlier in the dry environment when compared to the wet environment.. Within the groups the senescent lines had a longer duration of GS3.

The harvest index of the lines decreased significantly due to moisture stress. The senescent groups in general had a higher HI in both the environments and the decrease in HI with moisture stress was lesser compared to the stay-green lines. Amongst the stay-green lines group3 showed the highest HI in both the environments and the least decrease in HI with stress which may be one of the reason for the high yields of that group. Moisture stress had a significant effect on the stalk weight of the lines under study. The stalk weight recorded was higher for the stay-green lines when compared to the senescent lines. Lower senescence rate contributed to higher stalk weight and linear senescence rate can be used for selecting lines with higher stalk weight. The grain yields were significantly more in the stay-green lines compared to the senescent lines across both the environments indicating a higher genetic potential as well as a high resistance to terminal moisture stress in the stay-green lines. A high senescence rate in the senescent lines causes a rapid decrease in the number and area of functional leaves which causes significant yield reduction. In the high stay-green lines 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 stay-green lines are better suited.

Under post-flowering drought stress conditions senescent lines showed 20 to 25 per cent lodging compared to less than 10 per cent lodging in the stay-green lines. Thus the stay-green trait has a direct benefit to sorghum by reducing moisture stress type lodging associated with premature leaf and stalk death. A significant negative correlation was observed between plant height and soft stalk($r = 0.75$) indicating that lines which were taller were more susceptible to soft stalk and its associated lodging. The charcoal rot parameters had a significant negative correlation with the linear rate of senescence.

The molecular analysis using the two RAPD primers revealed polymorphism between the lines showing contrasting terminal drought stress response. The RAPD primer *UBC176* showed two polymorphic bands while the primer *OPB8* showed one polymorphic band between the two parents. Hence the primers can be used in marker assisted selection of the staygreen trait.

LITERATURE CITED

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Al-Hamdani S H Murphy J M and Todd G W 1991 Stomatal conductance and CO2 assimilation as screening tools for drought resistance in sorghum. *Canadian Journal of Plant Science* **71:** 689-694

Arumuganathan K and Earle E D 1991 Nuclear DNA content of some important species. *Plant Molecular Biology Report* **9:** 208-218

Baldy C Konata J M Olufayo A Rupella P and Fern andes A 1993 Drought resistance of sorghum in a mediterranean climate and optimum management for limited water supply. *Science et Changements Planetaries Secheresse* **4:** 85-93

Bates L M and Hall A E 1982 Stomatal closure with soil water depltion not associated with changes in bulk water sattus. *Oecologia* **50:** 62-65

Beckman J and Soller J 1988 Selection of linkage between marker loci and loci affecting quantitative trait. *TAG* **79:** 228-236

Bennet J M 1979 Response of grain sorghum S bicolar L. Moench. University of Nebraska Lincoln Lincoln USA

Bennet W F and Tucker B 1986 Modern sorghum grain production. *Traduccion espanola Editorial Hemisferio Sur Argentina*

Berhan A M Hulbert S H Butler L G and Nennetzen J L 1993 Structure and evolution of two genomes of Sorghum bicolar and Zeamays. *TAG* **86:** 598-604

Bewazir A A A and Idle D B 1989 Drought resistance and root morphology in sorghum. *Plant and Soil* **119:** 217-221

Bidinger F R Mahalakshmi V and Rao G D P 1987 Assessment of drought resistance in pearl millet 1 Factors affecting yields under stress. *Australian Journal of Agronomic Research* **38:** 37-48

Binelli G Gianfranceschi L Stenhouse J and Busso C 1992 Similarity of maize and sorghum genomes as revealed by maize RFLP probes. *TAG* **84:** 10-16

Blackman P H and Davies W J 1985 Root to shoot communication in maize and the effects of soil drying. *Journal of Experimental Botany* **36:** 39-40

Blum A 1979 Principles and methodology of selecting for drought resistance in

sorghum. *Monoigraphie di Genetica agraria* **4:** 205-211

Blum A Poirkova H Gozlan G and Mayer J 1983 Chemical dessication of wheat plants as a simulator of post-anthesis stress 1 Effects on translocation and kernel growth. *Field Crops Research* **6:** 51-58

Blum A 1985 Breeding crop varieties for stress tolerance. *CRC Critical Reviews in Plant Sciences* **2:** 198-238

Blum A 1988 Productivity and drought resistance of genetically improved cultivars as compared with native l and races of sorghum. *Sorghum Newsletter* **31:** 41

Blum A Mayer J and Golan G 1989 agronomic and physiological assessment of genotypic variation for drought resistance in sorghum. *Australian journal of agricultural research* **40:** 49-61

Bohnert H J Nelson D E and Jensen R G 1995 Adaptation to environmental stresses. *Plant Cell* **7:** 1099-1111

Bolanos J and Edmeades G O 1988 CIMMYT's strategies in breeding for drought tolerance in tropical maize *In* "International Conference on Dry L and Farming" pp 349-364 Amervillo Texas USA

Botstien D While R L Skolnik M and Davis R W 1980 Construction of genetic linkage map in man using RFLP. *American Journal of Human Genetics* **32:** 314-331

Causton D R and Venus J C 1981 "The biometry of plant growth" EDward Arnold London

Clarke J M DePauw R M and Townley-Smith TF 1992 Evaluation of methods for quantification of drought tolerance in wheat. *Crop Science* **32:** 723-728

Cutler J M Shahan K W and Stepenkus P L 1980 Influence of water deficit on osmotic adjustment and leaf elongation in rice. *Crop Science* **20:** 314-318

Dhoble M V and Kale U V 1988 Recovery of drought resistance in sorghum genotypes. *Journal of Maharashtra Agricultural Universities* **13:** 118

Dogget H 1988 "Sorghum" 2/Ed Longman

Durley R C Kannagara T Seetharama N and Simpson G M 1983 Drought resistance in Sorghum bicolar 5 Genotypic differences in the concentration of free and conjugated abscisic acid pgaseic and indole-3-acetic acid in leaves of field

grown drought -stressed plants. *Canadian Journal of Plant Sciences* **63:** 131-145

Eastin J D 1972 "Efficiency of grain dry matter production in grain sorghum Report annual corn and sorghum research conference 27 - 7-17" American seed trade association Washington D C USA

Eastin J D Hultquist J H and Sullivan C Y 1973 Physiological maturity in grain sorghum. *Crop Science* **13:** 175-178

Fiscer R A and Maurer R 1978 Drought response in spring wheat cultivars 1 Grain yield response. *Australian Journal of Agricultural Research* **29:** 897-912

Fischer K S and Wilson I F 1976 Studies on grain production in Sorghum vulgare 1 The contribution of pre-flowering photosynthates to grain yield. *Australian Journal of Agriculture research* **22:** 33-37

Flower D J and Lodlow M M 1986 Contribution of osmotic adjustment to dehydration tolerance of water -stressed pigeon pea (Cajanus cajan*(L.) Millisp.) leaves *Plant Cell Environment* **9:** 33-40

Gaff D F 1980 "Protoplasmic tolerance to extreme water stress" Jhon Wiley and Sons Newyork

Geric T J and Miller F R 1984 Photoperiod and Temperature effects on Tropically and temperately adapted sorghum. *Field Crops Research* **9:** 29-40

Hay R K M and Walker A J 1989 "An introduction to physiology of crop yields" Longman Scientific and Technical

Henson I E Mahalakshmi V Alagarswamy G and Bidinger F R 1984 The effct of flowering on stomatal response to water stress in pearl millet. *Journal of Experimental Botany* **35:** 219-226

Henzell R G McRee K J Banel C H M V and Shertz K F 1976 Sorghum genotypic variability in stomatal sensitivity and leaf water deficits. *Crop Science* **161:** 660-661

Hsiao T C O'Toole X Yambon E B and Turner N C 1984 Influence of osmotic adjustment on leaf rolling and tissue death in rice (Oryza sativa L.). *Plant Physiology* **75:** 253-341

Hulbert S H Richter T E Axtell J D and Bennetzen J L 1990 Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. *Proceedings National Academy of Sciences USU* **87:** 4251-4255

Hurvitz S 1958 "Principles of Agronomy - The field crop and its environment"

Hurvitz S 1959 "Principles of Agronomy"

Jones M M and Rawson H M 1979 Influence of rate of development of leaf water deficits up on photosynthesis leaf conductance water use efficiency and osmotic potential in sorghum. *Plant Physiology* **61:** 122-126

Jones M M and Turner N C 1980 Osmotic adjustment in expanding and fully expanding leaves of sunflower in response to water deficits. *Australian Journal of Plant Physiology* **7:** 181-192

Jordan W R and Sullivan C Y 1982 Reaction and resistance of grain sorghun to heat and drought IN sorghum in the eighties *In* "International Symposium on Sorghun" L R House L K Mughozo and J M Peacock eds. Vol 1

Kannagara T Durley R C Simpson G M and Stout D G 1982 Drought resistance in sorghum bicolar4 Harmonal changes in relation to drought stress in field -grown plants. *Canadian Journal of Plant Science* **62:** 317-330

Kannagara T Seetharama N Durley R C and Simpson G M 1983 Changes in endogenous levels of growth regulators of plants grown across an irrigation gradient.*Canadian Journal of Plant Sciences* **63:** 147-155

Khizzah B W and Miller F R 1992 Correlations between sorghum components of drought resistance and various agronomic charecteristics at four locations. *Sorghum Newsletter* **33:** 54

Khizzah B W Newton R J MIller F R Rosenow D T and Cothern J T 1992 A study of some physiological components of drought resistance in some inbred sorghum lines and progenies subjected to prolonged water stress *In* "International Sorghum and Millet CRSP Conference" pp 261-262 INTSORMIL Publication tEXAS

Kreig D R 1983 Sorghum *In* "Crop water relations" I D Teare and M M Peet eds. pp 547 Jhon Wiley and sons

Kuiper P S C 1977 "Inleiding tot de plantenfisiologie"

Lauder E S Green P Abrahamson A Barlow A Daly W T Lincoln S E and Newbung L 1987 Mapmaker an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* **1:** 174-181

Legget J M 1991

Levitt J 1980 "Responses of plants to environmental stresses" 2 edition/Ed Academic Press

Lewis R B Hiller F A and Jordan W R 1974 Susceptibility of grain sorghum to water deficit at three growth stages. *Agronomy Journal* **66:** 89

Lewitt J 1972 "Response of plants to environmmental stresses" Academic press Newyork

Lira M d Br ando A R d Tabose J N and Brito G Q d 1989 Preliminary studies of drought resistance in genotypes of forage sorghum. *Revista da Sociedade Brasileira de Zootecnia* **18:** 1-12

Ludllow M M fisher M J and Wilson J R 1985 Stomatal adjustment to water defecits in three tropical grasses and a tropical legume grown in controlled conditions and the field. *Australian Journal of Plant Physiology* **12:** 131-149

Ludlow M M and Muchow R C 1990 A critical evaluation of traits for improving crop yields in water limited environments. *Advances in Agronomy* **43:** 107-153

Ludlow M M 1990 "Adaptive significance of stomatal responce to water stress" Wiley interscience

Ma H T 1991 "Studies on high yielding breeding and potential applications of RFLP in sorghum" Rockfeller Foundation Conference New York

Maiti R K Raju P S Reddy B V S and Peacock J M 1989 Evaluation of techniques to screen for drought resistance in sorghum seedlings. *Turrialba* **39:** 106-110

Martin G B Bromnon C Sheubert S H Chungwongse J Frary A Ganal M W Spirey R Wu T and Tanksley S D 1993 MAp based cloning of a Protiease-k gene conferring disease resistance in tomato. *Science* **262:** 1432-1436

Meyer R F and Boyer J S 1981 Osmoregulation solute distribution and growth in soya bean seedlings having low water potentials. *Planta* **151:** 482-489

Miller F R Duncan R R and Bockholt A J 1981 Descriptive comparision of senescent and nonsenescent sorghum genotypes. *Agronomy Jurrnal* **73:** 849-853

Morgan J M 1984 Osmoregulation and water stress in higher plants. *Annual Review of Plant Physiology* **35:**

Nooden L D 1988 "The phenomenon of senescence and aging" Aacdemic press Newyork

O'Toole J C and Chang T T 1978 "Drought and rice improvement in perspective" Rep No 14 278 IRRI Research paper series

Pereira M G Lee M Bramel-cox P Woodman W Doebley J and Whitkus R 1994 Construction of RFLP map in sorghum and comparitive mapping in maize. *Genome* **37:** 226-243

Rajaraman V 1990 Computer oriented numerical methods

Rosenow D T and Clark L E 1983 Drought tolerance in sorghum In proceedings 36 annual Corn and sorghum research conference. American seed trade association, Chicago

Rosenow D T 1987 Breeding sorghum for drought resistance *In* "Internatinal Drought Symposium" J M Menyonga and T Bezuneh eds. pp 83-89 Nairobi Kenya

Rosenow D T 1994 Evaluation for drought and disease resistance in sorghum for use in marker -assisted selection *In* "Proceeding s of an ODA plant sciences research programme" J R Witcombe and R R Duncan eds. pp 27-31 Norwich UK

Rosenow D T and Clark L E 1995 Drought and lodging resistance for a quality sorghum crop *In* "Proceedings of 15 Annual Corn and Sorghum Industry Research Conference" pp 82-97

Saghai-Maroof M A Soliman K M Jorgensen R A and Allard R W 1984 Ribosamal DNA spacer-length polymorphism in Barley mendilian inheritance chromosomal location and population dynamics. *Proceedings National Academy of Sciences USA* **81:** 8014-8018

Salam M A 1995 Studies on drought resistance in Sorghum bicolor L Moench Leaf water parameters in different growth stages. *Bodenkultur* **46:** 107-117

Santamaria J M Leedlow M M and Fukai S 1986 Drought resistance traits in hybrids and lines of Sorghum bicolor *In* "First Australian Sorghum Conference" M A Foale and R G Henzell eds. pp 4127-4144 Gatton Queensland Austrtalia

Santamaria J M Ludlow M M and Fukai S 1990 Contribution of osmotic adjustment to grain yield in SbicolarL. Moench under water limited conditions 1 Water stress before anthesis. *Australian Journal of Agriculture Research* **41:** 51-65

SAS Institute I 1990 SAS Users Guide Statistics SAS Institute Gary NC

Seetharama N and Bidinger F R 1977 "Sorghum drought resistance" International Sorghum Workshop ICRISAT Hyderabad

Seetharama N Reddy B V S Peacock J M and Bidinger F R 1982 Drought resistance in crops with emphasis on rice Los Banos IRRI

Sinha S K 1987 "Drought resistance in crop plants a critical physiological and biochemical assessment" Wiley interscience Newyork

Stout D G Kannagara T and Simpson G M 1978 Drought resistance of Sorghum bicolar water and stress effects on growth. *Canadian journal of plant sciences* **58:** 225-233

Stuber C W Lincoln S E Wolff D W Helentjaris T and L ander S E 1992 Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred linesusing molecular markers. *Genetics* **132:** 823-839

Takami S Rawson H M and Turner N C 1982 Leaf expansion of five sun flower Helianthus annus. cultivars in relation to water deficits. *Plant Cell Environment* **5:** 279-287

Tanksley S D 1993 Mapping polygenes. *Annual Review of Genetics* **27:** 205-223

Tanksley S D Ganal M W and Martin G B 1995 Chromosome l anding a paradigm for map based gene cloning in plants with large genome. *TAG* **11:** 63-68

Tao Y Z McIntyre C L and Henzell R G 1996 Application of molecular markers to Australian sorghum breeding programs 1 Construction of a RFLP map using sorghum recombinant inbred lines *In* "Proccedings Third Australian Sorghum Conference"

Thomas H and Stoddart J L 1980 Leaf Senescence. *Annual Review of Plant Physiology* **31:** 83-111

Thomas H and Smart C M 1993 Crops that stay green. *Annals of applied biology* **123:** 193-219

Tuinstra M R Gorte E M Goldsbrough P B and Ejeta G 1996 Identificatin of Quantitative Trait Loci Associated with Pre-flowering drought Tolerance in Sorghum. *Crop Science* **36:** 1337-1344

Turner N C 1979 "Drought resistance and adaptation to water deficits in crop plants" Jhon Wiley and Sons Newyork

V anderlip R L and Reeves H E 1974 Growth stages of sorghum (Sorghum bicolor (L.) Moench.). *Agronomy Journal* **64:** 13-16

vanOosterom E J Jayach andran R and Bidinger F R 1996 Diallele analysis of the stay-green trait and its components in sorghum. *Crop Science* **36:** 549-555

Wall J S and Ross W M 1970 "Sorghum production and utilisation" Conneticut USA

Walulu R S Rosenow D T Webster D B and Nygugen H T 1994 Inheritance of saty-green trait in sorghum. *Crop Science* **34:** 970-972

Walulu R S Rosenow D T Wester D B and Nguyen H T 1994 Inheritance of staygreen trait in sorghum. *Crop Science* **34:** 970-972

Wenzel W G 1987 Green house tests for drought resistance in grain sorghum. *Angewandte Botanik* **61:** 529-537

Wenzel W G 1988 Relationship between yield base temperature and drought resistance in grain sorghum. *South African Journal of plant and soil* **5:** 150-154

Wenzel W G 1989 Growth rate and transpirational water loss of cut seedlings as selection criteria for drought resistance in grain sorghum *Angew andte Botanik* **63:** 7-15

Wenzel W G 1990 The inheritance of drought resistance characteristics in grain sorghum seedlings. *South African Journal of Plant and Soil* **8:** 169-171

Williams J G K Hanafey M K and Tingey SV 1992 Genetic analysis using RAPD markers. *Methods in Enzymology* **218** : 704-740

Wittenbach V A 1977 Induced senescence of intact wheat seedlings and its reversibility. *Plant Physiology* **59:** 1039-1042

Wlimer C and Pantoja O 1992 "The plasma membrane and tonoplast of guard cell membranes" Kluwer Academic Press

Xu W W Castra O Rosenow D T Mullet J E and Nguyen H T 1994 Progress towards molecular mapping of drought resistance traits in sorghum. *International Sorghum and Millets Newsletter* **35:** 91-94

Xu W W Magill C W Schertz K F and Hart G E 1994 A RFLP linkage map of S bicolorL. Moench. *TAG* **89:** 139-145

Xu W W Rosenow D T and Nguyen H T 1994 Staygreen trait in sorghm visual rating

and objective measurement. *Internatioinal Soghum and Millets Newsletter* 46-47

Yang W Nadolska-Orezyk K V Wood D T Premchandra G S 1995 Near isogenic lines of maize deferring for glycinebetaine. *Plant Physiology* **107** : 621-630

Zartman R E and Woyewodzic R T 1979 Root distribution patterns of two hybrid grain sorghums under field conditions. *Agronomy Journal* **71:** 325-328

Zhang J and Kirkham M B 1991 Ethylene production by two genotypes of sorghum varying in drought resistance. *Cereal Research Communications* **19:** 357