GENE ACTION AND GENE EXPRESSION ANALYSIS IN YARDLONG BEAN (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdcourt) FOR DROUGHT TOLERANCE

by

RAHANA S.N.
(2016-21-010)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Gene action and gene expression analysis in yard long bean (*Vigna unguiculata* ssp. *sesquipedalis* (l.) Verdcourt) for drought tolerance"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Vellayani,
Date: 28.01.2022

Rahana S.N.
(2016-21-010)

CERTIFICATE

Certified that this thesis entitled **"Gene action and gene expression analysis in yard long bean (*Vigna unguiculata* ssp. *sesquipedalis* (l.) Verdcourt) for drought tolerance"** is a record of research work done independently by Mrs. Rahana S.N. (2016-21-010) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellayani,
Date: 28.01.2022

Dr. Beena Thomas
(Major advisor, Advisory Committee)
Assistant Professor
Department of Plant Breeding and Genetics
College of Agriculture, Vellayani
Thiruvananthapuram

CERTIFICATE

We, the undersigned members of the advisory committee of Mrs. Rahana S.N. (2016-21-010), a candidate for the degree of **Doctor of Philosophy in Agriculture** with major in Plant Breeding and Genetics, agree that the thesis entitled **"Gene action and gene expression analysis in yard long bean (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdcourt) for drought tolerance"** may be submitted by Mrs. Rahana S.N. (2016-21-010), in partial fulfilment of the requirement for the degree.

Dr. Beena Thomas
(Chairman, Advisory Committee)
Assistant Professor
Department of Plant Breeding and Genetics
College of Agriculture, Vellayani
Thiruvananthapuram-695 522

Dr. Arya K.
Professor and Head
Department of Plant Breeding and Genetics
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522

Dr. Roy Stephen
Professor
Department of Plant Physiology
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522

Dr. Soni K. B.
Professor
Department of Plant Biotechnology
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522

Mr. Jayakrishnakumar V.
Associate Professor
Department of Agronomy
College of Agriculture, Vellayani,
Thiruvananthapuram-695522

EXTERNAL EXAMINER
24/11/22
Dr. A. JOHN JOEL, Ph.D.,
Professor (PBG)
Centre for Plant Molecular Biology
Tamil Nadu Agricultural University
Coimbatore - 641 003

Dedicated to

My beloved parents

(Sainudeen & Nabeesa)

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daughter Diya

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LIST OF ABBREVIATIONS

Acc	Accession
. DNA	Deoxyribo Nucleic Acid
Mb	Mega base
FAO	Food and Agricultural Organization
qRT-PCR	Quantitative real time PCR
PWP	Permanent Wilting Percentage
IBPGR	International Board for Plant Genetic Resources
RLW	Relative Leaf Water Content
ψ_L	Leaf Water Potential
g/m^2	$Gram/meter^2$
PCR	Polymerase Chain Reaction
SAS	Statistical Analysis Software
UV-VIS	Ultraviolet-Visible
dNTPs	Deoxynucleotide Triphosphates
CD	Critical Difference
SE(m)	Standard Error (Mean)
G	Genotype
L x T	Line x Tester
%	per cent
^0C	Degree Celsius
m H_2O moles m^{-2} s^{-1}	milli H_2O moles $meter^{-2}$ $second^{-1}$
µ moles/g tissue	micro moles/gram tissue
cm	centimeter
g	gram
cm^3	cubic centimeter
ml	milliliter

µl	microlitre
ng/µl	nanogram/microlitre
mM	millimolar
pM	picomolar
nm	nanometer
bp	base pairs
rpm	rotations per minute
et al.	and other co-workers
$Plant^{-1}$	per plant
i.e.	that is
FYM	Farm Yard Manure
Kg	kilo grams
cDNA	Complementary DNA
Fig	Figures
gca	General Combining Ability
sca	Specific Combining Ability
RBD	Randomized Block Design
CRD	Completely Randomized Design
RNase	Ribonuclease

INTRODUCTION

1. INTRODUCTION

Yard long bean (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdcourt) is a dicotyledonous vegetable legume that belongs to the family Fabaceae. It is a diploid (2n=2x=22) self pollinated annual species with less than one per cent outcrossing. The plant is a vigorous climber that is primarily grown for its immature pods that are white, light green, dark green or brownish red which are consumed in cooked form. Being a leguminous vegetable, it enriches soil fertility by fixing atmospheric nitrogen and is an integral part of sustainable agriculture.

Yard long bean has been grown in the tropics and subtropics of the world since ancient times. According to Verdcourt (1970), *Vigna unguiculata* has five subspecies, the most common of which is *V. unguiculata* subsp. *unguiculata* (bush cowpea). *V. unguiculata* subsp. *cylindrica* (grain cowpea) and *V. unguiculata* subsp. *sesquipedalis* (yard long bean) are found in India and South East Asia. *V. unguiculata* subsp. *dekindtiana* (black eyed pea) and *V. unguiculata* subsp. *mensensis* are wild genotypes restricted to Africa.

Yard long bean evolved from the common cowpea and the epicentre of its genetic diversity is considered to be in South East Asia. In this region, the crop is widely grown in South China, Indonesia, Taiwan, Thailand and Philippines. Because of its long, slender and succulent pods it is also known as asparagus bean, Chinese long bean, long podded cowpea, pea bean, snake bean, and string bean. It is cultivated for its tender pods relished as vegetable, a good source of vitamin A, B and C, protein, fibre and other minerals. The pod is highly nutritious, being rich in protein (3.5 g), carotene (564 mg), calcium (72 mg), phosphorus (59 mg), iron (2.5 mg), vitamin B1 (0.07 mg), vitamin B2 (0.09 mg) and vitamin C (24 mg) per 100 g of edible pods. It is also good source of sodium, potassium, magnesium and micronutrients (Ano and Ubochi, 2008).

The crop is cultivated on around 7.7 million hectares of land across India mainly in Kerala, Karnataka, and Maharashtra. Yard long bean has several vernacular names in Kerala which include 'Achinga Payar', 'Kuruthola Payar', 'Pathinettumaniayan', 'Vallipayar' etc. It is a highly remunerative vegetable crop in the state because of its huge demand for its long green pods. The crop is grown in all the seasons, giving farmers a steady income across the year. It is an important vegetable crop in Kerala, extensively cultivated throughout the state covering an area of 5803 ha in 2018-19 (FIB, 2021).

Despite high economic value, yard long bean production in Kerala is much less by a number of biotic and abiotic factors, moisture stress being the major constraint that drastically reduces the yield. Irregular rainfall, particularly early in the season, has adverse effect on crop development. Drought stress occurring at the seedling stage could be detrimental to cowpea production (Verbree *et al.*,2015). Therefore, developing improved high yielding varieties of yard long bean with drought resistance/tolerance is crucial for its sustainable production in Kerala. Yard long bean has been in cultivation in Kerala since ancient times, which has resulted in rich and diverse domestic germplasm. This existing repository of genetic diversity must be conserved, documented and screened to utilise valuable genes including drought tolerance.

Water scarcity is reported to be the most serious challenge to sustainable agriculture, with global temperatures expected to rise by 1.5–5.9° C this century (Chadha *et al.*, 2019). Despite having a wet climate, the frequency of drought years in Kerala has been rising in recent decades. Droughts in Kerala in 2003, 2013 and 2016 are instances of climatic fluctuations wreaking havoc on the state's agricultural production (Abhilash *et al.*, 2019). Extreme flood and drought events, which are of common occurrence in Kerala, are challenges posed by the climate change that affects our farmers livelihood security. Crop varieties that adapt to

extreme climatic conditions can help to mitigate climate change risks to a large extent. The FAO (2015) emphasised the important role of plant genetic resources in reducing the impacts of climate change, ensuring sustainability in cultivation and ultimately in achieving food security.

The essential requirement for resistance/tolerance to drought in plants is their ability to continue to function near normal under water stress conditions. Drought tolerance is a complex trait. Several factors and mechanisms operate independently or jointly to enable plants to cope with stresses of drought. A combination of morphological, physiological and molecular drought responsive traits is utilized for screening drought tolerant lines.

The ability of drought tolerant line to transmit the associated adaptive traits to its progenies can be determined using line x tester analysis. Estimation and analysis of heterosis and combining ability help in the genetic evaluation of genotypes, selection of suitable parents and in the designing of breeding procedures to develop drought tolerant crop varieties. In addition, the analysis of the gene expression pattern of drought responsive genes in tolerant genotypes improves selection efficiency and aids in the understanding of the molecular mechanism involved in moisture stress tolerance.

In this context, the present research was conducted with the following objectives,

- To identify the drought tolerant yard long bean genotypes from the available germplasm.
- To study the gene action of the selected lines through line x tester analysis under induced water stress condition.
- To estimate and analyse the gene expression of drought responsive gene based on the available/reported genes associated with drought tolerance in drought tolerant genotype.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The present study involved the evaluation of yard long bean germplasm for drought tolerance, assessing the combining ability and nature of gene action of selected genotypes through line x tester analysis and analysing the gene expression of drought responsive genes in the drought tolerant genotype. An effort has been made to review relevant literature in this section on various aspects related to the present study under the following topics,

2.1 Evaluation of yard long bean germplasm for drought tolerance

2.2 Studies on morphological and physiological parameters

2.3 Studies on combining ability

2.4 Studies on nature of gene action

2.5 Studies on heterosis

2.6 Studies on gene expression

2.1. Evaluation of yard long bean germplasm for drought tolerance

Yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) belongs to the family Fabaceae formerly known by Leguminosae. The yardlong bean (subspecies *sesquipedalis*), catjang (subspecies *catjang*) and cowpea (subspecies *unguiculata*) were classified by Verdcourt (1970). Which were reclassified as cultivar groups *sesquipedalis, biflora* and *unguiculata* respectively under *Vigna. Vigna unguiculata* subsp. *unguiculata,* further five cultivar groups enumerated namely *unguiculata, sesquipedalis, biflora* (or *catjang*), *textilis* and *melanophthalmus* (Devan *et al.*, 2021). However, ssp. *sesquipedalis* is likely to be derived from domesticated ssp. *unguiculata* upon subjected to intensive selection for vegetable pod qualities and climbing growth characteristics after it was brought to Asia from sub-Saharan Africa (Xu *et al.*, 2010).

The tender long pods of yard long bean are used as a vegetable in eastern and southern Asia, it is regarded as one of the top ten vegetables of Asia, it is distinguished by its very long immature snap pods (0.5–1 m), small kidney shaped beans and creeping habit. The immature pods are of high quality, low cost source of vegetable protein and are rich in vitamin A and C, fibre and other minerals (Ano and Ubochi, 2008).

The quality of the pods in terms of firmness and sweetness are the most important factor that decides the buyer's acceptance (Kongjaimun *et al.* 2013). Due to the low requirement for cultivation management and its high nutritional value, yard long bean is one of the top crops that help combat malnutrition and food insecurity in most developing countries (Xia *et al.*, 2019).

Yard long bean is called alternatively as Chinese long bean, string bean, snake bean, snake pea, snap pea, bodi, pea-bean, asparagus bean and borboti. It is strictly a self-pollinated crop due to its cleistogamous nature of flowers (Ullah *et al.*, 2011).

Yard long bean has become a vital component of sustainable agriculture in the tropical marginal lands due to its rapid growing habit (Varghese and Celine, 2015). The crop is widely cultivated and a remunerative vegetable traditionally grown in Kerala as consumer demand is increasing. Produces delicious edible pods and beans that are popular for their delicate flavor and nutritional value (Damayanti *et al.*, 2009).

The production and productivity of yard long bean is mainly constrained by low yield due to its sensitive to adverse climatic conditions and biotic factors (Sarutayophat *et al.*, 2007). This warm season crop can tolerate heat, low rainfall and arid soils, but the pods become short and fibrous with low soil moisture. Moisture stress is the major constraint that drastically reduces both quality and yield of yard long bean (Lestari *et al.*, 2019). Reports showed that impacts of drought on crops such as cowpea have been acute in tropical and subtropical regions (Carvalho *et al.*, 2017).

Narayanan *et al.* (2014) defined drought as the inadequacy of water availability, including precipitation and soil moisture storage capacity, in quantity and distribution during the life cycle of crop to restrict in expression of full genetic yield potential. Under drought conditions, water stress develops in the plants as the demand exceeds supply of water. To counteract these environmental constraints, plants have evolved several defense mechanisms (Hakim *et al.*, 2018).

According to Lestari *et al.* (2019) plants cope with drought experience through several mechanisms namely avoidance, tolerance, recovery and escape. Drought tolerance is the ability of plants to function at low water potential by maintaining high fitness in drought conditions and contribute to yield stability (Wu *et al.*, 2010; Heschel and Riginos, 2005).

Drought stress is a serious environmental threat that limits growth, production and crop productivity than any other abiotic stress factor (Mahantesh *et al.,* 2018). Development of climate resilient crop varieties utilising natural and genetic resources that can quickly adapt to various climate related changes is considered vital for sustainable agriculture (Rao, 2015). Cultivars that can tolerate limited water supplies at early vegetative growth could be an affordable solution to overcome drought conditions (Ravelombola *et al.*, 2018).

Yard long bean being a highly self pollinated crop, different breeding procedures like pure line selection, mass selection, bulk selection, pedigree selection, single seed descent and backcrossing methods were employed to develop improved varieties. The genetic base of most of the improved varieties were narrow and the farmer's adoption of high yielding varieties results in a loss of genetic diversity (Boukar *et al.*, 2016).

Despite the existence of morphological variability in the germplasm for several traits, still genetic diversity noted to be limited in Asian ssp *sesquipedalis* germplasm. More exploration of germplasm resources is needed to enhance the genetic diversity for yard long bean breeding initiatives (Fang *et al.*, 2007).

The crop vulnerability to biotic and abiotic stress has increased due to the narrow genetic base of elite germplasm. Knowledge and use of available germplasm are critical for broadening cultivar genetic bases and sustaining improvement (Singh, 2001).

The genetic variability available within the several yard long bean accessions introduced has yet to be completely studied and tested. Information on extent of variability among these collections for traits of economic importance is lacking. Evaluation and characterization of such genotypes are required to fully exploit this genetic wealth for future possibilities (Rout *et al.*, 2018).

Information on existing germplasm and assessment of its genetic variability would enhance development of cultivars for adaptation to specific production constraints (Magashi *et al.*, 2019).

There is a need to identify new sources of longer drought tolerance among different selected genotypes and the level of heritability of such traits under drought condition (Ahamed *et al.*, 2014).

Precise germplasm screening and use of tolerant lines in breeding programs has been a primary approach in developing superior cultivars that are tolerant to various abiotic stresses (Mutava *et al.*, 2011).

Breeding for drought tolerance were usually performed by selectively crossing a drought tolerant strain with a high yielding variety. From the segregating population, plants with good combination of target traits under water limited conditions were selected for drought tolerance in breeding (Kumar *et al.*, 2008).

(Hajjar and Hodgkin, 2007) reported successful release of a drought and heat tolerant variety of chickpea (BG1103) after introgression from *Cicer reticulatum* Ladiz. The variety showed superior yield and pod filling under drought stress.

Several methods have been adopted to measure the level of drought tolerance in germplasm and for the selection of segregated breeding materials. Singh *et al.* (1999) suggested a simple wooden box screening method for discriminating drought tolerant and susceptible cowpea in the seedling stage. After the establishment of seedlings irrigation was withhold. Watering resumed until all the susceptible lines appeared permanently wilted. Based on the days taken to wilting and percent recovery, the varieties were rated as drought tolerant or susceptible.

Anyia and Herzog (2004) examined ten cowpea genotypes for their drought tolerance. Genotypes were grown in a growth chamber under well-watered conditions up to early flowering and then subjected to water deficit. Water deficit was induced by withholding irrigation until the soil water potential was −75 kPa, which was then maintained for 10 days.

Muchero *et al.* (2008) conducted pot experiments for screening cowpea genotypes against drought stress. The test could differentiate between 14 cowpea genotypes that exhibit significant genetic variation to drought stress at the seedling stage. They conclude that seedling stage test as a reliable technique for screening a large number of genotypes for drought tolerance and easy to conduct under controlled conditions.

Moisture stress tolerant plants, according to Cabuslay *et al.* (2002), were able to retain tissue water content, survive a decline in tissue water level and recover completely upon rewatering. Twenty accessions were identified with higher levels of drought tolerance than others, out of 1300 accessions evaluated for drought tolerance (Fatokun *et al.*, 2012)

At the seedling stage, Ajayi *et al.* (2018) tested eleven cowpea accessions for drought tolerance. Each pot watered with 250 ml of water per day until the first trifoliate leaf had fully expanded. Drought was imposed on the 16th day of sowing for 21 days when about 90% of the most susceptible accession have completely wilted or perished. After twenty one days of stress, watering was

resumed. After 14 days, percentage plant recovery, stem regrowth and stem greenness were used to distinguish drought tolerant and sensitive accessions.

Ravelombola *et al.* (2018) evaluated drought related traits of 30 cowpea genotypes at seedling stage grown within boxes. Drought stress was imposed by withholding irrigation when the first trifoliate was completely expanded and continued until some genotypes were completely dead, indicating susceptibility to drought stress. Soil moisture measure within boxes was recorded using moisture meter every third day. Observed that plants with good tolerance at early vegetative phase were able to withstand drought stress at a later stage of plant development.

Lestari *et al.* (2019) screened yard long bean varieties against drought stress at 50% and 100% of the field capacity to understand the drought tolerant mechanism. Plants raised in pots were well watered for 30 days after sowing. Drought treatment was imposed by stop watering until the moisture content was less than 50% field capacity while the control plants were maintained at 100%.

Magashi *et al.* (2019) screened seven varieties of cowpea for water stress tolerance using box screening method arranged in CRD with three replications. Watering continued for three weeks of sowing, after which watering was completely withdrawal. The data were collected at 28 days, 34 days and 40 days after sowing. The result obtained revealed significant difference in most of the quantitative traits studied and conclude that all the genotypes contained significant drought tolerance.

Nkoana *et al.* (2019) conducted a plastic box experiment to assess the genetic potential for drought tolerance in 28 cowpea germplasm accessions, including two controls. Three week old genotypes were subjected to a 5 week water stress treatment to assess their physiological response by leaf wilting index, relative water content and proline content. The genotypes responded differently

to drought stress after rewatering, indicated that the cowpea species had enough genetic variability which can be used in drought stress breeding.

Cataloguing of the germplasm

Pungulani *et al.* (2012) employed cluster analysis to group seedlings of cowpea under drought stress. Doumbia *et al.* (2013) performed a comparative study of 94 accessions of cowpea germplasm diversity using morphological characteristics. Twelve qualitative and twenty quantitative traits such as flower color, growth habit, seed shape, day 50% flowering, plant height, seed length and seed weight were used to assess collections. Accessions were classified based on their morphological relationships using unweighted pair group average cluster analysis. Results showed a relatively low level of genetic diversity between and within germplasms.

Rambabu *et al.* (2016) characterized forty-one genotypes of yard long bean based on morphological and yield related traits. The study revealed considerable variability in the genotypes for most of the traits like growth habit, flower colour, pod colour, seed colour and seed eye pattern.

Lovely *et al.* (2017) studied the nature and magnitude of genetic divergence among fifty genotypes of yard long bean. Based on nine important traits, all the genotypes were grouped into four clusters with genotypes from different geographic locations being grouped in the same clusters. Pod yield per plant contributed the maximum towards divergence.

While studying drought tolerance in cowpea varieties, Magashi *et al.* (2019) observed variability in the qualitative traits *viz.*, growth habit (spreading and erect), flower colour (white and violet), seed coat colour (brown and white), seed shape (kidney and rhomboid), seed texture (smooth and rough) and eye colour (brown and black).

Ajayi *et al.* (2018) evaluated genotypic differences among ten cowpea accessions using IBPGR descriptors. A dendrogram was constructed after cluster

analysis based on the ranking of morphological changes, showed the distribution of accessions into three groups indicating the existence of variability among them for drought tolerance. Susceptible accessions of cowpea were clearly separated by the dendrogram.

Six open pollinated yard long bean genotypes were agro-morphologically characterised by Pandey *et al.* (2020) using IBPGR descriptors to assess the variability among the genotypes. They reported significant difference among the genotypes for number of pods per plant and pod yield while no significant differences noted in plant vigour and uniformity scores.

Sultana *et al.* (2020) studied the genetic diversity of seven genotypes of yard long bean. The genotypes were clustered into 3 groups with the highest of inter-cluster distance between cluster I and III while the lowest between cluster II and III. The genotypes of cluster I exhibited higher mean performance while lower in cluster III for important traits including pod yield per plant

Widyawan *et al.* (2020) conducted a IRAP (Inter retrotransposon amplified polymorphism) marker based genetic diversity analysis on 16 yard long bean genotypes. Cluster analysis was performed to construct a dendrogram based on genetic similarities and the 16 genotypes were categorized into four clusters. The results revealed narrow genetic diversity among the genotypes.

Devan *et al.* (2021) characterized forty yard long bean genotypes on morphological traits such as plant type, growth habit, pod colour, pod length, pod shape, seed per pod and seed colour as per the NBPGR guidelines and observed adequate genetic variability among the genotypes. Cluster analysis was carried out using Mahalanobis D^2 statistics and grouped the genotypes into various clusters.

2.2 Studies on morphological and physiological parameters

Plants are exposed to a range of biotic and abiotic stresses throughout their life cycle. Under such conditions plants morphology as well as physiology

get altered which led to reduction in plant growth and development (Rahdari and Hoseini, 2012).

Drought, a multidimensional stress adversely affects the crop productivity and yield (Farahani *et al.*, 2009). To combat drought stress, plants deploy varied morphological, physiochemical and molecular changes to enhance water uptake and storage, reduce water loss and avoid wilting (Farooq *et al.*, 2009).

Understanding the drought resistant mechanism is important before adopting strategies for imparting drought tolerance in plants (Kaur *et al.*, 2021).

Biometric observations

Yield under stress is the primary trait for selection in breeding programs for drought prone environment. Several secondary traits which are associated with yield under stress are also adopted to measure the level of drought tolerance in plants. These include traits like leaf membrane stability, stomatal behaviour and conductance, leaf wilting scales, osmotic adjustment and root characters Ajayi *et al.* (2018).

Some of the secondary and generally accepted traits associated with drought tolerance selection are flowering date, root length, root density, osmotic adjustment, membrane stability, leaf relative water content, water use efficiency, drought responsible index, maturity date, harvest index, canopy temperature etc (Lafitte *et al.*, 2003).

Traits involving plant greenness, wilted plants, percentage of dead plants and recovery rate after rewatering were recorded for screening drought tolerance in cowpea seedlings (Ravelombola *et al.*, 2018).

According to Abayomi and Abidoye (2009) water deficit delays flowering in cowpea crops. They reported significant variation in days to flowering among drought stressed genotypes which otherwise showed similar flowering time under irrigated condition.

Early flowering has been reported as a drought adaptation mechanism in cowpea, allowing for quick recovery, significant pod production and increased production of second flush of pods after drought stress (Hall *et al.*, 2000).

According to Mitra (2001) flowering time is an important trait to select for drought escape. Positive associations exist between plasticity of yield and flowering time across different levels of water availability. Plants accelerate the flowering age and harvest age to escape from drought stress.

Pantuwan *et al.* (2001) reported that delayed flowering under moisture stress can be an effective indicator of cultivars susceptibility to drought and an integrative trait in identifying drought.

Trait correlation have been utilised in indirect selection for breeding high yielding plants under drought condition (Diouf, 2011). Magashi *et al.* (2019) in their variability studies in cowpea under water stress reported a positive relationship between pod length, number of pods per plant and days to 50% flowering with yield.

Tewolde *et al.* (1991) tested three cowpea genotypes for water stress tolerance. Given 6 irrigations over the entire growing period, 4 irrigations between sowing and early pod filling and 2 irrigations during seeding establishment. They concluded that moisture stress causes decreased seed per pod, dry matter and seed yield per plant, but did not affect seed weight and harvest index.

Drought caused a reduction in plant height in legumes (Fening *et al.*, 2009). They stated that a lack of water impairs the mitotic process and causes increased senescence. Reduced cell turgor inhibits cell division, elongation and expansion, resulting in a reduction in plant height.

Madhukumar (2006) through path analysis revealed that number of pods per plant and pod weight were the primary yield contributing characters due to their high direct effect on pod yield in yard long bean.

Through path analysis Sulthana *et al.* (2020) revealed that days to first flowering, days to maturity, number of pods per plant, pod weight and number of seeds per pod had direct positive effect on pod yield per plant. Devan *et al.* (2021) reported correlation and positive association of pod length, pod width and pods per plant with pod yield per plant in yard long bean.

Vidhya (2000) suggested that while selecting for yield improvement in yard long bean, number of pods per plant and pod weight should be included as these traits exhibited significant genotypic correlation with high direct effect on pod yield.

Lakshmi (2016) evaluated eight parents and 28 F1's from a half diallel cross. Reported significant difference among the treatments for all traits except seeds per pod for parents. Among parents, VS 29 recorded the highest yield (848.74 g plant$^{-1)}$ and pods plant^{-1} (56.67). The highest pod weight (27 g) and pod length (66.28 cm) was recorded in VS 50. Among the hybrids, highest yield was recorded in VS 34 x VS 50 (1414.55 g plant^{-1}). VS 34 x VS 13 recorded maximum number of pods plant^{-1} (107.17). Highest pod weight was recorded in VS 50 x VS 16 (30.67 g) whereas VS 54 x VS 26 had the maximum pod length (71.27 cm).

In yard long bean vine length, pod length, pod girth, number of pods per plant and pod weight were positively and significantly correlated with yield per plant (Bhagavati *et al.*, 2019; Kumar and Devi, 2009 and Kutty *et al.*, 2003)

Lovely (2005) studied the genetic basis and inheritance pattern of yield in fifty genotypes of yard long bean. Reported characters pod weight, pod length, pods per plant, pod breadth and stem length had positive direct effects on yield , which indicates selection of genotypes based on these characters can be effective for improving yield of the crop.

Ahmed and Suliman (2010) reported that water deficit significantly reduced the mean number of pod in cowpea cultivar, suggesting that this variable

is a sensitive indicator of drought tolerance. Drought stress during flowering reduced the pod filling and the number of pods.

Litty (2015) reported high variability in thirty yard long bean accessions for pod yield per plant. Damarany (2019) studied yield and drought tolerance in different cultivars of cowpea. He found significant differences in water stress tolerance among the cutivars.

In yard long bean number of pods per plant was found to be positively correlated with yield and reported as one of the yield attributing parameters by Pandey *et al.* (2020).

Ajayi *et al.* (2018) found that from the day of moisture stress imposition till 14th day, plant height increased in all accessions of cowpea seedlings, after which it remained constant until day 21 of stress. In cowpea Lestari *et al.* (2019) reported that moisture stress results in reduced plant height and the susceptible types as more sensitive.

Harvest index is a measure of production efficiency of the plants in translocating its total photosynthates from vegetative tissues to the economic and non-economic sinks. Yield under drought stress is a function of biomass production and harvest index at the vegetative and reproductive stage (Haunsajirao, 2017).

Edmeades *et al.* (1999) reported a positive gain in harvest index showing that yield gains were due to better photosynthates mobilization to ears under drought stress.

Mereena (1989) evaluated sixteen cowpea accessions for drought tolerance. Based on the studies on variability, correlation and path analysis it was concluded that a plant type suited for drought conditions should be early flowering with more number of pods per plant and high harvest index.

Yerima *et al.* (2013) suggested that while selecting superior genotypes for drought tolerance pod yield, harvest index and water use efficiency should be considered as selection criteria.

Crop duration are often used to evaluate earliness, a useful selection trait for drought avoidance that can be easily phenotyped (Rauf *et al.*, 2016).

Mohamed *et al.* (2002) reported the importance of a deep and vigorous root system for maintaining yield under drought stress in bean. The drought tolerant genotypes generally increase the photosynthates allocation for root elongation under drought stress.

Survival under drought stress reflects on the capacity of the root to function. Root growth rate, root volume, root depth and root dry weight are traits related to drought avoidance mechanism. The drought tolerant genotype should have greater root as compared to drought susceptible genotype (Yue *et al.*, 2006).

Hayatu and Mukhtar (2010) evaluated seven cowpea genotypes for their physiological responses to drought resistance with treatments under control, moderate and severe water stress condition. The results showed that water stress significantly reduced chlorophyll content and above ground biomass. At severe water stress, most of the genotypes recorded lower biomass and water use efficiency. Observed a general increase in root biomass in moderate and severe water stress condition. Increases in the root biomass recorded more under moderate stress.

Increased rooting depth and density, determined using Archimedes' method, would increase the plant capacity to extract water. When plants are subjected to moderate stress, it produces longer roots to absorb moisture from deeper layers. During drought, rooting depth is an important parameter for water acquisition (Lynch, 2013).

According to Hall (2013) water-use efficiency, deeper rooting and heat tolerance are important traits to be considered in cowpea for adaptation to

drought. Plants increase water uptake by forming long roots to promote their survival under water deficit condition (Wang *et al.*, 2020).

Physiological observations

Drought tolerance may be broadly defined as the ability of plants to withstand water deficit while maintaining appropriate physiological activities. Drought reported to impair physiological processes such as photosynthesis, accumulation of lipids and transcript expression (Hajibabaee *et al.*, 2012).

Physiological and biochemical parameters that correlate with yield under extreme moisture stress conditions can be used to select drought tolerant plants during the breeding process (Xiong and Ishithani, 2006). Tolerance to stress involves at least two mechanisms, osmotic adjustment and changes in the elastic properties of tissues (Zlatev and Lidon, 2012).

Osmotic adjustment is an effective mechanism of drought resistance which help to maintain cell turgor as the water potential decreases, enabling water uptake and the maintenance of plant metabolic activity and therefore growth and productivity (Martiinez *et al.*, 2007)

Plants survive the drought by enhancing their root density, reducing their transpiration rate, reduction in stomatal conductance, reduction in assimilate partitioning, slow wilting and delayed senescence. Expression of major osmoprotectants and transcription factors helps plants to increase their tolerance to water deficit (Shahzad *et al*, 2016).

The levels of proline, abscisic acid, nitrate reductase, stomatal resistance, water potential and transpiration rate in various crop plants at different growth stages have been reported as important physiological and biochemical traits useful for determination of drought tolerance (Narayanan *et al.*, 2014).

Osmoregulation is an adaptive mechanism for plants to survive under stress condition. Proline is a multifunctional protein and an important osmolyte

that accumulates both under stress and non-stress conditions in plants. Proline interacts with enzymes to preserve protein structure and enzyme activities and plays a critical role in protecting photosynthetic activity under osmotic stress. High proline level is a reliable index for drought tolerance in genotypes (Kavikishor *et al.*, 2015).

According to Noori *et al.* (2018) during extreme drought stress, the catalase enzyme, chlorophyll content and relative water content were found to be reduced whereas the peroxidase enzyme, electrolyte leakage and proline content were increased.

Somal and Yapa, (1998) investigated the effect of different types of stresses on free proline content of leaves of cowpea. Drought stress enhanced the proline levels. Regression analysis of data indicated a good linear relationship between drought stress and proline concentration (r=0.91).

Production and accumulation of proline is an adaptive response in plant tissue during drought and can be used a metabolic marker in relation to stress (Caballero *et al.*, 2005).

When compared to sensitive yard long bean genotypes, Ananthraju and Muthiah (2008) found that tolerant genotypes accumulate higher proline levels as well as higher overall biomass and pod yield.

Lestari *et al.* (2019) observed accumulation of proline due to drought stress and reported that drought tolerant yard long bean plants accumulate more proline than the sensitive type.

Nkoana *et al.* (2019) reported that drought stress caused an increase in proline content across cowpea accession as compared to irrigated. Analyses of variance showed highly significant differences in response to moisture stress among the cowpea accessions for proline content.

Drought stress causes degradation of cell membrane and the maintenance of membrane stability is a physiological process that allows plants to survive under the stress. The ability to limit membrane damage and to regain membrane integrity and activity quickly upon rehydration were used for the evaluation of tolerance to various stresses in plants (Bewley, 1979).

The amount of electrolyte leakage from leaf segments is used to assess the membrane stability. Membrane stability and percentage leakage are physiological index widely used for the evaluation of drought and temperature tolerance (Blum and Ebercon, 1981).

Lower membrane stability or higher injury reflects the extent of membrane lipid peroxidation, which in turn is a consequence of higher susceptibility to oxidative stress due to various environmental stresses including drought (Leibler *et al.*, 1986).

Electrolyte leakage was found to be greater in a susceptible accession than in a drought tolerant accession by Premachandra and Shimada (1987). Electrolyte leakages can be measured directly with an electric conductivity meter.

Drought induces stomatal closure and decreases the CO2 concentration in leaf mesophyll tissue and results in an accumulation of NADPH. Under such conditions, oxygen acts as an alternate acceptor of electrons resulting in the formation of superoxide radical ($O_2^{\cdot}$). Reactive oxygen species (ROS) such as superoxide anion radicals (O_2^{-}), hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and alkoxy radicals (RO) are potentially toxic compounds. Reactive oxygen species cause lipid peroxidation and consequently membrane injuries, protein degradation, enzyme inactivation thus induce oxidative stress (Foyer and Shigeoka, 2011).

Under optimal conditions plants synthesize ROS neutralizing substances including non-enzymatic and enzymatic antioxidants such as superoxide dismutase, ascorbate peroxidase, guajkol peroxidase, glutathione reductase,

catalase and metabolites to cope with reactive oxygen species thus minimizing oxidative damage (Sairam *et al.*, 2005).

Tolerant genotypes were not only able to retain sufficient water under drought but also generate low molecular weight antioxidants such as ascorbic acid, carotenoids, tocopherols and glutathione to protect plant cells from oxidative damage (Lovaas, 1997).

Ascorbic acid (vitamin C) is a major antioxidant and redox having substantial potential in modulating a number of fundamental functions in plants both under stress and non-stress conditions. Ascorbic acid content is an indication of stress tolerance ability of plants and its high value indicates that stress did not have much effect on tolerant plants (Matamoros *et al.*, 2006).

One method of measuring plant water stress is by sensing the infrared radiation released by the leaf. According to Jones and Corlett (1992), leaf temperature is related to plant water stress level and genotypes with high drought tolerance scores consistently stayed the coolest under stress.

Canopy temperature is an indicator of plant water status. A cooler canopy reported to be a measure of drought tolerance, low leaf temperature indicates maintenance of higher transpiration (Lafitte *et al.*, 2003). Genotypes with a cooler canopy temperature under drought stress or a higher canopy temperature depression (CTD), use more of the available water in the soil to avoid excessive dehydration (Ludlow and Muchow, 1990)

Relative water content reflects the water status of plant indicating the metabolic activity in tissues. The capacity to maintain higher relative water content under moisture stress condition is obviously a drought resistance mechanism (Kramer and Boyer, 1995). The genotypes maintaining higher relative water content accumulates more solutes and had higher photosynthesis and higher recovery upon stress relief (Jha and Singh, 1997).

The maintenance of plant water status more than plant functions, controls crop performance under drought. Higher values of RWC and osmotic adjustment confers for better growth and development of plant (Blum *et al.*, 2001).

Anyia and Herzog (2004) reported that stomata closure and a reduction in stomatal conductance maintained a high relative water content of leaves in several cowpea genotypes subjected to water stress. Under drought stress, high assimilation rate was found to be associated with high RWC.

Drought resistant bean cultivars were observed to maintain high relative water content under drought condition. Explained that these plants have the ability to accumulate large amounts of proline and other osmotic compounds, which support in water potential reduction and osmotic adjustment (Zlatev, 2005).

Water content is a standard metric of moisture status in plants that is expressed as relative water content (RWC). Relative water content compares the quantity of water in a leaf to the maximum amount that the leaf can hold at full turgidity and regarded as a suitable measure of plant water status under stress (Haunsajirao, 2017).

Lestari *et al.* (2019) reported decrease in the relative leaf water content of all the yard long bean varieties subjected to water stress. High yielding variety, Brawijaya Ungu-3 maintained relatively higher leaf water potential and relative leaf water content under water stress.

Yerima *et al.* (2013) reported genotypic difference for water use efficiency (WUE) as an important selection criterion for screening drought tolerance genotypes. Under soil moisture stress, drought resistant cowpea genotypes had higher water use efficiency than drought susceptible genotypes explained the genotypic variation for soil moisture extraction capacity from deep soil.

Anyia and Herzog (2004) observed variation in water use efficiency and stomata conductance of cowpea genotypes in response to water deficit. Water deficit improved the WUE .of two genotypes (IFH 27-8 and Lobia) by approximately 20% but caused moderate to huge reductions in most genotypes.

Cowpeas have stomata that are very sensitive to soil drying, partially closing before any changes in leaf water potential were detected. Low stomatal conductance during low soil water deficit is an alternate drought adaptive mechanism contributing to decrease transpiration and in maintaining low water potential (Bates and Hall, 1981).

Lestari *et al.* (2019) suggested the decreased evapotranspiration and increased number of closed stomata as the drought avoidance mechanism and the increasing proline buildup as the drought tolerance mechanism in yard long bean.

Ajayi *et al.* (2018) measured stomatal conductance in all drought stressed accessions and found substantial variance in all yard long bean genotypes, with the highest value (926.70 mmol $m^{-2}s^{-1}$) in accession AC03 and the lowest (70.19 mmol $m^{-2}s^{-1}$) in AC05.

According to Lawrent *et al.* (2013) leaf wilting remains one of the best indicators of drought stress in plants, as it reduces the complexities associated with drought in crops.

Ajayi *et al.* (2018) reported permanent wilting percentage as one of the effective screening traits for drought tolerance. Percentage permanent wilting was measured at different intervals (14 and 21 days) until 90% of most susceptible accessions were entirely wilted. The technique revealed heritable differences among the tested genotypes as regards their reaction to drought stress.

In cowpea plant greenness was recorded 4 weeks after first imposing drought stress when the susceptible genotype was completely dead. Recovery rate corresponded to the number of plants that fully recovered after one week of

rewatering. Rewatering was conducted when the susceptible genotypes were completely dead (Ravelombola *et al.* 2018).

Ajayi *et al.* (2018) assessed drought stressed cowpea seedlings for stem greenness and regrowth after 14 days of rewatering. The percentage of plant recovery was estimated based on the score and found to be ranged from 0.00 to 36.67%.

Stem greenness and plant recovery percentage appeared to be a reliable indicator for screening cowpea accession for drought tolerance, which also correlated significantly and positively with relative water content and proline content (Nkoana *et al.*, 2019).

Ravelombola *et al.* (2020) reported plant greenness score and recovery rate as accurate parameters for assessing drought tolerance at seedling stage in cowpea. Data recorded on a per plant basis. They reported that drought tolerant genotypes were slow wilting, whereas those that were more drought susceptible were fast wilting.

2.3 Studies on combining ability

Sprague and Tatum (1942) gave the concept of combining ability and proposed the idea of partitioning genetic variation into variance due to general combining ability (gca) and specific combining ability (sca). The ability of a genotype to produce superior progenies upon crossing is termed as combining ability. The success of crop improvement depends to a great extent on the types of parents used, their diversities for desired characters and their combining ability.

The line × tester analysis method introduced by Kempthorne (1957) is an important mating system and tool available to estimate the combining ability to assess differences among the genotypes and explain the genetic mechanism ie., nature and magnitude of gene actions involved. It has an important role to select parents and in assessing heterosis for identifying promising crosses in early

generation, that can give transgressive segregants in later segregating generations. It helps to decide breeding methods to be followed to choose desirable individuals (Salgotra *et al.*, 2009).

Choice of best parents is a pre-requisite in all crop breeding programmes. Evaluation of parents for their transmission potential for yield and yield components will gave a way for better selection. All available parents with high order of performance may not be able to transmit their superior traits to their progenies. Hence selection of desirable parents based on their combining ability is used in crop improvement programmes. Line × tester analysis is one of the most powerful tools that aids in selecting suitable parents and crosses with high specific combining ability (SCA) for exploitation in pedigree breeding (Rashid *et al.*, 2007).

General combining ability (gca) means the ability of a breeding line to produce superior progeny in a series of crosses and is the result of additive gene action. Specific combining ability (sca) is the performance of an inbred line in specific cross combination and is the result of non additive gene action. Non additive type gene actions is not reliably fixable whereas additive type of gene actions or complementary type epistatic gene interactions are reliably fixable (Nadarajan *et al.*, 2016).

Mishra *et al.* (1987) indicated the importance of both gca and sca for days to 50 per cent flowering in line x tester analysis involving four testers and ten lines of cowpea. A line x tester analysis to estimate the combining ability of cowpea varieties revealed the predominance of non-additive gene action for number of pods per plant (Kumar, 1993).

Based on line x tester analysis in cowpea Madhusuda *et al.* (1995) identified good general combiners for pod yield and seed yield per plant and both additive and non-additive genetic variances were found important in the inheritance of quantitative traits with a preponderance of non-additive gene effects in most cases.

Jeena and Arora (2001) reported the predominance of non-additive gene action for pods per plant, yield per plant, plant height, 100 seed weight and days to maturity in chickpea. Equal importance of additive as well as non-additive genetic variances were revealed for seeds per pod and primary branches per plant.

In a line x tester analysis in cowpea, Pal *et al.* (2002) found that ADCP-13, Red Seeded, Kala Zamal and Pusa Komal were good general combiners for days to 50 per cent flowering.

Philip (2004) reported significant gca effects for grain yield per plant, pods per plant, inflorescence per plant, pod length and seeds per pod in cowpea. Significant estimates of heterosis for inflorescence per plant, pods per inflorescence and grain yield was observed.

Manivannan and Sekar (2005) studied the combining ability for yield and quality traits in a line x tester analysis of cowpea. They found highly significant additive variance in the line IC 201099 for the characters like pod yield per plant, number of pods per plant, pod length and pod weight. The tester, Arka Garima showed the maximum additive variance for days to first flowering, pod weight, pod length and pod yield. The study recommended two hybrids namely, IC 201099 x Arka Garima and IC 201099 x Co-2 for heterosis breeding.

Selvakumar *et al.* (2014) carried out combining ability analysis among hybrids obtained from 11 selected cowpea lines and recorded the highly significant gca and sca effects for all the character studied. The parents GC 3, RC 101, Vyjayanthi and Vellayani Jyothika were identified as promising based on gca effects. However, the superior hybrids for yield and related traits were GC 3 x Vellayani local, GC 3 x Vellayani Jyothika, ACM 05-07 x VBN 2, ACM 05-07 x Vyjayanthi, RC 101 x Vellayani Jyothika and ACM 05-02 x Vyjayanthi.

Sanjeev *et al.* (2015) studied gene action and combining ability effects for fodder yield and its component characters in a line x tester analysis of fodder cowpea. In this study, the predominance of non additive gene action was

recorded for all the characters studied. The promising lines identified were CPD-31, MFC-09-09 and EC-458505 while the promising testers were NBC-2, IC-1071 and EC-170578-1-1.

Lakshmi (2016) evaluated eight parents and 28 F1's from a half diallel cross for yield and quality characters. Reported that VS 50 was the best general combiner for days to first flowering, pod weight, seeds per pod, yield per plant and days to harvest. The estimates of specific combining ability effects revealed VS 34 x VS 50, VS 34 x VS 13, VS 50 x VS 26, VS 54 x VS 26 and VS 16 x VS 38 the most promising crosses for yield and pods per plant. Based on the mean performance, specific combining ability and standard heterosis, the hybrids VS 34 x VS, VS 50 x VS 26 and VS 34 x VS 13 were found as the most promising.

The yield potential of hybrid depends on the magnitude of heterosis that in turn is influenced by the genetic distance and combining ability of the parental lines. Specific breeding procedures such as recurrent selection been exploited to improve the combining ability of the breeding lines (Rauf *et al.*, 2016).

Lovely and Kumar (2021) in a partial diallel cross of yard long bean reported significant differences of analysis of variances due to specific combining ability and general combining ability. The parents VS41, VS43 and VS47 have significant general combining ability. For pod yield per plant, the crosses VS-44 x VS-47, VS-9 x VS-43 and VS-43 x VS-47 exhibited the high SCA effect.

2.4 Studies on nature of gene action

Gene action refers to the behaviour or mode of expression of genes in a genetic population. Knowledge of gene action helps in the selection of parents for use in the hybridization programmes and also in the choice of appropriate breeding procedure for the genetic improvement of various quantitative characters. Insight into the nature of gene action involved in the expression of various quantitative characters is essential for starting a systematic breeding programme (Singh and Narayanan, 2015).

Gene action could be dominant, recessive, sex-linked or by chromosomal aberrations. A combination of such gene actions results in the observable phenotype of an organism. It is of two types, additive and non - additive gene action. Additive gene action included additive genetic variance and additive x additive type of epistatic variance controlled by gca effects. The dominance genetic variance, additive x dominance and dominance x dominance types of epistatic variance comes under non - additive gene action controlled by sca effects. The relative proportion of gca to sca variance shows the predominance of additive or non-additive gene effects. Additive variance can be fixable using the selection procedures, while non-additive variance is not fixable and this can be improved through heterosis breeding (Nadarajan *et al.* 2016).

Additive variance which results from the cumulative effect of minor genes or from their interaction is selectable through simple breeding procedures such as mass selection or pedigree selection. Interaction among alleles and genes also gives rise to dominance and epistatic effects. Dominance variance is the deviation of heterozygote genotypes from the average effect of the parents, while epistatic variance is due to complex interaction. Both variances are not selectable in segregating generations (Rauf *et al.*, 2016).

Gene action can be measured in terms of components of genetic variance or combining ability variance and effects (Singh and Narayanan, 2015). Use of combining ability as a measure of the type of gene action was suggested by Sprague and Tatum (1942) in maize. Gene models were also suggested to evaluate the additive and dominance gene effects by Comstock and Robinson (1948) and Mather (1948).

In 10 x 10 diallel analysis of cowpea, Sobha (1994) observed the predominance of both additive and non-additive gene action for plant height, primary branches, days to flowering, pod length, pod weight, pods per plant, 100 seed weight and yield per plant.

Renjana (2006) emphasized the importance of dominance gene action in controlling the quantitative and biochemical characters of yard long bean by combining ability study and components of variation due to gca and sca.

Sharma *et al.* (2013) studied the genetics of pod character in vegetable cowpea using line x tester analysis and reported that additive gene action control both the pod length and pod weight whereas non additive gene action controls the number of pods per cluster and number of pods per plant.

Lakshmi (2016) in a diallel analysis of yard long bean reported that the estimates of sca variance was higher than gca variance for pod length, pod breadth, pods per plant, stem length and yield per plant indicates the importance of non-additive gene action in the expression of the traits.

Rout *et al.* (2018) reported high estimates of heritability coupled with high genetic advance for characters such as leaf area, pod yield per hectare, pod yield per plant and vine length. Kumar and Devi (2009) reported high heritability coupled with high genetic advance for pods per plant, pod yield per plant, pods per cluster and pod weight, indicating the additive gene action and suggests the possibility of genetic improvement through selection.

George and Sarada (2019) estimated gene action through generation mean analysis in yard long bean for vegetative and yield characters. Reported dominance gene action for pod length, pod weight, vine length, days to first flowering, pods per plant, days to harvest and yield per plant.

Devan *et al.* (2021) observed high heritability and high genetic advance mean for seed weight, pod yield, pod width, pod length, pods per plant and vine length traits in yard long bean suggesting additive gene action in the expression of the traits.

Lovely and Kumar (2021) in a partial diallel cross of yard long bean reported predominance of dominant gene action for days to flowering, pod

length, pod weight, pod breadth, pods per plant, pod yield per plant and for root weight suggesting non-allelic complimentary gene action in the expression of the traits.

2.5 Studies on heterosis

Balanced gene combinations which were more adaptive to environmental conditions and useful from the agriculture point can be obtained through heterosis breeding. Heterosis, also known as hybrid vigour, describes the phenomenon in which an F_1 population obtained by crossing of two genetically different individuals, with enhanced or decreased vigour compared to the parents.

Thomas Fairchild was the first to identify and report this improved vigour of hybrids in 1716. Joseph Koelreuter was the first to conduct plant hybridization in a scientifically sound manner and to report on the benefits of outcrossing. G.H.Shull developed the concept of heterosis in 1914 as a consequence of evidences on the prevalence of hybrid vigour in several crops.

Bhushana *et al.* (2000) studied heterosis in 36 hybrids produced through line x tester mating designs and it showed maximum heterosis over mid parental value for number of pods per plant. Significant positive heterosis was observed for seed yield per plant, number of primary branches per plant, pod length and weight. Significant negative heterosis was observed for days to 50 per cent flowering in cowpea.

Pal *et al.* (2002) found that the F_1 hybrids like NDCP-13 x Arka Garima, Cowpea Local x Cowpea-263, Red seeded x Pusa Komal were superior performers for green pod yield because they recorded significant heterobeltiosis of 77.38, 70.73, 70.44 and relative heterosis of 94.00, 88.42 and 85.95%.

A line x tester analysis of heterosis in cowpea, Haibatpure *et al.* (2003) reported that heterosis in yield was due to heterosis for number of pods plant^{-1}, seeds pod^{-1}, number of branches plant^{-1} and 100 seed weight. They identified the

superior hybrids for grain yield over mid parent were TC 2000-2 × GC-2, TC 2000-2 × GC-3 and TC 2000-2 × GC-4.

Monneveux *et al.* (2006) observed that hybrids had greater buffering capacity against reduction of yield under drought stress than lines due to their heterozygous genetic back grounds and the magnitude of heterosis was found to increase under drought stress.

In a line x tester study, Patil and Gosavi (2007) reported the heterobeltiosis for following characters viz. days to maturity (-13.25 to 5.53%), plant height (-67.29 to 6.36%), number of pods plant^{-1} (-52.73 to 41.47%), pod length (-63.38 to 26.65%), number of seeds pod^{-1} (-25.11 to 111.72%) and seed yield plant^{-1} (-36.55 to 103.41%).

According to Ushakumari *et al.* (2010) two hybrids of cowpea Lola x VBN1 and Sarika x CO 4 exhibited significant standard heterosis over the variety CO (CP) 7 for seed per pod, cluster per plant, pods per plant and 50% flowering. The hybrid TC 49-1 x CO 2 had maximum standard heterosis for plant height and clusters per plant.

Yadav *et al.* (2010) conducted heterosis study in 8 x 8 diallel mating system in cowpea and reported that the hybrid IC 201085 x CO 4 had the highest heterobeltiosis for green pod yield (34.90%). The extent of heterobeltiosis for different characters were -47.84 to 16.34% (plant height), -20.79 to 7.59% (days to 50% flowering), -24.37 to 9.15% (pod length), -53.37 to 39.91% (pods plant^{-1}), -34.05 to 15.25 %,) and -59.91 to 34.90% (pod yield plant^{-1}). The heterosis for pod yield was due to the heterosis of yield components and these characters were mainly controlled by non-additive gene action.

In a 8 x 8 diallel analysis (excluding the reciprocals) in vegetable cowpea, Kadam *et al.* (2013) reported high per se performance and significant positive standard heterosis for green pod yield and its attributes were exhibited by GC-0203 x Anand Cowpea-1 (111.17 g and 21.72%), Subhra x GC-4 (99.40 g and

8.83%), GC-0203 x GC-0502 (96.13 g and 5.26%) and GC-0502 x Pusa Komal (95.93 g and 5.04%).

Sharma *et al.* (2013) conducted a line x tester analysis for studying the pod characters in vegetable cowpea and revealed that the hybrids ICP-38 x Arka Garima (23.82%), ICP-45 x Pusa Komal (10.37%) and ICP-42 x Indira Hari (86.45%) showed the highest heterosis over mid-parent, better parent and standard check respectively for pod length. For pod weight and pods plant^{-1} hybrids ICP-42 x Arka Garima (36.14%, 5.61% and 89.00%) and ICP-54 x Indira Hari (74.20%, 60.92% and 27.44%) exhibited the highest heterosis over mid-parent, better parent and standard check respectively.

Gudadhe *et al.* (2015) carried out the heterosis studies in vegetable cowpea and recorded the maximum heterobeltiosis of 56.07% and 50.75% for pod yield plant^{-1} and pods peduncle^{-1} respectively. Moreover, the maximum standard heterosis of 87.66% for pod yield plant^{-1}, 47.07% for pods plant^{-1}, 46.24% for pod length and 27.32% for pods peduncle^{-1}. The hybrids Chikhali local x Pusa Phalguni, Chikhali local x Gadchiroli 4, Chikhali local x Pusa Komal, Gadchiroli 4 x Pusa Phalguni and Gadchiroli 4 x GADCP 3 showed the maximum significant standard heterosis for pod yield and its component characters.

Lakshmi (2016) in a diallel analysis of yard long bean studied relative heterosis, heterobeltiosis and standard heterosis for yield and quality characters. Significant positive heterosis was observed for pods per plant, pod length, pod weight and yield per plant. Significant negative heterosis was observed for days to 50 per cent flowering. The hybrid VS 34 x VS 50 showed highest standard heterosis for yield. On the basis of mean performance, sca effects and heterosis magnitude, superior hybrids were found.

Rauf *et al.* (2016) while evaluating performance of hybrids observed that crosses between tolerant and sensitive hybrids were more effective under drought

stress, which may be due to the diverse genetic back ground of the inbred lines which resulted in increased heterosis.

One of the most important techniques for crop improvement is hybridisation programme, success of which mainly depends on the genetic diversity of the parents chosen for the trait. To produce higher heterotic effects in yard long bean genetically diverse parents have to be crossed (Asoontha, 2017).

George and Sarada (2019) emphasized the scope of heterosis breeding and hybridization followed by selection for exploitation of hybrid vigour in yard long bean. Being a self pollinated crop, heterosis breeding is considered to be one of the most effective ways to obtain variability in yard long bean. Knowledge of heterosis estimates will aid in the identification of hybrids that can lead to superior transgressive segregants in segregating generations.

Talape *et al.* (2020) assesses heterosis in 18 hybrids of cowpea obtained through L x T cross and reported the highest magnitude of heterosis for seed yield per plant. Observed that the crosses showing high heterosis and high per se performance involved the parents possessing high x high, high x low, low x low combining ability indicating importance of additive and non-additive genetic variance. The highest value of heterobeltiosis and standard heterosis were 52.16 and 42.80 per cent respectively.

The success of hybridization is largely determined by the genetically diverse parents, to produce heterotic combinations (Devan *et al.*, 2021). Among the twenty-five crosses studied, Lovely and Kumar (2021) observed significant positive standard heterosis for pod length, pod width, pods per plant and pod weight in yard long bean.

2.6 Studies on gene expression

Xia *et al.* (2019) reported a 632.8 Mb genome assembly (549.81 Mb non-N size) in yard long bean based on the whole genome shotgun sequencing

strategy with a total of 42,609 protein coding genes and 3579 nonprotein coding genes were predicted from the assembly.

In spite of intensive investigation on the problem of water deficit tolerance, many of its aspect remain to be explored. Water deficit induces expression of particular genes associated with adaptive responses of stressed plants (Zlatev and Lidon, 2012). Understanding the functions of the genes is critical to know the molecular mechanisms governing plant stress response and tolerance, ultimately leading to enhancement of stress tolerance in crops through genetic manipulation (Shinozaki and Yamaguchi, 2006).

Detailed studies on the molecular physiology of abiotic stress tolerance in yard long bean might identify key genes that could improve the breeding process for the development of tolerant lines (Suma *et al.*, 2021).

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product. Regulation of gene expression is vital to allow a cell to produce the gene products when it needs which gives cells the flexibility to adapt to a variable environment. Gene expression analysis were exploited to understand the differential pattern of gene expression and to identify drought responsive genes (whose expression increases under drought stress) and drought inducible genes (which only express during drought stress) (Rauf *et al.*, 2016).

Real-time quantitative PCR (qPCR) has been widely used as the most reliable method to measure gene expression, due to its high accuracy and specificity. A commonly used technique for detecting and quantifying expression profiles of selected genes (Deepak *et al.*,2007).

The drought related responses in plants are of a complex nature and result from genomic re-organization and alterations in gene expression. Drought tolerance involved induction of several transcription factors and drought responsive genes leads to synthesis of stress proteins, regulation of water

channels and production of osmolytes that are essential for maintenance of osmotic balance at the cellular level (Kaur *et al.*, 2021).

Constitutive accumulation, by overexpression of the responsible gene, of a cellular osmolytes is regarded as a serious approach in increasing crop drought resistance (Bohnert *et al.*, 1995).

Huang *et al.* (2008) identified 2000 drought stress responsive genes in *Arabidopsis thaliana,* the expression of which increases several folds during stress treatment. Seki *et al.* (2002) identified that drought stress increased the expression of 277 genes in Arabidopsis, 22 of which were also induced by cold and heat.

A number of drought inducible genes have been identified and isolated from cowpea by differential screening (Boukar *et al.*,2016). Important drought related genes reported in cowpea are CPRD12 and CPRD46 (Iuchi *et al.*, 1996a) and CPRD8, CPRD14 and CPRD22 (Iuchi *et al.*, 1996b). A stress inducible gene VuNCED1, encodes to 9-cis-epoxycarotenoid dioxygenase, involved in Abscisic Acid biosynthesis under water stress, associated with stress tolerance mechanism in drought tolerant cowpea (Iuchi *et al.*, 2000).

Muchero *et al.* (2009) identified 10 QTLs associated with drought tolerance in cowpea. Some of these QTLs coincided with genes involved in stem greenness and recovery dry weight following drought stress. Indicating the role of these traits in imparting tolerance to drought in cowpea.

QTLs associated with drought response mechanisms in cowpea have been identified with various traits such as root characters, membrane stability, osmotic adjustment and morphological and physiological traits where tolerance is measured as yield under drought, which can be used to understand the genetics of drought tolerance in legumes (Lonardi *et al.*, 2019).

Cantale *et al.* (2007) employed quantitative RT-PCR to monitor the expression profile of the TdDRF1 gene, (Triticum durum Dehydration Responsive Factor 1) a DREB homologous gene, in wheat cultivars varying in their drought tolerance/susceptibility. The relative expression profiles demonstrate that water stress as genotype dependent, with tolerant and susceptible genotypes exhibiting peculiar expression pattern. Suggested that dehydration condition has an effect on the expression patterns of a transcription factor encoding gene.

DREBs (dehydration responsive element binding) are important plant transcription factors (TFs) that regulate the expression of many stress inducible genes mostly in an ABA-independent manner and play a critical role in improving the abiotic stress tolerance of plants. qRT-PCR gene expression profile of DREB genes from four common bean subjected to drought stress indicates significant up and downregulation of the genes in comparison to the control samples (Konzen *et al.*, 2019).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The major objective of the investigation entitled "Gene action and gene expression analysis in yard long bean (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdcourt) for drought tolerance" was to identify drought tolerant genotypes of yard long bean under water stress condition. The experiments were carried out at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during the period 2017-2019. This chapter provides information about the materials used and the methods adopted in the study.

3.1 EXPERIMENTAL DETAILS

3.1.1 Location

The geographical coordinates are 8°5'N latitude, 76°9'E longitude and at an altitude of 29 m above mean sea level. The predominant soil type at the experimental location was red loam of Vellayani series, which was texturally classified as sandy clay loam.

Experiment I

3.1.2 SCREENING GERMPLASM FOR DROUGHT TOLERANCE AT THE SEEDLING STAGE IN FIELD

The materials for the study consisted of 100 genotypes of yard long bean collected from different cultivated areas of Kerala and collections from a previous project of the Department of Plant Breeding & Genetics, College of Agriculture, Vellayani entitled "Collection, conservation and genetic improvement of traditional land races and obsolete varieties of major vegetables in Kerala". The test entries, designated by treatment numbers G_1 to G_{100}, were evaluated in the field for drought tolerance from March to April 2018. The genotypes were planted in Completely Randomized Design with two replications and raised in progeny rows with five plants per row in the field. The source of collection of genotypes are provided in table 1..

Table.1 List of yard long bean genotypes used and their sources of collection

Treatment No.	Genotypes	Source
G1	Acc. 5	Olericulture Department, COH, KAU
G2	Acc. 32	Olericulture Department, COH, KAU
G3	Acc. 1112	Olericulture Department, COH, KAU
G4	Acc.1337	Olericulture Department, COH, KAU
G5	Acc.1339	Olericulture Department, COH, KAU
G6	Adoor local	Pathanamthitta Dist.
G7	Alathoor local	Palakkad Dist.
G8	Alenchery local	Kollam Dist.
G9	Alleppy local I	Alappuzha Dist.
G10	Alleppy local II	Alappuzha Dist.
G11	Alleppy local III	Alappuzha Dist.
G12	Ambalapuzha local	Alappuzha Dist.
G13	Anchal local I	Kollam Dist.
G14	Anchal local II	Kollam Dist.
G15	Aranmula local	Pathanamthitta Dist.
G16	Aryanadu local	Thiruvananthapuram Dist.
G17	Athirapally local	Thrissur Dist.
G18	Attappady local	Palakkad Dist.
G19	Ayira local	Thiruvananthapuram Dist.
G20	Ayyanthole local	Thrissur Dist.
G21	Chenkottukonam local II	Thiruvananthapuram Dist.
G22	Cherthala local I	Alappuzha Dist.
G23	Cherthala local II	Alappuzha Dist.
G24	Elamadu local I	Kollam Dist.
G25	Elamadu local II	Kollam Dist.
G26	Haripad local	Alappuzha Dist.

G27	Idukki local I	Idukki Dist.
G28	Idukki local II	Idukki Dist.
G29	Kadambarakonam local	Idukki Dist.
G30	Kallicaud local	Idukki Dist.
G31	Kallicaud local II	Idukki Dist.
G32	Kalliyoor local	Thiruvananthapuram Dist.
G33	Kandalloor local	Alappuzha Dist.
G34	Kanjikuzhi local	Kottayam Dist.
G35	Kasaragod local	Kasaragod Dist.
G36	Kattampally local	Kollam Dist.
G37	Kayamkulam local	Alappuzha Dist.
G38	Kilimanoor local	Thiruvananthapuram Dist.
G39	Kochi local	Ernakulam Dist.
G40	Kollam local I	Kollam Dist.
G41	Kollam local III	Kollam Dist.
G42	Kollam local IV	Kollam Dist.
G43	Kollamcode local	Thiruvananthapuram Dist.
G44	Koovappally local	Kottayam Dist.
G45	Kottarakara local I	Kollam Dist.
G46	Kottayam local I	Kottayam Dist.
G47	Kottayam local II	Kottayam Dist.
G48	Kottayam thattathi local	Kottayam Dist.
G49	Kozha local	Kottayam Dist.
G50	Kulashegarapuram local	Kollam Dist.
G51	Kulathupuzha local I	Kollam Dist.
G52	Kulathupuzha local II	Kollam Dist.
G53	Kundamankadavu local	Thiruvananthapuram Dist.
G54	Kumil local	Idukki Dist.
G55	Madur local	Kasaragod Dist.
G56	Malappuram local II	Malappuram Dist.

G57	Manjeri local	Malappuram Dist.
G58	Mavelikkara local	Alappuzha Dist.
G59	Mukkola local	Thiruvananthapuram Dist.
G60	Muttathukonam local	Pathanamthitta Dist.
G61	Nellad local I	Ernakulam Dist.
G62	Nellad local II	Ernakulam Dist.
G63	Nellad local III	Ernakulam Dist.
G64	Nellad local VI	Ernakulam Dist.
G65	Nellanadu local	Thiruvananthapuram Dist.
G66	Nellanadu local I	Thiruvananthapuram Dist.
G67	Nellanadu local II	Thiruvananthapuram Dist.
G68	Nenmara local I	Palakkad Dist.
G69	Nenmara local II	Palakkad Dist.
G70	Nenmara local III	Palakkad Dist.
G71	Nenmara local IV	Palakkad Dist.
G72	Nenmeni local	Wayanad Dist.
G73	Neyyattinkara local	Thiruvananthapuram Dist.
G74	Nilamel local	Kollam Dist.
G75	Nileswaram local	Kasaragod Dist.
G76	Ochira local I	Kollam Dist.
G77	Ochira local II	Kollam Dist.
G78	Omallur local I	Pathanamthitta Dist.
G79	Omallur local II	Pathanamthitta Dist.
G80	Ooramana local	Ernakulam Dist.
G81	Pachalloor local	Thiruvananthapuram Dist.
G82	Padavalam payar	Thiruvananthapuram Dist.
G83	Palakkad local I	Palakkad Dist.
G84	Palakkad local II	Palakkad Dist.
G85	Palakkad local III	Palakkad Dist.
G86	Palode local	Thiruvananthapuram Dist.

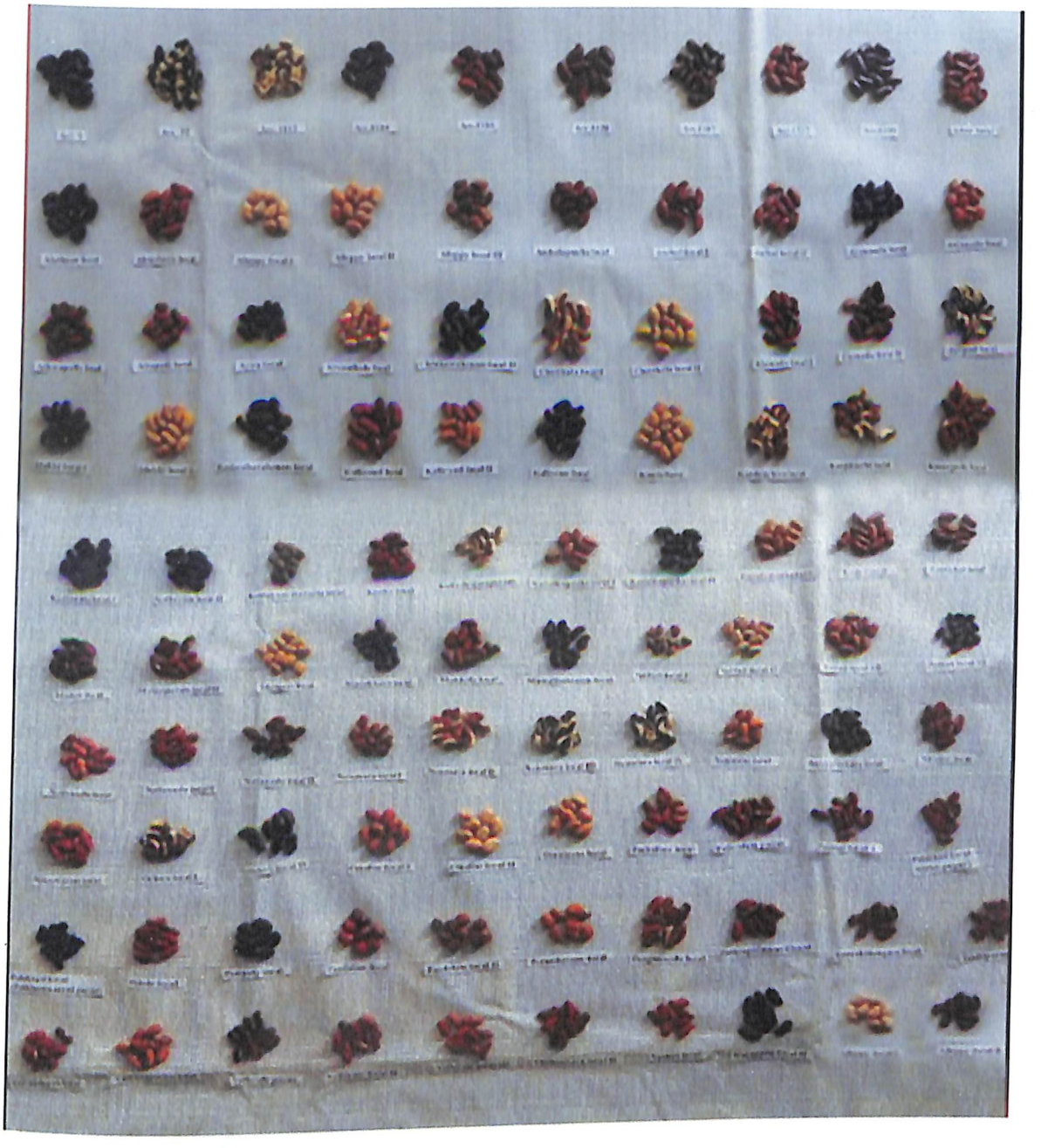

Plate 1. Seeds of germplasm used in the study

Plate 2. Field view of germplasm screening from experiment I

G87	Pampady local	Kottayam Dist.
G88	Perumbavoor local	Ernakulam Dist
G89	Pongumoodu local	Thiruvananthapuram Dist.
G90	Puthenpeedikayil local	Kottayam Dist.
G91	Ramankulangara local	Kollam Dist.
G92	Sakthipuram local	Thiruvananthapuram Dist.
G93	Trivandrum local	Thiruvananthapuram Dist.
G94	Vamanapuram local	Thiruvananthapuram Dist.
G95	Vellavalli payar	Kollam Dist.
G96	Vellayani local II	Thiruvananthapuram Dist.
G97	Vellayani local III	Thiruvananthapuram Dist.
G98	Vlathankara local II	Thiruvananthapuram Dist.
G99	Vythiri local	Wayanad Dist.
G100	Wayanadu local II	Wayanad Dist.

Seedlings were grown upto three weeks under well-irrigated conditions for establishment. To induce seedling stage moisture stress, irrigation was stopped after 21 to 25 days of initial seedling growth. Moisture was withheld until the plants showed signs of significant wilting, at which point 75 percent of genotypes were irreversibly wilted. The number of days required to reach the critical stress level was recorded. Later irrigation was restored in order to ensure the survival of the tolerant lines. After two weeks, the percentage of regeneration was calculated. The genotypes were classified as drought tolerant or susceptible based on their relative leaf water content, permanent wilting percentage, days to reach critical stress and recovery percentage.

Experiment-II

3.1.3 EVALUATION OF THE SELECTED GENOTYPES FOR DROUGHT TOLERANCE

Fifteen tolerant genotypes selected from experiment I were evaluated in the rain out shelter for confirmation of their moisture stress tolerance. Three replications of the genotypes were planted in grow bags in CRD. The land was

Plate 3. Evaluation of genotypes in rain out shelter from experiment II

well prepared, incorporating farmyard manure at 20 t ha^{-1}. Fertilizers were applied as per package of practices recommendations of Kerala Agricultural University. Water stress was imposed from flowering onwards by restricting the irrigation to once in four days at 10mm depth. Moisture content of the soil was estimated by using the thermogravimetric method. Morphological and physiological observations were made at various stages of plant development.

3.1.3.1. Cataloguing of the Germplasm

Based on the evaluation in the rain out shelter selected fifteen drought tolerant genotypes were morphologically described using IBPGR descriptor (IBPGR, 1983) for the cowpea (Appendix I). To understand the levels of similarity and dissimilarity among genotypes, the morphological descriptor data were subjected to cluster analysis using Ward's minimum variance clustering.

Experiment-III

3.1.4 PART I : DEVELOPMENT OF HYBRIDS

Seven drought tolerant genotypes selected from experiment II and three high yielding commercial varieties (Gitika, Vellayani Jyothika and Lola) were selected as lines and testers respectively. The parents were raised in crossing block by following all the recommended agronomic and crop management practices. Each of seven lines were crossed with three testers in Line x Tester (L x T) mating design to generate 21 crosses (Plate 4).

3.1.4.1. Procedure for crossing

Production of hybrids was done by the technique of artificial pollination as suggested by Myers (1996). Flower buds of the lines expected to open on the next day morning was chosen for emasculation on previous day evening. The bud was held firmly but gently between the thumb and forefinger with the keel petal on the upper side. Use small pointed forceps to cut two-thirds the width of the unopened bud in the center of the bud starting from its straight edge. Hold the upper portion of the folded petals by the thumb and index finger and gently tear off the cut segment. This leaves the upper portion of the style, stigma and

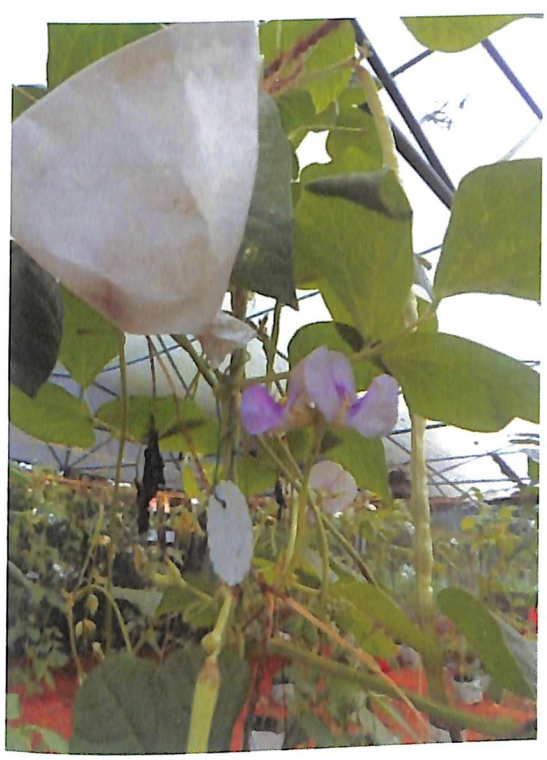

Plate 4. Raising parents and development of hybrids

stamens free. Remove all the anther sacs with scissors or forceps without any damage to style and stigma. Paper covers were used to protect the emasculated flowers.

Pollination was done in the next morning using freshly opened flowers of the tester parent. The standard and wing petal of the male flower were removed. The keel petal was gently pressed to expose the stamens covered with pollen grains. This as such was used as a brush to dust the pollen on to the stigma of the emasculated flower. The pollinated flower was then covered and the cover was retained for another 2-3 days. Proper tagging was done with all the required data. At the time of maturity, pods from crossed flowers were carefully collected crosswise, dried and stored for further studies.

3.1.5. PART II: FIELD EXPERIMENT FOR EVALUATION OF F_1 AND PARENTS

The twenty one hybrids developed through L x T along with their parents and a standard check (Arka Mangla) were evaluated for moisture stress tolerance in a field experiment. All the entries were raised in a randomized block design with 3 replications during summer season 2020. In each replication, all the entries were sown in a row with a spacing of 1.5 x 0.45 m. Recommended fertilizers and agronomic measures were followed as per the Package of Practices of Kerala Agricultural University under irrigated condition to obtain good crop stand.

3.2 Observations

3.2.1 Biometric Observations (Experiment I, II and III)

Data on the following characters were recorded from observations on five randomly selected plants from each replication and mean values were calculated.

3.2.1.1 Days to 50% flowering

Number of days taken from sowing to 50 percent flowering of the plants were recorded

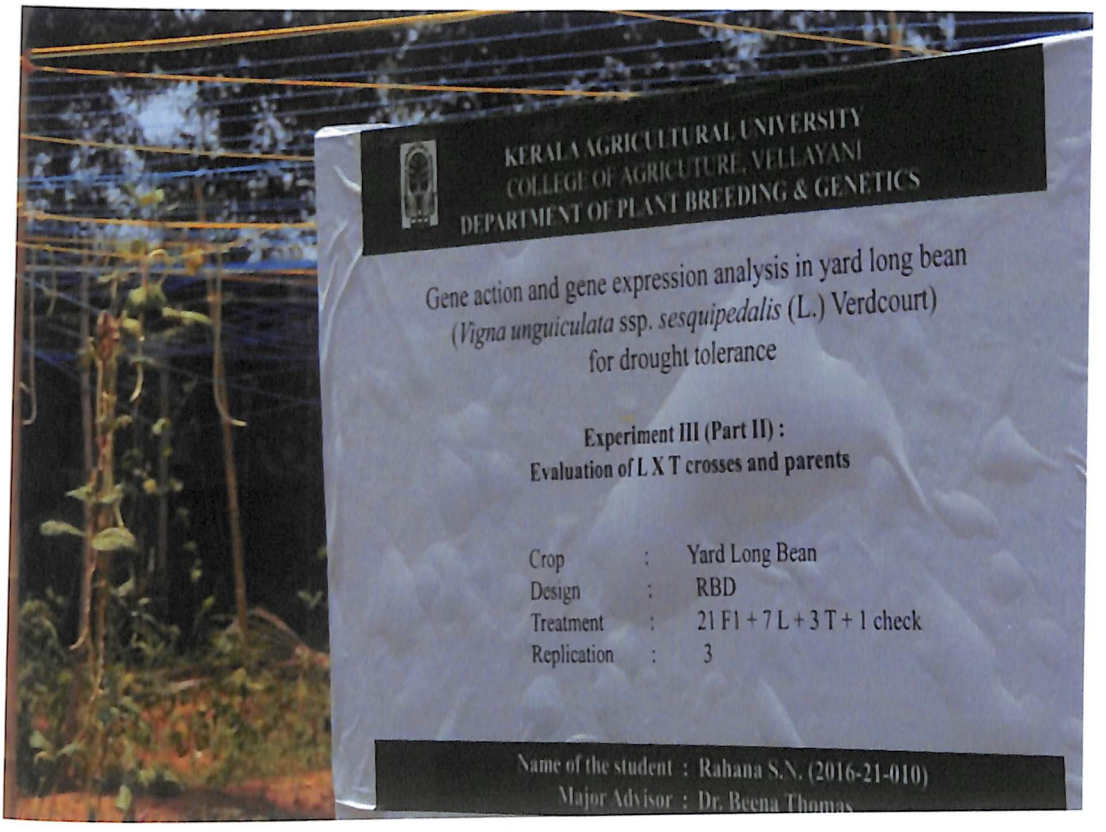

Plate 5. Field evaluation of L X T crosses and parents

3.2.1.2 Pod length (cm)

Length of five randomly selected individual pods were recorded from each observational plant

3.2.1.3 Pod width (mm)

Width of five randomly selected individual pods were recorded from each observational plant.

3.2.1.4 Pod weight (g)

Weight of five randomly selected individual pods were recorded from each observational plant.

3.2.1.5 Pods per plant

Pods obtained in each harvest from each of the observational plants were counted and recorded.

3.2.1.6 Yield per plant (g)

Weight of pods from observational plants were recorded after each harvest. Total weight of pods of each observational plant was calculated and recorded.

3.2.1.7 Vine length (m)

Length of the vine from the base of the plant to the terminal bud was measured and recorded.

3.2.1.8 Harvest Index (%)

Harvest index for each observational plant was calculated based on fresh weight basis by using the following formula.

$$\text{Harvest Index} = \frac{\text{Economic yield}}{\text{Biological yield}}$$

Total pod yield from each observational plant was recorded as the economic yield and fresh weight of all the other plant parts and the pod yield were considered as biological yield.

3.2.1.9 Crop duration (days)

Number of days taken from first harvest to last harvest was recorded.

3.2.1.10 Root depth (cm)

Rooting depth was measured from the base of the plant (collar region) to the tip of the longest root in 'cm'.

3.2.1.11 Root volume (cm^3)

Root volume was determined based on Archimedes principle by water displacement method. Roots after removing from soil carefully, cleaned thoroughly and were immersed in a measuring cylinder. The amount of water getting displaced while immersing the root was noted. The difference of the two readings gave root volume in cubic centimeter.

3.2.2 Physiological Observations

3.2.2.1 Proline content (µ moles/g)

Proline content was estimated as per the procedure described by Bates *et al.* (1973). A known amount (0.5g) of mid-leaf portion was homogenized with 10ml of 3% aqueous sulphosalicylic acid and centrifuged at 3000 rpm for 15 minutes. 2ml of the supernatant was taken and mixed with an equal amount of glacial acetic acid and acid ninhydrin. The contents were allowed to react at 100^0C for one hour in water bath. The reaction was terminated by keeping it in ice bath for 10 min. The reaction mixture was mixed with 4ml toluene using vortex mixture for 20 – 30 seconds. The chromophore containing toluene was aspirated from aqueous phase, warmed to room temperature and the optical density was read at 520 nm with toluene as blank. A standard curve was drawn using concentration verses absorbance. The concentration of proline was determined from graph and expressed as µ moles/g tissue.

Concentration of proline= {[(µg proline/ml) x ml toluene]/115.5} x (5/g sample)

where 115.5 is the molecular weight of proline.

3.2.2.2 Percentage leakage (%) and Membrane integrity (%)

Fully expanded leaves with their petiole are excised and intact in water to regain the turgidity by incubating in distilled water for 45 minutes. The leaves kept to wilt for three hours after taking the weight of turgid leaves. Leaf punches of 1 cm were taken after 40-60 percent loss of the fresh weight. Leaf punches are washed for 1 to 2 minutes to leach out their solutes from cut ends, blotted on a clean filter paper. Ten leaf punches were incubated in 20 ml distilled water for three hours. Initial leakage of the solute was recorded its absorbance at 273 nm. Final absorbance of the bathing medium was recoded at 273 nm after incubating in hot water bath (100°C) for 15 minutes.

$$\% \text{ Leakage} = \frac{\text{Initial absorbance of bathing medium}}{\text{Final absorbance of bathing medium}} \text{ X } 100$$

Membrane integrity (%) = 100- % leakage

3.2.2.3 Ascorbic acid (mg/100 g)

Estimation of ascorbic acid was done as per the procedure described by Sadasivam and Manickam (2016). Ascorbic acid otherwise known as vitamin C is present mainly in fresh vegetables and fruits. It is a water soluble and heat labile vitamin. In the volumetric method ascorbic acid reduced the 2,6-dichlorophenol indophenol dye to a colourless leuco-base. The ascorbic acid gets oxidised to dehydroascorbic acid. The dye is prepared by mixing 42 mg sodium bicarbonate and 52 mg 2,6-dichlorophenol indophenol and make up the volume to 200 ml. The dye is blue in colour while the end point is the appearance of pink colour. The dye is pink coloured in acid medium. Oxalic acid is used as the titrating medium. The amount of dye consumed is equivalent to the amount of ascorbic acid.

Procedure

For preparing standard solution, pipette out 5 ml of the working standard solution of oxalic acid into a 100 ml conical flask. Add 10 ml of 4% oxalic acid and titrate against the dye (V_1 ml). For sample analysis, ascorbic acid present in fresh pods (1 g) is extracted in 4% oxalic acid and made upto 100 ml volume and

centrifuge. Pipette out 5 ml of the supernatant, add 10 ml of 4% oxalic acid and titrate against the dye (V_2 ml).

Calculation

$$\text{Amount of ascorbic acid mg/100g sample} = \frac{0.5\text{ mg}}{\text{V1 ml}} \text{ X } \frac{\text{V2}}{\text{5ml}} \text{ X } \frac{\text{100 ml}}{\text{weight of the sample}} \text{ X 100}$$

3.2.2.4 Canopy temperature (^{0}C)

Canopy temperature was measured by using infra-red thermometer at 12 noon and expressed in degree Celsius

3.2.2.5 Relative water content (%)

Relative leaf water content (RWC) was measured based on the method described by Turner (1981). RWC measurement was taken from fully expanded leaves. A known weight of the sample was taken and then the leaf discs were immersed in distilled water for about 2 hours. After 2 hours, the leaves were removed from water and the adhering water was blotted off and the turgid weight was recorded. The samples were dried in oven at 70°C for about 48 hours and dry weight was recorded. The relative leaf water content was calculated using the following formula and expressed as per cent.

$$\text{Relative Water Content} = \frac{\text{Fresh Weight} - \text{Dry Weight}}{\text{Turgid Weight} - \text{Dry Weight}} \text{ X 100}$$

3.2.2.6 Water requirement (m3)

Water requirement is the quantity of water supplied at various stages of the crop growth.

3.2.2.7 Water Use Efficiency (kg/m^3)

Water use efficiency (WUE) is defined as the amount of water consumed to produce a unit weight of biomass.

$$\text{Water Use Efficiency} = \frac{\text{Yield}}{\text{Water Requirement}}$$

3.2.2.8 Stomatal conductance (m H_2O moles/m2/sec)

Stomatal conductance was measured at morning time between 9 am and 11 am using Infrared Gas Analyser (IRGA) and were expressed in m H_2O moles m^{-2} s^{-1}.

3.2.2.9 Permanent wilting percentage

The permanent wilting point is the point when there is no water available to the plant. The soil moisture content was estimated every 24 hours from the day of withholding irrigation until the peak wilting changes were observed. The soil moisture content at that point was recorded as permanent wilting percentage. Soil moisture was determined by gravimetric method, where a known weight of the fresh soil samples collected were oven dried at 105 ^{O}C until constant dry weight was obtained and the loss in weight was expressed as percentage.

Soil moisture = (fresh weight - dry weight) / dry weight

3.2.2.10 Number of days for reaching critical stress level

Withdrawing irrigation after 21 to 25 days of initial seedling growth induced seedling stage moisture stress. Moisture was withheld until the plants showed signs of significant wilting or had a relative leaf water content of 65 percent. The number of days it took to reach the critical stress level was recorded.

3.2.2.11 Plant recovery percentage

The number of plants recovered per genotype after rewatering following a period of moisture stress.

3.2.3 Soil moisture studies in the field

Soil moisture was determined by gravimetric method, where a known weight of the fresh soil samples collected from the plant rows were oven dried at 105°C until constant dry weight was obtained and the loss in weight was expressed as percentage. The field study was conducted during March when the average day time temperature ranged from 30°C - 34°C. In the field seedling were raised in progeny rows with five plants per row.

Experiment-IV

3.3. MOLECULAR ANALYSIS

3.3.1 Gene expression study using Quantitative Real-Time PCR

In the present study, Quantitative Real-Time PCR (qRT-PCR) assay was performed for determining quantitative changes in gene expression for drought

tolerance at the molecular level. Selected drought tolerant and susceptible yard long bean genotypes and hybrids from the study grown under water stress and control conditions were used for the gene expression analysis. The control plants were watered regularly.

3.3.2 Primer design

The primer pairs for the major drought responsive genes DREB1 and NCED1 were designed using Primer3Plus software (http://www.bioinformatics.nl/primer3plus). The software comprises of an input box for sequence information and they are pasted in FASTA or EMBL format. The region required for amplification was selected using '[ ]' indicated below the box. Entered the Default parameters such as length: 20-22 bp, GC%: 50-60%, melting temperature: 55-65^{0}c etc.

VuUbq involved in protein ubiquitination pathway served as an internal control (reference gene) to normalize the data. The specificity of primers was checked through NCBI Primer-BLAST software. The software was specifically designed by NCBI which utilizes the BLAST program and global alignment algorithm to pick primers against the target gene sequence.

3.3.3 Isolation of RNA

Total RNA was isolated from the leaf tissue using the total RNA isolation kit according to the manufacture instruction (Product code 10296010; Invitrogen, USA). The reagents, glassware, forceps, mortar and pestle, microtips and microfuge tubes were autoclaved. All the reagents used were prepared using DEPC (Diethyl pyrocarbonate) treated water. DEPC is also called diethyl dicarbonate, used to inactivate RNase enzymes in water. The DEPC treated water was prepared by adding 1 ml of DEPC to 1 litre of water (0.1%) for at least 2 hours at 37°C and then autoclaved at least 15 min to inactivate traces of DEPC.

Total RNA was extracted from the leaf samples of both control and stress induced yard long bean. The samples were ground in a chilled mortar and pestle that had been wiped with RNAase zap to eliminate all traces of RNAase. 100 mg

of the leaf sample was ground into fine powder using liquid nitrogen. 1ml of TRIzol reagent was added to the powdered samples and mixed gently to homogenize the mixture and incubated at ambient temperature for 5 minutes. Addition of TRIzol solution causes the disruption of cells and the release of RNA.

The content was transferred to pre-chilled microfuge tube. 200 µl of chloroform was added which was shaken vigorously for about 15 seconds and incubated for 5 minutes at room temperature. The microfuge tubes were kept in ice for 10 minutes and centrifuged at 12,000 g for 15 minutes at 4^0C. Chloroform extraction following centrifugation, holds the RNA in the upper aqueous phase of the microfuge tube. The interphase and the lower organic phase contain proteins and DNA. The aqueous phase was transferred to a sterile microfuge tube (1.5 ml). 500 µl of 100% ice cold isopropanol was added to the tube and mixed by inverting the tube slowly. It was incubated at room temperature for 10 minutes and again centrifuged at 12,000 g for 10 minutes at 4°C. Supernatant was discarded and pellet thus obtained was washed with 200 µl of 75% of ethanol. It was then centrifuged at 7,500 g for 5 minutes at 4°C. The supernatant was removed and the RNA pellet was air dried in the laminar air flow chamber. The pellet suspended in 40 µl of sterile TE buffer and kept for incubation at about 55-60^0C for 10 minutes. For further usage, the extracted RNA was stored at -80^0C.

3.3.4 RNA quantification and assessment of quality

Quantification and quality assessment of the isolated RNA samples were determined by using the Qubit HS RNA assay kit (Thermo Fisher Scientific) with a Qubit 3.0 Fluorometer (Qubit® 3.0; Life Technologies, USA), following manufacturer's instructions. Qubit system is supplied with fluorescent dyes that bind specifically to RNA, reading it precisely and provide more accurate quantification.

The RNA assay kit contains concentrated assay reagent, dilution buffer and prediluted RNA standards. The Qubit® RNA HS Assay requires 2 standards. The final volume in each tube must be 200 µl. Each standard tube requires 190 µl

of Qubit® working solution and each sample tube requires anywhere from 180–199 µl. The sample tube was prepared by diluting the reagent (1µl) using the buffer (198 µl) provided and adding1µl of sample. The samples were incubated in dark condition for 5 minutes and read the concentration using the Qubit® Fluorometer. The isolated RNA was resuspended in sterile TE buffer and stored at -80^{0}C.

The quality of the RNA samples was determined by absorbance in spectrophotometer at the wavelength of 260 and 280 nm. A_{260}/A_{280} ratio indicated the quality of RNA. Ratio ~ 2, indicates good quality RNA.

3.3.5 Complementary DNA synthesis (cDNA)

The RNA of both control and stress plants were converted to complementary DNA using the cDNA preparation kit (Thermoscientific Verso cDNA Synthesis kit, Product code- AB1453A) according to the manufacturer's protocol. The reaction mixtures and RNA were maintained in ice throughout the preparation to avoid degradation. The kit contained verso reverse transcriptase which could generate long cDNA strands, oligo dT primer, random hexamer, RNAase inhibitor to protect RNA templates from degradation and RT enhancer which help to remove DNA contamination. A 20 µl reaction mix was prepared using the following components:

Components	Volume (µl)
cDNA synthesis buffer	4
dNTP mix	2
RT enhancer	1
Verso enzyme mix	1
Template (RNA) + primer mix	2
Nuclease free water	10
Total volume	20

The reaction mix was briefly centrifuged and subjected for PCR. The tube was incubated for 30 minutes at 42^0C (1 cycle) followed by another incubation at 92^0C for 2 minutes using a reverse- transcriptase PCR and the synthesized cDNA samples were maintained at -20^0C.

3.3.6 Quantitative Real-Time PCR

The synthesized cDNA was diluted tenfold before being utilised as a template for qRT-PCR. The cDNA was used as templates in PCR with Ubiquitin primer as a control and other primers to be studied.

The cocktail was prepared as follows:

Sl.No.	Components	Volume added per tube (µl)
1	10 X Taq buffer	2.50
2	dNTPs (10 mM)	0.60
3	Primer forward	1.00
4	Primer reverse	1.00
5	Template (cDNA)	1.00
6	Taq polymerase	0.25
7	milliQ water	18.65
	Total	25.00

Reaction set up : Real Time PCR was performed in a thermal cycler (Light cycler 96, Roche, Switzerland) with SYBR Green Master Mix (Applied Biosystem, Life technologies) was programmed as follows:

	Temperature		Time	Cycles
Initial denaturation	95 °C		2 min	1
Denaturation	94 °C		10 sec	35
Annealing	55 °C		1 min	
Extension	72 °C		1 min	
Final extension	72 °C		7 min	1
Hold	4 °C			

Two gene specific primer sets were used and all the reactions were performed in triplicate. Ubiquitin gene was kept as the internal control.

3.3.7 Gene expression analysis

The result obtained are expressed as fold change *i.e.* increase or decrease in expression of genes. The relative changes in gene expression from real-time quantitative PCR experiments were analysed using the $\Delta\Delta C_T$ method (Rao *et al.*, 2013). The calculation is as follows:

ΔC_T (Control) = C_T (Target gene of control) - C_T (Reference gene)

ΔC_T (Stress) = C_T (Target gene of treatment) - C_T (Reference gene)

$\Delta\Delta C_T = \Delta C_T$ (Stress) - ΔC_T (Control)

The fold difference between the stress and control in expression of genes were calculated by $2^{-\Delta\Delta CT}$ to relate the drought responsiveness of genotypes.

3.3.8 Electrophoresis

The PCR products were electrophoresed in 2% agarose gel and bands visualized under UV. The cDNA amplified by the real time PCR was Eluted and purified from agarose gel using Quagen elution kit 8550 according to instruction manual.

3.3.9 Sequence analysis

The PCR products were sequenced. Sanger Sequencing was used to determine the nucleotide sequence of the cDNA. The sequences were subjected to in silico analysis, using Nucleotide BLAST. The deduction of the nucleotide sequence and the homology of the sequences with other known sequences in the NCBI database were carried out using BLAST (http://www.ncbi.nlm.nih.org/Blast). Following analysis, the sequences generated by the study were submitted to the BankIt NCBI (https://submit.ncbi.nlm.nih.gov/).

3.4. Statistical Analysis

The mean value for each treatment per replication from experiment I and II were subjected to analysis of variance for completely randomized design as described by Panse and Sukhatme (1985) using the SAS program (SAS institute Inc., 1990). The data after statistical analysis were used to test the significance of difference among genotypes and interpretation of the results.

3.4.1 Completely Randomized Design (CRD)

CRD analysis was performed by using the following formulae.

ANOVA for CRD analysis

Sources	df	Sum of Squares (SS)	Mean sum of Squares (MSS)	Observed F
Between Treatments	t-1	SST	MST	MST/MSE
Within treatments (Error)	t(r-1)	SSE	MSE	
Total	tr-1	TSS		

Standard Error difference (SE(d)) = $\sqrt{\frac{2MSE}{ri}}$

C.D. = t X SE (d)

and t is the critical t value for error degrees of freedom at 5% level.

3.4.2 Descriptive Statistical Analysis

The descriptive statistics for each character were calculated as follows:

3.4.2.1 Range

It records highest and lowest value in the observed value for each character in parents and the segregating populations.

3.4.2.2 Arithmetic Mean

It is calculated by the following formula:

$X = \Sigma X/N$

Where,

ΣX =sum of all the observations,

N = total number of observations.

3.4.2.3 Standard Deviation

$S.D = \sqrt{\Sigma(X-\underline{X})^2/N}$

Where,

X = Individual reading

$\bar{X}$ = mean

N = sample size

3.4.2.4 Standard Error

$S.E = S.D/\sqrt{N}$

Where,

S.D =Standard Deviation

N = total number of observations.

3.4.3 Analysis of Variance (ANOVA)

The mean data of quantitative characters collected from parents, hybrids and checks of experiment III were subjected to statistical analysis to estimate the combining ability of parents and hybrids and heterosis of hybrids using SAS statistical analysis software.

The mean data of 21 hybrids and their parents for each quantitative character were tabulated and analysis of variance, estimation of standard error and critical difference were worked out individually for all the characters by adopting the method suggested by Panse and Sukhatme (1985) as given below:

Source	df	SS	MS	Expected MS
Replication	(r-1)	RSS	RMS	

Genotypes	(g-1)	GSS	GMS (MS_1)	$\sigma^2e + r\sigma^2g$
Error	(r-1)(g-1)	ESS	EMS (MS_2)	σ^2e
Total	(rg-1)	TSS		

Where,

r = Number of replications g = Number of genotypes or varieties

MS_1 = Mean squares for genotype (*i.e.* variance and error variance)

MS_2 = Mean squares for error variance

σ^2e = Environmental variance (error variance) σ^2g = Genotypic variance

Significance of treatment mean squares and replication mean squares were tested by comparing with error mean squares and referring 'F' table values at 5 and 1 per cent levels of probability. Critical difference (CD) = SE (D) ×'t' at error degrees of freedom at 5 % level.

3.4.4 Estimation of combining ability

The combining ability analysis was assessed by line × tester method described by Kempthorne (1956). The general combining ability of the parents and specific combining ability of the hybrids were estimated. The mean squares due to different sources of variations and their genetic expectations were estimated as follows:

Table 2. Analysis of variance for Line x Tester design

Source	Df	SS	MS	Expectation of mean squares
Replication	(r-1)	RSS		
Crosses	(lt-1)	CSS		
Lines	(l-1)	LSS	MS_1	$\sigma^2e + r(Cov.FS - 2Cov.HS) - rt(Cov.HS)$
Testers	(t-1)	TSS	MS_2	$\sigma^2e + r(Cov.FS - 2Cov.HS) - rl(Cov.HS)$
L x T interactions	(l-t)(t-1)	LxTSS	MS_3	$\sigma^2e + r(Cov.FS - 2Cov.HS)$
Error	(r-1)(lt-1)	ESS	MS_4	σ^2e
Total	(rlt-1)	CSS		

Where,

r - Number of replications

l - Number of lines

t - Number of testers

Cov.HS - Covariance of Half sib

Cov.FS - Covariance of Full sib

From the genetic expectation of mean squares, the covariances of full sibs (Cov.F.S) and half sibs (Cov.H.S) were estimated as given below:

$$\text{Cov. HS} = \frac{MS_1 + MS_2 - 2MS_3}{r(1+t)}$$

$$\text{Cov. FS} - 2\text{Cov. HS} = \frac{[MS_3 - MS_4]}{r}$$

From the above parameters, general and specific combining ability variances were computed as follows:

GCA variance (σ^2 GCA) = Cov.HS

SCA variance (σ^2 SCA) = Cov.FS – 2Cov.HS

3.4.5 Gene Action

From the above variances of GCA and SCA the gene action was calculated as follows (Assuming there is no epistasis, when the parents are inbreds or purelines):

Additive genetic variance, σ^2 D = 2Cov.HS or 2 σ^2GCA

Non-additive (dominance genetic variance), σ^2H = Cov.FS – 2Cov.HS or σ^2 SCA

σ^2GCA/ σ^2SCA = 1 (equal proportion)

σ^2GCA/ σ^2SCA greater than 1 (additive)

σ^2GCA/ σ^2SCA less than 1 (dominance or non-additive)

If GCA variances are higher than SCA variances, preponderance of additive gene action and progeny selection will be effective.

If SCA variances are higher, preponderance of non-additive gene action (dominance and epistasis), heterosis breeding will be effective.

If both are equal, both are equally important and reciprocal recurrent selection may be used.

3.4.6 Proportional contribution of lines, testers and their interaction to total variance

$$\text{Contribution of lines} = \frac{\text{SS (lines)}}{\text{SS (hybrids)}} \times 100$$

$$\text{Contribution of testers} = \frac{\text{SS (testers)}}{\text{SS (hybrids)}} \times 100$$

$$\text{Contribution of interaction} = \frac{\text{SS (l x t)}}{\text{SS (hybrids)}} \times 100$$

3.4.7 Combining ability effects

The variation among the hybrids was further partitioned into genetic components attributed to general combining ability (gca) and specific combining ability (sca) as per the method suggested by Kempthorne (1956).

The gca and sca effects of parents and hybrids were estimated based on the following model.

$$X_{ijk} = \mu + g_i + g_j + S_{ij} + e_{ijk}$$

Where,

X_{ijk} - Value of ijk^{th} observation

μ - Population mean

g_i - gca effect of i^{th} line

g_j - gca effect of j^{th} tester

S_{ij} - sca effect of ij^{th} cross

e_{ijk} - Error effects associated with ijk^{th} observation

i, j and k - Number of lines, testers and replications respectively

The individual effects of gca and sca were obtained from the two way table of lines vs testers in which each figure was calculated as follows:

$$\mu = \frac{x_{...}}{rlt}$$

$$\text{gca effect of lines } (g_i) = \frac{x_{i...}}{rt} - \frac{x_{...}}{rlt}$$

gca effect of testers $(g_j) = \frac{x_{.j.}}{rl} - \frac{x_{...}}{rlt}$

sca effect of hybrids $(g_j) = \frac{x_{ij.}}{r} - \frac{x_{i..}}{rt} - \frac{x_{.j.}}{rl} - \frac{x_{...}}{rlt}$

Where,

X... - Sum of all the hybrid combinations over replications

$X_{i..}$ - Sum of i^{th} line over 't' testers and 'r'replications

$X_{.j.}$ - Sum of j^{th} tester over 'l' lines and 'r' replications

$X_{ij.}$ - Sum of ij^{th} hybrid over 'r'replications

l,t and r - Number of lines, testers and replications respectively

The significance of various effects was tested using 't'test. This calculated 't'value can be compared with table't' value at error degrees of freedom.

$$t_{cal} = \frac{\text{Effect}}{SE}$$

The standard errors pertaining to gca effects of parents and sca effects of crosses were calculated using the following formulae:

SE (g_i) lines = $\sqrt{\frac{EMS}{rt}}$

SE (g_j) testers = $\sqrt{\frac{EMS}{rl}}$

SE (S_{ij}) crosses = $\sqrt{\frac{EMS}{r}}$

Where,

SE = Standard Error

EMS = Error Mean Square

't = Parameter / SE

This calculated 't' value can be compared with table 't' value at error degrees of freedom to test the significance.

3.4.8 Estimation of Heterosis

The overall mean value for each parent and hybrid for each character was taken for estimation of heterosis. The magnitude of heterosis in hybrids was

expressed as percentage increase or decrease of a character over mid parent (di), better parent (dii) and standard check (diii) and was estimated using the following formula (Turner ,1953):

a) Heterosis over mid parental value [Relative Heterosis, di]

Relative heterosis was estimated as per cent deviation of the mean F_1 performance over the mean performance of the mid parents.

$$\text{Relative Heterosis (di)} = \frac{\overline{F_1} - \overline{MP}}{\overline{MP}} \times 100$$

$\overline{F_1}$ - the mean performance of hybrid

$\overline{MP}$ - the mean mid parental value i.e.,$(P_1 + P_2) / 2$

P1, P2 - the mean values of the first and the second parent respectively.

b) Heterosis over better parent [Heterobeltiosis, dii]

Heterobeltiosis was estimated as per cent deviation of the mean F_1 performance over the mean performance of the better parent.

$$\text{Heterobeltiosis (dii)} = \frac{\overline{F_1} - \overline{BP}}{\overline{BP}} \times 100$$

Where,

$\overline{BP}$ - The mean of better parental value

c) Heterosis over the standard variety [Standard Heterosis, diii]

Standard heterosis was estimated as per cent deviation of the mean F1 performance over the mean performance of the standard variety or hybrid.

$$\text{Standard Heterosis (diii)} = = \frac{\overline{F_1} - \overline{SP}}{\overline{SP}} \times 100$$

Where,

$\overline{SP}$ - The mean of Standard check

The significance of Relative heterosis, Heterobeltiosis and Standard heterosis were tested by the formulae suggested by Turner (1953).

$$\text{'t' for Relative Heterosis} = \frac{\overline{F_1} - \overline{MP}}{\sqrt{\frac{\sigma_e^2}{r} \times \frac{3}{2}}} \times 100$$

't' for Heterobeltiosis = $\frac{\overline{F_1} - \overline{BP}}{\sqrt{\frac{\sigma_e^2}{r} \times 2}} \times 100$

't' for Standard Heterosis = $\frac{\overline{F_1} - \overline{SP}}{\sqrt{\frac{\sigma_e^2}{r} \times 2}} \times 100$

Where,

σ_e^2 - Error variance (EMS)

r - Number of replications

$$\text{Cov. FS} = \frac{[MS_1 + MS_2 + MS_3 - 3MS_4] + [6r\ \text{Cov. HS} - r(1+t)\ \text{Cov. HS}]}{3r}$$

$$\text{Cov. FS} - 2\text{Cov. HS} = \frac{[MS_3 - MS_4]}{r}$$

From the above parameters, general and specific combining ability variances were computed as follows:

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$$\text{Contribution of testers} = \frac{\text{SS (testers)}}{\text{SS (hybrids)}} \times 100$$

$$\text{Contribution of interaction} = \frac{\text{SS (l x t)}}{\text{SS (hybrids)}} \times 100$$

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The variation among the hybrids was further partitioned into genetic components attributed to general combining ability (gca) and specific combining ability (sca) as per the method suggested by Kempthorne (1956).

The gca and sca effects of parents and hybrids were estimated based on the following model.

$$X_{ijk} = \mu + g_i + g_j + S_{ij} + e_{ijk}$$

Where,

X_{ijk} - Value of ijk^{th} observation

μ - Population mean

g_i - gca effect of i^{th} line

g_j - gca effect of j^{th} tester

S_{ij} - sca effect of ij^{th} cross

e_{ijk} - Error effects associated with ijk^{th} observation

i, j and k - Number of lines, testers and replications respectively

The individual effects of gca and sca were obtained from the two way table of lines vs testers in which each figure was calculated as follows:

$$\mu = \frac{X\ldots}{rlt}$$

gca effect of lines $(g_i) = \frac{x_{i\ldots}}{rt} - \frac{x_{\ldots}}{rlt}$

gca effect of testers $(g_j) = \frac{x_{.j.}}{rl} - \frac{x_{\ldots}}{rlt}$

sca effect of hybrids $(g_j) = \frac{x_{ij.}}{r} - \frac{x_{i..}}{rt} - \frac{x_{.j.}}{rl} - \frac{x_{\ldots}}{rlt}$

Where,

X... - Sum of all the hybrid combinations over replications

$X_{i..}$ - Sum of i^{th} line over 't' testers and 'r'replications

$X_{.j.}$ - Sum of j^{th} tester over 'l' lines and 'r' replications

$X_{ij.}$ - Sum of ij^{th} hybrid over 'r'replications

l,t and r - Number of lines, testers and replications respectively

The significance of various effects was tested using 't'test. This calculated 't'value can be compared with table't' value at error degrees of freedom.

$$t_{cal} = \frac{Effect}{SE}$$

The standard errors pertaining to gca effects of parents and sca effects of crosses were calculated using the following formulae:

SE (g_i) lines = $\sqrt{\frac{EMS}{rt}}$

SE (g_j) testers = $\sqrt{\frac{EMS}{rl}}$

SE (S_{ij}) crosses = $\sqrt{\frac{EMS}{r}}$

Where,

SE = Standard Error

EMS = Error Mean Square

't = Parameter / SE

This calculated 't' value can be compared with table 't' value at error degrees of freedom to test the significance.

3.4.8 Estimation of Heterosis

The overall mean value for each parent and hybrid for each character was taken for estimation of heterosis. The magnitude of heterosis in hybrids was expressed as percentage increase or decrease of a character over mid parent (di), better parent (dii) and standard check (diii) and was estimated using the following formula (Turner ,1953):

d) Heterosis over mid parental value [Relative Heterosis, di]

Relative heterosis was estimated as per cent deviation of the mean F_1 performance over the mean performance of the mid parents.

$$\text{Relative Heterosis (di)} = \frac{\overline{F_1} - \overline{MP}}{\overline{MP}} \times 100$$

$\overline{F_1}$ - the mean performance of hybrid

$\overline{MP}$ - the mean mid parental value i.e.,$(P_1 + P_2) / 2$

P1, P2 - the mean values of the first and the second parent respectively.

e) Heterosis over better parent [Heterobeltiosis, dii]

Heterobeltiosis was estimated as per cent deviation of the mean F_1 performance over the mean performance of the better parent.

$$\text{Heterobeltiosis (dii)} = \frac{\overline{F_1} - \overline{BP}}{\overline{BP}} \times 100$$

Where,

$\overline{BP}$ - The mean of better parental value

f) Heterosis over the standard variety [Standard Heterosis, diii]

Standard heterosis was estimated as per cent deviation of the mean F1 performance over the mean performance of the standard variety or hybrid.

$$\text{Standard Heterosis (diii)} = = \frac{\overline{F_1} - \overline{SP}}{\overline{SP}} \times 100$$

Where,

$\overline{SP}$ - The mean of Standard check

The significance of Relative heterosis, Heterobeltiosis and Standard heterosis were tested by the formulae suggested by Turner (1953).

't' for Relative Heterosis = $\frac{\overline{F_1} - \overline{MP}}{\sqrt{\frac{\sigma_e^2}{r} \times \frac{3}{2}}} \times 100$

't' for Heterobeltiosis = $\frac{\overline{F_1} - \overline{BP}}{\sqrt{\frac{\sigma_e^2}{r} \times 2}} \times 100$

't' for Standard Heterosis = $\frac{\overline{F_1} - \overline{SP}}{\sqrt{\frac{\sigma_e^2}{r} \times 2}} \times 100$

Where,

σ_e^2 - Error variance (EMS)

r - Number of replications

RESULTS

4. RESULTS

The result obtained from various experiments of the present investigation are given below.

Experiment I

4.1 SCREENING GERMPLASM FOR DROUGHT TOLERANCE AT THE SEEDLING STAGE

The performance of hundred yard long bean genotypes were evaluated for drought tolerance in the field at the seedling stage (Plate 2). The recorded observations were analysed statistically and the results are presented.

4.1.1. Mean performance

The mean data of the 100 genotypes collected for all the drought responsive traits namely number of days for reaching critical stress level, relative leaf water content, permanent wilting percentage and plant recovery percentage are presented in the table 3. Results obtained showed significant differences among genotypes for all traits under drought stress, revealed a wide range of variation for all the characters. Fifteen drought tolerant genotypes were selected for the next level of field evaluation (table 4).

Number of days for reaching critical stress level varied from 3.5 to 9 days. The mean critical day for distinguishing water stress tolerant and susceptible genotype was shown to be 6days. The longest day for reaching the critical stress level was reported by genotype G46 (9 days).

The highest relative leaf water content (79.09%) was recorded in the genotype G14,which was on par with the genotypes G50 (78.42%),G89 (78.23%),G51 (77.68%) and G60 (77.36%).The relative water content was minimum for the genotype G61 (57.73%).

Table 3. Mean performance for drought tolerant traits in 100 yard long bean genotypes

Treatment No.	Genotypes	RLW	PWP	Days for reaching critical stress	Recovery percentage
G 1	Acc. 5	73.83	15.5	7.5	35
G 2	Acc. 32	63.225	15.45	7.5	5
G 3	Acc. 1112	64.59	19	3.5	0
G 4	Acc.1337	67.43	17.35	5.5	0
G 5	Acc.1339	69.41	14.3	8.5	35
G 6	Adoor local	69.03	14.25	8.5	40
G 7	Alathoor local	61.275	17.4	5.5	0
G 8	Alenchery local	60.36	15.5	8	0
G 9	Alleppy local I	61.29	19	4	0
G 10	Alleppy local II	60.66	19	4	0
G 11	Alleppy local III	65.17	19	4	0
G 12	Ambalapuzha local	61.48	18.2	4.5	0
G 13	Anchal local I	67.345	14.3	8.5	0
G 14	Anchal local II	79.095	14.4	6	30
G 15	Aranmula local	76.415	14.3	8.5	40
G 16	Aryanadu local	67.165	18.95	3.5	0
G 17	Athirapally local	61.4	16.7	6.5	0
G 18	Attappady local	62.28	18.95	4	0
G 19	Ayira local	60.07	16.7	6.5	5
G 20	Ayyanthole local	59.495	18.15	5	0
G 21	Chenkottukonam local II	69.385	19	3.5	10
G 22	Cherthala local I	66.33	19	3.5	0
G 23	Cherthala local II	62.345	19	4	0
G 24	Elamadu local I	76.33	14.3	8.5	40
G 25	Elamadu local II	63.14	19	3.5	0
G 26	Haripad local	62.4	19	4	0
G 27	Idukki local I	67.49	19	3.5	0
G 28	Idukki local II	65.275	16.7	6.5	5
G 29	Kadambarakonam local	70.525	19	3.5	0
G 30	Kallicaud local	63.42	18.2	4.5	0
G 31	Kallicaud local II	68.53	19	4	0
G 32	Kalliyoor local	71.91	15.5	8	15
G 33	Kandalloor local	62.38	19	4	0
G 34	Kanjikuzhi local	68.255	19	3.5	0
G 35	Kasargode local	64.025	14.3	8.5	5
G 36	Kattampally local	75.285	15.45	8	25
G 37	Kayamkulam local	60.285	18.2	4.5	0

Table 3. Continued

Treatment No.	Genotypes	RLW	PWP	Days for reaching critical stress	Recovery percentage
G 38	Kilimanoor local	71.695	15.5	7.5	10
G 39	Kochi local	66.075	19	3.5	0
G 40	Kollam local I	68.6	19	4	0
G 41	Kollam local III	67.38	19	4	0
G 42	Kollam local IV	69.23	14.3	8.5	25
G 43	Kollamcode local	66.735	19	3.5	0
G 44	Koovappally local	64.305	17.35	5.5	0
G 45	Kottarakara local I	75.615	15.5	7.5	30
G 46	Kottayam local I	67.815	14.3	9	35
G 47	Kottayam local II	64.365	17.35	5.5	0
G 48	Kottayam thattathi local	66.82	19	3.5	0
G 49	Kozha local	73.66	15.5	7.5	15
G 50	Kulashegarapuram local	78.425	14.65	7	40
G 51	Kulathupuzha local I	77.68	15.5	8	35
G 52	Kulathupuzha local II	60.27	15.5	7.5	0
G 53	Kundamankadavu local	63.265	19	4	0
G 54	Kumil local	59.71	18.2	4.5	5
G 55	Madur local	62.38	17.4	6	10
G 56	Malappuram local II	64.39	17.4	5.5	0
G 57	Manjeri local	63.275	19	3.5	0
G 58	Mavelikara local	61.775	19	3.5	0
G 59	Mukkola local	63.325	18.95	3.5	0
G 60	Muttathukonam local	77.365	14.3	8.5	35
G 61	Nellad local I	57.735	19	3.5	0
G 62	Nellad local II	59.165	19	3.5	0
G 63	Nellad local III	63.265	18.2	5	0
G 64	Nellad local VI	64.485	19	3.5	0
G 65	Nellanadu local	58.06	15.5	7.5	5
G 66	Nellanadu local I	57.77	16.7	7	10
G 67	Nellanadu local II	63.115	18.2	5	0
G 68	Nenmara local I	64.285	18.2	5	0
G 69	Nenmara local II	65.25	16.65	6.5	0
G 70	Nenmara local III	66.4	19	4	0
G 71	Nenmara local IV	58.445	17.35	5.5	0
G 72	Nenmeni local	62.675	19	3.5	0
G 73	Neyyattinkara local	61.35	15.5	7.5	5
G 74	Nilamel local	75.365	14.3	8.5	35

Table 3. Continued

Treatment No.	Genotypes	RLW	PWP	Days for reaching critical stress	Recovery percentage
G 75	Nileswaram local	58.415	17.4	6	0
G 76	Ochira local I	63.605	17.4	6	0
G 77	Ochira local II	59.845	19	4	0
G 78	Omallur local I	61.835	19	3.5	0
G 79	Omallur local II	61.855	18.95	4	0
G 80	Ooramana local	64.71	18.2	4.5	0
G 81	Pachalloor local	63.49	16.7	6.5	5
G 82	Padavalampayar	61.015	18.2	4.5	0
G 83	Palakkad local I	58.65	17.35	5.5	0
G 84	Palakkad local II	67.37	17.4	6	0
G 85	Palakkad local III	61.66	15.45	8	5
G 86	Palode local	58.835	18.15	5	0
G 87	Pampady local	61.83	18.95	3.5	0
G 88	Perumbavoor local	62.5	19	4	0
G 89	Pongumoodu local	78.235	14.3	8.5	45
G 90	Puthenpeedikayil local	63.475	15.45	8	0
G 91	Ramankulangara local	61.385	15.5	8	0
G 92	Sakthipuram local	68.6	14.3	8.5	10
G 93	Trivandrum local	69.295	17.35	6	0
G 94	Vamanapuram local	68.85	18.2	5	0
G 95	Vellavallipayar	65.7	16.65	6.5	0
G 96	Vellayani local II	64.79	19	3.5	0
G 97	Vellayani local III	69.405	19	4	0
G 98	Vlathankara local II	62.235	19	4	0
G 99	Vythiri local	63.285	16.7	7	0
G 100	Wayanadu local II	63.44	18.2	5	0
Mean		65.44	17.33	5.56	6.5
SE		0.358	0.095	0.655	1.413
CD (0.05)		1.187	0.614	1.605	2.426

RLW- Relative leaf water, PWP- Permanent wilting percentage

Table 4. List of drought tolerant genotypes selected for experiment II

Treatment No.	Tolerant Genotypes	
A1	G1	Acc 5
A2	G5	Acc 1339
A3	G6	Adoor local
A4	G14	Anchal local II
A5	G15	Aranmula local
A6	G24	Elamadu local I
A7	G36	Kattampally local
A8	G42	Kollam local IV
A9	G45	Kottarakara local I
A10	G46	Kottayam local I
A11	G50	Kulashegarapuram local
A12	G51	Kulathupuzha local I
A13	G60	Muttathukonam local
A14	G74	Nilamel local
A15	G89	Pongamoodu local

a. Day 4

b. Day 6

c. Day 8

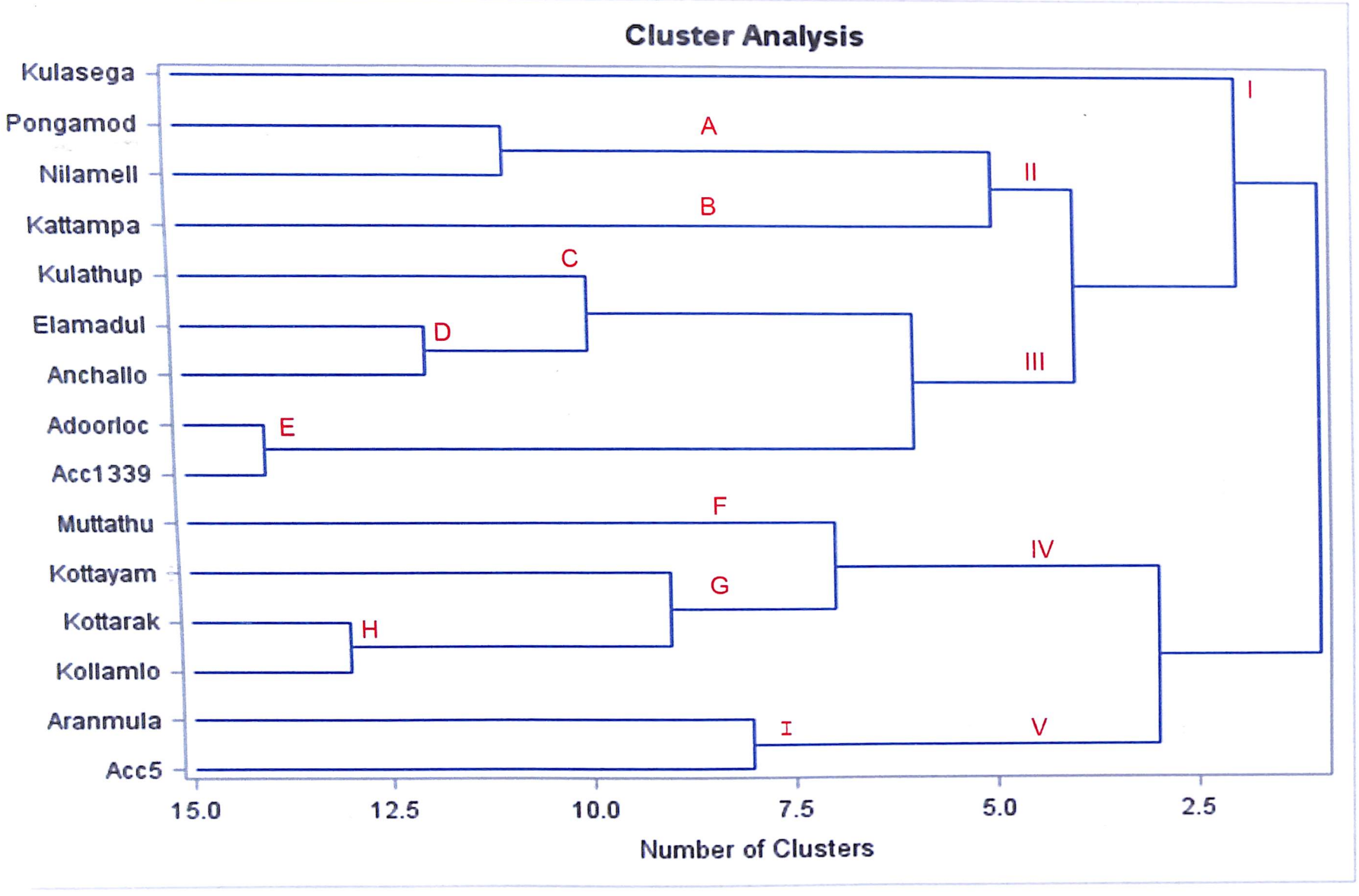
d. Day 9

Plate 6. View of seedlings after stress imposition

Plate 7. Drought tolerant (green) and susceptible (wilted) genotypes of yard long bean seedlings in field screening

Plate 8. Recovery of tolerant genotype on rewatering after drought stress

Fig 1. Dendrogram on the basis of morphological descriptor



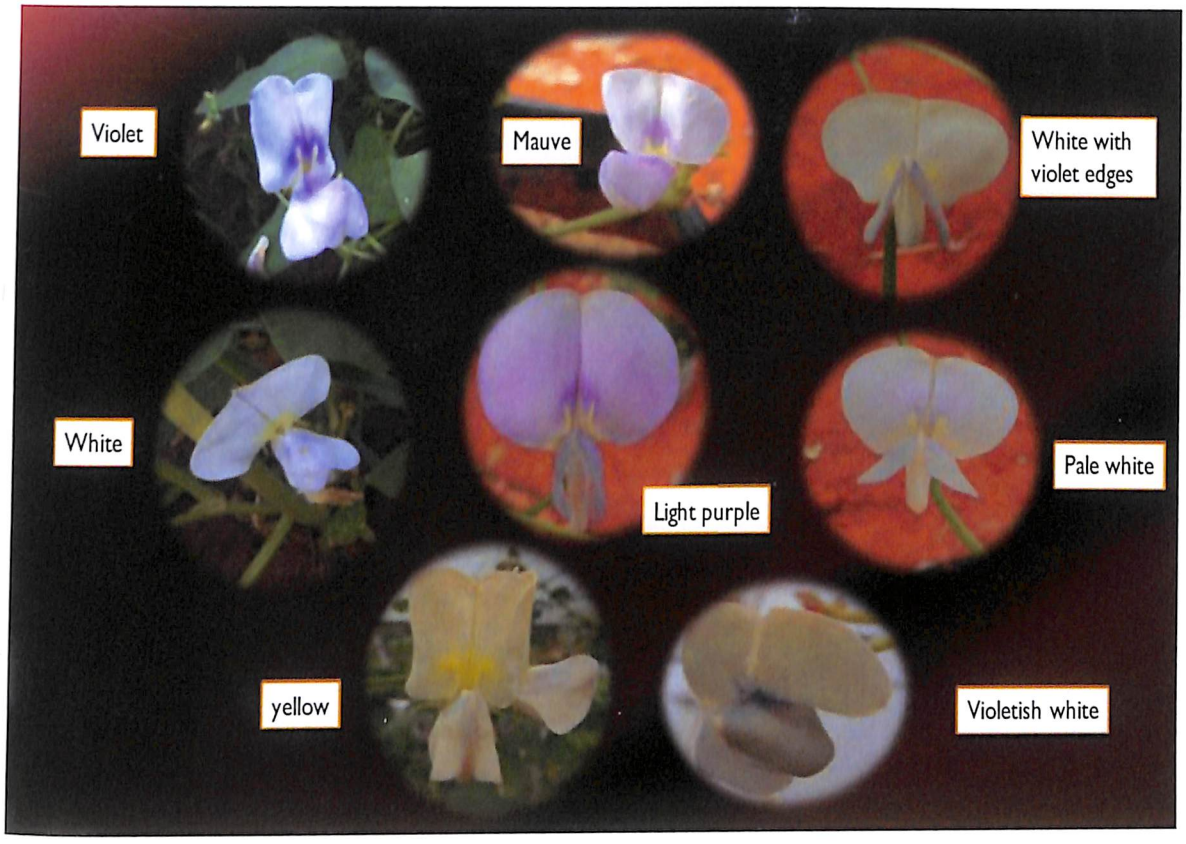


Plate 9. Variability in flower colour

Plate 10. Variability in seed characteristics

Permanent wilting percentage ranged from 14.25% (G6) to 19% (G98). Genotype G6 (14.25%) recorded the lowest PWP was significantly superior to the other genotypes.

Plant recovery percentage ranged from 0 to 45%. Genotype G89 (45%) recorded the highest plant recovery percentage.

Experiment-II

4.2 EVALUATION OF THE SELECTED GENOTYPES FOR DROUGHT TOLERANCE

4.2.1Cataloguing of the Germplasm

Fifteen tolerant genotypes selected from experiment I were described morphologically by using IBPGR descriptor for the cowpea (table 5).The genotypes were scored for 22 characters according to the descriptor (Appendix I).

All the genotypes had indeterminate growth pattern with pronounced twining tendency. Except Kulashegarapuram local all other genotypes have moderate plant pigmentation at the base and tips of petioles. Six genotypes have violetish white flower, five genotype have mauve pink flower, one white, one light pink, one violet and one genotype showed yellow flower colour. For pod colour, thirteen genotypes were found to be light green, while two genotypes exhibited light green with purple tip. Twelve genotypes have straight pods while three genotypes recorded slightly curved pods.

Regarding seed coat colour, high variation was found among genotypes for this trait. Three genotypes showed black seed colour, two genotypes exhibited dark brown, one brown, one light brown, one black with mottled red, one brown with cream spot, one black with brown edges, one reddish brown, one buff, one off white with mottled black, one cream with grey splash and one genotype had dark brown with long white speckled seed coat. Only kidney shaped seeds were noticed in all the fifteen genotypes. Seven genotypes have smooth seed testa texture, another seven have smooth to rough texture and five genotypes have rough testa

Table 5. Morphological characterisation of 15 yard long bean genotypes using IBPGR descriptors

Genotypes	Growth pattern	Twinning tendency	Plant pigmentation	Flower colour	Pod colour	Pod curvature	Seed Eye Colour	Testa texture	Seed shape	Seed Eye Pattern	Seed coat colour
Acc 5	2	4	3	VW	LGWPT	1	0	3	1	0	Black with mottled red
Acc 1339	2	4	3	VW	LG	1	5	1	1	1	Brown with cream spot
Adoor local	2	4	3	VW	LG	1	5	1	1	1	Dark brown
Anchal local II	2	4	3	MP	LG	1	5	3	1	1	Dark brown
Aranmula local	2	4	3	MP	LGWPT	2	0	3	1	0	Black with brown edges
Elamadu local I	2	4	3	VW	LG	1	5	3	1	1	Reddish brown
Kattampally local	2	4	3	MP	LG	2	2	1	1	1	Buff
Kollam local IV	2	4	3	W	LG	1	0	3	1	0	Off white with mottled black
Kottarakara local I	2	4	3	LP	LG	1	0	3	1	0	Black
Kottayam local I	2	4	3	V	LG	1	0	1	1	0	Black
Kulashegarapuram local	2	4	0	Y	LG	2	5	1	1	1	Cream with grey splash
Kulathupuzha local I	2	4	3	MP	LG	1	5	3	1	1	Brown
Muttathukonam local	2	4	3	VW	LG	1	0	1	1	0	Black
Nilamel local	2	4	3	MP	LG	1	5	5	1	1	Light Brown
Pongamoodu local	2	4	3	VW	LG	1	5	1	1	1	Dark brown with long white speckles

Table 6. Mean values of quantitative traits among 15 yardlong bean genotypes using IBPGR descriptors

Genotypes	Leaf length (cm)	Leaf width (cm)	Plant height (m)	Days to 50% flowering	Peduncle length (mm)	Pod length (cm)	Pod width (mm)	No.of Locules per pod	Pods per plant	100 seed weight (g)	Yield per plant (g)
Acc 5	11.00	7.33	4.17	49.33	14.07	46.30	9.2	19.8	39.67	14.95	674.67
Acc 1339	12.00	11.33	3.52	48.67	23.80	51.42	9.2	19.8	34.33	14.49	500.33
Adoor local	11.33	8.67	3.61	41.33	16.47	51.00	9.8	19.2	49.33	15.84	457.33
Anchal local II	14.33	12.67	4.69	40.00	12.87	55.00	9.8	20.1	45.67	15.66	805.33
Aranmula local	11.67	9.00	4.01	41.00	21.13	49.39	9.0	20.9	54.33	11.4	898.67
Elamadu local I	12.67	9.67	3.89	42.67	16.40	50.25	9.2	20.0	45.00	14.61	662.33
Kattampally local	14.67	12.67	3.92	40.00	16.47	33.03	9.1	17.8	26.67	16.33	947.33
Kollam local IV	13.00	10.33	4.73	43.33	17.33	40.22	9.0	20.3	30.67	12.68	516.00
Kottarakara local I	13.67	12.00	4.88	40.33	17.47	61.64	9.8	25.1	53.33	15.09	486.00
Kottayam local I	14.67	11.33	4.33	46.67	18.53	58.52	9.8	21.9	45.33	14.35	558.67
Kulashegarapuram local	14.33	10.33	4.40	41.33	17.87	37.13	7.3	17.8	50.00	9.06	996.33
Kulathupuzha local I	12.00	7.67	3.54	40.00	14.60	49.22	10.3	22.9	38.67	11.41	563.33
Muttathukonam local	12.67	7.00	4.45	40.33	16.47	51.27	9.6	22.9	46.00	13.05	870.67
Nilamel local	18.33	12.33	4.61	43.33	19.13	48.69	9.5	19.9	42.00	15.01	867.33
Pongamoodu local	13.33	10.67	3.97	41.33	18.40	60.53	12.1	21.7	56.00	15.22	1161.67

texture. Only nine of the fifteen genotypes have a very small eye pattern, while in others its absent. Of this nine, eight genotypes have black seed eye colour and one had grey seed eye colour.

The result based on the mean performance of the quantitative traits among the selected genotypes using IBPGR descriptors were given in table 6. Among the genotypes minimum leaf length was recorded by Acc 5 (11 cm) and maximum by Nilamel local (18.33 cm). The genotypes Muttathukonam local recorded minimum leaf width (7 cm) and maximum by Anchal local II and Kattampally local (12.67 cm). Maximum plant height was recorded by Kottarakara local I (4.88 m) and minimum by Acc 1339 (3.52 cm). The genotypes Anchal local II, Kattampally local and Kulathupuzha local I showed minimum days to 50% flowering (40 days) while maximum (49.33 days) was recorded in Acc 5. Peduncle length was highest in Acc 1339 (23.80 mm) and lowest in Anchal local II (12,87 mm).

Regarding pod characters, for pod length the genotype Pongamoodu local recorded maximum pod length (60.33 cm) while Kattampally local showed the minimum pod length (33.03 cm). Pod width was highest in Pongamoodu local (12.1 mm) and lowest in Kulashegarapuram local (7.3 mm). Among the genotypes Kulashegarapuram local and Kattampally local showed the lowest number of locules per pod (17.8) while the highest number of locules per pod was recorded in Kottarakara local I (25.1). For pods per plant the genotype Pongamoodu local exhibited the maximum (56.00) while Kattampally local recorded the minimum (26.67). The genotype Kattampally local recorded the maximum 100 seed weight (16.33 g) and Kulashegarapuram local recorded the minimum 100 seed weight (9.06 g). The genotype Pongamoodu local showed the highest yield per plant (1161,67 g) while Adoor local the lowest yield was noted in Adoor local.

To understand the levels of similarity and dissimilarity among genotypes the morphological descriptor data were subjected to cluster analysis using Ward's minimum variance clustering (table 7). The clustering of 15 selected genotypes is depicted in Fig 1.

Table 7. Distance Matrix

No.	Genotypes	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15
1	A1	0.0000														
2	A2	4.1446	0.0000													
3	A3	4.1762	0.2960	0.0000												
4	A4	3.9169	1.7287	1.7031	0.0000											
5	A5	2.6500	4.8657	4.8386	4.5289	0.0000										
6	A6	3.9991	1.8135	1.6883	0.8668	4.5730	0.0000									
7	A7	5.7785	3.2033	3.1061	3.4274	5.0315	3.3833	0.0000								
8	A8	3.5826	3.5788	3.4668	2.8839	3.8858	2.9099	4.8177	0.0000							
9	A9	3.997	3.9719	3.8486	3.2106	4.1128	3.3029	4.9674	0.6988	0.0000						
10	A10	4.6134	4.0101	3.8880	3.8486	4.6281	4.0267	4.9172	2.0471	1.7031	0.0000					
11	A11	7.3179	5.9285	5.8316	5.7359	6.3792	5.8953	4.9825	5.8141	5.6158	5.2759	0.0000				
12	A12	4.7001	2.9172	2.6824	2.0723	4.8558	1.6099	3.5157	2.7755	2.9099	3.6015	5.4619	0.0000			
13	A13	3.8563	3.1095	2.9504	3.4067	4.2677	3.1309	4.5796	2.0471	2.4712	2.5322	6.1199	3.1249	0.0000		
14	A14	5.8100	3.1622	2.9172	3.9508	5.8922	3.6818	3.626	4.2798	4.3480	3.6619	5.4859	3.1761	3.1942	0.0000	
15	A15	5.2627	2.9605	2.6644	3.1622	5.3790	2.6066	3.4661	3.5788	3.7447	3.7852	5.6250	1.8031	2.8135	1.7287	0.0000

Cluster analysis revealed five clusters indicating the existence of much variability among them and absence of duplication. At 0.767 R2 dendrogram showed the distribution of accessions into five groups. Classified the fifteen genotypes into 5 major clusters based on morphological characteristics; cluster I, cluster II, cluster III, cluster IV and cluster V. Among the 15 genotypes, only Kulashegarapuram local got divided into cluster I standing quite distinct from the rest of the genotypes. The second cluster divided into two groups II A and II B. Pongamoodu local and Nilamel local fell into cluster II A. Cluster II B include Kattampally local.

Cluster III into three groups III C, III D and III E. Cluster III C consist of Kulathupuzha local only. Cluster III D include Elamadu local and Anchal local II. Cluster III E got separated from group III with Adoor local and Acc 1339. Cluster IV divided into three groups IV F, IV G and IV H. IV F include only Muttathukonam local, IV G with Kottayam local and IV H with kottarakara local and Kollam local. Cluster V contained Aranmula local and Acc 5 which stood out from the rest of the group.

4.2.2Evaluation of genotype in rain shelter for drought tolerance

Fifteen tolerant genotypes selected from experiment I were evaluated in the rain shelter for confirmation of their moisture stress tolerance. Statistical analysis revealed very high variations among the accessions for all the morphological and physiological parameters during drought stress period.

4.2.2.1 Biometric evaluation

Mean performance of the 15 genotypes for biometric traits namely days to 50% flowering, pod length, pod width, pod weight, pods per plant, yield per plant, vine length, harvest index, crop duration, root depth and root volume in yard long bean under moisture stress condition are presented in table 8.

Table 8. Mean performance for 11 biometric characters in 15 yardlong bean genotypes under water stress

Genotype		Days to 50% flowering	Pod length (cm)	Pod width (mm)	Pod weight (g)	Pods per plant	Yield per plant (g)	Vine length (m)	Harvest Index (%)	Crop duration	Root depth (cm)	Root volume (cm3)
No.	Name											
A1	Acc 5	52.08	34.50	7.6	13.87	35.80	545.53	2.88	0.349	109.42	57.30	20.36
A2	Acc 1339	52.50	33.97	7.8	16.63	26.42	488.58	2.20	0.349	107.50	21.92	17.00
A3	Adoor local	43.33	44.80	8.2	12.27	28.19	395.35	2.44	0.361	97.92	53.83	21.00
A4	Anchal local II	41.75	52.80	6.5	18.03	41.75	770.05	3.93	0.375	98.83	69.50	24.00
A5	Aranmula local	42.18	40.03	9.9	16.20	45.89	783.04	3.19	0.366	93.25	87.25	23.58
A6	Elamadu local I	47.83	42.47	7.7	13.47	35.25	526.09	2.59	0.345	102.83	75.25	23.50
A7	Kattampally local	40.90	26.00	8.4	13.77	53.25	813.43	3.28	0.359	89.17	57.42	25.08
A8	Kollam local IV	50.92	29.37	7.6	7.37	46.17	394.46	2.99	0.348	98.92	58.25	23.00
A9	Kottarakara local I	42.42	52.90	8.9	11.93	34.18	462.20	3.62	0.368	95.25	115.75	28.00
A10	Kottayam local I	51.00	42.47	8.2	11.40	32.75	427.93	2.92	0.345	99.00	56.75	25.75
A11	Kulashegarapuram local	42.49	31.93	7.1	11.83	58.64	819.26	3.78	0.371	81.25	78.08	21.25
A12	Kulathupuzha local I	42.25	32.17	7.7	13.30	32.15	480.70	2.25	0.365	94.75	51.42	20.50
A13	Muttathukonam local	41.42	45.23	8.4	12.93	50.90	752.95	3.82	0.369	90.17	56.50	19.92
A14	Nilamel local	43.75	41.90	8.5	16.83	44.73	814.75	4.03	0.369	89.00	79.17	23.75
A15	Pongamoodu local	41.83	55.33	8.7	22.03	46.83	1070.43	3.46	0.378	75.58	95.92	28.42
General Mean		45.11	40.39	8.08	14.12	40.86	636.32	3.16	0.361	94.86	67.62	23.01
SE		0.56	0.90	0.21	0.46	0.61	13.65	0.09	0.001	0.61	2.17	0.25
CD (0.05)		1.64	2.62	0.61	1.35	1.79	39.74	0.28	0.003	1.78	6.31	0.72

Table 9: Mean performance for 9 physiological characters in 15 yardlong bean genotypes under water stress

Genotype		Proline content (µmoles/g)	Membrane integrity (%)	Percentage leakage (%)	Ascorbic acid (mg/100 g)	Canopy temperature (^{0}C)	Relative water content (%)	Water Use Efficiency (kg/m^3)	Water requirement (m^3)	Stomatal conductance (m H_2O moles/m2/sec)
No.	Name									
A1	Acc 5	3.10	80.20	19.80	12.00	31.14	70.67	5.51	0.099	178.97
A2	Acc 1339	2.57	86.31	13.69	13.33	30.65	65.00	5.72	0.085	166.87
A3	Adoor local	2.60	83.76	16.24	13.00	30.49	66.00	7.65	0.052	208.00
A4	Anchal local II	3.84	89.70	10.30	12.67	29.87	76.00	8.79	0.088	301.63
A5	Aranmula local	3.50	88.40	11.60	13.00	30.21	75.33	8.82	0.089	249.10
A6	Elamadu local I	3.18	77.69	22.31	11.67	30.88	71.67	5.68	0.093	169.37
A7	Kattampally local	4.31	88.80	11.23	12.00	29.12	75.00	7.38	0.110	171.77
A8	Kollam local IV	2.49	87.40	12.60	12.33	30.40	66.00	7.49	0.053	279.43
A9	Kottarakara local I	3.55	80.50	19.50	12.33	30.54	75.33	7.40	0.063	248.47
A10	Kottayam local I	2.58	83.03	16.97	11.67	30.58	65.00	5.99	0.072	182.40
A11	Kulashegarapuram local	3.89	89.50	10.47	13.00	29.58	75.33	9.64	0.085	330.37
A12	Kulathupuzha local I	3.28	89.64	10.36	12.00	31.24	74.33	7.22	0.067	257.97
A13	Muttathukonam local	3.02	87.40	12.64	12.00	29.74	76.33	8.34	0.091	337.13
A14	Nilamel local	4.04	88.90	11.09	13.67	29.02	72.67	9.42	0.087	313.70
A15	Pongamoodu local	2.99	89.69	10.31	13.00	29.18	76.67	9.77	0.110	279.07
General Mean		3.26	86.06	13.94	12.51	30.18	72.09	7.65	0.083	244.95
SE		0.17	0.43	0.42	0.40	0.12	1.34	0.16	0.003	4.05
CD (0.05)		0.49	1.23	1.24	1.16	0.36	3.92	0.47	0.008	11.82

Table 10. ANOVA of characters of 15 yardlong bean genotypes under water stress

Sl.No.	Characters	Mean square		
		Treatment	Error	F
I. Biometrical traits				
1	Vine length	1.1256	0.0287	39.16**
2	Days to 50% flowering	57.5045	0.9567	60.10**
3	Pod length	242.4483	2.4456	99.14**
4	Pod girth	1.8626	0.1321	14.10**
5	Pod weight	35.3759	0.6466	54.71**
6	Pods per plant	275.935	1.141	241.79**
7	Yield per plant	127474.849	558.725	228.15**
8	Harvest Index	0.00038	0.00000287	132.49**
9	Crop duration	244.784	1.1228	218.01**
10	Root depth	1476.141	14.0675	104.93**
11	Root volume	28.7637	0.1868	153.90**
II. Physiological traits				
12	Proline content	1.0083	0.0868	11.61**
13	Membrane integrity	48.263	0.542	88.997**
14	Percentage leakage	48.262	0.541	89.201**
15	Ascorbic acid	1.1841	0.4793	2.47**
16	Canopy temperature	1.554	0.046	33.535**
17	Relative water content	59.2603	5.4555	10.86**
18	Water Use Efficiency	6.360	0.078	81.074**
19	Water requirement	0.001	0.00002	49.66**
20	Stomatal conductance	11376.07	49.417	230.21**

** Significant at 1% level

Days to fifty per cent flowering ranged from 40.90 to 52.50. The lowest days for fifty per cent flowering were recorded in the genotype A7 (40.90 days) which is on par with A13 (41.41), A4 (41.75), A15 (41.83), A5 (42.17), A12 (42.17), A9 (42.41) and A11 (42.48). Genotype A1 recorded the highest days to fifty per cent flowering with mean of 52.08 days.

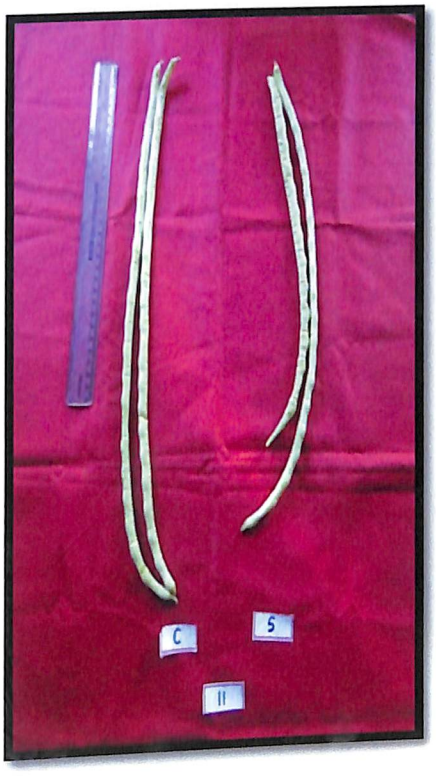
Pod length was the highest for genotype A15 (55.33) which was on par with A9 (52.90) and A4 (52.80). The lowest pod length was recorded by the genotype A7 (26.00). For pod width the highest was recorded by the genotype A5 (9.90) followed by A9 (8.90), A15 (8.66), A14 (8.46), A7 (8.40) and A13 (8.36) and lowest by A4 (6.53) followed by A11 (7.10).

The highest pod weight was exhibited by the genotype A15 (22.03) followed by A4 (18.03) and A14 (16.83). The genotype A8 (7.37) recorded the lowest pod weight.The genotype A11 (58.64) showed the highest pods per plant and lowest for A2 (26.42). Maximum yield per plant was recorded by the genotype A15 (1070.43) followed by A11 (819.26), A14 (814.75), A7 (813.43) and A5 (783.04). The lowest yield per plant was exhibited by A8 (394.46).

For vine length, the genotype A14 (4.03) showed the maximum which was on par with A4 (3.93), A13 (3.82) and A11 (3.78). The genotype A2 (2.20) recorded the lowest vine length. The highest harvest Index was observed in the genotype A15 (0.378) followed by A4 (0.375) and A11 (0.371). The lowest harvest index was showed by A6 and A10 (0.345). The lowest crop duration was exhibited by the genotype A15 (75.58) followed by A11 (81.25) while the longest crop duration was showed by A1 (109.42).

The genotype A9 (115.75) recorded the highest root depth followed by A15 (95.92) while A2 (21.92) exhibited the lowest. Maximum root volume was showed in the genotype A15 (28.42) which is on par with A9 (28.00) while the minimum was recorded for A2 (17.00).

a. Acc 5



b. Kulashegarapuram local

c. Muttathukonam local

d. Kattampally local

Plate 11. Variability in pod characteristics under control and stress

Plate 12. Screening for drought tolerance in rain shelter

4.2.2.2 Physiological evaluation

Mean performance for physiological traits namely proline content, membrane integrity, percentage leakage, ascorbic acid, canopy temperature, relative water content, water requirement, water use efficiency and stomatal conductance in yard long bean under moisture stress condition are presented in table 10.

The highest proline content of the leaves was observed for the genotype A7 (4.31) which was on par with A14 (4.04), A11 (3.89) and A4 (3.84). The lowest proline was showed by the genotype A8 (2.49). The percentage leakage ranged from 10.30 (A4) to 22.31 (A6). The highest membrane integrity was recorded for the genotype A4 (89.70) which was on par with A15 (89.69), A12 (89.64), A11 (89.50), A14 (88.90) and A7 (88.80). The membrane integrity reported lowest in A6 (77.69).

Ascorbic acid content was recorded the highest in A4 (13.67) which was on par with A2 (13.33), A1 (13.00), A5 (13.00), A3 (13.00) and A4 (12.67) while the lowest was shown in A6 and A10 (11.76). The Lowest canopy temperature was exhibited by A14 (29.02) which was on par with A15 (29.18) and A7 (29.12) while the highest was recorded in A12 (31.24).

The genotype A15 (76.67) recorded the highest relative water content which was on par with A13 (76.33), A4(76.00), A5 (75.33) and A7 (75.00). The lowest relative water content was noted in A2 (65.00). The water requirement ranged from 0.052 (A3) to 0.110 (A7 and A15). The water use efficiency was exhibited highest by the genotype A15 (9.77) which was on par with A11 (9.64) and A14 (9.42) while the lowest was recorded in A1 (5.51). The lowest stomatal conductance was exhibited by the genotype A2 (166.87) which was on par with A6 (169.37) and A7 (171.77). The highest stomatal conductance was showed by A13 (337.13).

Table 11. Best drought tolerant genotypes based on mean performance under water stress

Sl.No.	Characters	Genotypes
1	Days to 50% flowering	A7, A13, A4, A15, A5, A12, A9, A11, A3, A14
2	Pod length	A15, A9, A4, A13, A3, A10, A6, A14
3	Pod width	A5, A9, A15, A14, A7, A13, A10, A3
4	Pod weight	A15, A4, A14, A2, A5
5	Pods per plant	A11, A7, A13, A8, A15, A5, A14, A1, A4, A6, A9
6	Yield per plant	A15, A11, A14, A7, A5, A4, A13
7	Vine length	A14, A4, A13, A11, A9, A15, A7, A5
8	Harvest Index	A15, A4, A11, A14, A13, A9, A5, A12, A3
9	Crop duration	A1, A2, A6, A10, A8, A4, A3
10	Root depth	A9, A15, A5, A14, A11, A6, A4
11	Root volume	A15, A9, A10, A7, A4, A14, A5, A6
12	Proline content	A7, A14, A11, A4, A9, A5, A12
13	Membrane integrity	A4, A15, A12, A11, A14, A7, A5, A8, A13, A2
14	Percentage leakage	A4, A15, A12, A11, A14, A7, A5, A8, A13, A2
15	Ascorbic acid	A14, A2, A11, A15, A3, A5, A4
16	Canopy temperature	A14, A7, A15, A11, A13, A4, A5
17	Relative water content	A15, A13, A4, A5, A9, A11, A7, A12, A14
18	Water Use Efficiency	A15, A11, A14, A5, A4, A13, A3
19	Water requirement	A3, A8, A9, A12, A10
20	Stomatal conductance	A2, A6, A7, A1, A10, A3

Table 12. Frequency of the genotypes for different traits

Genotype		Frequency
No.	Name	
A1	Acc 5	3
A2	Acc 1339	6
A3	Adoor local	9
A4	**Anchal local II**	**17**
A5	**Aranmula local**	**16**
A6	Elamadu local I	6
A7	**Kattampally local**	**12**
A8	Kollam local IV	5
A9	Kottarakara local I	11
A10	Kottayam local I	6
A11	**Kulashegarapuramlocal**	**13**
A12	Kulathupuzha local I	6
A13	**Muttathukonam local**	**12**
A14	**Nilamel local**	**17**
A15	**Pongamoodu local**	**16**

Table 13. Details of lines and testers in L X T cross

No.	Parents		
Lines		Testers	
L_1	Anchal local II	T_1	Gitika
L_2	Aranmula local	T_2	Lola
L_3	Kattampally local	T3	VellayaniJyothika
L_4	Kulashegarapuramlocal		
L_5	Muttathukonam local		
L_6	Nilamel local		
L_7	Pongamoodu local		

The top seven genotypes with high yield and drought tolerance (table 12) based on biometric and physiological evaluations were selected as parents for further hybridisation in experiment III.

Experiment-III

4.3 PART I: DEVELOPMENT OF HYBRIDS

The experimental material consisted of seven drought tolerant genotypes selected from experiment II and three high yielding commercial varieties (Gitika, Vellayani Jyothika and Lola) as lines and testers respectively (table 13). The ten parents were raised in crossing block and each of the seven lines were crossed with three testers in Line x Tester pattern. The seeds from 21 F1's were collected at maturity and used for field evaluation.

4.4 PART II: FIELD EXPERIMENT FOR EVALUATION OF F_1 AND PARENTS

Twenty one hybrids along with their parents and check (Arka Mangla) were evaluated for moisture stress tolerance in the field. The details of the twenty one hybrid combinations in L x T are presented in table 14. The data were subjected to line x tester analysis. The statistical analysis on mean performance, combining ability, heterosis and gene action were carried out.

4.4.1 ANALYSIS OF VARIANCE

Analysis of variance of RBD for morphological and physiological traits under water stress condition are presented in Table 15a and 15b. The genotypic effect was significant for all the characters studied, indicating the presence of sufficient variability in the experimental material.

4.4.2 MEAN PERFORMANCE

The mean performance of parents and hybrids for all the sixteen traits under water stress are presented in tables 16a and 16b. The character wise results of mean performance are presented below.

Table 14. Details of crosses made in Line x Tester

Sl.No.	Code No.	Cross combination
1	$L_1 \times T_1$	Anchal local II x Gitika
2	$L_1 \times T_2$	Anchal local II xLola
3	$L_1 \times T_3$	Anchal local II xVellayaniJyothika
4	$L_2 \times T_1$	Aranmula localxGitika
5	$L_2 \times T_2$	Aranmula localxLola
6	$L_2 \times T_3$	Aranmula local xVellayaniJyothika
7	$L_3 \times T_1$	Kattampally local x Gitika
8	$L_3 \times T_2$	Kattampally local xLola
9	$L_3 \times T_3$	Kattampally local xVellayaniJyothika
10	$L_4 \times T_1$	Kulashegarapuramlocal xGitika
11	$L_4 \times T_2$	Kulashegarapuramlocal xLola
12	$L_4 \times T_3$	KulashegarapuramlocalxVellayaniJyothika
13	$L_5 \times T_1$	Muttathukonam local xGitika
14	$L_5 \times T_2$	Muttathukonam local xLola
15	$L_5 \times T_3$	Muttathukonam local xVellayaniJyothika
16	$L_6 \times T_1$	Nilamel local xGitika
17	$L_6 \times T_2$	Nilamel local xLola
18	$L_6 \times T_3$	Nilamel local xVellayaniJyothika
19	$L_7 \times T_1$	Pongamoodu local xGitika
20	$L_7 \times T_2$	Pongamoodu local xLola
21	$L_7 \times T_3$	Pongamoodu local xVellayaniJyothika

Table 15a. Analysis of variance for parents and crosses for 8 morphological traits

Sources	df	Mean square							
		Days to 50% flowering	Pod length	Pod girth	Pod weight	Pods per plant	Yield per plant	Crop Duration	Harvest Index
Replication	2	1.736	0.197	4.664	0.7802	0.2197	3.980	5.349	1.072
Genotypes	30	84.09**	106.23**	12.74**	218.71**	273.02**	440.34**	210.78**	59.53**
Error	60	0.6310	2.4157	0.2177	0.0870	0.5696	275.76	0.1916	0.000008

*Significant at 5 per cent level** Significant at 1 per cent level

Table 15b. Analysis of variance for parents and crosses for 8 physiological traits

Sources	df	Mean square							
		Proline content	Membrane integrity	Percentage leakage	Ascorbic acid	Canopy temperature	Relative water content	Water Use Efficiency	Stomatal Conductance
Replication	2	1.563	1.459	1.486	0.2955	1.637	1.747	0.2576	0.9315
Genotypes	30	5042.12**	96.71**	95.01**	10.16**	93.93**	46.99**	216.77**	3555.16**
Error	60	0.0004	0.1860	0.1894	0.4730	0.0477	6.0792	0.0455	2.9018

*Significant at 5 per cent level** Significant at 1 per cent level

Table 16a. Mean performance of parents and hybrids for morphological traits under water stress

Traits / Parents	Days to 50% flowering	Pod length	Pod girth	Pod weight	Pods per plant	Yield per plant	Crop duration	Harvest Index
L1	39.60	51.87	6.60	18.17	42.07	770.05	98.13	0.362
L2	42.80	40.93	8.73	16.40	47.40	783.04	93.33	0.353
L3	39.27	26.33	7.80	15.50	52.13	813.43	89.33	0.361
L4	42.33	31.93	7.67	15.15	53.73	819.26	82.20	0.367
L5	44.13	43.33	6.27	14.57	49.13	752.95	90.07	0.359
L6	45.93	42.20	8.20	17.33	46.73	814.75	89.27	0.359
L7	45.93	51.20	8.80	22.76	46.80	1070.43	79.00	0.374
T1	43.67	42.80	9.07	12.07	49.33	601.04	89.33	0.352
T2	43.20	35.73	7.13	11.24	50.87	576.48	88.47	0.341
T3	39.87	34.80	8.13	11.63	53.87	631.79	87.07	0.341
L1 X T1	50.40	32.20	6.33	17.23	45.00	781.23	85.53	0.331
L1 X T2	51.53	28.47	7.00	17.92	43.80	790.10	84.27	0.338
L1 X T3	39.33	41.53	8.07	19.32	57.20	1109.06	90.20	0.338
L2 X T1	47.27	30.00	7.20	18.05	47.53	863.16	87.07	0.349
L2 X T2	49.93	29.00	9.13	18.69	58.20	1092.77	91.60	0.357
L2 X T3	49.13	22.53	6.93	18.42	37.27	691.46	84.47	0.332
L3 X T1	41.33	44.20	8.67	16.69	62.33	1045.69	86.07	0.358
L3 X T2	39.80	38.07	8.40	16.83	53.53	905.38	89.53	0.361
L3 X T3	40.47	46.33	9.60	19.81	66.13	1315.28	84.33	0.381
L4 X T1	47.53	19.13	7.00	18.51	52.40	974.91	85.33	0.348
L4 X T2	38.67	27.40	8.33	19.37	64.87	1261.46	82.47	0.377
L4 X T3	39.53	29.13	6.60	18.53	61.87	1152.08	84.07	0.367
L5 X T1	40.80	29.40	6.60	18.84	58.60	1108.98	86.27	0.369
L5 X T2	39.20	23.00	7.20	18.77	61.27	1155.05	89.13	0.363
L5 X T3	39.87	21.13	6.00	17.85	52.87	948.75	88.27	0.349
L6 X T1	51.73	31.60	6.13	15.27	51.80	795.55	84.13	0.348
L6 X T2	39.60	53.53	8.27	19.23	66.07	1274.77	84.60	0.374
L6 X T3	43.07	43.47	7.73	14.43	57.47	835.11	89.13	0.352
L7 X T1	45.67	39.67	7.40	19.26	55.53	1075.20	87.53	0.361
L7 X T2	49.13	32.20	7.67	16.77	47.87	808.02	90.07	0.344
L7 X T3	47.33	30.60	7.53	16.93	48.93	833.34	87.20	0.345
Mean	43.95	35.65	7.59	17.19	53.09	922.19	87.33	0.356
SE	0.62	2.41	0.22	0.08	0.57	274.90	0.19	0.000008
CD (0.05)	1.28	2.53	0.76	0.48	1.23	27.06	0.71	0.005

Table 16b. Mean performance of parents and hybrids for physiological traits under water stress

Traits / Parents	Proline content	Membrane Integrity	Percentage leakage	Ascorbic Acid	Canopy Temp.	Relative Water Content	Water Use Efficiency	Stomatal Conductance
L1	3.43	88.86	11.14	13.00	29.63	81	9.33	302.30
L2	3.62	89.69	10.31	14.33	30.20	77	8.50	249.83
L3	4.43	85.69	14.31	13.33	29.13	75	7.57	172.13
L4	4.46	88.30	11.70	12.67	29.63	82	9.67	320.87
L5	3.04	85.30	14.70	12.33	30.50	78	9.07	337.57
L6	3.62	87.95	12.05	14.00	28.93	76	9.23	314.70
L7	2.71	89.42	10.58	14.67	29.17	84	9.80	279.43
T1	2.24	81.84	18.16	12.33	31.67	53	3.40	324.57
T2	1.89	82.03	17.97	12.33	30.87	56	3.90	294.13
T3	2.05	82.21	17.79	12.67	31.23	51	4.93	317.23
L1 X T1	2.49	85.45	14.55	12.67	31.73	65	5.87	290.73
L1 X T2	2.15	85.19	14.81	12.33	31.43	66	5.73	296.13
L1 X T3	3.55	88.55	11.45	13.67	30.37	71	6.93	435.47
L2 X T1	2.15	91.28	8.72	15.33	28.10	79	6.70	394.63
L2 X T2	3.46	86.44	13.56	16.00	28.77	81	8.33	431.10
L2 X T3	3.73	85.32	14.68	12.33	31.60	66	5.37	286.67
L3 X T1	2.25	85.09	14.91	14.33	28.37	71	6.83	347.13
L3 X T2	3.92	88.28	11.72	12.33	30.57	88	10.63	368.57
L3 X T3	4.28	87.95	12.05	15.67	28.57	86	9.37	432.83
L4 X T1	2.77	84.05	15.95	12.67	31.83	68	6.57	296.73
L4 X T2	4.26	90.74	9.26	13.67	28.33	83	8.67	414.50
L4 X T3	3.04	88.87	11.13	13.33	28.93	92	7.83	393.07
L5 X T1	4.44	85.29	14.71	14.67	30.53	82	6.70	377.30
L5 X T2	4.05	87.21	12.79	17.33	30.97	76	8.63	358.47
L5 X T3	2.40	89.11	10.89	13.33	31.23	71	6.90	342.53
L6 X T1	3.13	84.26	15.74	12.67	31.10	69	5.87	291.43
L6 X T2	4.51	86.52	13.48	13.33	28.27	75	9.87	395.10
L6 X T3	2.78	87.14	12.86	14.33	28.30	82	8.67	294.10
L7 X T1	3.79	88.28	11.72	12.33	29.67	80	7.57	331.57
L7 X T2	2.17	85.23	14.77	13.67	30.50	69	6.13	293.30
L7 X T3	2.46	85.12	14.88	12.67	31.13	69	6.70	307.17
Mean	3.18	86.59	13.41	13.58	30.07	72.91	7.41	332.49
SE	0.0005	0.18	0.18	0.47	0.05	5.89	0.05	2.81
CD	0.03	0.69	0.69	1.12	0.35	3.96	0.35	2.74

4.4.2.1 Days to 50% flowering

The mean performance of parents for days to 50% flowering ranged from 39.27 (L3) to 45.93 (L6 and L7). The mean performance of hybris for days to 50% flowering ranged from 38.67 (L4 x T2) to 51.73 (L6 x T1) with general mean value of 43.95 days. Among the hybrids, minimum value was shown by L4 x T2 (38.67) which was statistically on par with L5 x T2, L1 x T3, L4 x T3, L6 x T2, L3 x T2 and L5 x T3.

4.4.2.2 Pod length

For pod length the mean performance of parents ranged from 26.33 (L3) to 51.87 (L1). The mean performance of hybrids for pod length ranged from 19.13 (L4 x T1) to 53.53 (L6 x T2) with general mean value of 35.65. Among the hybrids, seven crosses viz., L6 x T2, L3 x T3, L3 x T1, L6 x T3, L1 x T3, L7 x T1 and L3 x T2 recorded higher performance than general mean.

4.4.2.3 Pod girth

The mean performance of parents for pod girth ranged from 6.27 (L5) to 9.07 (T1). The mean performance of hybrids for pod girth ranged from 6.00 (L5 x T3) to 9.60 (L3 x T3) with general mean value of 7.59. Among the hybrids, nine crosses viz., L7 x T2, L6 x T3, L1 x T3, L6 x T2, L4 x T2, L3 x T2, L3 x T1, L2 x T2 and L3 x T3 recorded higher performance than general mean.

4.4.2.4 Pod weight

For pod weight the mean performance of parents ranged from 11.24 (T2) to 22.76 (L7). The mean performance of hybrids for pod weight ranged from 14.43 (L6 x T3) to 19.81 (L3 x T3) with general mean value of 17.19. Among the hybrids, fifteen crosses viz., L3 x T3, L4 x T2, L1 x T3, L7 x T1, L6 x T2, L5 x T1, L5 x T2, L2 x T2, L4 x T3, L4 x T1, L2 x T3, L2 x T1, L1 x T2, L5 x T3 and L1 x T1 recorded higher performance than general mean.

4.4.2.5 Pods per plant

For pods per plant the mean performance of parents ranged from 42.07 (L1) to 53.87 (T3). The mean performance of hybrids for pods per plant ranged from 37.27 (L2 x T3) to 66.13 (L3 x T3) with general mean value of 53.09. Hybrid L6 x T2 is found to be on par with L3 x T3. Among the hybrids, twelve

crosses viz., L3 x T3, L6 x T2, L4 x T2, L3 x T1, L4 x T3, L5 x T2, L5 x T1, L2 x T2, L6 x T3, L1 x T3, L7 x T1 and L3 x T2 recorded higher performance than general mean.

4.4.2.6 Yield per plant

The mean performance of parents for yield per plant ranged from 576.48 (T2) to 1070.43 (L7). The mean performance of hybrids for yield per plant ranged from 691.46 (L2 x T3) to 1315.28 (L3 x T3) with general mean value of 922.19. Among the hybrids, twelve crosses viz., L3 x T3, L6 x T2, L4 x T2, L5 x T2, L4 x T3, L1 x T3, L5 x T1, L2 x T2, L7 x T1, L3 x T1, L4 x T1 and L5 x T3 recorded higher performance than general mean.

4.4.2.7 Crop Duration

For crop duration the mean performance of parents ranged from 79.00 (L7) to 98.13 (L1). The mean performance of hybrids for crop duration ranged from 82.47 (L4 x T2) to 91.60 (L2 x T2) with general mean value of 87.33. Among the hybrids, eight crosses viz., L2 x T2, L1 x T3, L7 x T2, L3 x T2, L6 x T3, L5 x T2, L5 x T3 and L7 x T1 recorded lower performance than general mean.

4.4.2.8 Harvest Index

For harvest index the mean performance of parents ranged from 0.341 (T3) to 0.374 (L7). The mean performance of hybrids for harvest index ranged from 0.331 (L1 x T1) to 0.381 (L3 x T3) with general mean value of 0.356. Among the hybrids, ten crosses viz., L3 x T3, L4 x T2, L6 x T2, L5 x T1, L4 x T3, L5 x T2, L7 x T1, L3 x T2, L3 x T1 and L2 x T2 recorded higher performance than general mean.

4.4.2 9 Proline content

The mean performance of parents for proline content ranged from 1.89 (T2) to 4.46 (L4). The mean performance of hybrids for proline content ranged from 2.15 (L2 x T1) to 4.51 (L6 x T2) with general mean value of 3.18. Among the hybrids, ten crosses viz., L6 x T2, L5 x T1, L3 x T3, L4 x T2, L5 x T2, L3 x T2, L7 x T1, L2 x T3, L1 x T3 and L2 x T2 recorded higher performance than general mean.

4.4.2.10 Membrane Integrity

The mean performance of parents for membrane integrity ranged from 81.84 (T1) to 89.69 (L2). The mean performance of hybrids for membrane integrity ranged from 84.05 (L4 x T1) to 91.28 (L2 x T1) with general mean value of 86.59. Among hybrids, maximum value was shown by L2 x T1 which was on par with L4 x T2. Among the hybrids, ten crosses viz., L2 x T1, L4 x T2, L5 x T3, L4 x T3, L1 x T3, L7 x T1, L3 x T2, L3 x T3, L5 x T2 and L6 x T3 recorded higher performance than general mean.

4.4.2.11 Percentage leakage

For percentage leakage the mean performance of parents ranged from 10.31 (L2) to 18.16 (T1). The mean performance of hybrids for percentage leakage ranged from 8.72 (L2 x T1) to 15.95 (L4 x T1) with general mean value of 13.41. Among hybrids, minimum value was shown by L2 x T1 (8.72) which was on par with L4 x T2 (9.26). Among the hybrids, ten crosses viz., L2 x T1, L4 x x T2, L5 x T3, L4 x T3, L1 x T3, L3 x T2, L7 x T1, L3 x T3, L5 x T2 and L6 x T3 recorded lower performance than general mean.

4.4.2.12 Ascorbic Acid

For ascorbic acid the mean performance of parents ranged from 12.33 (T2) to 14.67 (L7). The mean performance of hybrids for ascorbic acid ranged from 12.33 (L3 x T2) to 17.33 (L5 x T2) with general mean value of 13.58. Among the hybrids, ten crosses viz., L4 x T2, L1 x T3, L7 x T2, L3 x T1, L6 x T3, L5 x T1, L2 x T1, L3 x T3, L2 x T2 and L5 x T2 recorded higher performance than general mean. Crosses L2 x T2, L3 x T3 and L2 x T1 are statistically on par with high ascorbic acid content.

4.4.2.13 Canopy Temperature

The mean performance of parents for canopy temperature ranged from 28.93 (L6) to 31.67 (T1). The mean performance of hybrids for canopy temperature ranged from 28.10 (L2 x T1) to 31.83 (L4 x T1) with general mean value of 30.07. Among the hybrids, nine crosses viz., L2 x T1, L6 x T2, L6 x T3, L4 x T2, L3 x T1, L3 x T3, L2 x T2, L4 x T3 and L7 x T1 recorded lower temperature than general mean. Among the hybrids, minimum value was shown

by L2 x T1 (28.10) which was statistically on par with L6 x T2, L6 x T3, L4 x T2 and L3 x T1.

4.4.2.14 Relative Water Content

For relative water content the mean performance of parents ranged from 51 (T3) to 84 (L7). The mean performance of hybrids for relative water content ranged from 65 (L1 x T1) to 92 (L4 x T3) with general mean value of 72.91. Among hybrids, higher relative water content was recorded in L4 x T3 (92) which was on par with L3 x T2 (88.33). Among the hybrids, eleven crosses viz., L4 x T3, L3 x T2, L3 x T3, L4 x T2, L5 x T1, L6 x T3, L2 x T2, L7 x T1, L2 x T1, L5 x T2 and L6 x T2 recorded higher performance than general mean.

4.4.2.15 Water Use Efficiency

For water use efficiency the mean performance of parents ranged from 3.40 (T1) to 9.80 (L7). The mean performance of hybrids for water use efficiency ranged from 5.37 (L2 x T3) to 10.63 (L3 x T2) with general mean value of 7.41. Among the hybrids, nine crosses viz., L3 x T2, L6 x T2, L3 x T3, L6 x T3, L4 x T2, L5 x T2, L2 x T2, L4 x T3 and L7 x T1 recorded higher performance than general mean.

4.4.2.16 Stomatal Conductance

The mean performance of parents for stomatal conductance ranged from 172.13 (L3) to 337.57 (L5). The mean performance of hybrids for stomatal conductance ranged from 286.67 (L2 x T3) to 434.47 (L1 x T3) with general mean value of 332.49. Among the hybrids, nine crosses viz., L2 x T3, L1 x T1, L6 x T1, L7 x T2, L6 x T3, L1 x T2, L4 x T1, L7 x T3 and L7 x T1 recorded low stomatal conductance than general mean.

4.4.3 COMBINING ABILITY

Significant variation was showed among lines, testers and hybrids, hence gca and sca were calculated. The analysis of combining ability are given in table 17a and 17b. Significant differences were observed in both gca and sca effects. The general combining ability (gca) effects of parents are presented in the tables 18a and 18b. Specific combining ability (sca) effects of hybrids for all the sixteen characters are presented in the tables 19a and 19b.

Table 17a. Analysis of variance of combining ability for morphological traits (MSE)

Source	df	Days to 50% flowering	Pod length	Pod girth	Pod weight	Pods per plant	Yield per plant	Crop Duration	Harvest Index
Genotypes	30	84.09	106.23	12.74	218.71	273.02	440.34	210.78	59.53
Lines	6	2.55	4.05	2.60	0.936	1.73	0.841	0.956	2.66
Testers	2	1.66	0.059	2.76	0.229	0.344	0.394	0.504	0.855
L x T	12	71.03	55.63	7.91	73.84	278.84	414.99	106.20	47.99
Parents	9	28.37	81.12	12.22	424.65	70.04	219.60	439.15	38.20
Crosses	20	108.84	101.44	13.11	66.74	322.45	370.19	99.54	71.29
Parents vs Crosses	1	90.45	427.96	9.90	1404.59	1111.14	3830.11	380.06	16.25
Error	60	0.631	2.415	0.217	0.087	0.569	275.76	0.191	0.000008

Table 17b. Analysis of variance of combining ability for physiological traits (MSE)

Source	df	Proline content	Membrane integrity	Percentage leakage	Ascorbic acid	Canopy temperature	Relative water content	Water Use Efficiency	Stomatal Conductance
Genotypes	30	5042.12	96.71	95.01	10.16	93.93	46.99	216.77	3555.16
Lines	6	0.412	0.283	0.282	1.117	1.011	1.051	1.88	0.59
Testers	2	0.506	0.444	0.450	0.326	0.111	0.383	3.65	0.574
L x T	12	6261.93	94.34	92.51	12.73	126.13	32.95	91.27	3533.15
Parents	9	5997.33	161.82	159.13	4.89	56.56	79.66	411.37	2473.36
Crosses	20	4848.58	68.81	67.51	12.32	115.31	31.42	139.73	2955.65
Parents vs Crosses	1	315.83	68.79	68.11	14.41	2.88	64.21	6.19	25281.48
Error	60	0.0004	0.186	0.189	0.473	0.0477	6.079	0.045	2.902

Table 18a. General combining abilities (GCA) effects of lines and testers for morphological traits

Parents	Days to 50% flowering	Pod length	Pod girth	Pod weight	Pods per plant	Yield per plant	Crop Duration	Harvest Index
Lines								
L1	2.7397**	1.0857*	-0.3810*	0.2206*	-6.1206**	-97.8270**	-0.0603	-0.0190**
L2	4.4286**	-5.8032**	0.2413	0.4429**	-7.1206**	-108.8381**	0.9841**	-0.0084**
L3	-3.8159**	9.8857**	1.3746**	-0.1571	5.8794**	97.4952**	-0.0825	0.0121**
L4	-2.4381**	-7.7587**	-0.2032	0.8540**	4.9238**	138.1730**	-2.7714**	0.0097**
L5	-4.3937**	-8.4698**	-0.9143**	0.5429**	2.7905**	79.6063**	1.1619**	0.0060**
L6	0.4508	9.8857**	-0.1365	-1.6238**	3.6571**	-22.8159**	-0.7714**	0.0039**
L7	3.0286**	1.1746*	0.0190	-0.2794**	-4.0095**	-85.7937**	1.5397**	-0.0043**
SE	0.2648	0.5181	0.1555	0.0983	0.2516	5.5354	0.1459	0.0010
t 2.000 (5%)	.529	1.036	0.311	0.197	0.503	11.070	0.292	0.002
t 2.660 (1%)	0.704	1.378	0.413	0.261	0.669	14.724	0.388	0.003
Testers								
T1	2.0413**	-0.6667	-0.4667*	-0.2397*	-1.4730**	-42.0476**	-0.7365**	-0.0024*
T2	-0.3683*	0.1143	0.4857*	0.2841*	1.7270**	49.7667**	0.6540**	0.0048**
T3	-1.6730**	0.5524	-0.0190	-0.0444	-0.2540	-7.7190*	0.0825	-0.0024*
SE	0.1733	0.3392	0.1018	0.0644	0.1647	3.6238	0.0955	0.0006
t 2.000 (5%)	0.347	0.678	0.204	0.129	0.329	7.248	0.191	0.001
t 2.660 (1%)	0.922	1.805	0.542	0.343	0.876	19.279	0.508	0.003

Significant at 5% level *Significant at 1% level **

Table 18b. General combining abilities (GCA) effects of lines and testers for physiological traits

Parents	Proline content	Membrane integrity	Percentage leakage	Ascorbic acid	Canopy temperature	Relative water content	Water Use Efficiency	Stomatal Conductance
Lines								
L1	-0.4950**	-0.525**	0.5248**	-0.8571**	1.1635**	-8.2381**	-1.2444**	-10.5810**
L2	-0.1158**	0.763**	-0.7542**	0.8095**	-0.5254**	-0.5714	-0.6222**	19.4413**
L3	0.2540**	0.186	-0.1823	0.3651	-0.8476**	6.0952**	1.5222**	31.4857**
L4	0.1277**	0.963**	-0.9664**	-0.5238*	-0.3143**	5.0952**	0.2667**	16.7413**
L5	0.4021**	0.275*	-0.2822	1.3651**	0.8968**	0.7619	-0.0111	8.0746**
L6	0.2458**	-0.959**	0.9503**	-0.3016	-0.7921**	-0.2381	0.7111**	-24.4810**
L7	-0.4188**	-0.703**	0.7100**	-0.8571**	0.4190**	-2.9048**	-0.6222**	-40.6810**
SE	0.0069	0.119	0.1451	0.2293	0.0728	0.8219	0.0711	0.5678
t 2.000 (5%)	0.014	0.238	0.290	0.459	0.146	1.644	0.142	1.136
t 2.660 (1%)	0.018	0.317	0.386	0.610	0.194	2.186	0.189	1.510
Testers								
T1	-0.2249**	-0.673**	0.6788**	-0.2222	0.1762**	-2.2063**	-0.8365**	-18.5683**
T2	0.2760**	0.165*	-0.1656	0.3492*	-0.1810**	1.1746**	0.8635**	13.9508**
T3	-0.0510**	0.508**	-0.5133**	-0.1270	0.0048	1.0317	-0.0270	4.6175**
SE	0.0045	0.069	0.0950	0.1501	0.0477	0.5380	0.0465	0.3717
t 2.000 (5%)	0.009	0.138	0.190	0.300	0.095	1.076	0.093	0.743
t 2.660 (1%)	0.012	0.184	0.253	0.399	0.127	1.431	0.124	0.989

Significant at 5% level * Significant at 1% level **

Table 19a. Specific combining ability (SCA) effects of hybrids for morphological traits under water stress

Hybrids	Days to 50% flowering	Pod length	Pod girth	Pod weight	Pods per plant	Yield per plant	Crop Duration	Harvest Index
L1 x T1	1.269**	-1.200	-0.333	-0.683**	-2.194**	-70.164**	-0.397	-0.002
L1 x T2	4.813**	-5.714**	-0.619*	-0.506**	-6.594**	-153.144**	-3.054**	-0.002
L1 x T3	-6.083**	6.914**	0.952**	1.189**	8.787**	223.308**	3.451**	0.005**
L2 x T1	-3.552**	3.489**	-0.089	-0.071	1.340*	22.748*	0.092	0.006**
L2 x T2	1.524**	1.708	0.892**	0.005	8.806**	160.533**	3.235**	0.006**
L2 x T3	2.029**	-5.197**	-0.803**	0.067	-10.146**	-183.281**	-3.327**	-0.012**
L3 x T1	-1.241**	2.000*	0.244	-0.838**	3.140**	-1.052	0.159	-0.007**
L3 x T2	-0.365	-4.914**	-0.975**	-1.229**	-8.860**	-233.167**	2.235**	-0.011**
L3 x T3	1.606**	2.914**	0.730**	2.067**	5.721**	234.219**	-2.394**	0.017**
L4 x T1	3.581**	-5.422**	0.156	-0.049	-5.838**	-112.497**	2.1143**	-0.013**
L4 x T2	-2.876**	2.064*	0.537	0.260	3.429**	82.189**	-2.143**	0.009**
L4 x T3	-0.705	3.359**	-0.692*	-0.211	2.410**	30.308**	0.029	0.005**
L5 x T1	-1.197**	5.556**	0.467	0.595**	2.495**	80.103**	-0.886**	0.011**
L5 x T2	-0.387	-1.625	0.114	0.005	1.962**	34.356**	0.591*	-0.002
L5 x T3	1.584**	-3.930**	-0.581*	-0.600**	-4.457**	-114.459**	0.295	-0.009**
L6 x T1	4.892**	-10.600**	-0.778**	-0.805**	-5.171**	-130.875**	-1.086**	-0.008**
L6 x T2	-4.832**	10.552**	0.403	2.638**	5.895**	256.511**	-2.010**	0.011**
L6 x T3	-0.060	0.048	0.375	-1.833**	-0.724	-125.637**	3.095**	-0.004*
L7 x T1	-3.752**	6.178**	0.333	1.851**	6.229**	211.737**	0.003	0.013**
L7 x T2	2.124**	-2.070*	-0.352	-1.173**	-4.638**	-147.278**	1.146**	-0.011**
L7 x T3	1.629**	-4.108**	0.019	-0.678**	-1.591**	-64.459**	-1.149**	-0.002
SE	0.459	0.897	0.269	0.170	0.436	9.588	0.253	0.002
CD (5%)	0.917	1.795	0.539	0.341	0.871	19.175	0.505	0.000
CD (1%)	1.219	2.387	0.717	0.453	1.159	25.503	0.672	0.000

Significant at 5% level *: Significant at 1% level **

Table 19b. Specific combining ability (SCA) effects of hybrids for physiological traits under water stress

Hybrids	Proline content	Membrane integrity	Percentage leakage	Ascorbic acid	Canopy temperature	Relative water content	Water Use Efficiency	Stomatal Conductance
L1 x T1	-0.018	-0.267	0.279	0.000	0.379**	-0.238	0.525**	-31.476**
L1 x T2	-0.854**	-1.375**	1.356**	-0.905*	0.437**	-2.286	-1.308**	-58.595**
L1 x T3	0.873**	1.642**	-1.635**	0.905*	-0.816**	2.524	0.783**	90.071**
L2 x T1	-0.742**	4.278**	-4.287**	1.000*	-1.565**	5.762**	0.737**	42.402**
L2 x T2	0.076**	-1.404**	1.422**	1.095**	-0.541**	4.381**	0.670**	46.349**
L2 x T3	0.666**	-2.874**	2.865**	-2.095**	2.106**	-10.143**	-1.406**	-88.751**
L3 x T1	-1.009**	-1.338**	1.335**	0.444	-0.976**	-8.571**	-1.275**	-17.143**
L3 x T2	0.158**	1.010**	-1.022**	-2.127**	1.581**	5.381**	0.825**	-28.229**
L3 x T3	0.850**	0.328	-0.313	1.683**	-0.605**	3.190*	0.449**	45.371**
L4 x T1	-0.360**	-3.159**	3.179**	-0.333	1.957**	-10.905**	-0.286*	-52.798**
L4 x T2	0.626**	2.690**	-2.678**	0.095	-1.186**	0.714	0.114	32.449**
L4 x T3	-0.266**	0.469	-0.502**	0.238	-0.771**	10.190**	0.171	20.349**
L5 x T1	1.035**	-1.232**	1.224**	-0.222	-0.554**	8.095**	0.125	36.435**
L5 x T2	0.144**	-0.157	0.167	1.873**	0.237	-1.952	0.359**	-14.917**
L5 x T3	-1.179**	1.389**	-1.390**	-1.651**	0.317*	-6.143**	-0.484**	-21.517**
L6 x T1	-0.116**	-1.032**	1.024**	-0.556	1.702**	-3.905**	-1.430**	-16.876**
L6 x T2	0.761**	0.381	-0.367*	-0.460	-0.775**	-1.619	0.870**	54.271**
L6 x T3	-0.645**	0.651*	-0.657**	1.016*	-0.927**	5.524**	0.560**	-37.395**
L7 x T1	1.210**	2.750**	-2.754**	-0.333	-0.943**	9.762**	1.603**	39.457**
L7 x T2	-0.911**	-1.145**	1.122**	0.429	0.248	-4.619**	-1.530**	-31.329**
L7 x T3	-0.299**	-1.604**	1.632**	-0.095	0.695**	-5.143**	-0.073	-8.129
SE	0.012	0.251	0.170	0.397	0.126	1.4235	0.123	0.984
CD (5%)	0.024	0.503	0.367	0.794	0.252	2.847	0.246	1.967
CD (1%)	0.316	0.669	0.340	1.056	0.335	3.7865	0.328	2.616

Significant at 5% level *: Significant at 1% level **

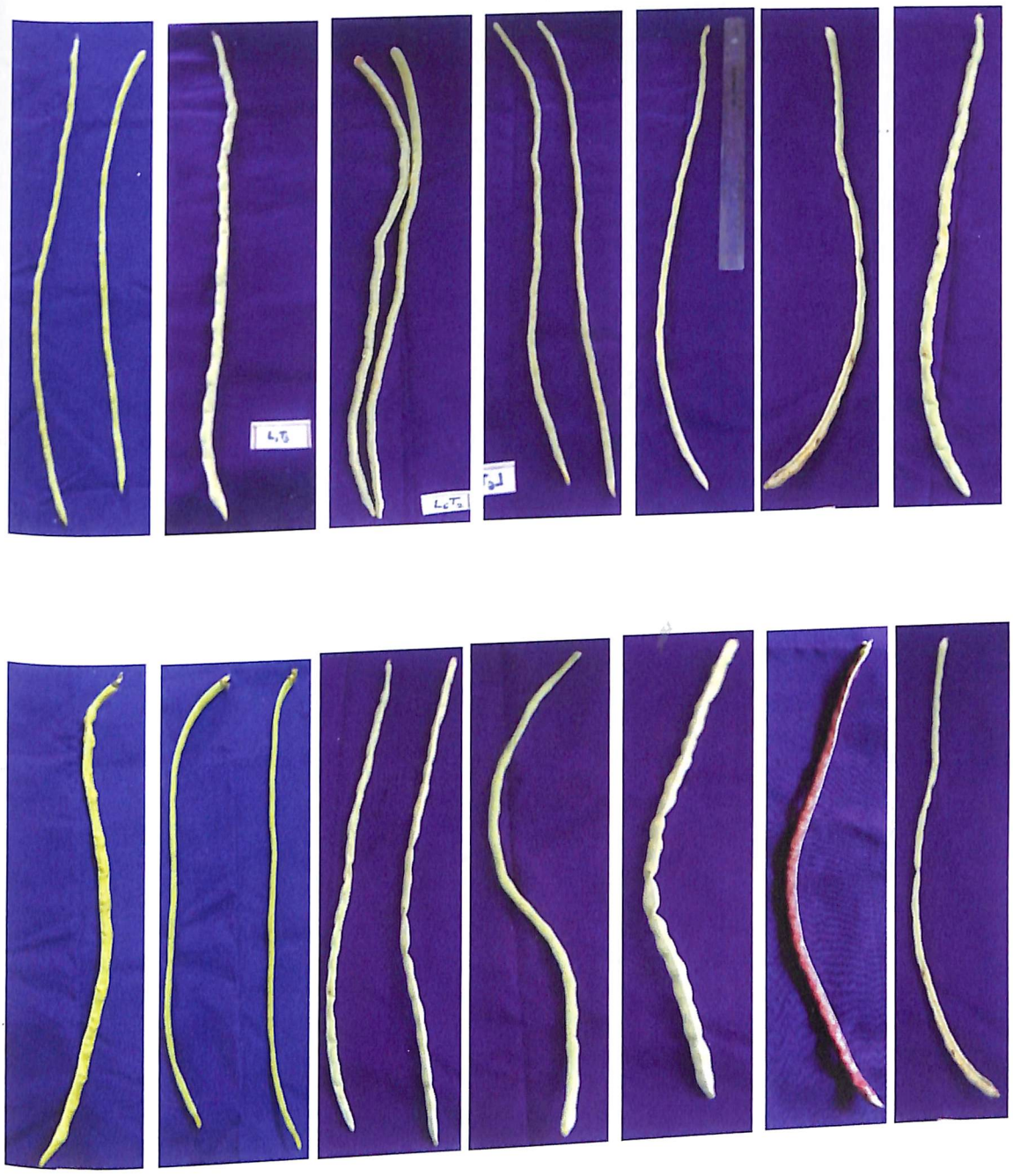


Plate 13. Variability in pod traits among the hybrids

4.4.3.1 Days to 50% flowering

For days to 50% flowering general combining ability effects of lines varied from -4.3937 (L5) to 4.4286 (L2). All lines except L6 had significant general combining ability effects for days to 50% flowering. Lines L5 (-4.3937), L3 (-3.8159) and L4 (-2.4381) had significant negative gca effects whereas L2 (4.4286), L7 (3.0286) and L1 (2.7397) showed significant positive gca effects. Among the testers T3 (-1.6730) and T2 (-0.3683) showed significant negative gca effects whereas T1(2.0413) showed significant positive gca effect. The testers differed significantly from each other.

The sca effects had a range between -6.083 in L1 x T3 to 4.892 in L6 x T1 for days to 50% flowering. Out of twenty one hybrids studied, seven crosses recorded negatively significant sca effects, while ten crosses exhibited significantly positive sca effects. Significant negative sca effects were shown by L1 x T3, L6 x T2, L7 x T1, L2 x T1, L4 x T2, L3 x T1 and L5 x T1. Positive significant sca effects were exhibited by L6 x T1, L1 x T2, L4 x T1, L7 x T2, L2 x T3, L7 x T3, L3 x T3, L5 x T3, L2 x T2 and L1 x T1.

4.4.3.2 Pod length

For pod length general combining ability effects of lines varied from -8.4698 (L5) to 9.8857 (L3 and L6). All the seven lines had significant general combining ability effects for pod length. Lines L3 (9.8857), L6 (9.8857), L7 (1.1746) and L1 (1.0857) had significant positive gca effects whereas L5 (-8.4698), L4 (-7.7587) and L2 (-5.8032) showed significant negative gca effects. None of the testers had significant gca effects for pod length. Among the testers T3 (0.5524) and T2 (0.1143) showed positive gca effects whereas T1(-0.6667) showed negative gca effect.

The sca effects had a range between -10.600 (L6 x T1) to 10.552 (L6 x T2) for pod length. Nine crosses recorded positive significant sca effects, while eight crosses exhibited significantly negative sca effects. Significant positive sca effects were shown by L6 x T2, L1 x T3, L7 x T1, L5 x T1, L2 x T1, L4 x T3, L3

x T3, L4 x T2 and L2 x T2. Negative significant sca effects were exhibited by L6 x T1, L1 x T2, L4 x T1, L2 x T3, L3 x T2, L7 x T3, L5 x T3 and L7 x T2.

4.4.3.3 Pod girth

For pod girth general combining ability effects of lines varied from -0.9143 (L5) to 1.3746 (L3). The line L3 (1.3746) had significant positive gca effect whereas L5 (-0.9143) and L1 (-0.3810) showed significant negative gca effects. Among the testers T2 (0.4857) showed significant positive gca effect whereas T1(-0.4667) showed significant negative gca effect.

The sca effects had a range between -0.975 (L3 x T2) to 0.952 (L1 x T3) for pod girth. Three crosses recorded positive significant sca effects, while six crosses exhibited significantly negative sca effects. Significant positive sca effects were shown by L1 x T3, L2 x T2 and L3 x T3. Negative significant sca effects were exhibited by L3 x T2, L2 x T3, L6 x T1, L4 x T3, L1 x T2 and L5 x T3.

4.4.3.4 Pod weight

For pod weight general combining ability effects of lines varied from -1.6238 (L6) to 0.8540 (L4). All the lines except L3 had significant general combining ability effects for pod weight. Lines L4 (0.8540), L5 (0.5429), L2 (0.4429) and L1 (0.2206) had significant positive gca effects whereas L6 (-1.6238) and L7 (-0.2794) showed significant negative gca effects. Among the testers T2 (0.2841) showed significant positive gca effect whereas T1 (-0.2397) showed significant negative gca effects.

The sca effects had a range between -1.833 (L6 x T3) to 2.638 (L6 x T2) for pod weight. Five crosses recorded positive significant sca effects, while nine crosses exhibited significantly negative sca effects. Significant positive sca effects were shown by L6 x T2, L3 x T3, L7 x T1, L1 x T3 and L5 x T1. Negative significant sca effects were exhibited by L6 x T3, L3 x T2, L7 x T2, L3 x T1, L6 x T1, L1 x T1, L7 x T3, L5 x T3 and L1 x T2.

4.4.3.5 Pods per plant

For pods per plant general combining ability effects of lines varied from -7.1206 (L2) to 5.8794 (L3). All the seven lines had significant general combining ability effects. Lines L3 (5.8794), L4 (4.9238), L6 (3.6571) and L5 (2.7905) had significant positive gca effects whereas L2 (-7.1206), L1 (-6.1206) and L7 (-4.0095) showed significant negative gca effects. Among the testers T2 (1.7270) showed significant positive gca effect whereas T1 (-1.4730) showed significant negative gca effect.

The sca effects had a range between -10.146 (L2 x T3) to 8.806 (L2 x T2) for pods per plant. Out of twenty one hybrids studied, eleven crosses recorded positive significant sca effects, while nine crosses exhibited significantly negative sca effects. Significant positive sca effects were shown by L2 x T2, L1 x T3, L7 x T1, L6 x T2, L3 x T3, L4 x T2, L3 x T1, L5 x T1, L4 x T3, L5 x T2 and L2 x T1. Negative significant sca effects were exhibited by L2 x T3, L3 x T2, L1 x T2, L4 x T1, L6 x T1, L7 x T2, L5 x T3, L1 x T1 and L7 x T3.

4.4.3.6 Yield per plant

For yield per plant general combining ability effects of lines varied from -108.8381 (L2) to 138.1730 (L4). All the seven lines had significant general combining ability effects for yield per plant. Lines L4 (138.1730), L3 (97.4952) and L5 (79.6063) had significant positive gca effects whereas L2 (-108.8381), L1 (-97.8270), L7 (-85.7937) and L6 (-22.8159) showed significant negative gca effects. Among the testers T2 (49.7667) showed significant positive gca effect whereas T1 (-42.0476) and T3 (-7.7190) showed significant negative gca effects. The testers differed significantly from each other.

The sca effects had a range between -233.167 (L3 x T2) to (256.511) in L6 x T2 for yield per plant. Ten crosses recorded positive significant sca effects, while ten crosses exhibited significantly negative sca effects. Significant positive sca effects were shown by L6 x T2, L3 x T3, L1 x T3, L7 x T1, L2 x T2, L4 x T2, L5 x T1, L5 x T2, L4 x T3 and L2 x T1. Negative significant sca effects were

exhibited by L3 x T2, L2 x T3, L1 x T2, L7 x T2, L6 x T1, L6 x T3, L5 x T3, L4 x T1, L1 x T1 and L7 x T3.

4.4.3.7 Crop Duration

For crop duration general combining ability effects of lines varied from -2.7714 (L4) to 1.5397 (L7). Lines L4 (-2.7714) and L6 (-0.7714) had significant negative gca effects whereas L7 (1.5397), L5 (1.1619) and L2 (0.9841) showed significant positive gca effects. Among the testers T1 (-0.7365) showed significant negative gca effect while T2 (0.6540) showed significant positive gca effect.

The sca effects had a range between -3.327 (L2 x T3) to 3.451 (L1 x T3) for crop duration. Out of twenty one hybrids studied, eight crosses recorded negative significant sca effects, while seven crosses exhibited significantly positive sca effects. Significant negative sca effects were shown by L2 x T3, L1 x T2, L3 x T3, L4 x T2, L6 x T2, L7 x T3, L6 x T1 and L5 x T1. Positive significant sca effects were exhibited by L1 x T3, L2 x T2, L6 x T3, L3 x T2, L4 x T1, L7 x T2 and L5 x T2.

4.4.3.8 Harvest Index

General combining ability effects of lines for harvest index varied from -0.0190 (L1) to 0.0121 (L3). All the seven lines had significant general combining ability effects for harvest index. Lines L3 (0.0121), L4 (0.0097), L5 (0.0060) and L6 (0.0039) had significant positive gca effects whereas L1 (-0.0190), L2 (-0.0084) and L7 (-0.0043) showed significant negative gca effects. Among the testers T2 (0.0048) showed significant positive gca effect whereas T1(-0.0024) and T3 (-0.0024) showed significant negative gca effects. The testers differed significantly from each other.

The sca effects had a range between -0.013 (L4 x T1) to 0.017 (L3 x T3) for harvest index. Nine crosses recorded positive significant sca effects, while eight crosses exhibited significantly negative sca effects. Significant positive sca effects were shown by L3 x T3, L7 x T1, L5 x T1, L6 x T2, L4 x T2, L2 x T1, L2

x T2, L1 x T3 and L4 x T3. Negative significant sca effects were exhibited by L4 x T1, L2 x T3, L3 x T2, L7 x T2, L5 x T3, L6 x T1, L3 x T1 and L6 x T3.

4.4.3.9 Proline content

For proline content general combining ability effects of lines varied from -0.4950 (L1) to 0.4021 (L5). All the seven lines had significant general combining ability effects. Lines L5 (0.4021), L6 (0.2458), L3 (0.2540) and L4 (0.1277) had significant positive gca effects whereas L1 (-0.4950), L7 (-0.4188) and L2 (-0.1158) showed significant negative gca effects. Among the testers T2 (0.2760) showed significant positive gca effect whereas T1(-0.2249) and T3 (-0.0510) showed significant negative gca effects. The testers differed significantly from each other.

The sca effects had a range between -1.179 in L5 x T3 to 1.210 in L7 x T1 for proline content. Out of twenty one hybrids studied, ten crosses recorded positive significant sca effects, while ten crosses exhibited significantly negative sca effects. Significant positive sca effects were shown by L7 x T1, L5 x T1, L1 x T3, L3 x T3, L6 x T2, L2 x T3, L4 x T2, L3 x T2, L5 x T2 and L2 x T2. Negative significant sca effects were exhibited by L5 x T3, L3 x T1, L7 x T2, L1 x T2, L2 x T1, L6 x T3, L4 x T1, L7 x T3, L4 x T3 and L6 x T1.

4.4.3.10 Membrane Integrity

General combining ability effects of lines for membrane integrity varied from -0.4950 (L6) to 0.4021 (L4). Lines L4 (0.963), L2 (0.763) and L5 (0.275) had significant positive gca effects whereas L6 (-0.959), L7 (-0.703) and L1 (-0.525) showed significant negative gca effects. Among the testers T3 (0.508) and T2 (0.165) showed significant positive gca effect whereas T1(-0.673) showed significant negative gca effects. The testers differed significantly from each other.

The sca effects had a range between -3.159 (L4 x T1) to 4.278 (L2 x T1) for membrane integrity. Seven crosses recorded positive significant sca effects, while nine crosses exhibited significantly negative sca effects. Significant positive sca effects were shown by L2 x T1, L7 x T1, L4 x T2, L1 x T3, L5 x T3, L3 x T2

and L6 x T3. Negative significant sca effects were exhibited by L4 x T1, L2 x T3, L7 x T3, L2 x T2, L1 x T2, L3 x T1, L5 x T1, L7 x T2 and L6 x T1.

4.4.3.11 Percentage leakage

For percentage leakage general combining ability effects of lines varied from -0.9664 (L4) to 0.9503 (L6). Lines L4 (-0.9664) and L2 (-0.7542) had significant negative gca effects whereas L6 (0.9503), L7 (0.7100) and L1 (0.5248) showed significant positive gca effects. Among the testers T3(-0.5133) showed significant negative gca effect while T1 (0.6788) showed significant positive gca effect.

The sca effects had a range between -4.287 (L2 x T1) to 3.179 (L4 x T1) for percentage leakage. Nine crosses recorded negative significant sca effects, while nine crosses exhibited significantly positive sca effects. Significant negative sca effects were shown by L2 x T1, L7 x T1, L4 x T2, L1 x T3, L5 x T3, L3 x T2, L6 x T3, L4 x T3 and L6 x T2. Positive significant sca effects were exhibited by L4 x T1, L2 x T3, L7 x T3, L2 x T2, L1 x T2, L3 x T1, L5 x T1, L7 x T2 and L6 x T1.

4.4.3.12 Ascorbic Acid

General combining ability effects of lines for ascorbic acid varied from -0.8571 (L1 and L7) to 1.3651 (L5). Lines L5 (1.3651) and L2 (0.8095) had significant positive gca effects whereas L1 (-0.8571), L7 (-0.8571) and L4 (-0.5238) showed significant negative gca effects. Among the testers only T2 (0.3492) showed significant positive gca effect.

The sca effects had a range between -2.127 (L3 x T2) to 1.873 (L5 x T2) for ascorbic acid. Out of twenty one hybrids studied, six crosses recorded positive significant sca effects, while four crosses exhibited significantly negative sca effects. Significant positive sca effects were shown by L5 x T2, L3 x T3, L2 x T2, L2 x T1, L1 x T3 and L6 x T3. Negative significant sca effects were exhibited by L3 x T2, L2 x T3, L5 x T3 and L1 x T2.

4.4.3.13 Canopy Temperature

For canopy temperature, general combining ability effects of lines varied from -0.8476 (L3) to 1.1635 (L1). All the seven lines had significant general combining ability effects for canopy temperature. Lines L3 (-0.8476), L6 (-0.7921), L2 (-0.5254) and L4 (-0.3143) had significant negative gca effects whereas L1 (1.1635), L5 (0.8968) and L7 (0.4190) showed significant positive gca effects. Among the testers T2 (-0.1810) showed significant negative gca effect while T1 (0.1762) showed significant positive gca effect.

The sca effects had a range between -1.565 in L2 x T1 to 2.106 in L2 x T3 for canopy temperature. Eleven crosses recorded negative significant sca effects, while eight crosses exhibited significantly positive sca effects. Significant negative sca effects were shown by L2 x T1, L4 x T2, L3 x T1, L7 x T1, L6 x T3, L1 x T3, L6 x T2, L4 x T3, L3 x T3, L5 x T1 and L2 x T2. Positive significant sca effects were exhibited by L2 x T3, L4 x T1, L6 x T1, L3 x T2, L7 x T3, L1 x T2, L1 x T1 and L5 x T3.

4.4.3.14 Relative Water Content

For relative water content, general combining ability effects of lines varied from -8.2381 (L1) to 6.0952 (L3). Lines L3 (6.0952) and L4 (5.0952) had significant positive gca effects whereas L1 (-8.2381) and L7 (-2.9048) showed significant negative gca effects. Among the testers T2 (1.1746) showed significant positive gca effect whereas T1 (-2.2063) showed significant negative gca effects.

The sca effects had a range between -10.905 (L4 x T1) to 10.190 (L4 x T3) for relative water content. Eight crosses recorded positive significant sca effects, while seven crosses exhibited significantly negative sca effects. Significant positive sca effects were shown by L4 x T3, L7 x T1, L5 x T1, L2 x T1, L6 x T3, L3 x T2, L2 x T 2 and L3 x T3. Negative significant sca effects were exhibited by L4 x T1, L2 x T3, L3 x T1, L5 x T3, L7 x T3, L7 x T2 and L6 x T1.

4.4.3.15 Water Use Efficiency

For water use efficiency, general combining ability effects of lines varied from -1.2444 (L2) to 1.5222 (L3). Lines L3 (1.5222), L6 (0.7111) and L4

(0.2667) had significant positive gca effects whereas L1 (-1.2444), L2 (-0.6222) and L7 (-0.6222) showed significant negative gca effects. Among the testers T2 (0.8635) showed significant positive gca effect whereas T1 (-0.8365) showed significant negative gca effects.

The sca effects had a range between -1.530 in L7 x T2 to 1.603 in L7 x T1 for water use efficiency. Ten crosses recorded positive significant sca effects, while seven crosses exhibited significantly negative sca effects. Significant positive sca effects were shown by L7 x T1, L6 x T2, L3 x T2, L1 x T3, L2 x T1, L2 x T2, L6 x T3, L1 x T1, L3 x T3 and L5 x T2. Negative significant sca effects were exhibited by L7 x T2, L6 x T1, L2 x T3, L1 x T2, L3 x T1, L5 x T3, and L4 x T1.

4.4.3.16 Stomatal Conductance

General combining ability effects of lines for stomatal conductance varied from -40.6810 (L7) to 31.4857 (L3). All the seven lines had significant general combining ability effects for stomatal conductance. Lines L7 (-40.6810), L6 (-24.4810) and L1 (-10.5810) had significant negative gca effects whereas L3 (31.4857), L2 (19.4413), L4 (16.7413) and L5 (8.0746) showed significant positive gca effects. Among the testers T1 (-18.5683) showed significant negative gca effect while T2 (13.9508) and T3 (4.6175) showed significant positive gca effect. The testers differed significantly from each other.

The sca effects had a range between -88.751 (L2 x T3) to 90.071 (L1 x T3) for stomatal conductance. Out of twenty one hybrids studied, eleven crosses recorded negative significant sca effects, while nine crosses exhibited significantly positive sca effects. Significant negative sca effects were shown by L2 x T3, L1 x T2, L4 x T1, L6 x T3, L1 x T1, L7 x T2, L5 x T3, L3 x T2, L3 x T1, L6 x T1 and L5 x T2. Positive significant sca effects were exhibited by L1 x T3, L6 x T2, L3 x T3, L2 x T2, L2 x T1, L7 x T1, L5 x T1, L4 x T2 and L4 x T3.

4.4.4 GENE ACTION

The magnitude of genetic variance for all the sixteen characters are presented in the table 20. The dominance variance was higher than the additive variance for all the traits under study. The ratio of additive variance to dominance variance was less than unity for all the morphological and physiological traits. The sca variances was also higher for all the character, shows the predominance of non-additive gene action (dominance and epistasis) in the expression of these characters. Non-additive variance is not fixable and this can be improved through heterosis breeding.

4.4.5 HETEROSIS

The heterosis percentage expressed by twenty one hybrids obtained from L xT cross for sixteen characters were estimated as its superiority over mid parent (di), standard check (dii) and better parent (diii) values. The standard heterosis was estimated based on the check Arka Mangala (table 21 to 28). Manifestation of heterosis was found in both positive and negative directions for all traits.

4.4.5.1 Days to 50% flowering

The range of relative heterosis for days to 50% flowering was from -11.14 (L6 x T2) to 24.48 (L1 x T2). Out of twenty one crosses, seven crosses showed negative and significant relative heterosis while nine crosses showed positive and significant relative heterosis. The best five F1's showing relative heterosis in negative direction were L6 x T2 (-11.14), L5 x T2 (-10.23), L4 x T2 (-9.59), L5 x T1 (-7.06) and L5 x T3 (-5.08).

The range of standard heterosis was from -19.78 (L4 x T2) to 7.33 (L6 x T1). Arka Mangala recorded a mean value of 48.2. Twelve crosses showed negative and significant standard heterosis while four crosses showed positive and significant standard heterosis. The best crosses showing significant heterosis over check in negative directions were L4 x T2 (-19.78), L5 x T2 (-18.67), L1 x T3 (-18.40), L4 x T3 (-17.98) and L6 x T2 (-17.84).

Table 20: Magnitude of genetic variance of various characters under water stress

Characters	σ^2GCA	σ^2SCA	$\frac{\sigma^2 GCA}{\sigma^2 SCA}$	σ^2A	σ^2D	$\frac{\sigma^2 A}{\sigma^2 D}$
Days to 50% flowering	0.6214	14.73	0.0422	1.2428	29.46	0.0422
Pod length	2.8822	43.99	0.0655	5.7644	87.98	0.0655
Pod girth	0.0295	0.5021	0.0588	0.0590	1.0042	0.0588
Pod weight	-0.0161	2.1115	-0.0076	-0.0322	4.2230	-0.0076
Pods per plant	0.6469	52.75	0.0123	1.2938	105.51	0.0123
Yield per plant	-321.69	38055.13	-0.0085	-643.38	76110.27	-0.0085
Crop duration	-0.0332	6.7193	-0.0049	-0.0664	13.4386	-0.0049
Harvest Index	0.0000	0.0001	0.0000	0.0000	0.0002	0.0000
Proline	-0.0158	0.8936	-0.0177	-0.0316	1.7872	-0.0177
Membrane Integrity	-0.0097	0.4249	-0.0228	-0.0194	0.8498	-0.0228
Leakage %	-0.1233	5.7777	-0.0213	-0.2466	11.5554	-0.0213
Ascorbic acid	-0.0050	1.8502	-0.0027	-0.0100	3.7004	-0.0027
Canopy Temperature	-0.0135	1.9908	-0.0068	-0.0270	3.9816	-0.0068
Relative Water Content	-0.2418	64.7514	-0.0037	-0.4836	129.5028	-0.0037
Water Use Efficiency	0.0574	1.3691	0.0419	0.1148	2.7382	0.0419
Stomatal conductance	-43.64	3416.53	-0.0128	-87.28	6833.05	-0.0128

Table 21. Heterosis (%) for days to 50% flowering and pod length under water stress

Sl. No	Hybrids	Days to 50% flowering			Pod length		
		RH	SH	HB	RH	SH	HB
1.	L1 x t1	21.06**	4.56**	27.27**	-31.97**	-31.78**	-37.92**
2.	L1 X T2	24.48**	6.92**	30.14**	-35.01**	-39.69**	-45.12**
3.	L1 X T3	-1.01 ns	-18.40**	-0.67 ns	-4.15 ns	-12.01**	-19.92**
4.	L2 X T1	9.33**	-1.94 ns	10.44**	-28.34**	-36.44**	-29.91**
5.	L2 X T2	16.12**	3.60**	16.67**	-24.35**	-38.56**	-29.15**
6.	L2 X T3	18.87**	1.94 ns	23.24**	-40.49**	-52.26**	-44.95**
7.	L3 X T1	-0.32 ns	-14.25**	5.26**	27.87**	-6.36*	3.27 ns
8.	L3 X T2	-3.48*	-17.43**	1.36 ns	22.66**	-19.35**	6.53**
9.	L3 X T3	2.28 ns	-16.04**	3.06*	51.58**	-1.84**	33.14**
10.	L4 X T1	10.54**	-1.38 ns	12.28**	-48.80**	-59.46**	-55.30**
11.	L4 X T2	-9.59**	-19.78**	-8.66**	-19.01**	-41.95**	-23.32**
12.	L4 X T3	-3.81**	-17.98**	-0.84 ns	-12.69**	-38.28**	-16.28**
13.	L5 X T1	-7.06**	-15.35**	-6.57**	-31.73**	-37.71**	-32.15**
14.	L5 X T2	-10.23**	-18.67**	-9.26**	-41.82**	-51.27**	-46.92**
15.	L5 X T3	-5.08**	-17.29**	0.00 ns	-45.90**	-55.23**	-51.23**
16.	L6 X T1	15.48**	7.33**	18.47**	-25.65**	-33.05**	-26.17**
17.	L6 X T2	-11.14**	-17.84**	-8.33**	37.38**	13.42**	26.86**
18.	L6 X T3	0.39 ns	-10.65**	8.03**	12.90**	-7.91**	3.00**
19.	L7 X T1	1.94 ns	-5.26**	4.58**	-15.60**	-15.96**	-22.53**
20.	L7 X T2	10.25**	1.94 ns	13.74**	-25.92**	-31.78**	-37.11**
21.	L7 X T3	10.33**	-1.80 ns	18.73**	-28.84**	-35.17**	-40.23**
CD		1.11	1.28	1.28	2.19	2.53	2.53

RH-Relative heterosis SH-Standard heterosis HB-heterobeltiosis

Significant at 5% level *: Significant at 1% level **

Table 22. Heterosis (%) for pod girth and pod weight under water stress

Sl. No	Hybrids	Pod girth			Pod weight		
		RH	SH	HB	RH	SH	HB
1.	L1 X T1	-19.15**	-4.04 ns	-30.15**	13.94**	-7.80**	-5.20**
2.	L1 X T2	1.94 ns	6.06 ns	-1.87 ns	21.85**	-4.09**	-1.38 ns
3.	L1 X T3	9.50*	22.22**	-0.82 ns	29.68**	3.43**	6.34**
4.	L2 X T1	-19.10**	9.09 ns	-20.59**	26.81**	-3.39**	10.05**
5.	L2 X T2	15.13**	38.38**	4.58 ns	35.18**	0.01 ns	13.92**
6.	L2 X T3	-17.79**	5.05 ns	-20.61**	31.39**	-1.42 ns	12.28**
7.	L3 X T1	2.77 ns	31.31**	-4.41 ns	21.11**	-10.65**	7.69**
8.	L3 X T2	12.50**	27.27**	7.69 ns	25.83**	-9.94**	8.54**
9.	L3 X T3	20.50**	45.45**	18.03**	46.06**	6.06**	27.83**
10.	L4 X T1	-16.33**	6.06 ns	-22.79**	36.01**	-0.93 ns	22.16**
11.	L4 X T2	12.61**	26.26**	8.70 ns	46.81**	3.70**	27.87**
12.	L4 X T3	-16.46**	0.00 ns	-18.85**	38.38**	-0.81 ns	22.31**
13.	L5 X T1	-13.91**	0.00 ns	-27.21**	41.41**	0.81 ns	29.24**
14.	L5 X T2	7.46 ns	9.09*	0.93 ns	45.44**	0.49 ns	28.82**
15.	L5 X T3	-16.67**	-9.09*	-26.23**	36.25**	-4.45**	22.49**
16.	L6 X T1	-28.96**	-7.07 ns	-32.35**	3.91**	-18.25**	-11.88**
17.	L6 X T2	7.83 ns	25.25**	0.81 ns	34.57**	2.91*	10.93**
18.	L6 X T3	-5.31 ns	17.17**	-5.69 ns	-0.32 ns	-22.74**	-16.72**
19.	L7 X T1	-17.16**	12.12*	-18.38**	10.60**	3.08*	-15.38**
20.	L7 X T2	-3.77 ns	16.16**	-12.88**	-1.34 ns	-10.23**	-26.30**
21.	L7 X T3	-11.02**	14.14*	-14.39**	-1.53 ns	-9.38**	-25.60**
CD		0.66	0.76	0.76	0.41	0.48	0.48

RH-Relative heterosis SH-Standard heterosis HB-heterobeltiosis
*Significant at 5% level **Significant at 1% level

Table 23. Heterosis (%) for pods per plant and yield per plant under water stress

Sl. No	Hybrids	Pods per plant			Yield per plant		
		RH	SH	HB	RH	SH	HB
1.	L1 X T1	-1.53 ns	-20.21**	-8.78**	13.96**	-26.24**	1.45 ns
2.	L1 X T2	-5.74**	-22.34**	-13.89**	17.35**	-25.40**	2.60 ns
3.	L1 X T3	19.25**	1.42 ns	6.19**	58.23**	4.71**	44.02**
4.	L2 X T1	-1.72 ns	-15.72**	-3.65**	24.73**	-18.51**	10.23**
5.	L2 X T2	18.45**	3.19**	14.42**	60.76**	3.17**	39.55**
6.	L2 X T3	-26.40**	-33.92**	-30.82**	-2.26 ns	-34.72**	-11.70**
7.	L3 X T1	22.86**	10.52**	19.57**	47.86**	-1.27 ns	28.55**
8.	L3 X T2	3.95**	-5.08**	2.69*	30.28**	-14.52**	11.30**
9.	L3 X T3	24.78**	17.26**	22.77**	82.02**	24.18**	61.70**
10.	L4 X T1	1.68 ns	-7.09**	-2.48**	37.28**	-7.96**	19.00**
11.	L4 X T2	24.03**	15.01**	20.72**	80.76**	19.10**	53.97**
12.	L4 X T3	14.99**	9.69**	14.85**	58.79**	8.77**	40.62**
13.	L5 X T1	19.03**	3.90**	18.78**	63.81**	4.70**	47.29**
14.	L5 X T2	22.53**	8.63**	20.45**	73.77**	9.05**	53.40**
15.	L5 X T3	2.65*	-6.26**	-1.86 ns	37.03**	-10.43**	26.01**
16.	L6 X T1	7.84**	-8.16**	5.00**	12.38**	-24.89**	-2.36 ns
17.	L6 X T2	35.38**	17.14**	29.88**	83.26**	20.35**	56.46**
18.	L6 X T3	14.25**	1.89 ns	6.68**	15.46**	-21.16**	2.50 ns
19.	L7 X T1	15.53**	-1.54 ns	12.57**	28.65**	1.51**	0.45 ns
20.	L7 X T2	-1.98 ns	-15.13**	-5.90**	-1.87 ns	-23.71**	-24.51**
21.	L7 X T3	-2.78*	-13.24**	-9.16**	-2.09 ns	-21.32**	-22.15**
CD		1.06	1.23	1.23	23.42	27.05	27.05

RH-Relative heterosis SH-Standard heterosis HB-heterobeltiosis

*Significant at 5% level **Significant at 1% level

Table 24. Heterosis (%) for crop duration and harvest index under water stress

Sl. No	Hybrids	Crop duration			Harvest index		
		RH	SH	HB	RH	SH	HB
1.	L1 X T1	-8.75**	-1.76**	-4.25**	-7.3212**	-9.0823**	-8.5710**
2.	L1 X T2	-9.68**	-3.22**	-4.75**	-3.8334**	-7.0594**	-6.5368**
3.	L1 X T3	-2.59**	3.60**	3.60**	-3.8420**	-7.0946**	-6.5722**
4.	L2 X T1	-4.67**	0.00 ns	-2.54**	-0.9106 ns	-3.9339**	-1.1046 ns
5.	L2 X T2	0.77*	5.21**	3.54**	2.7888**	-1.8423**	1.0485 ns
6.	L2 X T3	-6.36**	-2.99**	-2.99**	-4.4873**	-8.8173**	-6.1319**
7.	L3 X T1	-3.66**	-1.15**	-3.66**	0.2970**	-1.6592*	-1.0063 ns
8.	L3 X T2	0.71*	2.83**	1.21**	2.6563**	-0.8390 ns	-0.1806 ns
9.	L3 X T3	-4.38**	-3.14**	-3.14**	8.5796**	4.8524**	5.5485**
10.	L4 X T1	-0.51 ns	-1.99**	3.81**	-3.1340**	-4.2226**	-5.1649**
11.	L4 X T2	-3.36**	-5.28**	0.32**	6.4940**	3.7483**	2.7276**
12.	L4 X T3	-0.67 ns	-3.45**	2.27**	3.5065**	0.8089 ns	-0.1829 ns
13.	L5 X T1	-3.83**	-0.92*	-3.43**	3.8022**	1.4534*	2.7732**
14.	L5 X T2	-0.15 ns	2.37**	0.75**	3.6608**	-0.1925 ns	1.1060 ns
15.	L5 X T3	-0.34 ns	1.38**	1.38**	-0.2172 ns	-3.9542**	-2.7046**
16.	L6 X T1	-5.79**	-3.37**	-5.75**	-2.0429**	-4.2194**	-3.0537**
17.	L6 X T2	-4.80**	-2.83**	-4.37**	6.8527**	2.9245**	4.1772**
18.	L6 X T3	1.10*	2.37**	2.37**	0.5984 ns	-3.1279**	-1.9489**
19.	L7 X T1	4.00**	0.54 ns	10.80**	-0.5863 ns	-0.7453 ns	-3.5625**
20.	L7 X T2	7.56**	3.45**	14.01**	-3.8669**	-5.4189**	-8.1035**
21.	L7 X T3	5.02**	0.15 ns	10.38**	-3.4753**	-5.0606**	-7.7553**
CD		0.61	0.71	0.71	0.0041	0.0048	0.0048

RH-Relative heterosis SH-Standard heterosis HB-heterobeltiosis

*Significant at 5% level **Significant at 1% level

Table 25. Heterosis (%) for proline and membrane integrity under water stress

Sl. No	Hybrids	Proline			Membrane integrity		
		RH	SH	HB	RH	SH	HB
1.	L1 X T1	-12.073**	-4.229**	-27.331**	0.119 ns	1.442**	-3.838**
2.	L1 X T2	-18.920**	-17.125**	-37.116**	-0.300 ns	1.129**	-4.135**
3.	L1 X T3	29.859**	36.714**	3.736**	3.527**	5.123**	-0.349 ns
4.	L2 X T1	-26.717**	-17.495**	-40.694**	6.430**	8.356**	1.774**
5.	L2 X T2	25.855**	33.251**	-4.216**	0.679 ns	2.613**	-3.620**
6.	L2 X T3	31.595**	43.363**	3.052**	-0.733*	1.281**	-4.871**
7.	L3 X T1	-32.514**	-13.525**	-49.199**	1.584**	1.010*	-0.696 ns
8.	L3 X T2	24.052**	50.636**	-11.507**	5.277**	4.800**	3.030**
9.	L3 X T3	32.275**	64.676**	-3.259**	4.765**	4.403**	2.639**
10.	L4 X T1	-17.209**	6.581**	-37.825**	-1.194**	-0.221 ns	-4.806**
11.	L4 X T2	34.194**	63.754**	-4.472**	6.556**	7.725**	2.774**
12.	L4 X T3	-6.572**	16.872**	-31.821**	4.246**	5.501**	0.653 ns
13.	L5 X T1	68.430**	70.785**	46.289**	2.066**	1.254**	-0.003 ns
14.	L5 X T2	64.516**	55.782**	33.438**	4.246**	3.533**	2.248**
15.	L5 X T3	-5.572**	-7.691**	-20.931**	6.394**	5.781**	4.468**
16.	L6 X T1	6.894**	20.493**	-13.558**	-0.743*	0.029 ns	-4.189**
17.	L6 X T2	63.652**	73.495**	24.465**	1.804**	2.709**	-1.622**
18.	L6 X T3	-2.048**	6.846**	-23.349**	2.421**	3.441**	-0.921*
19.	L7 X T1	53.528**	45.946**	40.236**	3.102**	4.803**	-1.267**
20.	L7 X T2	-5.362**	-16.384**	-19.655**	-0.570 ns	1.182**	-4.678**
21.	L7 X T3	3.474**	-5.404**	-9.105**	-0.804*	1.050 *	-4.802**
CD		0.0300	0.0346	0.0346	0.598	0.691	0.691

RH-Relative heterosis SH-Standard heterosis HB-heterobeltiosis

*Significant at 5% level **Significant at 1% level

Table 26. Heterosis (%) for percentage leakage and ascorbic acid under water stress

Sl. No	Hybrids	Percentage leakage			Ascorbic acid		
		RH	SH	HB	RH	SH	HB
1.	L1 X T1	-0.694 ns	-7.708**	30.628**	0.03 ns	-11.60**	-2.54 ns
2.	L1 X T2	1.763 ns	-6.034**	32.998**	-2.63 ns	-13.95**	-5.13 ns
3.	L1 X T3	-20.860**	-27.378**	2.789 ns	6.49 ns	-4.65 ns	5.13 ns
4.	L2 X T1	-38.730**	-44.655**	-15.424**	15.00**	6.98 ns	6.98 ns
5.	L2 X T2	-4.119 ns	-13.965**	31.475**	20.00**	11.63**	11.63**
6.	L2 X T3	4.485*	-6.844**	42.357**	-8.64*	-13.95**	-13.95**
7.	L3 X T1	-8.171**	-5.398*	4.167 ns	11.69*	0.00 ns	7.50 ns
8.	L3 X T2	-27.409**	-25.652**	-18.134**	-3.90 ns	-13.95**	-7.50 ns
9.	L3 X T3	-24.915**	-23.528**	-15.796**	20.51**	9.30*	17.50**
10.	L4 X T1	6.799**	1.180 ns	36.257**	1.33 ns	-11.63**	0.00 ns
11.	L4 X T2	-37.627**	-41.282**	-20.926**	9.33*	-4.65 ns	7.89 ns
12.	L4 X T3	-24.546**	-29.400**	-4.925 ns	5.26 ns	-6.98 ns	5.26 ns
13.	L5 X T1	-10.507**	-6.702**	0.016 ns	18.92**	2.33 ns	18.92**
14.	L5 X T2	-21.741**	-18.882**	-13.040**	40.54**	20.93**	40.54**
15.	L5 X T3	-32.960**	-30.896**	-25.919**	6.67 ns	-6.98 ns	5.26 ns
16.	L6 X T1	4.174*	-0.154 ns	30.568**	-3.80 ns	-11.63*	-9.52*
17.	L6 X T2	-10.210**	-14.478**	11.837**	1.27 ns	-6.98 ns	-4.76 ns
18.	L6 X T3	-13.800**	-18.391**	6.719*	7.50*	0.00 ns	2.38 ns
19.	L7 X T1	-18.482**	-25.669**	10.700**	-8.64*	-13.95**	-15.91**
20.	L7 X T2	3.421 ns	-6.316**	39.522**	1.23 ns	-4.65 ns	-6.82 ns
21.	L7 X T3	4.864*	-5.610**	40.574**	-7.32*	-11.63*	-13.64**
CD		0.6001	0.6930	0.6930	0.97	1.11	1.11

RH-Relative heterosis SH-Standard heterosis HB-heterobeltiosis

*Significant at 5% level **Significant at 1% level

Table 27. Heterosis (%) for canopy temperature and relative water content under water stress

Sl. No	Hybrids	Canopy temperature			Relative water		
		RH	SH	HB	RH	SH	HB
1.	L1 X T1	3.535**	2.586**	7.087**	-3.23 ns	-6.25*	-19.75**
2.	L1 X T2	3.912**	1.616**	6.074**	-3.40 ns	-4.33 ns	-18.11**
3.	L1 X T3	-0.219 ns	-1.832**	2.475**	7.85**	2.40 ns	-12.35**
4.	L2 X T1	-9.159**	-9.159**	-6.954**	21.03**	13.46**	2.61 ns
5.	L2 X T2	-5.786**	-7.004**	-4.746**	21.30**	16.35**	5.22*
6.	L2 X T3	2.876**	2.155**	4.636**	3.66 ns	-4.81 ns	-13.91**
7.	L3 X T1	-6.689**	-8.297**	-2.632**	10.94**	2.40 ns	-4.91 ns
8.	L3 X T2	1.889**	-1.185*	4.920**	34.86**	27.40**	18.30**
9.	L3 X T3	-5.356**	-7.651**	-1.945**	37.23**	24.04**	15.18**
10.	L4 X T1	3.861**	2.909**	7.424**	-0.25 ns	-2.40 ns	-17.81**
11.	L4 X T2	-6.336**	-8.405**	-4.387**	19.23**	19.23**	0.40 ns
12.	L4 X T3	-4.929**	-6.466**	-2.362**	38.35**	32.69**	11.74**
13.	L5 X T1	-1.769**	-1.293*	0.109 ns	25.06**	18.75**	5.11*
14.	L5 X T2	0.923**	0.108 ns	1.530**	12.38**	9.13**	-3.40 ns
15.	L5 X T3	1.188**	0.970 ns	2.404**	10.59**	2.88 ns	-8.94**
16.	L6 X T1	2.640**	0.539 ns	7.488**	7.22**	0.00 ns	-8.77**
17.	L6 X T2	-5.463**	-8.621**	-2.304**	13.35**	8.17**	-1.32 ns
18.	L6 X T3	-5.928**	-8.513**	-2.189**	29.47**	18.27**	7.89**
19.	L7 X T1	-2.466**	-4.095**	1.714**	17.27**	15.87**	-3.98 ns
20.	L7 X T2	1.610**	-1.401*	4.571**	-0.95 ns	0.00 ns	-17.13**
21.	L7 X T3	3.091**	0.647 ns	6.743**	2.23 ns	-0.96 ns	-17.93**
CD		0.305	0.352	0.352	3.43	3.96	3.96

RH-Relative heterosis SH-Standard heterosis HB-heterobeltiosis
*Significant at 5% level **Significant at 1% level

Table 28. Heterosis (%) for water use efficiency and stomatal conductance under water stress

Sl. No	Hybrids	Water use efficiency			Stomatal conductance		
		RH	SH	HB	RH	SH	HB
1.	L1 X T1	-7.85**	-2.22 ns	-37.14**	-7.24**	-16.55**	-3.83**
2.	L1 X T2	-13.35**	-4.44 ns	-38.57**	-0.70 ns	-15.00**	0.68 ns
3.	L1 X T3	-2.80 ns	15.56**	-25.71**	40.58**	24.99**	44.05**
4.	L2 X T1	12.61**	11.67**	-21.18**	37.41**	13.27**	57.96**
5.	L2 X T2	34.41**	38.89**	-1.96 ns	58.50**	23.74**	72.56**
6.	L2 X T3	-20.10**	-10.56**	-36.86**	1.11*	-17.72**	14.74**
7.	L3 X T1	24.62**	13.89**	-9.69**	39.78**	-0.36 ns	101.67**
8.	L3 X T2	85.47**	77.22**	40.53**	58.09**	5.79**	114.12**
9.	L3 X T3	49.87**	56.11**	23.79**	76.90**	24.23**	151.45**
10.	L4 X T1	0.51 ns	9.44**	-32.07**	-8.05**	-14.83**	-7.52**
11.	L4 X T2	27.76**	44.44**	-10.34**	34.80**	18.97**	40.92**
12.	L4 X T3	7.31**	30.56**	-18.97**	23.20**	12.82**	23.90**
13.	L5 X T1	7.49**	11.67**	-26.10**	13.96**	8.30**	16.25**
14.	L5 X T2	33.16**	43.89**	-4.78*	13.49**	2.89**	21.87**
15.	L5 X T3	-1.43 ns	15.00**	-23.90**	4.62**	-1.68**	7.98**
16.	L6 X T1	-7.12**	-2.22 ns	-36.46**	-8.82**	-16.35**	-7.39**
17.	L6 X T2	50.25**	64.44**	6.86**	29.79**	13.40**	34.33**
18.	L6 X T3	22.35**	44.44**	-6.14**	-6.92**	-15.59**	-6.55**
19.	L7 X T1	14.65**	26.11**	-22.79**	9.79**	-4.83**	18.66**
20.	L7 X T2	-10.46**	2.22 ns	-37.41**	2.27**	-15.82**	4.96**
21.	L7 X T3	-9.05**	11.67**	-31.63**	2.96**	-11.84**	9.92**
CD		0.305	0.352	0.352	2.37	2.74	2.74

RH-Relative heterosis SH-Standard heterosis HB-heterobeltiosis
*Significant at 5% level **Significant at 1% level

The range of heterobeltiosis was from -9.26 (L5 x T2) to 30.14 (L1 x T2). The best crosses showing heterobeltiosis in negative direction were L5 x T2 (-9.26), L4 x T2 (-8.66), L6 x T2 (-8.33) and L5 x T1 (-6.57). While thirteen crosses showed positive and significant heterosis over better parent for days to flowering.

4.4.5.2 Pod length

The range of relative heterosis for pod length was from -48.80 (L4 x T1) to 51.58 (L3 x T3). Five crosses showed positive and significant relative heterosis. The best five F1's showing relative heterosis in positive direction were L3 x T3 (51.58), L6 x T2 (37.38), L3 x T1 (27.87), L3 x T2 (22.66) and L6 x T3 (12.90). While fifteen crosses showed negative and significant relative heterosis for pod length.

The range of standard heterosis was from -59.46 (L4 x T1) to 13.42 (L6 x T2). Arka Mangala recorded a mean value of 47.2. One cross showed positive and significant standard heterosis. The best cross showing significant heterosis over check in positive directions was L6 x T2 (13.42). Twenty crosses showed negative and significant standard heterosis for pod length.

The range of heterobeltiosis was from-55.30 (L4 x T1) to 33.14 (L3 x T3). Four crosses showed positive and significant heterosis. The best crosses showing heterobeltiosis in positive direction were L3 x T3 (33.14), L6 x T2 (26.86), L3 x T2 (6.53) and L6 x T3 (3.00).

4.4.5.3 Pod girth

The range of relative heterosis for pod girth was from -28.96 (L6 x T1) to 20.50 (L3 x T3). Five crosses showed positive and significant relative heterosis. Ten crosses showed negative and significant relative heterosis. The best crosses showing relative heterosis in positive direction were L3 x T3 (20.50), L2 x T2 (15.13), L4 x T2 (12.61), L3 x T2 (12.50) and L1 x T3 (9.50).

The range of standard heterosis was from -7.07 (L6 x T1) to 45.45 (L3 x T3). Arka Mangala recorded a mean value of 6.6. Twelve crosses showed positive

and significant standard heterosis. The best five crosses showing significant heterosis over check in positive directions were L3 x T3 (45.45), L2 x T2 (38.38), L3 x T1 (31.31), L3 x T2 (27.27) and L4 x T2 (26.26).

The range of heterobeltiosis was from -32.35 (L6 x T1) to 18.03 (L3 x T3). One cross showed positive and significant heterosis. Eleven crosses showed negative and significant heterosis over better parent. The best cross showing heterobeltiosis in positive direction was L3 x T3 (18.03).

4.4.5.4 Pod weight

The range of relative heterosis for pod weight was from -1.53 (L7 x T3) to 46.81 (L4 x T2). Eighteen crosses showed positive and significant relative heterosis. The best five F1'S showing relative heterosis in positive direction were L4 x T2 (46.81), L3 x T3 (46.06), L5 x T2 (45.44), L5 x T1 (41.41) and L4 x T3 (38.38).

The range of standard heterosis was from -22.74 (L6 x T3) to 6.06 (L3 x T3). Arka Mangala recorded a mean value of 18.7. Five crosses showed positive and significant standard heterosis while ten crosses showed negative and significant standard heterosis. The best crosses showing significant heterosis over check in positive directions were L3 x T3 (6.06), L4 x T2 (3.70), L1 x T3 (3.43), L7 x T1 (3.08) and L6 x T2 (2.91).

The range of heterobeltiosis was from -26.30 (L7 x T2) to 29.24 (L5 x T1). Fourteen crosses showed positive and significant heterosis while six crosses showed negative and significant heterosis over better parent. The best five F1's showing heterobeltiosis in positive direction were L5 x T1 (29.24), L5 x T2 (28.82), L4 x T2 (27.87), L3 x T3 (27.83) and L5 x T3 (22.49).

4.4.5.5 Pods per plant

For pods per plant the range of relative heterosis was from -26.40 (L2 x T3) to 35.38 (L6 x T2). Thirteen crosses showed positive and significant relative heterosis. The best five F1's showing relative heterosis in positive direction were L3 x T3 (24.78), L4 x T2 (24.03), L3 x T1 (22.86), L5 x T2 (22.53) and L1 x T3 (19.25).

The range of standard heterosis for pods per plant was from -33.92 (L2 x T3) to 17.26 (L3 x T3). Arka Mangala recorded a mean value of 56.4. Eight crosses showed positive and significant standard heterosis. The best crosses showing significant heterosis over check in positive directions were L3 x T3 (17.26), L6 x T2 (17.14), L4 x T2 (15.01), L3 x T1 (10.52) and L4 x T3 (9.69).

The range of heterobeltiosis was from -30.82 (L2 x T3) to 29.88 (L6 x T2). Thirteen crosses showed positive and significant heterosis while seven crosses showed negative and significant heterosis over better parent for pods per plant. The best five F1's showing heterobeltiosis in positive direction were L6 x T2 (29.88), L3 x T3 (22.77), L4 x T2 (20.72), L5 x T2 (20.45) and L3 x T1 (19.57).

4.4.5.6 Yield per plant

The range of relative heterosis for yield per plant was from -2.26 (L2 x T3) to 83.26 (L6 x T2). Eighteen crosses showed positive and significant relative heterosis. The best five F1'S showing relative heterosis in positive direction were L6 x T2 (83.26), L3 x T3 (82.02), L4 x T2 (80.76), L5 x T2 (73.77) and L5 x T1 63.81().

The range of standard heterosis was from -34.72 (L2 x T3) to 24.18 (L3 x T3). Arka Mangala recorded a mean value of 1059.2. Nine crosses showed positive and significant standard heterosis while eleven crosses showed negative and significant standard heterosis for yield per plant. The best crosses showing significant heterosis over check in positive directions were L3 x T3 (24.18), L6 x T2 (20.35), L4 x T2 (19.10), L5 x T2 (9.05) and L4 x T3 (8.77).

The range of heterobeltiosis was from -24.51 (L7 x T2) to 61.70 (L3 x T3). Twelve crosses showed positive and significant heterosis. The best five F1's showing heterobeltiosis in positive direction were L6 x T2 (56.46), L4 x T2 (53.97), L5 x T2 (53.40), L5 x T1 (47.29) and L1 x T3 (44.02).

4.4.5.7 Crop Duration

For crop duration the range of relative heterosis for crop duration was from -9.68 (L1 x T2) to 7.56 (L7 x T2). Eleven crosses showed negative and

significant relative heterosis while six crosses showed positive and significant relative heterosis. The best five F1's showing relative heterosis in negative direction were L1 x T2 (-9.68), L1 x T1 (-8.75), L2 x T3 (-6.36), L6 x T1 (-5.79) and L6 x T2 (-4.80).

The range of standard heterosis was from -5.28 (L4 x T2) to 5.21 (L2 x T2). Arka Mangala recorded a mean value of 87.1. Eleven crosses showed negative and significant standard heterosis. The best crosses showing significant heterosis over check in negative directions were L4 x T2 (-5.28), L4 x T3 (-3.45), L6 x T1 (-3.37), L1 x T2 (-3.22) and L3 x T3 (-3.14).

The range of heterobeltiosis was from -5.75 (L6 x T1) to 14.01 (L7 x T2). Nine crosses showed negative and significant heterosis. The best five F1's showing heterobeltiosis in negative direction were L6 x T1 (-5.75), L1 x T2 (-4.75), L6 x T2 (-4.37), L1 x T1 (-4.25) and L3 x T1 (-3.66).

4.4.5.8 Harvest Index

For harvest index the range of relative heterosis was from -7.3212 (L1 x T1) to 8.5796 (L3 x T3). Nine crosses showed positive and significant relative heterosis. The best five F1's showing relative heterosis in positive direction were L3 x T3 (8.5796), L6 x T2 (6.8527), L4 x T2 (6.4940), L5 x T1 (3.8022) and L5 x T2 (3.6608).

The range of standard heterosis was from -9.0823 (L1 x T1) to 4.8524 (L3 x T3). Arka Mangala recorded a mean value of 0.364. Four crosses showed positive and significant standard heterosis while thirteen crosses showed negative and significant standard heterosis for harvest index. The best crosses showing significant heterosis over check in positive directions were L3 x T3 (4.8524), L4 x T2 (3.7483), L6 x T2 (2.9245) and L5 x T1 (1.4534).

The range of heterobeltiosis was from -8.5710 (L1 x T1) to 5.5485 (L3 x T3). Four crosses showed positive and significant heterosis. The best crosses showing heterobeltiosis in positive direction were L3 x T3 (5.5485), L6 x T2 (4.1772), L5 x T1 (2.7732) and L4 x T2 (2.7276).

4.4.5.9 Proline content

The range of relative heterosis for proline content was from -32.514 (L3 x T1) to 68.430 (L5 x T1). Twelve crosses showed positive and significant relative heterosis. The best five F1's showing relative heterosis in positive direction were L5 x T1 (68.430), L5 x T2 (64.516), L6 x T2 (63.652), L7 x T1 (53.528) and L4 x T2 (34.194).

The range of standard heterosis was from -17.495 (L2 x T1) to 73.495 (L6 x T2). Arka Mangala recorded a mean value of 2.60. Fourteen crosses showed positive and significant standard heterosis while seven crosses showed negative and significant standard heterosis for proline content. The best crosses showing significant heterosis over check in positive directions were L6 x T2 (73.495), L5 x T1 (70.785), L3 x T3 (64.676), L4 x T2 (63.754) and L5 x T2 (55.782).

The range of heterobeltiosis was from -49.199 (L3 x T1) to 46.289 (L5 x T1). Six crosses showed positive and significant heterosis. The best five F1's showing heterobeltiosis in positive direction were L5 x T1 (46.289), L7 x T1 (40.236), L5 x T2 (33.438), L6 x T2 (24.465) and L1 x T3 (3.736).

4.4.5.10 Membrane Integrity

For membrane integrity the range of relative heterosis was from -1.194 (L4 x T1) to 6.556 (L4 x T2). Thirteen crosses showed positive and significant relative heterosis. The best five F1's showing relative heterosis in positive direction were L4 x T2 (6.556), L2 x T1 (6.430), L5 x T3 (6.394), L3 x T2 (5.277) and L3 x T3 (4.765).

The range of standard heterosis was from -0.221 (L4 x T1) to 8.356 (L2 x T1). Arka Mangala recorded a mean value of 84.2. Nineteen crosses showed positive and significant standard heterosis. The best crosses showing significant heterosis over check in positive directions were L2 x T1 (8.356), L4 x T2 (7.725), L5 x T3 (5.781), L4 x T3 (5.501) and L1 x T3 (5.123).

The range of heterobeltiosis was from -4.871 (L2 x T3) to 4.468 (L5 x T3). Six crosses showed positive and significant heterosis while eleven crosses showed negative and significant heterosis over better parent for membrane

integrity. The best five F1's showing heterobeltiosis in positive direction were L5 x T3 (4.468), L3 x T2 (3.030), L4 x T2 (2.774), L3 x T3 (2.639) and L5 x T2 (2.248).

4.4.5.11 Percentage leakage

For percentage leakage the range of relative heterosis was from -38.730 (L2 x T1) to 6.799 (L4 x T1). Thirteen crosses showed negative and significant relative heterosis while four crosses showed positive and significant relative heterosis for percentage leakage. The best five F1's showing relative heterosis in negative direction were L2 x T1 (-38.730), L4 x T2 (-37.627), L5 x T3 (-32.960), L3 x T2 (-27.409) and L3 x T3 (-24.915).

The range of standard heterosis was from -44.655 (L2 x T1) to 1.180 (L4 x T1). Arka Mangala recorded a mean value of 15.8. Nineteen crosses showed negative and significant standard heterosis. The best crosses showing significant heterosis over check in negative directions were L2 x T1 (-44.655), L4 x T2 (-41.282), L5 x T3 (-30.896), L4 x T3 (-29.400) and L1 x T3 (-27.388).

The range of heterobeltiosis was from -25.919 (L5 x T3) to 42.357 (L2 x T3). Seven crosses showed negative and significant heterosis. The best five F1's showing heterobeltiosis in negative direction were L5 x T3 (-25.919), L4 x T2 (-20.926), L3 x T2 (-18.134), L3 x T3 (-15.796) and L2 x T1 (-15.424).

4.4.5.12 Ascorbic Acid

The range of relative heterosis for ascorbic acid was from -8.64 (L2 x T3) to 40.54 (L5 x T2). Eight crosses showed positive and significant relative heterosis. The best crosses showing relative heterosis in positive direction were L5 x T2 (40.54), L3 x T3 (20.51), L2 x T2 (20.00), L5 x T1 (18.92) and L2 x T1 (15.00).

The range of standard heterosis was from -13.95 (L3 x T2) to 20.93 (L5 x T2). Arka Mangala recorded a mean value of 14.3. Three crosses showed positive and significant standard heterosis. The best crosses showing significant heterosis over check in positive directions were L5 x T2 (20.93), L2 x T2 (11.63) and L2 x T3 (9.30).

The range of heterobeltiosis was from -15.91 (L7 x T1) to 40.54 (L5 x T2). Four crosses showed positive and significant heterosis. The best crosses showing heterobeltiosis in positive direction were L5 x T2 (40.54), L5 x T1 (18.92), L3 x T3 (17.50) and L2 x T2 (11.63).

4.4.5.13 Canopy Temperature

The range of relative heterosis for canopy temperature was from -9.159 (L2 x T1) to 3.912 (L1 x T2). Ten crosses showed negative and significant relative heterosis. The best five F1's showing relative heterosis in negative direction were L2 x T1 (-9.159), L3 x T1 (-6.689), L4 x T2 (-6.336), L6 x T3 (-5.928) and L2 x T2 (-5.786).

The range of standard heterosis was from -9.159 (L2 x T1) to 2.909 (L4 x T1). Arka Mangala recorded a mean value of 30.9. Thirteen crosses showed negative and significant standard heterosis while four crosses showed positive and significant standard heterosis for canopy temperature. The best crosses showing significant heterosis over check in negative directions were L2 x T1 (-9.159), L6 x T2 (-8.621), L6 x T3 (-8.513), L4 x T2 (-8.405) and L3 x T1 (-8.297).

The range of heterobeltiosis was from -6.954 (L2 x T1) to 7.488 (L6 x T1). Eight crosses showed negative and significant heterosis. The best five F1's showing heterobeltiosis in negative direction were L2 x T1 (-6.954), L2 x T2 (-4.746), L4 x T2 (-4.387), L3 x T1 (-2.632) and L4 x T3 (-2.362).

4.4.5.14 Relative Water Content

For relative water content the range of relative heterosis was from -3.40 (L1 x T2) to 38.35 (L4 x T3). Fifteen crosses showed positive and significant relative heterosis. The best five F1's showing relative heterosis in positive direction were L4 x T3 (38.35), L3 x T3 (37.23), L3 x T2 (34.86), L6 x T3 (29.47) and L5 x T1 (25.06).

The range of standard heterosis was from -6.25 (L1 x T1) to 32.69 (L4 x T3). Arka Mangala recorded a mean value of 69.3. Eleven crosses showed positive and significant standard heterosis while one cross showed negative and significant standard heterosis for relative water content. The best crosses showing

significant heterosis over check in positive directions were L4 x T3 (32.69), L3 x T2 (27.40), L3 x T3 (24.04), L4 x T2 (19.23) and L5 x T1 (18.75).

The range of heterobeltiosis was from -19.75 (L1 x T1) to 18.30 (L3 x T2). Six crosses showed positive and significant heterosis. The best five F1's showing heterobeltiosis in positive direction were L3 x T2 (18.30), L3 x T3 (15.18), L4 x T3 (11.74), L6 x T3 (7.89) and L2 x T2 (5.22).

4.4.5.15 Water Use Efficiency

For water use efficiency the range of relative heterosis was from -20.10 (L2 x T3) to 85.47 (L3 x T2). Twelve crosses showed positive and significant relative heterosis while six crosses showed negative and significant relative heterosis. The best five F1's showing relative heterosis in positive direction were L3 x T2 (85.47), L6 x T2 (50.25), L3 x T3 (49.87), L2 x T2 (34.41) and L5 x T2 (33.16).

The range of standard heterosis was from -10.56 (L2 x T3) to 77.22 (L3 x T2). Arka Mangala recorded a mean value of 6. Sixteen crosses showed positive and significant standard heterosis. The best crosses showing significant heterosis over check in positive directions were L3 x T2 (77.22), L6 x T2 (64.44), L3 x T3 (56.11), L4 x T2 (44.44) and L6 x T3 (44.44).

The range of heterobeltiosis was from -38.57 (L1 x T2) to 40.53 (L3 x T2). Three crosses showed positive and significant heterosis. The best crosses showing heterobeltiosis in positive direction were L3 x T2 (40.53), L3 x T3 (23.79) and L6 x T2 (6.863).

4.4.5.16 Stomatal Conductance

For stomatal conductance the range of relative heterosis was from -8.82 (L6 x T1) to 76.90 (L3 x T3). Four crosses showed negative and significant relative heterosis while sixteen crosses showed positive and significant relative heterosis for stomatal conductance. The best crosses showing relative heterosis in negative direction were L6 x T1 (-8.82), L4 x T1 (-8.05), L1 x T1 (-7.24) and L6 x T3 (-6.92).

The range of standard heterosis was from -17.72 (L2 x T3) to 24.99 (L1 x T3). Arka Mangala recorded a mean value of 348.4. Ten crosses showed negative and significant standard heterosis. The best crosses showing significant heterosis over check in negative directions were L2 x T3 (-17.72), L1 x T1 (-16.55), L6 x T1 (-16.35), L7 x T2 (-15.82) and L6 x T3 (-15.59).

The range of heterobeltiosis was from -7.52 (L4 x T1) to 151.45 (L3 x T3). Out of twenty one crosses, four crosses showed negative and significant heterosis. The best crosses showing heterobeltiosis in negative direction were L4 x T1 (-7.52), L6 x T1 (-7.39), L6 x T3 (-6.55) and L1 x T1 (-3.83)

4.4.6 PROPORTIONAL CONTRIBUTION

The proportional contribution of lines, testers and crosses to total variance of the characters under study are presented in table 29.

Among the line the values ranged from 8.83 for membrane integrity to 66.77 for pod length. Among the testers, the value ranged from 0.33 pod length to 23.88 for water use efficiency. In the crosses, the values ranged from 32.9 for pod length to 85.06 for membrane integrity.

The crosses had contributed maximum to the total variance for most of the traits except days to 50% flowering, pod length, pod girth and harvest index. The testers had the least contribution to the total variance with respect to crosses and lines.

Experiment-IV

4.5 GENE EXPRESSION STUDY USING REAL TIME qRT-PCR

Quantitative Real-Time PCR was carried out for determining quantitative changes in gene expression for drought tolerance at the molecular level. qRT-PCR has been used to compare the differential expression of drought tolerance genes in the tolerant and susceptible genotypes. The genotypes and hybrid selected for gene expression study are given in table 34.

Table 29. Proportional contribution of lines, testers and line x tester to total variance

Sl.No.	Characters	Lines (%)	Testers (%)	Lines x testers (%)
1	Days to 50% flowering	49.99	10.86	39.15
2	Pod length	66.77	0.33	32.90
3	Pod girth	47.09	16.69	36.21
4	Pod weight	31.09	2.54	66.38
5	Pods per plant	45.13	2.98	51.88
6	Yield per plant	28.31	4.43	67.26
7	Crop duration	30.61	5.38	64.01
8	Harvest Index	53.86	5.76	40.39
9	Proline	15.97	6.54	77.49
10	Membrane Integrity	8.83	6.11	85.06
11	Leakage %	11.61	6.17	82.22
12	Ascorbic acid	34.63	3.38	61.99
13	Canopy Temperature	33.15	1.22	65.63
14	Relative Water Content	33.07	4.02	62.92
15	Water Use Efficiency	36.93	23.88	39.19
16	Stomatal conductance	21.41	6.86	71.72

Table 30. Best parents for important traits based on mean performance and gca

Sl. No.	Characters	Mean		*gca*		Mean and *gca*	
		Line	Tester	Line	Tester	Line	Tester
1	Days to 50% flowering	L_3, L_1, L_4, L_2	T_3, T_2, T_1	L_5, L_3, L_4	T_3, T_2	L_3, L_4	T_3, T_2
2	Pod length	L_1, L_7, L_5, L_6, L_2	T_1, T_2	L_3, L_6, L_1, L_7	-	L_1, L_7, L_6	-
3	Pod girth	L_7, L_2, L_6, L_3, L_4	T_1, T_3	L_3	T_2	L_3	-
4	Pod weight	L_7, L_1, L_6, L_2, L_3	-	L_4, L_5, L_2, L_1	T_2	L_1, L_2	-
5	Pods per plant	L_3, L_4	T_1, T_2, T_3	$L_3, L_4, L_6 L_5$	T_2	L_3, L_4	T_2
6	Yield per plant	$L_1, L_2, L_3 L_4, L_6, L_7$	-	L_4, L_3, L_5	T_2	L_3, L_4	-
7	Crop duration	L_7, L_4, L_3	T_3	L_4, L_6, L_3	T_1	L_3, L_4	-
8	Harvest Index	L_7, L_1, L_3, L_6, L_5	-	$L_3, L_5 L_6$	T_2	L_3, L_5, L_6	-
9	Proline	L_4, L_3, L_6, L_2, L_1	-	L_5, L_3, L_6, L_4	T_2	L_3, L_4, L_6	-
10	Membrane Integrity	L_2, L_7, L_1, L_4, L_6	-	L_4, L_2, L_5	T_3, T_2	L_2, L_4	-
11	Leakage %	L_2, L_7, L_1, L_4, L_6	-	L_4, L_2	T_3	L_2, L_4	-
12	Ascorbic acid	L_7, L_2, L_6, L_3	-	L_5, L_2	T_2	L_2	-
13	Canopy Temperature	L_6, L_3, L_7, L_4, L_1	-	L_3, L_6, L_2, L_4	T_2	L_3, L_4, L_6	-
14	Relative Water Content	$L_7, L_4, L_1, L_5, L_2, L_6, L_3$	-	L_3, L_4	T_2	L_3, L_4	-
15	Water Use Efficiency	$L_7, L_1, L_6, L_5, L_2, L_3$	-	$L_3, L_6,$	T_2	L_3, L_6	-
16	Stomatal conductance	$L_3, L_2, L_7, L_1, L_6, L_4$	T_2, T_3, T_1	L_7, L_6, L_1	T_1	L_1, L_6, L_7	T_1

Table 31: Frequency of the superior parent for different traits

Sl.No.	Genotype	No.of times
1	L1	3
2	L2	4
3	L3	10
4	L4	9
5	L5	1
6	L6	6
7	L7	2
8	T1	1
9	T2	2
10	T3	1

Table 32. Best hybrids for important traits based on mean performance, *sca* and heterobeltiosis

Sl.No	Characters	mean	*sca*	Heterobeltiosis	Superior hybrids
1	Days to 50% flowering	$L_4 \times T_2$, $L_5 \times T_2$, $L_1 \times T_3$, $L_4 \times T_3$, $L_6 \times T_2$, $L_3 \times T_2$, $L_5 \times T_3$ $L_3 \times T_3$, $L_3 \times T_1$, $L_6 \times T_3$	$L_1 \times T_3$, $L_2 \times T_1$, $L_3 \times T_1$, $L_4 \times T_2$, $L_5 \times T_1$, $L_6 \times T_2$, $L_7 \times T_1$	$L_4 \times T_2$, $L_5 \times T_1$, $L_5 \times T_2$, $L_6 \times T_2$	$L_4 \times T_2$, $L_6 \times T_2$
2	Pod length	$L_6 \times T_2$, $L_3 \times T_3$, $L_3 \times T_1$, $L_6 \times T_3$, $L_1 \times T_3$, $L_7 \times T_1$, $L_3 \times T_2$	$L_1 \times T_3$, $L_2 \times T_1$, $L_3 \times T_1$, $L_3 \times T_3$, $L_4 \times T_2$, $L_4 \times T_3$, $L_5 \times T_1$, $L_6 \times T_2$, $L_7 \times T_1$	$L_3 \times T_2$, $L_3 \times T_3$, $L_6 \times T_2$, $L_6 \times T_3$	$L_3 \times T_3$, $L_6 \times T_2$
3	Pod girth	$L_7 \times T_2$, $L_6 \times T_3$, $L_1 \times T_3$, $L_6 \times T_2$, $L_4 \times T_2$, $L_3 \times T_2$, $L_3 \times T_1$, $L_2 \times T_2$, $L_3 \times T_3$	$L_1 \times T_3$, $L_2 \times T_2$, $L_3 \times T_3$	$L_3 \times T_3$	$L_3 \times T_3$
4	Pod weight	$L_3 \times T_3$, $L_4 \times T_2$, $L_1 \times T_3$, $L_7 \times T_1$, $L_6 \times T_2$, $L_5 \times T_1$, $L_5 \times T_2$, $L_2 \times T_2$, $L_4 \times T_3$, $L_4 \times T_1$, $L_2 \times T_3$, $L_2 \times T_1$, $L_1 \times T_2$, $L_5 \times T_3$, $L_1 \times T_1$	$L_1 \times T_3$, $L_3 \times T_3$, $L_5 \times T_1$, $L_6 \times T_2$, $L_7 \times T_1$	$L_1 \times T_3$, $L_2 \times T_1$, $L_2 \times T_2$, $L_2 \times T_3$, $L_3 \times T_1$, $L_3 \times T_2$, $L_3 \times T_3$, $L_4 \times T_1$, $L_4 \times T_2$, $L_4 \times T_3$, $L_5 \times T_1$, $L_5 \times T_2$, $L_5 \times T_3$, $L_6 \times T_2$	$L_1 \times T_3$, $L_3 \times T_3$, $L_5 \times T_1$, $L_6 \times T_2$
5	Pods per plant	$L_3 \times T_3$, $L_6 \times T_2$, $L_4 \times T_2$, $L_3 \times T_1$, $L_4 \times T_3$, $L_5 \times T_2$, $L_5 \times T_1$, $L_2 \times T_2$, $L_6 \times T_3$, $L_1 \times T_3$, $L_7 \times T_1$, $L_3 \times T_2$	$L_1 \times T_3$, $L_2 \times T_1$, $L_2 \times T_2$, $L_3 \times T_1$, $L_3 \times T_3$, $L_4 \times T_2$, $L_4 \times T_3$, $L_5 \times T_1$, $L_5 \times T_2$, $L_6 \times T_2$, $L_7 \times T_1$	$L_1 \times T_3$, $L_2 \times T_2$, $L_3 \times T_1$, $L_3 \times T_2$, $L_3 \times T_3$, $L_4 \times T_2$, $L_4 \times T_3$, $L_5 \times T_1$, $L_5 \times T_2$, $L_6 \times T_1$, $L_6 \times T_2$, $L_6 \times T_3$, $L_7 \times T_1$	$L_1 \times T_3$, $L_2 \times T_2$, $L_3 \times T_1$, $L_3 \times T_3$, $L_4 \times T_2$, $L_4 \times T_3$, $L_5 \times T_1$, $L_5 \times T_2$, $L_6 \times T_2$, $L_7 \times T_1$
6	Yield per plant	$L_3 \times T_3$, $L_6 \times T_2$, $L_4 \times T_2$, $L_5 \times T_2$, $L_4 \times T_3$, $L_1 \times T_3$, $L_5 \times T_1$, $L_2 \times T_2$, $L_7 \times T_1$, $L_3 \times T_1$, $L_4 \times T_1$, $L_5 \times T_3$	$L_1 \times T_3$, $L_2 \times T_1$, $L_2 \times T_2$, $L_3 \times T_3$, $L_4 \times T_2$, $L_4 \times T_3$, $L_5 \times T_1$, $L_5 \times T_2$, $L_6 \times T_2$, $L_7 \times T_1$	$L_1 \times T_3$, $L_2 \times T_1$, $L_2 \times T_2$, $L_3 \times T_1$, $L_3 \times T_2$, $L_3 \times T_3$, $L_4 \times T_1$, $L_4 \times T_2$, $L_4 \times T_3$, $L_5 \times T_1$, $L_5 \times T_2$, $L_5 \times T_3$, $L_6 \times T_2$	$L_1 \times T_3$, $L_2 \times T_2$, $L_3 \times T_3$, $L_4 \times T_2$, $L_4 \times T_3$, $L_5 \times T_1$, $L_5 \times T_2$, $L_6 \times T_2$
7	Crop duration	$L_4 \times T_2$, $L_4 \times T_3$, $L_6 \times T_1$, $L_1 \times T_2$, $L_3 \times T_3$, $L_2 \times T_3$, $L_6 \times T_2$, $L_4 \times T_1$, $L_1 \times T_1$, $L_3 \times T_1$, $L_5 \times T_1$, $L_2 \times T_1$, $L_7 \times T_3$	$L_1 \times T_2$, $L_2 \times T_3$, $L_3 \times T_3$, $L_4 \times T_2$, $L_5 \times T_1$, $L_6 \times T_1$, $L_6 \times T_2$, $L_7 \times T_3$	$L_1 \times T_1$, $L_1 \times T_2$, $L_2 \times T_1$, $L_2 \times T_3$, $L_3 \times T_1$, $L_3 \times T_3$, $L_5 \times T_1$, $L_6 \times T_1$, $L_6 \times T_2$	$L_1 \times T_2$, $L_2 \times T_3$, $L_3 \times T_3$, $L_5 \times T_1$, $L_6 \times T_1$, $L_6 \times T_2$

Table 32. Continued

8	Harvest Index	$L_3 \times T_3$, $L_4 \times T_2$, $L_6 \times T_2$, $L_5 \times T_1$, $L_4 \times T_3$, $L_5 \times T_2$, $L_7 \times T_1$, $L_3 \times T_2$, $L_3 \times T_1$, $L_2 \times T_2$	$L_1 \times T_3$, $L_2 \times T_1$, $L_2 \times T_2$, $L_3 \times T_3$, $L_4 \times T_2$, $L_4 \times T_3$, $L_5 \times T_1$, $L_6 \times T_2$, $L_7 \times T_1$	$L_3 \times T_3$, $L_4 \times T_2$, $L_5 \times T_1$, $L_6 \times T_2$	$L_3 \times T_3$, $L_4 \times T_2$, $L_5 \times T_1$, $L_6 \times T_2$
9	Proline	$L_6 \times T_2$, $L_5 \times T_1$, $L_3 \times T_3$, $L_4 \times T_2$, $L_5 \times T_2$, $L_3 \times T_2$, $L_7 \times T_1$, $L_2 \times T_3$, $L_1 \times T_3$, $L_2 \times T_2$	$L_1 \times T_3$, $L_2 \times T_2$, $L_2 \times T_3$, $L_3 \times T_2$, $L_3 \times T_3$, $L_4 \times T_2$, $L_5 \times T_1$, $L_5 \times T_2$, $L_6 \times T_2$, $L_7 \times T_1$	$L_1 \times T_3$, $L_2 \times T_3$, $L_5 \times T_1$, $L_5 \times T_2$, $L_6 \times T_2$, $L_7 \times T_1$	$L_1 \times T_3$, $L_2 \times T_3$, $L_5 \times T_1$, $L_5 \times T_2$, $L_6 \times T_2$, $L_7 \times T_1$
10	Membrane Integrity	$L_2 \times T_1$, $L_4 \times T_2$, $L_5 \times T_3$, $L_4 \times T_3$, $L_1 \times T_3$, $L_7 \times T_1$, $L_3 \times T_2$, $L_3 \times T_3$, $L_5 \times T_2$, $L_6 \times T_3$	$L_1 \times T_3$, $L_2 \times T_1$, $L_3 \times T_2$, $L_4 \times T_2$, $L_5 \times T_3$, $L_6 \times T_3$, $L_7 \times T_1$	$L_2 \times T_1$, $L_3 \times T_2$, $L_3 \times T_3$, $L_4 \times T_2$, $L_5 \times T_2$, $L_5 \times T_3$	$L_2 \times T_1$, $L_3 \times T_2$, $L_4 \times T_2$, $L_5 \times T_3$
11	Leakage %	$L_2 \times T_1$, $L_4 \times T_2$, $L_5 \times T_3$, $L_4 \times T_3$, $L_1 \times T_3$, $L_7 \times T_1$, $L_3 \times T_2$, $L_3 \times T_3$, $L_5 \times T_2$, $L_6 \times T_3$	$L_1 \times T_3$, $L_2 \times T_1$, $L_3 \times T_2$, $L_4 \times T_2$, $L_4 \times T_3$, $L_5 \times T_3$, $L_6 \times T_2$, $L_6 \times T_3$, $L_7 \times T_1$	$L_2 \times T_1$, $L_3 \times T_2$, $L_3 \times T_3$, $L_4 \times T_2$, $L_5 \times T_2$, $L_5 \times T_3$	$L_2 \times T_1$, $L_3 \times T_2$, $L_4 \times T_2$, $L_5 \times T_3$
12	Ascorbic acid	$L_5 \times T_2$, $L_2 \times T_2$, $L_3 \times T_3$, $L_2 \times T_1$, $L_5 \times T_1$, $L_6 \times T_3$, $L_3 \times T_1$, $L_7 \times T_2$, $L_1 \times T_3$, $L_4 \times T_2$	$L_1 \times T_3$, $L_2 \times T_1$, $L_2 \times T_2$, $L_3 \times T_3$, $L_5 \times T_2$, $L_6 \times T_3$	$L_2 \times T_2$, $L_3 \times T_3$, $L_5 \times T_1$, $L_5 \times T_2$	$L_2 \times T_2$, $L_3 \times T_3$, $L_5 \times T_2$
13	Canopy Temperature	$L_2 \times T_1$, $L_6 \times T_2$, $L_6 \times T_3$, $L_4 \times T_2$, $L_3 \times T_1$, $L_3 \times T_3$, $L_2 \times T_2$, $L_4 \times T_3$, $L_7 \times T_1$	$L_1 \times T_3$, $L_2 \times T_1$, $L_2 \times T_2$, $L_3 \times T_1$, $L_3 \times T_3$, $L_4 \times T_2$, $L_4 \times T_3$, $L_5 \times T_1$, $L_6 \times T_2$, $L_6 \times T_3$, $L_7 \times T_1$	$L_2 \times T_1$, $L_2 \times T_2$, $L_3 \times T_1$, $L_3 \times T_3$, $L_4 \times T_2$, $L_4 \times T_3$, $L_6 \times T_2$, $L_6 \times T_3$	$L_2 \times T_1$, $L_2 \times T_2$, $L_3 \times T_1$, $L_3 \times T_3$, $L_4 \times T_2$, $L_4 \times T_3$, $L_6 \times T_2$, $L_6 \times T_3$
14	Relative Water Content	$L_4 \times T_3$, $L_3 \times T_2$, $L_3 \times T_3$, $L_4 \times T_2$, $L_5 \times T_1$, $L_6 \times T_3$, $L_2 \times T_2$, $L_7 \times T_1$, $L_2 \times T_1$, $L_5 \times T_2$, $L_6 \times T_2$	$L_2 \times T_1$, $L_2 \times T_2$, $L_3 \times T_2$, $L_3 \times T_3$, $L_4 \times T_3$, $L_5 \times T_1$, $L_6 \times T_3$, $L_7 \times T_1$	$L_2 \times T_2$, $L_3 \times T_2$, $L_3 \times T_3$, $L_4 \times T_3$, $L_5 \times T_1$, $L_6 \times T_3$	$L_2 \times T_2$, $L_3 \times T_2$, $L_3 \times T_3$, $L_4 \times T_3$, $L_5 \times T_1$, $L_6 \times T_3$
15	Water Use Efficiency	$L_3 \times T_2$, $L_6 \times T_2$, $L_3 \times T_3$, $L_6 \times T_3$, $L_4 \times T_2$, $L_5 \times T_2$, $L_2 \times T_2$, $L_4 \times T_3$, $L_7 \times T_1$	$L_1 \times T_1$, $L_1 \times T_3$, $L_2 \times T_1$, $L_2 \times T_2$, $L_3 \times T_2$, $L_3 \times T_3$, $L_5 \times T_2$, $L_6 \times T_2$, $L_6 \times T_3$	$L_3 \times T_2$, $L_3 \times T_3$, $L_6 \times T_2$	$L_3 \times T_2$, $L_3 \times T_3$, $L_6 \times T_2$
16	Stomatal conductance	$L_2 \times T_3$, $L_1 \times T_1$, $L_6 \times T_1$, $L_7 \times T_2$, $L_6 \times T_3$, $L_1 \times T_2$, $L_4 \times T_1$, $L_7 \times T_3$, $L_7 \times T_1$	$L_1 \times T_1$, $L_1 \times T_2$, $L_2 \times T_3$, $L_3 \times T_1$, $L_3 \times T_2$, $L_4 \times T_1$, $L_5 \times T_2$, $L_5 \times T_3$, $L_6 \times T_1$, $L_6 \times T_3$, $L_7 \times T_2$	$L_1 \times T_1$, $L_4 \times T_1$, $L_6 \times T_1$, $L_6 \times T_3$	$L_1 \times T_1$, $L_4 \times T_1$, $L_6 \times T_1$, $L_6 \times T_3$

Table 33: Frequency of the crosses performed superior for the different traits

Sl.No.	Hybrids	No.of times
1	L1 X T1	1
2	L1 X T2	1
3	L1 X T3	4
4	L2 X T1	3
5	L2 X T2	5
6	L2 X T3	2
7	L3 X T1	2
8	L3 X T2	4
9	L3 X T3	11
10	L4 X T1	1
11	L4 X T2	7
12	L4 X T3	4
13	L5 X T1	7
14	L5 X T2	4
15	L5 X T3	2
16	L6 X T1	2
17	L6 X T2	10
18	L6 X T3	3
19	L7 X T1	2
20	L7 X T2	0
21	L7 X T3	0

Table 34. List of genotype/hybrid selected for real-time PCR

Genoptype	Code	Remark
Ayyanthole local	SP	Susceptible genotype from initial germplasm screening (Experiment I)
Kattampally local x Vellayani Jyothika (L_3 x T_3)	LT	Drought tolerant hybrid selected with high SCA from Experiment III
Anchal local II	L	Best drought tolerant genotype selected from experiment II
Lola	T	Tester selected with high GCA from experiment III
Arka Mangala	CK	The check used in the study

4.5.1 RNA quantification and assessment of quality

Total RNA was isolated from the leaf samples of both control and stress induced plant tissue by following the TRIzol RNA isolation protocol. Quantity of RNA was determined in Qubit 3.0 Fluorometer, using the Qubit HS RNA assay kit which contain fluorescent dyes that bind specifically to RNA, provides more accurate quantification. The quality of the RNA samples was determined by absorbance in spectrophotometer at the wavelength of 260 and 280 nm. The concentration of RNA was in the range of 1097 to 2091 µg/ml with the purity level within the range of 1.9 to 2.1.

Table 35. Quality and quantity of RNA extracted

Sample	A 260 / A 280	RNA concentration (µg/ml)
SP control	2.0	1135
SP stress	2.1	1260
LT control	2.1	1097
LT stress	1.9	1780
L control	1.9	1703
L stress	2.0	1690
T control	2.1	2091
T stress	2.0	1748
CK control	2.1	1128
CK stress	1.9	1851

4.5.2 Sequences of the primers designed

DREB and NCED are candidate genes involved in conferring tolerance under water stress. The primer pairs for the major drought responsive genes were designed using Primer3Plus software. The designed primers and associated parameters were provided in the table 36.

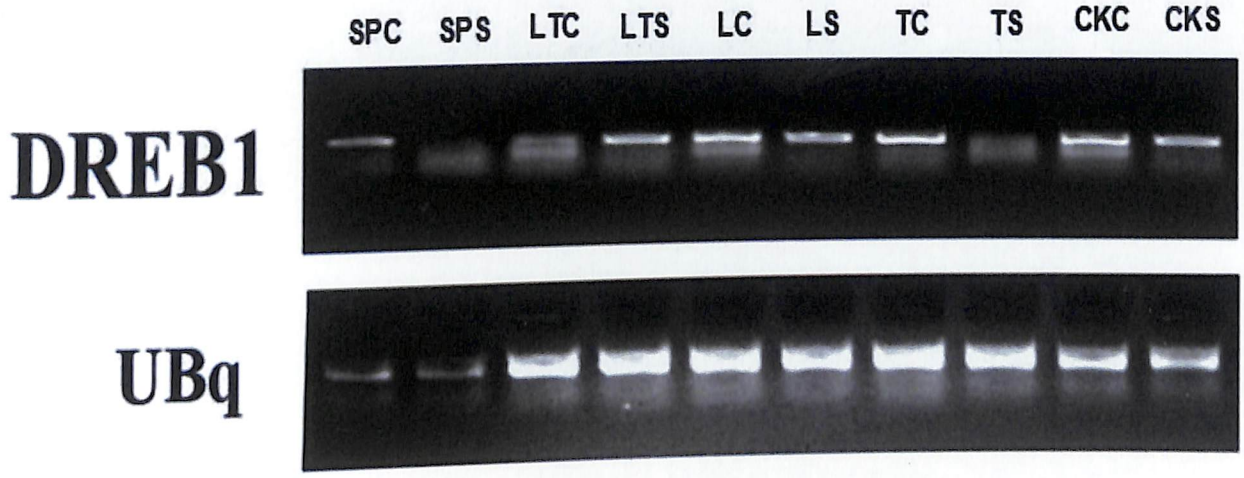
Table 36. Primer sequence designed for the drought responsive genes

Oligo name	Sequence	Primer length	Amplicon size (bp)	GC content (%)	Melting temperature (Tm)
VuNCED1	F- GGG GAG CCT CTG TTT CTT CC R- TAG GGA ACA CGA GAG GGG AG	20	158	60	61.0
VuDREB1	F- GGA AGA AGT TCC GGG AGA CG R- GCG ACA TCA GCA CCA TGT TC	20	405	58	60.6
VuUbq	F-AGA AAA GCC CCC AAG TGT TC R- CTG CCA TCT CCT TCT TCA GC	20	161	55	59.3

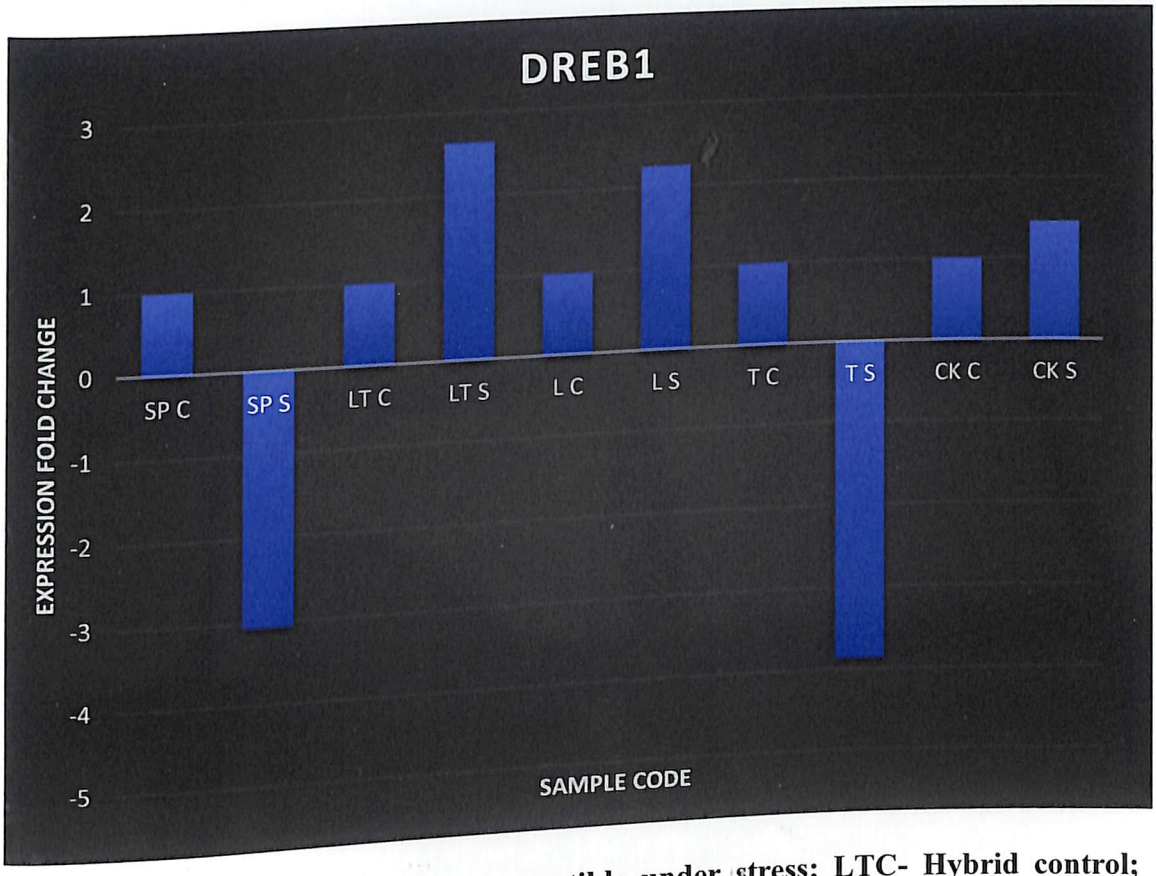
Gene	Function
NCED1	Encode 9-cis-epoxycarotenoid dioxygenase is a key enzyme in abscisic acid (ABA) biosynthesis and involved in the response to drought stress
DREB1	Dehydration responsive element binding - important transcription factors (TFs) that regulate the expression of many stress-inducible genes. Play critical role in enhancing drought stress resistance in cowpea
Ubq	A House keeping gene, Ubiquitin is found in almost all cellular tissues which helps to regulate the processes of other proteins in the body

4.5.3 Relative gene expression analysis

The RNA of control and stress plants were converted to complementary DNA (cDNA). Expression levels of genes linked with DREB and NCED in the selected genotypes were studied using the quantitative real time PCR. A differential expression of drought related genes was seen in tolerant and susceptible genotypes (Plate 15).



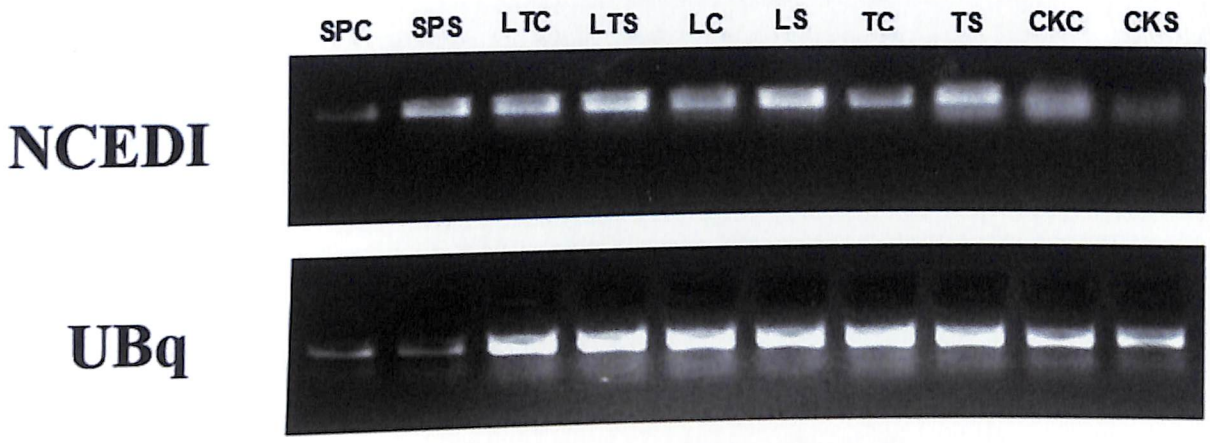
a. Gel image of RT-q PCR for DREB 1 gene



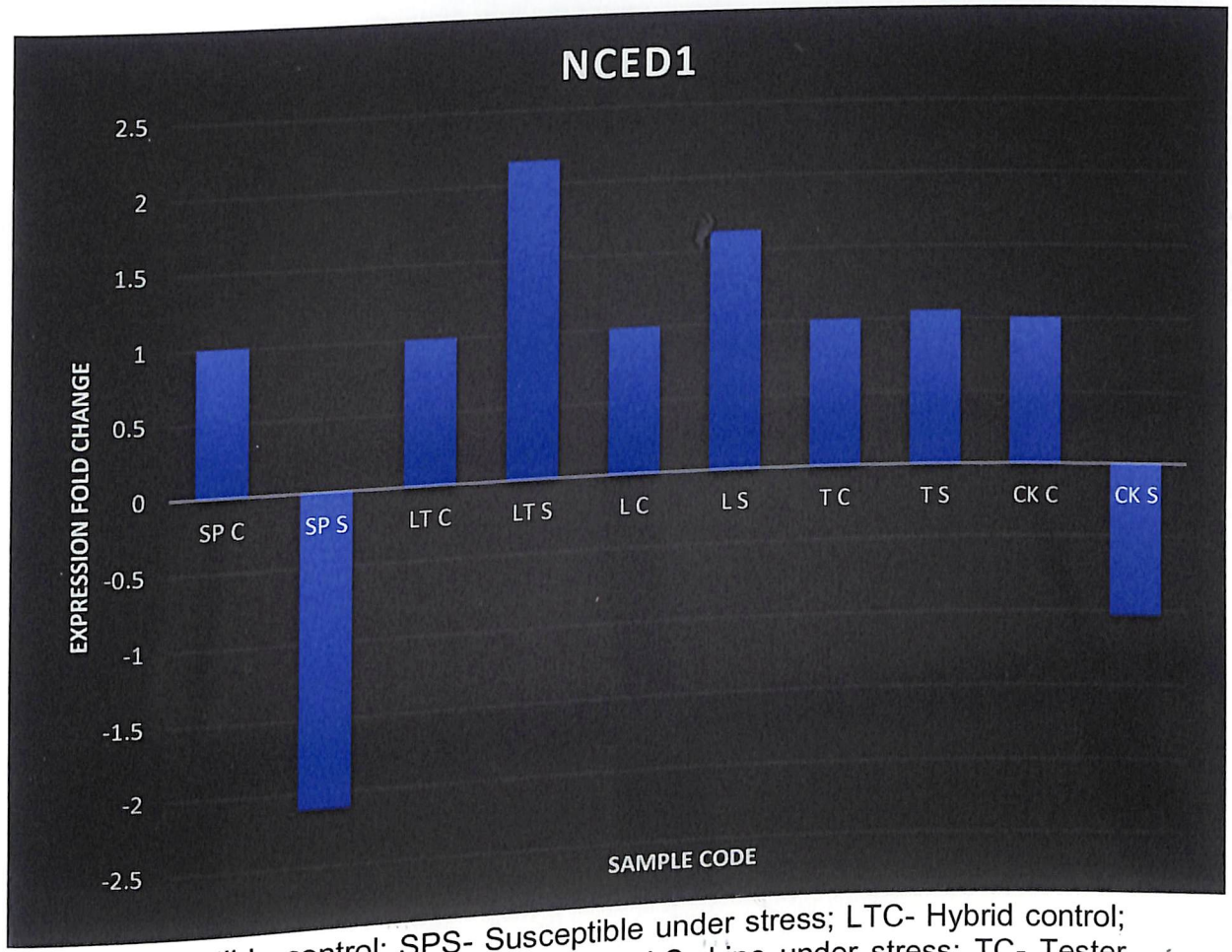
SPC- Suscesptible control; SPS- Susceptible under stress; LTC- Hybrid control; LTS- Hybrid under stress; LC- Line control; LS- Line under stress; TC- Tester control; TS- Tester under stress; CKC- Check control; CKS-Check under stress

b. Relative Expression profile of DREB1 gene

Plate 14 . Expression profile of DREB1 gene



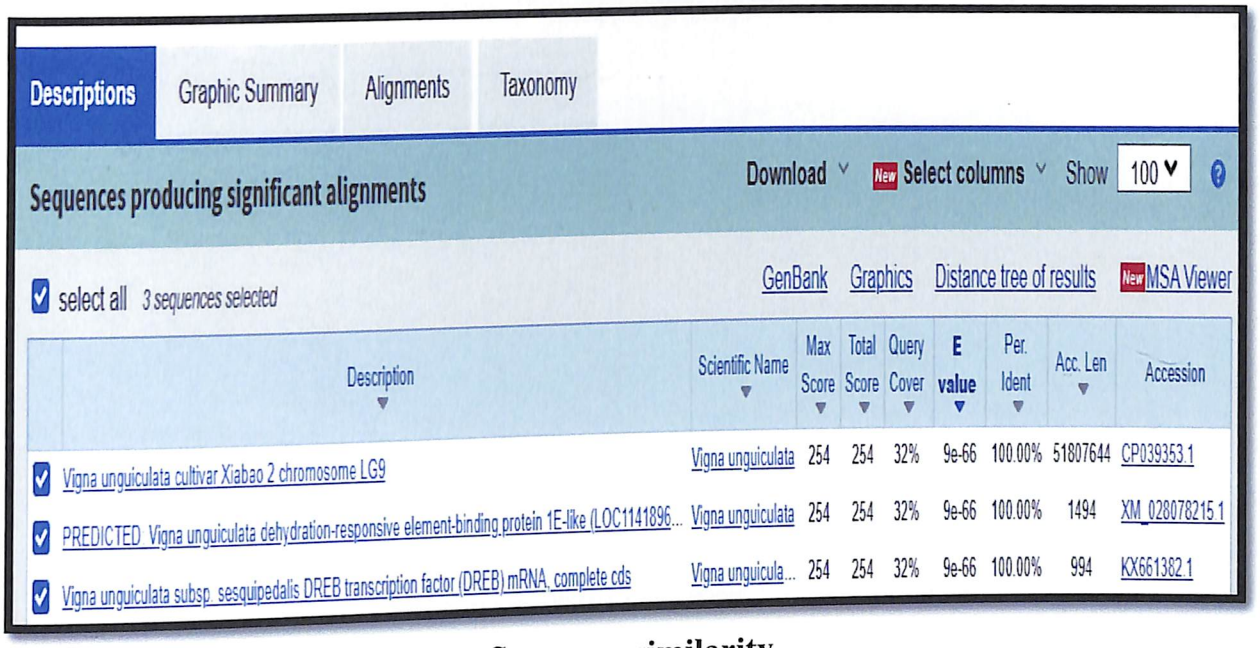
a. Gel image of RT-q PCR for NCED1 gene



SPC- Susceptible control; SPS- Susceptible under stress; LTC- Hybrid control; LTS- Hybrid under stress; LC- Line control; LS- Line under stress; TC- Tester control; TS- Tester under stress; CKC- Check control; CKS-Check under stress

b. Relative Expression profile ofNCED1 gene

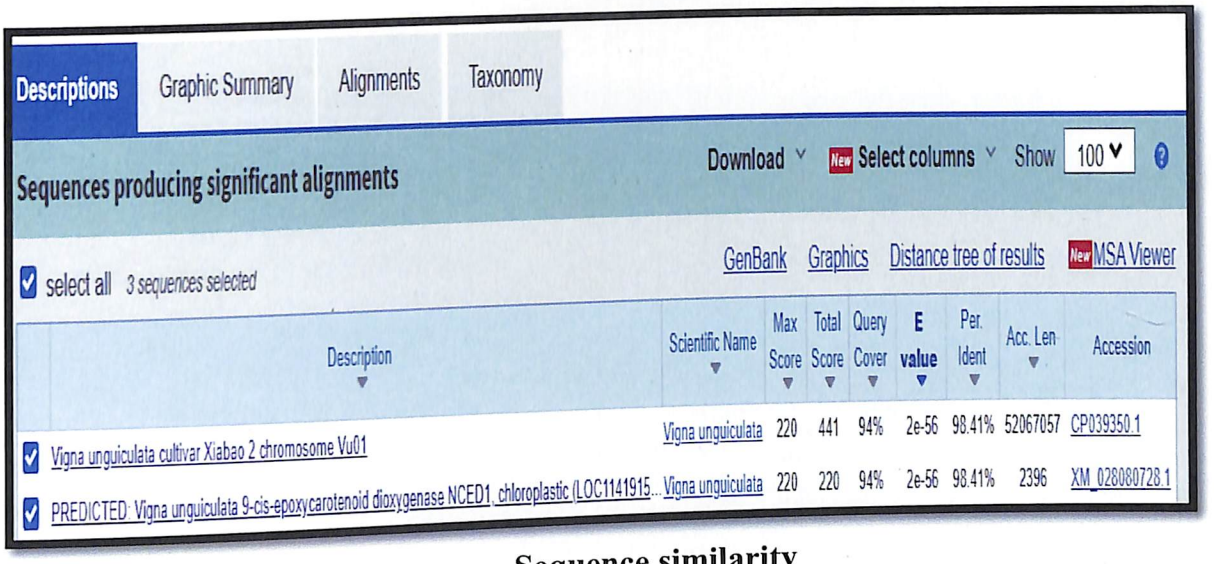
Plate 15 . Expression profile of NCED1 gene



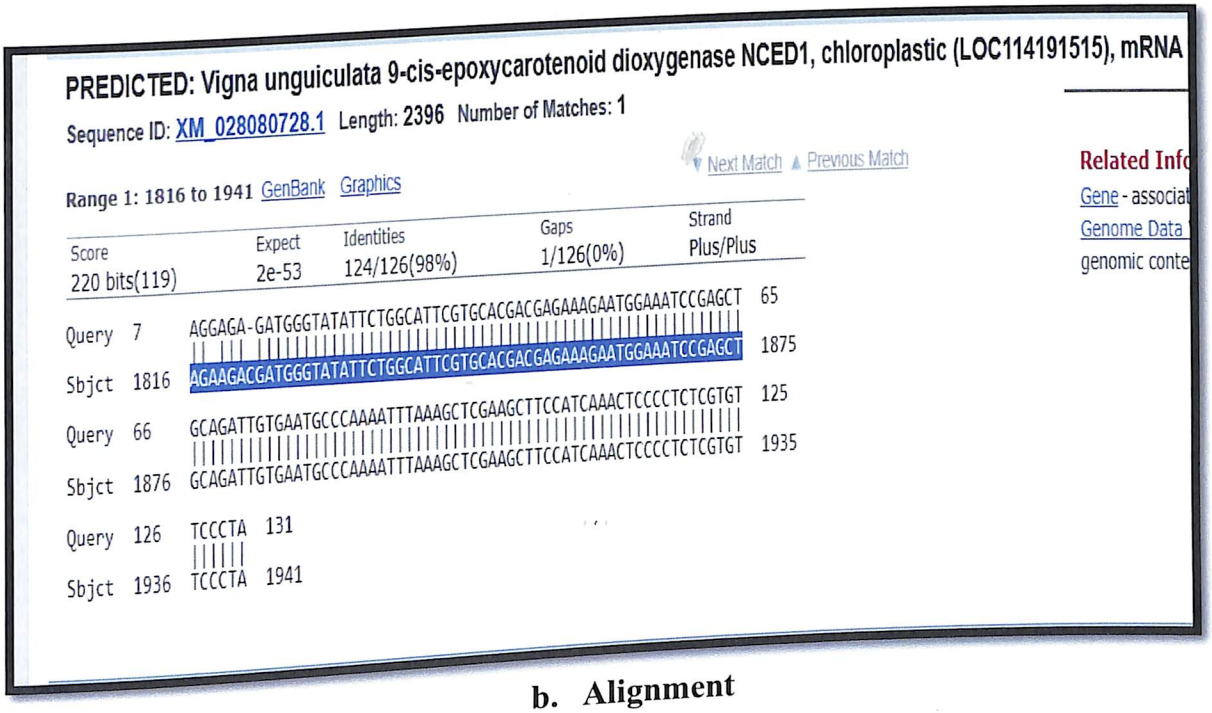
a. Sequence similarity

b. Alignment

Plate 16. Result of nucleotide BLAST of the 657 bp sequence generated by DREB



a. Sequence similarity



b. Alignment

Plate17. Result of nucleotide BLAST of the 132 bp sequence generated by NCED1

The relative expression of genotypes demonstrates varying degree of tolerance to drought stress. Upregulation of gene expression was noted in tolerant hybrid and parental genotype, while downregulation of drought responsive genes in susceptible genotype. qRT-PCR analysis showed that all the two genes were upregulated in drought stressed plants.

The relative expression profile using DREB1 gene shows 1.6373, 1.2477 and 0.4424 fold increase in expression of stressed genotype of Kattampally local x Vellayani Jyothika hybrid, Anchal local II and Arka Mangala respectively, as compare to its control. While a decrease in fold of -2.0596 and -2.8568 were observed in the stressed genotype of Ayyanthole local and in the tester Lola respectively in comparison to its control.

For NCED1 gene, the expression of stressed genotypes of Kattampally local x Vellayani Jyothika hybrid, Anchal local II and Lola exhibited increase in fold of 1.1548, 0.6269 and 0.0511 respectively, as compared to its control. While a decrease in fold of -1.1014 and -0.027 were observed in the stressed genotype of Ayyanthole local and Arka Mangala.

4.5.4 Sequencing of the amplicon

The PCR products were electrophoresed in 2% agarose gel and the bands visualized under UV (Plate 14). The cDNA bands amplified by the real time PCR had shown its suitability for direct sequencing. The PCR products were sequenced with the primers NCEDI & DREB1.

Gene	Sequence
NCED1	>VuNCED_VuNCED.F_27698-1_P3894, Raw Sequence (132 bp) GTGGGTAGGAGAGATGGGTATATTCTGGCATTCGTGCACGACGAGAAA GAATGGAAATCCGAGCTGCAGATTGTGAATGCCCAAAATTTAAAGCTC GAAGCTTCCATCAAACTCCCCTCTCGTGTTCCCTAA
DREB1	>VuDREB_DREB.F_27698-2_P3894, Raw Sequence (657 bp) TCACGGGGTTCGCGGCGTAGGCGGAGGGATCCGGCAGTGGGTGTGCG AGGTGCGCGAGCCCAATAAGAAGACTAGGATTTGGTTGGGGACCTTTC CCACGGCGGAGATGGCAGCGCGTGCGCACGACGTGGCTGCGCTGGCG CTTAGGGGAAGGTCGGCCTGTCTCAATTTCGCCGACTCCACGAATCGG TTACCGGTGCCGGCGACGGCGGATCCCCGGGACATTCAGAAGGCGGC GGCAGAGGCTGCAGAGGCGTTTCGGCCCGGTAATGAATCGGGAAAGG ATGATGATGCGGTGGTGGAGACGGTGGCGACAGCGACGGAAAATGAT GAAGAAAAAGAGATGGAGGATCTGAAGAACATGGTGCTGATGTCGCA TGCTTTGCCCCTCTCATTTCTGGACCTGACGCTTTAACTTTCTTCCCAAA TTATACTGACTTAAAATTTTTCTCAACTTCCTCAAAGATGAGCCCCGCA CGCTGACGATCAAGCCTTTCTCCCTATATGCTTGAACAACCTTACTTGA ACTTGTTTCTTTCCATCCTTCCCGACCCGTGATGGCGTTATGTTGCTCA ATTTTCTCACGGTTCAGCACCTCTGTCCTCCCTTGTCTACGCAGCGTTG GGTGAAGTTCCCATCCTTATCAACCCCCCATCT

4.5.5 Sequence analysis using BLAST

The sequence was subjected to in silico analysis, using Nucleotide BLAST (Basic Local Alignment Search Tool). In BLASTN the sequence DREB1 had shown for 100% similarity with Vigna unguiculata –DREB transcriptor factor with sequence ID: KX661382.1 (Plate 16). The sequence NCED1 had shown 98.41% similarity with Vigna unguiculata 9-cic-epoxycarotenoid NCED1, chloroplastic (LOC114191515) with accession number XM_028080728.1(Plate 17).

The two gene sequence data were submitted in the BankIt NCBI with accession number. VuDREB : MW066863 and VuNCED : MW066864

DISCUSSION

5. DISCUSSION

Yard long bean (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdcourt) is a highly remunerative vegetable crop in Kerala. The crop is mainly grown under rainfed conditions in Kerala. Despite all its economic and nutritional importance, its production is subjected to a wide range of biotic and abiotic constraints. Among the abiotic stresses, drought is an important factor that adversely affects crop growth and production. Extreme flood and drought events, which are frequent in Kerala, are challenges posed by the climate change that affects our farmer's livelihood security. Exploring new sources of variation of drought tolerance is essential for sustainably enhancing yard long production. In addition, little has been done regarding screening drought tolerance in yard long bean.

Breeding for drought tolerant cultivars is one of the cost effective ways to tackle the effects of water stress on crops. Drought stress is expected to be more severe in the coming years and drought affected areas may double in the year 2050 (Douglas *et al.* 2008). Providing farmers with crops that better withstand drought requires effective and strong breeding programs through the establishment of a better phenotyping and screening approach (Ravelombola *et al.*, 2018).

The present study was conducted to identify drought tolerant genotype from the available germplasm and to understand the nature and magnitude of gene action and gene expression involved in the inheritance of drought tolerance in yard long bean. The ultimate objective is to choose the best parents for hybridization to develop drought tolerant varieties. The experiments were taken up at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani from 2017 to 2019.

The study comprised four experiments. The first experiment dealt with the seedling stage evaluation of 100 yard long bean genotypes for drought tolerance. The fifteen selected genotypes from the first experiment were evaluated for drought tolerance in the second experiment by imposing moisture stress at the

reproductive stage. In the first part of the third experiment, LxT crosses were performed by using seven selected tolerant genotypes as lines with three popular yard long bean varieties as testers to generate twenty one hybrids. The genetic analysis of hybrids and parents were carried out in the second part of the experiment. Real time-PCR was conducted in the final experiment to analyze the expression of drought responsive genes in the tolerant hybrids and parents.

Experiment I

5.1 SCREENING GERMPLASM FOR DROUGHT TOLERANCE AT THE SEEDLING STAGE IN FIELD

For selecting drought tolerant genotypes, a detailed evaluation of the germplasm for attributes contributing to tolerance and yield is essential. In the present study, the initial screening of 100 yard long bean genotypes for drought tolerance at the seedling stage revealed significant differences among them. In yard long bean, seedling stage screening is found to be an effective method for identifying drought tolerant varieties. Almost identical reports have been reported by Singh *et al.* (1999), Ajayi *et al.* (2018) and Ravelombola *et al.* (2018) in cowpea.

Studies demonstrated that plant genotypes with some degree of drought tolerance at the seedling stage were able to withstand drought stress at later stages of development (Rzepkaplevnes *et al.*, 2009; Ravelombola *et al.*, 2018).

5.1.1 Mean performance

Based on the level of resistance displayed and symptoms of wilting from the day of imposition of water stress, genotypes were recorded for their degree of tolerance to water stress. Analysis of variance revealed significant differences among the genotypes for all the drought responsive traits namely number of days for reaching critical stress level, relative leaf water content, permanent wilting percentage and plant recovery percentage. The result indicates significant variability among the germplasm that could be exploited through selection.

Similar results of germplasm variability were noticed in yard long bean by Rambabu *et al.* (2016), Lovely *et al.* (2017), Gul *et al.* 2019 and Lestari *et al.* (2019).

The number of days for reaching critical stress levels varied from 3.5 to 9 days. The critical day for distinguishing water stress tolerant and susceptible genotype was found to be 6 days. The effect of stress beyond the 6th day of drought imposition got worsened in majority of the genotypes. Only one genotype G46 (Kottayam local I) could withstand tolerance upto 9 days. The critical day for distinguishing moisture stress tolerant genotypes in cowpea has been reported by Anyia and Herzog (2004) and Ajayi *et al.* (2018)

According to Kumar *et al.* (2014) genotypes that are tolerant to drought showed higher RWC than genotypes that are susceptible to drought. In the present study, significant difference in relative water content was found among the 100 yard long bean genotypes. The RWC values across stress treatments ranged from 57.7% to 79.1%. During the screening five genotypes recorded higher RWC which indicates their ability to maintain comparatively a higher water status under stress. Higher water status confers for better metabolic activity, growth and development of plants under moisture stress. These results are in line with the reports of Blum *et al.* (2001)

Ajayi *et al.* (2018) reported permanent wilting percentage as one of the best traits for screening dehydration tolerance in cowpea seedlings under controlled conditions. In the present study, analysis of variance for PWP recorded significant variation among 100 genotypes in the range of 14.25% (G6) to 19% (G98). The mean value was 17.33%. More than 60% of genotypes show above average PWP indicates their different drought adaptation capacity. The genotypes that have low PWP and better adaptation to drought stress across stress treatment were selected as drought tolerant.

Plant greenness score and recovery rate at the seedling stage have been reported as accurate parameters for evaluating drought tolerance in cowpea

(Ravelombola *et al.*, 2018). The percentage of recovery ranged from 0 to 45%. Nearly 70% of genotypes did not recover on rewatering after the imposition of moisture stress. The highest recovery rate was found in G89 (Pongamoodu local).

In the present investigation, out of the 100 genotypes screened 15 drought tolerant genotypes were identified based on their better performance in terms of high RLW, low PWP, more number of days for reaching critical stress level and high recovery percentage. The genotypes identified were G1 (Acc 5), G5 (Acc 1339), G6 (Adoor local), G14 (Anchal local II), G15 (Aranmula local), G24 (Elamadu local), G36 (Kattampally local), G42 (Kollam local), G45 (Kottarakara local), G46 (Kottayam local), G50 (Kulashegarapuram local), G51 (Kulathupuzha local), G60 (Muttathukonam local), G74 (Nilamel local) and G89 (Pongamoodu local).

Experiment-II

5.2 EVALUATION OF THE SELECTED GENOTYPES FOR DROUGHT TOLERANCE

5.2.1 Cataloguing of the Germplasm

Out of the 100 genotypes, fifteen drought tolerant genotypes that perform well in field conditions were carried to experiment II. The genotypes were planted and evaluated in grow bags in the rain shelter. The selected genotypes were morphologically characterized as per descriptors of NBPGR for cowpea. Morphological characterization helps in the effective utilization of germplasm in crop improvement programmes.

Data on 11 morphological and 11 quantitative traits were recorded. The greatest morphological variation was observed for seed coat colour, flower colour, pod curvature, seed eye pattern, seed eye colour and seed texta testure. However, no variation was observed for seed shape, growth pattern and twinning tendency. Similar results were reported by Rambabu *et al.* (2016), Pandey *et al.* (2020) and Devan *et al.* (2021) in yard long bean.

The result based on the mean performance of quantitative traits also exhibited significant variability among the genotypes. Almost identical reports have been reported by Vidhya (2000), Asoontha and Abraham (2017) and Toppo and Sahu (2020)

The descriptor data of the selected genotypes were subjected to cluster analysis to assess genetic diversity and identify duplication. The dendrogram indicates the existence of much variability among the fifteen genotypes. The fifteen genotypes were grouped into five major clusters based on morphological characteristics; cluster I, cluster II, cluster III, cluster IV and cluster V (Fig.1).

Cluster I consisted of only one genotype; Kulashegarapuram local featured yellow flowers, no plant pigmentation, a slightly curved pod, black eyed seeds and a cream seed coat with grey splashes, among other traits.

Cluster II consist of two sub clusters; A and B. Sub-cluster A consisted of closely related genotypes Pongamoodu local and Nilamel local which exhibited variations in flower colour, seed texture and seed colour. Kattampally local is the only genotype in sub-cluster B, with specific traits like pink flowers, brown eyed seeds and a buff seed coat.

Cluster III had three subclusters; C, D and E. Sub-cluster C contained only Kulathupuzha local. Sub-cluster D comprised of Elamadu local and Anchal local II, closely related genotypes with distinct trait variation in flower colour, seed texture, seed eye and coat colour. Sub-cluster E consisted of Adoor local and Acc 1339 highly associated genotypes with notable differences in seed coat colour.

Cluster IV consisted of three sub clusters; F, G and H. sub-cluster F contained only Muttathukonam local. Kottayam local was in sub-cluster G which was distinguished by its violet flower colour and smooth grey eyed black coat seeds. Similar genotypes Kottarakara local and Kollam local were grouped in sub-cluster H, with differences in flower colour and seed coat colour.

Cluster V included Aranmula and Acc.5, both had specific trait of light green pod with purple tip but varied in flower colour, pod curvature and seed coat colour.

Almost identical reports have been reported by Sultana *et al.* (2020), Widyawan *et al.* (2020) and Devan *et al.* (2021). The result revealed that there is considerable variability among the genotypes for most of the characters studied. The genotypes from different clusters that performed better in terms of drought tolerance and yield contributing attributes could be used in future hybridization programmes to recombine the desirable characters leading to enhancement in pod yield of yard long bean.

5.2.2 Biometric evaluation

The statistical analysis shows highly significant differences among the 15 genotypes for biometric traits namely days to 50% flowering, pod length, pod width, pod weight, pods per plant, yield per plant, vine length, harvest index, crop duration, root depth and root volume in yard long bean under moisture stress condition. Indicates considerable variability among the tested yard long bean genotypes for drought tolerance. The existence of similar variability under water stress was reported by Tewolde *et al.*(1991), Ahmed and Suliman (2010) and Magashi *et al.(*2019).

Yield under stress is the primary trait for selection in a breeding programme for drought tolerance. The ability to maintain photosynthesis under water stress is of major importance in drought tolerance. Hence traits associated with yield under stress are considered to measure the level of drought tolerance. Under water stress, biometric parameters associated to yield were shown to be lowered when compared to control plants. Magashi *et al.* (2019) also reported similar results in yard long bean.

Magashi *et al.* (2019) in their variability studies in yard long bean under water stress reported a positive relationship between pod length, number of pods

per plant and days to 50% flowering with yield. Lestari *et al.* (2019) reported that moisture stress results in reduced plant height and the susceptible types as more sensitive.

Positive association of pod length, pod width and the number of pods per plant with pod yield were reported by Lovely 2005, Jithesh 2009, Vavilapalli and Celine 2014, Rambabu *et al.* 2016 and Bhagavati *et al.* 2019. The drought tolerant genotype should have greater root as compared to the drought susceptible genotype (Yue *et al.*, 2006). Improvement in drought tolerance of lines was due to greater partitioning of the root mass to the deeper soil profiles and increased ability to extract moisture from those depths. The result indicate that here is a significant increase in root volume and depth among the tolerant genotypes compare to the susceptible ones.

In the study, selection was carried out based on these linked traits since they are in the desired direction. The drought tolerant genotypes were selected based on early flowering, increased pod length, pod width, pod weight, pods per plant, yield per plant, vine length, harvest index, root depth and root volume and with reduced crop duration.

The maximum yield under water stress was recorded for A15 (Pongamoodu local), A11 (Kulashegarapuram local), A14 (Nilamel local), A7 (Kattampally local) and A5 (Aranmula local). All of these genotypes also have improved drought tolerant biometric characteristics.

5.2.3 Physiological evaluation

Results obtained revealed significant genotypic differences among yard long bean for physiological traits under moisture stress condition. Physiological and biochemical parameters that correlate with yield under extreme moisture stress conditions can be used to select drought tolerant plants during the breeding process (Xiong and Ishithani, 2006).

Drought tolerant genotypes were chosen based on physiological measurements that showed high proline level, ascorbic acid, relative water content, water requirement and water use efficiency while low values were preferred for percentage leakage, canopy temperature and stomatal conductance.

A high level of proline and ascorbic acid in plants is an adaptive response to drought. Accumulation of proline confers tolerance to abiotic stress and oxidative stress and plays a critical role in protecting photosynthetic activity under osmotic stress. Ananthraju and Muthiah (2008) reported that tolerant genotypes accumulate higher proline levels, biomass and pod yield. In this study, the highest proline content was observed for the genotypes A7 (Kattampally local) A14 (Nilamel local), A11 (Kulashegarapuram local) and A4 (Anchal local II). While high Ascorbic acid content was observed in A4 (Anchal local II), A2 (Acc 1339), A1 (Acc 5), A5 (Aranmula local) and A3 (Adoor local)

Drought stress causes injury to the cell membrane, resulting in electrolytic leakage. Genotypes with less electrolyte leakage correlate with tolerance to plant stress. Lower membrane stability or higher injury reflects the higher susceptibility of genotype to oxidative stress. In this study, the tolerant genotypes were found to have a low value of percentage leakage and high membrane integrity which may be due to the less damage of cell membrane under water stress. This is in line with the reports of Leibler *et al.* (1986) and Premachandra and Shimada (1987).

A cooler canopy is reported to be a measure of drought tolerance, low leaf temperature indicates maintenance of higher transpiration (Lafitte *et al.*, 2003). Low canopy temperature was exhibited by A14 (Nilamel local), A15 (Pongamoodu local) and A7 (Kattampally local). For relative water content genotypes with higher values were considered drought tolerant. The genotypes A15 (Pongamoodu local), A13 (Muttathukonam local), A4 (Anchal local II), A5 (Aranmula local) and A7 (Kattampally local) recorded the higher relative water content. This is in line with the reports of Jha and Singh (1997).

Drought resistant genotypes had high water use efficiency and water requirement than susceptible genotypes which may be due to the variation for moisture extraction capacity from deep soil. The water use efficiency was exhibited highest by the genotype A15 (Pongamoodu local), A11 (Kulashegarapuram local) and A14 (Nilamel local). This is in line with the reports of Yerima *et al.* (2013).

Drought avoidance is the ability of plants to maintain tissue hydrated at high water potential by reducing the water loss from plants. This mechanism of drought avoidance is related to stomatal characteristics. Drought induces stomatal closure thus minimizing the water loss and maintaining a better plant water status. Plants with low stomatal conductance aid moisture stress tolerance. The lowest stomatal conductance was exhibited by the genotype A2 (Acc 1339), A6 (Elamadu local I) and A7 (Kattampally local). The results are in accordance with reports of Lestari *et al.* (2019).

Based on biometric and physiological evaluations, the top seven genotypes with high yield and drought tolerance were selected as parents for further hybridization in experiment III: A4 (Anchal local II), A5 (Aranmula local), A7 (Kattampally local), A11 (Kulashegarapuram local), A13 (Muttathukonam local), A14 (Nilamel local), and A15 (Pongamoodu local).

Experiment-III

5.3 PART I: DEVELOPMENT OF HYBRIDS

Choice of parents or crosses is important in determining success from hybridization. Line x tester analysis developed by Kempthorne in 1957 is one of the breeding strategies for predicting the general combining ability of parents and the selection of suitable parents and crosses with high specific combining ability. It provides information on the genetic mechanisms that control major quantitative traits (Salgotra *et al.*, 2009). In the present investigation line x tester was carried out to evaluate the drought tolerance of parents and hybrids on the basis of mean

performance, gca of parents and sca of hybrids and to understand the gene action controlling the expression of tolerance traits.

5.4. PART II: FIELD EXPERIMENT FOR EVALUATION OF F1 AND PARENTS

5.4.1 Analysis of variance

The significant difference among the genotypes for all the sixteen characters was tested by analysing the different components of variance. The results revealed that variation due to genotypes was found to be significant for all the sixteen characters studied, which indicate the presence of sufficient variability among the genotypes for improvement. The finding was in accordance with the result of Lovely and Kumar (2021) and Renjana (2006).

5.4.2 Combining ability

All available parents with a high order of performance may not be able to transmit their superior traits to their progenies. Combining ability assess the relative genotype ability to transmit desirable trait to its hybrids and evaluates hybrids in terms of their genetic value. Hence selection of desirable parents based on their combining ability is used in crop improvement programmes. A total of 31 entries *viz.* seven lines, three testers and their twenty one hybrids were studied for sixteen characters.

Among the three testers, T2 was found to be a better general combiner for two characters namely days to 50% flowering and pods per plant while T1 show better gca for only stomatal conductance and T3 for stomatal conductance.

From the combining ability analysis, it is found that line L3 (Kattampally local) had a significant gca effect for ten characters while line L4 (Kulashegarapuram local) had a significant gca effect for nine characters. The results imply that these genotypes are outstanding general combiners and can be exploited as parents for drought tolerance breeding in yard long bean.

Among the 21 crosses evaluated, L3 X T3 (Kattampally local x Vellayani Jyothika) showed a significant sca effect for eleven characters while cross L6 X T2 (Nilamel local x Lola) exhibited a significant sca effect for ten characters.

5.4.3 Gene action

Combining ability analysis provides information about the nature and magnitude of different types of gene action governing various quantitative traits (Sprague and Tatum,1942). The nature of gene action helps in deciding the breeding procedures for the genetic improvement of such characters. The variance of general combining ability (gca) and specific combining ability (sca) effects provides a measure of variation due to additive and dominance (non-additive) gene action respectively. Additive gene action provides fixable variation, whereas non-additive gene action includes effects of dominance and epistasis, which cannot be fixed.

The magnitude of genetic variance for all the sixteen characters shows that the dominance variance was higher than the additive variance for all the traits. The ratio of additive variance to dominance variance was less than unity for all the morphological and physiological traits studied. The higher sca variances for all the character, shows the predominance of non-additive gene action (dominance and epistasis) in the expression of the characters namely days to 50% flowering, pod length, pod girth, pod weight, pods per plant, yield per plant, harvest index, crop duration, proline content, membrane integrity, percentage leakage, ascorbic acid, canopy temperature, relative water content, water use efficiency and stomatal conductance. Non-additive variance is not fixable and this can be improved through heterosis breeding. The existence of significant amount of dominance variance is essential for undertaking a heterosis breeding programme. The findings were in accordance with the result of Lovely and Kumar (2021), George and Sarada (2019) and Renjana (2006).

The predominance of non-additive gene action in the inheritance of the traits under study indicated that heterosis breeding and recombination breeding

with the postponement of selection to later generations will be ideal for obtaining genotypes with superior drought tolerance.

5.4.4 Heterosis

Information on the magnitude of heterosis is a prerequisite in the development of hybrids. A good hybrid should manifest a high amount of heterosis for commercial exploitation. The existence of a significant amount of dominance variance is essential for undertaking heterosis breeding. Even a small magnitude of heterosis for a trait is desirable for its improvement. A high estimate of heterosis is a result of high genetic diversity among parents creates the possibility of identifying high yield transgressive segregants in the population (Singh, 2001).

The heterosis percentage expressed by twenty one hybrids for sixteen characters were estimated as its superiority over mid parent (relative heterosis), standard check (standard heterosis) and better parent (heterobeltiosis) values. Manifestation of heterosis was found in both positive and negative directions. Except for days to 50% flowering, percentage of leakage, crop duration, canopy temperature and stomatal conductance positive heterosis was preferred for rest of the parameters. This is in accordance with the earlier reports of Asoontha (2017), Litty (2015) and Madhukumar (2006).

Yield is an important trait considered for the selection of drought tolerant genotypes. Pod yield showed remarkable variation among the hybrids. For superior recombinants in heterosis breeding, high heterosis for yield combined with high heterosis for yield contributing traits need to be considered (Kadam *et al.*, 2013)

In biometric evaluation crosses that showed significant heterosis in all the three heterosis, namely relative heterosis, standard heterosis, and heterobeltiosis, are given as follows: for days to 50% flowering significant negative heterosis observed in the crosses L5 X T2, L4 X T2 and L6 X T2; for pod length significant

positive heterosis is in L6 X T3; for pod girth significant positive heterosis is in L3 X T3; for pod weight significant positive heterosis is found in L4 X T2 and L3 X T3; for pods per plant significant positive heterosis is observed in L3 X T3, L4 X T2 and L3 X T1; for crop duration significant negative heterosis is found in L6 X T1 and L1 X T2 and for harvest index significant positive heterosis is in L3 X T3, L6 X T2, L5 X T1 and L4 X T2

In physiological evaluation crosses that showed significant heterosis in all the three heterosis, namely relative heterosis, standard heterosis, and heterobeltiosis, are given as follows: for proline significant positive heterosis is observed in the crosses L5 X T1, L5 X T2 and L6 X T2; for ascorbic acid significant positive heterosis is in the crosses L5 X T2 and L2 X T2; for membrane integrity significant positive heterosis is found in L5 X T3 and L4 X T2; for percentage leakage significant negative heterosis is observed in L2 X T1, L5 X T3 and L4 X T2; for canopy temperature significant negative heterosis is in L2 X T1, L4 X T2 and L3 X T1; for relative water content significant positive heterosis is found in L4 X T3, L3 X T3 and L3 X T2; for water use efficiency significant positive heterosis is observed in L3 X T2, L6 X T2 and L3 X T3 and for stomatal conductance negative heterosis is found in L6 X T1, L6 X T3 and L1 X T1.

Among the 21 crosses evaluated, L4 X T2 (Kulashegarapuram local x Lola) showed significant heterosis for eight characters while the crosses L3 X T3 (Kattampally local x Vellayani Jyothika) and L6 X T2 (Nilamel local x Lola) exhibited significant sca effect for six characters.

5.4.5 Selection of hybrids

Based on the mean performance, sca effect and heterobeltiosis the cross L3 x T3 (Kattampally local x Vellayani Jyothika) was identified as one of the most promising cross combination followed by L6 x T2 (Nilamel local x Lola) and L4 x T2 (Kulashegarapuram x Lola). While analysing the drought tolerance behavior in these hybrids the activity of relative water content and water use

efficiency was found to be increased than their parents and other crosses. The data recorded for traits like pod length, pod girth, pod weight, pods per plant, pod yield and canopy temperature were also higher in these crosses. The results imply that the characters like pod length, pod girth, pod weight, pods per plant, pod yield, canopy temperature, relative water content and water use efficiency can be used as predictive criterion to indicate the degree of drought tolerance in yard long bean.

The genetic analysis suggested hybridization as the best strategy for the improvement of drought tolerance traits in yard long bean. All the hybrids manifest a significant amount of dominance variance for commercial exploitation. The existence of a significant amount of dominance variance emphasized the scope of heterosis breeding and hybridization followed by selection for exploitation of hybrid vigour in yard long bean. Based on the mean performance, sca effect and heterobeltiosis the crosses L3 x T3 (Kattampally local x Vellayani Jyothika), L6 x T2 (Nilamel local x Lola) and L4 x T2 (Kulashegarapuram x Lola) as desirable recombinants for drought tolerance and yield in yard long bean. Hence these crosses can be considered to be more desirable to be grown under a water shortage condition for increasing yield per unit area.

Experiment-IV

5.5 Gene expression

Water stress is likely to affect metabolic processes. Control and stability of metabolic processes during water stress necessitate significant changes in post transcriptional mechanisms that facilitate regulatory flexibility which is essential for timely stress response and adaptation. According to Cantale *et al.* (2007) dehydration has an impact on the expression patterns of transcription factor encoding gene. The overexpression of different drought responsive genes in plants confers tolerance to abiotic stress and protects plants against oxidative stress.

In the present study, real-time quantitative PCR assay was performed for determining quantitative changes in gene expression for drought tolerance at the

molecular level. Total RNA was isolated from the leaf samples of both control and stress induced plant tissue. The concentration of RNA was in the range of 1097 to 2091 µg/ml with the purity level within the range of 1.9 to 2.1. Based on the available sequences of DREBI and NCEDI genes in Vigna and related species primer pairs were designed using Primer3Plus software. Expression studies were done using Real–time PCR with DREBI and NCEDI primers with cDNA obtained from RNA isolated.

The PCR products were electrophoresed in 2% agarose gel and bands visualized under UV. qRT-PCR gene expression profile of DREB1 and NCEDI genes showed significant up and downregulation of the genes in drought tolerant and susceptible genotypes and hybrids in comparison to the control samples. Both genes showed elevated expression in tolerant hybrid and parental genotypes, while downregulation was observed in susceptible genotype. The elevated expression of DREBs and NCED1 genes under drought suggested that the increased expression potentially results in enhanced tolerance. The results were found to be consistent with the field studies. Similar results were also reported by Konzen *et al.* (2019) in common bean. The overexpression of the genes in the tolerant hybrid and parents also confirmed the role of DREB1 and NCED1 in enabling better drought tolerance.

The amplified products of the genes were purified from the gel and sequenced. The sequence was subjected to in silico analysis, using Nucleotide BLAST. The two gene sequence data were submitted in the BankIt NCBI.

SUMMARY

SUMMARY

Yard long bean (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdcourt) is an important legume vegetable in Kerala. Moisture stress is a major abiotic constraint that limits yard long bean production. To tackle this challenge, high yielding varieties of yard long bean with drought tolerance must be developed. Yard long bean has been in cultivation in Kerala since ancient times, which has resulted in rich and diverse domestic germplasm. This existing repository of genetic diversity can be screened to utilise the valuable genes including that conferring drought tolerance.

In this context, the present study entitled "Gene action and gene expression analysis in yard long bean (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdcourt) for drought tolerance" was undertaken as an initial step for the development of drought tolerant yard long bean varieties. The objective of the study was to identify the drought tolerant yard long bean genotypes from the available germplasm and to study the gene action of the selected lines through line x tester analysis under induced water stress conditions and evaluation of the drought tolerant genotype at the molecular level by analyzing the expression of drought responsive genes. Four experiments were carried out in order to reach the objectives.

In experiment I, 100 genotypes of yard long bean (G1 to G100) were evaluated during summer for drought tolerance in the field at the seedling stage. The materials were collected from different cultivated areas of Kerala. The moisture stress was imposed by withholding irrigation and later irrigation was restored in order to ensure the survival of the tolerant lines. The results of the analysis showed significant variations among genotypes, indicating the possibility of selection of desirable genetic material for further improvement. The relative water content ranged from 57.7% to 79.1%. The number of days for reaching critical stress level is found to be 6. Out of the 100 genotypes screened, 15 drought tolerant genotypes were identified based on their better performance in terms of high RLW, low PWP, more number of days for reaching critical stress

level and high recovery percentage. The genotypes identified were G1 (Acc 5), G5 (Acc 1339), G6 (Adoor local), G14 (Anchal local II), G15 (Aranmula local), G24 (Elamadu local), G36 (Kattampally local), G42 (Kollam local), G45 (Kottarakara local), G46 (Kottayam local), G50 (Kulashegarapuram local), G51 (Kulathupuzha local), G60 (Muttathukonam local), G74 (Nilamel local) and G89 (Pongamoodu local).

The selected fifteen drought tolerant genotypes that perform well in field conditions were carried to experiment II. The genotypes were planted and evaluated in grow bags in the rain shelter. The selected genotypes were morphologically described using IBPGR descriptor for the cowpea. To understand the levels of similarity and dissimilarity among genotypes the morphological descriptor data were subjected to cluster analysis using Ward's minimum variance clustering. Cluster analysis revealed five clusters indicating the existence of variability among them and the absence of duplication.

In experiment II, water stress was imposed from flowering onwards by restricting the irrigation to once in four days at 10mm depth. To assess moisture stress tolerance,11 biometric and 9 physiological characteristics were used. Analysis of variance was found to be significant for all the genotypes evaluated. Drought tolerance is a complex trait, several factors and mechanisms operate independently or jointly to enable plants to cope with drought stress. Drought tolerant genotypes were thus identified based on their superior mean performance across the 20 parameters. Anchal local II, Aranmula local, Kattampally local, Kulashegarapuram local, Muttathukonam local, Nilamel local and Pongamoodu local were identified as the best performing genotypes.

Experiment III was divided into two parts: first, the development of F1's in Line x Tester mating design and second, the evaluation of F1's and parents in the field under induced water stress conditions. Seven drought tolerant genotypes selected from experiment II and three high yielding commercial varieties (Gitika, Vellayani Jyothika and Lola) were selected as lines and testers respectively. The ten parents were crossed in Line x Tester pattern. Twenty one hybrids along with their parents and check (Arka Mangla) were evaluated for moisture stress

tolerance in the field. The performance was evaluated based on eight morphological and eight physiological parameters.

Mean performance, combining ability, gene action and heterosis were estimated. Based on the mean performance and gca effects, L3 (Kattampally local) andL4 (Kulashegarapuram local) was found to be good general combiners among lines and T2 (Lola) among testers. Based on the mean performance, sca effect and heterobeltiosis the crosses L3 x T3 (Kattampally local x Vellayani Jyothika), L6 x T2 (Nilamel local x Lola) and L4 x T2 (Kulashegarapuram x Lola) were identified as desirable recombinants for drought tolerance and yield under water stress.

From the variances of gca and sca, the gene action was calculated. The ratio of gca variance to sca variance was lower than unity for all the traits under study. The high magnitude of sca variance alone indicates the predominance of non-additive gene action in the inheritance of drought tolerant traits in yard long bean. Non-additive type of gene action suggests that hybridization is the best strategy for improving drought tolerance character in yard long bean. Based on gene action, suitable breeding methods for developing drought tolerant varieties include pureline selection, mass selection, hybridization and selection which include mainly pedigree breeding.

In experiment IV, a Real-time quantitative PCR assay was performed for determining quantitative changes in the expression of DREB1 and NCED1 genes for drought tolerance at the molecular level. Total RNA was isolated from the leaf samples of both control and stress induced plant tissue. The concentration of RNA was in the range of 1097 to 2091 µg/ml with the purity level within the range of 1.9 to 2.1. Based on the available sequences of DREBI and NCEDI genes in Vigna and related species primer pairs were designed using Primer3Plus software. Expression studies were done using Real time PCR with DREBI and NCEDI primers with cDNA obtained from RNA isolated.

The PCR products were electrophoresed in 2% agarose gel and bands visualized under UV. qRT-PCR gene expression profile of DREB1 and NCEDI genes showed significant up and downregulation of the genes in drought tolerant

and susceptible genotypes and hybrids in comparison to the control samples. Both genes showed elevated expression in tolerant hybrid and parental genotypes, while downregulation was observed in susceptible genotype. The elevated expression of DREBs and NCED1 genes under drought suggested their possible role in enhanced tolerance. The results were found to be consistent with the field studies. The overexpression of the genes in the tolerant hybrid and parents confirmed the role of DREB1 and NCED1 in enabling better drought tolerance. The PCR products of these were purified from the gel and sequenced. The sequence was subjected to in silico analysis, using Nucleotide BLAST. The two gene sequence data were submitted in the BankIt NCBI.

The study was successful in identifying drought tolerant genotype and crosses conferring drought tolerance in yard long bean. The tolerant genotypes i.e., L4 (Kulashegarapuram local) and L3 (Kattampally local) are identified as outstanding general combiners and can be exploited as parents for drought tolerance breeding in yard long bean. Based on the mean performance, sca effect and heterobeltiosis the crosses L3 x T3 (Kattampally local x Vellayani Jyothika), L6 x T2 (Nilamel local x Lola) and L4 x T2 (Kulashegarapuram x Lola) as promising combinations for drought tolerance and yield under water stress in yard long bean.

The elevated expression of DREBs and NCED1 genes in tolerant hybrids and genotypes in gene expression analysis indicates the increased drought tolerance ability which was in conformity with the field studies. All the hybrids manifest a significant amount of dominance variance for commercial exploitation. The identified genotypes can be used for the isolation of purelines with enhanced drought tolerance. The transgressive segregants from the identified crosses can be used for the development of drought tolerant high yielding cultivars in the future. The work can be continued with the identified genotypes and crosses for the development of climate smart drought tolerant varieties of yard long bean.

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Appendix

Appendix

Cow pea Descriptor (IBPGR, 1983)

1. VEGETATIVE CHARACTERS

a. Growth pattern

1. Determinate
2. Indeterminate

b. Twinning tendency

1. None
2. Slight
3. Intermediate
4. Pronounced

c. Plant pigmentation

Recorded for stem, branches, petioles and peduncles in the 6th week after sowing

0 - None

1 - Very slight

3 - Moderate at the base and tips of petioles

5 - Intermediate

7 - Extensive

9 - solid

d. Leaf length (cm)

To be measured on central leaf of 5th fully grown leaf (average of 5 random plants) - quantitative

e. Leaf width (cm)

To be measured on central leaf of 5th fully grown leaf (average of 5 random plants) – quantitative

g. Plant height (m)

To be measured from the ground to the tip of the plant at the maturity of the crop (average of 5 random plants) – quantitative.

2. INFLORESCENCE AND FRUIT CHARACTERS

a. Flower colour

1. White
2. Violet
3. Mauve- pink
4. Others (specify)

b. Days to 50% flowering

To be recorded as the number of days from planting to the day when 50% of the plants in a row flowered - quantitative.

c. Peduncle length (cm)

Recorded when peduncles have grown full length. Mean length of 5 peduncle, one from each of 5 randomly selected plants - quantitative.

d. Pod colour

Of mature pod

1. Pale tan or straw
2. Dark tan
3. Dark brown
4. Black or dark purple
5. Other (specify)

e. Pod length (cm)

To be recorded as average of 10 random mature pods - quantitative.

f. Pod width (cm)

To be recorded as average of 10 random mature pods - quantitative.

g. Pod curvature

Of mature pods

1. Straight
2. Slightly curved
3. Curved
4. Coiled

h. Pod weight (g)

To be recorded as average of 10 random mature pods - quantitative.

i. Pods per plant

Mean number of mature pods from 10 randomly selected plants

j. Number of locules per pod

To be recorded as average of 10 random mature pods - quantitative.

k. Yield per plant (g)

Average of 5 random plants, on maturity – quantitative.

3. SEED CHARACTERS

a. Seed coat colour

1. White
2. Apricot buff
3. Red
4. Deep red
5. Brown
6. Black
7. Capusine buff
8. Mottled brown
9. Buff
10. Mottled grey
11. Mottled red
12. Others (Specify)

b. Seed shape

1. Kidney
2. Ovoid
3. Crowded
4. Globose
5. Rhomboid
6. Others (Specify)

c. Seed eye pattern

The shape of the pigment pattern which surrounds the hilum.

0 - Absent

1 - Very small

2 - Kabba group

3 - Narrow eye

4 - Small eye

5 - Holstein group

6 - Watson group

7 - Self coloured

8 - Others (Specify)

d. Seed eye colour

0 - Eye absent

1 – Brown splash or grey

2 – Tan Brown

3 - Red

4 – Green

5 – Blue to black

6 – Blue to black spots or mottle

7 – Speckled

8 – Mottled

9 – Mottled and speckled

10 – Others (Specify)

e. Seed weight (g)

Weight of 100 random seeds in grams (average of 5 random plants) - quantitative.

f. Texta texture

1- Smooth

3 - Smooth to rough

5 - Rough (fine reticulation)

7- Rough to wrinkled

9 -Wrinkled (coarse folds on the testa)

GENE ACTION AND GENE EXPRESSION ANALYSIS IN YARD LONG BEAN (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdcourt) FOR DROUGHT TOLERANCE

by

RAHANA S.N.
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Abstract of the thesis

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COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA.

2022

ABSTRACT

Yard long bean (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdcourt) is a highly remunerative legume vegetable of Kerala. Due to climate change and erratic rainfall, in summer season the crop growth and pod production is heavily affected by moisture stress. Development of high yielding varieties of yard long bean with drought tolerance is essential for its sustainable production. In this context, the present study entitled "Gene action and gene expression analysis in yard long bean (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdcourt) for drought tolerance" was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, with an objective to identify drought tolerant genotype from the available germplasm and to understand the nature and magnitude of gene action and gene expression involved in the inheritance of drought tolerance in yard long bean.

The study comprised four experiments. First experiment dealt with the seedling stage evaluation of 100 yard long bean genotypes for drought tolerance in field. The moisture stress was imposed by withholding irrigation and later irrigation was restored in order to ensure the survival of the tolerant lines. The results of the analysis showed significant variations among genotypes. Out of the 100 genotypes screened, 15 drought tolerant genotypes were identified based on their better performance in terms of high RLW, low PWP, more number of days for reaching critical stress level and high recovery percentage. The genotypes identified were G1 (Acc 5), G5 (Acc 1339), G6 (Adoor local), G14 (Anchal local II), G15 (Aranmula local), G24 (Elamadu local), G36 (Kattampally local), G42 (Kollam local), G45 (Kottarakara local), G46 (Kottayam local), G50 (Kulashegarapuram local), G51 (Kulathupuzha local), G60 (Muttathukonam local), G74 (Nilamel local) and G89 (Pongamoodu local).

The fifteen selected genotypes from the first experiment were evaluated for drought tolerance in the second experiment by imposing moisture stress at the reproductive stage. Based on the biometric and physiological evaluations, the top seven genotypes with high yield and drought tolerance A4 (Anchal local II), A5

(Aranmula local), A7 (Kattampally local), A11 (Kulashegarapuram local), A13 (Muttathukonam local), A14 (Nilamel local), and A15 (Pongamoodu local) were selected as parents for further hybridization in experiment III.

In the third experiment, LxT crosses were performed by using seven selected tolerant genotypes as lines with three popular yard long bean varieties as testers to generate twenty one hybrids. The genetic analysis of hybrids and parents were evaluated based on eight morphological and eight physiological parameters. Mean performance, combining ability, gene action and heterosis were estimated. Based on the mean performance and gca effects, L4 (Kulashegarapuram local) and L3 (Kattampally local) are identified as outstanding general combiners and can be exploited as parents for drought tolerance breeding in yard long bean. Three superior crosses, Kattampally local x Vellayani Jyothika (L3 x T3), Nilamel local x Lola (L6 x T2) and Kulashegarapuram local x Lola (L4 x T2) were identified as promising combinations for drought tolerance and yield under water stress.

In the final experiment quantitative real time PCR was conducted to analyze the gene expression of drought responsive genes in tolerant hybrids and parents. The elevated expression of DREBs and NCED1 genes in tolerant hybrids and genotypes in gene expression analysis reflects the increased drought tolerance ability of those genotypes. The gene expression analysis was in conformity with the field studies.

All the hybrids manifested significant amount of dominance variance for commercial exploitation. Existence of significant amount of dominance variance and non-additive gene action suggests that hybridization as the best strategy for improving the drought tolerance character in yard long bean. The identified genotypes can be used for isolation of purelines with enhanced drought tolerance and the transgressive segregants from the identified crosses can be used for the development of drought tolerant high yielding cultivars in the future. The work can be continued with the identified genotypes and crosses for the development of climate smart drought tolerant varieties of yard long bean.

വള്ളിപ്പയർ (വിഗ്ന അങ്കിക്കുലേറ്റാ എസ്എസ്പി. സെസ്കിപെഡലിസ് (എൽ.) വെർഡ്കോർട്ട്) കേരളത്തിലെ ഉയർന്ന വരുമാനമുള്ള പയർവർഗ്ഗ പച്ചക്കറിയാണ്. കാലാവസ്ഥാ വ്യതിയാനവും ക്രമരഹിതമായ മഴയും കാരണം, വേനൽക്കാലത്ത് വിളകളുടെ വളർച്ചയെയും കായ്കളുടെ ഉൽപാദനത്തെയും ഈർപ്പത്തിൻറ സമ്മർദ്ദം സാരമായി ബാധിക്കുന്നു. സുസ്ഥിര ഉൽപ്പാദനത്തിന് വരൾച്ച സഹിഷ്ണുതയോടെ ഉയർന്ന വിളവ് നൽകുന്ന വള്ളിപ്പയർ വികസിപ്പിക്കേണ്ടത് അത്യാവശ്യമാണ്. ഈ പശ്ചാത്തലത്തിൽ, "വരൾച്ച സഹിഷ്ണുതയ്ക്കായി വള്ളിപ്പയർ (വിഗ്ന അങ്കിക്കുലേറ്റാ എസ്എസ്പി. സെസ്കിപെഡലിസ് (എൽ.) വെർഡ്കോർട്ട്) ജീൻ പ്രവർത്തനവും ജീൻ എക്സ്പ്രഷൻ വിശകലനവും " എന്ന തലക്കെട്ടിലുള്ള ഇപ്പോഴത്തെ പഠനം അഗ്രികൾച്ചർ കോളേജിലെ പ്ലാന്റ് ബ്രീഡിംഗ് ആൻഡ് ജനറ്റിക്സ് വകുപ്പിൽ നടത്തി. വള്ളിപ്പയറിലെ ലഭ്യമായ ജനിതകരൂപങ്ങളിൽ നിന്ന് വരൾച്ചയെ പ്രതിരോധിക്കുന്ന ജനിതകരൂപം തിരിച്ചറിയാനും വരൾച്ച പ്രതിരോധനത്തിൽ ഉൾപ്പെട്ടിരിക്കുന്ന ജീൻ പ്രവർത്തനത്തിന്റെയും ജീൻ എക്സ്പ്രഷന്റെയും സ്വഭാവവും വ്യാപ്തിയും മനസ്സിലാക്കുകയും ചെയ്യുക.

പഠനം നാല് പരീക്ഷണങ്ങൾ ഉൾക്കൊള്ളുന്നു. ആദ്യ പരീക്ഷണം വയലിലെ വരൾച്ചയെ സഹിഷ്ണുതയ്ക്കായി 100 യാർഡ് നീളമുള്ള ബീൻ ജനിതകരൂപങ്ങളുടെ തൈകളുടെ ഘട്ടം വിലയിരുത്തി. ജലസേചനം തടഞ്ഞുകൊണ്ട് ഈർപ്പത്തിന്റെ സമ്മർദ്ദം അടിച്ചേൽപ്പിക്കുകയും പിന്നീട് ജലസേചനം പുനഃസ്ഥാപിക്കുകയും ചെയ്തു, സഹിഷ്ണുതയുള്ള ലൈനുകളുടെ നിലനിൽപ്പ് ഉറപ്പാക്കാൻ. വിശകലനത്തിന്റെ ഫലങ്ങൾ ജനിതകരൂപങ്ങളിൽ കാര്യമായ വ്യത്യാസങ്ങൾ കാണിച്ചു. സ്ക്രീൻ ചെയ്ത 100 ജനിതകരൂപങ്ങളിൽ, ഉയർന്ന ആർ.എൽ.ഡബ്ല്യു, കുറഞ്ഞ പി.ഡബ്ല്യു.പി, ഗുരുതരമായ സ്ട്രെസ് ലെവലിലെത്താനുള്ള കൂടുതൽ ദിവസങ്ങൾ, ഉയർന്ന വീണ്ടെടുക്കൽ ശതമാനം എന്നിവയിലെ മികച്ച പ്രകടനത്തെ അടിസ്ഥാനമാക്കി 15 വരൾച്ചയെ പ്രതിരോധിക്കുന്ന

ജനിതകരൂപങ്ങളെ തിരിച്ചറിഞ്ഞു. ജി1 (എസിസി 5), ജി5 (എസിസി 1339), ജി6 (അടൂർ ലോക്കൽ), ജി14 (അഞ്ചൽ ലോക്കൽ II), ജി15 (ആറന്മുള ലോക്കൽ), ജി24 (ഇളമാട് ലോക്കൽ), ജി36 (കാട്ടാമ്പള്ളി ലോക്കൽ), ജി42 (കൊല്ലം) എന്നിവയാണ് തിരിച്ചറിഞ്ഞ ജനിതകരൂപങ്ങൾ. ലോക്കൽ), ജി45 (കൊട്ടാരക്കര ലോക്കൽ), ജി 46 (കോട്ടയം ലോക്കൽ), ജി 50 (കുലശേഖരപുരം ലോക്കൽ), ജി 51 (കുളത്തൂപ്പുഴ ലോക്കൽ), ജി 60 (മുട്ടത്തുകോണം ലോക്കൽ), ജി 74 (നിലമേൽ ലോക്കൽ), ജി 89 (പോങ്ങമൂട് ലോക്കൽ).

ആദ്യ പരീക്ഷണത്തിൽ നിന്ന് തിരഞ്ഞെടുത്ത പതിനഞ്ച് ജനിതകരൂപങ്ങൾ പ്രത്യുൽപ്പാദന ഘട്ടത്തിൽ ഈർപ്പം സമ്മർദ്ദം ചെലുത്തി രണ്ടാമത്തെ പരീക്ഷണത്തിൽ വരൾച്ചയെ സഹിഷ്ണുതയ്ക്കായി വിലയിരുത്തി. ബയോമെട്രിക്, ഫിസിയോളജിക്കൽ മൂല്യനിർണ്ണയങ്ങളെ അടിസ്ഥാനമാക്കി, ഉയർന്ന വിളവും വരൾച്ച സഹിഷ്ണുതയും ഉള്ള മികച്ച ഏഴ് ജനിതകരൂപങ്ങൾ എ) 4 (അഞ്ചൽ ലോക്കൽ II), എ)5 (ആറന്മുള ലോക്കൽ), എ)7 (കാട്ടാമ്പള്ളി ലോക്കൽ), എ)11 (കുലശേഖരപുരം ലോക്കൽ), എ)13 (മുട്ടത്തുകോണം ലോക്കൽ), എ)14 (നിലമേൽ ലോക്കൽ), എ)15 (പോങ്ങമൂട് ലോക്കൽ) എന്നിവരെ പരീക്ഷണം III-ൽ കൂടുതൽ ഹൈബ്രിഡൈസേഷനായി മാതാപിതാക്കളായി തിരഞ്ഞെടുത്തു.

മൂന്നാമത്തെ പരീക്ഷണത്തിൽ, തിരഞ്ഞെടുത്ത ഏഴ് സഹിഷ്ണുത ജനിതകരൂപങ്ങൾ ഇരുപത്തിയൊന്ന് സങ്കരയിനങ്ങൾ സൃഷ്ടിക്കുന്നതിനായി മൂന്ന് ജനപ്രിയ വള്ളിപ്പയർ ഇനങ്ങളെ ടെസ്റ്റുകളായി ഉപയോഗിച്ചാണ് എൽ x റ്റി ക്രോസുകൾ നടത്തിയത്. എട്ട് മോർഫോളജിക്കൽ, എട്ട് ഫിസിയോളജിക്കൽ പാരാമീറ്ററുകൾ അടിസ്ഥാനമാക്കിയാണ് സങ്കരയിനങ്ങളുടെയും മാതാപിതാക്കളുടെയും ജനിതക വിശകലനം വിലയിരുത്തിയത്. ശരാശരി പ്രകടനം, സംയോജന ശേഷി, ജീൻ പ്രവർത്തനം, ഹെറ്ററോസിസ് എന്നിവ കണക്കാക്കി. ശരാശരി പ്രകടനവും gca ഇഫക്റ്റുകളും അടിസ്ഥാനമാക്കി, എൽ4 (കുലശേഖരപുരം ലോക്കൽ), എൽ3 (കാട്ടാമ്പള്ളി ലോക്കൽ) എന്നിവ മികച്ച പൊതു സംയോജനങ്ങളായി തിരിച്ചറിയപ്പെടുന്നു, കൂടാതെ മുറ്റത്തെ നീളമുള്ള ബീനിൽ വരൾച്ച

സഹിഷ്ണുത പ്രജനനത്തിനായി മാതാപിതാക്കളായി പ്രയോജനപ്പെടുത്താം. കാട്ടാമ്പള്ളി ലോക്കൽ x വെള്ളായണി ജ്യോതിക (എൽ3 x റ്റി3), നിലമേൽ ലോക്കൽ x ലോല (എൽ6 x റ്റി2), കുലശേഖരപുരം ലോക്കൽ x ലോല (എൽ4 x റ്റി2) എന്നീ മൂന്ന് സുപ്പീരിയർ ക്രോസുകൾ വരൾച്ചയെ അതിജീവിക്കുന്നതിനും ജലസമ്മർദ്ദത്തിൽ വിളവു നൽകുന്നതിനുമുള്ള വാഗ്ദാന സംയോജനങ്ങളായി തിരിച്ചറിഞ്ഞു.

അവസാന പരീക്ഷണത്തിൽ, സഹിഷ്ണുതയുള്ള സങ്കരയിനങ്ങളിലും മാതാപിതാക്കളിലും വരൾച്ചയെ പ്രതികരിക്കുന്ന ജീനുകളുടെ ജീൻ എക്സ്പ്രഷൻ വിശകലനം ചെയ്യുന്നതിനായി ക്വാണ്ടിറ്റേറ്റീവ് റിയൽ ടൈം പിസിആർ നടത്തി. സഹിഷ്ണുതയുള്ള സങ്കരയിനങ്ങളിലെ ഡിആർഇബികളുടെയും എൻസിഇഡി1 ജീനുകളുടെയും ഉയർന്ന എക്സ്പ്രഷൻ, ജീൻ എക്സ്പ്രഷൻ വിശകലനത്തിലെ ജനിതകരൂപങ്ങൾ, ആ ജനിതകരൂപങ്ങളുടെ വർദ്ധിച്ച വരൾച്ച സഹിഷ്ണുത കഴിവിനെ പ്രതിഫലിപ്പിക്കുന്നു. ജീൻ എക്സ്പ്രഷൻ വിശകലനം ഫീൽഡ് പഠനങ്ങളുമായി പൊരുത്തപ്പെടുന്നതായിരുന്നു

എല്ലാ സങ്കരയിനങ്ങളും വാണിജ്യപരമായ ചൂഷണത്തിന് കാര്യമായ ആധിപത്യ വ്യതിയാനം പ്രകടമാക്കി. ഗണ്യമായ അളവിലുള്ള ആധിപത്യ വ്യതിയാനവും അഡിറ്റീവ് അല്ലാത്ത ജീൻ പ്രവർത്തനവും സൂചിപ്പിക്കുന്നത് വള്ളിപ്പയർ വരൾച്ച സഹിഷ്ണുത മെച്ചപ്പെടുത്തുന്നതിനുള്ള മികച്ച തന്ത്രമാണ് സങ്കരീകരണം. വർദ്ധിപ്പിച്ച വരൾച്ച സഹിഷ്ണുതയോടെ പ്യുർലൈനുകളെ വേർതിരിക്കുന്നതിന് തിരിച്ചറിഞ്ഞ ജനിതകരൂപങ്ങൾ ഉപയോഗിക്കാനും തിരിച്ചറിഞ്ഞ സങ്കരയിനങ്ങ നിന്നുള്ള അതിരുകടന്ന വേർതിരിവുകൾ ഭാവിയിൽ വരൾച്ചയെ അതിജീവിക്കുന്ന ഉയർന്ന വിളവ് തരുന്ന ഇനങ്ങളുടെ വികസനത്തിന് ഉപയോഗിക്കാനും കഴിയും. കാലാവസ്ഥാ സ്മാർട്ട് വരൾച്ചയെ സഹിഷ്ണുതയുള്ള വള്ളിപ്പയർ വികസിപ്പിക്കുന്നതിന് തിരിച്ചറിഞ്ഞ ജനിതകരൂപങ്ങളും സങ്കരയിനങ്ങളും ഉപയോഗിച്ച് പ്രവർത്തനം തുടരാം.