IDENTIFICATION AND VALIDATION OF QTL CONTROLLING TRAITS RELATED TO DROUGHT TOLERANCE IN THE RECOMBINANT INBRED POPULATION OF THE CROSS NRCG 12568 × NRCG 12326 IN GROUNDNUT (*Arachis hypogaea* L.)

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DEPARTMENT OF GENETICS AND PLANT BREEDING
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE
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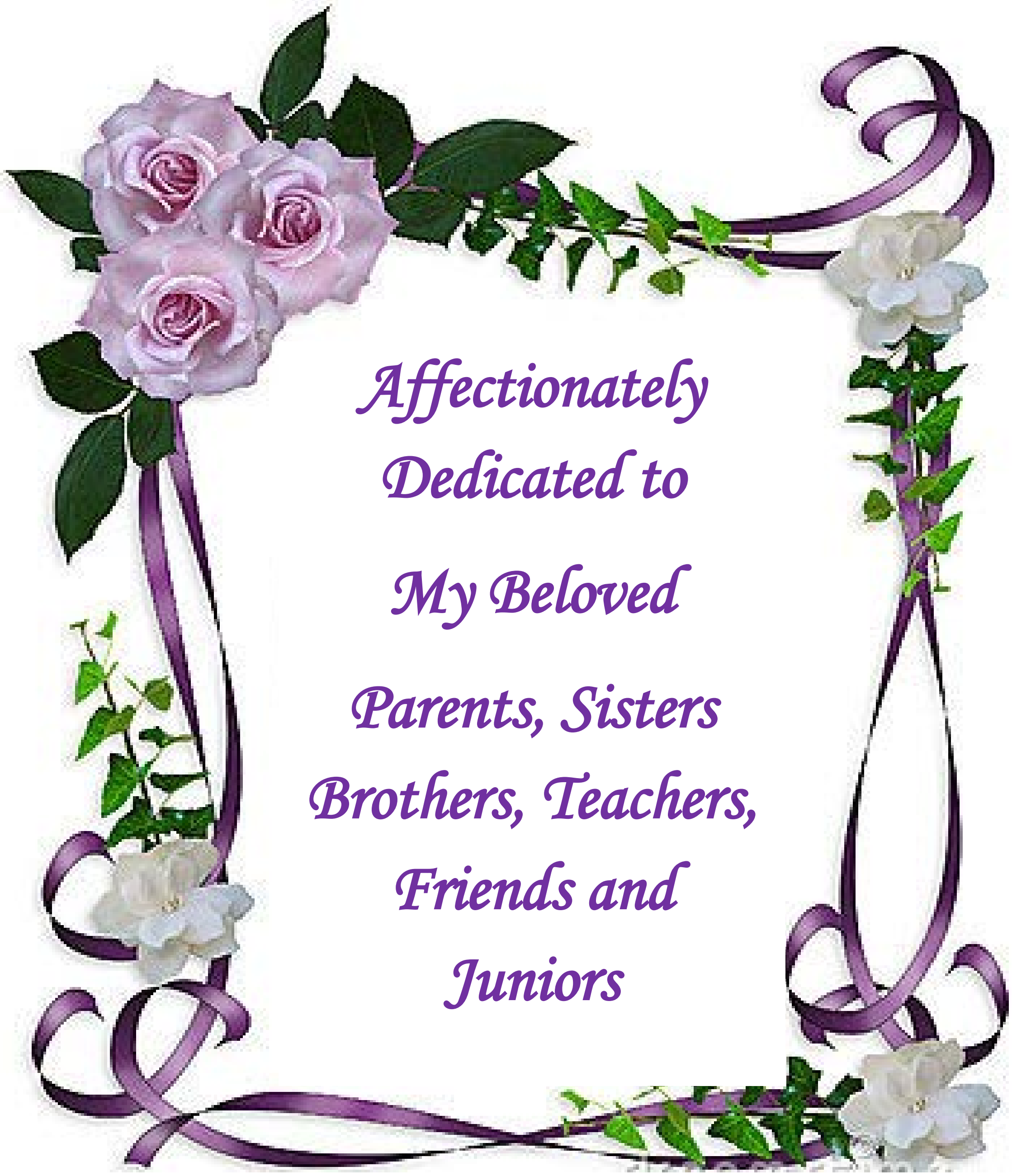

Affectionately
Dedicated to

My Beloved

Parents, Sisters
Brothers, Teachers,
Friends and
Juniors

DEPARTMENT OF GENETICS AND PLANT BREEDING
UNIVERSITY OF AGRICUTURAL SCIENCES
BANGALORE

CERTIFICATE

This is to certify that the thesis entitled **IDENTIFICATION AND VALIDATION OF QTL CONTROLLING TRAITS RELATED TO DROUGHT TOLERANCE IN THE RECOMBINANT INBRED POPULATION OF THE CROSS NRCG 12568 × NRCG 12326 IN GROUNDNUT (*Arachis hypogaea* L.)** submitted in partial fulfilment of the requirement for the degree of **DOCTOR OF PHILOSOPHY in GENETICS AND PLANT BREEDING** to the University of Agricultural Sciences, Bangalore, is a *bona-fide* record of research work done by **Mr. BHARATH KUMAR P. JAMBAGI, ID No. PALB-6061** during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bengaluru
December, 2020

(D. L. SAVITHRAMMA)
Major Advisor

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.... Omission of any names doesn't mean lack of gratitude. Ending is inevitable, for all good work it is time to end the acknowledgement.

Bengaluru

December, 2020 **(Bharath Kumar P. Jambagi)**

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BHARATH KUMAR P. JAMBAGI

ABSTRACT

An investigation was carried out with the experimental material consisting of 147 RILs developed from the cross NRCG-12568 × NRCG-12326 during summer 2017 and 2018 at GKVK, Bengaluru under four different conditions *viz*., Well-watered (WW), Water stress (WS) I -with holding irrigation from 30-45 DAS (flowering period), Water stress II -with holding irrigation from 45-65 DAS (flowering and peg initiation stage), water stress III -with holding irrigation from 65-85 DAS (peg penetration and pod development stage) in augmented design including checks; TMV-2 and GKVK-5. Significant differences among RILs were observed for most of the traits in both the conditions. SCMR, SLA, pods plant^{-1} and pod yield plant^{-1} showed more genetic variation exhibiting moderate to high heritability with high genetic advance. Four out of 15 drought tolerant indices tested *viz*., MP, GMP, HMP and STI were found to be better indicators of drought tolerance. RIL133, RIL126, RIL145 and RIL25 showed higher pod yield in WW and WS conditions in comparison to GKVK 5 across years and locations based on *per se* performance, biplot analysis and ranking method. A linkage map was developed with 172 SSR markers on 20 linkage groups using genotypic data of 147 RILs. The length of linkage map spanned 2212.87 cM with an average of 11.12 cM inter-maker distance. Twenty-one QTLs were detected with 6.27-13.55% of phenotypic variance explained (PVE) in WW and WS environments. Two major QTLs each for SCMR and days to 50 *per cent* flowering, three major QTLs for SLA were detected in WS condition at LOD 3.0. A total of 38 Di-QTL interactions with more than 5% PVE were identified at LOD 5.0. The QTLs identified in the study can be utilized for marker assisted back cross breeding after validation. Stable RILs can be tested in multilocations or could be used in future breeding for drought tolerance.

December, 2020

Department of Genetics and Plant Breeding — **D. L. SAVITHRAMMA**

UAS, GKVK, Bengaluru-560065 — (Major advisor)

ನೆಲಗಡಲೆ ತಳಿಗಳಾದ ಎನ್.ಆರ್.ಸಿ.ಜಿ.–12568 × ಎನ್.ಆರ್.ಸಿ.ಜಿ.–12326 ರ ಪುನರ್ ಸಂಯೋಜಿತ ಒಳಸಂಕರಣ ತಳಿಗಳಲ್ಲಿ ಬರ ಸಹಿಷ್ಣುತೆಗೆ ಸಂಬಂಧಿಸಿದ ನಿಯಂತ್ರಣ ಗುಣಲಕ್ಷಣಗಳುಳ್ಳ ಸ್ಥಿರ ವಂಶವಾಹಿ ಗುಂಪುಗಳ ಗುರುತಿಸುವಿಕೆ ಮತ್ತು ಮೌಲ್ಯಮಾಪನ ಕುರಿತಾದ ಸಂಶೋಧನೆ

ಭರತ್ ಕುಮಾರ್ ಪಿ. ಜಂಬಗಿ

ಪ್ರಬಂಧ ಸಾರಾಂಶ

ಕಡಲೆಕಾಯಿ ಬೆಳೆಯಲ್ಲಿ ಪುನರ್ ಸಂಯೋಜಿತ ಒಳ ಸಂಕರಣ ತಳಿಗಳಲ್ಲಿ ನೀರಿನ ಬಳಕೆ ಸಾಮರ್ಥ್ಯಕ್ಕೆ ಸಂಬಂಧಿಸಿದ ಗುಣಗಳಿಗಾಗಿ ಸಮಗ್ರ ವಂಶವಾಹಿ ನಕ್ಷೆ ಅಭಿವೃದ್ಧಿ ಪಡಿಸುವುದು ಮತ್ತು ಸ್ಥಿರ ವಂಶವಾಹಿ ಗುಂಪುಗಳನ್ನು ಗುರುತಿಸುವ ಕುರಿತು ಸಂಶೋಧನೆಯನ್ನು ನಡೆಸಲಾಯಿತು. ಈ ಸಂಶೋಧನೆಗಾಗಿ ಎನ್.ಆರ್.ಸಿ.ಜಿ.–12568 × ಎನ್.ಆರ್.ಸಿ.ಜಿ.–12326 ಸಂಕರಣದಿಂದ ಅಭಿವೃದ್ಧಿ ಪಡಿಸಿದ ಪುನರ್ ಸಂಯೋಜಿತ ಒಳ ಸಂಕರಣ ತಳಿಗಳು, ಅವುಗಳ ತಂದೆ–ತಾಯಿ ತಳಿಗಳು ಮತ್ತು ಎರಡು ಹೋಲಿಕೆ ತಳಿಗಳನ್ನು ಬಳಸಲಾಯಿತು. ಈ ತಳಿಗಳನ್ನು ಸತತ ಎರಡು ಬೇಸಿಗೆ ಕಾಲದಲ್ಲಿ ನಾಲ್ಕು ವಿಭಿನ್ನ ಪರಿಸ್ಥಿತಿಗಳಲ್ಲಿ ಅಂದರೆ, ಚೆನ್ನಾಗಿ ನೀರಾಯಿಸಿದ, ನೀರಿನ ಒತ್ತಡ ಪರಿಸ್ಥಿತಿ I-30 ರಿಂದ 45 ದಿನಗಳವರೆಗೆ ನೀರು ಹಿಡಿದಿಟ್ಟುಕೊಳ್ಳುವುದು (ಹೂ ಬಿಡುವ ಅವಧಿ), ನೀರಿನ ಒತ್ತಡ ಪರಿಸ್ಥಿತಿ II-45 ರಿಂದ 65 ದಿನಗಳವರೆಗೆ ನೀರು ಹಿಡಿದಿಟ್ಟುಕೊಳ್ಳುವುದು (ಪೆಗ್ ನುಗ್ಗುವಿಕೆ ಮತ್ತು ಕಾಯಿ ಬಿಡುವ ಹಂತ), ನೀರಿನ ಒತ್ತಡ ಪರಿಸ್ಥಿತಿ III-65 ರಿಂದ 85 ದಿನಗಳವರೆಗೆ ನೀರು ಹಿಡಿದಿಟ್ಟುಕೊಳ್ಳುವುದರೊಂದಿಗೆ (ಕಾಯಿ ಬಲಿಯುವ ಹಂತ) ಜಿ.ಕೆ.ವಿ.ಕೆ ಬೆಂಗಳೂರು 2017 ಮತ್ತು 2018 ರಲ್ಲಿ ಅಭಿವೃದ್ಧಿ ಪಡಿಸಿದ ವಿನ್ಯಾಸದಲ್ಲಿ ಮೌಲ್ಯಮಾಪನ ಮಾಡಲಾಯಿತು. ಮೇಲೆ ತಿಳಿಸಲಾದ ಎಲ್ಲಾ ಪರಿಸ್ಥಿತಿಗಳಲ್ಲಿ ಹೆಚ್ಚಿನ ಗುಣಲಕ್ಷಣಗಳಿಗೆ ಪುನರ್ ಸಂಯೋಜಿತ ಒಳ ಸಂಕರಣ ತಳಿಗಳ ನಡುವೆ ಗಮನಾರ್ಹ ವ್ಯತ್ಯಾಸಗಳು ಕಂಡುಬಂದವು. ಗುಣಲಕ್ಷಣಗಳಾದ (ನೀರಿನ ಒತ್ತಡದಲ್ಲಿ), ಪತ್ರಹರಿತ್ತು (SCMR), ನಿರ್ದಿಷ್ಟ ಎಲೆ ವಿಸ್ತೀರ್ಣ, ಗಿಡವೊಂದಕ್ಕೆ ಕಡಲೆಕಾಯಿಗಳ ಸಂಖ್ಯೆ ಮತ್ತು ಗಿಡವೊಂದಕ್ಕೆ ಕಾಯಿಗಳ ಇಳುವರಿ ಹೆಚ್ಚಿನ ವ್ಯತ್ಯಾಸವನ್ನು ತೋರಿದವು ಹಾಗೂ ಅವುಗಳ ಅನುವಂಶಿಕತೆಯೂ ಸಹ ಹೆಚ್ಚಾಗಿ ಕಂಡುಬಂದಿದೆ. ಹದಿನೈದು ಬರಗಾಲದ ಸಹಿಷ್ಣು ಸೂಚ್ಯಾಂಕಗಳ ಪೈಕಿ ನಾಲ್ಕು ಸೂಚ್ಯಂಕಗಳಾದ ಸರಾಸರಿ ಉತ್ಪಾದಕತೆ, ಜಾಮಿತಿಯ ಉತ್ಪಾದಕತೆ, ಸಾಮರಸ್ಯ ಉತ್ಪಾದಕತೆ ಮತ್ತು ಒತ್ತಡಕ್ಕೆ ಒಳಗಾಗಬಹುದಾದ ಸೂಚ್ಯಂಕಗಳು ಉತ್ತಮವೆಂದು ಗುರುತಿಸಲ್ಪಟ್ಟವು. ಜಿಕೆವಿಕೆ 5 ಕ್ಕೆ ಹೋಲಿಸಿದರೆ, ಪುನರ್ ಸಂಯೋಜಿತ ಒಳ ಸಂಕರಣ ತಳಿ ಸಂಖ್ಯೆಗಳಾದ 113, 126, 145 ಮತ್ತು 25 ಚೆನ್ನಾಗಿ ನೀರಾಯಿಸಿದ ಮತ್ತು ನೀರಿನ ಒತ್ತಡ ಸ್ಥಿತಿಗಳಲ್ಲಿ ಹೆಚ್ಚಿನ ಕಡಲೆಕಾಯಿ ಇಳುವರಿಯನ್ನು ನೀಡಿದವು ಹಾಗೂ ಸರಾಸರಿ ಕಡಲೆಕಾಯಿ ಇಳುವರಿ, ದ್ವಿನಕ್ಷೆ ಮತ್ತು ಶ್ರೇಯಾಂಕ ವಿಧಾನಗಳನ್ನು ಆಧರಿಸಿ ಉತ್ತಮವೆಂದು ಗುರುತಿಸಲಾಗಿದೆ. 147 ಒಳ ಸಂಕರಣ ತಳಿಗಳ ಅನುವಂಶಿಕ ಧಾತುಗಳ ಅಂಕಿ ಸಂಖ್ಯೆಯ ಮಾಹಿತಿಯನ್ನು ಬಳಸಿಕೊಂಡು 20 ಅನುವಂಶಿಕ ಸಂಪರ್ಕ ಗುಂಪುಗಳಲ್ಲಿ 172 (ಸರಳ ಅನುಕ್ರಮ ಪುನರಾವರ್ತಿಸಿ) ಎಸ್.ಎಸ್.ಆರ್. ಅನುವಂಶಿಕ ಸೂಚಕಗಳೊಂದಿಗೆ ಸಂಯೋಜಿತ ಸಂಪರ್ಕ ನಕ್ಷೆಯನ್ನು ಅಭಿವೃದ್ಧಿ ಪಡಿಸಲಾಯಿತು. ಅನುವಂಶಿಕ ನಕ್ಷೆಯ ಉದ್ದವು 2212.87cM ಆಗಿದ್ದು, ಸರಾಸರಿ 11.12cM ಅಂತರವನ್ನು ಹೊಂದಿದೆ. ಪ್ರಧಾನ–ವಂಶವಾಹಿ ಪರಿಮಾಣಾತ್ಮಕ ಗುಣಸ್ಥಾನಗಳ ವಿಶ್ಲೇಷಣೆಯು 21 ಗುಣಸ್ಥಾನಗಳನ್ನು 6.27-13.55% ರಷ್ಟು ಪ್ರಕಟ ಲಕ್ಷಣ ವ್ಯತ್ಯಾಸದೊಂದಿಗೆ ಒತ್ತಡ ಮತ್ತು ಒತ್ತಡರಹಿತ ಪರಿಸರದಲ್ಲಿ ವಿವರಿಸಿದೆ. ಒಟ್ಟಾರೆ ಪತ್ರಹರಿತ್ತುಗಾಗಿ ಮತ್ತು 50 ಪ್ರತಿಶತ ಹೂಬಿಡುವ ಗುಣಲಕ್ಷಣಕ್ಕಾಗಿ ತಲಾ ಎರಡು ಪ್ರಧಾನ–ವಂಶವಾಹಿ ಪರಿಮಾಣಾತ್ಮಕ ಗುಣಸ್ಥಾನಗಳು ಮತ್ತು ನಿರ್ದಿಷ್ಟ ಎಲೆ ವಿಸ್ತೀರ್ಣಕ್ಕಾಗಿ ಮೂರು ಪ್ರಧಾನ–ವಂಶವಾಹಿ ಪರಿಮಾಣಾತ್ಮಕ ಗುಣಸ್ಥಾನಗಳ ಸಂವಹನಗಳನ್ನು LOD ಸ್ಕೋರ್ 3.0ರಲ್ಲಿ ವರ್ಷಗಳ ಹಾಗೂ ತೇವಾಂಶ ಪರಿಸ್ಥಿತಿಗಳಲ್ಲಿ ಸ್ಥಿರ ವಂಶವಾಹಿ ಪರಿಮಾಣಾತ್ಮಕ ಗುಣಸ್ಥಾನಗಳೆಂದು ಪತ್ತೆಹಚ್ಚಲಾಗಿದೆ. ಒಟ್ಟು 38 ದ್ವಿ–ವಂಶವಾಹಿ ಪರಿಮಾಣಾತ್ಮಕ ಗುಣಸ್ಥಾನಗಳ ಸಂವಹನಗಳನ್ನು ಶೇ.5 ಕ್ಕಿಂತ ಹೆಚ್ಚು ಪ್ರಕಟ ಲಕ್ಷಣ ವ್ಯತ್ಯಾಸದೊಂದಿಗೆ LOD ಸ್ಕೋರ್ ಶೇ.5.0ರಲ್ಲಿ ಕಂಡುಹಿಡಿಯಲಾಯಿತು. ಸ್ಥಿರವಾದ ಪ್ರಧಾನ–ವಂಶವಾಹಿ ಪರಿಮಾಣಾತ್ಮಕ ಗುಣಸ್ಥಾನಗಳನ್ನು ಮುಂದೆ ಧೃಡೀಕರಿಸಿ ನಂತರ ತಳಿ ಅಭಿವೃದ್ಧಿಯಲ್ಲಿ ಬಳಸಬಹುದಾಗಿದೆ. ಸಂಯೋಜಿತ ಸ್ಥಿರ ಒಳ ಸಂಕರಣಗಳ ತಳಿಗಳನ್ನು ಮುಂದೆ ಹೆಚ್ಚಿನ ಸ್ಥಳಗಳಲ್ಲಿ ಪ್ರಯೋಗಕ್ಕೆ ಒಳಪಡಿಸಿ ಪರೀಕ್ಷಿಸಬಹುದು ಮತ್ತು ಬರಸಹಿಷ್ಣು ತಳಿ ಅಭಿವೃದ್ಧಿಯಲ್ಲಿ ಬಳಸಬಹುದಾಗಿದೆ.

ಡಿಸೆಂಬರ್, ೨೦೨೦
ಅನುವಂಶೀಯ ಸಸ್ಯತಳಿ ಅಭಿವೃದ್ಧಿ ಶಾಸ್ತ್ರ ವಿಭಾಗ
ಕೃ.ವಿ.ವಿ., ಗಾಂ.ಕೃ.ವಿ.ಕೇ., ಬೆಂಗಳೂರು– ೫೬೦ ೦೬೫

ಡಿ. ಎಲ್. ಸಾವಿತ್ರಮ್ಮ
(ಮುಖ್ಯ ಸಲಹೆಗಾರರು)

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%	:	*per cent*
ANOVA	:	Analysis of Variance
ATI	:	Abiotic Tolerance Index
BIS	:	Before Initiation of Stress
BRS	:	Before Release of Stress
C. D.	:	Critical Difference
C.V.	:	Coefficient of Variation
Cm	:	Centimeter (s)
DAS	:	Days After Sowing
DFF	:	Days to Fifty *per cent* Flowering
DI	:	Drought Resistance Index
DTI	:	Drought Tolerant Indices
GAM	:	Genetic Advance as *per cent* Mean
GCV	:	Genotypic Coefficient of Variation
GMP	:	Geometric Mean Productivity
h^2_{bs}	:	Heritability (Broad Sense)
HMP	:	Harmonic Mean Productivity
LG	:	Linkage Group
LOD	:	Logarithm of Odds
MP	:	Mean Productivity
No.	:	Number (s)
PC	:	Principal Component
PCA	:	Principal Component Analysis
PCV	:	Phenotypic Coefficient of Variation
PVE	:	Phenotypic Variation Explained

QTL	:	Quantitative Trait Loci
RDI	:	Relative Drought Index
RILs	:	Recombinant Inbred Lines
RM	:	Rank Mean
RS	:	Rank Sum
RWC	:	Relative Water Content
S.Em±	:	Standard Error of Mean
SCMR	:	SPAD Chlorophyll Meter Readings
SDR	:	Standard Deviation Of Rank
SLA	:	Specific Leaf Area
SMA	:	Single Marker Analysis
SNPI	:	Stress Non Stress Productive Index
SSI	:	Stress Susceptibility Index
SSPI	:	Stress Susceptibility Percentage Index
STI	:	Stress Tolerance Index
TOL	:	Tolerance Index
WS1	:	Water Stress 1
WS2	:	Water Stress 2
WS3	:	Water Stress 3
WUE	:	Water Use Efficiency
WW	:	Well Watered
YI	:	Yield Index
YSI	:	Yield Stability Index

I INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important oilseed, food, fodder and four-foliate legume crop grown in both tropical and subtropical regions of the world. It originated in South America through hybridization of two diploid wild species due to spontaneous chromosome duplication to form an allotetraploid (AABB, $2n = 4x = 40$) (Kochert *et al.*, 1996). The most agreed to hypothesis stated that *A. duranensis* as the probable A genome donor and *A. ipaensis* as the B genome donor. It belongs to the family *Leguminosae* (sub family: *Fabacea*e), self-pollinating, allotetraploid having A and B genomes with basic chromosome number ten (Stebbins 1957; Stalker and Dalmacio, 1986) with the genome size of 2800 Mb (Guo *et al.*, 2009).

It is grown in more than 100 countries covering an area of 27.66 million hectares with an annual production of 43.98 million tonnes and productivity of 1590 kg ha^{-1} (FAO, 2018). Largest producers of groundnut are China followed by India, Nigeria, and United States. In India, groundnut is cultivated in an area of 5.80 million hectares with production of 6.85 million tonnes and productivity of 1182 kg ha^{-1} (FAO, 2018). The productivity of groundnut in India is low (1554 kg ha^{-1}) compared to USA (4397 kg ha^{-1}), China (3492 kg ha^{-1}), Israel (7389 kg ha^{-1}), and Argentina (2848 kg ha^{-1}) (FAO, 2018). In India, Gujarat and Andhra Pradesh ranks first and second respectively, both in area and production followed by Karnataka in area and Tamil Nadu in production. In Karnataka, it is grown in an area of 0.65 million hectares with production of 0.51 million tonnes and productivity of 769 kg ha^{-1} (Indiastat, 2018).

Globally, in many groundnut producing areas irrigation is unavailable or impractical. Moreover, due to agro-ecological changes, the crop is facing high risk of moisture stress ever before. In the contemporary times, with the ever surging realization of the fear of the catastrophic effects of global climate change, an inflated insistence on world food security and its impact at the regional stages specifically, have come to the front line of the scientific community. Thus, crop growth and development, water use and yield under normal conditions are majorly established by weather conditions during the growing season of the crop. Unexpectedly, with slight diversions from the regular weather conditions, the proficiency of notably applied inputs and food production is gravely undermined. At the moment, food security is influenced based on the ability to increase crop production levels with less availability/usage of water.

Drought is related with moisture stress and high temperature, which obtrudes various detrimental effects on plant growth and development. The complexity of drought tolerance mechanisms elucidates the steady progress of yield improvement in drought prone environments. Further, plant responses to drought stress are species-specific and show significant phenological variation. The understanding of the full interaction of a complex collection of drought adaptation traits is very arduous than discerning the functioning of each trait alone. The accurate assessment of a cultivars adoption to drought is not possible from a single season evaluation. Similarly, there is no single trait that breeders can take advantage of to improve the productivity of a given crop under water-limiting conditions. Any endeavor implemented to attenuate the loss due to drought could be beneficial to intensify the food production in the country.

It has been acknowledged that crop improvement for increased yield levels can be realized very efficiently by identifying characters like escape mechanism, avoidance or tolerance water stress by plants. Therefore, the plants have acquired various morphological and physiological adaptations which help in their survival under dreadful situations like moisture stress thereby completing its life cycle. The most economic/feasible and productive way of employing crops in drought-prone areas is by screening and selection of plants with substantial water stress tolerance amalgamated with appropriate crop management practices to reduce water loss. There are several physiological approaches to sustain the productivity under water limited conditions namely water use efficiency (WUE). WUE is one such physiological trait; it is the amount of dry matter produced per unit of water transpired. Increase in WUE may be a way to increase productivity.

WUE is the amount of biomass produced per unit of water transpired (Passioura, 1986). It is associated with drought avoidance mechanisms adopted by plants under water stress conditions (Anyia and Herzog, 2004). WUE is positively associated with SCMR

(SPAD chlorophyll meter readings), but it is negatively related with specific leaf area (SLA) (Sheshashayee *et al*., 2006; Songsri *et al*., 2009). As the scope to achieve a high level of transpiration (T) under drought-prone environment, increasing WUE will offer an avenue for maintaining high yield in water stress.

Being mostly a rainy season crop, groundnut is exposed to intermittent water stress during gaps in rainfall, or terminal water stress at the end of the season when the rains are over. In these situations of water limitation, the strategy so far proposed to improve tolerance levels in groundnut for drought was realized by identifying lines with high WUE (Udayakumar *et al.*, 1998). The level of variations for WUE in many crop species was assessed by different methods. Methods adopted to quantify the variation in WUE are gas exchange studies and gravimetry, but these methods are laborious. Thus, use of simple, reliable and surrogate traits *viz*., specific leaf area (SLA), SPAD chlorophyll meter readings (SCMR) were suggested as indirect measures for drought tolerance in groundnut (Talwar *et al*., 2004), blackgram, greengram (Sudhakar *et al*., 2006) and rice (Renuka Devi *et al.*, 2013).

Several efforts through conventional breeding have been made to enhance crop productivity, but the results were not promising. However, there is increasing demand for groundnut for higher productivity whereas conventional breeding is inadequate to address the complex genetic behavior of the yield and other desired traits. Groundnut being tetraploid in nature makes it even more complex to understand the genetic behavior of various traits, polyploidy nature coupled with narrow genetic base is another bane to groundnut breeders. Because of these impediments and also due to presence of linkage drag, very meager genetic diversification activities have been conducted via interspecific hybridization between cultivated groundnut and other species of section *Arachis*. Removal of the linkage drag is a very tiresome and extensive process which may result in obtaining moderate/less than moderate level of resistance present in wild relatives of *Arachis*.

Recent advances in crop genomics offers tools to assist drought tolerance breeding (Varshney *et al.*, 2005, 2006). The identification/locating of genomic regions coupled with drought tolerance shall invariably enable the plant breeders to develop improved groundnut cultivars with higher levels of drought tolerance using marker-assisted selection (MAS) (Ribaut *et al.,* 1996). In order to implement marker-assisted selection, it is necessary to recognize those fractions of the genome related with the trait of interest, accurate phenotyping methods, a highly saturated molecular marker-based genetic linkage maps, and most importantly, identify genomic segments/quantitative trait loci linked with the desired trait of interest. Discovery of such QTLs shall invariably accelerate the process of introgression of productive traits into desired varieties, especially those governed by polygenic inheritance, such as drought.

Previous studies reported several QTL regions controlling the drought tolerance related traits. To cite, five QTLs in soybean were recognized for WUE (Mian *et al.*, 1998). In the cereal crop wheat, five QTLs for drought tolerance were identified by Dashti *et al.* (2007). However, in current years the handiness of advanced mapping populations such as RILs and fairly huge number of polymorphic molecular markers, linkage-mapping based marker analysis is gaining importance to locate the QTLs for drought tolerance related traits (Gautami *et al.*, 2011; Ravi *et al.*, 2011; Varshney *et al.*, 2009), resistance to foliar disease (Khedikar *et al.*, 2010; Sujay *et al.*, 2012) and nutritional quality traits (Sarvamangala *et al.*, 2011). Breeding for WUE traits coupled with the QTLs identified for the same can enhance the level of drought tolerance in the groundnut. In consideration to all these efforts, an investigation was structured to identify/locate QTLs for surrogate traits of water use efficiency, pod yield and yield related traits in groundnut RIL population. Therefore, the present investigation has been framed with the following objectives.

i. Identification of stable moisture stress tolerant RIL’s for productivity *per se* traits

ii. Development of SSR marker-based linkage map in the cross NRCG 12568 × NRCG 12326

iii. Validation of linked QTL’s for moisture stress tolerance and identification of novel QTL’s in the RIL’s derived from NRCG 12568 × NRCG 12326

iv. Identification of QTL’s for moisture stress tolerance stable across pre- and post-flowering moisture stress production environments

II REVIEW OF LITERATURE

Drought is a complex trait, involving several climatic, edaphic and agronomic factors namely; unpredictable rainfall, high temperature, high levels of solar radiation and poor soil characteristics prevalent in groundnut-growing tropical areas making it extremely difficult to predict intensity of drought stress. Further, plant responses to drought stress are species-specific and show significant phenological variation. While the direct selection for yield can be effective for specific adaptation but its application in larger breeding programmes catering for a wider range of environments is becoming increasingly limited and it depends on a comprehensive understanding of the nature and extent of G × E interactions for the physiological traits contributing to yield, especially under water-limited environments (Ludlow and Muchow, 1990; Sheshshayee *et al.*, 2003).

In recent years, crop physiology and genomics have led to new insights in drought tolerance providing breeders with new knowledge and tools for plant improvement (Tuberosa and Salvi, 2006). Use of molecular markers accelerates plant breeding enormously in areas including disease resistance and drought tolerance. The availability of consensus genetic maps facilitated localization of genes controlling both simple and complex traits, as well mapping QTLs of agronomic interest in many crops through marker assisted selection (Paterson *et al.,* 1991; Winter and Kahl, 1995). The present study was initiated to understand the combinations of morphological and physiological traits responsible for optimal adaptation under drought stress condition and to develop a molecular genetic map of groundnut using SSR markers in RIL population. Thus, an effort to understand the work done by earlier workers in this direction has been reviewed under the following headings:

2.1 Studies on water use efficiency

    2.1.1 SPAD chlorophyll meter reading (SCMR) and Specific leaf area (cm^2/g)

2.2 Genetic variability, Heritability and Genetic Advance

    2.2.1 Growth and yield parameters

2.2.2 Physiological traits

2.3 Identification of drought tolerant lines based on biplot and rank sum method using drought tolerant indices

2.3.1 Principal component analysis

2.3.2 Biplot analysis

2.3.3 Rank sum method

2.4 Molecular markers and genetic mapping in groundnut for various traits

2.5 QTL mapping approach

2.1 Studies on water use efficiency

Identification of physiological traits contributing to superior performance of crop plants under drought conditions has been a long-term goal of plant scientists. For drought tolerance breeding, Water use efficiency is an important parameter correlated with SPAD chlorophyll meter reading (SCMR) and specific leaf area (SLA). Hence, SCMR and SLA can be used as surrogate traits for selecting lines with high WUE. In order to improve SCMR and SLA, and in turn WUE in groundnut, a good knowledge of the genetic system controlling the expressions of these traits is essential for the selection of most appropriate and efficient breeding procedure.

Water use efficiency is one such trait that can contribute to productivity when water resources are scarce. The yield model proposed by Passioura (1986) i.e., (Grain yield = Transpiration × Water Use Efficiency × Harvest Index) reveals that water use efficiency is an important parameter influencing the biomass production. Water use efficiency has often been examined from various points of view in different context by hydrologists, agronomists, and physiologists. Physiologically, water use efficiency (WUE) is defined at either single leaf level or at whole plant level or at canopy level. Existence of genetic variability between and within species is necessary for successful exploitation of WUE through breeding programme. Variability in WUE is mainly determined by any one of the following three methods

1) At canopy level, under field conditions using crop growth and yield model. This technique is more employed in perennial tree species.
2) At whole plant (canopy) level in small pots or big containers (field mini lysimeter) by adopting gravimetric technique
3) At single leaf levels by adopting the gas exchange approach. Several studies were carried out for addressing the variation in WUE which are reviewed and listed below.

In container experiments, genetic variability among the peanut plants with respect to WUE ranged from 2.46 g dry matter/kg of water to 3.71g dry matter/kg of water (Wright *et al*., 1988). The variability in WUE was attributed to variation in total dry production than that of water use. In ten genotypes of groundnut, Nageswara Rao *et al*. (1993) observed a significant variability among genotypes for WUE that ranged between 1.38 to 2.50 g dry matter/kg of water. Hebbar *et al*. (1994) studied 14 Spanish bunch groundnut genotypes under two different moisture regimes (at field capacity and 60 *per cent* capacity) reporting a significant variability in WUE between genotypes and moisture regimes.

2.1.1 SPAD chlorophyll meter reading and Specific leaf area (cm^2/g)

The observed variations in WUE is mainly attributed to two physiological parameters namely transpiration and photosynthetic capacity. Among several factors that determine variation in WUE, the carboxylation efficiency regulated by the enzyme RuBisCo seems to be the most significant. It has been demonstrated that $\Delta^{13}C$ and RuBisCo content are related in groundnut (Nageswara Rao *et al*., 1995). RuBisCo level has a direct association with leaf nitrogen (amount of nitrogen per unit leaf mass per unit area) and photosynthetic efficiency (Nageswara Rao *et al*., 2001). Hence, it can be hypothesized that any rapid measure of specific leaf nitrogen (SLN) can also serve as surrogate trait for transpiration efficiency.

The light absorbance and/or transmittance characteristics of a leaf can be exploited to determine the leaf chlorophyll content (Balasubramanian *et al*., 2000; Takebe *et al*., 1990). The ability to maintain chlorophyll density under water deficit condition has been suggested as drought tolerance mechanism in groundnut (Arunyanark *et al.*, 2008; Sheshshayee *et al.*, 2006). The SCMR is an indicator of the photo-synthetically active light transmittance characteristic of the leaf, which is dependent on unit amount of chlorophyll per unit leaf area.

Specific leaf area (SLA) is one of the physiological traits in plant analysis which is defined as the ratio of leaf area to leaf dry weight. It is an indirect measure of leaf expansion. Higher SLA indicates higher leaf area per unit biomass and larger surface area for transpiration. At the same time, higher SLA denotes lesser leaf thickness and hence smaller photosynthesis capacity. Therefore, an inverse relationship between SLA and water use efficiency has been observed (Wright *et al.*, 1994). More convincing evidence suggests that SLA can be an alternate surrogate came with the studies linking SLA and carbon isotope discrimination. SLA also exhibited positive association with kernel yield (Jayalakshmi *et al.*, 1999); Harvest index (Arjunan *et al.*, 1997) and pod yield (Jayalakshmi *et al.*, 2000) whereas it recorded a negative correlation with nitrogen content per unit leaf area i.e. Specific leaf nitrogen (Nageswara Rao and Wright, 1994).

Wright *et al.* (1994) and Nageswara Rao & Wright (1994) reported a positive correlation between specific leaf area and $\Delta^{13}C$ in groundnut and negative relationship between SLA and transpiration efficiency (TE) suggesting that SLA can be used as a alternate to estimate genetic variability for TE among groundnut genotypes. Although a close correlation between SLA and $\Delta^{13}C$ varied with ‘r’ ranging from 0.71 to 0.94 over a range of groundnut genotypes and environments (Wright *et al.*, 1994). Therefore, it can be inferred that SLA might be influenced by factors such as time of sampling, leaf age (Hammer and Wright, 1994; Nageswara Rao *et al.*, 1995, 2001) as well as accuracy of the measurement.

The SLA values measured on 60 DAS showed a negative trend with SCMR but were not significant, whereas SLA recorded on 110 DAS showed strong significant negative relationship with SCMR in diverse germplasm lines of groundnut (Shashidhar, 2002).

Richardson *et al*. (2002) revealed that thicker leaves (low SLA) usually have higher chlorophyll per unit area and hence have a greater photosynthetic capacity compared with thinner leaves.

Talwar *et al.* (2004) showed that SLA exhibited significant genotypic variations in seven genotypes of *virginia* groundnut lines and SLA was also negatively correlated with total chlorophyll content. Another study conducted by Rekha (2005), revealed that there was a negative association between the SLA and SCMR in 12 crosses of groundnut.

A significant positive relationship between SCMR and chlorophyll content has been reported in many crop species including groundnut (Nageswara Rao *et al.*, 2001; Sheshshayee *et al.,* 2006). Further, Dwyer *et al*. (1995) demonstrated that SCMR is related to the leaf nitrogen status. Therefore, the close relationships observed among SCMR, SLA and SLN (Nageswara Rao *et al*., 2001) implied that SCMR could be used as a rapid, low cost and *insitu* technique to screen for transpiration efficiency in large breeding populations.

Sheshashayee *et al*. (2006) reported significant positive relationship between SCMR and transpiration efficiency. They also found significant inverse relationship between SCMR and $\Delta^{13}C$. They opined that SLA is an indirect measure of leaf expansion. Higher SLA indicates higher leaf area per unit biomass and hence larger surface area for transpiration.

Nigam and Aruna (2008) reported that SCMR and SLA had low G × E interaction, indicating that these traits are stable across a wide range of environments. These traits showed highly significant negative correlation between SCMR and SLA and the relationship was insensitive to crop stage and season in groundnut.

Songsri *et al.* (2008a) conducted an experiment to assess the heritability, correlations between drought resistance traits and agronomic traits. From the results it is evident that heritability for biomass, pod yield, HI, SLA and SCMR were high. Correlation between SLA and SCMR was found negatively significant, while SCMR was positively correlated related with pod yield and seed size.

Jongrungklang *et al*. (2008) studied the effect of drought on quantitative traits and observed reduced total dry matter, pod dry weight, harvest index, WUE and SLA, but increased SCMR and canopy temperature. The positive correlation of WUE with SCMR was observed. This indicates higher SCMR and lower SLA are desiable under drought conditions.

Songsri *et al*. (2009) conducted an experiment under different water regimes to assess the relationship between SLA and SCMR. Results revealed that groundnut genotypes having ability to maintain high SCMR and lower SLA under drought stress were more tolerant to drought and hence maintains higher WUE under severe drought conditions.

Boontang *et al.* (2010) in a split plot design consisting of ten groundnut genotypes under two water regimes *viz.*, field capacity and 1/3rd available water, identified the released cultivar KKU-60 as the drought tolerant genotype by using SCMR, SLW and pod yield as selection criteria.

Upadhyaya *et al.* (2011) evaluated two crosses *viz.*, ICG 72439 × ICG 9418 and ICG 67669 × Chico, and their reciprocals to determine the gene action controlling the inheritance of SCMR and SLA in groundnut. They emphasized importance of additive effects in both the crosses for SCMR at 80 DAS whereas predominance of dominance effects with duplicate epistasis for SCMR at 60 DAS and SLA at both stages in both the crosses suggesting that selection could be effective even in early generations for SCMR at 80 DAS, whereas, selection in later generations for SCMR at 60 DAS and SLA at both stages in both crosses are advisable / effective.

Thakur *et al*. (2013) evaluated 25 groundnut genotypes in a randomized complete block design in three replications and identified genotypes *viz.*, ICGV-99171, ICGV-98089, ICGV-97100, Baidehi, ICGV-00440 and B-4 for drought tolerance with high pod yield by using the traits such as SCMR, SLA, root to shoot ratio and drought tolerance score. They also suggested that these genotypes could be used as parents in breeding programmes for developing drought tolerant groundnut cultivars.

Koolachart *et al*. (2013) conducted field experiment at Khon Kaen University using split plot design during 2010-11 and 2011-12. He revealed that the groundnut genotypes with high SCMR, RWC, leaf area index and stomatal conductance, low SLA and canopy temperature and high drought tolerant index (DTI) for pods plant^{-1}, DTI for number of seeds pod^{-1} and DTI for 100-seed weight could maintain high pod yield under drought conditions. The results suggested that ability to maintain physiological traits and yield components could aid groundnut genotypes in sustaining high pod yield under stress conditions and identified the genotypes *viz.*, ICGV 98324, ICGV 98348 and Tifton 8 for water stress conditions.

Bera *et al*. (2014) noticed the variance due to genotype × environment interactions for SCMR was lower than SLA along with high heritability, indicating that SCMR was a more stable parameter than SLA in *Arachis glabrata*.

The results obtained from the study conducted by Janila *et al*. (2015) on six generations of four groundnut crosses showed significant additive effects for SCMR and SLA in all the crosses indicating possible gains through selection. Relative contribution of additive effects was high for both, SCMR (58 to 93 %) and SLA (63 to 91 %). The low heritability of SCMR and SLA indicates environmental influence on these traits.

2.2 Genetic variability, Heritability and Genetic advance as *per cent* mean

The basic key to bring about the genetic upgrading in a crop is to utilize the available or created genetic variability. Genetic variability is the basic requirement for crop improvement as this provides wider scope for selection. Thus, effectiveness of selection is dependent upon the nature, extent and magnitude of genetic variability present in material and the extent to which it is heritable. The genetic coefficient of variability gives a useful measure of the magnitude of genetic variance present in the population. The degree of success depends on the magnitude of heritability [h^2_b] as it measures the relative amount of the heritable portion of variability. Genetic advance (GA) under selection gives an idea about how much of the genetic gain obtained was due to selection. Hence, the estimates of genetic variability, heritability and genetic advance had an immense value in identifying the superior genotypes. A brief review of work done on variability, heritability and genetic advance in groundnut is presented here under.

2.2.1 Growth and yield parameters

Sharma and Varshney (1995) noticed high GCV, PCV, heritability and genetic advance for harvest index and its component traits *viz.*, pod yield per plant, branches per plant, 100-seed weight and sound mature kernel *per cent* in groundnut.

Varman and Raveendran (1996) observed high heritability for plant height, number of branches per plant, number of mature pods per plant, number of pegs per plant and 100- kernel weight. They also revealed low genetic advance for harvest index and kernel yield per plant and low heritability for shelling out turn and oil content in groundnut. They further noted that kernel weight and number of mature pods are the most important contributing traits to pod yield and oil content, respectively.

Naik *et al.* (2000) study revealed high GCV for pod weight plant^{-1}, 100-kernel weight and plant height suggesting that selection for these characters would be more effective and low GCV was observed for primary branches plant^{-1}, pods plant^{-1}, shelling percentage and sound mature kernel percentage. High heritability [$h^2{}_b$)] for 100-kernel weight and primary branches^{-1} indicated that they were least influenced by environment and also recorded medium heritability values for plant height and pod weight plant^{-1} and high GAM for pod weight plant^{-1} and 100-kernel weight.

Vijayasekhar (2002) reported high GCV, PCV, heritability and genetic advance as per cent of mean for harvest index, 100-seed weight, kernel yield per plant and shelling percentage. On contrary, low estimates of variability, heritability and genetic advance were recorded for primary branches plant^{-1}, days to maturity, plant height, pods plant^{-1}, mature pods plant^{-1} and oil content.

A field evaluation with 48 diverse large seeded groundnut genotypes was conducted by Parameshwarappa *et al.* (2005), results revealed higher genetic variability for primary branches, pod yield per plant, kernel yield and plant height and noticed considerable variability for number of pods, 100-kernel weight, seed size and protein content and low variability for oil content and sound mature kernels. High heritability coupled with high genetic advance was noticed with respect to kernel yield, sound mature kernels and 100-kernel weight indicating that the traits are controlled by additive genes for these characters and reported low genetic advance for kernel size and oil content indicating that these traits were influenced by environment.

Suneetha *et al.* (2005) reported high heritability coupled with high genetic advance as *per cent* of mean for mature pods and immature pods, while high heritability coupled with moderate GAM was observed for days to 50 *per cent* flowering, plant height, 100-seed weight and pod yield plant^{-1}. On contrary, high heritability coupled with low GAM was noticed for number of primary branches and shelling percentage.

Genetic variability, heritability and genetic advance were estimated in 163 genotypes of groundnut for pod yield and its attributes by Singh (2005). From the results, it is evident that high heritability and high percentage of GAM for pods per plant and pod yield plant^{-1} indicating the traits are under control of additive genes and improvement in yield could be brought about by selection based on phenotype.

Korat *et al.* (2009) tested 80 diverse bunch type groundnut genotypes during summer 2006 for 14 yield contributing characters and observed high PCV, GCV and heritability coupled with high genetic advance as *per cent* of mean for secondary branches plant^{-1} indicates that this trait is mainly governed by additive gene action and responsive to selection for further improvement.

Wunna *et al.* (2009) evaluated 90 $F_{4:5}$ derived lines from four groundnut crosses under field capacity and one third available water. They reported high heritability for biomass production, pod yield number of pods per plant and 100 seed weight for all crosses under both water regimes.

Raut *et al.* (2010) evaluated six crosses of F_2 population along with nine parental lines in groundnut and results revealed high estimates of GCV, PCV and genetic advance for days to flowering, number of primary branches per plant, plant height, number of mature pods per plant, number of immature pods plant^{-1}, kernel yield plant^{-1} and pod yield plant^{-1} in most of the crosses indicating that the trait is under the influence of additive gene action and scope for improvement through simple selection is apt.

Shinde *et al.* (2010) observed highly significant differences among the genotypes for all the yield contributing characters except for number of primary branches plant^{-1}. High estimates of GCV and PCV for pod yield plant^{-1}, number of immature pods plant^{-1}, number of mature pods plant^{-1} and biological yield plant^{-1} indicating the large extent of genetic variability and less role of environment in the expression of these traits. High GCV, high heritability coupled with high genetic advance for pod yield plant^{-1} and number of mature pods plant^{-1}. Hence results suggested that these characters were mainly under the influence of additive gene action indicating the ample scope for improvement in these traits through simple selection.

Pradhan and Patra (2011) evaluated 460 germplasm lines of groundnut in four different seasons for estimation of phenotypic variation, heritability and genetic advance among pod yield and yield components. Low GCV was observed for shelling percentage and moderate GCV for 100-pod weight and 100- kernel weight, whereas high heritability coupled with high genetic advance as *per cent* of mean for 100- pod weight and 100- kernel weight.

Jogloy *et al.* (2011) studied 200 lines derived from ten crosses (F_6 generation) in groundnut to evaluate heritability for pod yield and its attributes. High heritability estimates were recorded for days to maturity and harvest index while, low to moderate heritability was observed for pod yield.

Zaman *et al.* (2011) evaluated 34 genotypes during *rabi* 2009-2010 for estimation of genetic variability and genetic parameters for 11 morphological characters and observed highly significant variations among the genotypes for all the characters studied. The highest GCV was observed for kernel yield hectare^{-1} followed by kernel yield plant^{-1}, branches plant^{-1}, immature and mature nuts plant^{-1}, 100-kernel weight and plant height. The highest heritability was observed for kernel yield plant^{-1}, followed by kernel yield hectare^{-1}, 100-kernel weight, immature and mature nuts plant^{-1}, and branches plant^{-1} while, high values of genetic advance were recorded for all the characters except days to maturity and days to 50 *per cent* flowering.

A review of literature on variability for quantitative characters in groundnut indicates the existence of high degree of variability for most of characters studied. Though there were reports of high genetic variability for plant height, shelling percentage, pod yield plant^{-1}, reports contradicting the above and quoting low or medium variability for these characters were also available which are listed in Table 1. Some of the reports on genotypic and phenotypic coefficient of variation for some quantitative characters in groundnut are given in Table 2.

High heritability for plant height, pod yield plant^{-1}, shelling percentage, primary branches, kernel yield, 100-seed weight were earlier reported. However, contradicting reports stating moderate to low heritability for plant height, pod yield per plant, shelling *per cent*, were also observed by many researchers (Table 3).

High genetic advance was reported for plant height, number of primary branches, and pod yield per plant, kernel yield, sound mature kernels, and 100-kernel weight. However, for shelling per cent and 100-kernel weight contrasting reports stating medium to low heritability were available and are presented in Table 4.

2.2.2 Physiological traits

Efficient utilization of the physiological traits for improving drought resistance in a breeding program requires an understanding of the inheritance and genetic relationship of the trait that is available for selection. Series of experiment were conducted to estimate the heritability of physiological traits for drought resistance under different drought conditions (Cruickshank *et al.*, 2004; Hubick *et al.*, 1988; Ntare and Williams, 1998a, 1998b; Nigam *et al.*, 2005; Puangbut *et al.*, 2011; Songsri *et al.*, 2008a).

Table 1. Literature cited for variability studies of growth and yield parameters in groundnut

Sl. No.	Characters	Status of variability	References
1	Days to 50% flowering	High	Sumathi and Ramanathan (1995a), Uddin *et al*. (1995), John *et al*. (2008), Raut *et al*. (2010), John and Reddy (2014)
		Low	Ganesan and Sudhakar (1995), Ravi Kumar (2005), Korat *et al.* (2009), Vishnuvardhan *et al.* (2012), Satish (2014), Thirumala Rao *et al*. (2014), Ganapati Mukri *et al*. (2014), Salih *et al*. (2014)
2	Plant height (cm)	High	Habib *et al.* (1985), Alam *et al.* (1985a), Kandaswamy *et al.* (1986), Patil and Bhaskar (1987), Reddy *et al.* (1987), Kale and Dhoble (1988), Prasanthi *et al.* (1990), Mishra and Yadava (1992), Sumathi and Ramanathan (1995a), Uddin *et al.* (1995), Mishra *et al*. (2000), Naik *et al*. (2000), Suryanarayan *et al*. (2001), Nath and Alam (2002), Makan *et al*. (2003), John *et al*. (2007), Apte *et al.* (2008), John *et al.* (2008), Thaware *et al.* (2008), Raut *et al.* (2010), Shinde *et al.* (2010), Mukesh *et al*. (2014), Yadav *et al*. (2014), Vasanti *et al*. (2015), Thirumala Rao *et al*. (2014)
		Moderate	Ganesan and Sudhakar (1995), Naik *et al.* (2000), Ravi Kumar (2005), Korat *et al.* (2009), Mothilal (2004), Channayya *et al*. (2011)
3	Branches plant^{-1}	High	Uddin *et al.* (1995), Thaware *et al.* (2008), Raut *et al*. (2010)
4	Pods plant^{-1}	High	Ravi Kumar (2005), John *et al*. (2007), Nath and Alam (2002), Jatti *et al*. (2008),John *et al*. (2008), Savaliya *et al*. (2009), Sanjeevakumar *et al*. (2015), Thirumala Rao *et al*. (2014), Ganapati Mukri *et al*. (2014) Mahalakshmi *et al*. (2018)
		Moderate	Vasanthi *et al*. (2003)
		Low	Mishra *et al.* (2000), Naik *et al.* (2000)
5	Kernel yield plant^{-1} (g)	High	Wang *et al.* (1987), Lu *et al.* (1988), Mishra and Yadava (1992), Reddy and Gupta (1992), Kumar and Rajamani (2004), Kavani *et al*. (2004), Golakia *et al*. (2005), John *et al*. (2007), Jatti *et al.* (2008), John *et al*. (2008), Sumathi and Muralidharan (2009), Raut *et al.* (2010), Zaman *et al.* (2011), Narasimhulu *et al*. (2012), Azharudheen *et al.* (2010), Sanjeevakumar *et al*. (2015), Mahalakshmi *et al*. (2018)
		Moderate	Shoba *et al.* (2009), Sharma and Gupta (2011)

Sl. No.	Characters	Status of variability	References
6	Pod yield plant^{-1} (g)	High	Deshmukh *et al*. (1986), Kandaswami *et al.* (1986), Reddy *et al.* (1986), Reddy *et al.* (1987), Lu *et al.* (1988), Patil and Bhaskar (1987), Manoharan *et al.* (1990), Vaddoria and Patel (1990), Reddy and Gupta (1992), Bansal *et al.* (1992), Mishra and Yadava (1992), Pathirana (1993), Sharma and Varshney (1995), Varman and Raveendran (1996), Gowda *et al*. (1996), Bashir *et al.* (1998), Yadav *et al.* (1998), Rudraswamy *et al.* (1999), Mishra *et al*. (2000), Naik *et al*. (2000), Nath and Alam (2002),Abhay *et al.* (2002), Makan Lal *et al*. (2003), Kavani *et al*. (2004), John *et al*. (2005), Ravi Kumar (2005), Golakia *et al*. (2005), John *et al*. (2006), John *et al.* (2007), Jatti *et al*. (2008), John *et al.* (2008), Thaware *et al*. (2008), Savaliya *et al.* (2009), Sumathi and Muralidharan (2009), Khote *et al*. (2009), Raut *et al.* (2010), Narasimhulu *et al*. (2012), Mukesh *et al*. (2014), Yadav *et al*. (2014), Satish *et al*. (2014), Satyanarayan *et al*. (2014), Savaliya *et al*. (2009) Sanjeevakumar *et al*. (2015), Mahalakshmi *et al*. (2018)
		Moderate	Kale and Dhoble (1988), Uddin *et al.* (1995), Naik *et al.* (2000), Vasanthi *et al*. (2003), Mothilal (2004), Parameswarappa *et al*. (2005), Korat *et al.* (2009), Channayya *et al*. (2011), Jonah *et al*. (2012)
		Low	Badwal *et al.* (1967), Wynne and Rawling (1978)
7	Shelling percentage	High	Manoharan *et al*. (1990), Varman and Paramashivam (1992), Reddy and Gupta (1992), Bhat (1995), Uddin *et al*. (1995), Singh and Singh (1998), Rudraswamy *et al.* (1999), Singh *et al.* (1996), Mather and Manivel (2000), Abhay *et al.* (2002), Yadav *et al*. (2014), Mahalakshmi *et al*. (2018)
		Moderate	Reddy (1994), John *et al*. (2008), Vishnuvardhan *et al*. (2013)
		Low	Reddy *et al*. (1986), Nadaf and Habib (1987), Sumathi and Ramanathan (1995a), Gowda *et al.* (1996), Sarala and Gowda (1998), Mishra *et al*. (2000), Naik *et al.* (2000), Yogendraprasad *et al.* (2002), Kumar and Rajamani (2004), Ravi Kumar (2005), Narasimhulu *et al*. (2012), Jonah *et al.* (2012), Parameswarappa *et al*. (2005), Satyanarayan *et al*. (2014), Vishnuvardhan *et al*. (2013), Channayya *et al*. (2011), Thirumala Rao *et al*. (2014), Ganapati Mukri *et al*. (2014)

Table 2. Reports on genotypic coefficient of variation and phenotypic coefficient of variation for quantitative traits in groundnut

Sl. No.	Characters	GCV	PCV	References
1	Days to 50% flowering	High	High	Raut *et al*. (2010)
		Medium	Medium	Sunday and Omolayo (2013)
		Low	Low	Venkateshmurthy (2005), Channayya (2009), Korat *et al*. (2009), John *et al*. (2011), Zaman *et al*. (2011), Rao *et al*. (2012), Vishnuvardhan *et al*. (2012)
		Low	High	Thakur *et al*. (2011)
		High	-	Suryanarayana Reddy *et al.* (2001), Thaware *et al.* (2008),
		High	High	Kandswamy *et al.* (1986), Makhan *et al.* (2003), Venkateshmurthy (2005), John *et al*. (2007), Apte *et al*. (2008), Korat *et al*. (2009), Raut *et al*. (2010), Sudha *et al*. (2012), Sunday and Omolayo (2013)
		Medium	Medium	Venkateshmurthy (2005), John *et al.* (2006), Kavera (2008), Channayya (2009), Zaman *et al.* (2011), Vishnuvardhan *et al*. (2012), Rao *et al*. (2012),
		Low	Medium	Kavera (2008), Sarvamangala (2009), John *et al.* (2011)
		Low	High	Thakur *et al.* (2011)
		-	High	Nath and Alam (2002)
2	Primary branches plant^{-1}	High	High	Raut *et al*. (2010), Sudha *et al.* (2012)
		Medium	Medium	Channayya (2009), Korat *et al.* (2009), Vishnuvardhan *et al.* (2012)
		Low	Medium	John *et al.* (2011)
		-	Low	Rudraswamy *et al.* (1999)
		High	-	Venkataramana *et al.* (2001)
		High	High	John *et al.* (2007), Kavera (2008), Raut *et al.* (2010), Rao *et al.* (2012)

Sl. No.	**Characters**	**GCV**	**PCV**	**References**
4	Kernel yield plant^{-1} (g)	Low	Low	Apte *et al.* (2008)
		Low	Medium	John *et al.* (2011)
		Medium	Medium	Jonah *et al.* (2012)
		High	Medium	Venkateshmurthy (2005)
		High	-	Chuahan and Shukla (1985)
		High	High	Kandswamy *et al.* (1986), Sharma and Varshney (1995), Makhan *et al.* (2003), Venkateshmurthy (2005), John *et al* (2006), John *et al.* (2007), Kavera (2008), Raut *et al.* (2010), Zaman *et al.* (2011), Jonah *et al.* (2012), Sudha *et al.* (2012), Rao *et al*. (2012), Sunday and Omolayo (2013)
5	Pod yield plant^{-1} (g)	-	High	Kale and Dhoble (1988), Nath and Alam (2002)
		-	Low	Rudraswamy *et al.* (1999)
		Low	Medium	John *et al*. (2011)
		Medium	High	Venkateshmurthy (2005), Kavera (2008), Sarvamangala (2009), Azharudheen (2010), Vishnuvardhan *et al.* (2012)
		Medium	Medium	Korat *et al.* (2009)
		Medium	Low	Azharudheen (2010)
		-	Low	Rudraswamy *et al.* (1999)
		Low	Medium	Vishnuvardhan *et al.* (2012)
6	Shelling percentage	High	High	Kandswamy *et al*. (1986)
		Medium	Medium	Zaman *et al.* (2011)
		Low	Low	Venkateshmurthy (2005), Kavera (2008), Channayya (2009), Sarvamangala (2009), Korat *et al*. (2009), Azharudheen (2010), John *et al*. (2011), Nageshwar Rao *et al.* (2012)

Table 3. Literature cited for quantitative traits in groundnut on degree of heritability

Sl. No.	Characters	Degree of heritability	References
1	Days to 50% flowering	High	Suneetha *et al*. (2004), Venkateshmurthy (2005), Korat *et al*. (2009), John *et al*. (2011), Vishnuvardhan *et al*. (2012), Rao *et al*. (2012), Sunday and Omolayo (2013), Patil A. S., *et al*. (2014), John and Reddy (2014), and Satish (2014), Upadhyaya *et al*. (2014), Sukruth *et al*. (2014), Sanjeevakumar *et al*. (2015), Mahalakshmi *et al*. (2018)
		Moderate	Vaddoria and Patel (1990), Thakur *et al*. (2011), Zaman *et al*. (2011), Thirumala Rao *et al*. (2014), Ganapati Mukri *et al*. (2014)
		Low	Venkateshmurthy (2005), Channayya (2009), Alam *et al*. (2013)
2	Plant height (cm)	High	Ganesan and Sudhakar (1995), Uddin *et al*. (1995), Varman and Raveendran (1996), Nath and Alam (2002), Makhan *et al*. (2003), Suneetha *et al*. (2004), Ravi Kumar (2005), Venkateshmurthy (2005), John *et al* (2006), John *et al*. (2007), Apte *et al*. (2008), John *et al*. (2008), Jatti *et al*. (2008), Thaware *et al*. (2008), Kavera (2008), Channayya (2009), Channayya *et al*. (2011), Zaman *et al*. (2011), John *et al*. (2012), Sudha *et al*. (2012), Rao *et al*. (2012), Sunday and Omolayo (2013), Thirumala Rao *et al*. (2014), Patil A. S., *et al*. (2014), Yadav *et al*. (2014), Vasanti *et al*. (2015), Sanjeevakumar *et al*. (2015),
		Moderate	Alam *et al*. (1985a), Prasanthi *et al*. (1990), Sumathi and Ramanathan (1995a), Mishra *et al*. (2000), Naik *et al*. (2000), Suryanarayan *et al*. (2001), Thakur *et al*. (2011), Vishnuvardhan *et al*. (2012), Ganapati Mukri *et al*. (2014),
		Low	Sarvamangala (2009), John *et al*. (2011)
3	Primary branches plant^{-1}	High	Dixit *et al*. (1970), Raman and Sreerangaswamy (1970), Kandaswamy *et al*. (1986), Kuriakose and Joseph (1986), Ganeshan and Sudhakar (1995), Channayya (2009), Korat *et al*. (2009), Sudha *et al*. (2012)
		Moderate	John *et al*. (2011), Vishnuvardhan *et al*. (2012)
4	Pods plant^{-1}	High	Nath and Alam (2002), Suneetha *et al*. (2004), Ravi Kumar (2005), John *et al*. (2007), John *et al*. (2008) and Savaliya (2009), Sanjeevakumar *et al*. (2015), Mahalakshmi *et al*. (2018)
		Moderate	Vasanthi *et al*. (2003), John *et al*. (2011), Thirumala Rao *et al*. (2014)

Sl. No.	**Characters**	**Degree of heritability**	**References**
5	Kernel yield plant^{-1} (g)	High	Bansal *et al*. (1992), Kumar and Rajamani (2004), Ravi Kumar (2005), Golakia *et al*. (2005), John *et al*. (2007), John *et al*. (2008), Kavera (2008), Sumathi and Muralidharan (2009), Zaman *et al*. (2011), Rao *et al*. (2012), John *et al*. (2012), Mahalakshmi *et al*. (2018)
		Low	John *et al*. (2011)
6	Pod yield plant^{-1} (g)	High	Reddy (1986), Bansal *et al*. (1992), Mishra and Yadava (1992), Yadava (1992), Reddy and Gupta (1992), Sharma and Varshney (1995), Varman and Raveendran (1996), Singh and Singh (1998), Prakash *et al*. (2000), Venkataraman *et al*. (2001), Yogendraprasad *et al*. (2002), Nath and Alam (2002), Suneetha *et al*. (2004), Venkateshmurthy (2005), Parameswarappa *et al*. (2005), John *et al*. (2005), Golakia *et al*. (2005), John *et al* (2006), John *et al*. (2007), Apte *et al*. (2008), John *et al*. (2008), Jatti *et al*. (2008), Korat *et al*. (2009), Sumathi and Muralidharan (2009), Savaliya *et al*. (2009), Raut *et al*. (2010), Zaman *et al*. (2011), Rao *et al*. (2012), Jonah *et al*. (2012), Sudha *et al*. (2012), Sunday and Omolayo (2013), Alam *et al*. (2013), Yadav *et al*. (2014), Satish *et al*. (2014), Satyanarayan *et al*. (2014), Sanjeevakumar *et al*. (2015), Mahalakshmi *et al*. (2018)
		Moderate	Basu *et al*. (1986a), Sumathi and Ramanathan (1995a**),** Singh (1998), Mishra *et al*. (2000), Naik *et al*. (2000), Vasanthi *et al*. (2003), Kavera (2008), Vishnuvardhan *et al*. (2012) Alam *et al*. (2013)
		Low	Wang *et al*. (1987), Manoharan *et al*. (1990), Reddy and Gupta (1992), Bhat (1995), Ntrae and Waliyar (1999), Upadhyaya *et al*. (2005), Painawadee *et al*. (2009), Sarvamangala (2009), Azharudheen (2010), John *et al*. (2011)
7	Shelling percentage	High	Sandhu and Khehra (1977), Uddin *et al*. (1995), Vasanthi *et al*. (1998b), Varman and Raveendram (1996), Singh *et al*. (1998), Ntare and Waliyar (1999), Yogendraprasad *et al*. (2002), Nath and Alam (2002), Suneetha *et al*. (2004), Ravi Kumar (2005), Venkateshmurthy (2005), Parameswarappa *et al*. (2005), Upadhyaya *et al*. (2005), John *et al*. (2007), Kavera (2008), Sumathi and Muralidharan (2009), Savaliya *et al*. (2009), Korat *et al*. (2009), Channayya *et al*. (2011), Zaman *et al*. (2011), Jonah *et al*. (2012) Satyanarayan *et al*. (2014), Yadav *et al*. (2014), Thirumala Rao *et al*. (2014), Sanjeevakumar *et al*. (2015), Mahalakshmi *et al*. (2018)
		Moderate	Venkateshmurthy (2005), Thakur *et al*. (2011), Rao *et al*. (2012), Vishnuvardhan *et al*. (2012), Vishnuvardhan *et al*. (2013), Ganapati Mukri *et al*. (2014)
		Low	Swamy Rao *et al*. (1988), Manoharan *et al*. (1990), Varman and Raveendran (1996), Sarvamangala (2009), Azharudheen (2010), John *et al*. (2011), Mukesh *et al*. (2014)

Table 4. Reviews on genetic advance for growth and yield traits in groundnut

Sl. No.	Characters	Degree of genetic advance	References
1	Days to 50% flowering	High	Korat *et al.* (2009), Raut *et al.* (2010), Zaman *et al.* (2011), Vishnuvardhan *et al.* (2012)
		Moderate	Suneetha *et al*. (2004), Ravi Kumar (2005), John *et al.* (2011)
		Low	Monoharan *et al*. (1990), Vaddoria and Patel (1990, Venkateshmurthy (2005), Channayya (2009), John *et al*. (2011), Thakur *et al*. (2011), Zaman *et al*. (2011), Vishnuvardhan *et al*. (2012), Rao *et al*. (2012), Salih *et al*. (2014), Vishnuvardhan *et al*. (2013), John and Reddy (2014), John *et al*. (2014), Satish (2014), Thirumala Rao *et al*. (2014), Ganapati Mukri *et al*. (2014)
2	Plant height (cm)	High	Deshmukh *et al.* (1986), Kandaswamy *et al.* (1986), Reddy *et al.* (1987), Kale and Dhoble (1988), Manoharan *et al.* (1990), Uddin *et al.* (1995), Varman and Raveendran (1996), Suryanarayana Reddy *et al.* (2001), Nath and Alam (2002), Apte *et al.* (2008), John *et al.* (2008), Jatti *et al*. (2008), Thaware *et al.* (2008), Raut *et al.* (2010), Rao *et al.* (2012), Yusuf *et al*. (2017)
		Moderate	Suneetha *et al*. (2004), Ganapati Mukri *et al*. (2014), Mahalakshmi *et al*. (2018)
		Low	Venkateshmurthy (2005), Channayya (2009), John *et al*. (2011), Thakur *et al.* (2011), Vishnuvardhan *et al.* (2012)
3	Primary branches plant^{-1}	High	Kuriakose and Joseph (1986), Ganeshan and Sudhakar (1995), Uddin *et al.* (1995), Raut *et al.* (2010)
		Low	Channayya (2009), John *et al.* (2011), Vishnuvardhan *et al.* (2012)
4	Pods plant^{-1}	High	Yusuf *et al*. (2017)
		Moderate	Vasanthi *et al*. (2003), Balaraju and Kenchangoudar (2016)
5	Kernel yield plant^{-1} (g)	High	Reddy *et al.* (1987), Kale and Dhoble (1988), Sumathi and Ramanathan (1995a), Kumar and Rajamani (2004), Ravi Kumar (2005), John *et al*. (2007), John *et al.* (2008), Raut *et al.* (2010), Rao *et al.* (2012) Zaman *et al*. (2011), Balaraju and Kenchangoudar (2016), Mahalakshmi *et al.* (2018)
		Moderate	Sumathi and Muralidharan (2009), Bhargavi *et al*. (2017), Jonah *et al.* (2012)
		Low	Varman and Raveendran (1996), Venkateshmurthy (2005), John *et al.* (2011)

Sl. No.	**Characters**	**Degree of genetic advance**	**References**
6	Pod yield plant^{-1} (g)	High	Chauhan and Shukla (1985), Alam *et al.* (1985a), Deshmukh *et al.* (1986), Kandaswami *et al.* (1986), Reddy *et al.* (1987), Kale and Dhoble (1988), Vaddoria and Patel (1990), Manoharan *et al.* (1990), Mishra and Yadava (1992) Reddy and Gupta (1992), Yadava (1992), Sharma and Varshney (1995), Ganesan and Sudhakar (1995), Sarala and Gowda (1998), Bashir *et al.* (1998), Prakash (2000), Mishra *et al*. (2000), Naik *et al*. (2000), Venkataraman *et al*. (2001), Nath and Alam (2002), John *et al*. (2005), Apte *et al.* (2008), John *et al*. (2007), Jatti *et al.* (2008), John *et al.* (2008), Savaliya *et al.* (2009), Sumathi and Muralidharan (2009), Raut *et al.* (2010), John *et al.* (2011), Zaman *et al*. (2011), Channayya *et al*. (2011), Rao *et al.* (2012) Jonah *et al*. (2012), Alam *et al*. (2013), Yadav *et al*. (2014), Satish *et al*. (2014) Sanjeevakumar *et al*. (2015), Mahalakshmi *et al*. (2018)
		Moderate	Vasanthi *et al*. (2003), Suneetha *et al*. (2004), Venkateshmurthy (2005), Parameswarappa *et al*. (2005), Balaraju and Kenchangoudar (2016)
		Low	Parameswarappa *et al*. (2005), John *et al* (2006), Sarvamangala *et al*., (2010), Vishnuvardhan *et al.* (2012), Satyanarayan *et al*. (2014)
7	Shelling percentage	High	Reddy *et al.* (1986), Reddy and Gupta (1992), Nath and Alam (2002), John *et al.* (2007), Savaliya *et al.* (2009), Sumathi and Muralidharan (2009), Channayya *et al*. (2011), Zaman *et al*. (2011), Yadav *et al*. (2014), Bhargavi *et al*. (2017) Mahalakshmi *et al*. (2018)
		Moderate	Reddy *et al.* (1987), Uddin *et al.* (1995), Ravi Kumar (2005), Thirumala Rao *et al*. (2014), Balaraju and Kenchangoudar (2016)
		Low	Badwal *et al.* (1967), Nadaf and Habib (1987), Alam *et al.* (1985a), Swamy Rao *et al.* (1988) Prashanthi *et al.* (1990), Manoharan *et al.* (1990), Vaddoria and Patel (1990), Sumathi and Ramanathan (1995a), Uddin *et al.* (1995), Varman and Raveendran (1996), Rudraswamy *et al.* (1999), Yogendraprasad *et al.* (2002), Suneetha *et al*. (2004), Parameswarappa *et al*. (2005), Venkateshmurthy (2005), Channayya (2009), Thakur *et al.* (2011), Jonah *et al*. (2012), Vishnuvardhan *et al*. (2012), Rao *et al.* (2012), Vishnuvardhan *et al*. (2013), Satyanarayan *et al.* (2014), Ganapati Mukri *et al*. (2014), Mahalakshmi *et al*. (2018)

Hubick *et al*. (1988) reported high heritability estimates for transpiration efficiency (TE) and especially for carbon isotope discrimination (D) and there was no significant G × E interaction for D. There are numerous reports on groundnut response to drought but most studies have been limited to small number of groundnut genotypes (e.g. Vorasoot *et al*., 2003). Other studies have used larger number of germplasm (e.g. thirty six, sixty, and one hundred twenty in Ndunguru *et al*., 1995; Jongrungklang *et al*., 2008 and Painawadee *et al*., 2009, respectively) revealed a significant genotypic variation for drought tolerance.

Nautiyal *et al*. (2002) reported significant variation in DTI in the field experiments conducted in three soil moisture regimes of adequate irrigation (W1), drought simulated under rain-out-shelter (W2) and rain-fed (W3) conditions and reported low heritability for SLA. Surihan *et al*. (2005) reported predominance of additive gene effects in determining the expression of SLA and HI in the crosses *viz.*, ICGV 86388 × IC 10, ICGV 86388 × KK 60-1 and IC 10 × KK 60-1 accounting for 80-95% of total genetic variability for SLA and 63-73% for HI.

Songsri *et al*. (2008a) found physiological traits for drought tolerance in groundnut with high heritability ($h^2 > 0.50$) under drought and well-watered conditions. Painawadee *et al*. (2009) conducted pot experiments for 128 F_3 progenies derived from the cross ICGV 98324 × KK - 4 in randomized complete block design with four replications and observed low to intermediate heritability (bs) ranging from 0.27 to 0.59 for root and drought resistance traits viz., SLA and SCMR.

Nandini *et al.* (2011) reported a moderate GCV and heritability along with high GAM for SLA, while low GCV, medium heritability with medium GAM for SPAD chlorophyll meter reading in groundnut.

Puangbut *et al*. (2011) reported high heritability estimates for SLA and SCMR under early season drought and recovery conditions, indicating that the selection for these traits could be achieved and also indicated their importance in contribution towards the pod yield.

Girdthai *et al*. (2012) evaluated 140 groundnut lines of $F_{4:6}$ and $F_{4:7}$ generations developed from four crosses (ICGV 98348 × Tainan 9, ICGV 98348 × KK60-3, ICGV 98353 × Tainan 9, and ICGV 98353 × KK60-3) under well-watered and terminal drought conditions. Reported higher heritability estimates for physiological traits compared to agronomic traits. Heritability for HI, SCMR and SLA ranged from 0.55 to 0.85, 0.72 to 0.91, and 0.61 to 0.90, respectively among the crosses. A remarkable level of variability was revealed by Thakur *et al*. (2013) for drought tolerance among the groundnut varieties, which is very essential for improving the performance under rainfed conditions.

2.3 Identification of drought tolerant lines based on biplot and rank sum method using drought tolerant indices

Selection / identification of drought tolerant genotypes is not easy, due to strong interaction between genotypes and environment as role of tolerance mechanisms is less known. Yield loss is the main concern of plant breeders, hence they emphasize on yield performance under stress conditions. Researchers adopted different methods to evaluate genetic parameters for drought tolerance. The drought indices provides a measure of drought based on yield losses under drought-conditions been used for screening drought tolerant genotypes (Mitra, 2001).

According to Fernandez (1992) the best criteria for selection in drought condition is to distinguish the genotypes having desirable and similar yield performance in stress and non-tress condition along with their co-linearity for yield in both conditions. On the other hand, drought resistance is defined by Hall (1993) as the relative yield of a genotype compared to other genotypes subjected to the same drought stress.

Several selection criteria have been proposed to select desirable genotypes based on their yield performance under stress and non-stress environments. Stress Tolerance Index (STI) was defined as a useful tool for determining high yield and stress tolerance potential of genotypes (Fernandez, 1992). Rosielle and Hamblin (1981) demonstrated that lower stress tolerance index (STI), i.e. yield in normal irrigation and drought condition were close to each other or plant is resistant to drought.

To improve the efficiency of STI a modified stress tolerance index (MSTI) was suggested by Farshadfar and Sutka (2002) which corrects the STI as a weight. It was calculated as K_1STI, where K_1 is a correction coefficient, which corrects the STI as a weight. Therefore, K_1STI and K_2STI are the optimal selection indices for non-stress and stressed conditions, respectively.

Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the differences in yield between stress and irrigated environments and mean productivity (MP) as the average yield of genotypes under stress and non-stress conditions. The genotypes with high amount of TOL shows susceptibility to stress whereas with high value of MP will be more desirable. The geometric mean productivity (GMP) is often used by breeders is based in relative performance, since drought stress can vary in severity in field environments over years (Fernandez, 1992), the genotypes with high value of this index will be more desirable. The harmonic mean (HM) was introduced by Kristin *et al*., (1997) and the genotypes with high HM value will be more desirable under drought condition.

The yield index (YI; suggested by Gavuzzi *et al.*, 1997) and yield stability index (YSI; suggested by Bouslama and Schapaugh (1984) are often used to evaluate the stability of genotypes under stress and non-stress conditions. The genotypes with high values can be regarded as stable genotypes under non-stress and stressed conditions. Blum (1988) defined new indices of drought resistance index (DI), which is commonly accepted to identify genotypes producing high yielding genotypes under both stress and non-stress conditions. Fischer *et al*., (1998) suggested that relative drought index (RDI) is positive indices for stress tolerance.

Fischer and Maurer (1978) suggested stress susceptibility index (SSI) for measurement of yield stability that apprehended the changes in both potential and actual yields in variable environments. SSI more or less than 1 indicate above and below-average susceptibility to drought stress, respectively. Moosavi *et al*. (2008) introduced abiotic tolerance index (ATI), stress susceptibility percentage index (SSPI) and stress non-stress production index (SNPI) for screening drought tolerant genotypes under stress and non-stress conditions. The genotypes with high these values of indices will be tolerant to drought stress.

2.3.1 Principal Component Analysis (PCA)

In plant breeding one of the important approaches is hybridization followed by selection. Selection of desirable parents is essential to enhance the genetic recombination for potential yield increase. In order to characterize and assess the diversity in germplasm, principal component analysis (PCA) is one of the best statistical tools. The PCA is a multivariate statistical technique to simplify and analyze the inter-relationship among a large set of variables into a relatively a small set of variables or components without losing any essential information of original data set.

Principal component analysis (PCA) involves a mathematical procedure that transforms a number of correlated variables into a (smaller) number of uncorrelated variables called *principal components* (Chatfield and Collis 1980). The first principal component accounts for as much of the variability presented in the data and each succeeding component accounts for as much of remaining variability as possible. The objectives of PCA are to discover or to reduce dimensionality of data set and to identify meaningful underlying variables (Jolliffe, 2002).

2.3.2 Biplot analysis

It is a better approach than a simple correlation analysis and is necessary to identify superior genotypes for both non-stress and stress conditions, because genotypes in biplot analysis are compared simultaneously for all the attributes. Biplot uses the first two main principal component analyses (PCA 1 and PCA 2) contributing to high level of *per cent* to display the relationship among different indices. Hence, selection of genotypes with high PCA 1 and low PCA 2 are suitable for both stress and non-stress environments (Amiri *et al*., 2014). Fernandez (1992) classified DTI's and genotypes according to their performance under non-stress and stress conditions; genotypes with high performance under both conditions (Group A), genotypes with high performance only under non-stress conditions (Group B), genotypes with high performance only under stressed condition (Group C) and genotypes with low performance under both conditions (Group D). Biplot analysis is the graphical representation of the results of principal component analysis.

2.3.3 Rank sum method

Ranks were assigned to genotypes for each drought tolerant index. Then the rank mean, standard deviation of rank and rank sum (RS) of each genotype values of all DTIs of each genotype were calculated. The RS is the total of rank mean and standard deviation of rank. A genotype with the highest value for each of the criteria such as Ys, Yp, STI, GMP, MP, HM, YSI and YI were assigned with rank of 1, while for genotypes with the lowest value for each of the indices namely SSI, SDI and TOL received a rank of 1 (Farshadfar *et al.,* 2012). The best tolerant genotypes were identified as having low rank mean, SDR and least in RS (Farshadfar *et al.*, 2012).

Ten wheat genotypes were studied for drought tolerant using five DTIs i.e. GMP, MP, STI, SSI and TOL. The correlation and principal component analysis indicated that the most suitable criteria for the identification of genotypes under irrigated and rainfed conditions were GMP, MP and STI indices. Three dimensional plots and biplots showed that TV2 was the high yielding drought tolerant genotype (Drikvand *et al*., 2012).

Zade *et al*. (2012) evaluated drought tolerance in castor bean genotypes based on their yield performance in stress and non-stress environments along with DTIs such as MP, TOL, GMP, HM, SSI and STI. The indices STI, HM, MP and GMP exhibited significant positive correlation with the yield performance in both conditions. The genotype 80-12-1 was selected as the drought tolerant genotype having high STI, HM, GMP and MP values, along with its positioning in distinct cluster.

Metwali *et al*. (2015) calculated seven selection indices (SSI, STI, TOL, MP, GMP, YSI and YI) of fifteen tomato cultivars for shoot fresh weight, Y_{pi} and Y_{si}, under normal and stressed conditions, respectively. The results indicated that GMP, MP, TOL and STI represented suitable indices for screening the drought tolerance of tomato cultivars. The cultivars C9, C5, C15 and C11 ranked among those with the highest STI and GMP, indicated their drought tolerance.

Brdar-Jokanović *et al*. (2017) evaluated 41 tomato (*L. esculentum*) populations by adopting 16 drought stress selection indices on the basis of tomato shoot and root dry weight yield determined at water stress and non-stress conditions. Increased root proportion in plant dry weight and number of leaves below the first flower branches were unchanged under drought stress. The results indicated that populations designated with numbers 126, 124, 131, 125, 128, 105, 101, 138, 110, 132 and 109 exhibited satisfactory level of drought tolerance at vegetative phase.

Anwar *et al*. (2011) evaluated 19 exotic genotypes for drought tolerance by estimating ten DTIs. The results indicated that the grain yield had significant positive correlation with MP, HMP, GMP, STI and K_1STI under irrigated and stressed condition. So they were the better predictor of potential yield in Yp and Ys than TOL, SSI and YSI.

Mohammadi *et al*. (2012) identified resistant wheat genotypes based on MP, HAM (Harmonic mean), GM, STI, TOL, SSI, RDI, YSI and grain yield under end-season drought and normal conditions. The results revealed a positive and significant correlation of grain yield with HAM, GMP, MP, STI and YI indices were more efficient DTIs in identifying drought tolerant lines and C-81-10 genotype identified as the most tolerant genotype to end-season drought stress based on MP, GMP, STI and HAM.

Zaheri and Bahraminejad (2012) assessed drought tolerance of oat genotypes and varieties using six DTIs namely; STI, GMP, MP, TOL, SSI and HAM calculated based on grain yield in rainfed and irrigated environments. The results indicated that MP, GMP, HAM and STI which are the most suitable indices to screen genotypes by their highly positive and significant correlation with Ys and Yp as drought tolerant genotypes.

Nouraein *et al*. (2013) evaluated drought tolerant RIL population in wheat using seven DTIs. The results based on biplot analysis exhibited in two components obtained from PCA of all DTIs indicated the most appropriate indices to identify stress tolerant genotypes were GMP, MP, and STI.

Bahrami *et al*. (2014) evaluated six DTIs namely SSI, YSI, TOL, MP, GMP, and STI based on seed yield under drought non-stress and stressed conditions to screen drought tolerance among 64 safflower genotypes. Results of calculated correlation coefficients, biplot and three-dimensional biplots based on PCA showed that GMP and

STI indices were able to discriminate between drought-sensitive and tolerant safflower genotypes for both stress and non-stress field conditions.

Peksen *et al*. (2014) studied drought cowpea cultivars using leaf and stomata characteristics, and drought tolerance indices such as TOL, MP, GMP, SSI, SI, STI, HAM, YI and YSI for each cowpea cultivars based on seed yield under stress and non-stress environment. Based on drought tolerance indices, Karagoz-86 was found more tolerant to drought stress than Akkiz-86 and it could be recommended for rainfed condition.

Sabaghnia and Janmohammadi (2014) evaluated chickpea genotypes for drought tolerance using twelve DTIs, superiority index, TOL, MP, GMP, HMP, STI, YI, YSI, SSI, K_1STI, K_2STI and relative reduction, computed from potential yield and stress yield in rainfed and irrigated conditions, respectively. The results revealed that FLIP 98-106C and Azad which had high yield and low rank of DTIs are better genotype for commercial cultivation.

Abraha *et al.* (2015) identified sorghum tolerant landraces using seven tolerance indices including STI, MP, GMP, SSI, TOL, YI and YSI based on grain yield under drought stress and irrigated conditions. Significant correlations between both yield with GMP, MP, STI and YI were recorded. Based on ranking, cluster and biplot analysis, accessions EG 885, EG 469, EG 481, EG 849, Hamelmalo, EG 836 and EG 711 were identified as superior genotypes for post-flowering drought tolerance.

2.4 Development of genetic linkage maps in groundnut

A linkage map may be thought as a ‘road map’ of the chromosomes derived from two different parents. Linkage map is a pictorial representation of the position and relative genetic distances between markers along chromosomes. The most important use of linkage maps is to identify chromosomal locations containing genes and QTLs associated with traits of interest. Construction of genetic linkage map is necessary to apply marker assisted selection tool in crop improvement programme. Although efforts were initiated towards developing genetic maps as early as 1993, genome mapping in groundnut has significantly increased since 2005. Initial genetic maps were developed based on mapping populations derived from the diverse parental genotypes. However, it is difficult to obtain useful linkage maps of cultivated groundnut as it presents extremely low levels of polymorphism due to single event hybridization followed by polyploidization. But recently explosion of robust molecular marker methods revealed significant amount of polymorphism in the crop (Table 5).

Halward *et al*. (1993) is a pioneer to construct the RFLP based linkage map in groundnut aimed to improving the cultivated species (*Arachis hypogaea*). 132 markers were screened for the segregation in population and 117 markers were mapped on 11 linkage groups. A total map distance of 1400 cM was covered with a 20 cM resolution. This map covers 80 *per cent* of the groundnut genome.

Burow *et al*. (2001) constructed the first molecular map representing entire tetraploid genome of groundnut. 370 RFLP loci were mapped on to 23 linkage groups spanning 2210 cM which was slightly greater than twice the length of (1063 cM) the diploid map (Garcia *et al*., 1995).

Milla (2003) constructed a genetic linkage map for an F_2 population of *A. kuhlmannii* × *A. digoi*. The map consisted of 102 AFLP markers grouped in to 12 linkage groups and spanning 1068.1 cM. Herselman *et al*. (2004) studied twenty putative markers were identified of which 12 mapped to five linkage groups covering a map distance of 139.4cM. A single recessive gene was mapped on linkage group1, 3.9cM from a marker originating from the susceptible parent that explained 76.1 *per cent* of the phenotype variation for aphid resistance. This study represents the first partial AFLP based genetic linkage map for cultivated peanut and this is the first report on identification of molecular markers linked to Aphid resistance to groundnut rosette disease (GRD).

Garcia *et al.* (2005) used a backcross population of *Arachis stenosperma* × (*Arachis stenosperma* × *A. cardenasii*) and 39 shared RFLPs and 167 RAPD loci to locate on the RFLP map. The RAPDs covered a total genetic length of 800 cm and mapped on to 11 linkage groups.

Moretzsohn *et al*. (2005) constructed a linkage map based on microsatellites using an F_2 population obtained from a cross between two diploid wild species with AA genome (*Arachis durocnesis* and *A. stenosperma*). The resulting linkage map consists of 11 linkage groups covering 1,230.89 cm of total map distance with an average distance of 7.24 cM between markers.

Gobbi *et al*. (2006) constructed B genome map of groundnut by using 94 SSR markers in F_2 population of 93 individuals obtained from the cross between *Arachis ipaensis* (KG 30076) and *Arachis magna* (KG 30097) both diploid species with B genome for map construction. Eleven linkage groups were obtained from 94 polymorphic micro-satellite markers covering a total distance of 754.8 cM. The size of linkage groups ranged from 5.6 to 130 cM.

Khedikar (2008) constructed a molecular genetic linkage map of cultivated groundnut in a mapping population consisting of 268 recombinant inbred lines obtained from a cross TAG-24 × GPBD-4 using 67 microsatellite markers. A total of 59 markers mapped on 13 linkage groups spanning 909.4 cM with an average marker interval of 15.25 cM.

To detect polymorphisms between the two parents Yueyou 13 and Zhenzhuhei, 141 SSR primer pairs, 127 genomic-SSR and 14 EST-SSR were used. A linkage genetic map was constructed with 131 SSR loci in 20 linkage groups, with a coverage of 679 cM and an average of 6.12 cM of inter-maker distance was constructed **(**Hong *et al*., 2008).

Cuc *et al*. (2008) studied thirty primers were SSRs while 16 primers were imperfect SSRs out of 46 primers which have shown polymorphism (44.2 %) when 104 primers were screened on 32 genotypes of the cultivated groundnut.

Out of 29 SSRs isolated, primer pairs were designed for 23 SSR loci, of which 14 (61 %) primer pairs yielded scorable amplicons. Eight (57 %) primer pairs were showed polymorphism among 23 groundnut genotypes (Gautami *et al*., 2009).

Table 5. Details of genetic maps constructed in *Arachis* species

Population	Population size	Marker loci mapped	References
A-genome genetic maps			
A. stenosperma × *A. cardenassi*	F_2	117 RFLPs	Halward *et al*., 1993
A. kuhlmanni × *A. diogoi*	179 F_2	102 AFLPs	Milla, 2003
A. stenosperma × (*A. stenosperma* × *A. cardenassi)*	44 BC_1F_1	167 RAPDs	Garcia *et al*., 2005
A. duranensis × *A. stenosperma*	F_2	170 SSRs	Moretzsohn *et al*., 2005
A. duranensis × *A. stenosperma*	93 F_2	369 markers (SSR, AFLP, SNP, RFLP, SCAR)	Leal-Bertioli *et al*., 2009
A duranensis × *A. duranensis*	94 F_2	2,319 markers (1,127 SNPs, 971 SSRs, 221 SSCPs)	Nagy *et al*., 2010
B-genome genetic maps			
A. ipäensis × *A. magna*	93F_2	149 SSRs	Gobbi *et al*., 2006 and Moretzsohn *et al*., 2009
A. batizocoi PI 298639 × *A. batizocoi* PI 468327	94 F_2	449 SSRs	Guo *et al*., 2009a
AB genome genetic maps			
A. hypogaea × *A. cardenasii*	46 F_{10}	RAPDs	Garcia *et al*., 1995
A. hypogaea × (*A. batizocoi* × (*A. cardenasii* × *A. diogoi))*	78 BC_1F_1	370 RFLPs	Burow *et al*., 2001
ICG 12991 × ICGV-SM 93541	200 F_2	12 AFLPs	Herselman *et al*., 2004
TAG 24 × ICGV 86031	318 RILs	191 SSRs	Varshney *et al*., 2009, Ravi *et al*., 2011
A. duranensis × (*A. ipäensis* × *A. duranensis)*	88BC_1F_1	298 SSRs	Foncéka *et al*., 2009
Yueyou 13 × Zhen Zhuhei	142 RILs	132 SSRs	Hong *et al*., 2010
Yueyou 13 × FU 95-5	84 RILs	109 SSRs	Hong *et al*., 2010
Yueyou 13 × J 11	136 RILs	46 SSRs	Hong *et al*., 2010
TAG 24 × GPBD 4	266 RILs	188 SSRs	Khedikar *et al*., 2010, Sujay *et al*., 2011
ICGS 44 × ICGS 76	188 RILs	82 SSRs	Gautami *et al*., 2011
ICGS 76 × CSMG 84-1	176 RILs	119 SSRs	Gautami *et al*., 2011
TG 26 × GPBD 4	146 RILs	181 SSRs	Sarvamangala *et al*., 2011 and Sujay *et al*., 2011
SunOleic 97R × NC94022	190 RILs	170 SSR, 2 CAPS	Chen *et al*., 2010
Tifrunner × GT-C20	158 RILs	238 SSR, 1 CAPS	Chen *et al*., 2010

Varshney *et al*. (2009) developed first SSR based linkage map in cultivated groundnut and also identified some QTLs related to drought tolerance traits by screening 1145 SSR markers and few unpublished markers on 318 F_8 RILs produced by crossing TAG 24 and ICGV 86031. Out of 1145 markers, 144 markers showed polymorphism (12.6 %) and these amplified a total of 150 loci. Polymorphism study revealed that 119 SSR markers were polymorphic (34 %) between the parents NRCG 12568 and NRCG 12326 for the trait Δ^{13}C (Nandini *et al*., 2010).

Ravi *et al*. (2011) in this study genotyping data obtained for 65 loci, were used to integrate into the framework map comprising of 135 loci (Varshney *et al*., 2009). Of the 65 loci tried, 56 loci got integrated into different linkage groups and nine markers remained unlinked. Thus, the present map has a total of 191 loci integrated into 22 linkage groups, covering a length of 1785.4 cM with an average of 9.34 cM between loci along the linkage groups. The 56 new loci got evenly distributed into 17 of the 22 LGs.

Varshney (2012) developed the consensus map based on 3 mapping populations segregating for drought related traits was constructed with 293 SSR loci distributed over 20 linkage groups, spanning 2,840.8 cM. Marker loci per linkage group ranged from 2-31 while the length of the linkage groups ranged from 6.3-293.4 cM with an average density of 11.08 cM/loci.

Sarvamangala *et al*. (2012) conducted study to screen more than 1000 SSR markers, a partial genetic linkage map comprising of 45 SSR loci on 8 linkage groups with an average inter-marker distance of 14.62 cM was developed. QTL analysis based on single marker analysis (SMA) and composite interval mapping identified some candidate SSR markers associated with major QTLs as well as several minor QTLs for the nutritional traits.

Qin *et al*. (2012) developed a comparative integrated map. A total of 324 markers were anchored on this integrated map covering 1,352.1 cM with 21 linkage groups (LGs). Combining information from duplicated loci between LGs and comparing with published diploid maps, seven homoeologous groups were defined and 17 LGs (A1–A10, B1–B4, B7, B8, and B9) were aligned to corresponding A-subgenome or B-subgenome of diploid progenitors.

Shirasawa *et al.* (2012) in his study used *in-silico* analysis that increased the efficiency of polymorphic marker development by more than 3- fold. In total, 926 (34.2 %) of 2,702 markers showed polymorphisms between parental lines of the mapping population. Linkage analysis of the 926 markers along with 253 polymorphic markers selected from 4,449 published markers generated 21 linkage groups covering 2,166.4 cM with 1,114 loci.

Wang *et al.* (2012) developed a genetic linkage map was constructed, consisting of 318 loci onto 21 linkage groups and covering a total of 1,674.4 cM, with an average distance of 5.3 cM between adjacent loci.

Shirasawa *et al.* (2013) developed genetic linkage maps: 10 linkage groups (LGs) of 544 cM with 597 loci for the A genome; 10 LGs of 461 cM with 798 loci for the B genome; and 20 LGs of 1442 cM with 1469 loci for the AB genome. The resultant maps plus 13 published maps were integrated into a consensus map covering 2651 cM with 3693 marker loci which was anchored to 20 consensus LGs corresponding to the A and B genomes.

Haung *et al.* (2015) developed a genetic linkage map for cultivated peanut containing 470 simple sequence repeat (SSR) loci, with a total length of 1877.3 cM and average distance of 4.0 cM between flanking markers. All of the loci were assigned to 20 linkage groups (LG) that were designated as A1 - A10 for the A sub-genome and B1–B10 for the B sub-genome.

2.5 QTL mapping approach

In recent years due to availability of advanced mapping populations such as RILs and relatively large number of molecular markers, linkage-mapping based marker analysis has been undertaken to identify the QTLs for various traits (Table 6) such as resistance to foliar disease (Khedikar *et al.*, 2010; Sujay *et al.*, 2011) and nutritional quality traits (Sarvamangala *et al*., 2011). By using multi-environment phenotyping data for drought tolerance traits, 153 main-effect and 25 epistatic QTLs were identified (Varshney *et al*., 2009; Ravi *et al*., 2011; Gautami *et al*., 2011). On the other hand, one major QTL each for leaf rust (55.2 % PVE, Khedikar *et al*., 2010; 82.96% PVE, Sujay *et al*., 2011) and LLS (67.98 % PVE, Sujay *et al*., 2011) was detected. Although in some cases like resistance to nematode (Nagy *et al*., 2010), leaf rust (Khedikar *et al*., 2010), LLS (Sujay *et al*., 2011) and high-oleate trait (Chu *et al*., 2009, Chen *et al*., 2010) diagnostic molecular markers are available for deployment in molecular breeding, tightly linked molecular markers for are yet to be identified for several other important traits like ELS, GRD, etc.

Several studies have reported identification of QTL for drought tolerance in groundnut and also other crops; however, in the case of groundnut, QTL study for drought tolerance traits has been conducted based on only one mapping population TAG-24 × ICGV-86031 (Varshney *et al*., 2009). Comprehensive QTL analysis led to the identification of a total of 117 small main-effect QTL (M-QTL) and 23 epistatic QTL (E-QTL) for drought-related traits (Ravi *et al*., 2011).

But recently, with the aim of understanding the genetic basis and identification of quantitative trait loci (QTL) for drought tolerance, two new recombinant inbred line (RIL) mapping populations, namely ICGS 76 9 CSMG 84-1 (RIL-2) and ICGS 44 9 ICGS 76 (RIL-3) were used to develop a consensus map with 293 SSR loci distributed over 20 linkage groups, spanning 2, 840.8 cM. QTL analysis identified 153 main effect QTL (M-QTL) and 25 epistatic QTL (E-QTL) for drought-tolerance-related traits and the localization of these QTL on the consensus map provided 16 genomic regions that contained 125 QTL (Gauthami *et al*., 2012).

Several studies were conducted in the past that reported identification of QTLs for drought tolerance or related traits in other crops also. For instance, in soybean, 5 QTLs were identified for WUE in an F_2 population with 14–20% phenotypic variation explained (PVE) (Mian *et al*., 1998). In case of wheat, Dashti *et al*. (2007) identified five QTLs for drought tolerance with 13–34% PVE. In another study, 47 QTLs for different plant stress indicators in rice with 5–59% PVE were identified.

Table 6. List of QTLs identified for some economically important traits in groundnut

Trait	QTLs identified	PVE (%)	References
Biotic stress			
Late leaf spot (LLS)	27	1.70-82.96	Khedikar *et al*., 2010, Sujay *et al*., 2011
Resistance to *Aspergillus flavus*	6	6.2-22.7	Liang *et al*., 2009a
Aphid vector groundnut rosette disease	8	1.18-76.16	Herselman *et al*., 2004
Abiotic stress			
Transpiration (T)	15	4.36-18.17	Varshney *et al*., 2009, Ravi *et al*., 2011, Gautami *et al*., 2011
Transpiration efficiency (TE)	14	4.47-18.12	Varshney *et al*., 2009, Ravi *et al*., 2011, Gautami *et al*., 2011
Specific leaf area (SLA)	13	3.48-13.29	Varshney *et al*., 2009, Ravi *et al*., 2011
Leaf area (LA)	4	7.24-11.51	Varshney *et al*., 2009, Ravi *et al*., 2011
SPAD chlorophyll meter reading (SCMR)	29	5.72-19.53	Varshney *et al*., 2009, Ravi *et al*., 2011
Biomass	7	4.25-20.32	Varshney *et al*., 2009, Ravi *et al*., 2011
Canopy conductance (ISC)	7	3.28-22.24	Varshney *et al*., 2009, Ravi *et al*., 2011
Total dry matter (TDM)	7	4.34-22.39	Varshney *et al*., 2009, Ravi *et al*., 2011, Gautami *et al*., 2011
Agronomic traits			
Shoot dry weight (ShDW)	11	5.03-22.09	Varshney *et al*., 2009, Ravi *et al*., 2011, Gautami *et al*., 2011
Pod weight (PW)	7	4.17-8.73	Varshney *et al*., 2009, Ravi *et al*., 2011
Seed weight (SW)	5	4.18-8.22	Varshney *et al*., 2009, Ravi *et al*., 2011
Haulm weight (HW)	6	3.78-33.66	Varshney *et al*., 2009, Ravi *et al*., 2011
Harvest index (HI)	3	6.39-40.10	Gautami *et al*., 2011
Pod mass/ plant	3	13.1-18.3	Liang *et al*., 2009
Mature pods/ plant	3	11.9-12.3	Liang *et al*., 2009
Branches/ plant	7	8.1-17.3	Liang *et al*., 2009
Height of main axis	7	8.2-12.8	Liang *et al*., 2009
Stem diameter	4	7.8-24.1	Liang *et al*., 2009
Leaf length, width and length / width ratio	7	12.4-18.9	Liang *et al*., 2009
Yield parameters	5	9.19-17.69	Selvaraj *et al*., 2009
Oil content (%)	7	1.5-9.5	Liang *et al*., 2009, Selvaraj *et al*., 2009, Sarvamangala *et al*., 2011
Oil quality (%)	10	1-4-9.7	Sarvamangala *et al*., 2011
Protein content (%)	10	1.5-13.4	Liang *et al*., 2009, Sarvamangala *et al*., 2011

III MATERIAL AND METHODS

Development of overall drought tolerance in a plant is the collective expression of many plant characteristics in the appropriate environment. By taking these things into consideration in the present study, an attempt was made to identify the superior RILs derived from a cross NRCG 12568 × NRCG 12326 based on evaluation for high pod yield along with physiological traits under different water regimes. The material and methods to address the objectives framed in the research are presented under the following headings.

3.1 Experimental material

The experimental material for the present study consisted of 147 RILs of F_{11} generation derived from the NRCG 12568 × NRCG 12326 cross, which were evaluated for two consecutive summer seasons along with the parents and checks for quantitative and physiological traits under different water regimes.

3.1.1 Development of mapping population

Around 250 groundnut germplasm lines were evaluated in field for growth, yield and physiological parameters like SCMR, SLA and $\Delta^{13}C$. Out of 250 genotypes, few genotypes contrasting for pod yield, traits related to WUE like SCMR, SLA, $\Delta^{13}C$ were selected to carry out hybridization. Selection for high WUE with high yield was the criteria followed during the selection process in segregating generations.

From the germplasm, three lines *viz*., NRCG 12473, NRCG 12326 and NRCG 12568 contrasting for SLA, SCMR, $\Delta^{13}C$, pod yield and related traits were selected (Table 7). The mapping population was developed at University of Agricultural Sciences, GKVK, Bengaluru. The F_1's derived from the cross NRCG 12568 × NRCG 12326 were selfed to produce F_2 and advanced through Single Seed Descent (SSD) method till F_6 generation. Schematic representation of development of mapping population was given in the Plate 1.

3.2 Soil moisture content and meteorological condition

Soil moisture content was determined using lysimeter method at a depth of 0 – 15 cm during 30, 45, 60 and 75 DAS to verify level of moisture content in well watered (WW) and water stress (WS) conditions. Rainfall, relative humidity (RH), vapour pressure, maximum and minimum temperature were recorded daily from the day of sowing until harvest at Main Agricultural Research Station, University of Agricultural Sciences, Bengaluru at a distance of 200 m from the experimental field.

Table 7. Contrasting features of parents in the present study

Sl. No.	Features	NRCG 12568 (P_1)	NRCG 12326 (P_2)
1.	Pods	16.20 - 23.80	8.80 - 13.20
2.	Pod yield plant^{-1} (g)	10.50 - 17.90	6.50 - 9.60
3.	Kernel yield plant^{-1} (g)	6.20 - 11.00	4.00 - 6.98
4.	Shelling percentage	51.12 - 73.79	59.15 - 76.83
5.	Δ^{13} C	12.67	18.97
6.	SCMR	48.24 - 55.65	40.45 - 52.27
7.	SLA (cm^{2}g^{-1})	99.70 - 108.34	113.71 - 126.04

3.3 Climate and weather

Geographically, University of Agricultural Sciences, GKVK, Bengaluru is located at 13° 05'' N latitude and 77° 34'' E longitude and an altitude of 924 m above mean sea level. The annual rainfall ranges from 528 mm to 1374.4 mm with the mean of 915.8 mm. The weather data during the crop growth period of both the years is presented in figure 1 and 2 (Appendix 1 and 2).

3.4 Methods

3.4.1 Phenotyping for drought tolerance and quantitative traits

The F_{11} RIL population along with parents *viz.*, NRCG 12568 and NRCG 12326 and checks were evaluated under water stress and control conditions for yield contributing traits, physiological traits and quality traits during summer 2017 and 2018

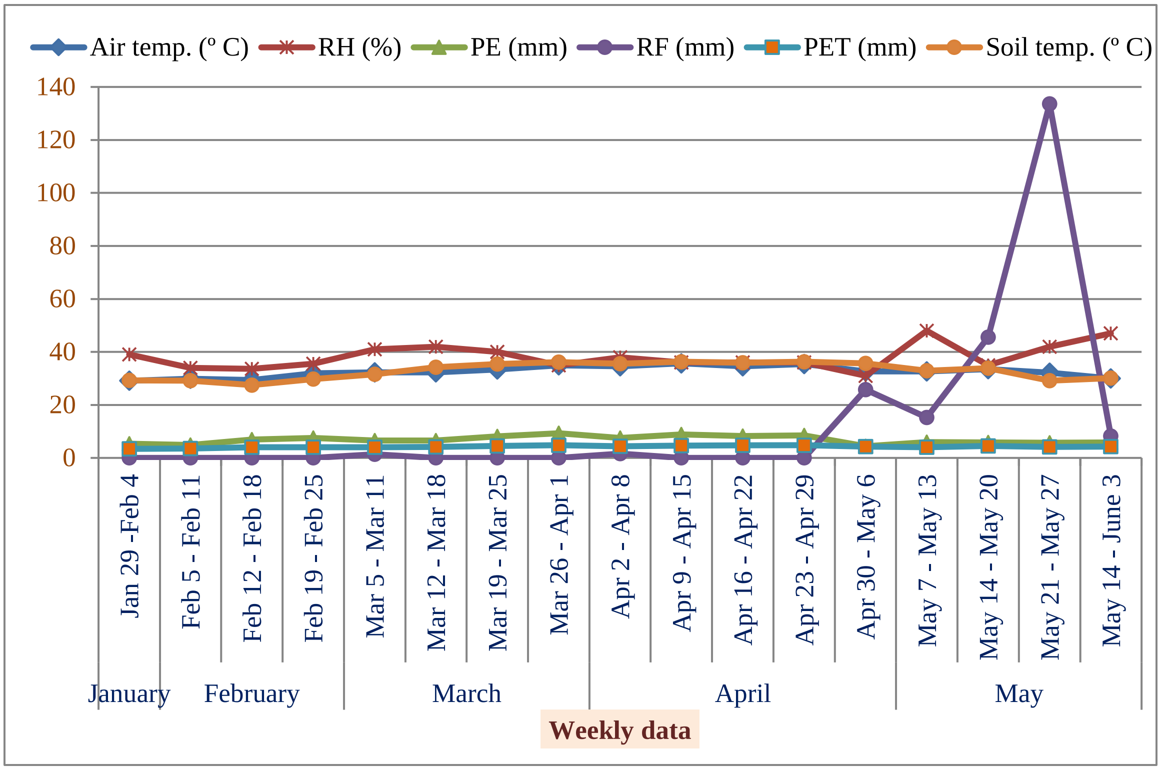


Fig. 1. Weekly weather data of *Summer* 2017 at GKVK, UAS, Bengaluru

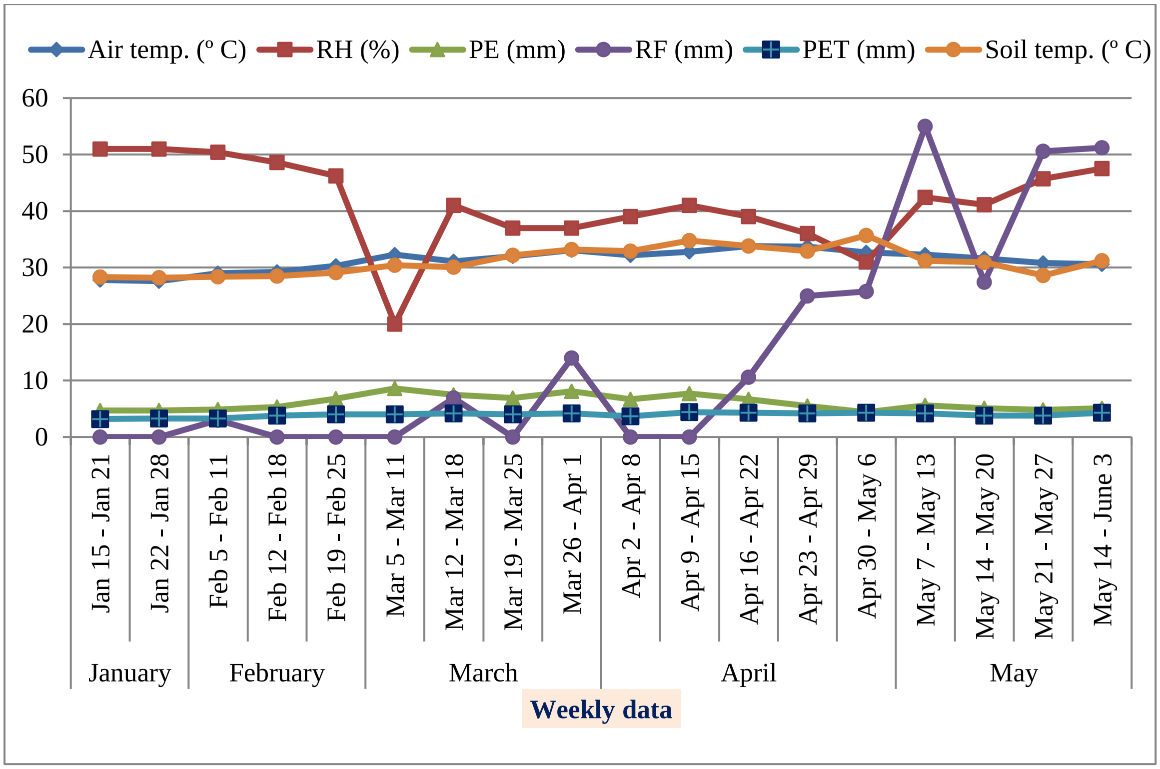
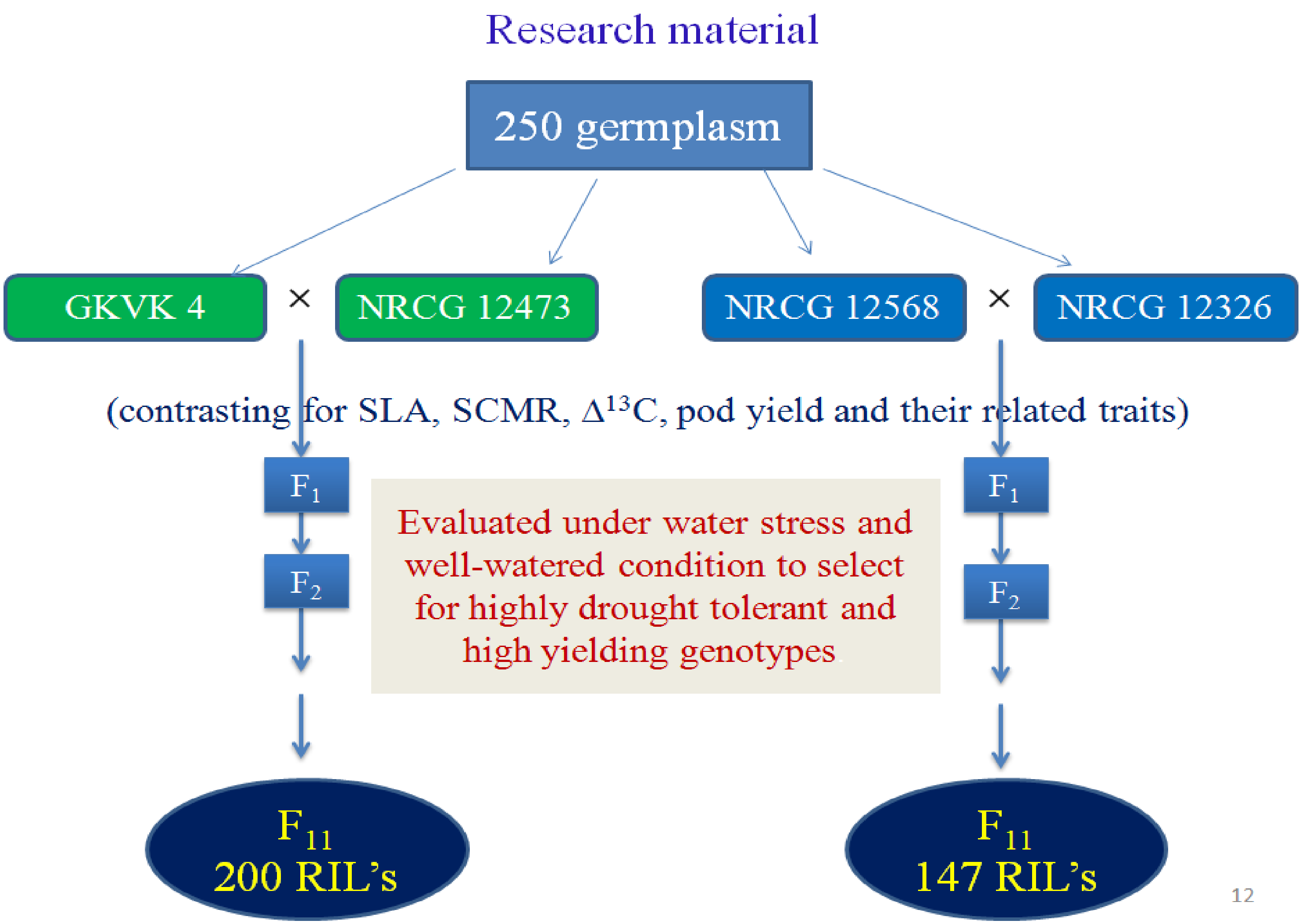


Fig. 2. Weekly weather data of *Summer* 2018 at GKVK, UAS, Bengaluru



Research material

250 germplasm

GKVK 4 × NRCG 12473

NRCG 12568 × NRCG 12326

(contrasting for SLA, SCMR, $\Delta^{13}C$, pod yield and their related traits)

F_1

F_2

Evaluated under water stress and well-watered condition to select for highly drought tolerant and high yielding genotypes

F_1

F_2

F_{11}
200 RIL’s

F_{11}
147 RIL’s

12

Plate 1. Schematic representation of development of NRCG 12568 × NRCG 12326 mapping population

(Plate 2 & 3). In the first year, field evaluation of 147 (F_{11}) RILs was carried out during summer 2017 in augmented design along with parents and checks *viz.*, GKVK 5 and TMV 2 at K-Block, Department of Genetics & Plant Breeding, UAS, GKVK, Bengaluru in three experiments *viz*., well-watered (WW), water stress-II (WS2); withholding irrigation from 45-65 DAS (flowering and peg initiation stage), water stress-III (WS3)-withholding irrigation from 65-85 DAS (peg penetration and pod development stage). One more stress experiment was included for second year field evaluation by withholding irrigation from 30-45 DAS i.e. water stress-I (WS1) in order to understand the responsiveness of the RILs for water stress during flowering to 50% flowering period and also to identify the stress responsive QTLs along with the RILs which are capable of withstanding the water stress between 30-45 DAS. In both the seasons sowing was done in one row of 2 m length with a spacing of 30 cm between rows and 10 cm between the plants. All recommended package of practices was followed for better establishment of plant population.

3.4.2 Imposing the water stress conditions

In the field, irrigation to both WW and WS plots was given equal period of intervals up to the time of flowering. Later, when stress was imposed to WS plots in different water regimes at specific crop growth stage. Irrigation was supplied to the WW plot at 7-10 days interval regularly. Harvesting was done at physiological maturity stage of the crop in the experimental plots. Comparison of RILs at different intervals before and after release of stress in WW and WS condition are shown in Plate 4 and 5.

3.4.3 Observations recorded

Observations were recorded on five randomly selected plants from each RIL for the following morphometric traits, surrogate traits of WUE like; SLA and SCMR, pod yield and yield attributing traits in F_{11} and F_{13} RIL population. The characters studied and techniques adopted to record the observations are given below.

Days to 50 *per cent* flowering (DFF)

Number of days taken from the date of sowing to the date on which 50 *per cent* of plants in each treatment flowered was recorded as days to 50 per cent flowering.

Plant height (cm)

Plant height was measured as length of the main axis from ground level to the apical leaflet recorded at crop maturity stage and expressed in cm.

Primary branches per plant

Numbers of branches on main axis (n + 1) was counted on five randomly selected plants and recorded.

Pods per plant

The total number of matured pods produced in each plant was counted and recorded at the time of harvest.

Pod yield per plant (g)

Dry pod weight in each treatment was recorded and expressed in grams.

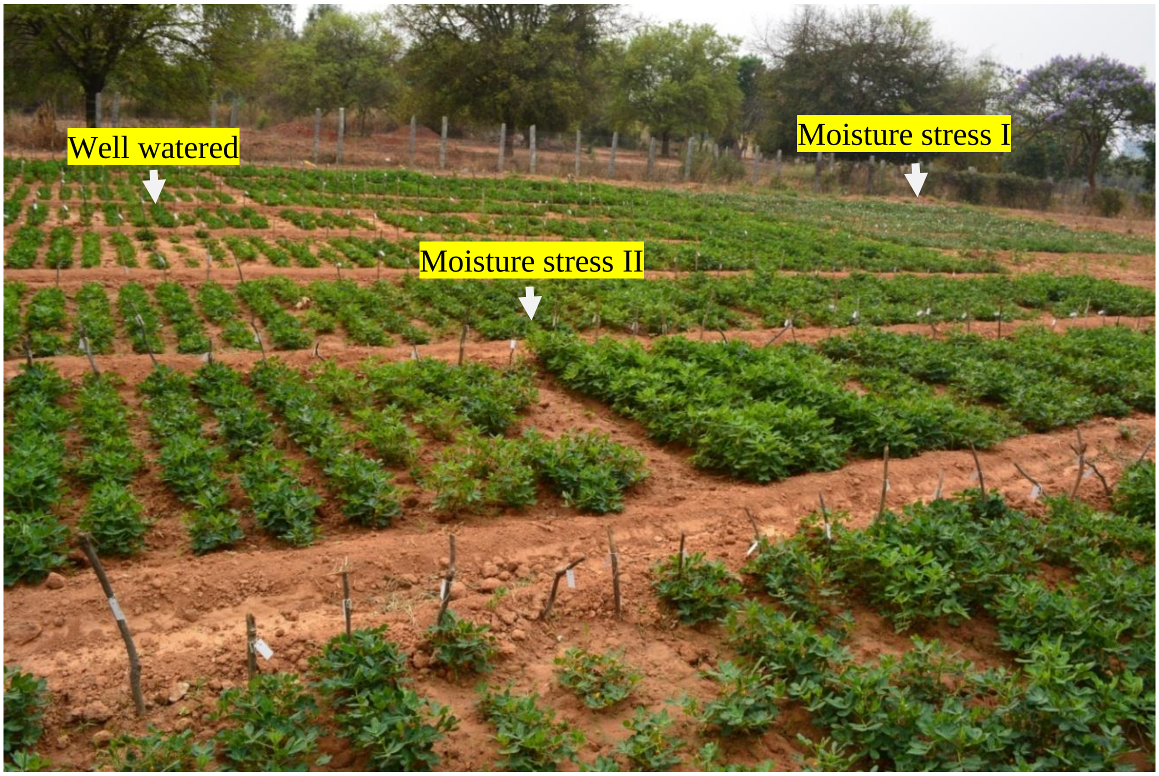
Kernel yield per plant (g)

Dry kernel weight of each treatment after shelling was recorded and expressed in grams.

Shelling *per cent*

All the dried pods from five plants of each RIL were weighed separately (pod yield) and shelled. Then, the weight of kernels recovered after shelling the pods was taken (kernel yield). The shelling percentage was calculated using following formula;

$$\text{Shelling percentage} = \frac{\text{Dry kernel yield}}{\text{Pod yield}} \times 100$$



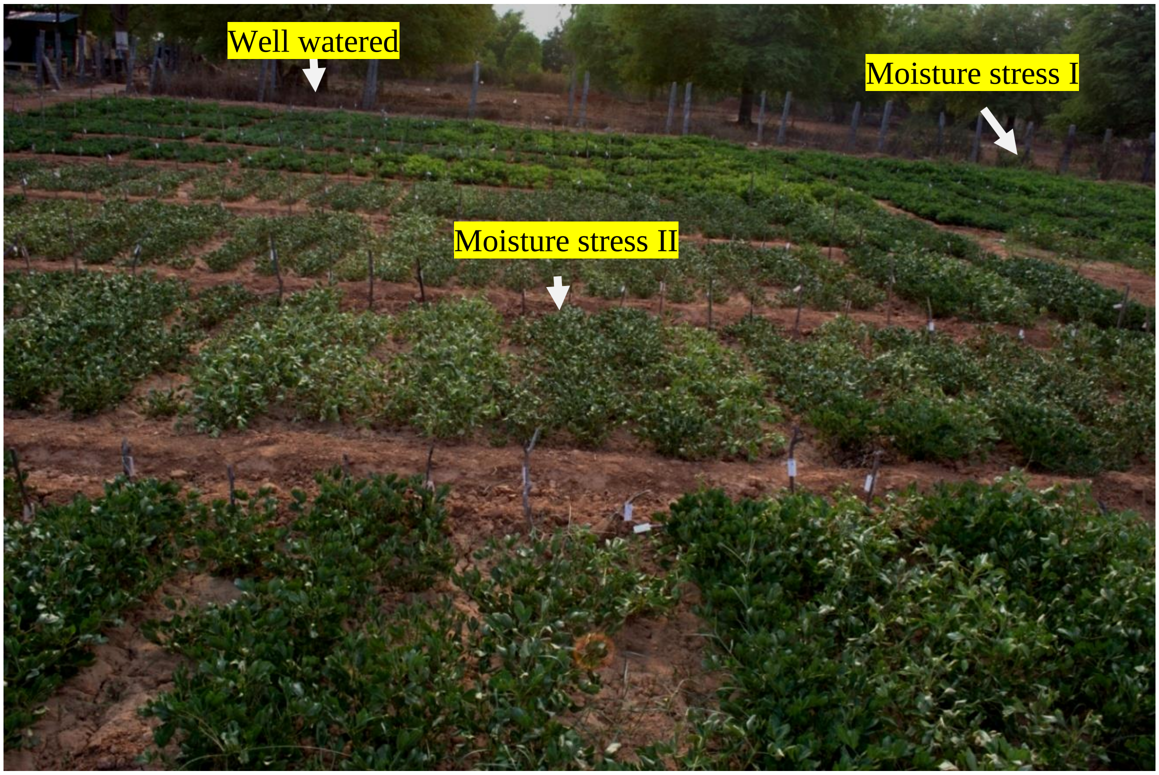


Plate 2. General view of the experimental plot under both the stress conditions during summer 2017

Plate 3. **General view of the experimental plot under both well watered and water stress conditions (WS1, WS2, WS3) during summer 2018**

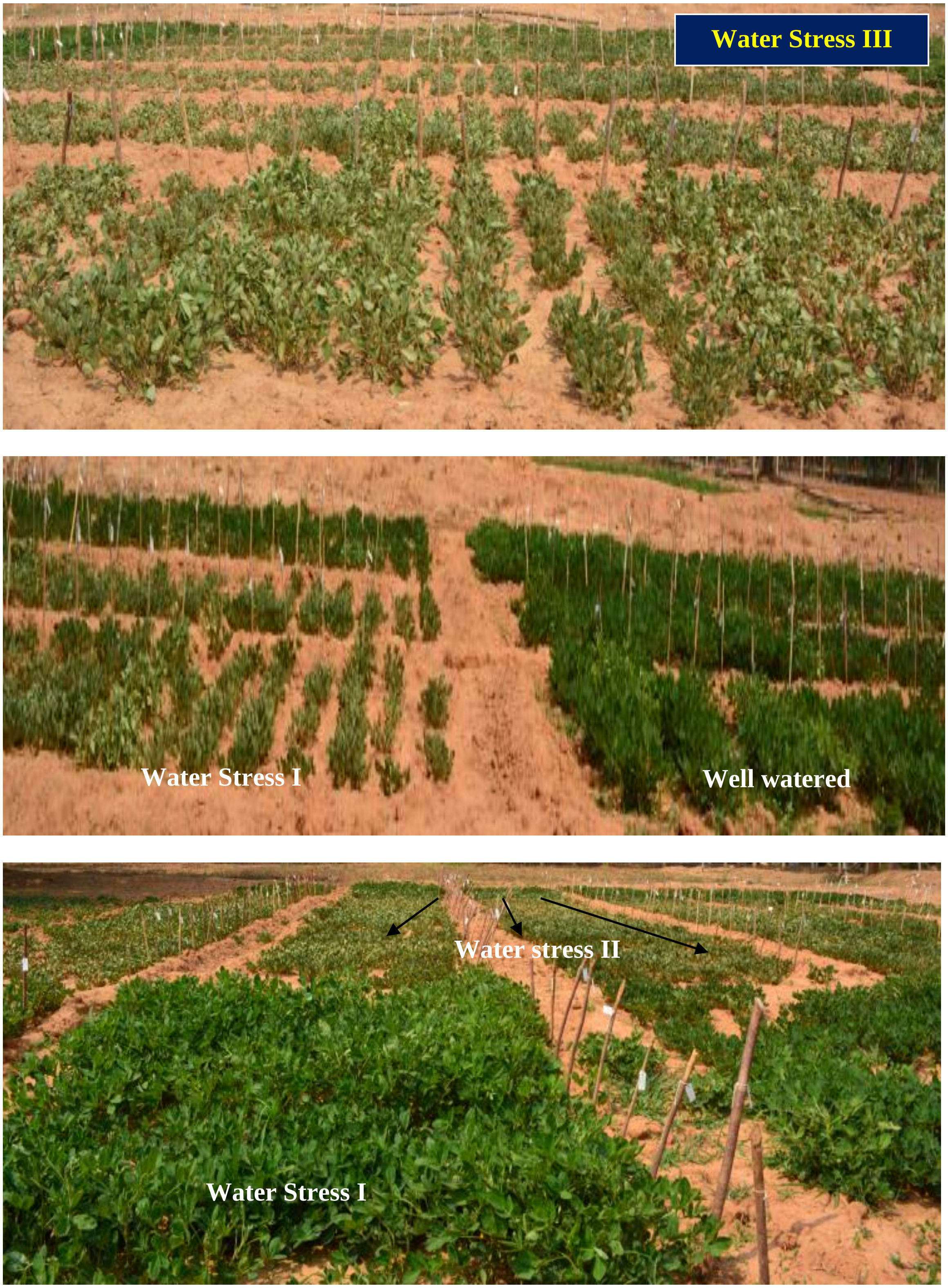


Plate 4. Comparison of water stress-imposed plots with well watered plot before release of stress

Last day of the stress imposed

A day after releasing stress

Two days after releasing stress

Plate 5. Photos indicating stress imposed on RIL population and its recovery after releasing stress

Sound mature kernel percentage (SMK %)

Mature sound and healthy seeds were separated and counted from the total number of seeds. Sound mature kernel percentage was calculated using the formula given below;

$$\text{SMK \%} = \frac{\text{Number of sound mature kernels}}{\text{Total number of kernels}} \times 100$$

Specific leaf area ($cm^2\ g^{-1}$) (SLA)

Fully expanded third leaf from the main axis was collected to measure the leaf area using leaf area meter. Then the leaves were oven dried at 70°C for 3 days and the dry weight of the leaf was accurately measured using a sensitive balance. The SLA was calculated using the following formula (Evans, 1972).

$$\text{SLA} = \text{Leaf area } (cm^2) / \text{ leaf dry weight (g)}$$

SPAD chlorophyll meter reading (SCMR)

Amongst several leaf characters, leaf thickness and chlorophyll content determines the leaf transmittance. Leaf nitrogen content normally influences the leaf chlorophyll content. A device has been developed by Minolta company, New Jersey USA (SPAD-502) which measures the light attenuation at 430 nm (the peak wavelength for chlorophyll a and chlorophyll b) and at 750 nm (near infrared) with no transmittance. The unit value measured by the chlorophyll meter is termed as SCMR (SPAD chlorophyll meter reading) which provides information on the relative amount of leaf chlorophyll content. The SPAD meter (Soil Plant Analytical Development) is a simple hand held instrument, which operates with DC power of three Volts.

The third leaf from the plant apex was selected to record the SCMR. Selected leaf was clamped avoiding the mid rib region into the sensor head of SPAD meter. A gentle stroke was given to record the SPAD reading and the average of such four times was considered. Since groundnut has tetra foliate leaf, SCMR was recorded in all the four leaflets and the average value was recorded. The SCMR was recorded under normal sunlight between 8.00 a.m. to 10.00 a.m

3.5 Statistical analysis

Observations recorded on five randomly selected plants and mean of F_{11} and F_{13} RIL populations on quantitative traits were subjected to statistical analysis. Different statistical methods were used to assess variability and identification of drought tolerance RILs are detailed below;

3.5.1 Descriptive statistics, genetic parameters, heritability and GAM

Genetic variability among the germplasm accessions was assessed using first-degree statistics such as mean, range and standardised range. Genetic parameters such as phenotypic coefficient of variance (PCV) and genotypic coefficient of variance (GCV) were computed (Burton and De vane, 1953) to enable comparison of phenotypic and genotypic variance across the traits. Broad-sense heritability (h^2) (Lush, 1945) and expected genetic advance as *per cent* mean (GAM) was estimated (Johnson *et al*., 1955) using mean trait values of each germplasm accession.

a. Mean

Arithmetic mean is defined as the sum of all observations divided by the total number of individual's added (n) was calculated by using the following formula;

$$\text{Mean} = \Sigma X_i/n$$

Where,

X_i = i^{th} observation of a population

n = Total number of observations

Mean was obtained for each trait separately for RIL populations.

b. Standard Error

It is the measure of uncontrolled variation present in sample, which is estimated by dividing the standard deviation by square root of number of observations in the sample and is denoted by SE.

$$SE = SD/\sqrt{n}$$

Where,

SD = Standard deviation

n = No. of observations

c. Range

The minimum and maximum values on the basis of all the accessions mean were used to indicate the limit of range for a given character.

Absolute range (R) was estimated as, R= (Min-Max).

d. Standardized range (SR) was estimated as,

$$SR = \frac{[(\text{Highest trait value - Lowest trait value})]}{\text{Trait mean}}$$

e. Critical difference

It is the least significant difference equal to or greater than the mean of genotypes in which all the differences are significant.

CD = S.E.(d) × t value (at error d.f.)

f. Phenotypic and Genotypic Co-efficient of Variation (PCV and GCV)

Both phenotypic and genotypic co-efficient of variation for all the characters were estimated using the formula of Burton and De Vane (1953).

Phenotypic Coefficient of Variation (PCV): PCV (%) = $\frac{\sqrt{\sigma^2 p}}{X} \times 100$

Genotypic Coefficient of Variation (GCV): GCV (%)= $\frac{\sqrt{\sigma^2 g}}{X} \times 100$

Where,

σ^2g= Genotypic variance

σ^2p = Phenotypic variance

$\overline{X}$ = Mean

PCV and GCV were classified as shown below by following the method suggested by Robinson *et al*., (1949):

a. 0-10 % - Low
b. 10-20 % - Moderate
c. >20 % - High

g. Heritability in broad sense (h^2_{bs})

Heritability (broad sense) was estimated for all the characters as the ratio of genotypic variance to the total variance as suggested by Johnson *et al*., (1955).

$$h^2{}_{bs} = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Where,

h^2_{bs} = Heritability (Broad sense) expressed in *per cent*
$\sigma^2 g$ = Genotypic variance
$\sigma^2 p$ = Phenotypic variance

The heritability was categorized as suggested by Robinson *et al.*, 1949

0 – 30 % = low
30 – 60 % = moderate
60 – 90 % = high

h. Genetic Advance as *per cent* mean:

The Genetic Advance was predicted by using the formula; $GA = h^2 \times \sigma_p \times k$ (Lush, 1949 and Johnson *et al.*, 1955)

Where, h^2 = heritability in broad sense
σ_P = standard deviation of phenotypic variance
k = selection differential at 5 % (2.06)

$$\text{GA as } \textit{per cent} \text{ mean (GAM)} = \frac{\text{GA}}{\text{Trait mean}} \times 100$$

The GA as *per cent* mean (GAM) was categorized as suggested by Johnson *et al.* (1955) and the same is given below:

0 – 10 % = Low
10 – 20 % = Moderate
20 % & above = High

3.5.2 Analysis of variance for augmented design

Analysis of variance was performed to partition the total phenotypic variation among the RILs and check entries in to sources attributable to 'RILs + check entries', 'RILs', 'check entries' and 'RILs *vs* check entries' following Augmented design (Federer, 1956) (Table 8) using INDOSTAT 8.5 software package (WINSTAT, 2010).

Table 8. Structure of ANOVA as per augmented design (Federer, 1956)

Source of variation	**df**	**MSS**	**'F' ratio**
Blocks (b)	(b-1)	MSS(B)	MSS(B)/ EMSS
Entries (e) (RILs + check entries)	(e-1)	MSS(E)	MSS(E)/EMSS
RILs (g)	(g-1)	MSS (G)	MSS(G)/EMSS
Check entries (c)	(c-1)	MSS (C)	MSS(C)/EMSS
RILs *vs* Check varieties	(g-1) (c1)	MSS (GC)	MSS(GC)/EMSS
Error	(c-1) (b-1)	EMSS	

Where,

b = number of blocks
e = number of entries
g = number of germplasm accessions
c= number of check entries

The effect of each block (B_j) was computed as, $B_j = X_j - X_{..}$

Where,

X_j = Trait means of check entries in j^{th} block
$X_{..}$= Trait mean of all the checks in all the blocks.

Bj was used to adjust the trait means of the accessions relevant to the block. Thus, trait means of each accession evaluated in j^{th} block was adjusted by subtracting the block effect B_j of the j^{th} block from actual trait value of the accession.

3.5.3 Identification of drought tolerant groundnut RILs based on principal component analysis, biplot analysis and ranking method

Loss of yield is the main concern of plant breeders and hence they emphasize yield performance under stressed conditions. However, the complexity of yield caused by large genotype–season and genotype–location interactions make it difficult to select stress-tolerant genotypes and variation in yield potential can arise from factors related to adaptation rather than drought tolerance *per se* (Nazari and Pakniyat, 2010).

Identification of a standard evaluation assay is the most critical problem to select drought-resistant genotypes and ultimately for elucidating the internal genetic mechanisms. Over recent years, several evaluation methods and selection criteria based on statistical analysis were proposed and reviewed, to evaluate response of crop genotypes to water stress in association with yield (Blum, 1984).

Drought resistance is defined by Hall (1993) as the relative yield of a genotype compared to other genotypes subjected to similar kind of drought stress. Drought susceptibility of a genotype is often measured as a function of reduction in yield under drought stress (Blum, 1988) whilst the values are confounded with differential yield potential of genotypes (Ramirez & Kelly, 1998). Several drought indices which provide a measure of drought, based on loss of yield under drought-conditions in comparison to normal conditions have been used for screening drought-tolerant genotypes (Mitra, 2001).

3.5.3.1 Drought tolerant indices computed based on pod yield under water stress (Y_S) and well-watered (Y_P) condition

Previously developed and reported indices (Table 9) were estimated to quantify the responses of RILs to pod yield under well-watered (Y_p) and water-stress (Y_s) condition. These indices consider the ability and performance of the RILs under stress and non-stress environments. All the indices were estimated based on the extent of reduction in pod yield plant^{-1} of the RILs evaluated under well-watered environment to those evaluated under water stress environment.

Table 9. List of drought tolerant indices (DTI) computed among groundnut RIL population

Name of DTI	Abbrev.	Formula	Reference
Tolerance index	TOL	$Yp - Ys$	Rosielle and Hamblin (1981)
Mean productivity	MP	$(Yp + Ys)/2$	Rosielle and Hamblin (1981)
Geometric mean productivity	GMP	$\sqrt{(Ys \times Yp)}$	Fernandez (1992)
Harmonic mean productivity	HMP	$2 * (Yp \times Ys)/(Yp + Ys)$	Fernandez (1992)
Stress tolerance index	STI	$(Ys \times Yp)/(\bar{Y}p^2)$	Fischer and Maurer (1978)
Relative drought tolerance	RDI	$(Ys/Yp)\ /\ (\bar{Y}s/\bar{Y}p)$	Fischer *et al*. (1998)
Abiotic tolerance index	ATI	$[(Yp - Ys)/\ (\bar{Y}p/\bar{Y}s) \times \surd(Yp \times Ys)$	Moosavi *et al*. (2008)
Stress susceptibility percentage index	SSPI	$((Yp - Ys/2(\bar{Y}p)) \times 100$	Moosavi *et al*. (2008)
Stress non-stress production index	SNPI	$[\sqrt[3]{Yp + Ys}/(Yp - Ys)] \times [\sqrt[3]{}Yp \times Ys \times Ys]$	Moosavi *et al*. (2008)
Yield index	YI	$(Ys)/(\bar{Y}s)$	Gavuzzi *et al*. (1997)
Yield stability index	YSI	Ys/Yp	Bouslama and Schapaugh (1984)
Modified STI	K_1STI	$Yp^2/\bar{Y}p^2$	Farshadfar and Sutka (2002)
Modified STI	K_2STI	$Ys^2/\bar{Y}s^2$	Farshadfar and Sutka (2002)
Drought resistance index	DI	$(Ys \times (Ys/Yp))/\ \bar{Y}s$	Lan (1998)
Stress susceptibility index	SSI	$(1 - (Ys/Yp))/(1 - (\ \bar{Y}s)/\ \bar{Y}p))$	Fischer and Maurer (1978)

Where,

Y_P: Pod yield under well-watered (WW)　Y_S: Pod yield under water stress (WS)

$\bar{Y}_P$: Grand mean of pod yield under WW　$\bar{Y}_S$: Grand mean of pod yield under WS

3.5.3.2 Criteria to identify desirable indices for effective discrimination of the RILs for responses to water stress

Statistics such as standardized range (SR) and phenotypic coefficient of variation (PCV) for pod yield plant^{-1} were estimated as

$$SR = \frac{[(\text{Highest trait value - Lowest trait value})]}{\text{Trait mean}}$$

$$PCV = \frac{\text{Trait standard deviation}}{\text{Trait mean}} \times 100$$

3.5.3.3 Criteria to identify desirable indices for selection of water stress tolerant RILs

Two criteria were used to identify desirable indices for selection of water stress tolerant RILs in all the experiments. The desirable indices were identified as those (1) which effectively discriminated the genotypes for responses to waters stress and (2) which were significantly correlated (with high magnitude) with mean pod yield plant^{-1}of RILs evaluated under both WW and WS conditions. As water stress tolerant RILs varied with the indices, rank sum (RS) method (Farshadfar *et al.*, 2012) combining all the indices was identified as desirable based on above mentioned two criteria used to select WS tolerant RILs.

3.5.3.4 Correlation between DTIs and pod yield under well watered (Y_P) and water stress (Y_S) condition

The Pearson correlation coefficients (r) were computed between fifteen DTIs and fruit yield under well-watered and water-stress conditions among 147 groundnut RILs by using variance and covariance components as suggested by Al-Jibouri *et al.* (1958). Analysis was done using XLSTAT 2014. The best DTI was selected according to Mitra (2001), who revealed that a suitable index must have a highly significant correlation with pod yield under both the conditions.

Quantitative drought resistance indices estimated for each genotype provides a measure of drought, based on yield loss under water-stress condition in comparison to well-watered condition have been used for screening drought-tolerant genotypes (Mitra, 2001). The principal component analysis (PCA) with biplot display was used to identify tolerant and high yielding genotypes (Giancarla *et al.*, 2010).

3.5.3.5 Principal Component Analysis (PCA)

PCA is a multivariate statistical method, originated with Pearson (1901) as a means of fitting planes by orthogonal least square but was later proposed by Hotelling (1933) for the purpose of analyzing correlation structure. It is a sort of multivariate analysis where canonical vectors or roots representing different axes of differentiation and amount of variation accounted for by each of such axes, respectively are derived (Rao, 1952). PCA attempts to describe 'the total variation in a multivariate sample with fewer variables than in the original data set.

PCA selects linear combinations of the attributes, which retain the highest proportion of unexplained variability between the units. These linear combinations (the principal components) can explain the relationships between units and attributes and by restriction; the dimensionality of the problem is reduced. The major axis or vector is called the principal component 1 (PC 1) and subsequent orthogonal axes are numbered sequentially (PC 2, PC 3 ... PCn). Each PC axis is defined in terms of linear transformation of original variable scores. Coefficients of each transformation equation form a set of eigen vector and the total variance accounted by each equation is called the eigen value. The eigen values exceeding 1.0 and the corresponding PC has inherently more information than any single variable alone. A significant PC interprets the percentage of total variation in the data set. The data matrix for all the N objects of the population over p attributes is DN_{xp}.

Therefore principal component analysis serves the following objectives:

a) It measures divergence between varieties in terms of spatial distance rather than quantifying it as a D^2 dose. It gives group constellations as varietal distribution in two way pictorial graph fixes the relative position of each variety.

b) It determines the effective number of axes differentiation- primary, secondary, tertiary or so based on number of canonical vectors (z_1, z_2, z_3 or further).

c) It also indicates the characters important for divergence of each axis. The largest element in each vector (z_1, z_2 etc.) constitutes the greatest contributors.

Computational procedures

Introduced by Pearson (1901) and Hotelling (1933) to describe the variation in a set of multivariate data in terms of a set of uncorrelated variables. We typically have a data matrix of 'n' observations on 'p' correlated variables x_1, x_2......x_p. PCA looks for a transformation of the x_i into 'p' new variables Y_i that are uncorrelated

Looking for a transformation of the data matrix X (nxp) such that

$$Y= \delta^T X= \delta_1 X + \delta_2 X + .. + \delta_p X_P$$

Where $\delta= (\delta_1, \delta_2,.. \delta_p)^T$ is a column vector of weights with $\delta^2 + \delta\ p \delta 2^2 + .. + = 1$

Maximize the variance of the projection of the observations on the Y variables

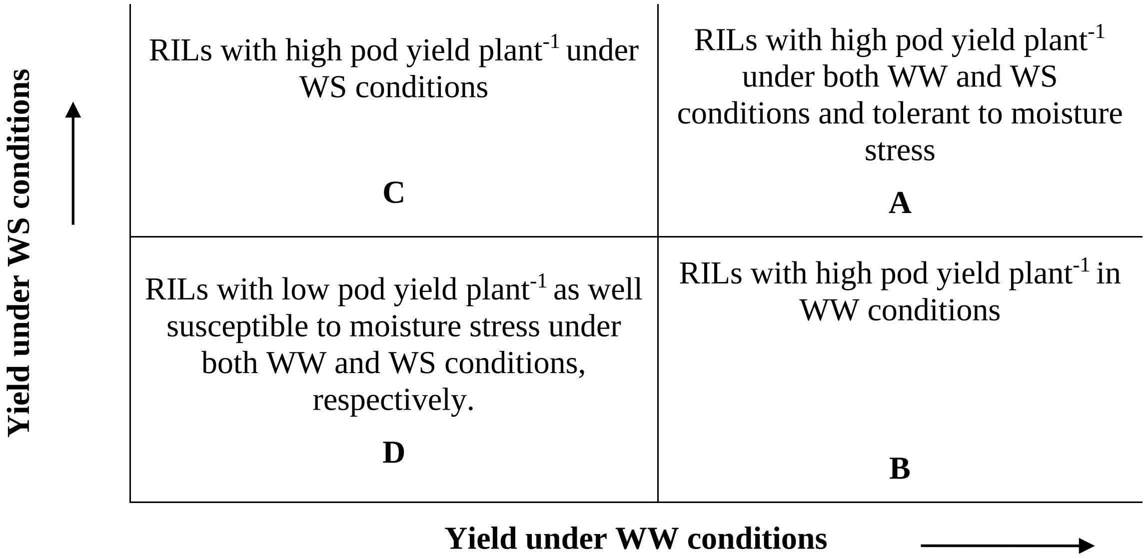
Find δ so that Var ($\delta^T X$) = δT Var (X) is maximal

The matrix C=Var(X) is the covariance matrix of the X_i variables

Corresponding to the largest γ is given by the eigen vector δ The direction of eigen value of matrix C. The second vector that is orthogonal (uncorrelated) to the first is the one that has the second highest variance which comes to be the eigen vector corresponding to the second eigen value and so on …. New variables Y_i that are linear combination of the original variables (xi): Y_i = ai1x1+ ai2x2 +….+ aipxp ; i = 1...p The new variables Y_i are derived in decreasing order of importance; they are called 'principal components' calculating eigen values and eigen vectors are found by solving the equation λ. PCA is useful for finding new, more informative, uncorrelated features; it reduces dimensionality by rejecting low variance features.

3.5.3.6 Biplot analysis

Biplot analysis was performed by using first two principal component analysis (PCA) - which were computed based on the correlation matrix using data from fifteen drought tolerance indices along with pod yield under control and water stressed condition by XLSTAT 2014, Copyright Addinsoft 1995-2014 (http://www. xlstat.com) as followed by Iqbal *et al.* (2014). RILs were categorized into four groups (A, B, C and D) based on their performance in well-watered and water-stressed environments: Group A - accessions expressing uniform superiority in well-watered and water-stressed conditions, Group B - accessions that perform favourably only in well-watered conditions, Group C - accessions that gives relatively higher yield only in water-stressed condition and Group D - accessions perform poorly in both the conditions.



RILs with high pod yield plant^{-1} under WS conditions **C**	RILs with high pod yield plant^{-1} under both WW and WS conditions and tolerant to moisture stress **A**
RILs with low pod yield plant^{-1} as well susceptible to moisture stress under both WW and WS conditions, respectively. **D**	RILs with high pod yield plant^{-1} in WW conditions **B**

Fig. 3: Categorization of the RILs based on response criteria into different groups (A, B, C and D) for drought tolerance and susceptibility

The optimal selection criterion should distinguish accessions showing superiority in both conditions from the other three groups. Three dimensional graphs were drawn by plotting pod yield plant^{-1} against the RILs in all experiment under WW and WS conditions on X-axis and Y-axis, respectively and indices on Z-axis to group the genotypes into classes A, B, C and D responses. These graphs were plotted using 'Statistica' software v. 12.

3.5.3.7 Ranking Method

By virtue of the formulae used to estimate the indices, higher the magnitude of the indices identified as desirable, higher is the WS tolerance of the RILs. Considering this relationship between magnitude of the indices and WS tolerance of the RILs, the RILs with highest and lowest magnitudes of indices estimated based on pod yield plant^{-1} were assigned rank '1' and highest rank, respectively. The ranks of each genotype were summed across all the indices. The rank mean (RM) and standard deviation of ranks (SDR) were estimated. Rank sum (RS) was calculated as RS = RM + SDR. The genotypes with lowest and highest magnitudes of RS were declared as most WS tolerant and least WS tolerant, respectively.

3.6 Assigning parental polymorphism and genotyping for linkage map construction and QTL analysis

3.6.1 DNA extraction from parents and mapping populations

Young leaves (20-25 days old) were collected from the parents and F_{12} populations (NRCG 12568 and NRCG 12326) and genomic DNA was extracted following Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1987). The following steps were used to extract the DNA.

1. Two grams of fresh young leaves from shoot apex was harvested and crushed in liquid nitrogen in a pre-cooled pestle and mortar to make fine powder.
2. The powder was transferred to a 2 ml sterile polypropylene (eppendorf) tube containing 1 ml of extraction buffer (2 % w/v CTAB, 1M Tris HCl, 5M NaCl, 0.5M EDTA, pH 8.0), pre-warmed to 65 °C and 2-3µl of β – Mercaptoethanol was added to it.
3. The samples were incubated at 65°C for 30-60 minutes in a waterbath with intermittent mixing of tubes at every 15 minutes.
4. Then the mixture was centrifuged at 12000 rpm for 15-18 minutes at 4°C.

5. Slowly the supernatant was pipetted out into 2ml eppendorf tube and 500-600µl solution containing chloroform: isoamyl alcohol (24:1 v/v) was added to it and the mixture was shaken well and centrifuged at 12000 rpm for 20 minutes.
6. After centrifugation upper aqueous phase (supernatant) was carefully transferred to new sterile 2ml eppendorf tube and 500 µl freshly prepared phenol: chloroform: isoamyl alcohol (25:24:1) and mixed thoroughly. Then again it was centrifuged at 12000 rpm for 20 minutes.
7. Carefully upper aqueous phase (supernatant) was transferred to new sterile 2ml eppendorf tube and chilled iso-propanol (500-600µl or equal volume of sample) and 3M sodium acetate (40-50 µl) was added and the mixture was kept in −20°C freezer overnight.
8. Next day, tubes were shaken and centrifuged at 14000 rpm for 22 minutes to pellet the DNA.
9. The supernatant was decanted and the pellet was washed with freshly prepared 70 % ethanol (50-100 µl) by centrifuging at12000 rpm for 10-15 minutes.
10. The ethanol was decanted off and the DNA pellet was kept for drying for at least 4-5 hrs. After drying, pellet was suspended by adding 30-50 µl 1X TE buffer (1M Tris-HCl, 0.5M EDTA maintained at pH 8) depending on the size of the pellet. When DNA was fully dissolved, 5 µl of RNAse was added (stock 10 mg/ ml) and incubated at 37 °C for an hour or at room temperature overnight.
11. The extracted DNA samples were stored at -20°C.

3.6.2 DNA quality and quantity estimation

To assess the quality of DNA, samples were separated on 0.8 *per cent* agarose gel in 1x TBE buffer (For 250 ml 10x TBE – 30.27 g of Trisbase, 12.82 g of boric acid and 4 ml of 0.5 M EDTA) and stained with 5µl ethidium bromide (10 mg/ml stock) per 100 ml of gel and checked for shearing of DNA and contamination of RNA. The quantity of DNA was assessed by comparing the band intensity with that of λ DNA.

3.6.3 Optimization of annealing temperature and polymerase chain reaction (PCR) amplification

The parents of RIL mapping populations were genotyped using 612 genomic and groundnut specific SSR markers which are Di and Tri-nucleotide repeats (Appendix 1). For optimization of annealing temperature, PCR was carried out in 10 µl reaction mixture consisting of template DNA and other components required for amplification of the SSR priming regions of genomic DNA (Table 10). Polymerase chain reaction (PCR) for all the primer pairs were performed in 10 µl volume following a touchdown PCR profile in an Eppendorf Master cycler Gradient. Touchdown PCR amplification cycle consisted of an initial denaturation (5 minutes at 95 °C) followed by an initial 35 cycles. The thermo profile for the PCR reaction was set as shown in Table 11. The PCR amplicons of the linked markers were visualized by using 2 *per cent* of each metaphor + agarose gel.

Table 10. PCR reactions for the targeted microsatellites loci used for genotyping of NRCG 12568 × NRCG 12326 RIL population

Components	Concentration per µl	PCR reaction (10 µl)
Primers (F + R) (Eurofins, Netherland)	10 pmol	2.00 µl
dNTP's (Sigma Aldrich, India)	1 mM	1.00 µl
$MgCl_2$	0.5 mM	1.00 µl
PCR buffer (Hi-Media Laboratories, India)	10x	1.00 µl
Taq polymerase (Hi-Media Laboratories, India)	5U/µl	0.30 µl
Double distilled water/ Nuclease free water	-	3.70 µl
Template DNA	5 ng/ µl	1 µl

3.6.4 Separation and visualization of PCR product

The SSR markers which successfully amplified their complementary priming genomic regions of the parents were used to identify those polymorphic between parents (NRSC 12568 and NRCG 12326). Out of 612, 36 amplicons were separated on metaphor + agarose (2 % + 2 %) and 162 amplicons on MultiNa ((Microchip Electrophoresis

System for DNA / RNA Analysis, Shimadzu Biotech, Tokyo, Japan). The protocol of MultiNA is given below.

1. Isolated DNA of 147 RILs was used to prepare PCR reaction mixture for genotyping 198 polymorphic SSR primers.
2. A SYBR GOLD solution prepared by mixing 1 µl SYBR GOLD (Invitrogen) and 99 µl 1X TE buffer in 2ml eppendorf tube.
3. Separation buffer solution mixture was prepared by mixing 2475 µl separation buffer DNA-500 from Shimazdu with 25 µl solution of SYBR GOLD solution in the separation buffer tube.
4. From the marker solution DNA-500 (Shimazdu), 280 µl was pipette out in marker solution tube.
5. DNA ladder of prepared by dissloving 1 µl DNA ladder 25bp (Invitrogen) having volume of 50 µl was with 49 µl 1X TE buffer in a small eppendorf tube.
6. The PCR products in PCR plate was loaded in the MultiNA along with all other solutions.
7. The MultiNA was run for 210 minutes with four chips for reading the samples.

Metaphor agarose gel electrophoresis (MAGE) is another approach to separate alleles of microsatellite markers (Abdurakhmonov *et al*., 2007). Metaphor agarose is an intermediate melting temperature agarose (75° C) that provides twice the resolution capabilities of the finest sieving agarose products. Using submarine gel electrophoresis, metaphor agarose gives high resolution separation of 20 to 800 bp DNA fragments that differ in size by 2 *per cent*, which approximates the resolution of polyacrylamide gels. Metaphor agarose gels (2 % to 4 %) made in TBE and stained with ethidium bromide are ideal for resolving SSRs (Anonymous, 2007). Separation of SSR alleles for two groundnut cultivars with a size difference of five base pairs by using 2 *per cent* standard agarose and 2 *per cent* metaphor agarose was achieved by Asif *et al*., 2008. Hence, MAGE is a reliable and appropriate approach for identification of small length polymorphisms while screening large number of samples. In the present study, 2 *per cent*

Hi-media agarose and 2 *per cent* metaphor agarose (Metaphor™ Agarose - Lonza) were used for gel electrophoresis.

Table 11. Touch-down PCR profile for the targeted microsatellite loci used for genotyping of NRCG 12568 × NRCG 12326 RIL population

Sl. No	**Steps**	**Temperature (°C)**		**Time**	
		60-55 (56)	**65-60 (59)**		
1	Initial denaturation	95	95	15 min	5 cycles
2	Denaturation	94	94	20 sec	
3	Annealing	**60**	**65**	20 sec	
4	Primer extension	**72**	**72**	30 sec	
5	Denaturation	**94**	**94**	20 sec	35 cycles
6	Annealing	**56**	**59**	20 sec	
7	Primer extension	**72**	**72**	30 sec	
8	Final extension	**72**	**72**	20 sec	
9	Hold at (°C)	**4**	**4**	20 sec	

3.6.5 Construction of linkage map and tagging of genes and QTLs

3.6.5.1 Phenotypic evaluation of mapping population

Mapping population was developed as mentioned in the section 3.1.1. To identify the QTLs for yield and drought related traits totally 147 RILs were evaluated for physiological and yield related traits for two seasons of summer 2017 and 2018 under irrigated and water stress conditions. Phenotyping for all traits was done in mapping population as mentioned in the subsection 3.4.3

3.6.5.2 Assessment of parental polymorphism

Initially the parents NRCG 12568 and NRCG 12326 were screened for polymorphism using a total of 612 SSR primers. Out of 612 primers, 198 primers were found polymorphic between the parents (Appendix III).

3.6.5.3 Marker analysis of mapping population

DNA was isolated as per the procedure mentioned in the section 3.6.1 from 147 RILs. Totally 198 polymorphic SSR markers between the parents were selected to screen against the 141 RILs. DNA quality check and polymerase chain reaction was carried out as per the procedure mentioned in the sub section 3.6.2. and 3.6.3. Obtained PCR products were run on horizontal electrophoresis with 4% metaphore agarose gel to assure the clear band appearance. The marker data generated represents the alleles, and were scored as follows.

1. “A” or 2 - Homozygote at marker locus as that of recurrent parent (NRCG 12568).
2. “B” or 0 - Homozygote at marker locus as that of donor parent (NRCG 12326).
3. “U” or -1 - Missing data for the individual at the locus

3.6.5.4 Construction of genetic linkage map

Using genotyping data on 141 RILs, linkage map was constructed with the help of software QTL IciMapping ver 4.0 (Wang *et al.*, 2014). For the map construction, markers were assigned to the linkage group using a LOD threshold of 3 and the maximum distance between markers of 50 cM. Commends ‘group’, ‘order’ and ‘ripple’ were used to organize markers on linkage groups. Linkage groups were oriented by checking markers against known marker positions on genetic maps constructed by Shirasawa *et al*. (2011), Shirasawa *et al*. (2012) Koilkonda *et al*. (2011) and Moretzsohn *et al*. (2005).

3.6.5.5 Single marker analysis

The phenotypic data of surrogate traits of WUE, pods yield and yield related traits and genotypic data of RIL population derived from NRCG 12568 × NRCG 12326 were used to detect SSR markers linked to QTL at LOD score threshold of 2.5 following single marker analysis (SMA) implemented using ‘QTL IciMapping software version 4.0.

3.6.5.6 Identification of QTL controlling surrogate traits of WUE, pod yield and yield related parameters

The phenotypic and genotypic data on randomly 147 $F_{11\ \&\ 13}$ RIL population derived from cross NRCG 12568 × NRCG 12326 of groundnut was used to detect, locate and estimate size effects and unravel the mode of action of the QTL/s controlling traits related to WUE, pods yield and yield related attributes. QTLs were detected at LOD threshold of 2.5 following inclusive composite interval mapping (ICIM) implemented using ‘QTL IciMapping’ software version 4.0. Among the available ones, ‘QTL IciMapping’ is user friendly and supports detection of QTLs based on different kinds of mapping populations.

QTL IciMapping works on the property of additivity of a QTL and epistatic effects between the QTLs. Additive effects of QTL can be completely absorbed by the two flanking markers variables and the epistatic effects between two QTL can be completely absorbed by the four marker-pair multiplication variables between the two pairs of flanking markers. Marker variables were considered in a linear model in ICIM for additive mapping and both marker variables and marker-pair multiplications were simultaneously considered for epistasis mapping. Two steps were included in ICIM. In the first step, step-wise regression was applied to identify the most significant regression variables in both cases but with different probability levels of entering and removing variables. In the second step, a one-dimensional scanning or interval mapping was conducted for mapping additive and a two-dimensional scanning was conducted for mapping digenic epistasis.

ICIM provides intuitive statistics for testing additive, dominance and epistasis and can be used for experimental population derived from two parental lines. The EM algorithm used in ICIM retains all advantages of CIM over IM, and avoids the possible increase of sampling variance and the complicated background marker selection process in CIM. Extensive stimulation using different genomes and various genetic modules indicate that ICIM has increased detection power, reduced false detection rate and less biased estimates of QTL effects compared to CI, in additive and dominance mapping.

Extensive simulation also show that ICIM is an efficient method for epistasis mapping and QTL epistasis networks can be identified no matter whether the two QTLs have any additive effects (user manual-QTL IciMapping). The LOD (Log of Odds) threshold was fixed at 2.5. Biological occurrence of recombination events in a particular genome is influenced by such events in an adjacent region. Since Kosambi mapping function accounts for such interference and multiple cross over events (Hartl and Jones, 1988; Kearsey and Pooney, 1996), the same was used to convert non-additive recombination fraction into additive distance units. Based on the *per cent* phenotypic variation explained by the detected QTL, they were classified into, minor QTL (With <10 % phenotypic variation) and major QTL (With > 10 % phenotypic variation) (Collard *et al*., 2005; Kou and Wang, 2010).

3.6.5.7 QTL validation

Validation of QTL controlling the traits is an essential step in MAS. The QTL validation in this study was performed from the QTLs identified in the cross NRCG 12473 × GKVK 4 and also from the earlier reported QTLs by the researchers in a different genetic background.

IV RESULTS AND DISCUSSION

The aim of plant breeding programme is to develop drought tolerant/high water use efficient cultivars with high productivity for overall crop improvement. Tolerance to drought is quite complex in nature and the networks involved are likely to be conditioned by many genes under strong environmental influence. Improvement through conventional breeding approaches is time-consuming and labour intensive due to its quantitative nature and difficulties in selection for drought tolerance. Recent advances in crop genomics offer tools for assisting breeding through identification and introgression of genomic regions associated with water use efficiency to develop improved cultivars with increased drought tolerance/WUE using marker-assisted selection. To identify the genomic regions suitable for marker-assisted breeding strategies, it is important to establish accurate phenotyping methods followed by development of dense genetic linkage maps/consensus maps and identification of quantitative trait loci (QTL) with traits of interest.

In an effort to improve groundnut for drought tolerance, the present investigation was carried out during 2017 and 2018 summer seasons at K-block UAS, GKVK, Bengaluru to assess the extent of genetic variability for quantitative characters, physiological traits and to identify superior recombinant inbred lines for traits related to water use efficiency and other pod yield traits among 147 RILs derived from the cross NRCG 12568 × NRCG 12326. The mapping population was genotyped with SSR markers at peanut molecular laboratory, Department of Genetics and Plant Breeding, GKVK, Bengaluru to construct a genetic linkage map and to identify QTLs for the traits studied. The results on phenotypic and genotypic evaluation of the RIL population and QTL detection are presented under the following headings;

4.1 Phenotyping of growth, pod yield related parameters and physiological traits in RILs of NRCG 12568 × NRCG 12326 evaluated during summer 2017 and 2018

- 4.1.1 Genetic variability for growth and pod yield related traits
- 4.1.2 Genetic variability for physiological traits
- 4.1.3 Effect of stress on pod yield of the RILs under different water regimes

4.1.4 Association among traits related to WUE and pod yield attributing characters

4.2 Identification of drought tolerant accessions based on drought tolerance indices using Biplot analysis and ranking method.

4.2.1 Drought tolerant indices

4.2.2 Identification of indices that most discriminate the RILs for responses to WS

4.2.3 Identification of water stress tolerant RILs based on rank sum method

4.2.4 Identification of drought tolerant groundnut RILs based on DTI

4.3 Construction of genetic linkage map

4.3.1 Parental polymorphism survey

4.3.2 Marker segregation

4.3.3 Construction of linkage map

4.3.4 Phenotyping data analysis

4.4 QTL mapping for traits related to drought tolerance and pod yield traits

4.4.1 Single marker analysis

4.4.2 Identification of QTLs for growth, yield related traits and surrogate traits of WUE

4.4.3 Di-QTL epistasis

4.4.4 Validation of QTL identified as surrogate traits of WUE and pod yield related traits

4.1 Phenotyping for quantitative and physiological traits in RILs of NRCG 12568 × NRCG 12326 evaluated during summer 2017 and 2018

4.1.1 Genetic variability for growth and pod yield related traits

4.1.1.1 Analysis of variance (ANOVA)

Analysis of variance for the quantitative traits among F_{11} (summer 2017) and F_{13} RILs (summer 2018) evaluated under different water regimes i.e. under well water (WW) throughout growth period and other experiment with water stress at 30-45 DAS, 45-65 DAS and 65-85 DAS for a period of 20 days revealed highly significant mean sum of squares attributable to 'RILs' and 'check varieties' for pod yield related parameters (Table 12 and 13).

Mean sum of squares of 'RILs *vs* check' varieties exhibited significant differences in all the experiments. These results suggest significant differences among the F_{11} and F_{13} RILs. However, RILs as a group significantly differed from the checks for all traits investigated in F_{11} and F_{13} under different water regimes. The results of ANOVA indicating the presence of genetic variability even after achieving maximum homogeneous and homozygosity and the choice of the material for the investigation was appropriate. Therefore, considerable development can be achieved for pod yield and yield attributes by simple selection. This was further supported by the fact that range was also quite wider for all the characters pointing out extreme recombinant inbred lines were available for selection.

However, analysis of variance by itself is inconclusive in explaining all the inherent genetic variability among the RILs. This is evident by partitioning the total variability inherent in the RILs from the phenotypic variance. Gopinath Jatti *et al*. (2008); Savitha (2012); Mallikarjun (2014) and Bhavya (2015) have also reported significant differences among the genotypes for the pod yield attributes in groundnut. Pooled ANOVA over three moisture regimes also indicated significant genetic differences for all the traits across two seasons (Table 14) among RILs, checks and also their interactions for all the traits studied. Though significant, low magnitude of correlation coefficient between performance of genotypes under different water regimes (Fig. 4, 5 and 6) in both the seasons suggested that genotype × environment interaction is largely of cross-over type.

4.1.1.2 Mean, range and components of variation

Mean and range of quantitative traits under WW and WS conditions under different water regimes during the summer 2017 and 2018 are presented in the Table 15. The nature and magnitude of variation for quantitative traits was assessed by phenotypi0c

Table 12. Analysis of variance for pod yield and its component traits in F_{11} recombinant inbred lines of the cross NRCG 12568 × NRCG 12326 in groundnut under water stress (WS) and well-watered condition (WW) during 2017

Source	Expt	df	DFF	PH (cm)	B/P	P/P	PY/P	KY/P	SP (%)	SMK (%)
Blocks (eliminating checks + RILs)	WW	6	0.56	1.89	0.19	8.68	11.92	5.99	12.23	32.94
	WS2	6	2.57	4.96	0.12	16.65 *	10.58	2.60	178.67 *	48.10
	WS3	6	1.74	38.90	0.33	52.87	44.39	15.51	64.19	14.37
RILs + Check	WW	150	4.97 *	58.11 ***	0.32	62.85 ***	35.91 ***	17.91 **	132.91 **	168.06 ***
	WS2	150	8.81 **	36.72 **	0.68 **	73.89 ***	44.82 ***	13.94 ***	136.25 *	133.11 **
	WS3	150	7.05 ** *	41.22 *	0.41 *	54.08	39.38	18.13	86.47	94.77 *
Checks	WW	3	12.43 **	446.78 ***	0.15	290.67 ***	215.88 ***	334.90 **	178.32 ***	1288.72 ***
	WS2	3	32.03 ***	235.52 ***	0.45	626.46 ***	422.90 ***	129.69 ***	16.08	406.46 ***
	WS3	3	26.98 ***	378.94 ***	2.07 ***	65.93	61.86	36.19 *	81.09	142.62 *
RILs	WW	146	4.63 *	45.41 ***	0.32	57.44 ***	29.21 **	12.49 *	124.94 *	145.29 ***
	WS2	146	8.35 *	32.87 **	0.68 **	57.89 ***	35.89 ***	11.35 ***	137.65 *	128.31 **
	WS3	146	6.53 **	32.94	0.38	46.26	36.42	15.35	77.21 *	94.01 *
Checks *vs.* RILs	WW	1	32.52 **	746.91 ***	0.07	168.42 ***	473.59 ***	327.56 ***	690.59 **	131.14 **
	WS2	1	6.75 ***	2.29	0.92 **	753.57 ***	213.32	45.54 ***	292.18 *	13.19
	WS3	1	22.49 **	237.73 **	0.50	1160.33 ***	403.52 ***	369.40 ***	1454.21 ***	64.24
Error	WW	18	2.17	5.84	0.27	8.77	8.23	5.63	49.60	13.01
	WS2	18	3.29	9.72	0.18	4.71	5.01	2.58	54.90	38.02
	WS3	18	1.85	18.79	0.20	31.07	21.84	11.29	37.26	40.41

*Significance @ P = 0.05 ** Significance @ P = 0.01 ***Significance @ P = 0.001

DFF- Days to 50% flowering PH- Plant height (cm) B/P- Branches plant^{-1} P/P- Pods plant^{-1}

PY/P- Pod yield plant^{-1}(g) KY/P- Kernel yield plant^{-1}(g) SP- Shelling percentage SMK- Sound Mature Kernel

Table 13. Analysis of variance for pod yield and its component traits in F_{13} recombinant inbred lines of the cross NRCG 12568 × NRCG 12326 in groundnut under water stress (WS) and well-watered condition (WW)

Source	Expt	df	DFF	PH (cm)	B/P	P/P	PY/P	KY/P	SP (%)	SMK (%)
Blocks (eliminating checks + RILs)	WW	6	3.79	3.92	0.06	32.47	20.62	12.88	43.41	146.52
	WS1	6	2.03	36.12	0.22	15.52	19.03	5.41	137.72	43.79
	WS2	6	5.071 *	5.84	0.59	0.96	0.78	0.65	11.46	37.12
	WS3	6	1.39	19.76	0.12	38.97	7.94	5.97	132.01	31.42
RILs + Check	WW	150	4.77	70.04 ***	0.34	50.93	22.63	8.54	45.35	165.09
	WS1	150	4.53 **	23.98	0.24	45.98 ***	19.67 *	5.50	79.30	58.93
	WS2	150	4.404 *	39.92 ***	0.31	49.49 ***	29.30 **	12.01	114.33	296.17 **
	WS3	150	1.04	40.73	0.11	39.50 *	15.91 **	5.66	104.18	315.56 *
Checks	WW	3	31.14 ***	193.88 ***	1.39 *	62.30	31.14	25.93	156.21	235.66
	WS1	3	17.57 ***	163.01 **	0.13	173.64 ***	34.09 *	11.84 *	31.33	44.27
	WS2	3	27.36 ***	142.81 ***	0.52	168.27 ***	144.01 ***	130.95 ***	236.43 *	128.69
	WS3	3	2.52	229.10 **	0.02	81.27 *	59.65 ***	30.84 ***	82.54	527.17 *
RILs	WW	146	4.23	63.57 ***	0.32	50.00	22.57	8.18	35.59	163.44
	WS1	146	4.24 **	18.71	0.24	43.26 ***	18.36 *	5.19	79.11	59.63
	WS2	146	3.96 *	37.73 **	0.30	46.62 ***	27.08 **	8.30	52.47	281.25 **
	WS3	146	1.01	37.04	0.11	38.75 *	15.11 **	5.13	91.42	306.54 *
Checks *vs.* RILs	WW	1	4.59 **	643.03 ***	0.37	151.4	6.41	9.15	1137.78	194.79
	WS1	1	8.71 *	377.22 **	0.24	60.45 **	167.18 ***	32.22 **	251.92	1.51
	WS2	1	0.07	50.13 *	0.28	111.97 **	9.41	197.53 ***	8779.39 ***	2976.75 ***
	WS3	1	1.61	13.79	0.01	23.34	1.60	6.84	2032.20 ***	997.74 *
Error	WW	18	2.98	14.65	0.39	45.35	28.61	15.90	81.72	176.42
	WS1	18	1.43	24.59	0.13	5.95	8.18	2.93	217.52	86.84
	WS2	18	1.90	10.22	0.47	8.95	9.51	9.21	66.75	87.56
	WS3	18	1.11	28.44	0.10	16.74	5.59	2.94	114.55	131.14

*Significance @ P =0.05 ** Significance @ P = 0.01 ***Significance @ P = 0.001

DFF- Days to 50% flowering

PH- Plant height (cm)

B/P- Branches plant^{-1}

P/P- Pods plant^{-1}

PY/P- Pod yield plant^{-1}(g)

KY/P- Kernel yield plant^{-1}(g)

SP- Shelling percentage

SMK- Sound Mature Kernel

Table 14. Pooled analysis of variance for pod yield and its component traits in F_{11} and F_{13} recombinant inbred lines of the cross NRCG 12568 × NRCG 12326 in groundnut under water stress (WS) and well-watered condition (WW)

Source	Expt	df	DFF	PH (cm)	B/P	P/P	PY/P	KY/P	SP (%)	SMK (%)
Blocks (eliminating checks + RILs)	WW	6	1.13	1.20	0.05	12.29	9.47	6.52	9.80	32.88
	WS2	6	3.21	4.23	0.25	3.66	2.54	0.90	64.86	13.56
	WS3	6	1.32	15.20	0.13	14.39	9.24	2.93	69.20	17.67
RILs + Check	WW	150	2.59 **	58.13 ***	0.19	35.41 **	18.01	8.47	42.03	81.63
	WS2	150	3.67 **	29.28 ***	0.27 *	37.762 ***	23.86 ***	8.55 ***	60.012 *	112.59 **
	WS3	150	2.09 *	23.23 *	0.19 **	24.66 **	15.09 **	6.64 **	47.34	116.48 **
Checks	WW	3	14.91 ***	299.48 ***	0.52 *	150.95 ***	101.72 ***	84.25 ***	236.20 **	585.67 ***
	WS2	3	29.21 ***	184.17 ***	0.43 *	359.67 ***	264.24 ***	129.45 ***	46.23	231.08 **
	WS3	3	11.36 ***	298.15 ***	0.57 ***	46.73 **	50.21 ***	30.35 ***	79.49	262.09 **
RILs	WW	146	2.25 *	48.81 ***	0.19	33.27 *	15.78	6.21	32.16	71.82
	WS2	146	3.16 *	26.17 **	0.26 *	30.91 ***	18.55 ***	5.39 *	50.65 *	106.49 **
	WS3	146	1.85 *	17.12	0.18 **	22.91 **	13.86 **	5.39 *	35.15	111.60 **
Checks *vs.* RILs	WW	1	15.39 ***	694.68 ***	0.03	0.11	92.61 **	111.81 ***	900.76 ***	1.56
	WS2	1	3.16 *	26.17 **	0.26 *	30.91 ***	18.55 ***	5.390 *	50.65 *	106.49 **
	WS3	1	9.03 **	91.44 **	0.17	213.64 ***	88.57 ***	119.18 ***	1730.62 ***	392.26 **
Error	WW	18	0.89	7.08	0.13	13.34	10.00	5.49	34.56	47.31
	WS2	18	1.28	6.86	0.13	4.10	3.59	2.17	25.70	30.36
	WS3	18	0.85	9.95	0.06	8.04	4.19	2.21	33.71	38.21

*Significance @ P =0.05   ** Significance @ P = 0.01   ***Significance @ P = 0.001

DFF- Days to 50% flowering   PH- Plant height (cm)   B/P- Branches plant^{-1} P/P- Pods plant^{-1}

PY/P- Pod yield plant^{-1}(g)   KY/P- Kernel yield plant^{-1}(g)   SP- Shelling percentage   SMK- Sound Mature Kernel

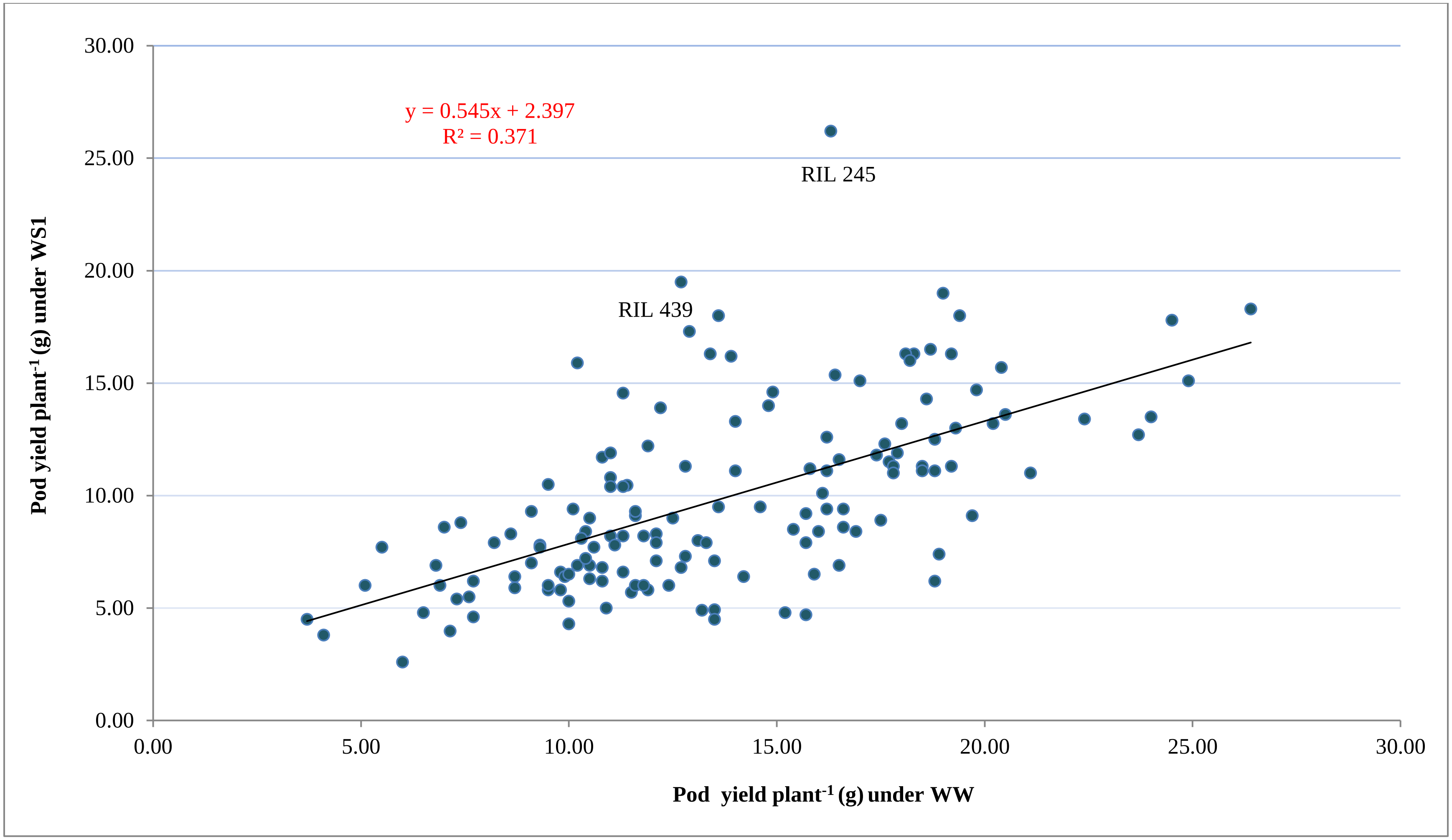


Fig. 4. Relationship between the RILs developed under water stress I and well watered conditions for pod yield plant^{-1}in groundnut during summer 2018

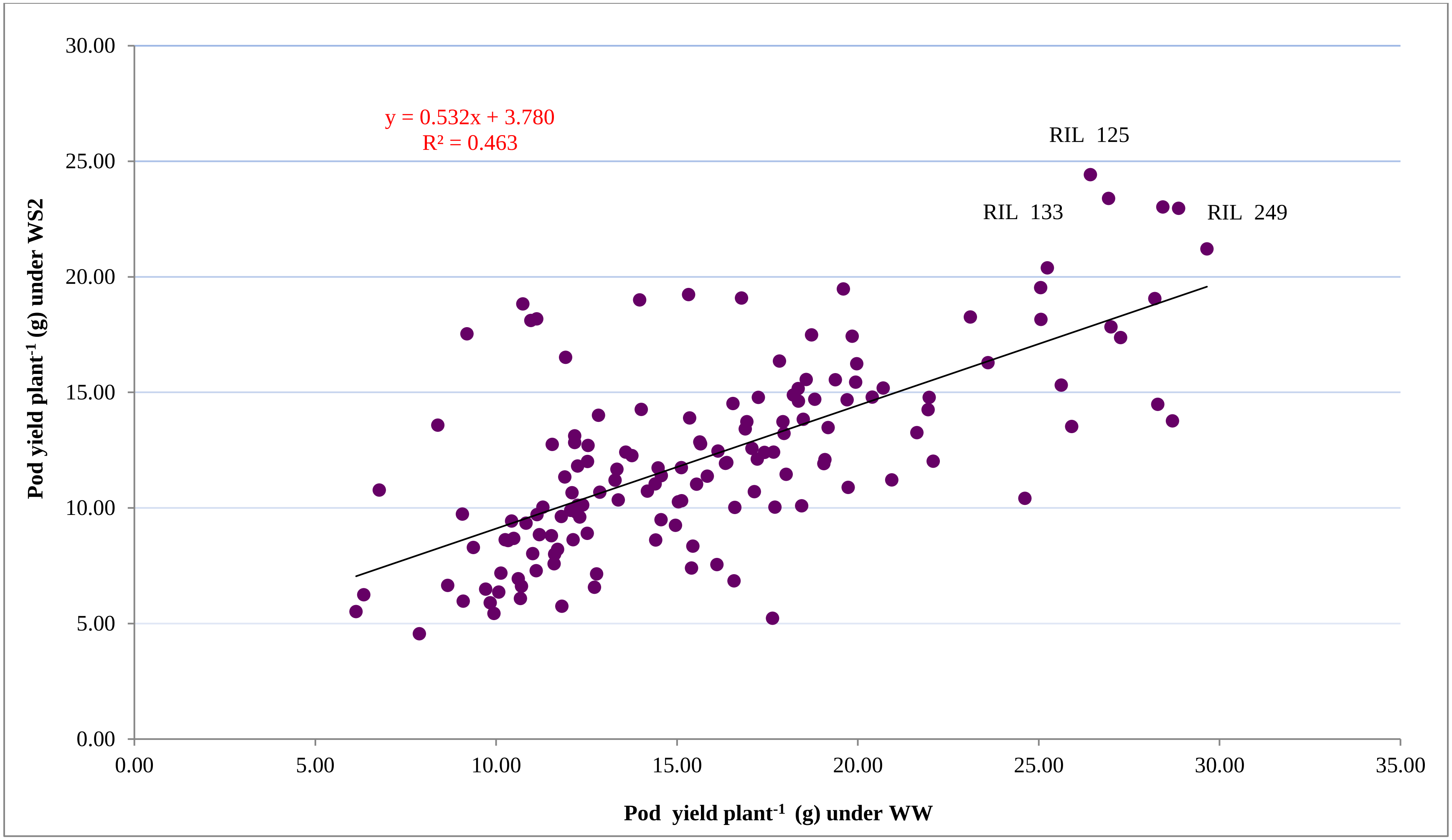


Fig. 5. Relationship between the RILs under water stress II and well watered conditions for pod yield plant^{-1} in groundnut (pooled data over two years)

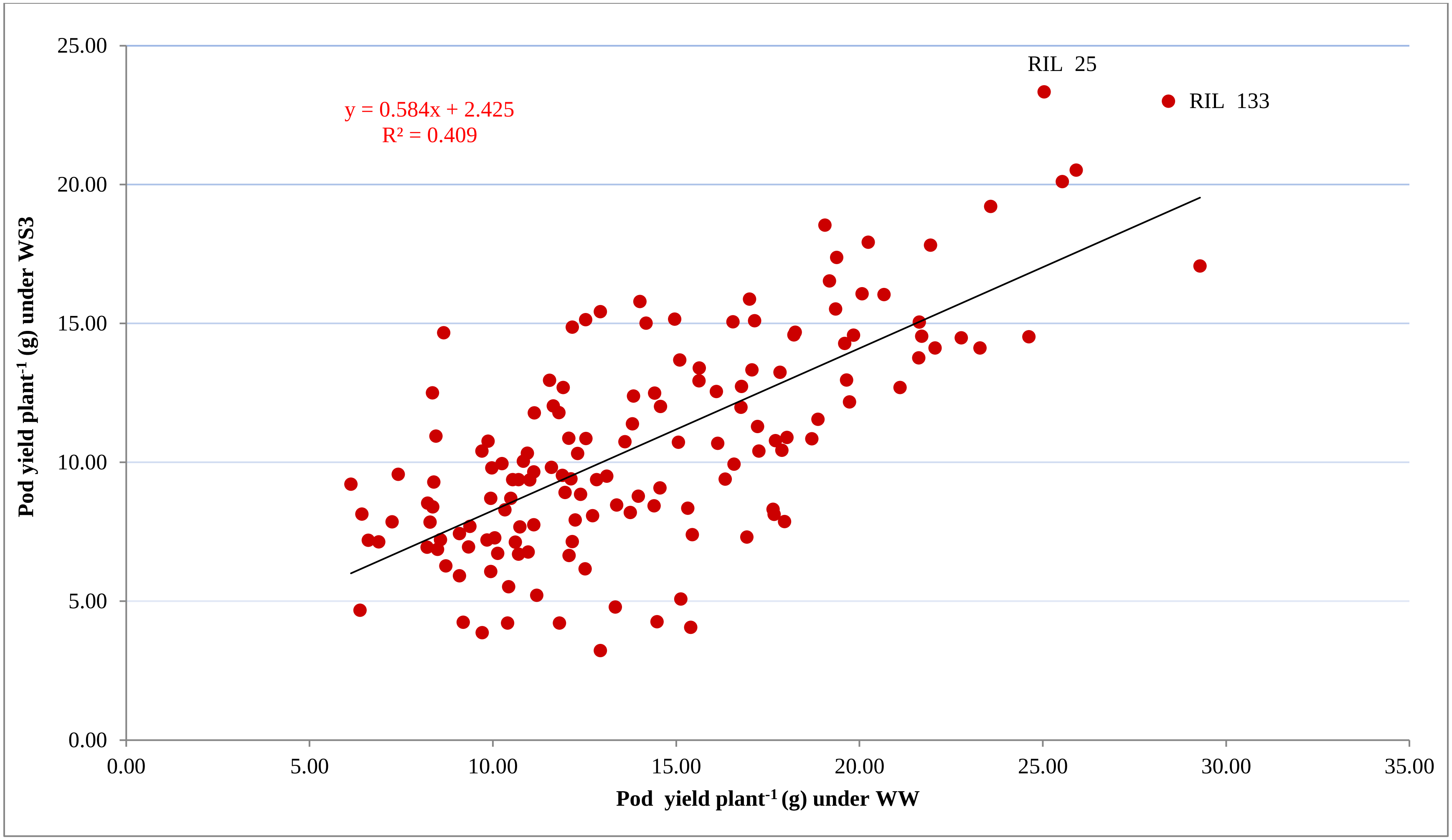


Fig. 6. Relationship between the RILs under water stress III and well watered conditions for pod yield plant^{-1} in groundnut (pooled data over two years)

coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability and genetic advance as *per cent* mean for the two consecutive summer seasons are presented in Table 16. Trait wise discussion with respect to genetic estimates and *per se* performance are described below

Days to fifty *per cent* flowering (DFF)

Days to fifty *per cent* flowering varied between the range of 38.00 - 48.00 with an average of 40.98 and 42.16 and a standardized range of 0.24 of F_{11} and F_{13} RILs respectively under well water conditions and it is coupled with moderate heritability (49.75 and 36.79) and GAM (15.19 and 12.64). Under water stress I conditions F_{13} RILs recorded a range of 36.00 - 46.00 with an average of 42.39 and standardized range of 0.24. The genetic estimate GCV was found lower than PCV indicated the role of environment and noticed high heritability (63.00) coupled with low GAM (16.02) indicated that trait is under the control of non-additive gene action (Table 15 and 16).

During water stress II conditions DFF ranged between 38.00 - 47.00 and 39.00 - 48.00 with an average of 41.14 and 42.25 having a standardized range of 0.22 and 0.21 in F_{11} and F_{13} RILs, respectively. The genetic estimate GCV was found less than PCV with narrow difference indicates the lower influence of environment and its supported by moderate heritability (57.29 and 48.61) with low GAM (17.96 and 14.53) indicated that the trait is under non - additive gene action. Under water stress III conditions F_{11} and F_{13} RILs for DFF ranged between 38.00 – 45.00 and 39.00 -48.00 with an average 41.87 and 41.33 having a standardized range of 1.45 and 2.38, respectively. The genetic estimate GCV was found lower than PCV indicated contribution of environment on trait expression. It recorded moderate heritability (58.78 and 48.71) coupled with moderate GAM (18.24 and 16.44) indicated that the trait is under the control of non-additive gene action. This concludes high influence of environment, inherited by non-additive gene action and less variability with respect to this trait. These results are in agreement with Thakur *et al*. (2011), Nageshwar Rao *et al*. (2012), Vishnuvardhan *et al*. (2012), Sunday and Omolayo (2013), Bhavya (2015), Anusha (2015) and Savita *et al*. (2017).

Table 15. Mean, range and standardized range of pod yield and its component traits in F_{11} and F_{13} RILs of the cross NRCG 12568 × NRCG 12326 in groundnut under different water regimes

Character	Expt.	Mean		Range		Standardized range	
		F_{11}	F_{13}	F_{11}	F_{13}	F_{11}	F_{13}
DFF	WW	40.98	42.16	38.00 - 48.00	38.00 - 48.00	0.24	0.24
	WS1	-	42.39	-	36.00 - 46.00	-	0.24
	WS2	41.14	42.45	38.00 - 47.00	39.00 - 48.00	0.22	0.21
	WS3	41.87	41.33	38.00 - 45.00	39.00 - 45.00	0.17	0.15
PH	WW	20.90	20.97	10.30 - 40.50	10.30 - 60.30	1.45	2.38
	WS1	-	20.73	-	10.30 - 30.40	-	0.97
	WS2	23.07	23.26	10.30 - 40.40	11.40 - 43.50	1.30	1.38
	WS3	31.57	23.23	20.30 - 50.40	10.96 - 40.71	0.95	1.28
B/P	WW	4.77	4.77	4.00 - 6.00	4.00 - 6.00	0.42	0.42
	WS1	-	4.96	-	4.00 - 5.68	-	0.34
	WS2	4.71	4.84	4.00 - 6.00	4.00 - 6.00	0.42	0.41
	WS3	4.78	4.30	4.00 - 6.00	3.75 - 5.13	0.41	0.32
P/P	WW	23.79	17.84	10.20 - 46.60	3.00 - 48.60	1.53	2.56
	WS1	-	15.08	-	2.40 - 34.20	-	2.11
	WS2	19.16	16.22	4.00 - 47.00	4.20 - 36.80	2.24	2.01
	WS3	17.13	12.21	4.40 - 35.00	1.40 - 35.80	1.78	2.82
PY/P	WW	13.75	10.95	5.58 - 29.50	2.10 - 36.20	1.74	3.11
	WS1	-	9.55	-	1.20 - 26.20	-	2.62
	WS2	13.06	10.78	2.78 - 36.18	2.00 - 30.40	2.56	2.63
	WS3	13.28	7.20	2.30 - 30.88	0.30 - 18.30	2.15	2.50
KY/P	WW	8.38	6.31	2.24 - 17.06	1.00 - 22.20	1.77	3.36
	WS1	-	5.09	-	0.60 - 12.60	-	2.36
	WS2	7.73	6.09	1.82 - 20.40	1.10 - 17.20	2.43	2.64
	WS3	8.35	4.01	1.76 - 18.32	0.10 - 11.70	1.98	2.89
SP	WW	61.02	57.54	23.33 - 85.53	37.04 - 75.68	1.02	0.67
	WS1	-	54.22	-	19.23 - 70.89	-	0.95
	WS2	60.54	57.00	24.72 - 79.05	38.27 - 83.33	0.89	0.79
	WS3	63.26	55.50	26.27 - 87.83	12.64 - 72.22	0.97	1.07
SMK	WW	72.94	71.07	41.43 - 94.05	40.23 - 92.96	0.72	0.74
	WS1	-	82.08	-	55.56 - 100.00	-	0.54
	WS2	76.16	64.37	35.74 - 96.00	19.35 - 98.41	0.79	1.23
	WS3	86.32	72.04	52.38 - 98.36	31.65 - 98.25	0.53	0.92

DFF- Days to 50% flowering
PH- Plant height (cm)
B/P- Branches plant^{-1}
P/P- Pods plant^{-1}
PY/P- Pod yield plant^{-1}
KY/P- Kernel yield plant^{-1}
SP- Shelling percentage
SMK- Sound Mature Kernel
WW – Well watered
WS – Water stress

Table 16. Genetic parameters for pod yield and its component traits in F_{11} and F_{13} RILs of the cross NRCG 12568 × NRCG 12326 in groundnut under different water regimes

Character	Expt.	GCV		PCV		h^2 (bs)		GAM	
		F_{11}	F_{13}	F_{11}	F_{13}	F_{11}	F_{13}	F_{11}	F_{13}
DFF	WW	3.58	2.48	5.07	4.78	49.75	36.79	15.19	12.64
	WS1	-	3.68	-	4.64		63.00	-	16.02
	WS2	5.10	3.16	6.74	4.53	57.29	48.61	17.96	14.53
	WS3	4.82	2.44	5.81	4.98	58.78	48.71	18.24	16.44
PH	WW	28.06	31.09	30.35	36.05	85.48	74.37	53.44	55.23
	WS1	-	17.88	-	21.29	-	26.27	-	11.52
	WS2	19.45	21.03	23.68	25.12	67.44	70.06	32.90	36.25
	WS3	11.11	11.77	17.66	25.79	39.57	20.83	14.39	11.07
B/P	WW	4.41	8.98	11.79	12.01	13.96	18.96	13.39	14.69
	WS1	-	6.48	-	9.63	-	45.35	-	18.99
	WS2	14.03	9.23	16.69	11.76	70.67	44.45	24.30	10.76
	WS3	8.28	1.78	12.55	7.59	43.53	35.52	11.25	10.86
P/P	WW	27.34	11.26	30.04	39.38	82.84	80.18	51.26	60.64
	WS1	-	37.76	-	41.08	-	84.51	-	71.50
	WS2	35.47	35.28	37.24	39.81	90.76	78.53	69.62	64.40
	WS3	21.21	35.83	38.83	49.07	49.83	53.33	23.86	33.90
PY/P	WW	31.05	32.87	37.41	44.14	68.92	42.45	33.10	20.42
	WS1	-	31.17	-	43.24	-	51.98	-	26.29
	WS2	39.68	36.25	43.22	46.17	84.26	61.63	75.03	28.62
	WS3	26.80	39.97	44.23	51.74	36.73	59.66	23.47	33.59
KY/P	WW	29.12	42.76	40.61	48.03	51.43	72.98	43.02	42.20
	WS1	-	27.50	-	43.43	-	40.09	-	25.87
	WS2	35.73	41.34	41.33	47.66	74.74	69.40	53.63	29.23
	WS3	22.49	34.43	46.11	54.87	53.80	49.38	22.60	24.52
SP	WW	13.26	9.76	17.58	11.21	56.90	26.31	20.60	12.25
	WS1	-	11.65	-	18.19	-	23.71	-	16.35
	WS2	13.86	8.76	18.41	12.93	56.71	52.83	21.50	16.08
	WS3	9.31	13.34	13.41	17.51	48.24	42.28	13.33	12.68
SMK	WW	14.70	15.65	15.51	18.08	69.84	56.83	28.70	22.54
	WS1	-	6.12	-	9.69	-	37.43	-	17.47
	WS2	11.63	20.15	14.17	24.85	67.36	65.78	19.67	33.67
	WS3	7.91	17.14	10.35	23.37	46.45	53.76	32.15	25.88

DFF- Days to 50% flowering
PH- Plant height (cm)
B/P- Branches plant^{-1}
P/P- Pods plant^{-1}
PY/P- Pod yield plant^{-1}(g)
KY/P- Kernel yield plant^{-1} (g)
SP- Shelling percentage
SMK- Sound Mature Kernel
WW – Well watered
WS – Water stress

Plant height (cm)

The plant height of RILs ranged between 10.30 - 40.50 and 10.30 - 60.30 with an average of 20.90 and 20.97 having a standardized range of 1.45 and 2.38 of F_{11} and F_{13} RILs respectively, under well water conditions, and its coupled with high heritability (85.48 and 74.37) and moderate GAM (33.44 and 35.23), while the difference between the GCV and PCV found less indicated the lower influence of environment and prevalence of additive gene action on plant height. Whereas, under water stress I plant height of RILs ranged between 10.30 cm-30.40 cm with an average of 20.73 cm and standardized range of 0.97cm in F_{13} RILs, it is coupled with high heritability (66.27) with low GAM (11.52) indicated that the selection is not effective at early stage as it inherited by non-additive genes and the lower difference between GCV and PCV indicated the less influence of environment on plant height. (Table 15 and 16).

During water stress II plant height of RILs varied between 10.30-40.40 and 11.40 – 43.50 with an average of 23.07 and 23.26 having an standardized range of 1.30 and 1.38 of F_{11} and F_{13} RILs, respectively and it is coupled with high heritability (67.44 and 70.06) with moderate GAM (32.90 and 36.25) indicated that the selection not effective at earlier stage as it inherited by non-additive genes and the difference between the GCV and PCV found less indicated the lower influence of environment on plant height. In case of water stress 3 varied between 20.30 – 50.40 and 10.96 and 40.71 with an average of 31.57 and 23.23 and recorded standardized range of 0.95 and 1.28 of F_{11} and F_{13} RILs respectively. The trait recorded moderate heritability(39.57 and 30.88) and low GAM (14.39 and 11.07) indicated that the trait is controlled by non-additive genes and the difference between GCV and PCV was found less indicate the negligible effect on the trait of interest (Table 15 and 16).

Hence the trait is less influenced by environment under well water and water stress condition and the selection is also not effective as it is controlled by non-additive genes. These results are found in accordance with the results of Sarvamangala (2009), John *et al.* (2011), Thakur *et al.* (2011), Vishnuvardhan *et al.* (2012), Ganapati Mukri *et al.* (2014) and Mahalakshmi *et al.* (2018) in groundnut.

Branches plant $^{-1}$

The number of branches plant^{-1} showed similar range of 4.0 - 6.0 and recorded mean of 4.77 with standardized range of 0.42 under well water conditions in both F_{11} and F_{13} generation RIL population (Table 15). The genetic parameters PCV was found higher than GCV indicating the interference of environment, while it is coupled with low broad sense heritability and low GAM where selection is not effective. Under water stress I conditions branches plant^{-1} of F_{13} RILs ranged between 4.0 - 5.68 with an average of 4.96 having standardized range of 0.34. The genetic estimate PCV was found higher than GCV indicated that trait is under the influence of environment and it recorded moderate heritability (45.35) coupled with low GAM (8.99) indicated that the selection is not effective as it controlled by additive genes (Table 15 and 16).

The number of branches plant^{-1} in F_{11} and F_{13} generation of RIL population during water stress II ranged between 4.0 - 6.0 with an average of 4.71 and 4.84 having standardized range of 0.41 and 0.42, respectively. The GCV was found lesser than PCV indicated the role of environment on the trait, whereas high heritability (70.67 and 44.45) coupled with low GAM indicated the selection is less effective. During water stress III the RILs in F_{11} and F_{13} generation recorded branches plant^{-1} with a range of 4.00 - 6.00 and 3.75 - 5.13 with an average of 4.78 and 4.30 along with the standardized range of 0.41 and 0.32, respectively. The GCV was found lower than PCV indicated the role non additive genes and presence of environment effect, moderate heritability (43.53 and 35.52) with low GAM (7.25 and 5.86) indicates selection is not effective (Table 15 & 16).

From the above results it is evident that the trait branches plant^{-1} under well water and water stress conditions are under the influence of environment and non-additive effects of genes hence selection is not effective. The results are found similar with the results of Channayya (2009), Korat *et al.* (2009), Sudha *et al.* (2012) John *et al.* (2011) and Vishnuvardhan *et al.* (2012) and Savita *et al.* (2014).

Pods Plant^{-1}

The pods plant^{-1} under well water conditions in both the generation i.e. F_{11} and F_{13} RILs recorded a range varying from 10.20 - 46.60 and 13.00 - 48.60 with an average of 23.79 and 17.84 having a standardized range of 1.53 and 2.56, respectively. The genetic estimate GCV was found lower than PCV in F_{11} coupled with high heritability (82.84) with high GAM (51.26 and 60.64) indicated the selection is effective. Under water stress I, pods plant^{-1} ranged from 12.40 - 34.20 with an average of 15.08 having standardized range of 2.11 in F_{13} generation. The PCV was found higher than GCV indicated the role of environment; high heritability (84.51) coupled with high GAM (71.50) indicated that selection is effective. (Table 15 and 16).

The pods plant^{-1} F_{11} and F_{13} RILs varied from 4.00-47.00 and 4.20-47.00 with an average of 19.16 and 16.22 having standardized range of 2.24 and 2.01, respectively in water stress II. For pods plant^{-1} the GCV was found lesser than PCV indicated the role of environment and it recorded high heritability (90.76 and 78.53) coupled with high GAM (69.62 and 64.40) indicated the selection for this trait is effective as its controlled by most of additive gene effects than non-additive effects under water stress II condition. During water stress III the pods plant^{-1} was ranged between 4.40 – 35.00 and 11.40 - 35.80 with an average of 17.13 and 12.21 with a standardized range of 1.78 and 2.82 in F_{11} and F_{13} RILs respectively. The PCV recorded higher value than GCV coupled with moderate heritability (49.83 and 53.33) and moderate GAM (23.86 and 33.90) indicated the role of environment and non-additive effects suggested that selection is less effective (Table 15 and 16).

It is evident from results that the pods plant^{-1} is controlled by non-additive gene action and influenced by environment under well water and water stress conditions except during water stress I. Similar results are found by John *et al.* (2011), Sunday and Omolayo (2013), Alam *et al*. (2013), Yadav *et al*. (2014), Satish *et al.* (2014), Satyanarayana *et al*. (2014), Sanjeevakumar *et al*. (2015) and Mahalakshmi *et al*. (2018).

Pod yield plant^{-1}(g)

The pod yield plant^{-1} ranged between 5.58 - 29.50 and 2.10 - 36.20 with mean yield of 13.75 and 10.95 having a standardized range of 1.74 and 3.11 in $F_{11}$and F_{13} RILs, respectively under well water conditions. The genetic estimate GCV was found lower than PCV indicating influence of environment and the heritability (48.92 and 42.45) and GAM (33.10 and 20.42) found moderate indicated the non-additive gene action. During water stress I the F_{13} RILs recorded pod yield plant^{-1}(g) from 1.20 - 26.20 with an average of 9.55 and standardized range of 2.62. The GCV was found lower than PCV indicated the role of environment on the trait and it is controlled by non-additive as it recorded higher heritability (51.98) with moderate GAM (26.29) (Table 15 and 16).

The F_{11} and F_{13} RILs recorded a wide range of 2.78 - 36.18 and 2.00 - 31.40 with an average yield of 13.06 and 10.78, respectively for pod yield plant^{-1}(g) and recorded standardized range of 2.56 and 2.63 under water stress II. The genetic estimate GCV was found lower than PCV indicated the influence of environment, high heritability (84.26 and 61.63) coupled with moderate GAM (45.03 and 28.62) indicated that the trait is under the control of non-additive gene action hence selection is not effective. During water stress III, pod yield plant^{-1}(g) ranged between 2.30 - 30.88 and 0.30 - 18.30 with an average of 13.28 and 7.28 having standardized range of 2.15 and 2.50 in F_{11} and F_{13} RILs, respectively. The PCV recorded higher value than GCV coupled with moderate heritability (56.73 and 59.66) with moderate GAM (23.47 and 33.59) indicated the role of environment and non-additive effects suggested that selection is less effective (Table 15 and 16).

The wide range of variation and standardized range in F_{11} and F_{13} RILs under both WW and WS condition suggested that trait may be improved by individual plant selection. The estimates of standardized range across traits provide clues about the occurrence of genotypes with extreme expression**.** It is clear from the results that pod yield plant^{-1} (g) is under the control of non-additive gene action. These results are comparable with those of Painawadee *et al.* (2009), Sarvamangala (2009), Zaman *et al*. (2011), Rao *et al*. (2012), Sunday and Omolayo (2013), Alam *et al*. (2013), Yadav *et al*.

(2014), Satish *et al*. (2014), Satyanarayana *et al*. (2014), Mallikarjun (2014), Bhavya (2015); Anusha (2015), Arun (2016), Sanjeevakumar *et al*. (2015) and Mahalakshmi *et al*. (2018).

Kernel yield plant^{-1} (g)

The kernel yield plant^{-1}(g) ranged between 2.24 - 17.06 and 1.00 - 22.20 with an average yield of 8.38 and 6.31 having standardized range of 1.77 and 3.36 in $F_{11}$and F_{13} RILs, respectively under well water conditions. The genetic estimate PCV was found higher than GCV indicating influence of environment and the heritability (51.43 and 72.98) and GAM (33.02 and 32.20) found moderate indicated the non-additive gene action suggesting selection is not effective. During water stress I, the F_{13} RILs recorded kernel yield plant^{-1}(g) from 0.60 - 12.60 with an average of 5.09 and standardized range of 2.36. The GCV was found lower than PCV indicated the role of environment on the trait and it is controlled by non-additive as it recorded moderate heritability (40.09) with moderate GAM (25.87) (Table 15 and 16).

The F_{11} and F_{13} RILs recoded a wide range of 1.82 - 20.40 and 1.10 - 17.20 with an average of 7.73 and 6.09, respectively for kernel yield plant^{-1}(g) and recorded standardized range of 2.43 and 2.654 under water stress II. The genetic estimate GCV was found lower than PCV indicated the influence of environment, high heritability (74.74 and 69.40) coupled with moderate GAM (23.63 and 29.23) indicated that the trait is under the control of non-additive gene action hence selection is not effective. During water stress III, kernel yield plant^{-1}(g) ranged between 1.76 - 18.32 and 1.10 - 17.20 with an average of 7.73 and 6.09 having a standardized range 2.43 and 2.64 in F_{11} and F_{13} RILs, respectively. The PCV recorded higher value than GCV coupled with moderate heritability (53.80 and 49.38) with moderate GAM (22.60 and 24.52) indicated the influence of environment on the trait and their non additive effects suggested that selection is less effective (Table 15 and 16).

The close correspondence between the estimates of GCV and PCV for kernel yield per plant in RILs indicated less environmental influence on expression of this trait. Hence, selection for these characters could be made based on RILs phenotypic performance. It is evident from the results that kernel yield plant^{-1}(g) is under the control of non-additive gene action. The results are comparable with the results of Rudraswamy *et al.* (1999), Apte *et al.* (2008), John *et al*. (2011), Venkateshmurthy (2005), Kavera (2008), Sarvamangala (2009), Azharudheen (2010) and Vishnuvardhan *et al.* (2012).

Shelling percentage (%)

Shelling percentage (%) ranged between 23.33 - 85.53 and 37.04 - 75.68 with a mean of 61.02 and 57.54 and recorded the standardized range of 1.02 and 0.67 in F_{11} and F_{13} RILs, respectively under well water condition. The PCV was found higher than GCV coupled with high heritability (56.91 and 56.31) and low GAM (20.60 and 12.25) indicating the role of environment and non-additive gene action. During water stress 1 the F_{13} RILs recorded a range of 19.23% -70.89% for shelling percentage with an average of 54.22% and standardized range of 0.95%. The genetic estimate GCV was found lower than PCV indicated the role of environment and noticed moderate heritability (43.71) coupled with low GAM (16.35) indicated that trait is under the control of non-additive gene action (Table 15 and 16).

Under water stress II conditions the shelling percentage of F_{11} and F_{13} RILs exhibited range of 24.72 - 79.05 and 38.27 - 83.33 with an average of 60.54 and 57.00 having standardized range of 0.97 and 1.07. The PCV was found higher than GCV indicated the role of environment and moderate heritability (48.24 and 42.28) coupled low GAM (21.50 and 16.08) suggesting that the trait is governed by non-additive gene action. During water stress III, the shelling percentage of F_{11} and F_{13} RILs ranged between 26.27 - 87.83 and 12.64 - 72.22 with an average of 63.26 and 55.50 and recorded a standardized range of 0.97 and 1.07. The GCV was lower than PCV inferring environmental influence on the trait, while low heritability (48.24 and 42.28) coupled with low GAM (13.33 and 12.68) indicated that the trait is controlled by non-additive genes and selection is not effective (Table 15 and 16).

The wide range of variation and standardized range in F_{11} and F_{13} RILs under both WW and WS condition suggesting that possibility of selection of RILs with extreme expressions. On the whole co-efficient of variation values indicated high variability and high heritability coupled with GAM indicate less amount of environmental influence and additive gene action was present in F_{11} and F_{13} RIL population. This indicates the existence of comparatively moderate variability, which can be exploited by improvement through further selection for this character. Hence, from the results it is evident that the trait shelling percentage is under the control of non-additive genes under well water and water stress conditions. The results are similar with the findings of Rudraswamy *et al.* (1999), Zaman *et al.* (2011), Sarvamangala (2009), Korat *et al*. (2009), Azharudheen (2010), John *et al*. (2011) and Nageshwar Rao *et al.* (2012) Mallikarjun (2014) reported moderate difference between GCV and PCV for shelling percentage in segregating generations.

Sound Mature Kernel (%)

The sound mature kernel (%) of RILs ranged between 41.43 - 94.05 and 40.23 - 92.93 with an average of 72.94 and 71.07 having standardized range of 0.72 and 0.74 in F_{11} and F_{13} RILs, respectively under well water conditions, and it is coupled with high heritability (69.84 and 56.83) and low GAM (15.70 and 12.54) indicated that the selection will be effective at earlier stage. the difference between the GCV and PCV found less indicated the lower influence of environment on sound mature kernels. Whereas, under water stress I sound mature kernel *per cent* in RILs ranged between 55.56 - 100.00 with an average of 82.08 having an standardized range of 0.54 in F_{13} RILs, it is coupled with moderate heritability (37.43) and low GAM (17.47) indicated that the selection not effective at earlier stage as it inherited by non-additive genes and the difference between the GCV and PCV found less indicated the lower influence of environment on the trait. (Table 15 and 16).

During water stress II, sound mature kernel of RILs varied between 35.74 - 96.00 and 19.35 - 98.41 with an average of 76.16 and 64.37 with an standardized range of 0.79 and 1.23 in F_{11} and F_{13} RILs, respectively and it is coupled with high heritability (67.36 and 65.78) and low GAM (19.67 and 17.67) indicated that the selection is not effective at earlier stage as it inherited by non-additive genes and the low correspondence between the GCV and PCV indicates the lower influence of environment. In case of water stress III, RILs varied between 52.38 - 98.36 and 31.65 - 98.25 with an average of 86.32 and

72.04 and recorded standardized range of 0.53 and 0.92 in F_{11} and F_{13} RILs, respectively. The trait recorded moderate heritability (46.50 and 53.76) and low GAM (12.15 and 15.88) indicated that the trait is controlled by non-additive genes and the difference between GCV and PCV was found lower, indicates the negligible effect on the trait. (Table 15 and 16).

The result suggests that presence of considerable amount of environmental effect and non-additive gene action for expression of SMK *per cent* trait under WS condition. The magnitude of variation revealed by GCV, PCV, heritability and genetic advance as *per cent* mean were moderate to high for all the traits except for SCMR and days to fifty *per cent* flowering. Wider gap between GCV and PCV indicate the influence of environment over expression of these characters. Hence, improvement through phenotypic selection will be more complex and markers are expected to improve the efficiency of selection. Hence the trait is less influenced by environment under well water and water stress condition and the selection is also not effective as it is controlled by non-additive genes these results are found similar with the results of Shinde *et al.* (2010), Jogloy *et al.* (2011), Pradhan and Patra (2011), Zaman *et al.* (2011), Vishnuvardhan *et al.* (2012), Bhavya (2015).

From the present investigation in F_{11} RIL and F_{13} RILs under WW and WS conditions, the magnitude of variation revealed by GCV, PCV, heritability and genetic advance as *per cent* mean were moderate to high for all the traits except for SCMR and days to fifty *per cent* flowering. Wider gap between GCV and PCV indicate the influence of environment over expression of these characters. Hence, improvement through phenotypic selection will be more complex and markers are expected to improve the efficiency of selection.

4.1.2 Genetic variability for physiological traits

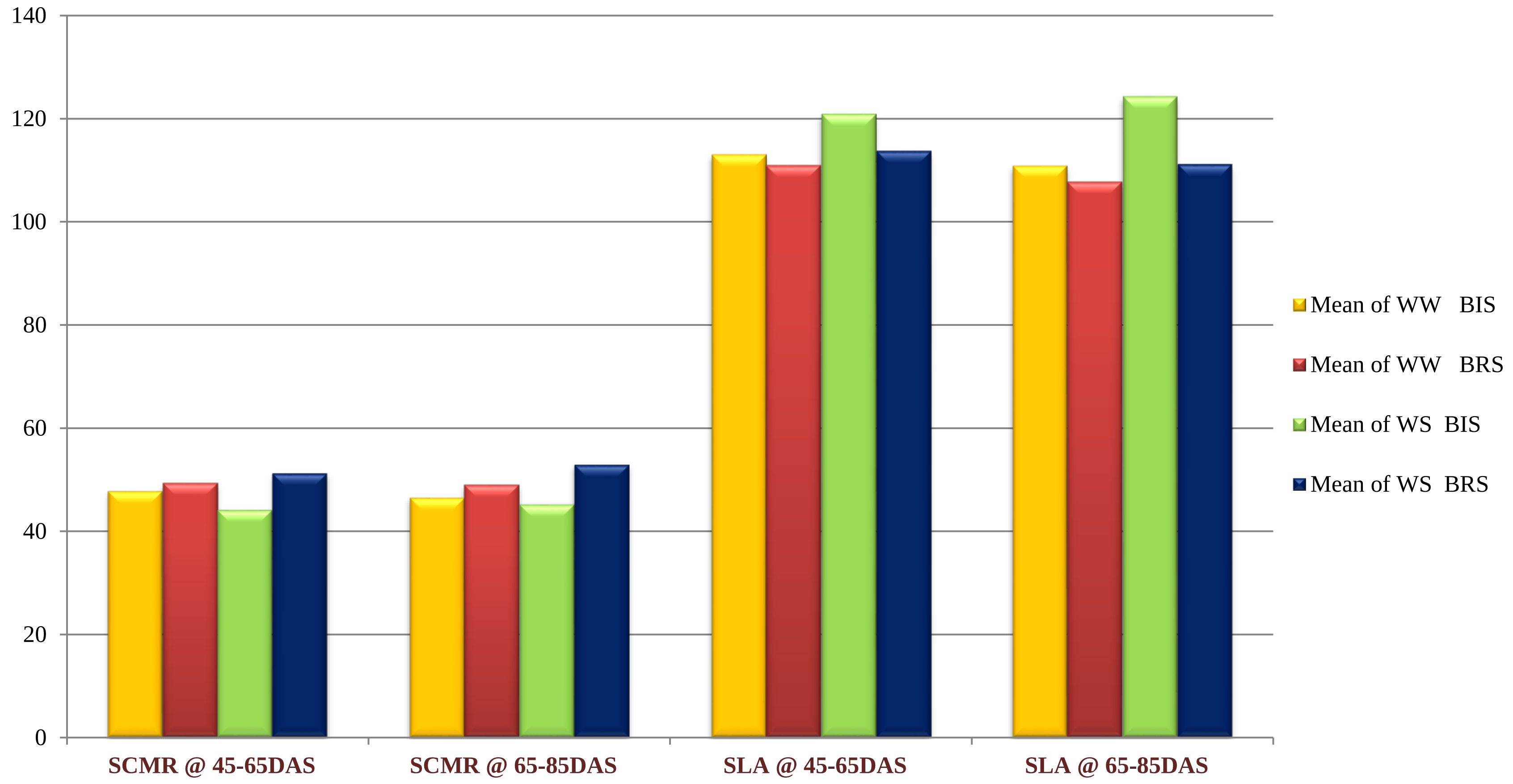
4.1.2.1 Analysis of variance (ANOVA)

Analysis of variance for the physiological traits among F_{11} (summer 2017) and F_{13} RILs (summer 2018) evaluated under different water regimes i.e. under well water (WW) throughout growth period and other experiment with water stress at 30-45 DAS, 45-65

DAS and 65-85 DAS for a period of 20 days revealed highly significant mean sum of squares attributable to ‘RILs’ and ‘check varieties’ for traits related WUE (Table 17 and 18). Mean sum of squares of ‘RILs *vs* check’ varieties exhibited significant differences in all the experiments. These results suggest that there is a significant difference among the F_{11} and F_{13} RILs. However, RILs as a group significantly differed from the checks for all traits investigated in F_{11} and F_{13} under different water regimes. The results of ANOVA indicated the presence of genetic variability even after achieving maximum homogeneous and homozygosity and the choice of the material for the investigation was appropriate. Therefore it could be used for considerable development for traits related WUE by simple selection. This was further supported by the fact that range was also quite wider for SLA and SCMR pointing out extreme recombinant inbred lines available for selection. However, analysis of variance by itself is inconclusive in explaining all the inherent genetic variability among the RILs. This is evident by partitioning the total variability inherent in the RILs from the phenotypic variance. Gopinath Jatti *et al*. (2008); Savita (2012); Mallikarjun (2014) and Bhavya (2015) have also reported significant differences among the genotypes for surrogate traits of WUE in groundnut. Pooled ANOVA across the two seasons (Table 19) indicated significant difference among RILs, checks and also their interactions for all the traits studied.

4.1.2.2 Mean, range and components of variation

Mean and range of physiological traits and pod yield per plant for WW and WS conditions during the two seasons of summer 2017 and 2018 are presented in Table 20. The mean pod yield per plant for both the seasons under WW and WS were not on par and this might be because of the environmental interaction over both the seasons targeting the performance of plants under different water regimes. Pod yield per plant and SLA showed relatively high range. The results indicate that these traits can be improved through individual plant selection. In the present study, the relative chlorophyll content of leaves was found lower in the initial stages of leaf development and increased as the dry matter accumulates and leaf matures. SCMR of the RILs under WS condition was higher compared to the SCMR under WW condition as indicated through mean for this trait at different stages of stress (Fig 7). Water stress also decreased the SLA but increased SCMR (Songsri *et al.*, 2009).



Note: WW- Well watered, WS- Water stress
BIS- Before initiation of stress, BRS- Before releasing of stress

Fig. 7. Mean of physiological traits in 147 RILs of NRCG 12568 × NRCG 12326 in groundnut under WW and WS conditions over two seasons

Table 17. Analysis of variance for physiological traits and pod yield of the F_{11} recombinant inbred lines of the cross NRCG 12568 × NRCG 12326 in groundnut under water stress (WS) and well-watered condition (WW) during summer 2017

Source		df	SCMR		SLA		Pod yield / plant
			BIS	BRS	BIS	BRS	
Blocks (eliminating checks + RILs)	WW	6	6.66	33.06	392.57	226.59	11.92
	WS2		15.55	6.00	101.20	422.89	10.58
	WS3		10.55	13.33	684.95 *	443.30	44.39
RILs + Check	WW	150	11.10	13.56	652.16 **	519.31	35.91 ***
	WS2		15.73	6.03	518.24	491.26 *	44.82 ***
	WS3		9.46	7.08	588.32 **	548.81	39.38
Checks	WW	3	100.26 **	18.79	813.16 *	492.84	215.88 ***
	WS2		62.77	3.89	1886.09 **	325.91	422.90 ***
	WS3		20.59	38.30	555.12	268.49	61.86
RILs	WW	146	8.86	7.83	651.83 **	505.36	29.21 **
	WS2		10.06	6.10	486.57	488.20 *	35.89 ***
	WS3		7.76	6.29	579.10 **	555.81	36.42
Checks vs. RILs	WW	1	69.30 *	835.54 ***	217.13	2635.14 *	473.59 ***
	WS2		701.54 ***	1.43	1039.49	1433.91 *	213.32
	WS3		223.22 ***	29.51	2033.15 **	368.43	403.52 ***
Error	WW	18	12.21	22.09	176.16	496.35	8.23
	WS2		21.34	4.08	332.60	198.23	5.01
	WS3		11.23	13.98	193.40	307.05	21.84

Note: *Significance @ P =0.05 ** Significance @ P = 0.01 ***Significance @ P = 0.001

BIS- Before initiation of stress, BRS- Before release of stress Results for WS1 is not pooled data

SCMR- SPAD Chlorophyll Meter Reading, SLA- Specific Leaf Area

Table 18. Analysis of variance for physiological traits and pod yield of the F_{13} recombinant inbred lines of the cross NRCG 12568 × NRCG 12326 in groundnut under water stress and well-watered condition during summer 2018

Source		**DF**	**SCMR**		**SLA**		**Pod yield / plant**
			BIS	**BRS**	**BIS**	**BRS**	
Blocks (eliminating checks + RILs)	WW	6	1.91	8.67	34.89	3791.154 *	20.62
	WS1		3.96	5.57	26.59	424.80	19.03
	WS2		5.99	19.31	1582.02	593.08	0.78
	WS3		13.48	36.975 **	886.22	21.14	7.94
RILs + Check	WW	150	6.38	10.91	141.86	1242.42	22.63
	WS1		5.99	8.39	128.15	1160.48 *	19.67 *
	WS2		20.59 **	20.31	2933.42	716.09	29.30 **
	WS3		16.82	98.02 ***	1206.43 *	119.648 *	15.91 **
Checks	WW	3	5.69	36.17 *	104.69	1141.53	31.14
	WS1		3.28	17.49	204.54	14556.96 ***	34.09 *
	WS2		12.69	4.48	7443.45	435.30	144.01 ***
	WS3		43.74	31.84 *	4663.28 ***	92.97	59.65 ***
RILs	WW	146	6.18	9.14	142.54	1247.72	22.57
	WS1		6.08	8.26	125.77	709.85	18.36 *
	WS2		17.05 **	19.51	2833.51	719.06	27.08 **
	WS3		15.90	99.17 ***	1133.69 *	118.855 *	15.11 **
Checks *vs*. RILs	WW	1	38.42 **	193.42 ***	154.39	770.39	6.41
	WS1		1.19	0.50	245.98	26763.68 ***	167.18 ***
	WS2		561.83 ***	184.21 *	3989.53	1124.01	9.41
	WS3		71.41 *	128.32 **	1456.28	315.45 *	1.60
Error	WW	18	3.40	8.68	109.89	1185.14	28.61
	WS1		3.81	7.52	71.07	582.87	8.18
	WS2		6.12	24.53	2667.01	381.58	9.51
	WS3		9.13	8.62	469.24	59.64	5.59

Note: *Significance @ P =0.05 ** Significance @ P = 0.01 ***Significance @ P = 0.001

BIS- Before initiation of stress BRS- Before release of stress Results for WS1 is not pooled data
SCMR- SPAD Chlorophyll Meter Reading, SLA- Specific Leaf Area

Table 19. Pooled analysis of variance for physiological traits and pod yield of the F_{11} / F_{13} RILs derived from NRCG 12568 × NRCG 12326 in groundnut over two seasons of summer 2017 and 2018

Source		**DF**	**SCMR**		**SLA**		**Pod yield / plant (g)**
			BIS	**BRS**	**BIS**	**BRS**	
Blocks (eliminating checks + RILs)	WW	6	1.66	7.07*	1382.17***	854.96	9.47
	WS1		2.38*	3.14*	150.73	382.51*	2.54
	WS2		0.90	8.23**	255.86*	323.13**	9.24
	WS3		2.56	5.82	867.60***	281.37*	18.01
RILs + Check	WW	150	35.72**	7.22	361.77*	515.05	23.86 ***
	WS1		10.93*	10.77	211.51	496.45	15.09 **
	WS2		5.66	6.43	556.68**	906.95	101.72 ***
	WS3		6.46**	25.20***	354.68**	426.13**	264.24 ***
Checks	WW	3	5.99	10.55	138.56	1661.21*	50.21 ***
	WS1		5.40	16.78**	102.34**	1104.36*	15.78
	WS2		1.80**	18.76	194.91	262.95*	18.55 ***
	WS3		2.31	4.06	566.53*	779.64**	13.86 **
RILs	WW	146	6.35**	7.11	367.65	489.09	92.61 **
	WS1		6.20	5.62	214.81*	487.74	18.55 ***
	WS2		5.74**	6.17	566.53***	860.34	88.57 ***
	WS3		6.56	25.67***	342.50**	390.71*	10.00
Checks *vs*. RILs	WW	1	33.09*	13.28	173.05*	867.14	3.59
	WS1		15.40	14.98*	56.19	329.73**	4.19
	WS2		5.81**	7.08	605.80**	255.25	9.47
	WS3		3.34*	18.72**	149.97**	153.82**	2.54
Error	WW	18	4.30	5.78	126.07	146.08	9.24
	WS1		5.94	10.92	125.06	73.29	18.01
	WS2		2.95	4.81	83.27	90.37	23.86 ***
	WS3		4.76	3.79	73.38	60.31	15.09 **

Note: *Significance @ P =0.05 ** Significance @ P = 0.01 ***Significance @ P = 0.001
BIS- Before initiation of stress BRS- Before release of stress Results for WS1 is not pooled data.
SCMR- SPAD Chlorophyll Meter Reading SLA- Specific Leaf Area

Table 20. Mean and range for physiological traits and pod yield plant^{-1} in groundnut RILs of NRCG 12568 × NRCG 12326 cross under WW and WS conditions during summer 2017 and 2018

Character	Mean of WW				Mean of WS				Range of WW				Range of WS			
	S – 2017		S - 2018		S - 2017		S - 2018		S - 2017		S - 2018		S - 2017		S - 2018	
	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS
SCMR @ 30-45DAS	-	-	48.2	49.0	-	-	42.0	47.9	-	-	40.82-53.25	37.73-62.10	-	-	35.75-49.25	31.47-60.43
SCMR @ 45-65DAS	46.9	48.2	49.0	50.4	40.5	42.0	48.2	38.7	39.60-55.75	40.82-53.25	37.73-72.10	36.27-60.23	33.70-50.25	35.75-49.25	33.27-57.30	31.55-47.20
SCMR @ 65-85 DAS	45.1	46.6	48.2	51.3	38.7	40.3	52.1	53.7	37.15-58.50	31.55-47.20	40.82-53.25	33.10-62.70	31.55-47.20	34.23-46.98	37.60-62.40	43.63-166.83
SLA @ 30-45DAS	-	-	43.0	78.2	-	-	51.9	97.6	-	-	20.61-148.55	50.14-224.11	-	-	32.47-116.11	38.18-158.91
SLA @ 45-65 DAS	106.8	107.5	111.1	108.4	106.1	106.8	101.2	145.0	69.36-115.91	59.07-110.43	50.14-141.21	46.37-190.64	69.36-115.91	59.07-110.43	20.11-125.73	47.69-152.22
SLA @ 65-85 DAS	113.5	114.2	71.9	107.4	112.8	113.5	129.4	132.5	73.71-129.46	62.78-123.65	36.91-93.50	36.90-134.23	67.81-175.05	74.52-195.86	53-131.62	70.47-172.90
Pod yield plant^{-1}	14.1		10.64		12.97		9.09		5.58-33.80		1.30-36.20		2.78-33.00		1.20-26.20	

Note: BIS- Before initiation of stress BRS- Before release of stress
SCMR- SPAD Chlorophyll Meter Reading SLA- Specific Leaf Area

The nature and magnitude of variation for quantitative traits was assessed by phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability and genetic advance for the two summer seasons (2017 and 2018) are presented in Table 21. Variability refers to the presence of genotypic and phenotypic differences among the individuals of plant population. Variability may be due to genetic constitution of the individuals of a population or due to environment in which they are grown. Selection is effective only when there is genetic variability among the individuals in a population. Hence, insight into the magnitude of genetic variability present in a population is of paramount importance to plant breeders. The total variability in the material cannot be inferred only by mean and range values. Hence, actual variance has been estimated for the characters to know the existing variability. Heritability and genetic advance are other important selection parameters. Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection than heritability estimates alone, it is not necessary that a character showing high heritability will also exhibit high genetic advance (Johnson *et al*., 1955).

Table 21. Genetic parameters for physiological traits in groundnut RILs developed from cross NRCG 12568 × NRCG 12326 during summer 2017 and 2018

Character	PCV (%)		GCV (%)		h^2_{bs}		GAM	
	2017	2018	2017	2018	2017	2018	2017	2018
SCMR @ 30 DAS	-	5.10	-	3.71	-	54.18	-	5.57
SCMR @ 45 DAS	4.29	5.10	3.67	3.71	74.81	54.18	6.46	5.57
SCMR @ 65 DAS	4.18	8.62	3.13	6.17	87.39	82.42	12.82	19.09
SCMR @ 85 DAS	8.54	8.38	4.64	6.55	80.43	82.33	15.22	10.52
SLA @ 30 DAS	-	10.94	-	10.79	-	96.66	-	21.92
SLA @ 45 DAS	5.18	10.85	3.98	10.37	60.57	93.41	6.29	20.40
SLA @ 65 DAS	9.93	7.72	3.74	5.48	14.51	51.59	2.90	8.01
SLA @ 85 DAS	5.25	4.25	3.75	2.20	19.59	27.51	5.54	2.36

SCMR- SPAD Chlorophyll Meter Reading,
SLA- Specific Leaf Area (cm^2/g)
PCV- Phenotypic coefficient of Variation
GCV- Genotypic coefficient of Variation
h^2 (broad sense) - Heritability
GAM- Genetic advance as *per cent* mean

Lower difference between phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) indicates less influence of environment on expression of all the traits studied (Table 21). Accordingly, Low PCV and GCV were recorded by the physiological traits measured while high PCV and GCV for pod yield per plant were recorded at all stages during both the seasons. The low genetic variation in the population and difference between PCV and GCV for SCMR and SLA indicated less prevalence of environment on these traits during both the seasons. The similar results of significant genotypic variation for the traits related to WUE was reported in numerous reports depending upon the material used for their study by Vorasoot *et al*. (2003), Painawadee *et al.* (2009), Srivalli *et al*. (2016) and Bhavya *et al*. (2017).

Higher estimates of heritability and low GAM for SCMR at 30-45 DAS was observed during summer 2018. High heritability and low GAM were recorded for SCMR at 45-65 DAS during both the seasons. High heritability and moderate GAM were recorded for SCMR at 65-85 DAS indicating the less influence of environment and wider scope for selection of these traits. Selection for drought tolerance based on SCMR could be effective only at later stages of stress imposition compared to the early stages. Similar kind of results of high heritability and moderate GAM was reported for SCMR by Painwadee *et al*. (2009), Srivalli *et al*. (2016) and John *et al*. (2011).

High heritability and moderate to high GAM was observed during summer 2018 for SLA at 30-45 DAS as well as at 45-65 DAS indicating the additive gene effects and selection for this trait could be effective in the early stages. The results were in accordance with the reports of high heritability and high GAM for SLA by Songsri *et al.* (2008a) and Savitha (2012). Low to moderate heritability along with low GAM were recorded for SLA at 65-85 DAS during summer 2017 and 2018 indicating the influence of environmental effects on the expression of SLA at this stage. Similar results of moderate $h^2_{(b)}$ and low GAM was reported by John *et al.* (2011). The heritability and GAM for pod yield per plant were moderate to high in well-watered and water stress conditions indicating the prevalence for selection. Differential heritability and GAM estimates for physiological traits and pod yield in the present study are because of varied stages and duration of drought stress imposition. The mechanisms of physiological responses to early season drought might be different from those for long duration drought, which have been reported previously (Songsri *et al.,* 2008b). Cruickshank *et al.* (2004) reported that broad sense heritability of drought tolerance traits were varied among groundnut crosses, traits and stages depending on levels of genetic variation for the trait and stage of imposition of drought stress.

4.1.3 Effect of stress under different water regimes on pod yield of the RILs

Water stress during crop growth period at certain interval considerably affected pod yield of RILs over both the season. The RILs varied widely for pod yield plant^{-1} under different water regimes relative to that under well-watered condition in both the seasons (Fig. 8, 9 and 10). However, pod yield in water stress I, RIL 245 and RIL 439 out-performed even exposed to early stages of the crop growth in comparison with the performance of the same RIL in well-watered conditions. In water stress II, RIL 125, RIL 133 and RIL 249 sustained the stress condition and yielded better than the same RIL in well-watered condition. In water stress III, RIL 25 and RIL 133 tolerated the stress condition imposed during pod development stage and yielded better than the same RIL in well-watered condition. Higher magnitudes of results were expected for pod yield per plant against RILs in well-watered conditions. Few RILs in the population performed better even under stress conditions, while few couldn’t cope up with the stress limits. But many RILs maintained to produce average pods and yield was neutralized. This genic action is majorly due expose of the RILs to intermittent moisture stress. Among the RILs exposed for intermittent stress conditions, RIL 133 withstand the moisture stress imposed during peg initiation, peg penetration and pod development stage infers its adapatibility nature for moisture stress across the critical growth stages in groundnut. The relative adaptability of RILs reflect their degree of moisture stress tolerance.

4.1.4 Association among traits related to WUE and pod yield attributing characters

It will be more meaningful if the structure of yield is probed through its component traits rather than directly. Hence, it is anticipated to break genetic barriers of the yield by the study of character association and such associations are best ascertained by phenotypic correlations. SCMR is correlated positively and significantly with pods per plant (0.38, 0.23 and 0.03), kernel yield per plant (0.33, 0.31 and 0.72) and it exhibited significant negative association with SLA (-0.24, -0.21 and -0.26) in RIL populations under WW, WS1 and WS2 respectively (Table 22).

Table 22. Phenotypic correlation coefficient for pod yield and traits related to water use efficiency under different water regimes in groundnut RILs developed from the cross NRCG 12568 × NRCG 12326

Traits	Water regimes	SCMR	SLA (cm^2/g)	Pods / plant (g)	Kernel yield / plant (g)	Pod yield / plant (g)
SCMR	WW	1	-0.24*	0.38**	0.33*	0.51**
	WS 1	1	-0.21*	0.23**	0.31*	0.42**
	WS 2	1	-0.26**	0.03**	0.10	0.53*
	WS 3	1	-0.29 **	0.63 **	0.72**	0.64**
SLA (cm^2/g)	WW		1	-0.09	-0.02	-0.16*
	WS 1		1	-0.47**	-0.02*	-0.24*
	WS 2		1	-0.56**	-0.12	-0.23*
	WS 3		1	-0.61**	-0.06	-0.16
Pods / plant (g)	WW			1	0.33*	0.90**
	WS 1			1	0.29**	0.73**
	WS 2			1	0.60**	0.97**
	WS 3			1	0.72**	0.92**
Kernel yield/ plant (g)	WW				1	0.38**
	WS 1				1	0.35*
	WS 2				1	0.46**
	WS 3				1	0.42**

Note: *Significance @ P = 0.05 ** Significance @ P = 0.01

The strong inverse relationship between the SLA and SCMR under well-watered and water deficit condition showed that these surrogate traits of WUE can be potentially used as selection tool. Therefore, selection for genotypes with high SCMR offers the

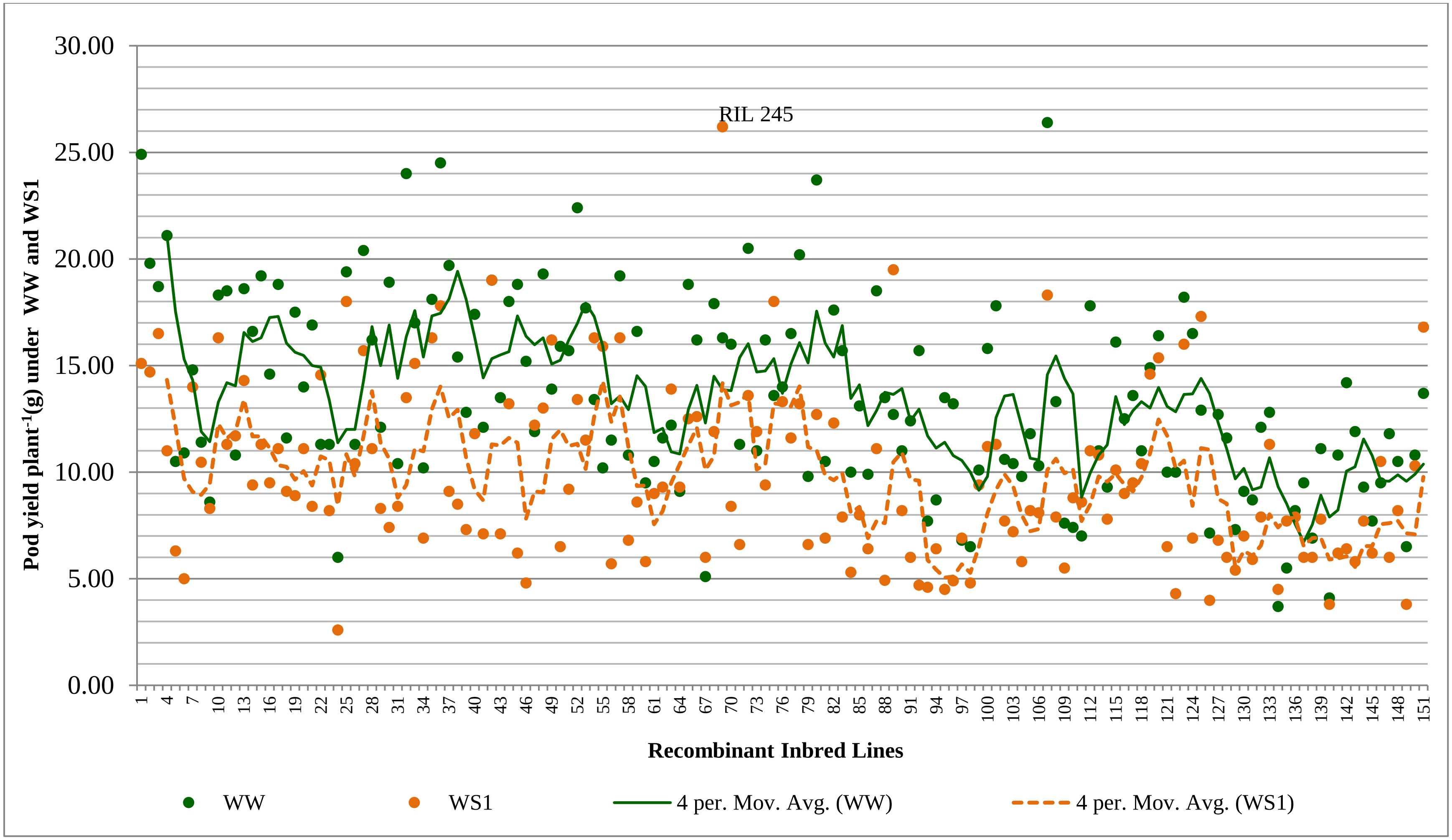


Fig. 8. **Responses of the recombinant inbred lines for pod yield plant^{-1} under well watered and water stress I condition in groundnut along with their parents and checks during summer 2018**

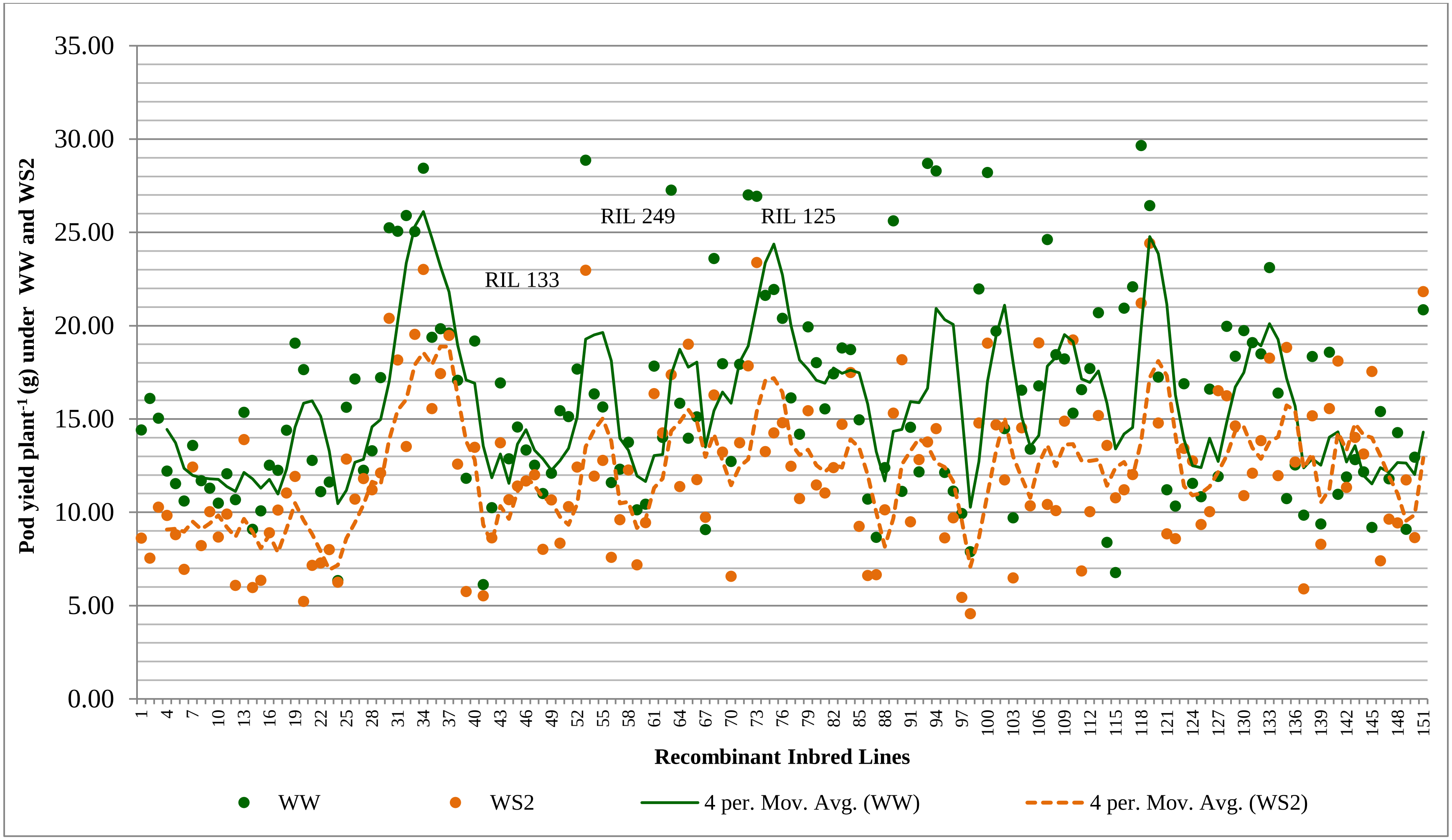


Fig. 9. Responses of the recombinant inbred lines pod yield plant^{-1} under well watered and Water stress II condition in groundnut (pooled data over two years) along with their parents and checks

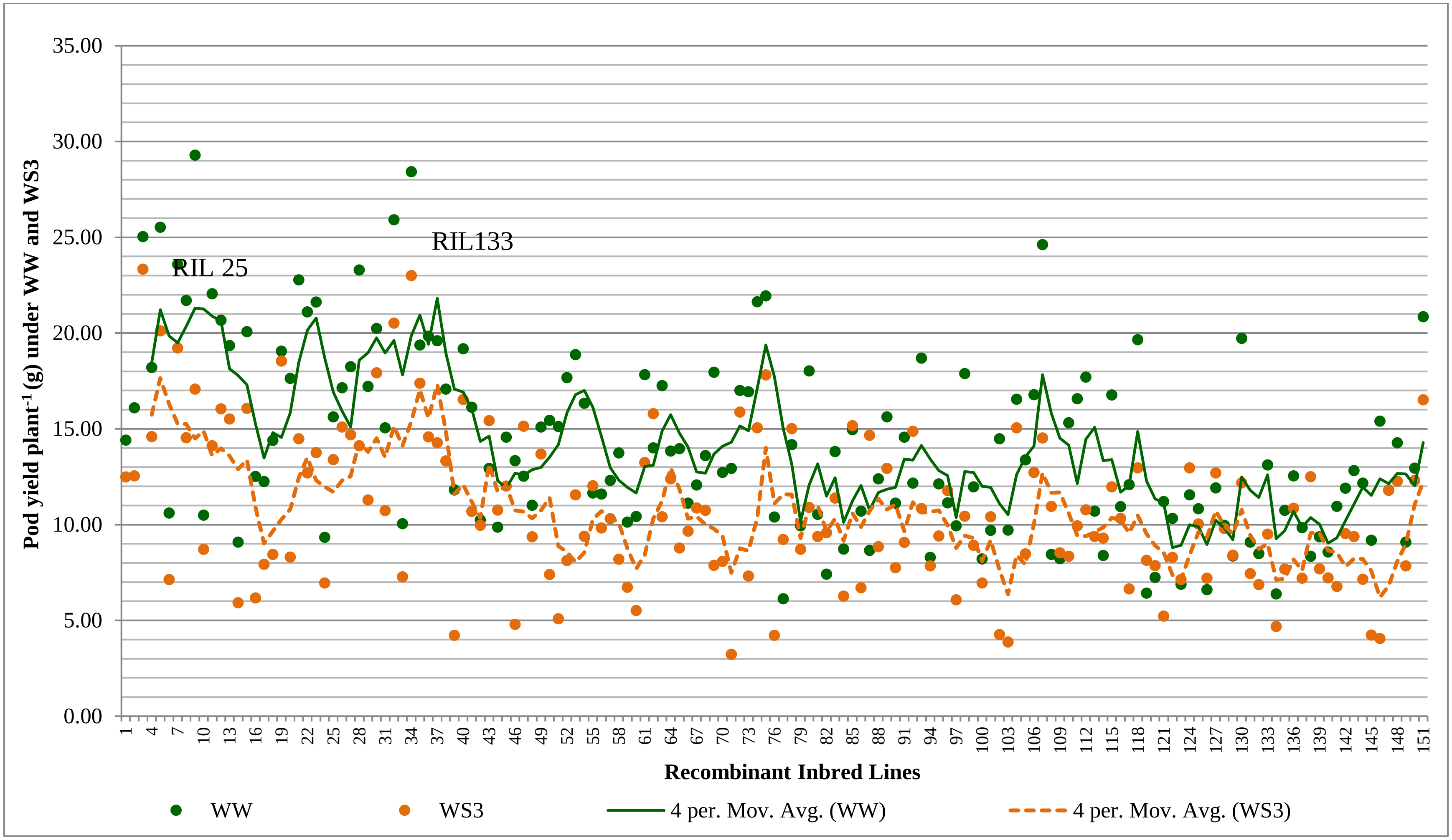


Fig. 10. **Responses of the RILs for pod yield plant^{-1} under well watered and Water stress III condition in groundnut (pooled data over two years) along with their parents and checks**

scope for simultaneous improvement of yield in groundnut as higher SCMR indicate high photosynthetic efficient genotypes. The results are in agreement with the reports of Farquhar *et al*. (1989); Wright (1994); Nageswaraa Rao *et al*. (2001); Upadhyaya (2005); Songsri *et al.* (2009); Rekha (2005); Mallikarjun (2014) and Kalariya *et al*. (2015).

SLA exhibited significant negative association with pods per plant (-0.47, -0.56 and -0.61) and pod yield per plant (-0.16, -0.24, 0.23 and -0.16) in WS1, WS2, WS3 of the RIL population respectively (Table 22). Thus, selecting plants with lower specific leaf area will lead to high pod yield coupled with high water use efficient genotypes. Similar types of results are found by Songri (2008a), Koolachart *et al.* (2013) and Bhavya (2015) Thi (2016) and Eradasappa (2018).

Pods per plant exhibited significant positive association with other yield attributing traits in both generations and both WW and WS condition in the cross NRCG 12568 × NRCG 12326 indicating selection for plants with more pods could simultaneously improve the pod yield. Savitha (2012) reported number of mature pods per plant seemed to play important role in maintaining higher pod yield under severe water stress conditions. Yadlapalli (2014) and Anusha (2015), Mallikarjun (2014), Jyothi (2018) and Cauvery (2019) also reported similar results in groundnut.

The present association study revealed that the surrogate traits for WUE, SCMR and SLA, and yield related parameters *viz*., pods per plant, shelling *per cent,* sound mature kernel *per cent* and kernel yield per plant were observed to be important yield contributing characters in groundnut irrespective of drought stress environment. Hence, selection criteria should consider these traits for improvement of genotypes with high pod yield and high-water use efficiency.

4.2 Identification of drought tolerant accessions based on drought tolerance indices, using Biplot analysis and ranking method.

4.2.1 Drought tolerant indices

Occurrence of drought stress and loss of yield are the main concern of plant breeders in rainfed farming systems and hence looking for reliable screening methods for yield performance and drought tolerance in water stress conditions. One possible way to ensure future food needs of the increasing world population should involve a better use of water by the development of crop varieties which needs less amount of water and more tolerance of crops to drought by Shao *et al*. (2006).

In the absence of an understanding of the special mechanisms of tolerance, the quantification of drought tolerance should be based on yield in both stress and non-stress environments that can lead to selection of high yielding genotypes under stress condition since, the response of selection under non-stress condition is maximal and heritability of the yield under these conditions is high (Talebi *et al*., 2009; Shirinzadeh *et al*., 2010 and Geravandi *et al*., 2011). Thus, drought indices which provide a measure of drought based on yield loss under drought conditions in comparison to normal conditions have been used for screening drought-tolerant genotypes (Mitra, 2001). These indices are based on drought resistance or susceptibility of genotypes (Fernandez, 1992).

A total of 15 reported indices were used in the present study. These 15 indices could be grouped into two classes, (1) stress susceptibility indices and (2) stress tolerance indices (Rosielle and Hamblin, 1981; Fernandez, 1992. All the 15 indices were used in the present study to quantify the genotypes for their degree of moisture stress tolerance. In the present study, the genotypes of both the experiments varied considerably for their abilities to tolerate, as revealed by wide variation in magnitudes of all the 15 indices based on both pod yield under stress and non-stress conditions. Thus, these indices are useful statistics for objective screening of the genotypes for their responses to water stress and degree of moisture stress tolerance. Several researchers across the crop groups have used these indices to quantify the responses and degree of tolerance of the genotypes to abiotic stresses. To quote a few, Safavi *et al.* (2015) in sunflower, Uday *et al.* (2016) in chickpea, and Bennani *et al.* (2016) and Bennani *et al.* (2017) in bread wheat have used different combinations of the indices to quantify the degree of tolerance of genotypes to abiotic stress.

4.2.2 Identification of indices that most discriminate the RILs for responses to WS

Good discrimination of genotypes helps effective selection of most desirable ones tolerant to moisture stress environment. Thus, identification of indices that most discriminate the genotypes for responses to moisture stress environment assumes importance in this context. In the present study, only eight of the 15 indices such as TOL, STI, ATI, SSPI, K_1STI, K_2STI, SNPI and SSI showed better ability than others to discriminate the RILs for their responses to moisture stress environments for pod yield plant^{-1} as indicated from high magnitude of estimates of both SR and PCV under different water regimes (Table 23). It is desirable to preferentially use these indices for screening the genotypes for responses to moisture stress environment in groundnut. Bennani *et al.* (2016) and Bennani *et al.* (2017) have also suggested one of these five indices, i.e., STI for discriminating bread wheat genotypes for their responses to drought stress. However, they used significant mean squares attributable to indices as criteria to identify those with better discriminating ability.

Pod yield under water stress (Ys) and well-watered (Yp) conditions were significantly and positively correlated with MP, GMP, STI and HMP indicating these criteria are able to discriminate genotypes that express uniform superiority in water stress and well-watered conditions from the others (Table 24, 25 and 26). Khalilzade and Karbalayi Khiavi (2002) and Farshadfar *et al*. (2001) believe that the most suitable indices for selection of drought tolerant cultivars, are indicators which show a relatively high correlation with yield in both stress and non-stress conditions. To investigate suitable drought tolerant indices, a suitable index must have a significant correlation with yield under both the conditions (Mitra, 2001). Thus, the above results indicated that these indices were more effective in identifying high yielding accessions under different irrigated conditions. However, drought tolerant indices MP, GMP and STI were suggested as the best indices because they had highest Pearson correlation coefficients (>0.7) under both normal and stressed conditions in RILs of groundnut. Talebi *et al*. (2010) reported in wheat that cultivars producing high yield in both well-watered and water stress conditions can be identified by STI, GMP and MP values. Pireivatlou *et al*. (2010) also noted that STI can be a reliable index for selecting high yielding genotypes.

Table 23. Estimates of measures of variability for various drought tolerant indices based on pod yield plant^{-1} under moisture stress environments over two seasons (pooled data)

Indices	Pod yield plant^{-1} (g)					
	WS1		WS2		WS3	
	Standardized Range	PCV (%)	Standardized Range	PCV (%)	Standardized Range	PCV (%)
TOL	3.81	62.30	4.05	81.29	4.34	82.40
MP	1.80	32.72	1.40	25.14	1.36	25.83
GMP	1.88	33.61	1.43	25.47	1.27	26.30
HMP	2.07	35.52	1.50	26.19	1.24	27.27
STI	3.42	70.09	3.23	52.80	2.70	53.34
RDI	1.75	78.37	1.12	27.05	1.21	27.54
ATI	3.03	78.58	4.64	81.43	5.40	81.49
SSPI	3.81	62.30	4.05	81.29	4.34	82.40
YI	2.62	44.90	1.69	30.74	1.31	31.19
YSI	1.75	78.37	1.12	27.05	1.21	27.54
K$_1$STI	2.18	63.68	3.50	58.33	3.98	61.89
K$_2$STI	2.26	70.09	3.67	62.85	2.42	59.52
DI	1.78	64.43	2.45	49.15	2.15	47.50
SNPI	11.61	58.43	16.77	51.48	12.33	61.39
SSI	3.46	55.02	2.81	68.06	2.92	66.31

TOL - Tolerance index
SSPI - Stress susceptibility percentage index
MP - Mean productivity
HAM - Harmonic mean
RDI - Relative drought index
YSI - Yield stability index
ATI - Abiotic tolerance index
Ys-Pod yield under stress condition

DI - Drought resistant index
K$_1$STI - Modified STI
STI - Stress tolerance indices
K$_2$STI - Modified STI
GMP -Geometric mean productivity
YI - Yield index
Yp - Pod yield under normal condition

Table 24. Correlation coefficients of the RIL population for drought tolerant indices and pod yield under WW and WS1 condition in groundnut

Variables	**TOL**	**MP**	**GMP**	**HMP**	**STI**	**RDI**	**ATI**	**SSPI**	**YI**	**YSI**	**K_1STI**	**K_2STI**	**DI**	**SNPI**	**SSI**	**Y_p**	**Ys**
TOL	1.00	0.10	0.02	-0.05	0.04	-0.78*	0.73*	0.98*	-0.63*	-0.78*	0.68*	-0.60*	-0.72*	0.53*	0.78*	0.71*	-0.63*
MP		1.00	0.97*	0.92*	0.95*	-0.02	-0.05	0.10	0.71*	-0.02	0.72*	0.69*	0.22*	-0.03	0.02	0.77*	0.71*
GMP			1.00	0.99*	0.97*	-0.05	0.01	0.02	0.75*	-0.05	0.61*	0.71*	0.16*	-0.04	0.05	0.70*	0.75*
HMP				1.00	0.95*	-0.07	0.05	-0.05	0.76*	-0.07	0.51*	0.70*	0.12	-0.05	0.07	0.62*	0.76*
STI					1.00	-0.05	0.01	0.04	0.72*	-0.05	0.64*	0.72*	0.16	-0.04	0.05	0.70*	0.72*
RDI						1.00	-0.88*	-0.78*	0.54*	0.96*	-0.36*	0.52*	0.93*	-0.39*	-0.90*	-0.51*	0.54*
ATI							1.00	0.73*	-0.56*	-0.88*	0.34*	-0.62*	-0.96*	0.43*	0.88*	0.43*	-0.56*
SSPI								1.00	-0.63*	-0.78*	0.68*	-0.60*	-0.72*	0.53*	0.78*	0.71*	-0.63*
YI									1.00	0.54*	0.08	0.96*	0.68*	-0.40*	-0.54*	0.10	0.90*
YSI										1.00	-0.36*	0.52*	0.93*	-0.39*	-0.90*	-0.51*	0.54*
K_1STI											1.00	0.09	-0.21*	0.27*	0.36*	0.94*	0.08
K_2STI												1.00	0.69*	-0.43*	-0.52*	0.10	0.96*
DI													1.00	-0.39*	-0.93*	-0.31*	0.68*
SNPI														1.00	0.39	0.31*	-0.40*
SSI															1.00	0.51*	-0.54*
Y_p																1.00	0.10
Y_s																	1.00

Note: *Significance @ P =0.05

Table 25: Correlation coefficients of the RIL population for drought tolerant indices and pod yield under WW and WS2 condition in groundnut pooled over two seasons

Variables	**TOL**	**MP**	**GMP**	**HMP**	**STI**	**RDI**	**ATI**	**SSPI**	**YI**	**YSI**	**K_1STI**	**K_2STI**	**DI**	**SNPI**	**SSI**	**Y_p**	**Y_s**
TOL	1	0.28*	0.16	0.05	0.16*	-0.91*	0.82*	0.92*	-0.29*	-0.91*	0.68*	-0.27*	-0.65*	-0.10	0.91*	0.67*	-0.29*
MP		1	0.99*	0.97*	0.98*	0.04	0.63*	0.28*	0.83*	0.04*	0.88*	0.82*	0.53*	-0.06	-0.04	0.94*	0.83*
GMP			1	0.99*	0.98*	0.16	0.56*	0.16*	0.89*	0.16	0.81*	0.88*	0.62*	-0.05	-0.16	0.84*	0.89*
HMP				1	0.98*	0.26*	0.48*	0.05	0.94*	0.26*	0.74*	0.92*	0.70*	-0.04	-0.26	0.77*	0.94*
STI					1	0.14	0.57*	0.16*	0.88*	0.14	0.82*	0.89*	0.61*	-0.05	-0.14	0.83*	0.88*
RDI						1	-0.62*	-0.91*	0.57*	0.94*	-0.36*	0.51*	0.84*	0.12	-0.91*	-0.37*	0.57*
ATI							1	0.82*	0.15	-0.62*	0.87*	0.14	-0.28*	-0.10	0.62*	0.86*	0.15
SSPI								1	-0.29*	-0.91*	0.68*	-0.27*	-0.65*	-0.10	0.91*	0.67*	-0.29
YI									1	0.57*	0.49*	0.98*	0.91*	0.65	-0.57*	0.51*	0.93*
YSI										1	-0.36*	0.51*	0.84*	0.12	-0.96*	-0.37*	0.57*
K_1STI											1	0.50*	0.10	-0.08	0.36*	0.98*	0.49*
K_2STI												1	0.88*	-0.01	-0.51*	0.52*	0.98*
DI													1	0.06	-0.84*	0.12	0.91*
SNPI														1	-0.12	-0.09	0.08
SSI															1	0.37*	-0.57*
Y_p																1	0.51
Y_s																	1

Note: *Significance @ P =0.05

Table 26. Correlation coefficients of the RIL population for drought tolerant indices and pod yield under WW and WS3 condition in groundnut pooled over two seasons

Variables	**TOL**	**MP**	**GMP**	**HMP**	**STI**	**RDI**	**ATI**	**SSPI**	**YI**	**YSI**	**K_1STI**	**K_2STI**	**DI**	**SNPI**	**SSI**	**Y_p**	**Y_s**
TOL	1	0.33*	0.18*	0.05	0.21*	-0.90*	0.74*	0.80*	-0.27*	-0.91*	0.71*	-0.23*	-0.63*	-0.12	0.90*	0.70*	-0.27*
MP		1	0.99*	0.95*	0.98*	0.01	0.73*	0.33*	0.82*	0.01	0.87*	0.83*	0.51*	0.15	-0.01	0.90*	0.82*
GMP			1	0.99*	0.99*	0.15	0.66*	0.18*	0.89*	0.15	0.79*	0.90*	0.62*	0.18*	-0.15	0.83*	0.89*
HMP				1	0.97*	0.27*	0.59*	0.05	0.94*	0.27*	0.71*	0.94*	0.70*	0.21*	-0.27*	0.74*	0.94*
STI					1	0.12	0.70*	0.21*	0.87*	0.12	0.82*	0.89*	0.58*	0.17*	-0.12	0.83*	0.87*
RDI						1	-0.51*	-0.91*	0.56*	0.93*	-0.39*	0.50*	0.84*	0.19*	-0.89*	-0.41*	0.56*
ATI							1	0.74*	0.29*	-0.51*	0.91*	0.30*	-0.15	0.10	0.51*	0.89*	0.29*
SSPI								1	-0.27*	-0.91*	0.71*	-0.23*	-0.63*	-0.12	0.90*	0.70*	-0.27*
YI									1	0.56*	0.46*	0.98*	0.90*	0.23*	-0.56	0.49*	0.90*
YSI										1	-0.39	0.50*	0.84*	0.19*	-0.98	-0.41	0.56*
K_1STI											1	0.49*	0.07	0.06	0.39*	0.98*	0.46*
K_2STI												1	0.88*	0.19*	-0.51*	0.52*	0.98*
DI													1	0.20*	-0.84*	0.11	0.91*
SNPI														1	-0.19	0.06	0.23*
SSI															1	0.40*	-0.56*
Y_p																1	0.49*
Y_s																	1

Note: *Significant @ P =0.05

Ilker *et al*. (2011) concluded that MP, GMP and STI values are convenient parameters to select high yielding wheat genotypes in both stress and non-stress conditions. Findings of Farshadfar *et al*. (2012) indicated that STI, GMP and MP values are better parameters to identify high yielding genotypes under both drought and favorable conditions. Thi *et al*. (2016) concluded that MP, GMP, HMP and STI were better drought tolerant indices under well-watered and water stress condition. Our findings reported that MP, GMP, HMP and STI values are better parameters to identify high yielding and drought tolerant groundnut RILs under both well-watered and water stress conditions.

4.2.3 Identification of water stress tolerant RILs based on rank sum method

In ranking method, the drought tolerance status of RILs was ranked using the mean figures for each RIL based on the average of the mean drought tolerance ratings and their standard deviations. Hence, to determine the most desirable drought tolerant RIL, rank mean (RM), Standard Deviation of Rank (SDR) and Rank Sum (RS) for each RIL was calculated based on rank of selected DTIs. Selection of genotypes for pod yield per plant under both water stress and well-watered conditions allow RILs to maintain ranks for high yields since the same RILs will be expected to perform well in either situation.

Among 15 DTIs, two separated subgroups were recorded. The first group was clustered for MP, GMP, HAM, STI, ATI, YI, K1STI, K2STI and DI which recorded lower values of rank for their higher values, while the remaining group (TOL, and SSPI) was observed with lower values of rank for their low values. The best tolerant genotypes were identified as having low rank mean, SDR and least in RS (Farshadfar *et al.*, 2012). Those accessions with low rank values of all DTIs denoted as drought tolerant. In consideration to all indices, RIL 126, RIL 249, RIL 133, RIL 145, RIL 129, RIL 539, RIL 248, RIL 233, RIL 258 and RIL 139 recorded the best mean rank and low standard deviation of ranks in well-watered and water stress condition, Hence rank sum (RS) method effectively combines all these three (MP, GMP and STI) indices into one was used to select water stress tolerant RIL (Table 27). The same procedures have been used to screen quantitative indicators of drought tolerance in wheat (Mohammadi *et al.*, 2012),

Table 27. Schematic description of rank sum (RS) method to identify tolerant RILs in groundnut based on hypothetical magnitude of three indices

RILs	MP	Rank	GMP	Rank	STI	Rank	Rank Mean (RM)	Standard deviation of ranks (SDR)	Rank sum (RS) (RM +SDR)
126	22.82	1	22.67	1	0.69	1	1.00	0.00	1.00
249	20.16	3	19.90	2	0.53	2	2.33	0.58	2.91
133	20.73	2	19.24	4	0.50	4	3.33	1.15	4.49
145	19.54	5	19.54	3	0.52	3	3.67	1.15	4.82
129	19.72	4	18.72	5	0.47	5	4.67	0.58	5.24
539	17.93	6	17.89	6	0.43	6	6.00	0.00	6.00
248	17.42	9	17.41	7	0.41	7	7.67	1.15	8.82
233	17.32	10	17.31	8	0.41	8	8.67	1.15	9.82
258	17.45	8	16.94	10	0.39	10	9.33	1.15	10.49
139	17.14	11	16.92	11	0.39	11	11.00	0.00	11.00
231	17.10	12	17.08	9	0.39	9	10.00	1.73	11.73
128	16.61	13	16.54	12	0.37	12	12.33	0.58	12.91
529	16.54	14	16.53	13	0.37	13	13.33	0.58	13.91
237	16.49	15	16.29	14	0.36	14	14.33	0.58	14.91
541	17.52	7	16.02	15	0.35	15	12.33	4.62	16.95
631	15.69	18	15.47	16	0.32	16	16.67	1.15	17.82
201	15.89	17	15.38	18	0.32	18	17.67	0.58	18.24
245	15.60	19	15.41	17	0.32	17	17.67	1.15	18.82
108	15.49	20	15.07	19	0.31	19	19.33	0.58	19.91

maize (Farshadfar and Sutka, 2002), rye (Farshadfar *et al*., 2012), groundnut (Savita, S. K., 2017; Eradasappa, 2018), tomato (Thi, 2016; Suresh, K., 2018), and Dolichos (Balaraju, 2018).

4.2.4 Identification of drought tolerant groundnut RILs based on DTIs.

4.2.4.1 Principal component and biplot analysis

In order to further investigate the interrelationships and repeatability among drought selection indices, as well as to distinguish tolerant RIL on the basis of several indices, principal component (PC) analysis has been performed and the corresponding biplot has been drawn (Fig. 11, 12 and 13). The first two principal components (PC) had eigen values more than unity and accounted for 85.60, 89.41 and 91.27 *per cent* of the total variance in the data (Table 28, 29 and 30) under WS1, WS2 and WS3 conditions The results of Makinde and Ariyo (2010) reported that only five of the 33 principal components had eigen values greater than three.

Anthony *et al.* (2011) also reported four of the nine principal components had eigen value greater than 1 and accounted for 76.92 *percent* of the total variation among 50 genotypes. Higher score of PC1 were in accordance with the higher rank of drought tolerance, whereas low score for PC1 showed drought sensitive genotypes. Therefore first component was named as drought tolerance. Further, the results showed high positive loadings of nine indices and maximum positive loadings was noticed in MP, HMP, STI, GMP, K_2STI, YI and pod yield under water stress condition. Negative loadings were reported for DI, RDI and YSI components of PC2 and therefore this component was named as drought sensitive. Thus, biplot was drawn based on the first two principal components (Fig. 1), which shows overview of inter-relationship among drought tolerant indices.

4.2.4.2 Biplot method of analysis based on principal component analysis

The relationships among different indices were graphically presented in biplot of first (PC 1) and second (PC 2) principal component analyses of 147 RILs of groundnut. The relationships, similarities and dissimilarities among DTIs can be explained based on indices correlation matrix from PCA using XLSTAT version 2017(XLSTAT solutions).

Table 28. Estimates of eigen values and percentage of variation under WS1 for 15 drought tolerant indices of F_{11} and F_{13} RIL population developed from the cross NRCG 12568 × NRCG 12326 in groundnut

Principal component (PCs)	Eigen value	Variability (%)	Cumulative (%)
1	7.601	50.675	50.675
2	5.239	34.926	85.602
3	1.133	7.553	93.155
4	0.658	4.385	97.540
5	0.239	1.596	99.136
6	0.071	0.475	99.611
7	0.041	0.276	99.886
8	0.013	0.087	99.973
9	0.004	0.024	99.997
10	0.000	0.002	100.000
11	0.000	0.000	100.000

Table 29. Estimates of eigen values and percentage of variation under WS2 for 15 drought tolerant indices of F_{11} and F_{13} RIL population developed from the cross NRCG 12568 × NRCG 12326 in groundnut

Principal component (PCs)	Eigen value	Variability (%)	Cumulative (%)
1	8.420	56.135	56.135
2	4.992	33.282	89.418
3	0.998	6.651	96.068
4	0.372	2.482	98.551
5	0.173	1.153	99.704
6	0.035	0.232	99.936
7	0.005	0.035	99.970
8	0.004	0.025	99.995
9	0.001	0.004	100.000
10	0.000	0.000	100.000
11	0.000	0.000	100.000

Table 30. Estimates of eigen values and percentage of variation under WS3 for 15 drought tolerant indices of F_{11} and F_{13} RIL population developed from the cross NRCG 12568 × NRCG 12326 in groundnut

Principal component (PCs)	Eigen value	Variability (%)	Cumulative (%)
1	8.547	56.979	56.979
2	5.144	34.293	91.272
3	0.709	4.729	96.001
4	0.298	1.984	97.985
5	0.264	1.757	99.742
6	0.031	0.206	99.948
7	0.004	0.030	99.977
8	0.002	0.013	99.990
9	0.001	0.009	100.000
10	0.000	0.000	100.000
11	0.000	0.000	100.000

The cosine of the angle between the index vectors represents their approximate positive (acute angles) or negative (obtuse angles) correlation. Overlapping index vectors refer to correlation coefficient of 1 and an identical ranking of accessions. As depicted in biplot and in accordance to Spearman's coefficients of rank correlation, high repeatability was found between MP, GMP, HAM, STI, K_1STI, and K_2STI, Thus, instead of these fifteen, calculating six indices would be sufficient for further studies. Thus, these best indices can be used to detect genotypes which have low water requirements and/or suffer less yield reduction by water shortage during their growth period, to be advised to cultivate in regions with limited water resources in order to enhance cultivated area and production efficiency.

For drought tolerance indices, the PC 1 and PC 2 axes justified 98.32 *per cent* of total variation and mainly distinguished the indices in three groups (DTIs with high correlation and acute angles were assigned in the same group). In addition, considering both axes simultaneously, three groups of associated indices have been identified: one consisting of YI and ATI refer to Group 1 (G1), the second consisting of MP, GMP, HAM, STI, K_1STI, and K_2STI in a Group 2 (G2), while the remaining indice was classified into single Group 3 (G3). Among these, group 2 consisted of major indices, MP, GMP, HAM, STI, K_1STI, and K_2STI and were strongly correlated with yield under both conditions indicating that these criteria are suitable for identification of drought tolerant genotypes.

Many authors believed that the most suitable indices for selection of drought tolerant genotypes are indicators, which had relatively high correlation with yield in both non-stress and stressed condition (Farshadfar *et al.*, 2001; Zare 2012; Amiri *et al.*, 2014; Jatav and Kandalkar, 2014 and Subhani *et al.*, 2015). The biplot analysis has become an often-used tool for data in plant breeding research; including those performed on tomatoes (Adalid *et al.*, 2010; Joshi *et al.*, 2011; Panthee *et al.*, 2013; Hernández *et al.*, 2014; Thi, 2016). As depicted in Fig. 1, the two moisture conditions (WW and WS) differed in terms of the analyzed relations with pod yield per plant. Besides the starting breeding material, it is of great importance to choose the selection criteria applied to distinguish desirable genotypes.

Regarding the principal components analysis, results for the indices and biplot were displayed based on the first two factors. The higher scores for PC1 and lower scores for PC2 were in accordance with the higher rank of drought tolerance. Biplot analysis (Fig. 11, 12 and 13) showed that RILs *viz*., RIL 126, RIL 139, RIL 108, RIL 191, RIL 133, RIL 249, RIL 529 and RIL 128 were considered to be most drought tolerant RILs. Zare (2012) used same method for identification of drought tolerant genotypes in barley. Similarly, in safflower by Bahrami *et al.* (2014), in Tomato by Thi (2016) and Suresh (2018), in groundnut by Savita (2017). The results of this study are comparable with Fernandez (1992); Kaya *et al.* (2002); Golabadi *et al.* (2006); Farshadfar *et al.*, (2012); Zare (2012); Bahrami *et al.* (2014); Brdar-Jokanović *et al.* (2014a and 2017).

Table 31. Superior RILs of groundnut from the cross NRCG 12568 × NRCG 12326 for WUE traits, yield and yield related traits under water stress condition across two seasons based on biplot analysis and ranking method

RIL No.	Pods/ plant	Pod yield/ plant (g)	Kernel yield / plant (g)	SCMR	SLA (cm^2/g)
541	40.90	24.62	14.10	49.10	102.17
126	27.30	20.24	13.41	51.27	113.25
139	27.40	19.84	12.01	48.29	94.81
145	25.30	19.60	11.24	48.09	103.99
108	33.00	19.06	12.53	50.29	90.76
273	25.40	18.02	10.61	48.51	126.31
231	26.60	17.83	9.69	48.30	91.55
191	31.40	17.67	10.76	49.92	87.69
133	26.50	17.26	10.04	46.04	102.98
125	29.30	17.22	10.08	49.74	126.89
248	25.90	17.00	10.80	49.17	100.16
249	23.00	16.93	11.06	48.96	109.94
539	27.70	16.78	9.02	46.84	109.76
529	26.20	16.55	9.18	46.53	100.24
128	24.10	15.06	7.38	50.55	108.63
25	18.40	15.04	8.55	52.04	127.44
Mean	13.79	8.85	4.82	47.58	112.23
SEm±	1.02	1.51	1.04	1.76	10.01
CD @ 5%	3.11	4.27	3.71	4.05	27.12

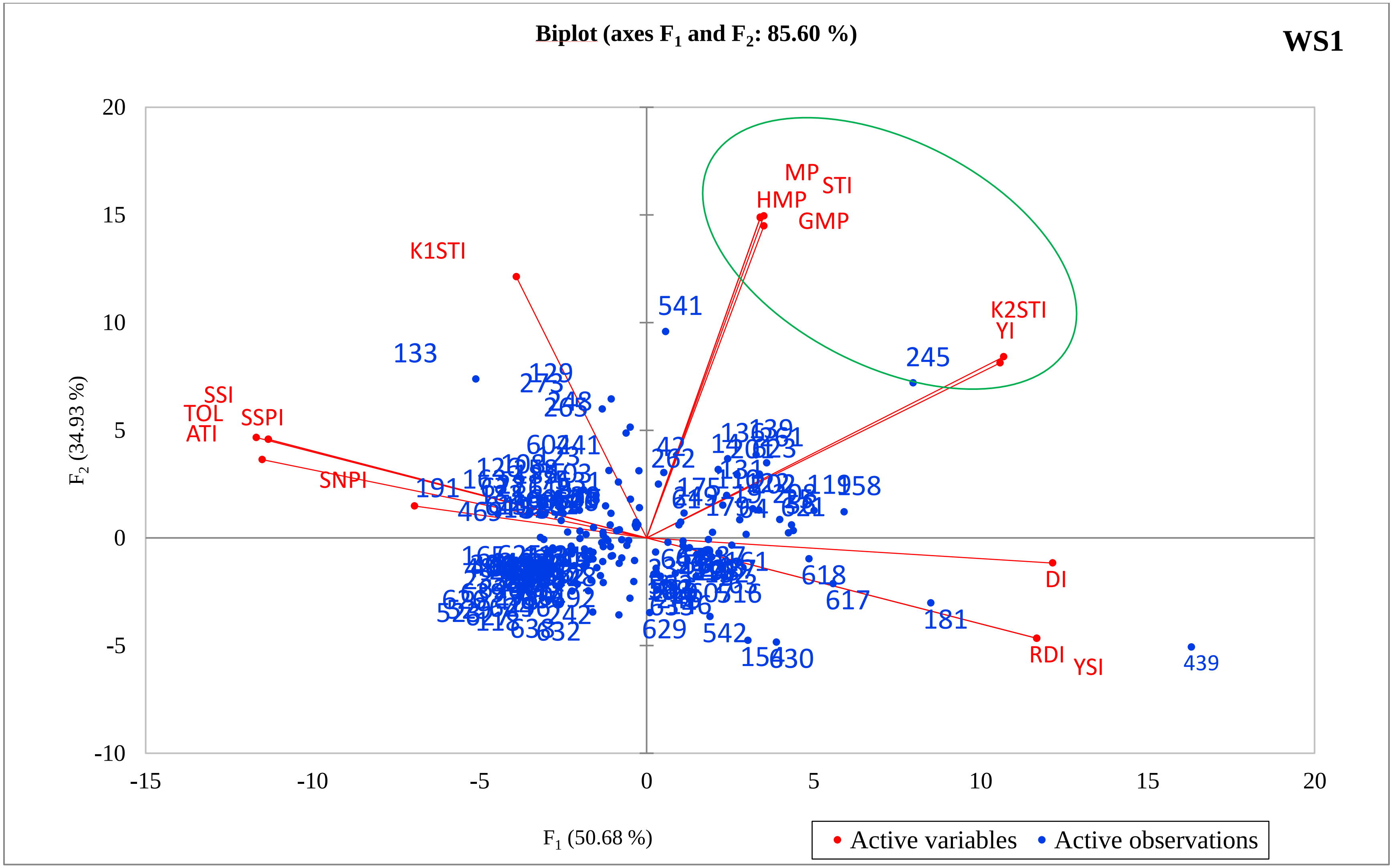


Fig. 11. Identification of drought tolerant RILs through indices using Biplot analysis under WW and WS1 conditions

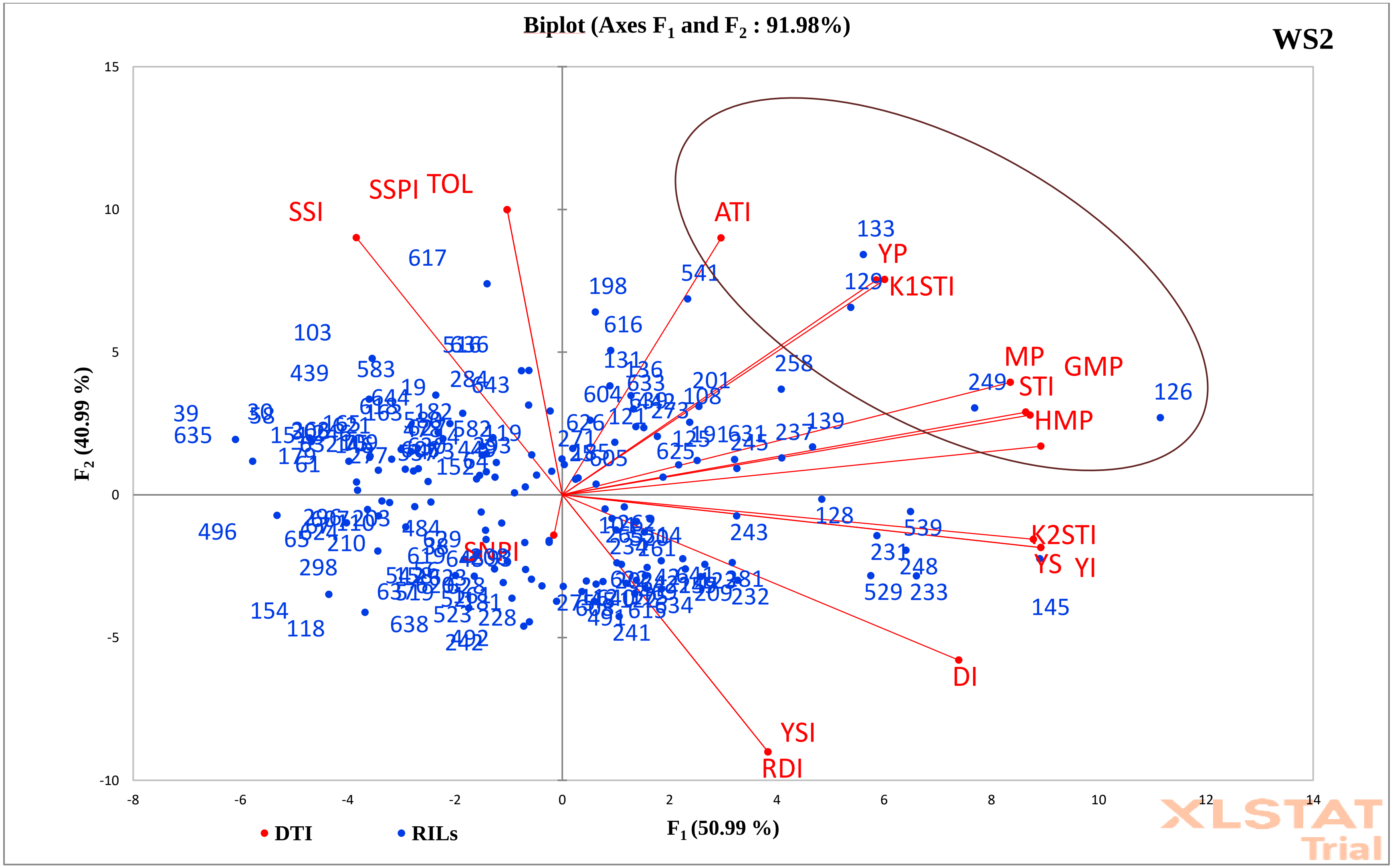


Fig. 12. Identification of drought tolerant RILs through indices using Biplot analysis under WW and WS2 conditions

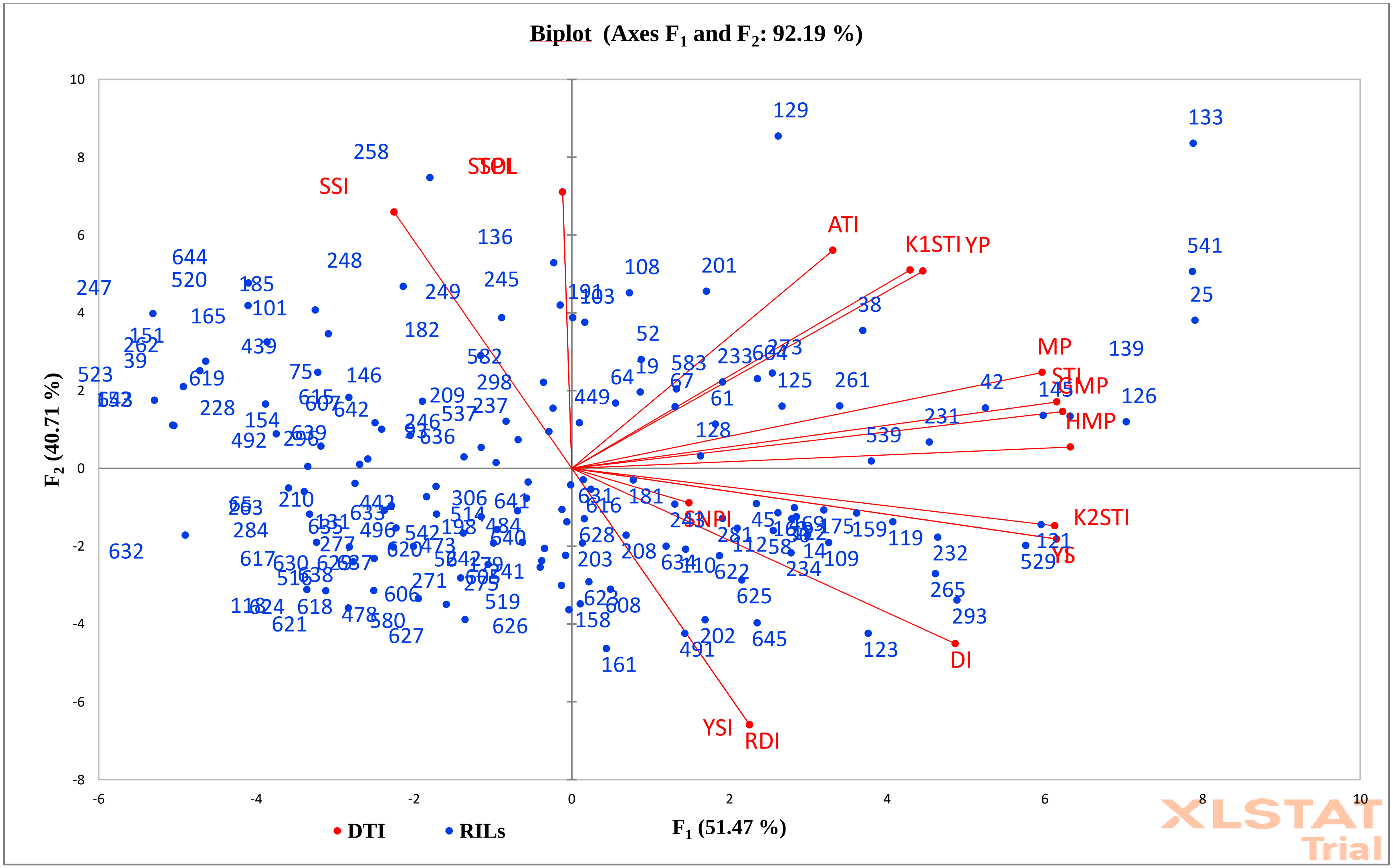


Fig. 13. Identification of drought tolerant RILs through indices using Biplot analysis under WW and WS3 conditions

Table 32. Superior RILs of groundnut from the cross NRCG 12568 × NRCG 12326 for surrogate traits of WUE and yield related traits under water stress condition across two seasons selected based on *per se* performance

RIL No.	Pods/ plant	Pod yield/ plant (g)	Kernel yield / plant (g)	SCMR	SLA (cm^2/g)
541	40.90	24.62	14.10	49.10	102.17
126	27.30	20.24	13.41	51.27	113.25
139	27.40	19.84	12.01	48.29	94.81
145	25.30	19.60	11.24	48.09	103.99
108	33.00	19.06	12.53	50.29	90.76
191	31.40	17.67	10.76	49.92	87.69
133	26.50	17.26	10.04	46.04	102.98
125	29.30	17.22	10.08	49.74	126.89
248	25.90	17.00	10.80	49.17	100.16
249	23.00	16.93	11.06	48.96	109.94
539	27.70	16.78	9.02	46.84	109.76
529	26.20	16.55	9.18	46.53	100.24
128	24.10	15.06	7.38	50.55	108.63
Mean	13.79	8.85	4.82	47.58	112.23
SEm±	1.02	1.51	1.04	1.76	10.01
CD @ 5%	3.11	4.27	3.71	4.05	27.12

4.2.4.3 Identification of superior RILs

In comparison with parents and checks, 17 superior RILs were identified over two seasons and under different water regimes *viz*., RIL 541, RIL 126, RIL 139, RIL 145, RIL 108, RIL 273, RIL 231, RIL 604, RIL 191, RIL 133, RIL 125, RIL 248, RIL 249, RIL 539, RIL 529, RIL 128 and RIL 25 were selected based on statistical approaches (Rank sum method and biplot analysis) and individual mean ± SE for surrogate traits of WUE, SLA and SCMR and pod yield and yield attributing characters, pod per plant, shelling percentage, sound mature kernel percentage and kernel yield per plant in RILs (Table 31).

In consideration to *per se* performance of the RIL population, 13 RILs (541, 126, 139, 145, 108, 191, 133, 125, 248, 249, 539, 529, 128) identified which outperformed in water stress condition (Table 32). These were common in superior RILs selected based on combination of ranking method and biplot analysis. While, RIL 273, RIL 231 and RIL 25 differed from selection based on *per se* performance. They also outperformed over parents and checks under water stress conditions, based on individual mean ± SE for surrogate traits of WUE (SLA and SCMR), pod yield and yield attributing characters.

The three RIL's 125, 133 and 25 showed consistent superior performance over the seasons under different water regimes. The superior performance of the RILs will be confirmed by evaluating in multilocations/ over seasons for higher WUE and high pod yield. These superior RILs could be exploited in future breeding programme.

4.3 Construction of genetic linkage map

RIL populations are ideally considered for QTL mapping because of several advantages over F_2 population. The major advantage of RIL is as each line is 'true-breeding' or homozygous line that can be multiplied and reproduced without genetic change occurring there by allowing for genotyping and phenotyping of many traits under various environmental conditions to be performed on the same material (Simon *et al.*, 2008). Further, the RIL population undergoes several rounds of meiosis before homozygosity is reached; the degree of recombination is higher compared to F_2 populations. Consequently, RIL populations show a higher resolution than maps generated from F_2 populations (Burr and Burr 1991). Seeds may be transferred between different laboratories for further linkage analysis and the addition of markers to existing maps ensuring that all collaborators examine identical material (Paterson 1991 and Young 1996). Therefore, RIL mapping population serves as 'eternal' resource for QTL mapping.

4.3.1 Parental polymorphism survey

A total of 612 SSR markers (both genomic and EST based) available in public domain (Appendix 3) and/or accessed from other sources/various collaborators were used

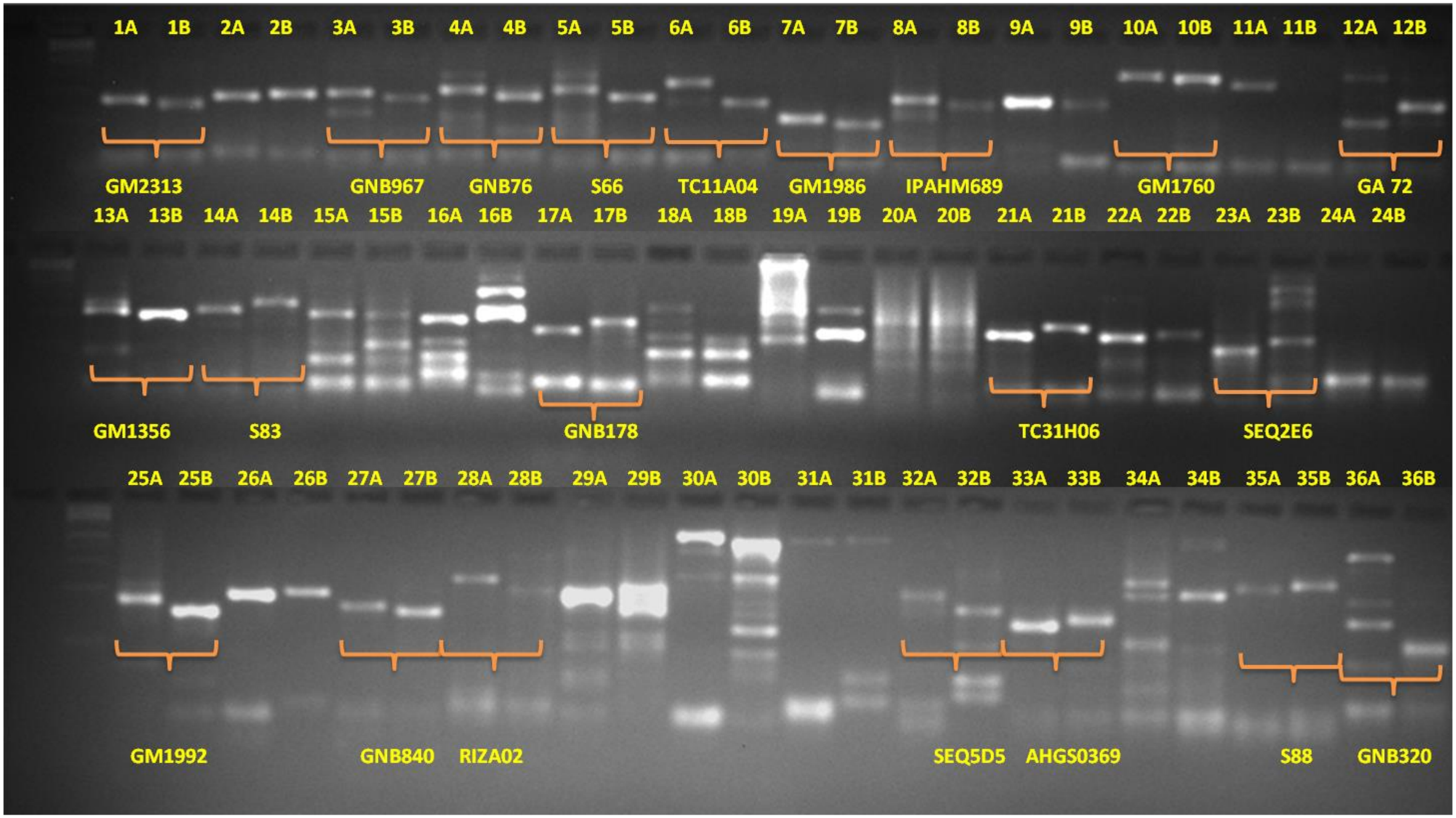


Plate 6. Gel picture depicting the parental polymorphism of few SSR markers in 4% Agarose; Metaphor + Agarose (2% + 2%)

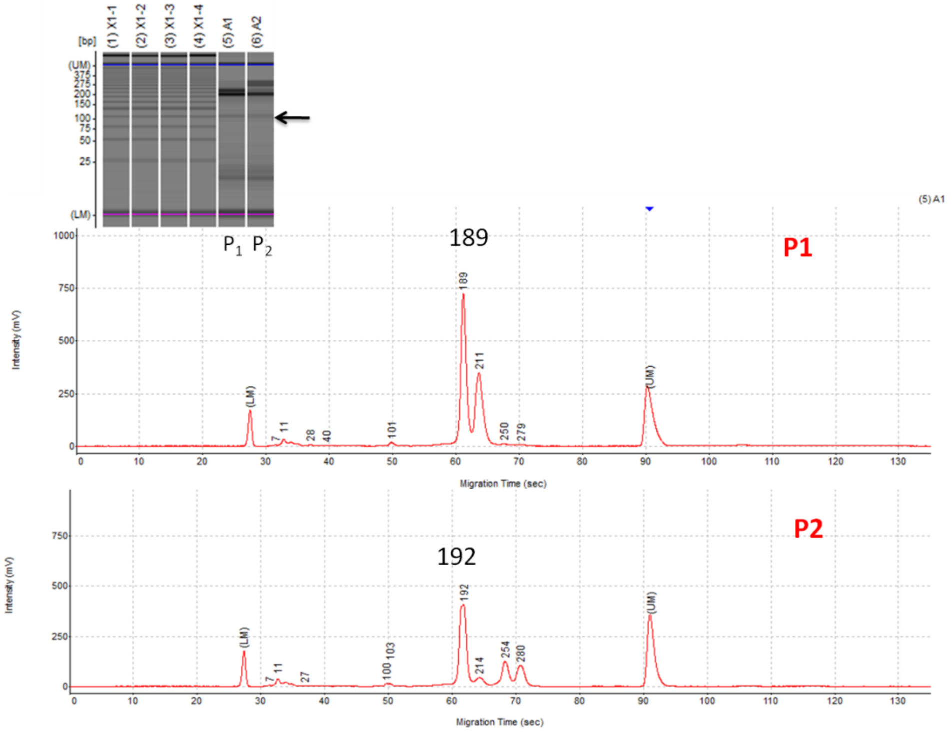


Plate 7. Gel pictures depicting the parental polymorphism of SSR markers RI2A06 in MultiNa

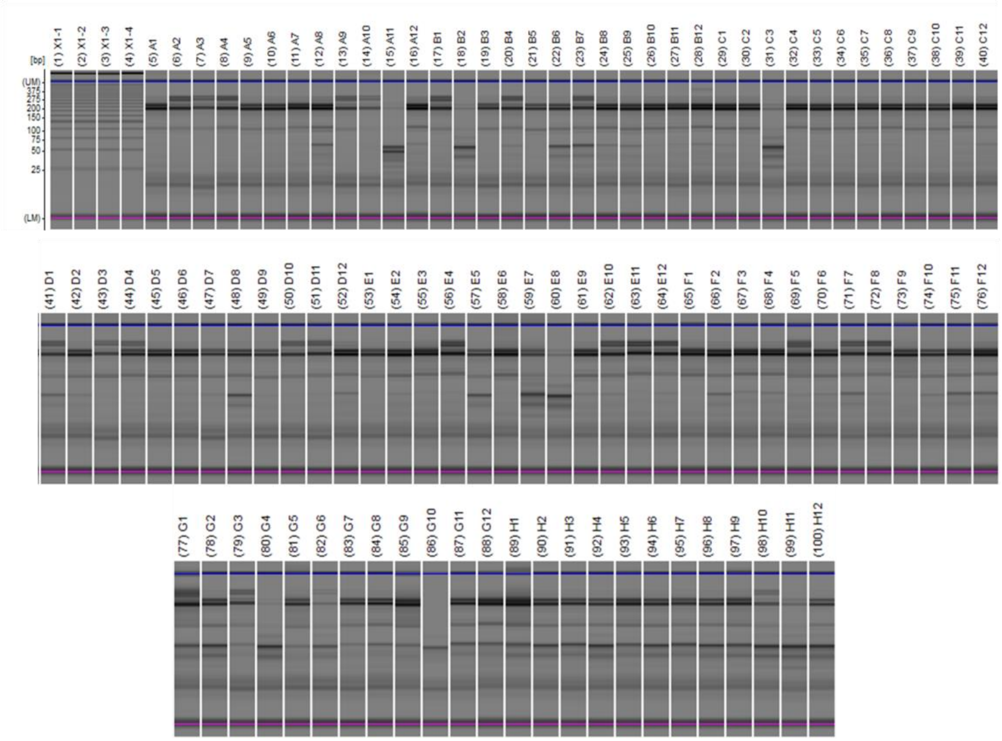


Plate 8. Genotyping with marker RI2A06 in the RIL population on capillary electrophoresis, MultiNa

to screen the polymorphism on the parental genotypes of the mapping populations NRCG 12568 × NRCG 12326 and were subsequently used in the present study for construction of linkage map and further for identifying QTLs associated with drought related traits.

Overall, after screening a total of 612 SSR markers on the parental genotypes of the mapping population, 198 polymorphic markers were obtained, out of which 172 markers were used for genotyping and linkage map construction (Plate 6 and 7).

4.3.2 Marker segregation

The genotypic data obtained for 198 SSR markers in 147 RILs was analyzed for marker segregation (Plate 8). The expected frequency of homozygous NRCG 12568 alleles and NRCG 12326 alleles in RIL population according to mendelian pattern i.e. in 1:1 ratio (equal proportion), respectively.

4.3.3 Construction of linkage map

Linkage map was developed based on the marker data produced from the RILs of NRCG 12568 × NRCG 12326. Out of 612 microsatellite markers used for screening the parents, 198 (32.25 %) were polymorphic (Appendix 4). Marker segregation data was used to construct the linkage map using QTL IciMapping v.4.0 (Wang *et al.,* 2014) software. The chi square test was employed to test segregation ratio for the markers. All the 172 polymorphic markers were used for linkage map construction, the markers exhibiting segregation distortion among the markers were not considered. Linkage groups were formed at a minimum LOD of 3.0 and a maximum distance of 50.0 cM, 172 markers were mapped on 20 linkage groups. The linkage map covered 2212.87 cM with an average marker distance of 11.12 cM. The number of markers mapped per linkage group ranged from 2 (LG-12 and LG16) to 23 (LG-1). The length of the linkage group varied from 12.64 cM (LG-12) to 370.07 cM (LG-1). The number of marker loci mapped on each linkage group along with the number of markers mapped, total length (cM) and average marker density (cM) was given in Table 33.

Recently, a few genetic maps based on RIL populations have been developed in cultivated groundnut (Varshney *et al*., 2009a; Hong *et al*., 2010; Khedikar *et al*., 2010;

Ravi *et al*., 2011 and Sarvamangla *et al*., 2011) and only one population namely TAG 24 × ICGV 86031 has been used for developing the genetic map and QTL analysis for drought tolerance traits. While, Gauthami (2012) used two RIL populations namely ICGS 76 × CSMG 84-1 and ICGS 44 × ICGS 76 segregating for drought tolerance together with the genetic map of TAG 24 × ICGV 86031 developed from the earlier studies (Ravi *et al*., 2011) for constructing a dense consensus map for drought tolerance traits. Savita *et al*. (2017) developed GKVK 4 × NRCG 12473 RIL population and identified QTLs for surrogate traits of WUE and pod yield related traits. In the present study a new mapping population of NRCG 12568 × NRCG 12326, segregating for several traits such as quantitative, physiological, root traits which are related to drought tolerance and other quality parameters was used for the construction of genetic linkage map. The stringent mapping parameters adopted for individual map construction resulted in 20 linkage groups. As a result of collaborative efforts made in last ten years worldwide, nearly 4,000 SSRs were developed by the groundnut community.

The parental genotypes of mapping population NRCG 12568 × NRCG 12326 were screened with a total of 612 SSR markers. However, low level of polymorphism was observed (32.35 %). This may be attributed mainly to a narrow genetic diversity in the cultivated groundnut gene pool (Young *et al*., 1996; Varshney *et al*., 2009; Hong *et al*., 2010; Ravi *et al*., 2011 and Sarvamangla *et al*., 2011). Availability of a high-density genetic map in a crop species is must to initiate genetical and molecular breeding activities. The newly developed map consists of 172 SSR markers distributed over 20 linkage groups. Two out of 20 linkage groups possessed more than two markers. LG-1 was the highest dense linkage group with 23 markers followed by LG-4 and LG-18 with 21 and 18 markers. LG-12 and LG-16 were very small with only two markers in each linkage groups (Fig. 14 and Table 33). These small linkage groups could be artificial and additional genetic markers are needed to improve the linkage analysis. The observed total map distance of the newly developed map (2212.87 cM) was less than the expected genome length of groundnut genome (2800 Mb/1C) representing the requirement for saturation of the linkage map with more number of SSR markers.

Table 33. Distribution of SSR markers on the linkage map of RIL population of the cross NRCG 12586 × NRCG 12326 in groundnut

Linkage group (LG)	No. of markers	Length (cM)	Average inter-marker distance (cM)
1	23	370.07	16.08
2	11	140.65	12.78
3	11	133.08	12.09
4	21	333.62	15.88
5	6	67.79	11.29
6	6	82.71	13.78
7	4	19.34	4.83
8	5	61.52	12.30
9	5	45.67	9.13
10	13	178.70	13.74
11	5	37.99	7.59
12	2	12.64	6.32
13	11	138.57	12.59
14	5	41.67	8.33
15	9	124.09	13.78
16	2	14.20	7.09
17	3	29.83	9.94
18	18	248.83	13.82
19	4	36.89	9.22
20	8	95.03	11.87
Total	**172**	**2212.87**	**11.12**

Note: cM- centimorgan

As groundnut is tetraploid crop species, few markers amplified more than one polymorphic locus. Amplification of more than one locus may be due to the polyploidy nature of the crop and has been reported in earlier studies (Hopkins *et al*., 1999; Krishna *et al*., 2004; Kottapalli *et al*., 2007; Varshney *et al*., 2009a; 2009b; Ravi *et al*., 2011 and Hong *et al*., 2010)

4.3.4 Phenotyping data analyses

In order to identify quantitative trait loci (QTLs) for drought related traits in mapping populations, the phenotyping was done for drought tolerance traits for two seasons of summer 2017 and 2018 in UAS, GKVK, Bengaluru. The phenotypic data was obtained for various quantitative, physiological traits related to drought tolerance. The phenotyping data of parents and RILs were subjected to analysis of variance (ANOVA). The results on ANOVA and genetic parameters were presented in the section 4.1.

4.4 QTL mapping for traits related to water use efficiency and pod yield traits

Single marker analysis was used to identify significant marker-trait association. Although SMA confounds size effects and location of QTL, it provides preliminary results that facilitate the use of more advanced analytical tools to detect and characterize QTL (Mulualeam and Bekelo, 2016). SMA indicated significant linkage of SSR markers with yield related traits (Table 34).

The SSR markers GI832 and SEQ18E7 located on LG 1 and LG 4 at 246.33 cM and 274.17 cM was significantly linked with days to 50% flowering, respectively and explained 8.35-8.59 *per cent* phenotypic variance (% PVE). Similarly, another SSR marker ‘GM 1992’ located on LG 12 at 12.64 cM was significantly linked with plant height and explained 8.22 *per cent* phenotypic variance (PVE %) with a negative additive effect of -1.65 in RIL population under WW condition (Table 34). SSR marker PM 31 located on LG 15 at 90.57 cM was significantly linked with specific leaf area and explained 11.33 *per cent* phenotypic variance with a negative additive effect of -8.63. Whereas, the SSR markers GM 2165 and SEQ5D5 located on LG 10 were significantly linked with loci controlling DFF and SPAD chlorophyll meter readings with phenotypic variation explained in population ranging from 5.55 -8.17 *per cent*. The marker SEQ5D5

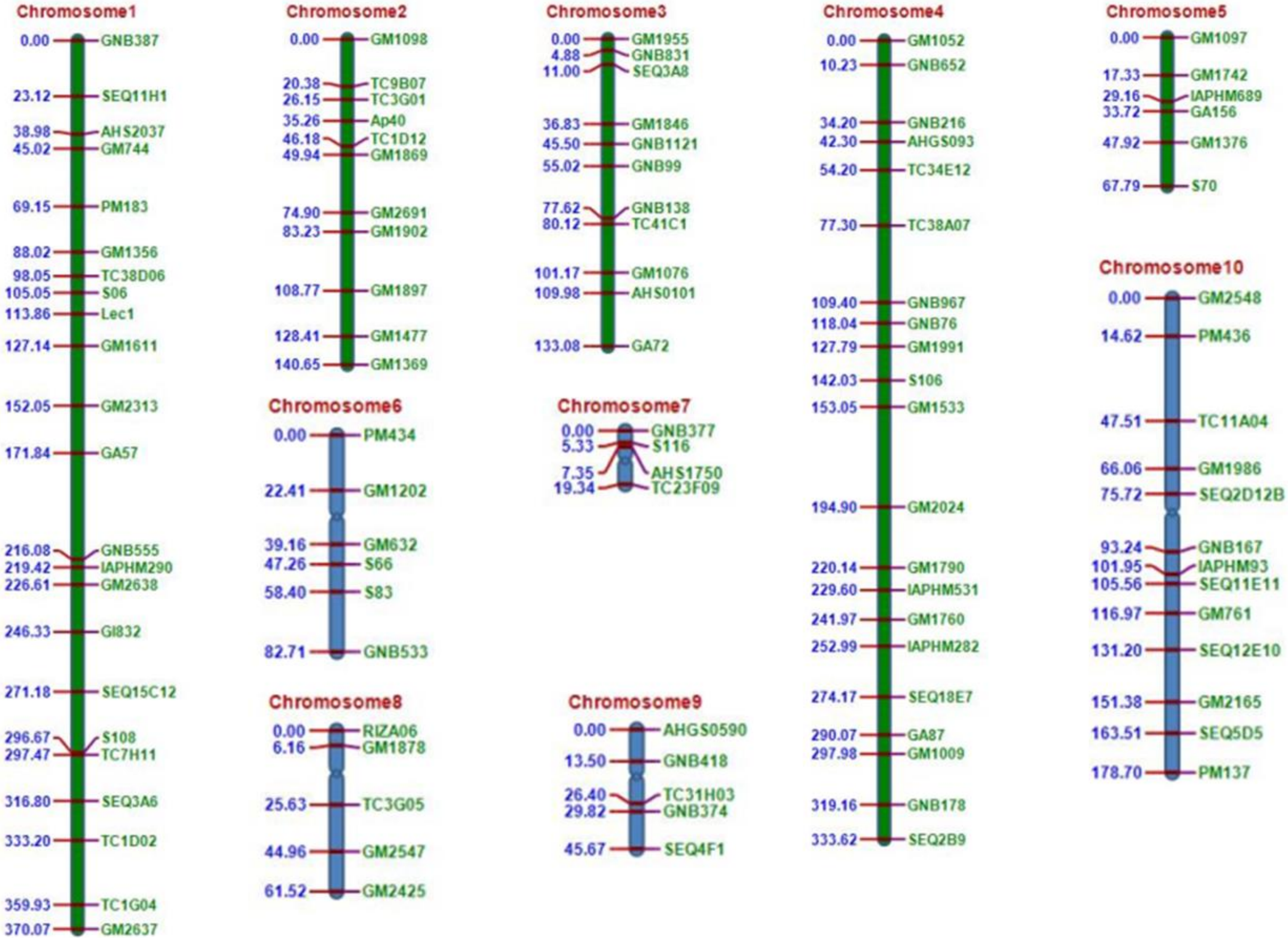
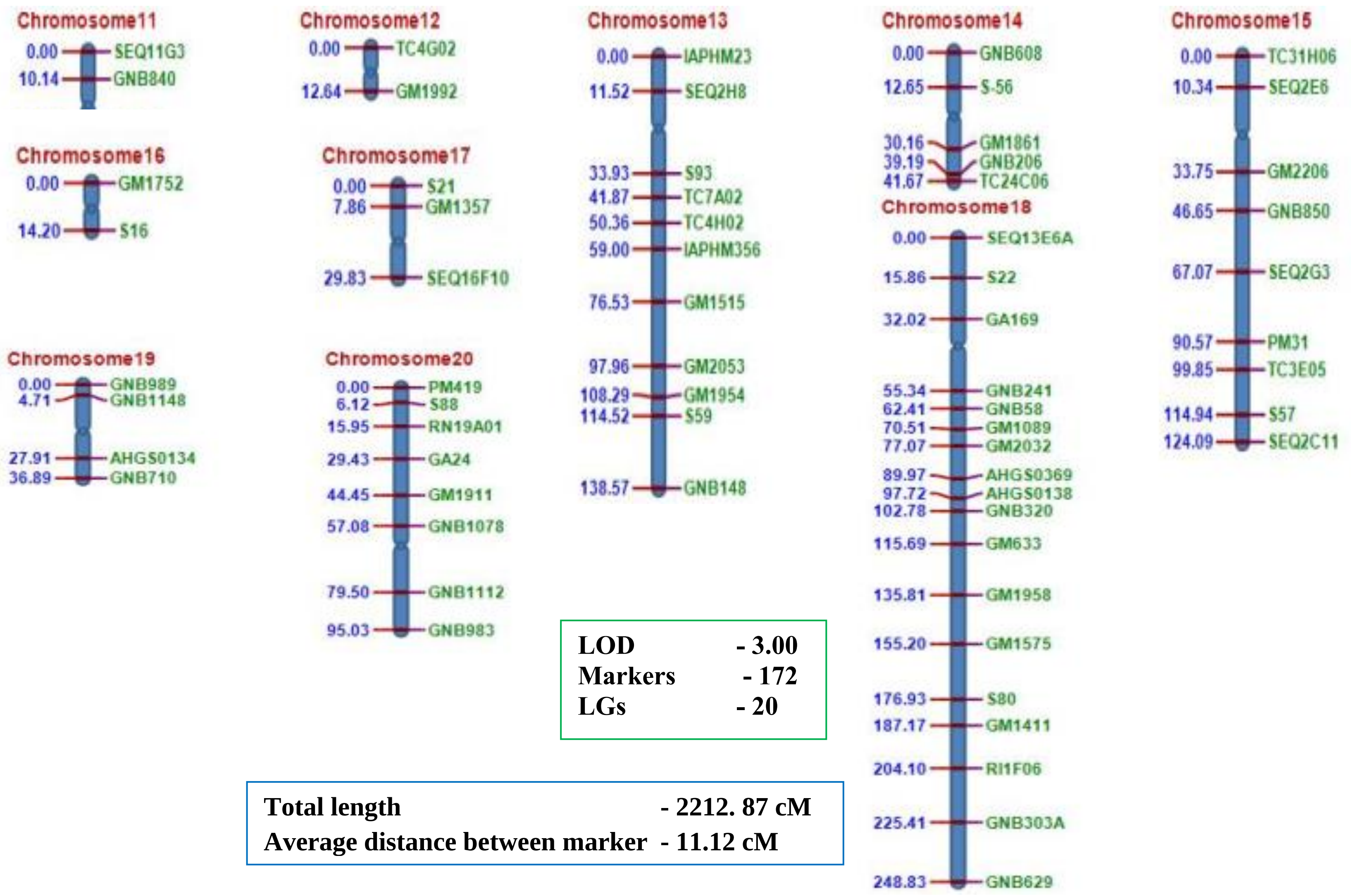


Fig. 14. Genetic linkage map of the cross NRCG 12568 × NRCG 12326



LOD - 3.00
Markers - 172
LGs - 20

Total length - 2212. 87 cM
Average distance between marker - 11.12 cM

Fig. 14. Genetic linkage map of the cross NRCG 12568 × NRCG 12326 (contd…)

is also linked to specific leaf area explaining 9.42 *per cent* phenotypic variance with negative additive effects of -8.63. Sarvamangala *et al*. (2011) reported a total of 25 markers were associated with agronomic traits (plant height and branches) with the phenotypic variation ranging from 2.11 to 14.72 *per cent*. Anitha *et al*. (2015) using SMA, identified six QTLs controlling 100- kernel weight, branches per plant, 100-pod weight, pod yield per plant, kernel yield per plant and oil yield per plant. These QTLs explained *per cent* phenotypic variation ranging from 4.20 to 12.40. Most of the linked SSR markers in the present study explained fairly lower magnitude of *per cent* PVE for days to 50% flowering, plant height, branches per plant, SLA and SCMR with *per cent* phenotypic variation ranging from 5.49 – 11.33. The large variations explained by the markers linked to genomic regions controlling these two traits are expected as these traits are controlled by monogenic/ oligogenic inheritance.

Table 34. Single marker analysis for surrogate traits of WUE, pod yield and yield related traits of RIL population of the cross NRCG12568 × NRCG 12326 in groundnut

Trait Name	Marker Name	Position	Linkage group	LOD	PVE (%)	Additive effects
Days to 50% flowering	GI832	246.33	1	3.33	8.35	-0.92
	SEQ18E7	274.17	4	3.43	8.59	0.94
	GM2165	151.38	10	2.70	6.85	0.84
	SEQ5D5	163.51	10	2.61	8.17	-0.38
Plant height	GM1992	12.64	12	2.62	8.22	-1.65
Branches/ plant	PM434	0.00	6	2.51	7.88	0.12
Specific leaf area (SLA)	PM31	90.57	15	3.68	11.33	-8.63
	GM2691	74.90	2	3.45	6.19	6.13
	GM1902	83.23	2	3.08	5.55	5.79
	SEQ5D5	163.51	10	3.03	9.42	5.95
SPAD Chlorophyll Meter readings (SCMR)	IAPHM689	29.16	5	2.95	9.18	0.70
	SEQ5D5	163.51	10	2.92	9.10	-1.19

Single marker analysis is the simplest tool and significance of phenotypic groups is based on regression models or ANOVA. It is preliminary and least informative and does not reveal the location and effects precisely. Hence, QTL mapping was undertaken based on the information on inter marker distance obtained in the linkage map with the help of IciMapping software which used inclusive composite interval mapping (ICIM).

4.4.2 Identification of quantitative trait loci (QTL) for growth, pod yield related traits and surrogate traits of WUE

QTLs for morphometric and physiological traits were identified by integrating the genotyping and phenotyping data of two seasons under different water regimes experiments and also the pooled data over seasons in each experiments for all the traits under study using the QTL IciMapping ver. 4.0 (Wang *et al.*, 2014) software by following Inclusive composite interval mapping (ICIM) method. Results on QTLs identified for various traits are presented in Table 35.

In present study totally 21 QTLs were identified for four different morphometric traits and two related to surrogate traits of water use efficiency recorded under three water regimes in two consecutive seasons in groundnut. The QTLs explaining more than 10% phenotypic variation were considered as major QTLs (Collard *et al*., 2005). Among all traits, there were no QTLs have been identified for traits like pods plant^{-1}, kernel yield plant^{-1}, pod yield plant^{-1} and sound mature kernels.

4.4.2.1 Growth and yield related traits

Total of 10 QTLs were detected for growth and yield related traits while considering the two generations and four water regimes and also pooled mean of F_{11} and F_{13} generations (under well-watered and water stress conditions). These QTLs individually explained 7.21 - 11.77 *per cent* of phenotypic variance (Table 35). The QTLs on linkage map associated with growth and yield related traits in NRCG 12568 × NRCG 12326 mapping population is presented in Fig 15.

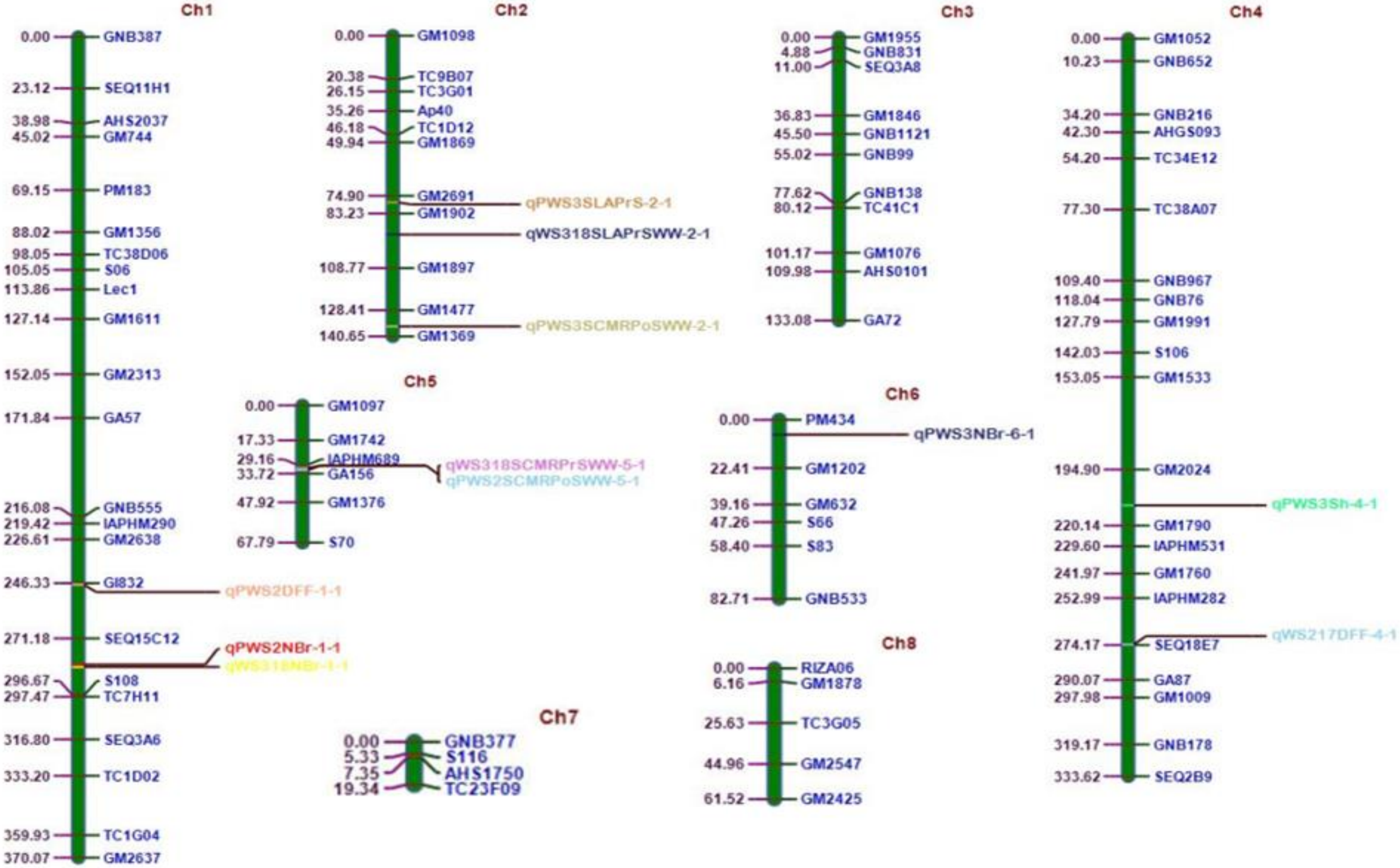


Fig. 15. QTL for traits related to WUE and yield related parameters identified on QTL map of groundnut

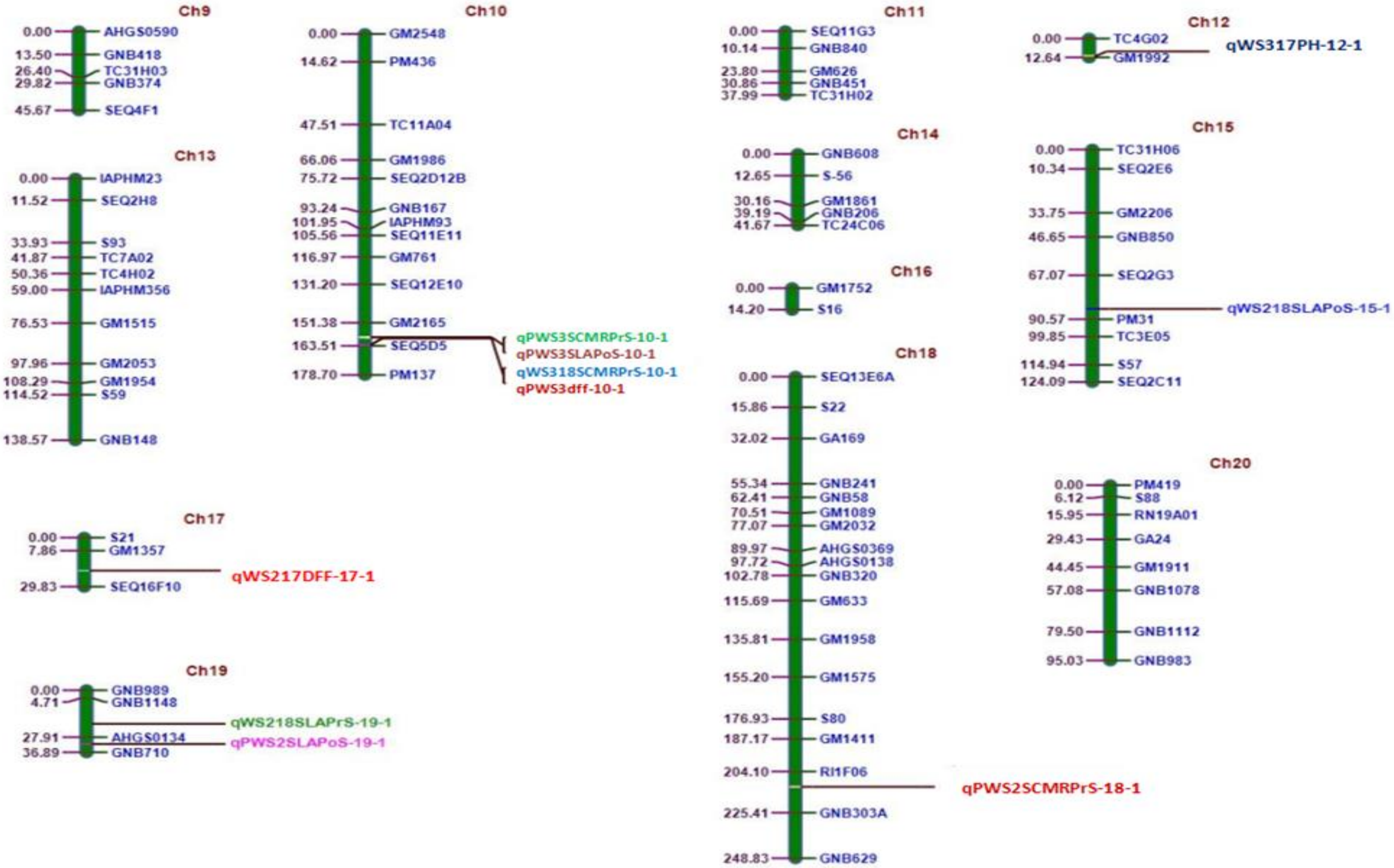


Fig. 15. QTL for traits related to WUE and yield related parameters identified on QTL map of groundnut (contd…)

Table 35. Linkage group, identity, position and size effects of QTL controlling surrogate traits of WUE in the RIL population of the cross NRCG12568 × NRCG 12326 in groundnut

	Trait Name	Linkage group	Position	Flanking markers (FM)		LOD	Left marker	Right marker	Distance between FM (cM)	PVE (%)	Additive effects
DFF	WS217DFF	4	274	IAPHM282	SEQ18E7	3.42	266.50	280.50	14.00	7.71	0.94
	WS217DFF	17	20	GM1357	SEQ16F10	3.18	10.50	29.00	18.50	11.77	1.16
	PWS3DFF	10	163	GM2165	SEQ5D5	2.60	155.50	170.50	15.00	8.35	-0.39
	PWS2DFF	1	247	GI832	SEQ15C12	3.35	240.50	255.50	15.00	10.65	-0.51
PH	WS317PH	12	12	TC4G02	GM1992	2.63	3.50	12.00	8.50	8.24	-1.70
NBr	WS318NBr	1	284	SEQ15C12	S108	2.56	270.50	295.50	25.00	7.21	0.13
	WS118NBr	16	9	GM1752	S16	2.62	0.00	14.00	14.00	7.61	-0.17
	PWS3NBr	6	7	PM434	GM1202	2.81	0.00	17.50	17.50	8.65	0.16
	PWS2NBr	1	283	SEQ15C12	S108	2.53	267.50	295.50	28.00	7.60	0.20
SH	PWS3Sh	4	211	GM2024	GM1790	2.50	197.50	225.50	28.00	7.93	-2.22
SLA	WS218SLAPrS	19	20	GNB1148	AHGS0134	2.69	5.50	27.50	22.00	9.33	-18.48
	WS218SLAPoS	15	85	SEQ2G3	PM31	4.04	74.50	89.50	15.00	12.62	-10.60
	WS318SLAPrSWW	2	93	GM1902	GM1897	3.12	83.50	103.50	20.00	8.76	11.93
	PWS2SLAPoS	19	32	AHGS0134	GNB710	2.53	28.50	36.00	7.50	7.66	-9.06
	PWS3SLAPrS	2	78	GM2691	GM1902	3.76	70.50	83.50	13.00	11.74	6.82
	PWS3SLAPoS	10	159	GM2165	SEQ5D5	3.29	151.50	169.50	18.00	10.04	6.85
SCMR	WS318SCMRPrS	10	163	GM2165	SEQ5D5	2.91	155.50	171.50	16.00	9.25	-1.21
	PWS2SCMRPrS	18	212	RI1F06	GNB303A	2.58	204.50	219.50	15.00	6.27	0.95
	PWS2SCMRPoSWW	5	32	IAPHM689	GA156	3.40	29.50	38.50	9.00	10.85	0.92
	PWS3SCMRPrS	10	159	GM2165	SEQ5D5	4.67	153.50	167.50	14.00	13.55	-1.06
	PWS3SCMRPoSWW	2	136	GM1477	GM1369	2.80	128.50	140.00	11.50	8.60	0.82

DFF- Days to 50% flowering
PH- Plant height (cm)
NBr- No. of Branches plant^{-1}
SH- Shelling percent
SLA- Specific leaf area (cm^2/g)
SCMR- SPAD Chlorophyll meter reading
PWS- Pooled water stress
PrS- Prior to stress
PoS- Post stress

Days to 50 *per cent* flowering

Four QTLs were detected for days to 50 % flowering LG1 (1 QTL), LG 4 (1 QTL), LG 10 (1 QTL) and LG 17 (1 QTL) (Table 35) with phenotypic variation ranging from 7.71 to 11.77 *per cent*. An M-QTL identified in F_{11} RILs, flanked by GM1357 – SEQ16F10 located on LG17 contributed maximum phenotypic variation 11.77 *per cent* with LOD 3.18. These results suggest that favorable allele of this QTL came from NRCG 12568 with positive additive effects of 1.16 (Table 35). Similarly, Another M-QTL was identified in pooled mean of water stress II conditions, flanked by GI832 – SEQ15C12 located on LG 1 contributed 10.65 *per cent* PVE at LOD 3.35. The favorable allele of this QTL came from the parent NRCG 12326 with negative additive effect of -0.52.

Plant height (cm)

One QTL ‘qWS317PH’was detected for plant height on LG 12 under water stress III in F_{11} RIL population. This was flanked by the markers TC4G02 and GM1992 explaining 8.24 *per cent* phenotypic variance with the LOD value of 2.63 and derived from parent NRCG 12326 contributing negative effects of -1.70 for the trait indicates this genomic region contribute to reduce the plant height in RIL population. Fonceka *et al*. (2012) identified five QTL together explained 40.9 *per cent* of phenotypic variation and responsible to increase in the plant height under well-watered condition. Huang *et al*. (2015) detected three QTLs for plant height and they explained 6.12 *per cent* - 8.80 *per cent* of PVE.

Primary branches per plant

Four QTLs were identified for branches per plant when considering the F_{13} RIL (WS1 and WS3) and pooled mean of F_{13} RILs under WS2 and WS3 condition (Table 35). The QTL flanked by SEQ15C12 – S108 on LG1 contributed PVE *per cent* (7.21 % and 7.60 %) with LOD score of 2.56 and 2.53 in F_{13} WS3 and pooled mean of F_{13} WS2 condition, respectively.

The favorable alleles of this QTL came from NRCG 12568 with positive additive effects of 0.13 and 0.20 respectively. The SSR marker PM434 is also found linked to genomic region controlling number of branches per plant in single marker analysis. One each QTL on LG 16 and 6 was identified in F_{13} WS1 and pooled mean of WS3 conditions with the phenotypic variation of 7.61 and 8.35 *per cent* contributing additive effects of -0.17 and 0.16, respectively (Table 35). These results explain that these alleles were contributed by both increasing and decreasing parents. Sarvamangala *et al*. (2012) reported one QTL for branches per plant explained the phenotypic variance of 2.10 *per cent* with an additive effect of 0.155.

Shelling *per cent*

Only one QTL was identified for shelling *per cent* under pooled water stress III condition (Table 35). The QTL was flanked by GM2024 - GM1790 located on LG 4 explained phenotypic variation of 7.93 *per cent* with LOD score of 2.50. This QTL might be responsible for increase in shelling *per cent* and the favorable alleles of this QTL came from NRCG 12326 with negative additive effect of -2.22. This QTL is specific to water stress condition. Sarvamangala *et al*. (2012) reported five QTLs for shelling *per cent* explained 1.90 *per cent* - 7.10 *per cent* with the positive additive effects. Anita *et al*. (2015) identified two QTLs for shelling *per cent* with the phenotypic variation ranging from 4.9 *per cent* to 6.9 *per cent*.

4.4.2.2 QTLs identified for surrogate traits of water use efficiency traits

To identify QTLs for drought tolerance, an extensive study was done in TAG 24 × ICGV 86031. Varshney *et al*. (2009a) and Ravi *et al*. (2011) identified several M-QTLs and a large number of E-QTLs for drought tolerance related traits in different seasons. Similarly Gauthami *et al.* (2012) also reported several M-QTLs and a large number of E-QTLs for drought tolerance related traits in their consensus map developed from the two new mapping populations ICGS 76 × CSMG 84-1 and ICGS 44 × ICGS 76 along with mapping population TAG 24 × ICGV 86031. Since the QTLs identified in all the previous studies on drought tolerance in groundnut revealed large number of QTLs with low phenotypic variance was imperative of complex nature of drought tolerance and its component traits, it is necessary to confirm these in new mapping populations and identify the new QTLs if any. In this context, QTL analysis for drought tolerance related traits was undertaken in the present study on the new mapping population of NRCG 12568 × NRCG 12326.

SPAD Chlorophyll Meter Readings (SCMR)

QTL IciMapping, using the inclusive composite interval mapping (ICIM) method, detected a total of eleven QTLs for traits related WUE, in the F_{11} and F_{13} RIL population evaluated under four water regimes (Table 35). Five QTLs for SCMR (SPAD chlorophyll meter reading under WW and WS conditions) were identified with phenotypic variance explained (PVE) 6.27 *per cent* (RI1F06 - GNB303A) to 13.35 *per cent* (GM2165 - SEQ5D5). Among these two major-QTL (M-QTL) detected on LG 5 and LG 10 flanked by markers IAPHM 689 - GA156 and GM2165 - SEQ5D5, respectively. QTL mapped on LG 10 explained greater phenotypic variance of 13.35 with negative additive effects (-1.06) at LOD score of 4.67 (Fig 15). Another QTL was mapped on LG 5 explained phenotypic variance of 10.35 with positive additive effects (0.92) at LOD score of 3.40. Three minor-QTLs were detected on LG10, LG18 and LG 2 with PVE ranged from 6.27 to 9.25 *per cent*. These QTLs explaining lower to moderate phenotypic variance. Hence, minor QTLs need to be validated across different genetic background and locations.

Specific Leaf Area

A total of six QTLs were detected for SLA. Among them three major QTL was identified for SLA (Specific leaf area under water stress) on LG15, LG 2 and LG 10. QTL on LG 15 located at 85 cM and flanked by SEQ2G3- PM31 with LOD score of 4.04 explains greater phenotypic variance of 12.62 *per cent* with negative additive effects of -10.60, indicating favorable alleles coming from high water use efficient parent (NRCG 12568). QTL mapped on LG 2 located at 78 cM flanked by markers GM 2691 and GM 1902 explains 11.74 *per cent* of phenotypic variation with positive additive effects (6.82) at LOD score 3.76 indicating favorable alleles coming from low water use efficient parent (NRCG 12326). QTL mapped on LG 10 located at 159 cM flanked by markers GM 2165 and SEQ5D5 explains 10.04 *per cent* of phenotypic variation with positive additive effects (6.85) at LOD score 3.29 indicating favorable alleles coming from low water use efficient parent (NRCG 12326).

Three minor-QTLs were detected on LG19 (2 QTLs) and LG 2 (1 QTL) with PVE ranged from 7.66 to 9.33 *per cent*. The allele linked to SSR markers GM 2165 and SEQ5D5 contributed to decrease SLA under water stress condition and increased SCMR under well-watered condition (Table 35). Varshney *et al*. (2009) identified eight QTLs for SLA and nine QTLs for SCMR with PVE *per cent* ranged from 3.5 to 17.6 *per cent* and 2.9 to 11.0 *per cent* respectively. Ravi *et al*. (2011) reported 13 QTLs for SLA and 29 QTLs for SCMR with PVE *per cent* ranges from 3.48 to 13.29 and 4.00 to 19.53.

In general, alleles with low to high additive effects were identified for majority of the traits under study. However, alleles with low to medium additive effects were detected in the earlier study using TAG 24 × ICGV 86031 (Ravi *et al*., 2011). The combination of these favorable alleles derived from both the tolerant (positive additive effect) and the susceptible (negative effect) parents may confer more tolerance to drought (Gauthami *et al.,* 2012). Alleles that improve the trait being derived from parents agronomically inferior have also been identified for several plant species (Xiao *et al*., 1998; Frary *et al*., 2004 and Yoon *et al*., 2006). Since QTLs with low to high phenotypic variation were detected in contrast with earlier study (Ravi *et al*., 2011), there is a need for identification of stable QTLs over seasons. Stable QTLs which are identified in two or more environments of the two water regimes across the two seasons were given in the Table 35.

A considerable number of QTLs were identified in the present study for drought related traits with low to high phenotypic variation for different yield and drought component traits suggesting that drought tolerance is governed by a large number of main effect QTLs and epistatic QTLs. In the present study a total 21 QTLs were identified for 9 traits evaluated in different water regimes on 20 linkage groups, among which 7 were major QTLs while remaining 14 were minor QTLs. Among a total of 10 QTLs identified for quantitative traits, 2 were major QTLs and 8 were minor QTLs. Similarly, five among eleven QTLs for physiological traits were found to be major QTLs identified under different water regimes.

Some of the genomic regions had QTLs mapped for various traits. Such regions between the markers which are common for various traits identified in the present study are given in Table 35. Understanding of relation between those traits can be useful for harnessing such QTLs (Gautami *et al.,* 2012).

The GM 2165 – SEQ5D5 (159-163cM) region on LG-10 harbored 4 QTLs for three different traits under four water regimes of two summer seasons. Under water stress conditions, genotypes favoring dense plant canopy (lower plant height but with more number of primary and secondary branches per plant), higher root growth and lower leaf thickness, it is expected to have greater photosynthetic capacity per unit leaf area (Nageswara Rao *et al*., 1995) and they can maintain water status and are likely to achieve higher yields under intermittent drought stress in that field experiment. As mentioned above, a recent finding indicated that lines having lower plant stature could be better adapted to intermittent stress conditions (Ratnakumar and Vadez 2011) by limiting the effect of stress on reproduction, thereby the link with the HI. LOD peak for various traits on LG-10 between GM 2165 – SEQ5D5 region ranged from 2.60 – 4.67. Another region between SEQ15C12 and S108 on LG-1 harbored two QTLs for primary branches per plant. These QTLs can be considered as stable QTLs as they have been identified across the seasons and also under different water regimes. These stable QTLs should be validated in the other material and can be used in further breeding programmes for improving the quality of groundnut under water limited conditions. Other QTLs which are specific in each season have to be validated in different WS conditions for dissecting the stable QTLs in all water limiting situations.

The stability and accuracy of QTLs are still affected by environmental factors, including the season and climatic conditions. Nevertheless, we detected several stable QTLs that are common across different years and environments as well as several pleiotropic QTLs. The co-localization of QTLs was observed for yield-related traits in this study, which is similar to that in other crops, such as soybean (Xie *et al*., 2014), rapeseed (Shi *et al*., 2009; Li *et al*., 2014; Liu., 2015), and rice (Zuo and Li, 2014). The significant pleiotropic QTLs suggest that these traits are influenced by several genes that control different aspects of complex metabolic pathways, and they might have resulted from the artificial selection and rapid evolution of multiple traits in peanut breeding (Yoshizawa *et al*., 2013).

Such clusters can be considered as hotspot genomic regions for further study and utilization in improving crop productivity through introgression of these genomic regions. Further studies are required to dissect these regions to identify tightly linked markers for the QTLs with high phenotypic variation as well as for the introgression either in the same genetic background for the improvement of crop productivity under water stressed conditions.

For the complex traits such as drought tolerance and yield which are controlled by several genes, many QTLs with moderate to high phenotypic variance are reported and those QTLs which are stable across the most of the conditions can be tackled through modern breeding approaches such as marker-assisted recurrent selection (MARS) or genomic selection (GS) (Ribaut and Ragot, 2007; Bernardo, 2008 and Varshney and Dubey, 2009). Since, majority of the components of drought, root and yield are correlated, clustering of QTLs controlling these different components at specific genomic region has much significance and of practical use for crop improvement for these traits. Therefore, such key genomic regions, containing QTLs for above mentioned traits may be harnessed through marker-assisted selection (MAS) approach to enhance drought tolerance in the elite cultivars/varieties.

4.4.3 Di-QTL epistasis

The majority of the studies suggested that quantitative variation is determined by a few QTLs with a relatively large effect and a large number of QTLs with smaller effects. Apart from main effects QTL, di-QTL which arise due to interactions of different loci in a particular cross also play a significant role in controlling a particular trait (Jannink 2007; Isobe *et al*., 2007). Therefore, to detect such interactions and QTL co-ordination in the present study, Di-QTL epistatic interaction analysis undertaken with QTL IciMapping software *ver* 4.0. Results revealed that several Di-QTLs in two generations, four water regimes and pooled mean of two generations.

QTL IciMapping detected total of 319 di-QTLs at LOD threshold of 5.00 for traits related WUE, pod yield and yield related parameters in F_{11}, F_{13} RILs under WW and WS condition and pooled analysis of both generations. Of these 13 di-QTLs identified for SCMR (PVE 0.99 to 11.77 %) and the interaction effects ranged from -32.81 to -2.28. A di-QTL detected for SLA explained PVE *per cent* 19.84 with negative interaction effects of -13.48. Twenty eight di-QTLs identified for days to fifty *per cent* flowering (DFF) with PVE ranged from 1.91 to 21.36 *per cent*. Whereas, no M-QTLs were detected for pods plant^{-1}, pod yield plant^{-1}, kernel yield plant^{-1} and sound mature kernel in any generations/different water regimes. The *per cent* PVE for plant height explained by eight di-QTLs interaction ranged from 5.07 *per cent* to 6.17 *per cent* and interaction effects ranged from -14.39 to 5.89; For pods per plant, *per cent* PVE explained by six di-QTLs interaction ranged from 5.35 to 18.52 and interaction effects ranged from -7.82 to 2.21; The di-QTL epistasis explained variation in kernel yield per plant ranging from 1.97 – 12.11 *per cent* PVE and the interaction effects ranged from -4.04 to 1.89; The *per cent* PVE in sound mature kernel percentage explained by 22 di-QTL interaction ranged from 1.90 – 19.84 and interaction effects ranged from 4.99 to 15.75.

For all the traits di-QTL interactions were detected (Fig. 16). These results indicate that di-QTL epistasis played a greater role in inheritance of most of the traits. These results are not surprising given that epistasis is more important for traits governed by several QTL with small/minor effects than for those governed by a few large main effects QTL (Bernardo, 2008). Epistatic QTL without individual effects have been reported in groundnut for SLA, SCMR and yield related parameters (Varshney *et al*., 2009; Ravi *et al*., 2011 and Goutami *et al*., 2012). A total of 38 Di-QTL interactions with > 5% PVE were detected at LOD score of 5.0. On the other hand in the present study, di-QTL interactions were not detected for traits such as branches per plant and shelling percentage, although main effects of QTL were detected. The di-QTL epistasis controlling the traits investigated in the present study is solely attributable to additive × additive type as these were detected in stabilized RIL population.

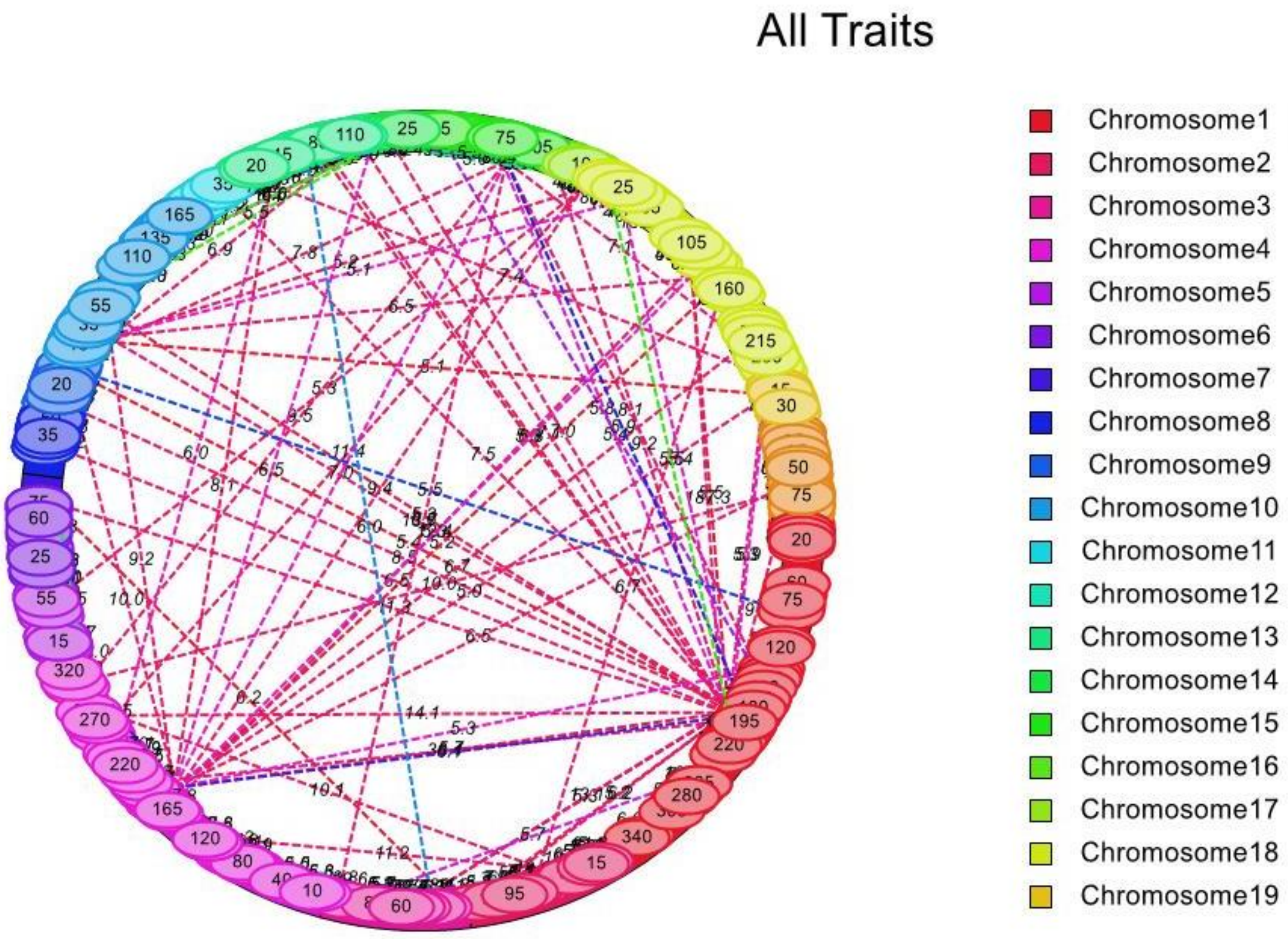


Fig. 16. **Significant epistatic interactions detected in NRCG 12568 and NRCG 12326 derived RIL population for traits related to WUE and pod yield in a cyclic graph**

4.4.4 Validation of QTL identified as WUE related traits and pod yield components

For effective implementation of MAS in the improvement of any quantitative traits, it is a pre-requisite to validate the QTL and their effects. One of the objectives of the present study was to validate the QTL identified by Eradasappa (2018) as surrogate traits of WUE and pod yield related traits in the cross GKVK 4 × NRCG 12473 and previous reported QTLs. Eradasappa (2018) reported two QTL for number of branches flanked by marker TC4G02 and SEQ9H8 located on LG 10 at 19 cM with 81.52 and 77.7 *per cent* PVE when mapped across the locations and water regimes. Similarly, two QTL were identified relative water content flanked by marker TC4E10-IPAHM 689 on LG 17 at 29 cM with 2.44 and 4.77 per cent PVE. The marker TC4G02 has been reported by Ravi *et al*. (2011) and Goutami *et al*. (2012) as marker linked to growth and yield component traits. In the present study, one QTL was identified with flanking markers TC4G02 – GM1992 on LG 12 at 12 cM explaining 8.24 PVE with LOD score of 2.63 for plant height trait. Another QTL was identified with flanking markers IPAHM 689 – GA 156 on LG 5 at 32 cM explaining 10.85 PVE with LOD score of 3.40 for SCMR trait. In both QTL identified one flanking marker is common differing to their size effects and location as reported. Interestingly, another flanking marker of QTL has been found mapped on the next or previous linkage group. Thus, indicating the saturation of the linkage map with few more markers to saturated linkage map, so that the distance between the markers come down and validate the identified QTL and its association with the trait. However, position and size effects of the QTL could vary for different RIL population as their genetic basis of these traits was mainly dependent on groundnut genotypes.

Outcome of the research

1. Three indices; MP, HMP and STI are effective for identifying moisture stress tolerant genotypes with high pod yield in groundnut.
2. In consideration with all different statistical procedures for screening drought tolerant lines; among all genotypes, RIL-126 and RIL-133 were identified as the genotype

with high and stable yield under moisture stress conditions in comparison of standard check GKVK 5.

3. RIL 126, RIL 139, RIL 108, RIL 191, RIL 133, RIL 249, RIL 529 and RIL 128 were found to be promising lines for further breeding programme.
4. The QTLs identified for traits viz., WS217DFF, WS218SLAPoS, PWS2DFF, PWS3SLAPoS, WS318SCMRPrS, PWS3SCMRPrS and WS218SLAPoS under water stress condition showed high phenotypic variation that could be used for marker assisted breeding of groundnut for improving the pod yield under water limited conditions.
5. Two markers are found to be related to more than one trait. The marker SEQ5D5 was found to be associated with PWS3SLAPoS, WS318SCMRPrS, PWS3SCMRPrS and PWS3DFF. Similarly, marker SEQ15C12 was related to WS318NBr, PWS2NBr and PWS2DFF.

Future line of work

1. A number of recombinant inbred lines were observed to be significantly superior for individual characters compared to the best parent and also checks which could be valuable material for trait specific breeding.
2. Superior drought tolerant RILs having higher pod yield under intermittent drought stress conditions without yield penalty under normal conditions identified in the present study by using a combination of characters *viz.*, quantitative and physiological traits during both the seasons may be further evaluated preferably over locations, seasons and in larger plot size to confirm their superiority and stability.
3. The best RILs identified for drought tolerance in the present study are RIL 125, 133 and 258 can be taken up for station trial and evaluated in multi locations basically for their pod yield potential and WUE. Later, once superiority is observed can be forwarded for farm trial. The line (s) can be released as a variety if a significant superiority over the checks is observed.
4. Identified RILs based on statistical approaches and *per se* performance can be tested for seedling vigour under moisture stress induced by PEG-6000. Further, phenotyping of the selected recombinant inbred lines for root traits and evaluation for yield and yield related traits.
5. Based on the overall results, any few drought tolerant lines can be taken over for transcriptomic analysis along with one susceptible line.
6. QTL region between the markers GM 2165-SEQ5D5 has to be validated for drought tolerance attributes, across locations and seasons and has to be fine mapped for use in map-based cloning or subjected to functional genomics approach to identify candidate genes.
7. All the major QTLs which are stable across seasons have to be validated and used for stacking through marker-assisted backcrossing (MABC).
8. Linkage map needs to be saturated with more markers, including SNP and DArT for further QTL detection.

V SUMMARY

Breeding through conventional approaches for increased water use efficiency (WUE) in groundnut is deployed on the basis of phenotypic selection for surrogate characters of WUE, pod yield and its component traits. It is rather less effective owing to their crop-stage specific expression, complex inheritance and significant cross-over genotype-by-environment interaction. The DNA markers owing to their crop stage non-specificity, simple inheritance and environment neutrality are proven to be powerful surrogates of such difficult-to-select traits. DNA marker aided identification and introgression of QTL into elite genetic background is expected to add up selection based on phenotype and enhance pace and breeding efficiency for high WUE in groundnut.

Objectives:

1. Identification of stable moisture stress tolerant RIL's for productivity *per se* traits
2. Development of SSR marker-based linkage map of the cross NRCG 12568 × NRCG 12326
3. Validation of linked QTL's for moisture stress tolerance and identification of novel QTL's in the RIL's derived from NRCG 12568 × NRCG 12326
4. Identification of QTL's for moisture stress tolerance stable across pre- and post-flowering moisture stress production environments

The present experimental material consists of 147 ($F_{11\ \&\ 13}$) recombinant inbred lines acquired from the cross NRCG 12568 and NRCG 12326. The two parents are contrasting for $\Delta^{13}C$, SLA, SCMR i.e. NRCG 12568 has low $\Delta^{13}C$, SLA with high SCMR while NRCG 12326 has high $\Delta^{13}C$, SLA with low SCMR. Significant positive correlations between SPAD cholorophyll meter reading and chlorophyll density indicates that high SCMR estimate possess a higher photosynthetic activity per unit area. SCMR is negatively associated with SLA on responses at a particular stage in the course of water deficit condition leading indispensable economic yield loss. SCMR increases and SLA turns down due to early season drought stress in groundnut.

Field evaluation of 147 ($F_{11\ \&\ 13}$) RILs was done during summer 2017 and 2018 in augmented design together with parents and checks *viz.*, GKVK 5 and TMV 2 at K-Block, Department of Genetics and Plant Breeding, in three experiments i.e., well-watered (WW), water stress-I (WS1) - withholding irrigation from 45-65 DAS (flowering to peg initiation stage), water stress-II (WS2) - withholding irrigation from 65-85 DAS (peg penetration and pod development stage). One more stress experiment was included for second year field evaluation by withholding irrigation from 30-45 DAS in order to understand the responsiveness of the RILs for water stress during flowering to 50% flowering period and also to identify the stress responsive QTLs along with the RILs which are capable of withstanding the water stress between 30-45 DAS.

The parents of the RIL mapping population were checked for polymorphism using 612 genomic SSR markers. The 147 RIL's derived from NRCG 12568 and NRCG 12326 were genotyped using a total of 198 polymorphic SSR markers. The linkage map was constructed availing genotypic information from the 172 polymorphic markers on 141 RILs at LOD 3.0 and were mapped on to 20 LGs. The SSR primer's genotypic data and phenotypic data were integrated to map QTLs controlling traits related to WUE, pod yield and yield associated parameters.

The entire length of the constructed linkage map spread over 2212.87 cM with an average density of 11.12 cM. The length of linkage map varied from 12.62 cM (LG 12) to 370.07 cM (LG 1). The highest number (23) of markers was mapped on to LG 1 and least number of markers (two) was mapped onto LG 12 and LG 16.

The trait means of every single recombinant inbred line and check's were used for statistical analysis, for the assessment of variability and identification of high water use efficient RILs. Drought tolerant RILs were identified based on drought tolerant indices estimates using pod yield under well-watered and water stress conditions. The linkage map was constructed by QTL IciMapping software version 4.0 using 172 SSR markers (after ignoring those with SD). The QTL controlling traits related to WUE, pod yield and yield linked parameters were mapped employing single marker analysis (SMA) and also through Inclusive Composite Interval Mapping (ICIM) availing QTL IciMapping software version 4.0.

- A total of 21 QTLs were perceived for surrogate traits of WUE (11) and yield related parameters (10) at LOD threshold of more than 2.5. Among 21 QTL, only 7 QTL are major QTL.

- In the present research investigation, more than one QTL has been spotted for traits under four water regimes. The QTLs identified for traits viz., WS217DFF, WS218SLAPoS, PWS2DFF, PWS3SLAPoS, WS318SCMRPrS, PWS3SCMRPrS and WS218SLAPoS under water stress condition showed higher phenotypic variation that could be employed for marker assisted breeding of groundnut for improving the pod yield under water limited conditions.

- Two markers are found affiliated to more than one trait. The marker SEQ5D5 was found to be interconnected with PWS3SLAPoS, WS318SCMRPrS, PWS3SCMRPrS and PWS3DFF. Similarly, marker SEQ15C12 was related to WS318NBr, PWS2NBr and PWS2DFF.

- This indicates probably same set of genes are controlling the expression of these traits. Moreover, these traits phenotypically may have more association with each other. Thus, these markers may be useful for high WUE and yield improvement scheme.

- In consideration with all different statistical procedures for screening drought tolerant lines; among all genotypes, RIL-126 and RIL-133 were identified as the genotype with high yield under moisture stress conditions in comparison of standard check GKVK 5.

- Three indices; MP, HMP and STI are effective for identifying moisture stress tolerant genotypes with high pod yield in groundnut.

- RIL 126, RIL 139, RIL 108, RIL 191, RIL 133, RIL 249, RIL 529 and RIL 128 were found to be promising lines for further breeding programme.

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Appendix 1. Weekly weather data of *summer* 2017 at UAS, GKVK, Bengaluru

Month	Week	Air temp. (º C)	RH (%)	PE (mm)	RF (mm)	PET (mm)	Soil temp. (º C)
January	Jan 29 -Feb 4	29.1	39.0	5.3	0.0	3.4	29.3
February	Feb 5 - Feb 11	29.9	34.0	4.9	0.0	3.6	29.1
	Feb 12 - Feb 18	29.4	33.6	6.9	0.0	4.0	27.5
	Feb 19 - Feb 25	31.9	35.5	7.5	0.0	4.0	29.8
March	Mar 5 - Mar 11	32.3	41.0	6.6	1.4	4.0	31.6
	Mar 12 - Mar 18	32.3	42.0	6.6	0.0	4.1	34.2
	Mar 19 - Mar 25	33.4	40.0	8.1	0.0	4.5	35.4
	Mar 26 - Apr 1	35.0	35.0	9.3	0.0	4.8	36.1
April	Apr 2 - Apr 8	34.6	38.0	7.5	1.6	4.4	35.6
	Apr 9 - Apr 15	35.7	36.0	8.8	0.0	4.6	36.3
	Apr 16 - Apr 22	34.6	36.0	8.3	0.0	4.7	35.9
	Apr 23 - Apr 29	35.4	36.0	8.5	0.0	4.8	36.3
	Apr 30 - May 6	32.7	31.0	4.4	25.8	4.3	35.7
May	May 7 - May 13	32.6	48.0	6.0	15.2	4.0	32.9
	May 14 - May 20	33.5	35.0	5.8	45.6	4.5	33.9
	May 21 - May 27	32.2	42.0	5.7	133.6	4.2	29.2
	May 14 - June 3	30.0	47.0	5.8	8.3	4.3	30.1

Appendix 2. Weekly weather data of *summer* 2018 at UAS, GKVK, Bengaluru

Month	Week	Air temp. (º C)	RH (%)	PE (mm)	RF (mm)	PET (mm)	Soil temp. (º C)
January	Jan 15 - Jan 21	27.8	51.0	4.7	0.0	3.2	28.3
	Jan 22 - Jan 28	27.6	51.0	4.7	0.0	3.3	28.2
February	Feb 5 - Feb 11	29.0	50.4	4.9	3.0	3.3	28.4
	Feb 12 - Feb 18	29.2	48.6	5.3	0.0	3.8	28.5
	Feb 19 - Feb 25	30.3	46.2	6.8	0.0	4.0	29.1
March	Mar 5 - Mar 11	32.3	20.0	8.6	0.0	4.0	30.4
	Mar 12 - Mar 18	31.1	41.0	7.5	6.9	4.2	30.1
	Mar 19 - Mar 25	32.0	37.0	6.9	0.0	4.0	32.2
	Mar 26 - Apr 1	33.1	37.0	8.1	14.0	4.2	33.2
April	Apr 2 - Apr 8	32.2	39.0	6.7	0.0	3.7	32.9
	Apr 9 - Apr 15	32.8	41.0	7.7	0.0	4.4	34.8
	Apr 16 - Apr 22	33.8	39.0	6.7	10.6	4.3	33.8
	Apr 23 - Apr 29	33.7	36.0	5.5	25.0	4.2	32.9
	Apr 30 - May 6	32.7	31.0	4.4	25.8	4.3	35.7
May	May 7 - May 13	32.3	42.4	5.6	55.0	4.2	31.2
	May 14 - May 20	31.6	41.1	5.1	27.4	3.8	30.9
	May 21 - May 27	30.8	45.7	4.8	50.6	3.8	28.6
	May 14 - June 3	30.6	47.5	5.1	51.2	4.3	31.2

Appendix 3. List of SSR markers used in the present study

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	Reverse Primer (R)	F (°C)	R (°C)
1	Ap40	CTGTTTGATCGCCGCTATG	GTCAAGTGCTTCCTCCGATG	59.4	56.7
2	PM179	TGAGTTGTGACGGCTTGTGT	CTGATGCATGTTTAGCACACTT	56.5	57.3
3	PM204	TGGGCCTAAACCCAACCTAT	CCACAAACAGTGCAGCAATC	57.3	57.3
4	PM346	AAAGGCGCACTCGATTCTAA	CGCACAGAAACATCAAGCAT	55.3	55.3
5	pPGSpPGPseq11G3	CCGCGTTGTTAAACCAGAAC	ATGGAGGATGTGAGTGGGAA	57.3	57.3
6	pPGSpPGPseq13A7	AATCCGACGCAATGATAAAAA	TCCCCTTATTGTTCCAGCAG	57.3	52
7	pPGSpPGPseq14H6	GCAACTAGGGTGTATGCCGT	CAACCCTATACACCGAGGGA	59.4	59.4
8	pPGSpPGPseq15F12	AAAGTCAACCGCTCACACTG	AGGGTTAGGATTTTGGGTGG	57.3	57.3
9	pPGSpPGPseq16C6	TTGCTACTAAGCCGAAAATGAAG	CTTGAAATTAACACATATGCACACA	56.4	57.1
10	pPGSpPGPseq17G6	AACGACAACGACAACGACAA	TCCACTATACAGTTGGGGGC	59.4	55.3
11	pPGSpPGPseq18E7	AACGTGCGTGGAAAGAGTTC	TGAGAGTGGTTTTTGTTGGTG	55.9	57.3
12	pPGSpPGPseq19A5	ATTCGTCTCCTTCTTTTGGC	TTTTGCTTCCAAATGGCTTC	53.2	55.3
13	pPGSpPGPseq19D9	TGTTGCCCACTGTTCTAATCA	TCAAATGGCATAGTCTCCCC	57.3	55.9
14	pPGPpPGPseq1B9	CGTTCTTTGCCGTTGATTCT	AGCACGCTCGTTCTCTCATT	57.3	55.3
15	pPGPpPGPseq2B9	GCAACATGCTCTGAATTTTGAC	TGTGCAACCCAATTCAATAACTT	55.3	56.5
16	pPGPpPGPseq2G4	TTCTTGGTTCCTTTGGCTTC	TGCTCAAGTGTCCTTATTGGTG	58.4	55.3
17	TC11B04	GATCTGAAGGCTCTGATACCAT	GATCTCAACCAGAACAGTATGC	58.4	58.4
18	TC1E06	ACCGTTACGAACGCTTTGTC	TCCCTCTCATACGACACCCT	59.4	57.3
19	TC2A02	CTCCCTTGTGGGTATGTGGT	GGCTCCCATTCATTCTCAAA	55.3	59.4
20	TC2C07	CACCACACTCCCAAGGTTTT	TCAAGAACGGCTCCAGAGTT	57.3	57.3
21	TC3E05	CACCACTTGAGTTGGTGAGG	CTTCTTCTTCTCCCGCAATG	57.3	59.4
22	TC4A02	ATTCAAATCGGAATGGCAAG	GAGCAAAGGGCGAATCTATG	57.3	53.2
23	TC4D09	TTGTGCTCTGCTCTTGGTTG	CTTGCTGGAGGAAACACACA	57.3	57.3
24	TC4E10	ACGTCATCTTCCCTCCTCCT	CCATTTTCTCCTCGAACCAA	55.3	59.4
25	TC4F12	GATCTTTCCGCCATTTTCTC	GGTGAATGACAGATGCTCCA	57.3	55.3
26	TC4G02	GATCCAACTGTGAATTGGGC	CACACCAGCAACAAGGAATC	57.3	57.3
27	TC7E04	GAAGGACCCCATCTATTCAAA	TCCGATTTCTCTCTCTCTCTCTC	60.6	55.9

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	Reverse Primer (R)	F (°C)	R (°C)
28	TC9B07	CCATCTCCTTCTTGACTTTAGCC	GTTCTCCAACCTCCTCCTTTTC	60.3	60.6
29	TC9H09	TTAGCGACAAAGGATGGTGAG	TAGGGACGAAAATAGGGACTGA	58.0	57.9
30	pPGPpPGPseq2E6	TACAGCATTGCCTTCTGGTG	CCTGGGCTGGGGTATTATTT	57.3	57.3
31	GM1483	GCTGTTACATGGGCATCATTT	TCATCAGAGACCCAAGATCCA	57.9	55.9
32	Ah26	GAAAATGATGCCATAAAGCGTA	AGTGTAACACCCCGTTAGCC	59.4	54.7
33	GM618	TGAAGGGAGGAAGAGACGAAT	CCTCTGTGCGACATGACTCTAA	60.3	57.9
34	GM626	CATCCAAAGCCAAAGTTCACA	GCTTAGCTTGCTTTGATTAGGG	58.4	55.9
35	GM632	TTCAATCATTTCACGTGTCAATC	AGTGCTAGGAGCCAGCAATTT	57.9	55.3
36	GM633	CAAAGTTTGCAGTGATTTTGTTG	AAATTTTCAGGTAAATCATTCTT	50.0	55.3
37	GM692	TGAGGCCGTCTTGTTTAGAGA	CCTCTTCCATCACCGTTCATA	57.9	57.9
38	IPAHM123	CGGAGACAGAACACAAACCA	TACCCTGAGCCTCTCTCTCG	61.4	57.3
39	IPAHM165	CAACACGTTCGCTTCCAGAT	TCACTCTCATTTCCGCCATT	55.3	57.3
40	IPAHM23	GTGTCTTTTCGTTCGCGATT	CGACTCTTAGGGTGGATTATAGTAAGA	61.9	55.3
41	IPAHM287	TCTAACCCTTCGGTTCATGG	TCACTATCCCATCCCTGCTC	59.4	57.3
42	IPAHM290	CCACCGCTGATGTGTAATTGTA	GACGTGTAGTTGAAAACAACAGTATCA	60.4	58.4
43	IPAHM97	TCAGCTGTCTCTCTGCTCCA	CTCCAAGCAAAAAGTTGAAGG	55.9	59.4
44	PM419	TGCGAGACCAGAGAGGAGAT	TAGCGGTTCAGAAACCCTTT	55.3	59.4
45	PM436	ACACTGCTGGTGGGATTTTC	ATGAAATGAGCAAGCGGAAC	55.3	57.3
46	RM2C1	GGTCAAGGCTACCCCTTCTACT	ATCCAATCCATCCAAGAAAGTG	56.5	62.1
47	S-54	GTGTGCCATGTAGGTGTGACTG	GTTTGCCCTCTTGTTTTCTCC	57.9	62.1
48	pPGPGPseq13E6	TGGCAATTTATTGATGCAGG	GTCACGTAATTGGATGCACG	57.3	53.2
49	pPGPpPGPseq7G2	ACTCCCGATGCACTTGAAAT	AACCTCTGTGCACTGTCCCT	59.4	55.3
50	AC3D07	TAGCTTCGATAACCAGGGAGAC	CCCTAACACTCGTTCATTCCTC	60.3	60.3
51	IPAHM103	GCATTCACCACCATAGTCCA	TCCTCTGACTTTCCTCCATCA	57.9	57.3
52	IPAHM105	CAGAGTTTGGGAATTGATGCT	GCCAGATCTGAGCAAGAACC	59.4	55.9
53	IPAHM121	GCTATATCCAGACAGCCATCG	GAGAAGGGTGGATTGAAGAAGA	58.4	59.8
54	IPAHM171c	CAACACAAGCCCACAACAAA	TCCATCATCACCCTCATCAA	55.3	55.3
55	IPAHM176	TTCAGCAAAAACATGCAAGG	TGCAATGAGTTATATTCACCTCTCC	59.7	53.2

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	**Reverse Primer (R)**	**F (°C)**	**R (°C)**
56	IPAHM272	TCCACTTTGGAGAAACAGGTG	CAAACCCCTGACTCGACCT	58.8	57.9
57	IPAHM282	AAGCCTTTGCGAATATAACCA	TGCAGGACTTGTATTTTGAGGA	56.5	54
58	IPAHM295	TGCTGGAATGGTAACTTTGCT	TAACCCCCTTCACTCCCTCT	59.4	55.9
59	IPAHM352	GGCGGGACACTTATCAGAGA	TTCCGTGTGACAGAAATACCC	57.9	59.4
60	IPAHM395	CAGAGTCAATGGCAGCGTAG	TCCTTCCCTCATCTAAAACCAA	56.5	59.4
61	IPAHM475	GTGATTTCCTGGTTGGTGCT	AGCCTCAGCTGGTTTTGCT	56.7	57.3
62	IPAHM531	TGCCAGGTTGCTGTAACAAA	CATACACGCTTTTCCCCTGT	57.3	55.3
63	PM137	AACCAATTCAACAAACCCAGT	GAAGATGGATGAAAACGGATG	55.9	54.0
64	PM183	TTCTAATGAAAACCGACAAGTTT	CGTGCCAATAGAGTTTTATACGG	58.9	53.5
65	PM36	ACTCGCCATAGCCAACAAAC	CATTCCCACAACTCCCACAT	57.3	57.3
66	pPGSPPGPseq11G7	CATGTCTCCATGAGCATTTCA	TGGATGTGGACAGCATATCG	57.3	55.9
67	pPGSPPGPseq17F6	CGTCGGATTTATCTGCCAGT	AGTAGGGGCAAGGGTTGATG	59.4	57.3
68	pPGSPPGPseq18G1	AATAGGTTGTGAAGCACGCA	TTCGGTGGTACTTTTAAGGCA	55.9	55.3
69	pPGSPPGPseq4E08	ACCATTGCACTTTGAAGCTCT	GCTTGGTTTGGGTTAGTTTGA	55.9	55.9
70	pPGSPPGPseq15C12	ACAATGCAATGACCGTTGTT	TTGTTGCATGAGAACGTGAA	53.2	53.2
71	pPGPPPGPseq7H6	CATCCTCACGGGAGTCAGAT	ATACCTACGCGTTGTGGAGC	59.4	59.4
72	RN16F05	CCTACCTCCTTGCCGTCCT	CCGATTCCTCCTTCTCTTCG	59.4	61.0
73	RN19A01	AACACACACCCCTTTGAT	AGGTGGTAAAACACAAGAGAG	55.9	51.4
74	TC0A01	CAGCTCATTTTTCACCTCCA	CCATAACCCCAAAAATGCAG	55.3	55.3
75	TC1B02	AACATGCATGCAAATGGAAA	GCCAAAGTCACTTGTTTGCTT	55.9	51.2
76	TC1D12	CCCTTTCATTCTCCCTTTCC	TTCTCCTGCACTAGGTTTCCA	57.9	57.3
77	TC1G04	TGCTGTGAGAGAAATGGCAG	GCGCATTCTTCGATTAAAGG	55.3	57.3
78	TC2E05	GAATTTATAAGGCGTGGCGA	CCATCCCTTCTTCCTTCACA	57.3	55.3
79	TC3A12	GCCCATATCAAGCTCCAAAA	TAGCCAGCGAAGGACTCAAT	57.3	55.3
80	TC3E02	TGAAAGATAGGTTTCGGTGGA	CAAACCGAAGGAGGAACTTG	57.3	55.9
81	TC4H07	CCTCCGTTGCTCTTCTGAAC	GATCAAGCACTTCAGACAATGG	58.4	59.4
82	TC5A07	GTTTGGTTCTCCCTCCTCCT	AGCCTCTTCATTCCCCTCAT	57.3	59.4
83	TC6H03	TCACAATCAGAGCTCCAACAA	CAGGTTCACCAGGAACGAGT	59.4	55.9

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	**Reverse Primer (R)**	**F (°C)**	**R (°C)**
84	TC7H11	AGGTTGGAACTATGGCTGATTG	CCAGTTTAGCATGTGTGGTTCA	58.4	58.4
85	TC9B08	GGTTGGGTTGAGAACAAGG	ACCCTCACCACTAACTCCATTA	58.4	56.7
86	GM1009	TTTCCTTCTTTCCCTTCTTCTTC	CGTTGTTGCCGTTAAACTGA	55.3	57.1
87	GM1536	AAAGCCCTGAAAAGAAAGCAG	TATGCATTTGCAGGTTCTGGT	55.9	55.9
88	GM1954	GAGGAGTGTGAGGTTCTGACG	TGGTTCATTGCATTTGCATAC	54.0	61.8
89	GM2009	CAAACGCATACACCCCATAAC	TTTGGTTCTCGTTTGTGTTTT	52.0	57.9
90	GM2079	GGCCAAGGAGAAGAAGAAAGA	GAAGGAGTAGTGGTGCTGCTG	61.8	57.9
91	GA1	GCGTGAAATGAGTGTTGTGAG	CATAGCCACCATAGACACCAAA	58.4	57.9
92	GA8	TGAAGGGAGGAAGAGACGAAT	CCTCTGTGCGACATGACTCTAA	60.3	57.9
93	GA24	AACGAAATATTTTGAGAAAGGAT	AGCATTAGCAACTCTAAGCTCAT	57.1	51.7
94	GA26	CCTCACTTCTTTTTGCATGGT	TGGAAAGGAAATGATTTGGTG	54	55.9
95	GA35	CAAAGTTTGCAGTGATTTTGTTG	AAATTTTCAGGTAAATCATTCTT	50	55.3
96	GA57	ATCGTCCTCGCAGGTTCTTA	CTTGATTTGGTAATGGGCTGA	55.9	57.3
97	GA72	ACTTTGGTGGCTTTCCTTCAT	TCTCTGTGCCCTCTTTCTTCA	57.9	55.9
98	GA87	CTTCACCTCCAAAATCAACCA	ACCGCTGACATTTGATTGTTC	55.9	55.9
99	GA110	GGAGAACCAGTGACGTGACATA	GGATTAATTCTGATACCATGAAAGG	58.1	60.3
100	GA131	ATGTCCTTGCCTTGTTTCGTT	TAGTTGGCGGTATGGCTTAGA	57.9	55.9
101	GA156	CTACTCCCTCTGCTGCTTCCT	TAGGGTTTCGTTGAGGAGGTT	57.9	61.8
102	GA161	TGAGGCCGTCTTGTTTAGAGA	CCTCTTCCATCACCGTTCATA	57.9	57.9
103	GA169	CTCCTTAACAATAAATCGAGTGATGA	CAACCCATTTGCCACCTCTAT	57.9	58.5
104	EM-31	AAAGTCCCATGAATGCTCTC	AGTAGAAAACACGGTAGCCA	55.3	55.3
105	EM-87	CATGCTCCTCCAATTTATTACG	CGAGACTTGAGTGCCTTGTTG	59.8	56.5
106	pPGPpPGPseq2A5	GGGAATAGCGAGATACATGTCAG	CAGGAGAGAAGGATTGTGCC	59.4	60.6
107	pPGPpPGPseq2C11	TGACCTCAATTTTGGGGAAG	GCCACTATTCATCGCGGTA	56.7	55.3
108	pPGPpPGPseq2D12B	AAGCTGAACGAACTCAAGGC	TGCAATGGGTACAATGCTAGA	55.9	57.3
109	pPGPpPGPseq2G3	ATTCACAAGGGGACAGTTGC	ATTCAAGCCTGGGAAACAGA	55.3	57.3
110	pPGPpPGPseq2H8	AAGCTAAGTGGGGTGGGAGT	AAGCAGTTTGCGTAAGCATTT	54	59.4
111	pPGPpPGPseq3A8	ATACGTGACTTGGGCCAGAC	AGTGAAAAATACACCCAACGAA	54.7	59.4

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	Reverse Primer (R)	F (°C)	R (°C)
112	pPGPpPGPseq3F5	TTCAGTTGTGATTCCACCCC	TTACATGGCCACTGACTAGAAGTT	59.3	57.3
113	pPGPpPGPseq4F1	CAGAAACACGCGTAGTGGAA	AGCCAGGTACCAACAAAACG	57.3	57.3
114	pPGPpPGPseq4H11	ATCACCATCAGAACGATCCC	TTTGTAGCCTTCTGGCGAGT	57.3	57.3
115	pPGPpPGPseq5D1	TGGCCAAAACAACTGATTGA	TCCCAACTTTTCCGTTCTTG	55.3	53.2
116	pPGPpPGPseq5D5	AAAAGAAAGACCTTCCCCGA	GCAGGTAATCTGCCGTGATT	57.3	55.3
117	pPGPpPGPseq7B3	TCTGTTTTCTCGTTCGAGCTG	ACCCACCTAGCATCATTTGC	57.3	57.9
118	pPGPpPGPseq8D9	TGAGTTTCCCCAAAAGGAGA	CAACAACAATACGGCCAACA	55.3	55.3
119	pPGPpPGPseq8E12	TCTGTTGAGAACCACCAGCA	GTGCTAGTTGCTTGACGCAC	59.4	57.3
120	pPGSpPGPseq10D4	ATCCCTGATTAGTGCAACGC	CGTAGGTGGTTTTAGGAGGG	59.4	57.3
121	pPGSpPGPseq11D4	CCCTTTTTCAAACAACCCAA	GGATTTTGCATTTGTAGTTGATAGC	58.1	53.2
122	pPGSpPGPseq11E11	CTGCTATATTCTGGGCGGAG	CGAGAAAACAGTTTGGGAGG	57.3	59.4
123	pPGSpPGPseq11H1	TTTGTGTTTAAGAAGGGGTGC	GCGGTCCAACATCCTTTTT	54.5	55.9
124	pPGSpPGPseq12E10	TGCTTTTAGAGGCTTTGCCA	GAAACTGCAACAGCAACAGAA	55.9	55.3
125	pPGSpPGPseq12E3	GACCAAAACAAAATTTGGAACA	CTCACACCAATCAGTCGACAA	57.8	52.8
126	pPGSpPGPseq14C11	CGTTGGGGACAAAAACGATA	TTTTCTTGAAACTCGTTGATATGG	55.9	55.3
127	pPGSpPGPseq14F4	ACGTTTAGTTGCTTGCGTGA	TGAATTCAAAGGAAAATGAAAAA	50.0	55.3
128	pPGSpPGPseq15E12	GCAGAACTAAGGTCGGCAAG	TCCGCCCTTTATTTTTGTGT	53.2	59.4
129	pPGSpPGPseq16F10	TGGAGGGAAAAACATTTTGG	CCTGGAGGGGTGAGAGGT	60.5	53.2
130	pPGSpPGPseq16G8	CTCAAAAAGCGCTTAGCCAC	CTGCCTACTGCCTACTGCCT	61.4	57.3
131	pPGSpPGPseq17E3	TTTCCTTTCAACCCTTCGTG	AATGAGACCAGCCAAAATGC	55.3	55.3
132	pPGSpPGPseq19E7	TAGCCGACCTAAACTTTGCG	GGAGATTCTTATGAATTGTGCG	56.5	57.3
133	pPGSpPGPseq19E9	ACTGCTTGCTCTCTTCCTCG	TTCCACCTATAAAATCAATGGTGA	55.9	59.4
134	pPGSpPGPseq9A7	TCAGCCATTCTGATTATGTAAGTTTT	TCTCAGTTTCCACGTTGAGC	57.3	56.9
135	Ah 4-20	ACCAAATAGGAGAGAGGGTTCT	CTCTCTTGCTGGTTCTTTATTAACTC	60.1	58.4
136	Ah-594	TGACCAAATCATCCCATCTTGC	CAGCACATCTCCAACTTTCTCCA	60.6	58.4
137	gi-716	AGTAGACCAAATTAAACCACACT	TAGATGATTGTGAATGTTTCTG	52.8	55.3
138	PM188	GGGCTTCACTGCTTTTGATT	TGCGACTTCTGAGAGGACAA	57.3	55.3
139	PM3	GAAAGAAATTATACACTCCAATTATGC	CGGCATGACAGCTCTATGTT	57.3	57.4

Sl. No.	**Primer**	**Nucleotide sequence**		**Anneling Temp**	
		Forward Primer (F)	**Reverse Primer (R)**	**F (°C)**	**R (°C)**
140	PM31	TTCGGCATGACAGCTCTAAG	GAAAGAAATTATACACTCCAATTATGC	57.4	57.3
141	PM32	AGTGTTGGGTGTGAAAGTGG	GGGACTCGGAACAGTGTTTATC	60.3	57.3
142	PM35	TGTGAAACCAAATCACTTTCATTC	TGGTGAAAAGAAAGGGGAAA	53.2	55.9
143	PM384	GGCGTGCCAATAGAGGTTTA	TGAAAACCAACAAGTTTAGTCTCTCT	58.5	57.3
144	PM73	GGGACTCGGAACAGTGTTTATC	AGTGTTGGGTGTGAAAGTGG	57.3	60.3
145	RI1F06	TGTCTCTCTTCCTTTCCTTGCT	CCTTTTGCTTCTTTGCTTCC	55.3	58.4
146	RI2A06	TTCCTAAACTGGAAGCGTGTTC	CAAGAGCTTTCTTCTGCCATTC	58.4	58.4
147	RN0x615	GGGCATTTAAGGGACAATGG	CCCGACCCACACATTTAACATA	58.4	57.3
148	TC11A04	ACTCTGCATGGATGGCTACAG	CATGTTCGGTTTCAAGTCTCAA	56.5	59.8
149	TC11H06	CCATGTGAGGTATCAGTAAAGAAAGG	CCACCAACAACATTGGATGAAT	56.5	61.6
150	TC1D02	GATCCAAAATCTCGCCTTGA	GCTGCTCTGCACAACAAGAA	57.3	55.3
151	TC4H02	ACCGCAAACTCATCCATCTC	GATAGCGTCAGAGGCAGAGG	61.4	57.3
152	TC5A06	TCGGTTTGGGAGACACTCTT	TTGTAAGCAGACGCCACATC	57.3	57.3
153	TC6E01	CTCCCTCGCTTCCTCTTTCT	ACGCATTAACCACACACCAA	55.3	59.4
154	pPGPpPGPseq4D2	GGTCACTTCTCTTTAAGACACGACT	TTTGAGGTTGCCAAATACCC	55.3	61.3
155	pPGPpPGPseq4G1	TTTGTGGTGCGTGTTCTTTC	TGTCAACGGCATCACATTTT	53.2	55.3
156	TC11F12	TGAGTCTGTGGAAGAATAAGAGAAG	TGAGTCTATCGCCGCCTAC	58.8	59.7
157	TC3B05	GGAGAAAACGCATTGGAACT	TTTGTCCCGTTGGGAATAGT	55.3	55.3
158	TC3G05	GATCCCAAGTCTCCAGAGGA	AACAACAAGGAGGCAGAGGA	57.3	59.4
159	TC7A02	CGAAAACGACACTATGAAACTGC	CCTTGGCTTACACGACTTCCT	59.8	58.9
160	gi-427	TCGAAATAGGTAGCTGAGTCTTGA	CATGGTGTTGACGTTAGGATTT	56.5	59.3
161	IPAHM 177	TCAGCGGAGAAGAAAACTAAGG	GAGGTGTTTGGAGAACTAGGATTT	59.3	58.4
162	IPAHM 229	TCAGCCTGCGAAACTAAGGT	TGGAGAACTAGGATCTCTTTTGTG	59.3	57.3
163	IPAHM 509	GCCTGCAAAGCTAAGGTTG	TGGAGAGTTAGGATTTCTGGCTA	58.9	56.7
164	IPAHM 689	GATGACAATAGCGACGAGCA	GTAAGCCTGCAGCAACAACA	57.3	57.3
165	IPAHM 93	TCCATCGTTAGTGGCACTGT	GTCGACTCCTGCCCAATCTA	59.4	57.3
166	PM238	CTCTCCTCTGCTCTGCACTG	ACAAGAACATGGGGATGAAGA	55.9	61.4
167	PM434	TTCGGCTGACAGCTCTAAG	GAAAGAAATTATACACTCCAATTATGC	57.4	56.7

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	Reverse Primer (R)	F (°C)	R (°C)
168	S-01	TGGACTAGACAAGGAACAACCA	GAGCCATGAGCACACAACAC	59.4	58.4
169	S-03	GCACCAATTTTGTCCCTGAT	AAGGGGTTTGCACGTAAATG	55.3	55.3
170	S-09	CGCTGTCCTTATCGAACCAT	CTCTCACTCGCGCTTTCTCT	59.4	57.3
171	S-11	ATGACGGCAGTAGCAGAAGC	TTGAGGAGAAGACGCTGTTG	57.3	59.4
172	S-16	TGGTAGTGGAGTCAGAGTGTGTG	GTTGCATTGCCCAACTCTTT	55.3	62.4
173	S-19	GCTCCACTAGTGCCGAAATC	CAGACACCCGGAGGCTTA	58.2	59.4
174	S-21	AGTCCTACTTGTGGGGGTTG	TCCCTTTTGCAGTGAAATCC	55.3	59.4
175	S-22	CGTGACAAACATGTGCTGCT	TTTTGGAATCTGTTTATGGGAAA	53.5	57.3
176	S-23	CTGGAAGTGGTCCTGTTGGT	GCTGCTCCTGTCTCTGGAAT	59.4	59.4
177	S-24	GGCAATGCACACGCTACTCT	CGTGAGGCGTGAGAGTTCAT	59.4	59.4
178	S-26	ACATGAGTGCCCAACTAGCC	TGCAGAGCTTCAACAACCAC	57.3	59.4
179	S-38	GGCAGCGAAGCACCCATTGTTA	GTAGGGTTGCGTTTCGTTTTCTTATCG	63.4	62.1
180	S-40	ACCCAACACTAGCCGCCACTGA	GCAACGCCTCCTCCTCTTCCTCTA	66.1	64
181	S-46	ATGGCGAATCGGAGGGTAGGTT	TCCAATCGTGCGTTTCAATCATCT	59.3	62.1
182	S-48	ATTCTGAGGCTGCTTCCCAAACT	CTGCCATGTAAGCCGTGAATAAG	60.6	60.6
183	S-49	GGCAGCGAAGCACCCATTGT	CGTTTCGTTTTCTTATCGCACTTC	59.3	61.4
184	S-52	CCCTGAGAATGAAAGAAAGAAACA	CAACCGCAGCGACGATAGATG	61.8	57.6
185	S-57	AGGGCGAAAGGCAGAGGAAGA	AAAGGGGTGAGACAGCCAATAACAT	61.3	61.8
186	S-59	TTGGTGGAAGCCCTAGAGTGAGTGAA	ATGGAAATGAAGCCGATAAGAGA	57.1	64.8
187	S-68	AATCAAGGTGGCAACTACAGC	AGACACTATACTTGCAACGAGGAT	59.3	57.9
188	S-70	CCTTTCCCATTCCATTAGC	GTCCGAGTTGAGGAACAACAA	57.9	54.5
189	S-72	TACAGCCCAAATGGAATGAGAA	GAGTTGGGAAGAAAGGATGAAGAT	59.3	56.5
190	S-73	AGTCCACTGAACCGAACACCAATC	TCCCTACCACCGAACGAAACAAT	60.6	62.7
191	S-76	ATACTGATAGATAGGGTCGAAGGAGAG	CAACGAAAGAAAAATAAGGACATAGTG	58.9	63.4
192	S-80	GGCGTCCCATTGCTTAC	AGAATGCGTTGATGTTATGAA	52	55.2
193	S-83	CTTGAACTTATTTTTGGTGGGTGAAC	CAAGGGAGAATGAAGAATGCTAAG	59.3	60.1
194	S-84	CAGCCAATATGTCACAACCCTAAT	CTCCCACTACAAATCTCCAATCAAT	59.7	59.3
195	S-86	TCCATGAGGGGTTATAGGTGTTT	GGGTGTATTTCTGAAGTTCCATTATC	60.1	58.9

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	Reverse Primer (R)	F (°C)	R (°C)
196	S-93	TTGGGGAAATACAGAATAACG	CTCCCACATCCCCACCAT	58.2	54
197	S-96	ATACACATTCCTCTCCATCTCCT	TTTTTCTTCCCTTTCTTCTTTCTA	54.2	58.9
198	S-101	AGTGCGTTTGGCTCATCA	ATTTCGTTCATTTAGTCCATAGA	53.5	53.7
199	S-108	GCTTACATTACACGTCATCTC	CCGAACTTACAGTTAGGAG	54.5	55.9
200	S-113	TTGCATGTAGGAAAGAAAGATT	TTGGATGTGGTGGTGATGT	54.5	52.8
201	S-118	TATATGATGCTTGATTGAGACT	CATGTAGAAGGCTTGGAGGGTAT	60.6	52.8
202	Ah 4-4	CGATTTCTTTACTGAGTGAG	ATTTTTTTGCTCCACACA	46.9	53.2
203	gi-832	GCCACTTTATTCTAAGCACTCC	AAGAGACCACACGCTCACA	56.7	58.4
204	pPGPpPGPseq3A6	TGCATCAGCAAGCTACATACG	GCGATTCACCATCAATCTCA	55.3	57.9
205	pPGSpPGPseq9H8	CTGGATACATCGACGCTGAG	GCGGTCCAATACTAACAAAATC	56.5	59.4
206	TC3G01	GACGGTAATCGTGCCCTAAA	TGCAGTAGTGGCAGCAGAAC	59.4	57.3
207	GM744	TGATGCCTGAGAGACTTTGGT	GACTCCTTCACCTCCCTAAGC	61.8	57.9
208	GM761	GCAATTGGAGAACGAGAAACA	GAATGCCTTCAAACCAGTCAA	55.9	55.9
209	GM822	CACGGAACCCAGATAAACTGA	ATCACCATCACCATCGTTGTC	57.9	57.9
210	GM840	GCAGCATACAAGCAATCCACT	TTTGCCATTTGCTGTTCTACC	55.9	57.9
211	GM1043	GAATTCAGCTTGTGGATTGGA	TTGTTGTTGTTAGGCCACCAC	57.9	55.9
212	GM1097	CGTTTGGCAATACCTGTGTTT	CCTCTTCCTCATCATCATCATC	58.4	55.9
213	GM1098	ACGACGATGACGATAACGAGT	AACCTCTTTTCCTCCTCTCACC	60.3	57.9
214	GM1202	GTGTGCCTTTTGAATCGTCAT	CGATTACGCGTTTTATGGCTA	55.9	55.9
215	GM1256	GACGGCCAGAGAGGTAGAGAG	AAGCAGACCAGGAGGATCTTG	59.8	63.1
216	GM1357	ACGCAATGCACATCCTTTAGA	CAGAAACAGGTGAAGGAGCTG	59.8	55.9
217	GM1369	TGAATTAGAAGAAGGCCAGCA	ATCCATCACCATCAGCTTCAC	57.9	55.9
218	GM1411	TTGTTCTCAACAGCAACAACG	AAGGTGCAAACTGCTCTGCT	57.3	55.9
219	GM1469	TTGCCGTCATCTGATTCTTCT	TCTCACCGGTCTCAGTGTTCT	59.8	55.9
220	GM1477	GTTTGTGTTTGTGCCGAACTT	CAAGCAACCCTTGATGTGTTA	55.9	55.9
221	GM1489	GGAAGATGTGGTTGCAAATTC	CTCCCAGCTATCAACTTCACG	59.8	55.9
222	GM1515	CAGATATCGCGAATCGAAGAG	TTCTTCTTGTGTTTGTGCTGCT	56.5	57.9
223	GM1533	CCATTAGCTGAAGGCATTGAA	CAAGATGGTGACGAGAAGAGC	59.8	55.9

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	**Reverse Primer (R)**	**F (°C)**	**R (°C)**
224	GM1562	TCTTCTAATCTTCGGTCAACAAT	TCATCAGATCCTCCAAAGCAC	57.9	55.3
225	GM1575	GCAATCGGTCATGGTTATTGT	GTCACTGAATGCCCAACTAGG	59.8	55.9
226	GM1577	GCGGTGTTGAAGTTGAAGAAG	TAACGCATTAACCACACACCA	55.9	57.9
227	GM1664	TGCTCAATTCAGCCTTCTTG	CTGCTGCCAATGGAAATTCT	55.3	55.3
228	GM1745	TGGAATTGGGATTTGGTTGT	CTCCTCCTGCTACTGCTGCT	61.4	53.2
229	GM1760	TGAAGAGCCATGTCAGATCG	AGGGCCCCAACAAGATAAGT	57.3	57.3
230	GM1773	CCCAACAGTAACGCACTTGA	GGGGTGTTGTTGTTTGTGTG	57.3	57.3
231	GM1834	GAAGCAAGAAACCAACCAAGTC	GTGATAAAGCGGCCACAATAG	57.9	58.4
232	GM1839	GAATCTGAGAGTGAAACAGAGCA	GAATTTGGGAAGACGAGGTTG	57.9	58.9
233	GM1842	TGTTGAGGTTGCTCTGTTGC	TCATTCCTTCATAATCAAATTCC	53.5	57.3
234	GM1845	TAACACAGTAACGCCACAACC	TGAGAAACTAAGTTAGGGCTGCT	58.9	57.9
235	GM1864	CAACACACCCAGTCACTCTCTC	TCCTTTCTGATGTTCTGTGTGTG	58.9	62.1
236	GM1869	ATAAACCCACACGGAAACCA	GCGGTTGATGTTCTTCTCTCTT	58.4	55.3
237	GM1879	TTCCGTTGTGTTGATCCTTTC	GTAACCGTGCATCTCTTGCTC	59.8	55.9
238	GM1907	CACTGTCCTCTTCCCTCACTCT	GGTGGACGAAGAAGAAGAAGAA	58.4	62.1
239	GM1911	CAGCTTTCTTTCAATTCATCCA	CACTTCGTGTTCTTCCTGCTC	59.8	54.7
240	GM1937	TTCATCCTCTGCTTCCTTTGA	TGACCAAACCCATCATCATCT	55.9	55.9
241	GM1949	GCACCAATAGAAAATGCCAAA	CAGCAACAGCAACAATTCTGA	55.9	54.0
242	GM1958	TGAAGTGTACGTGCAATGATCTC	GACGGACACTACTTCCATAGCC	62.1	58.9
243	GM1959	GTGTTCTCAGCCATCTTTTCG	GTGAAGGTGTTGTGAATGCAG	57.9	57.9
244	GM1960	TTTATTCTCCCTGAAATGACGA	CCCTCTTCTTCCTCCATCCTA	59.8	54.7
245	GM1977	CAAAGGAAAGACTTGGTTGTGTC	TTGGTCCAGAGACATGAAAGG	57.9	58.9
246	GM1986	GCTGCTGCAAGTCTTAAGGAA	AAAGTGTCAGGTGCAAAGCAT	55.9	57.9
247	GM2024	CATCATTACACGCGCTTCTT	AGAGGAGGAGGAGAAGCAGAA	59.8	55.3
248	GM2053	ACAAGGAAAACCCATCCAATC	ACGTGATGGATTCTTGTGGAG	57.9	55.9
249	GM2103	GCAACATGCCCTTAGACATACA	GCTTTCTCTTCTCGCTTCCTC	59.8	58.4
250	GM2165	CTACGCGCATCGCATAATATC	GTGAGATGGGGTTGGAGATTT	57.9	57.9
251	GM2206	TTCCTTCTCCAAAGTCCAAGC	GGAGGAGGGATGTAAGTACGG	61.8	57.9

Sl. No.	**Primer**	**Nucleotide sequence**		**Anneling Temp**	
		Forward Primer (F)	**Reverse Primer (R)**	**F (°C)**	**R (°C)**
252	GM2215	GAAATCGGAGTCGGAGAGGT	TCCCCTTCTTTCTTCGTTCTT	55.9	59.4
253	GM2348	ACACAAGAACCACCAAAAGCA	CAGCGCCATTTCTCAACTATC	57.9	55.9
254	GM2407	GCTGAGTCAGAAGTAAACGAGGA	ATCGGACGGAGAGAGAGTGAG	61.8	60.6
255	GM2444	CCCTGTTACACACAAGCCATT	TGAGCAAGTGTTAGCCATGAA	55.9	57.9
256	GM2478	TGTGTAGCCATGACTGCTGAG	GGAAGTTGCTCTCTTCCCTGT	59.8	59.8
257	GM2504	ACATCAATCCCTGCCTACCTC	TCGGATTCTGTTACCACCTCA	57.9	59.8
258	GM2522	GCATGGAATGTTGATGATGTG	AGAATGCAGAAAGTGCAGAGC	57.9	55.9
259	GM2528	TGCAAACTCAGTAGGAGCCATA	GCCAAACAGTAGAAGCTGGTG	59.8	58.4
260	GM2531	CAAGGATGTCCCAGATGATGT	GGACTCAATTTGTCGACCCTA	57.9	57.9
261	GM2602	ACTCGATTGGGAACTGAGGAT	TCTCGTCTCTGCCATTAGTACC	60.3	57.9
262	GM2603	ACTCGATTGGGAACTGAGGAT	CGTCTCTCGTCTCTGCCATTA	59.8	57.9
263	GM2605	ACTGCTGCCATGGTTGAGTTA	TTTCGCACTTTCTCAGTTTCC	55.9	57.9
264	GM2606	AGAAACACGCGTAGTGGAATG	TGCCGTTTACTCTCACTCACA	57.9	57.9
265	GM2623	AGTGGTATCAACGCAGAGTGG	AGAACCATGTTATGGCAGCTT	55.9	59.8
266	GM2638	ATGCTCTCAGTTCTTGCCTGA	CAGACATAACAGTCAGTTTCACC	58.9	57.9
267	GM2671	CTCTTAGAAGCCGCCATTACC	TCGCAACCACTAGAAACAGGT	57.9	59.8
268	GM2730	GGAGACGAGTTGTTGTTACCG	GGAAAGAGACCCCATCAACTC	59.8	59.8
269	GM2746	TCAACCTCAAGGGTGATTGTC	ACACAAACCCGCTCACTCTAA	57.9	57.9
270	GNB18	AAAAATTCTTCCTAGTTTACCCCC	CTCAACACAACAAGTGGAGCA	57.9	57.6
271	GNB38	TCCAGGGTCACTGTTCTTCC	CGTTGGTTTCATCAAAGGCT	55.3	59.4
272	GNB58	TCTGCTTTATACCACACTTTTTCC	ATTTCCCAAAATCCAGGGTC	55.3	57.6
273	GNB73	GAAGGTGAAGGGGAAGAACC	TACACTCTCTTCCGCCACCT	59.4	59.4
274	GNB98	GAGAAGGAGACAGAGTGTAAGGG	TTCATTCATTCACCGACTCATC	56.5	62.4
275	GNB100	TTCCTAGAGTGTTTTGGCTTACTG	AAAAACCTAATTATTCGACTTAAAGCA	55.9	59.3
276	GNB107	ATGGCACATGAACAGCAAAA	TCCTCTTGCAAGCAAAATCC	55.3	53.2
277	GNB126	AAAAGGAACATTGAACCTGGAA	GGTTTCGCAATCAGCAATTT	53.2	54.7
278	GNB136	GATTTAATGGGGGATGAGGG	TCACCCACACCCTTTTGATT	55.3	57.3
279	GNB145	CCCAAACCCACACTCTCCTA	AAAGGGTTTTTGGCATCTCC	55.3	59.4

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	Reverse Primer (R)	F (°C)	R (°C)
280	GNB155	AGAACGCGAGATTACGCAAC	GTCTGGGCGTCAGATTAGGA	59.7	57.3
281	GNB159	CGAATCGGAACACCGTACTT	TTTTAAATGTTTGGCCCAGG	53.2	57.3
282	GNB167	GGCAAATTTTTCACCTAGCG	TCAATACTTTCGTTGCATGGAT	54.7	55.3
283	GNB178	GGCATGGTTGGTTAGTCACA	TTACTGAGCATGCCCCTTTT	55.3	57.3
284	GNB181	TGGAACCAATAATGAAACTAGGC	TGAGAAGCTTGTGGACTTGG	57.3	57.1
285	GNB262	TGCGGTGAATAATATACTTAACAGTTT	TACACACATGCCTAGCCGAC	59.4	57.4
286	GNB284	AATGCGTCATTTAGGCAAGG	GCTCTGCATGGTAGGGTGTT	59.4	55.3
287	GNB303a	ATAGGGAGCTCTTGGGCATT	TTTTGCACCAGAAGTTGCAG	55.3	57.3
288	GNB317	GAAAAGCTTGCAAAATCGAGA	TCCTTCCATGTTGGTGAATG	55.3	54.0
289	GNB344	AGGAATTCAGTAGCACGGGA	CCAACACAAACAGCACCATC	57.6	57.3
290	GNB357	AGGTTTGCTTTGGGATGATG	CCGATAAAACCAGGCAAGAA	55.3	55.3
291	GNB378	TATCCCTAGGTTGTGGCAGC	ATCCCGTACAAACACTTGCC	57.3	59.4
292	GNB387	TGCATAGAAACTCACATTGGAA	CAATTGTAAACCTTCCCACCA	55.9	54.7
293	GNB392	AAAACAAACAAGCCAATCCTACA	TTCGTGGTGCTCTTGCTATG	57.3	55.3
294	GNB397	CGACTCAGGATGTTCGACCT	TCATTTGGTGGGCAGTGTAA	55.3	59.4
295	GNB428	AGGTGGTTGTTCTGACAGGC	GAAGGACAAACCACCCACAC	59.4	59.4
296	GNB461	TAATTATGCCATGCACTACCTATTTT	AAAGGATGCCAACTTTTAAGAAA	53.5	56.9
297	GNB464	TTTGAATTTTCACCTTCGTCA	TTGCTTCTGTGATGTCCGAG	57.3	52.0
298	GNB467	AATGGCATTCCTCACTTTGC	GCAAGAAAAAGGATGATGGG	55.3	55.3
299	GNB515	AAACTTGGACGTTGGCTTTG	CAGGGAAAAAGGCACTCTTG	57.3	55.3
300	GNB555	TCAGGGTGGGTTACCAACAT	TAATCCACATTGAACCGACG	55.3	57.3
301	GNB569	GCCTTGGATCCTTTTTACCC	TTGTTGGCTCTTGAAAGAATGA	54.7	57.3
302	GNB608	CGAGCTGGCTCAAATAATAAGAA	AAGTTCAGCTCATTAACTCGTAAAC	58.1	57.1
303	GNB643	CAGATTACCAATCCCAGCGT	TTTCTGGTTTGCACACTGCT	55.3	57.3
304	GNB667	TGTCCAACTCCTTTATCGCC	AGAGGCAGCCACTTCTTCAG	59.4	57.3
305	GNB679	TGGTTGGACTCAATTCGTGA	TCTGTTTTCACTCAAGCGGA	55.3	55.3
306	GNB682	TTGATTTGACCTAAAAATTCGG	TTTGTGCAAATAGGGTTAGGA	54.0	52.8
307	GNB712	TGAAGGGGGAAGAAGAAGAA	TTCGAATTCCCTCTCTCCTTA	55.9	55.3

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	Reverse Primer (R)	F (°C)	R (°C)
308	GNB716	TCAATTTCTTTCTGTTGTTGCAC	TGGGAGCAGAAAATCAGCTT	55.3	55.3
309	GNB733	GTGTCCGAATCTTTCACGGT	CGACATTAAAATGCCCGAAT	53.2	57.3
310	GNB775	TCAGCTGGTACAGGGGATTC	AGATTGGAGGGCCTAAGGAA	57.3	59.4
311	GNB782	GGGTCATTTTGTCCCATTTTC	TGGGTTGATAAAAATGTGCG	53.2	55.9
312	GNB840	GTTCACGTCCTTGGTCTGGT	TTGAATCTTCAAGAATTGGGG	54.0	59.4
313	GNB842	TGTCACGATTAAAAAGTTTCGG	TGGTAATTTTGCAGCATTATCTGT	55.9	54.7
314	GNB850	ATGTTAGCCCCACAAAGGGT	TCAATTTTACTCGAAGTGTCGG	56.5	57.3
315	GNB853	ACTCTAGGCTCGACCTGCAA	GCGAAGTTGAGATGTTGGCT	57.3	59.4
316	GM724	CGATCGAGCTTTTCTTCTCCT	ATGGCAATTTGATGTTTTGCT	52.0	57.9
317	GM986	AGCCAAAATGGGCTATACGAT	TTTCTTGTGGCTCTTGTGCTT	55.9	55.9
318	GM1330	CGTCCGAAGGAATTAGTTGAA	CTCCACCTTTTGGTGTTGAGA	57.9	55.9
319	GM1356	GGTATTTTGCCCTGAGGAAGA	CGCAGGTTTTATTTGCACAAG	55.9	57.9
320	GM1376	CACGGGTGACATTGGTTACAT	AGGTGCCACTTGGTATCCTTT	57.9	57.9
321	GM1416	TTCTTCTCTTTTCCCCAGCAC	TGGCTACTCCCTGAGACATTG	59.8	57.9
322	GM1494	CTTCGAAGAAAAGTGCATCG	GAAGACAGAAGACGAAGAGCGTA	60.6	55.3
323	GM1540	GAACTTCCTGAGCATGTGATG	GACGACGACGAGGAGGAG	60.5	57.9
324	GM1611	TCACACTCATGCACTCTTCCA	TTGTTATTGATGGCGGTTTTC	54.0	57.9
325	GM1623	TCTTTTCCTCTTTTAGACTCTGAGC	CTTCGACGAGAAGTGGAAGC	59.4	59.7
326	GM1630	TCCAAACCCTTAACCCTTCA	TGGCAATGCTTGGAGTATCA	55.3	55.3
327	GM1742	GCCTTGTTGCAATCATCACA	ACCTCCAACAGGAACATTGC	57.3	55.3
328	GM1771	AACCCTATCGCTTGGAAGGT	CCAAAGGAGAATGGTGAGGA	57.3	57.3
329	GM1790	CCCATAATTGTTTCACCACCA	GGAGAAACGAGTGGTTTGTACCT	60.6	55.9
330	GM1861	GGGAACTGATCACTGTCTTCG	AAAAGGAAGGCTAAAGCATGA	54.0	59.8
331	GM1878	TCAGTGGTTCAGTGCATCAAG	GTCCCTTGGTCATCTTCGATT	57.9	57.9
332	GM1899	GGCTCCGATTTCTACGTTTCT	GGAGCTCCGATGAGAAGCTAA	59.8	57.9
333	GM1902	TTTTCAGTTTCGCTCTGAACC	ATGGCCACTGATCCTGATAGA	57.9	55.9
334	GM1923	TTCCTTGTCCAGTTCCCATAA	TGGGGTTCAGTGATGAAGAAG	57.9	55.9
335	GM1964	AGATCTGGACCCATGGCTAGT	CTCTACGGCCACCACTACTTG	61.8	59.8

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	Reverse Primer (R)	F (°C)	R (°C)
336	GM1971	TTTTCTCCGAACCTTCCTTTC	AAGAAAAGAAGAGCAGCCACA	55.9	55.9
337	GM2006	TTACAGACACGAAAGGATGGTG	CAAGGAGCAGAGAAGCGTATG	59.8	58.4
338	GM2038	ACAAGGTTTAGGCATGGTGGT	CCTCCCTCACAACTCATCAAG	59.8	57.9
339	GM2313	GATGCTGCTAAATCGAGATGC	GTTGTTTTGTTTCACGCCAGT	55.9	57.9
340	GM2325	TCCCTAAAATAATTTCTGCCAAA	TCTTTTCTTCTGCTCCCCTTT	55.9	53.5
341	GM2452	CAACAAACATGCTGCTCAAAC	CAACAACAACAACCACCATTG	55.9	55.9
342	GM2513	GCATAGGCAATACCGCATATCT	CCCAATGTGTGATCAATTACGA	56.5	58.4
343	GM2548	TGTTTGGGGATAACAACTTGG	GAGGAGTTAGAGGCAGCGATT	59.8	55.9
344	GM2703	GCACATCTCAAAGTAGCCAAAA	GCCAAGGAATGTTTAGAAGCTG	58.4	56.5
345	GNB152	ATATTGCTCACCCTCCAGCA	AATGGCCGAACTAACTCCCT	57.3	57.3
346	GNB206	TCTAAGAGAGGGAGAGGGTTCA	TTCCTTTTCTTAATTTTCGAACTC	54.2	60.3
347	GNB241	CTCACAACCTTCGCAACTCA	TTGTTGGGAAGTAGAAGCGG	57.3	57.3
348	GNB374	TGCTAAATAAAAGGTAATTAGTGGCA	AACACTGAAACTTTGAGAGCACA	57.1	56.9
349	GNB500	GCATCACTTATGGTGTGTTGATT	TGGTTTTATTTGTTCACTACAACATTT	55.9	57.1
350	S-06	CCGGCTAGAGAATACACACACA	TCCTCCTTCCTCCTTGAACA	57.3	60.3
351	S-44	GGTGTTGAGGGATGGTTGTTCTAA	CTTTCCCGCCTCTCCCTCTC	63.5	61.0
352	S-56	CATAGGCGTCCCATTGCTTACAG	GATTACGCGCTCTTTCATTTG	55.9	62.4
353	S-66	ACCCCCATTGAGCGATTTG	AGTCCCATTGCCTTTCTTCTGTAT	59.3	56.7
354	S-82	TTGCAAAGTAGCGTTCAGAC	CATGGATGGCAGGACAAT	53.7	55.3
355	S-88	TCAAAGAAGCAATAAAAATC	CTCCACCGGCAAGCACCTC	63.1	47.1
356	S-106	GCCAGCATAGAAGCATAATAACA	GAGTAATAGTGAATCAATGAGAAGAGG	60.4	57.1
357	S-107	TTCAATAATCCAAACCTCATCA	CTGTTTGCGTTTTTCTACTCTG	56.5	52.8
358	S-116	GATTTGTTTTCTTCTTCGTTTTT	CATAATCCACTTCGCCCTAAT	55.9	51.7
359	PPGPseq17E03	TTTCCTTTCAACCCTTCGTG	AATGAGACCAGCCAAAATGC	55.3	55.3
360	pPGPseq19B01	TTGGTGATGGTGTTGGA	TTAAACCAGGCCAAAAGTGG	55.3	55.3
361	pPGPseq05D05	AAAAGAAAGACCTTCCCCGA	GCAGGTAATCTGCCGTGATT	57.3	55.3
362	pPGPseq18C05	GGACAGCCGGATGCTATTTA	ACATGAGTCCCTTTTCCCTT	55.3	57.3
363	pPGPseq02D12B	AAGCTGAACGAACTCAAGCG	TGCAATGGGTACAATGCTAGA	55.9	57.3

Sl. No.	**Primer**	**Nucleotide sequence**		**Anneling Temp**	
		Forward Primer (F)	**Reverse Primer (R)**	**F (°C)**	**R (°C)**
364	TC11H06	CCATGTGAGGTATCAGTAAAGAAAGG	CCACCAACAACATTGGATGAAT	56.5	61.6
365	TC6E01	CTCCCTCGCTTCCTCTTTCT	ACGCATTAACCACACACCAA	55.3	59.4
366	TC01A02	GCAATTTGCACATTATCCGA	CATGTTCGGTTTCAAGTCTCAA	56.5	53.5
367	TC11A04	ACTCTGCATGGATGGCTACAG	CATGTTCGGTTTCAAGTCTCAA	56.5	59.8
368	TC5A06	TCGGTTTGGGAGACACTCTT	TTGTAAGCAGACGCCACATC	57.3	57.3
369	TC7C06	GGCAGGGGAATAAAACTACTAACT	TTTTCCTTCCTTCTCCTTTGTC	56.5	59.3
370	TC4H02	ACCGCAAACTCATCCATCTC	GATAGCGTCAGAGGCAGAGG	61.4	57.3
371	Lec-1	CAAGCATCAACAACAACGA	GTCCGACCACATACAAGAGTT	57.8	52.4
372	IPAHM 108	CTTGTCAAACTCTGTGACTTAGCA	CATGAACAATTACACCCAGTCA	56.5	59.3
373	IPAHM 356	TTGGGATTGGATCCCTAAGA	CAACTACCCTTCTCTCCACCA	59.4	55.3
374	pPGPseq19G07	ATTCAATTCCTCTCTCCCCC	TCAATCAATCAATCGCAGGA	53.2	57.3
375	GM723	CAAACCCTAACCCAAATAAAACC	TTCACCCTTGAGTACCGGAAT	57.9	57.1
376	GM1073	TCCCATACTACCCCTTAGCTTTT	GAAAACAACCAAACCGAAGTT	54.0	58.9
377	GM1089	TTGGAACAAGGATGGAAAGAA	GTTTACGGTTGGCTTGTCAAA	55.9	54
378	GM1555	CGTAGACGTGAACCACTACCAA	CGCCTAGTGTCTCAGAAAACG	59.8	60.3
379	GM1565	CTCCGTACCTGAAATGATGAA	TGCTCACTGTTTCAACCTGAAT	56.5	55.9
380	GM1603	GGTTATGACATCTTGATTGGATG	AGATTGCAGCGAGAAGGAAGT	57.9	57.1
381	GM1609	TGAATTCCATTTCCATCATCAA	TGAATGTGTGTGAGGGAAAGAT	56.5	52.8
382	GM1752	CAACCGAATCTGCAAAGAAA	AGTGCATGTGATAGCCCCATGT	57.3	53.2
383	GM1846	GTGCGTAGTGAGTGGGTCCTA	CACGCAAACTCTCGATTGTAA	55.9	61.6
384	GM1863	CACACCCAGTCACTCTCTCTG	TCTGATGTTCTGTGTGTGGAGA	58.4	61.8
385	GM1867	GAGCAGACATGGTTGAATGGT	CACCTCAACGAAGAACTCAGC	59.8	57.9
386	GM1897	GCGGTTTCTGGTAGCTTGAA	CCAGGGACACAAACAGAAGAA	57.9	57.3
387	GM1901	GAAACACCGATATTTTCGATACA	TGACGAGCAAGTCATGTATGTG	58.4	55.3
388	GM1902	TTTTCAGTTTCGCTCTGAACC	ATGGCCACTGATCCTGATAGA	57.9	55.9
389	GM1992	GGAGAGTCGGTGAGAGGAGAG	CGCTCGTTTTCCTCTTTCATT	55.9	63.7
390	GM1954	GAGGAGTGTGAGGTTCTGACG	TGGTTCATTGCATTTGCATAC	54.0	61.8
391	GM1955	CACACTCTGTCCCCACCATAG	TAACCGCAAGATTCCAAAGAA	54.0	61.8

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	Reverse Primer (R)	F (°C)	R (°C)
392	GM1979	TCCAAGAGGAAGAGGGAAAAC	AAAACCCTTACTTCAACAAAAGA	53.5	57.9
393	GM1988	GGAGAATCAATTGGTGGAAGC	TCAGACATAGGTGGTGGTGAA	57.9	57.9
394	GM1989	GCTTGCCTGACTCATTCACTC	AATGTTCCTTTCCATCGAGGT	55.9	59.8
395	GM1991	GAAAATGATGCCGAGAAATGT	GGGGAGAGATGCAGAAAGAGA	59.8	54.0
396	GM1992	TGATGCTTGGTCAATGTATGTG	TTTCTCTGCTTGCGTCATTTT	54.0	56.5
397	GM1996	CATCCCATCATTTTCCCTCTT	TACAGTGAAGGTGGGATCCTG	59.8	55.9
398	GM2032	GCCGATGATGTACGTTTCTTC	GAGACGGCATGTCAAAAGAAT	55.9	57.9
399	GM2067	TCGCCAAGAAGAACAAAACAC	CTGGTCGAAGAAGGGTTCTCT	59.8	55.9
400	GM2084	CGCAGAAATGAACCGAAATTA	GGATGCATTCTTCTTCCTCCT	57.9	54.0
401	GM2120	TCCACTGCCACCTCTATCATC	TCCACCCACATGGACAGAAGC	59.8	59.8
402	GM2246	GCAATTTTGTGCACCCTTTT	CGCTTGACACCAATGAAGTCT	57.9	54.0
403	GM2250	TGATGAATCAGAAGCATGTGG	TCTTAATTGCTCCGAAACCAA	54.0	55.9
404	GM2262	GTGGTTGCATGGTTTTGACTT	AAGGCTTCTTGTGATTTGTGG	55.9	55.9
405	GM2301	GTAACCACAGCTGGCATGAAC	TCTTCAAGAACCCACCAACAC	57.9	59.8
406	GM2307	CCATGCAGGAAAGCAAGATAA	GCTGGTACATTCGGAGAGTGA	59.8	55.9
407	GM2313	GATGCTGCTAAATCGAGATGC	GTTGTTTTGTTTCACGCCAGT	55.9	57.9
408	GM2350	GACTGTGGTTGGTGGTTTT	CTCCTTGACCTCCTGGAGAAT	59.8	57.9
409	GM2425	AGGATCAACCACAACCAACAAC	TTCTGATCCCTCTTCTTGATCG	58.4	57.9
410	GM2482	TGTCCTTGACACTGAAGACCA	GACAAAGGAGAAGAGGCTTGC	59.8	57.9
411	GM2547	GATCGTGACTTTCACCCAAAA	GGAACCCAGGAGGTTGATATT	57.9	55.9
412	GM2584	ACATTTTCAGCACCCTTCCTT	ACTCGTCAATGTTCCCTTGTG	57.9	55.9
413	GM2589	TTTCAGCTACCCTTCTTCCAAA	CAAAAGTGAACCCCGTAAGAA	55.9	56.5
414	GM2637	ATGCTCTCAGTTCTTGCCTGA	AAGGAGCCAGCTAGCTACATAGT	60.6	57.9
415	GM2687	GACAAGAAGGAAGGCAGTCAA	TCAACCACCTTGTTGCTCTCT	57.9	57.9
416	GM2691	GAGAAGCTCGAATCCGTGTAA	TTGCCTGCTTCTTCTCTTCAC	57.9	57.9
417	GM1538	AGCTCCAGAGGCAAAGAAAAC	TGCATAGTGATGGTTCCTTCC	57.9	57.9
418	GM1919	TGGAAGGGGTCAGAAGAAGTT	CCGTGTCTCTCTCTTTCTCTCC	62.1	57.9
419	GM1076	AACAGACCAAAGGGTGTGTGA	TGCAGATTCTCACTTCCTAAGC	58.4	57.9

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	**Reverse Primer (R)**	**F (°C)**	**R (°C)**
420	GM1501	TCTCCAGTGTGTGTGTGATGA	TAAGAACCAAAATTGCGACCA	54.0	57.9
421	GM1502	TTCCTTTACACACACGCACAC	TGGAGGAAATGTAGGGAAAGG	57.9	57.9
422	TC1D02	GATCCAAAATCTCGCCTTGA	GCTGCTCTGCACAACAAGAA	57.3	55.3
423	GNB278/ARS710	GTGTGGATTTGGATATCGGG	GTCGTTGTCATTGTCGTC	57.3	57.3
424	GNB353/ARS715	CGATCCTAGACCCGATGAAA	TCGCGAAACTATAAGACCAACA	57.3	56.5
425	GNB418/ARS719	AATTAAAAGCGCAACGCTC	ATTCTCCCGCCAACCTTACT	53.2	57.3
426	GNB710/ARS724	TGAAGCCATCCACAGTTTGT	GCTCAGACAGGAGCACATGA	55.3	59.4
427	GNB816/ARS727	CAACACTAAGCCAGGCACAA	GGCAGCGGCTAGATTTCTTA	57.3	57.3
428	GNB831/ARS729	GGGATCCATGCCTTGAAAAT	CCAAATCAAACAAACAAAACA	55.3	50.9
429	GNB876/ARS734	TTGCTAATCACATTGTTGGTTT	TACCTGGCCCAGAAAAGAAA	52.8	55.3
430	GNB1078/ARS736	TCCCCACCAAGTACAGTCATC	ATGTTGAGTGTGGGTGGGAT	59.8	57.3
431	GNB1121/ARS737	TTTTTCACATTCAGTCATGCG	ATGCATCCCTTTTTGGTGAT	54.0	53.2
432	GNB2/ARS738	CTTGCTGTGGTGGATTTCCT	GCCTCCAATTTGCGAATCTA	57.3	55.3
433	GNB324/ARS749	TTCTCATGCTGCTGCTTTTT	CCATTTTAGCCAATTCTTTTTACA	53.2	54.2
434	GNB377/ARS752	TGAATACAAGCTATTTGGTGCAT	GGAGTGAGTGAAGAATTGTTGAAA	55.3	57.6
435	GNB519/ARS753	TTTATAGAGCGAAATCACAAGTAAA	TTCGGAACAAAGGTTATGGG	55.3	55.3
436	GNB619/ARS756	TAACCACAAGCAAGGCAACA	AATGGCTTCCAGAAGCTTGA	55.3	55.3
437	GNB695/ARS759	TCTGTGCTTTACCTTTCAGTTG	TCGAAGAGAACATGCTCAACA	56.5	55.9
438	GNB533/ARS754	GAGATGCGTCAAAGGGCTTA	TCTAAATGGGTGGAACAAGCG	57.3	57.3
439	GNB625/ARS757	TTCACGTATTGTCCAAGGCA	TGGTCTGAGGGTAGGGTGAG	55.3	61.4
440	GNB649/ARS758	GAAGCTTTCCATGCACCAGT	TGAAATCCAACAACCACAGGA	57.3	55.3
441	GNB703/ARS760	GCAACATAAAGTAATCAACAAGGG	CCACGTTTTGACCCAATTTT	57.6	53.2
442	GNB736/ARS762	CCTTTAACTTCCATGGGCCA	AACAAGCAAATCACACGCA	57.3	53.2
443	GNB1112/ARS768	CACTGGAGCGTCGACTGTTA	GCATGCTAGCTAAGAATACTACCAAA	59.4	60.1
444	GNB1152/ARS769	TTGATAATGTCTCCACAATTTGTAA	AGAAACAAACCTGGAAGGCCA	54.8	55.3
445	GNB65/ARS772	TTTTTCTGGACCAAGCCTCT	TGAATTCGGGCTACTGATCC	55.3	57.3
446	GNB76/ARS773	GGGAACGAATGAAGTAGGCA	GCATGGGTTTCAAGGTCTGT	57.3	57.3
447	GNB87/ARS774	GCCTGTAGCACTGCAAACAA	GGAATAGGGGCAAGAATGGT	57.3	57.3

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	Reverse Primer (R)	F (°C)	R (°C)
448	GNB138/ARS777	CAGCCCGATGAAGGAATAAA	CGACCTAAACTCCGTAGGCA	55.3	59.4
449	GNB218/ARS783	GCCATATTTCTGTCAAATCAAAA	TACCATCTGGTTTACCCCCA	53.5	57.3
450	GNB320/ARS785	GAATTCCTGGCTCGAACTTG	ACCCCTCCATTTCGTCTTCT	57.3	57.3
451	GNB325/ARS786	GCTGCACACGTCACCTTCTA	TGTTGCGATATGATGGAGGA	59.4	55.3
452	GNB371/ARS788	CGCTGCTAATTTATTATGGCAA	TGTAGCCTTAGGTCCCTTATTTTT	54.7	57.6
453	GNB523/ARS793	TCACAAAATGCTTGGAATGC	GAACTGAGCACAAACTCAGTAACAA	53.2	59.7
454	GNB486/ARS795	CATTGCAGTTTCCCAGGAGT	TAGCACTCAGTCGGTCTCCA	57.3	59.4
455	GNB580/ARS796	TTATTGGGACCAATTTGGGA	ATGCACTGAACATGGGCATA	53.2	55.3
456	GNB613/ARS797	TTTGTGACGTTTTGTTGGGA	AACGAAACGCACGGTTTTAG	53.2	55.3
457	GNB629/ARS798	ACGTTCAGATAAACCGGCAC	GCTGGGTGAAGAAACAAAGC	57.3	57.3
458	GNB651/ARS800	CCGTAAGCTTTTTATAACACACACA	GCAAAAACCTTTTAGCATACCA	58.1	54.7
459	GNB652/ARS801	CAAAGTCGCACAAAGTGGAA	AACTCCGCAGGCTGTGACTA	55.3	59.4
460	GNB827/ARS806	CAGGCTTAAACTCCGTGAGC	ACTTGGATGACCCGGTACAA	59.4	57.3
461	GNB887/ARS808	TCTGTTTGGCCGTTGAAAA	TCCAATCGTTCAATCACGTC	52.4	55.3
462	GNB904/ARS811	TGAGCTGTTATGTTTTCTCTGGG	AAATTCCAAGCCAAACACCA	58.9	53.2
463	GNB905/ARS812	TTTTCTTATGGCTTCGGTGG	AAAACTATTTTGAAAGGCAAAATTAAG	55.3	54.2
464	GNB877/ARS816	TCAGCGGCTACGATGAATAA	TGGGTATCCACAACCACAAA	55.3	55.3
465	GNB41	TTGATGTGGCATCATTAGCAA	TGCCAATTCAGCTTTCACTTT	54.0	54.0
466	GNB62	TATTGACTTGGCATTGGGGT	AAGTGTGGGGAAAACTGTGG	55.3	57.3
467	GNB99	TGGACATTTTAGTCTCCAAAAACA	TCTTAACGCAAACCAACTTGAA	55.9	54.7
468	GNB148	CTTTGAACTCCCCGGGTTCTC	TTGCACAGTCCCTCTGTACG	59.4	59.4
469	GNB216	TCTCTGTCGTCATAGGGTTAGGA	CCGAACAAATCAATTTAGCCA	60.6	54.0
470	GNB230	TAAAATATTGCTCGCCCTCG	AATTACACCCACTCCCTCC	55.3	59.4
471	GNB247	TTGGAAAGACGACTCGGACT	GGTGTCTTAGGAGGCCATGA	57.3	59.4
472	GNB274	TCCCCTGATTCAACAACTCC	TGCATACCTCTGATCCACAA	57.3	55.3
473	GNB380	ACGGACCAAAAATGCTAACG	ATGGCTGTAAACGGTTCGTC	55.3	57.3
474	GNB409	AATTGGACATTTTAGCCCCC	GCCAAGTTATGGTGTCTTCTCC	55.3	60.3
475	GNB451	GCTTTTGTCTTCCCTTTCCC	CACAAACCCCCTCTTCTTCA	57.3	57.3

Sl. No.	**Primer**	**Nucleotide sequence**		**Anneling Temp**	
		Forward Primer (F)	**Reverse Primer (R)**	**F (°C)**	**R (°C)**
476	GNB602	TTTCAAGTTTGAACATGATTTCG	CAATTGCACAATGAGGTTCTG	53.5	55.9
477	GNB616	GTGCCATCAATGGTTATGGA	CCAAACCTACTCCCTCTCCC	55.3	61.4
478	GNB620	CATCATGGAGGGCTTCCTTA	TTCAATCCCTTTTCAGGTCG	57.3	55.3
479	GNB817	TTTAAAACAAAGGGCGCATC	AAGGTGAGCCTGGAGCTTGA	53.2	57.3
480	GNB837	CGAAAACATCGATCTCATGC	GGTGCATGTTTTATCTTTACACGA	55.3	57.6
481	GNB873	TTTTGGCCATCAAATATTGTCTT	AAACATCGAGAGAATCAACCAAA	53.5	55.3
482	GNB901	TGCGTTTAGTTACCGTGTGC	CAGAGGTTACGACAGTTCTAAGGC	57.3	62.7
483	GNB967	CATTTGCTGAACAAGAGGCA	GCGTTGCCTTTCCTAAAAGA	55.3	57.3
484	GNB970	CAACGTCAAAGGTAGCAGCA	TTCGCATTCAGTCTCCCTCT	57.3	57.3
485	GNB983	TCGTTGGACTGCTACAAACG	TGGACACTTTGTAGTTCACCG	57.3	57.9
486	GNB988	CAGGTCCCAAACTCCCAATA	TCTGCGTGCTGATTCTTGTT	57.3	55.3
487	GNB989	GCTTTCAAACACAAACAAGCC	GCGCATCCCTTATTTTTCAT	55.9	53.2
488	GNB1040	TCACTTTTGAGTGTGCCTGC	TCCATTAGTGAGAATACCCCTAACAAG	57.3	60.1
489	GNB1096	CACCTTGACCGAAACACCTT	CGGTCTTAGGTGCCATTGAT	57.3	57.3
490	GNB1148	ACCTATGGGCCTATTGGGTC	TTTGTTTTGGATGGGAGAGG	59.4	55.3
491	AHGS0108	GGTGAGGGAAAGAATCCACA	ACAAGGGTGACTTTGTTGGC	57.3	57.3
492	AHGS0132	CAAATGTACCTTCGGCGATT	TTACGAACACCCCCTTTCTG	55.3	57.3
493	AHGS0134	GAATCTGCTGTGGACCGTCT	GCAGATGGAGAAGCCATGA	59.4	56.7
494	AHGS0138	CATATTGACGGTGATGGCAG	CCGACCCTAATCCTAATACAACA	57.3	58.9
495	AHGS0147	TAACAGCCGGATCAAACTCC	ACCACCACCTGCAATCACTT	57.3	57.3
496	AHGS0151	AGCAAATGAAGGTGAGGGTG	GCCCAAAAATGCTAACGAAG	57.3	55.3
497	AHGS0202	CAATTCGTTTATCACCCGCT	TTGCAACGACGAGTCTATCG	55.3	57.3
498	AHGS0230	GGAAGCATCACCCTTCAAAT	GAGTTTAGCATTTGATGAAAGTCA	55.3	55.9
499	AHGS0266	AGAGCTCGAAGAGGGAGCTT	TGATCTCCTCGCTTGGACA	59.4	57.3
500	AHGS0278	CGAACTCCAGTGACAGGCTC	ACCTGCCGTTGTTTCTCATC	61.4	57.3
501	AHGS0347	AACCAGTATCCCTTCCGCTT	AGTTTGTTGCCAGTTTCGCT	57.3	55.3
502	AHGS0357	CCTTTCCTTCCTTCATTCCC	AACATCTGCCAAATGCAACA	57.3	53.2
503	AHGS0369	ACGATTCGACGGAGAGAGAG	TTCAAACACAGAACCCCTCC	59.4	57.3

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	Reverse Primer (R)	F (°C)	R (°C)
504	AHGS0590	AAGCCCTTCTCCCTCACTTC	ATAGTGGACCTCAACCACGG	59.4	59.4
505	AHGS0599	AGATTGGTGGTGACAGAGGC	GCCATCGATGTAACCCTCAC	59.4	59.4
506	AHGS0695	TGGTGTGTTGGGAAATGAGA	ACAGCAGAATCTCGGGCTAA	55.3	57.3
507	AHGS0729	TGGTTGTTCTAACCCTTCGG	TCACTATCCCATCCCTGCTC	57.3	59.4
508	AHGS0798	CGTAGTTGGTGGTAGCCGAT	GAACCGTTAACCCTCTTCCC	59.4	59.4
509	AHGS0993	TGGAGAAACTGTGGGAAACC	TCACATATGCATTGACCTTTCA	57.3	54.7
510	AHS0116	ACAAGACCACCACCATAGCC	CGGTTATGAGGGGGAAGATT	59.4	57.3
511	AHS0161	TTTTTGCAGTCAATGGTGGA	TCCAGAGGTGGTGTTGTCAG	53.2	59.4
512	AHS0970	CATCATTTGCAAAAGCCCAC	GGGTGAGTCATTGTTTGGCT	55.3	57.3
513	AHS1684	GCTGCGGTTTCGATCCAT	GCGCCAGAGAGAGAGAGGAAA	56.0	59.4
514	AHS1750	ACAGAAGGCAAGGAAGTTCG	AGCGGTTTGAGGAGAGATGA	57.3	57.3
515	AHS1855	GCTGTCTCCAGGTTCTCAGC	AAGGTGGTCATCGACGGTAGG	61.4	59.4
516	AHS1943	GCAGTTGCAGTGCCAAAAT	CAGCAAGCTTGTCCTCCAAT	54.5	57.3
517	AHS2037	TGGCCTTGATTACTCTCGCT	ACAGGGGTCTGGAGGAAGTT	57.3	59.4
518	AHS2312	CTTACCCACCAATTCTTCGC	TTGCTTCAGCAAAGTCAACG	57.3	55.3
519	AHS2567	TTTTCTGGTGGATTTTTCGC	TCTGTGACTATGGCATCCGC	53.2	59.4
520	TC19B07	ACATGGTATCACGGTGTGAAAT	ACACAGAACAGCTCCTTGATTC	56.5	58.4
521	TC19B11	ATCTCTTCCAACAGTTTGGGG	ATGCATCGCAAACATCACTCT	57.9	55.9
522	TC19E01	ATCAGAAACAGAACCCTGGAGA	GGGGAAGAAGAAAGCGGA	58.4	56.0
523	TC20B05	GCATGTAAACTATGCAATCGCT	CAACAACTTATTCCACCAAATATCA	56.5	56.4
524	TC21C10	ACAGGATTGGGAAAATGTTGAG	CCGCCTCAATAACAATAACCTT	56.5	56.5
525	TC21D06	ATCCTTACCCCAAAGCAACG	TGGTGATGGAGTTGAATGAATG	57.3	56.5
526	TC23C08	AGCAGAGTGGAAAACGAAGAAG	GTCAGTTTGTGAATCGGGTTTT	58.4	56.5
527	TC23F04	CACGTGTAATAGTTGCTCAAAAT	TATATGCATCAGACTCTCCAGC	55.3	58.4
528	TC24B05	ATTGATACCTCTTTGCTCTCGC	TGAAACCCTAACTAGCTCGGAA	58.4	58.4
529	TC27H12	TAACGAATGTACATCAATCCCTG	TCTCACTTTGCACTCTCCTCAA	56.5	58.4
530	TC29C07	CTCTTCTGAGCTGGAGACAA	CTTAATTTCCACCCATACCA	57.3	53.2
531	TC30D04	TCCATGAAGTCAAGGTTCTGGT	TGTTCATCAACCAATTTGCATC	58.4	54.7

Sl. No.	**Primer**	**Nucleotide sequence**		**Anneling Temp**	
		Forward Primer (F)	**Reverse Primer (R)**	**F (°C)**	**R (°C)**
532	TC31G11	TTTTGAGACTGTTGGTGGTGTC	CACTCTGATCACGTGCAATACA	58.4	58.4
533	TC31H02	AGTCATGTCCATTTCATATACAG	TTTTGGACTTACACCCTCTA	55.3	53.2
534	TC31H06	CATGTGGATATCTTTATTTCCT	CAGAAGATTCAAACTGGTAAA	52.8	52.0
535	TC38D06	GGTTGTTGGAAGAAGACAAAGG	AGACTCCTCCTTCCCTGCAT	58.4	59.4
536	TC39F01	ATCTATAAATTGGAGGATGAC	CAAGATGAAAGCTCAAAA	52.0	46.9
537	TC41A10	GTTTTGCTTCCTAATAATAAAGG	ATTCCCAAACTCTCTTCTCTC	53.5	55.9
538	TC41C11	CACCAGAAAACATGAGAGCAAA	CATCCACCTTCGTGTTAACCTC	56.5	60.3
539	TC41E09	TTTAATTTTGACACGTTGGGTG	ACACAGCAAAGCAACAGATGAT	54.7	56.5
540	TC42A02	AGAAATTGTGGAAATGAAGGGA	CACTTACAGAGCTGTTTTCGCT	54.7	58.4
541	TC13C03	AGATGGTACCTGGAGAGTGGAA	TGTTGAGTTCACACCAAAACCT	60.3	56.5
542	TC13E05	TCCTCTGCTTCCTCTGTTTCTT	TGGCTGTGGCTTTAGGGTATAG	58.4	60.3
543	TC14B08	CACCAGCACAAACTTCTGAATC	CGCTTTTCTGTGTTTTGTGTTT	58.4	54.7
544	TC15F12	ACCCTGAAATCGTGTCTCTGTC	GGGCATAGAGGAGATCGAAGAC	60.3	62.1
545	TC16A10	TATCGGAGAACAAGCACACATC	CTCCAAAGTCCAAACACAAACA	58.4	56.5
546	TC16A10-1	AGCAGAGAGCAGAGTGGAAAAC	CTCCAAAGTCCAAACACAAACA	60.3	56.5
547	TC19A02	CAAACCATCTAGCATCCGACA	AGATTGTACACATGAAATTGGCA	58.4	55.3
548	TC19B07	ACATGGTATCACGGTGTGAAAT	ACACAGAACAGCTCCTTGATTC	56.5	58.4
549	TC19E01	ATCAGAAACAGAACCCTGGAGA	GGGGAAGAAGAAGCGGA	58.4	56.0
550	TC20B05	GCATGTAAACTATGCAATCGCT	CAACAACTTATTCCACCAAATATCA	56.5	56.4
551	TC20D05	CAGCACCACATGATTGTCTTTA	GATCAAACCCTCCATAATCGTA	56.5	56.5
552	TC20E08	AGGCGGGACAAAGATTACATTA	AAACTGGTGGCCAAAGCTATAA	56.5	56.5
553	TC21A09	AGCATGGATATTTGGAGGAAGA	TTAGCATTTCCCACATCACTTG	56.5	56.5
554	TC21C03	TAAAGTTTCCGTCGTTTTGTCA	AAAGATGAACAAGGTTGCAGAA	54.7	54.7
555	TC21D06	AACACCATTTGTTTTCCTCAGC	ATCTGGCATAGGAACGTTGAAA	56.5	56.5
556	TC21G01	TGATCGCACCTAGTGGAATTAG	AACATGCCTTCATCTGATTGTG	58.4	56.5
557	TC22G05	TATACTCTGATTTTCCCGCCAT	GAGCCATTCGATTAAGCTGTG)	56.5	57.9
558	TC22H12	TTCTAGGTCTTCCTCTGCCTTG	ACGCGATTGGATAGAATAAGGA	60.3	56.5
559	TC23B10	GTTCGGGTGAACAAGCACTAA	AAGTCACCTTCACTGCATATTGC	57.9	58.9

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	Reverse Primer (R)	F (°C)	R (°C)
560	TC23D04	TGTTCTCGTTCAGTACCTCCCT	GTAGCAAGCGTTAAGGACCTGT	60.3	60.3
561	TC23E04	TGGGTATCAAACAAAACAAGAGG	TCAAAGACAAACACCAAAACCTT	57.1	55.3
562	TC23E04-1	GAGAGAAAAGAGGGTAGCTTTGC	TCAAAGACAAACACCAAAACCTT	60.6	55.3
563	TC23F09	ATTCTCTCCAGTCTCAATCCCA	CCAATAGTGAAAGGCATCATCA	58.4	56.5
564	TC23H10	TCCCTTTGAGTCATTCATTGTG	CATCAGAGCTCCTTTTCCCTAA	56.5	58.4
565	TC24A06	CTGCACAATTAATCCTGGTGAA	AATGAAGTTTGGGGTCTGCTT	56.5	56.5
566	TC24B05	ATTGATACCTCTTTGCTCTCGC	TGAAACCCTAAACTAGCTCGGAA	58.4	58.4
567	TC24C06	TCAACGCTAAAGGTGGTGTAAA	TGGAAGGGCAAAAGAAGAAAG	56.5	56.5
568	TC24D06	TCCCTTCTACACAGACATCCAA	TTTCGCAGGAACTACTCATCTG	58.4	58.4
569	TC24D12	TGGATTTGATTTCATTAACCCC	TGGTGATGGAATTTCAATGG	54.7	55.9
570	TC24G10	AAGCTCATAACATTACCACGGG	GAGGGTGGAGAACTTGTTGGTTC	58.4	60.3
571	TC25B04	TGCTTGTGTATTGAGCTGTCCT	CATCTGCCAAGGTCCTAAAATC	58.4	58.4
572	TC25F03	AGATCGAGATGAGAAGCACACA	AATTATGCTTGGACTTTCCTGC	58.4	56.5
573	TC25G11	GCATCAGCTCCCATCAGTATCT	AATTGTTTGCAAGTATCATGCG	60.3	54.7
574	TC27H12	TAACGAATGACATCAATCCCTG	TCTCACTTTGCACTCTCCTCAA	56.5	58.4
575	TC28A12	TTGAAAGCGAGAGTTTTGAGAA	TCTCAGTTTCTTTGTCGCTCAT	54.7	56.5
576	TC28B01	ATTTATTGCCAAATCTGTCGCT	CATTGCCAACTGTTACTACCCA	54.7	58.4
577	TC28B07	CTTCCTGAACTTTACAAGGTAT	GATCCTGTAGAAAGAAGGTCTA	54.7	56.5
578	TC29C07	TGGAGACAACCAATGAAATC	CTTAATTTCCACCCATACCA	53.2	53.2
579	TC29H08	ATAGAGTGCTCCACACACACCC	TAATCGGAAGACCTTTACGACG	62.1	58.4
580	TC30D04	TCCATGAAGTCAAGGTTCTGGT	TGTTCATCAACCAATTTGCATC	58.4	54.7
581	TC31C09	TCAAACAGTTCATGAGCAATAACA	AGGAAGAATCAGTGAGCTACGC	55.9	60.3
582	TC31G11	TTTTGAGACTGTTGGTGGTGTC	CACTCTGATCACGTGCAATACA	58.4	58.4
583	TC31G11-1	ATCCTTTTGAGACTGTTG	ATCACGTGCAATACATTA	49.1	46.9
584	TC31H02	AGTCATGTCCATTTCATATACAG	TTTTGGACTTACACCCTCTA	55.3	53.2
585	TC31H03	TGTAAGTTGACTCTAATCCAGTG	GATCCATACACATTCAACATAAA	57.1	53.5
586	TC31H06	CATGTGGATATCTTTATTTCCT	CAGAAGATTCAAACTGGTAAA	52.8	52.0
587	TC34E12	GTTACGGATGCTTACACATTAT	TCCTACTATACCAAACATAACATAAC	54.7	56.9

Sl. No.	Primer	Nucleotide sequence		Anneling Temp	
		Forward Primer (F)	Reverse Primer (R)	F (°C)	R (°C)
588	TC35F05	GATCAGCGAGAGAGAGGG	CTACTCAACTTCTCCAAATATGC	58.2	57.1
589	TC36C02	TCCTTGGTGCTCCTAAAGTCAT	TACTGTCTGATCCACTGGTTGG	58.4	60.3
590	TC36C03	TGGCGAAGTAGTTTGAAAGGTT	AGCGAGAGAGCAAGAGAGAGAA	56.5	60.3
591	TC38A07	CAAAGCAAAAGATTTCACTGAG	AGTGCTCTTTCTGCATATGTTT	54.7	54.7
592	TC38F01	TTTATGCATCACTTCAAGCACGG	AGCTGTTGAGCTTGCATCCTAT	56.5	58.4
593	TC38H09	CCAGGTAAGATTTTCAGTTATT	AGAGAATTTAGAAGATAAGGTGA	52.8	53.5
594	TC39A10	GCTGCTAATCGAGATGCAAGTA	TTTGTTTCACGCCAGTAGATTTT	58.4	55.3
595	TC39B04	ATCTCGTTCATGCACACGTTAG	ACAGTACTTGGCGTTAAGAGGG	58.4	60.3
596	TC39C01	AGGCGCGGTGATAGAAAAC	GTTTTGCTGTTCGTCCCTAAAC	56.7	58.4
597	TC39E08	AACAGAAAACGTTGAGTTGGCT	AGAAGAAGAAGGAGGATGAGGAA	56.5	58.9
598	TC39F01	ATCTATAAATTGGAGGATGAC	CAAGATGAAAGCTCAAAA	52.0	46.9
599	TC39F08	TATGAGGTGTTTCCAGTTTCCA	GCAATCACAAGAAGATGGTTGA	56.5	56.5
600	TC40D04	TTCCTTCCTCATATTCCAACCA	ATACTATCTCCGCCTTCAACCA	56.5	58.4
601	TC41A05	TTTTCCATTACAAACGTTGCAC	GGCAAGTGAAGTAAATTGTTGCT	54.7	57.1
602	TC41A10	GTTTTGCTTCCTAATAATAAAGG	ATTCCCAAACTCTCTTCTCTC	53.5	55.9
603	TC41A11-1	GATCCAAGCAGTTGGTCAAAAT	GGTCCAAACTACCTAAGATGCG	56.5	60.3
604	TC41C11	CACCAGAAAACATGAGACCAAA	CATCCACCTTCGTGTTAACCTC	56.5	60.3
605	TC42A02	AGAAATTGTGGAAATGAAGGGA	CACTTACAGAGCTGTTTTCGCT	54.7	58.4
606	TC11H06	CCATGTGAGGTATCAGTAAAGAAAGG	CCACCAACAACATTGGATGAAT	56.5	61.6
607	TC6E01	CTCCCTCGCTTCCTCTTTCT	ACGCATTAACCACACACCAA	55.3	59.4
608	TC01A02	GCAATTTGCACATTATCCGA	CATGTTCGGTTTCAAGTCTCAA	56.5	53.5
609	TC11A04	ACTCTGCATGGATGGCTACAG	CATGTTCGGTTTCAAGTCTCAA	56.5	59.8
610	TC5A06	TCGGTTTGGGAGACACTCTT	TTGTAAGCAGACGCCACATC	57.3	57.3
611	TC7C06	GGCAGGGGAATAAAACTACTAACT	TTTTCCTTCCTTCTCCTTTGTC	56.5	59.3
612	TC1D02	GATCCAAAATCTCGCCTTGA	GCTGCTCTGCACAACAAGAA	57.3	55.3

Appendix 4. List of 198 polymorphic SSR markers for NRCG 12568 × NRCG 12326

Sl. No.	Primer	Sl. No.	Primer	Sl. No.	Primer	Sl. No.	Primer	Sl. No.	Primer
1	RIZA06	43	AHS1750	85	AHS0101	127	GA24	169	S-06
2	GM1878	44	GNB377	86	GA72	128	GM1911	170	TC38D06
3	TC3G05	45	TC23F09	87	GNB712	129	GNB1078	171	GM1356
4	GM2547	46	GNB989	88	TC31H06	130	GNB1112	172	GM1577
5	GM2425	47	GNB1148	89	SEQ2E6	131	GNB983	173	PM183
6	PM346	48	AHGS0134	90	GM2206	132	GM2605	174	GM744
7	GM1477	49	GNB710	91	GNB850	133	S-21	175	AHS2037
8	GM1369	50	TC4G02	92	SEQ2G3	134	GM1357	176	GNB616
9	GM1897	51	GM1992	93	PM31	135	SEQ16F10	177	SEQ11H1
10	GM1902	52	SEQ11G7	94	TC3E05	136	GNB629	178	GNB387
11	GM2691	53	GNB608	95	S-57	137	GNB303A	179	GNB842
12	TC1D12	54	S-56	96	SEQ2C11	138	RI1F06	180	GNB643
13	GM1869	55	GM1861	97	GM2637	139	GM1411	181	GM1771
14	Ap-40	56	GNB206	98	TC1G04	140	S-80	182	GM2548
15	TC3G01	57	TC24C06	99	TC1D02	141	GM1575	183	PM436
16	TC9B07	58	TC31H02	100	SEQ3A6	142	GM1958	184	TC11A04
17	GM1899	59	GNB451	101	TC7H11	143	GM633	185	GM1986
18	GM1098	60	GM626	102	S-108	144	GNB320	186	SEQ2D12B
19	GM1752	61	GNB840	103	GM1996	145	AHGS0138	187	GNB167
20	S-16	62	SEQ11G3	104	SEQ15C12	146	AHGS0369	188	IAPHM93
21	GM1533	63	PM434	105	GI832	147	GM2032	189	SEQ11E11
22	S-106	64	GM1202	106	GM2638	148	GM1089	190	GM761
23	GM1991	65	GM632	107	GNB555	149	GNB58	191	SEQ12E10
24	GNB76	66	S-66	108	IAPHM290	150	GNB241	192	GM2165
25	GNB967	67	S-83	109	S-107	151	GA169	193	SEQ5D5
26	TC27H12	68	GNB533	110	SEQ17E3	152	S-22	194	PM137
27	TC38A07	69	AHGS0590	111	GM1565	153	SEQ13E6A	195	GM2589
28	TC34E12	70	GNB418	112	GM1773	154	GM1489	196	TC41A11.1
29	AHGS093	71	TC31H03	113	IAPHM23	155	GM2024	197	S-06
30	GNB216	72	GNB374	114	SEQ2H8	156	GM1790	198	GM1902
31	GNB652	73	SEQ4F1	115	S-93	157	IAPHM531		
32	GM1052	74	GM2746	116	TC7A02	158	GM1760		
33	SEQ9H8	75	SEQ10D4	117	TC4H02	159	IAPHM282		
34	GM1097	76	GM1955	118	IAPHM356	160	SEQ18E7		
35	GM1742	77	GNB831	119	GM1515	161	GA87		
36	IAPHM689	78	SEQ3A8	120	GM2053	162	GM1009		
37	GA156	79	GM1846	121	GM1954	163	GNB178		
38	GM1376	80	GNB1121	122	S-59	164	SEQ2B9		
39	S-70	81	GNB99	123	GNB148	165	GA57		
40	GM1469	82	GNB138	124	PM419	166	GM2313		
41	SEQ14F4	83	TC41C1	125	S-88	167	GM1611		
42	S-116	84	GM1076	126	RN19A01	168	Lec-1		

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Genetic Variability for Physiological Traits and Pod Yield in Groundnut (*Arachis hypogaea* L.) RILs under different Water Stress Conditions

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ABSTRACT

Groundnut is grown under non-irrigated conditions in most of the areas and is vulnerable to the effects of seasonal drought. Methods for efficiently evaluating drought tolerance and understanding the inherent principles of traits related to drought tolerance are critical for the success of groundnut breeding programme that aim to improve it. Therefore, the objective of the research is to study the extent of genetic variability for physiological traits under different water regimes. A mapping population of 147 recombinant inbred lines (RILs) resulting from the cross of NRCG 12568 × NRCG 12326 were evaluated in four different stress conditions *viz.,* well watered (WS), water stress-I (WS1) -with holding irrigation from 30-45 DAS (flowering period), water stress-II (WS2) -with holding irrigation from 45-65 DAS (flowering and peg initiation stage), water stress-III (WS3) -with holding irrigation from 65-85 DAS (peg penetration and pod development stage) for drought tolerance over two years at University of Agricultural Sciences, Bangalore during summer 2017 and 2018 in augmented design. Data was recorded on physiological traits namely specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) before and after stress imposition at different water regimes along with well watered condition simultaneously. Low PCV and GCV were recorded by all the physiological traits measured at all stages across the seasons indicating low genetic variability in the population and difference between PCV and GCV for these traits indicated influence of environment on these traits during both the seasons. SCMR at 65-85 DAS and SLA at 30-45 DAS as well as at 45-65 DAS had moderate to high heritability and genetic advance as per cent of mean, indicating that selection for drought tolerance based on SCMR could be effective at later stages of intermittent stress. While based on SLA, selection could be effective only at the early stages of crop development.

Keywords: Groundnut, SCMR, SLA, Genetic variability, Water stress

GROUNDNUT (*Arachis hypogaea* L.) is an important oilseed crop for small holding farmer community of the semi-arid tropics of Asia and Sub-Saharan Africa. Drought is one of the main limiting factors for groundnut yield, especially during (1) germination and seedling stages, which determine plant survival and health and (2) pod development and maturity stages, which affect the final production. Further more, drought may easily aggravate *Aspergillus flavus* contamination, as well as infection with other diseases and insect pests. Therefore, it is vital to understand the mechanisms of drought tolerance in peanut and identifying drought tolerant lines.

Two-thirds of the global production occurs in rainfed regions of the semi-arid tropics where rainfall is generally erratic and insufficient, causing unpredictable drought stress, the most important constraint for groundnut production. Insufficient moisture at some point in the growing season is often the limiting factor in the production of a peanut crop. Additionally, the effects of drought can be economically devastating when it occurs at critical growth stages *i.e.,* during peg penetration and pod development stage. Water uptake is crucial during key stages like flowering and kernel filling at these stages can bring large yield benefits in groundnut.

Being mostly a rainy season crop, groundnut is exposed to intermittent water stress during gaps in rainfall, or at terminal water stress *i.e.,* at the end of the season when the rains are over. In these situations of water limitation, the strategy so far proposed to improve groundnut tolerance to drought is to identify lines with high water use efficiency.

In this context, WUE is defined as the ratio of dry matter production to water use, which provides a means to compare the variation among genotypes in their ability to produce dry matter in water-limiting conditions and thus to increase yield. Because groundnut production is habitually affected by drought, elevation of water use efficiency (WUE) is crucial to cope up with drought conditions. Soil plant analytical development (SPAD) chlorophyll meter reading (SCMR) and specific leaf area (SLA) are amongst easily assessable surrogates of WUE that can be used in breeding and selection schemes in crop plants. Thus, genetic variability is the pre-requisite for any successful breeding programme as the degree of response to selection depends on the quantum of variability. In the present study, genetic variability and heritability and genetic advance were studied for physiological traits and pod yield under different water regimes.

MATERIAL AND METHODS

The experimental material for the present study consisted of 147 recombinant inbred lines (RILs) derived from the cross NRCG 12568 × NRCG 12326. The two parents were contrasting for $\Delta^{13}C$ (delta C13), SCMR and SLA. NRCG 12568 has low $\Delta^{13}C$ (16.90) and NRCG 12326 has high $\Delta^{13}C$ (21.50) while SCMR and SLA were negatively correlated. The first season field evaluation of 147 (F_{11}) RILs was carried out during summer 2017 in augmented design along with parents and checks *viz.*, GKVK 5 and TMV 2 at K-Block, Department of Genetics and Plant Breeding, UAS, GKVK, Bengaluru under three water regimes *i.e.,*well-watered (WW), water stress-II (WS2) -with holding irrigation from 45-65 DAS (flowering and peg initiation stage), water stress-III (WS3) -with holding irrigation from 65-85 DAS (peg penetration and pod development stage) to evaluate the RILs for physiological traits *viz.,* SCMR and SLA. The second season field evaluation of RILs was repeated with an addition of stress period at 30-45 DAS (flowering period) during summer 2018 under both well watered and water stress conditions.

All the lines were sown in one row of 2 m length with a spacing of 0.3 m between rows and 0.1 m between the plants. The recommended package of practices was followed for raising a good crop. All the four experiments including well watered plot and water stress plots under different water regimes were maintained separately. Imposition of stress was initiated only at specific periods in water stress plots *i.e.,* during WS1 (30-45 DAS), WS2 (45-65 DAS) and WS3 (65-85 DAS) while, irrigation was supplied to the WW plot at 7-10 days interval.

The SPAD chlorophyll meter (Minolta SPAD-502 m, Tokyo, Japan) reading was recorded on each leaflet of the tetra foliate leaf of five selected plants along the midrib. While recording the SCMR care was taken to ensure that the SPAD meter sensor fully covers the leaf lamina and that interference from veins and midribs was avoided. The unit value measured by the chlorophyll meter is termed as SCMR which provides information on the relative amount of leaf chlorophyll.

For recording SLA, fully expanded third leaf from the main axis was collected to record the leaf area by using leaf area meter. Leaves were dried in an oven at 80 °C for at least 48 hours to determine the leaf dry weight. Immediately after drying, the leaves were weighed and the SLA was derived as leaf area per unit leaf dry weight ($cm^2 \; g^{-1}$). Observations on SCMR and SLA were simultaneously recorded in the well watered and water stress plots before and after imposition of stress. The SLA was calculated using the following formula (Evans, 1972).

$$\text{Specific Leaf Area (SLA)} = \frac{\text{Leaf area (cm}^2)}{\text{Leaf dry weight (g)}}$$

Statistical Analysis

Statistical analyses of the data were carriedout according to augmented design. Analysis of variance was carried out as per the method suggested by Yates (1937). The genotypic and phenotypic coefficient of variation (Burton and Devane, 1953), heritability in broad sense (Hanson *et al.,* 1956) and genetic advance as per cent of mean (Johnson *et al.,* 1955) were computed. The data was analyzed by Windowstat 8.5 *ver.* Software.

RESULTS AND DISCUSSION

Pooled analysis of variance carried out for two season data under different water stress conditions (Table 1) indicated significant differences among the RILs, season and also their interactions for most of the traits indicating the presence of genetic variability for all the traits studied. Mean and range of physiological traits and pod yield per plant for WW and WS conditions during the two seasons of summer 2017 and 2018 are presented in Table 2. In RILs the mean pod yield per plant over both the seasons under WW and WS were not on par, which might be because of the environmental interaction over both the seasons.

TABLE 1

Pooled analysis of variance for physiological traits and pod yield of RILs derived from NRCG 12568 × NRCG12326 in groundnut over two seasons of summer 2017 and 2018

Source	DF	DF	SCMR		SLA		Pod yield / plant (g)
			BIS	BRS	BIS	BRS	
Blocks (eliminating checks + RILs)	WW	6	1.66	7.07 *	1382.17 ***	854.96	26.45 **
	WS1		2.38 *	3.14 *	150.73	382.51 *	39.46
	WS2		0.90	8.23 **	255.86 *	323.13 **	33.05 *
	WS3		2.56	5.82	867.60 ***	281.37 *	31.16 **
RILs + Check	WW	150	35.72 **	7.22	361.77 *	515.05	17.21 *
	WS1		10.93 *	10.77	211.51	496.45	19.07 *
	WS2		5.66	6.43	556.68 **	906.95	20.04 *
	WS3		6.46 **	25.20 ***	354.68 **	426.13 **	13.97
Checks	WW	3	5.99	10.55	138.56	1661.21 *	17.23 ***
	WS1		5.40	16.78 **	102.34 **	1104.36 *	16.21
	WS2		1.80 **	18.76	194.91	262.95 *	42.96 *
	WS3		2.31	4.06	566.53 *	779.64 **	19.40 **
RILs	WW	146	6.35 **	7.11	367.65	489.09	16.83
	WS1		6.20	5.62	214.81 *	487.74	17.98
	WS2		5.74 **	6.17	566.53 ***	860.34	18.87 *
	WS3		6.56	25.67 ***	342.50 **	390.71 *	13.86
Checks *vs.* RILs	WW	1	33.09 *	13.28	173.05 *	867.14	73.00 **
	WS1		15.40	14.98 *	56.19	329.73 **	17.98
	WS2		5.81 **	7.08	605.80 **	255.25	21.44 **
	WS3		3.34 *	18.72 **	149.97 **	153.82 **	13.65 **
Error	WW	18	4.30	5.78	126.07	146.08	24.80
	WS1		5.94	10.92	125.06	73.29	39.01
	WS2		2.95	4.81	83.27	90.37	9.37
	WS3		4.76	3.79	73.38	60.31	15.74

Note: * Significant at 0.05 probability level, ** Significant at 0.01 probability level, *** Significant at 0.001 probability level
BIS - Before initiation of stress, BRS - Before release of stress, Results for WS1 is not pooled data

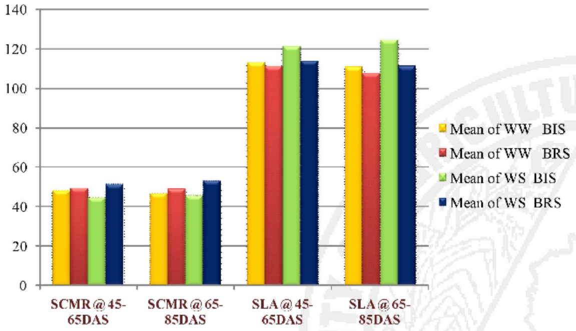
TABLE 2

Mean and range for physiological traits and pod yield per plant in RILs of NRCG 12568 × NRCG 12326 in groundnut under WW and WS conditions during summer 2017 and 2018

Character	Mean of WW				Mean of WS				Range of WW				Range of WS			
	S-2017		S-2018		S-2017		S-2018		S-2017		S-2018		S-2017		S-2018	
	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS
SCMR @ 30-45DAS	-	-	48.2	49.0	-	-	42.0	47.9	-	-	40.82-53.25	37.73-182.10	-	-	35.75 49.25	31.47-60.43
SCMR @ 45-65DAS	46.9	48.2	49.0	50.4	40.5	42.0	48.2	38.7	39.60-55.75	40.82-53.25	37.73-72.10	36.27-60.23	33.70-50.25	35.75-49.25	33.27-57.30	31.55-47.20
SCMR @ 65-85 DAS	45.1	46.6	48.2	51.3	38.7	40.3	52.1	53.7	37.15-58.50	31.55-47.20	40.82-53.25	33.10-62.70	31.55-47.20	34.23-46.98	37.60-62.40	43.63-166.83
SLA @ 30-45DAS	-	-	43.0	78.2	-	-	51.9	97.6	-	-	20.61-148.55	50.14-224.11	-	-	32.47 116.11	38.18-158.91
SLA @ 45-65 DAS	106.8	107.5	111.1	108.4	106.1	106.8	101.2	145.0	69.36-115.91	59.07-110.43	50.14-141.21	46.37-190.64	69.36-115.91	59.07-110.43	20.11-125.73	47.69-152.22
SLA @ 65-85 DAS	113.5	114.2	71.9	107.4	112.8	113.5	129.4	132.5	73.71-129.46	62.78-123.65	36.91-93.50	36.90-134.23	67.81-175.05	74.52-195.86	53-131.62	70.47-172.90
Pod yield per plant	14.1		10.6		WS2-12.97 WS3-10.13		WS1-9.09 WS2-10.09 WS3-6.94		5.58		1.30 36.20		WS2-2.78-36.18 WS3-2.30-33.00		SW1-1.20-26.20 WS2-2.00-30.80 WS3-1.5-20.30	

Note: " 66 " indicates no water stress experiment was taken up at 30-45 DAS during summer 2017

Pod yield per plant and SLA showed relatively high range. This indicating that these traits can be improved through individual plant selection. In the present study, the relative chlorophyll content of leaves was found lower in the initial stages of leaf development and increased as the dry matter accumulates and leaf matures. SCMR of the RILs under WS condition was higher compared to WW condition as indicated through mean at different stages of stress (Fig. 1). The chlorophyll density decides the photo synthetically active light-transmittance features of the leaf which is measured by the SCMR. Significant positive correlation between SCMR and chlorophyll density indicates that high SCMR value may have a higher photosynthetic activity per unit area. A strong negative relationship between WUE and SLA has been reported in several peanut studies, indicating that the genotypes with thicker leaves have greater WUE and leading to the conclusion that SLA can be used as a fast, relatively inexpensive technique for identifying and selecting genotypes with high WUE (Kalariya *et al*., 2015 and Mashamba *et al*., 2016). Water stress also decreased the SLA but increased SCMR.



Note : WW - Well watered, WS - Water stress, BIS - Before initiation of stress, BRS - Before releasing of stress

Fig. 1 : Mean of physiological traits in 147 RILs of NRCG 12568 x NRCG 12326 in groundnut under WW and WS conditions over two seasons

The estimates of mean performance, range, PCV (%) and GCV (%) heritability and genetic advance as per cent mean (GAM) of various characters are given in the Table 3. Lower difference between phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) indicates less influence of environment on expression of all the traits studied. Accordingly, Low PCV and GCV were recorded by the physiological traits measured while high PCV and GCV for pod yield per plant were recorded at all stages during the seasons (summer 2017 and 2018). The low genetic variation in the population and difference between PCV and GCV for SCMR and SLA indicated less prevalence of environment on these traits across the seasons. The similar results of significant genotypic

TABLE 3

Genetic parameters for physiological traits and pod yield in the RILs of NRCG 12568 × NRCG 12326 in groundnut during summer 2017 and 2018

Character	PCV (%)		GCV (%)		h^2 (broad sense)		GAM	
	S-2017	S-2018	S-2017	S-2018	S-2017	S-2018	S-2017	S-2018
SCMR @ 30-45 DAS	-	5.10	-	3.71	-	54.18	-	5.57
SCMR @ 45-65 DAS	4.29	5.10	3.67	3.71	74.81	54.42	6.46	5.57
SCMR @ 65-85 DAS	4.18	8.62	3.13	6.17	87.39	82.42	12.82	19.09
SLA @ 30-45 DAS	-	10.94	-	10.79	-	96.66	-	21.92
SLA @ 45-65 DAS	5.18	10.85	3.98	10.37	60.57	93.41	16.29	20.40
SLA @ 65-85 DAS	9.93	7.72	3.74	5.48	14.51	51.59	2.90	8.01
Pod yield^{-1} WW	34.12	44.94	24.19	44.23	44.70	28.20	36.87	45.76
Pod yield^{-1} WS1	-	48.55	-	45.57	-	88.14	-	35.87
Pod yield^{-1} WS2	48.23	47.76	25.55	27.71	58.80	53.68	29.65	31.67
Pod yield^{-1} WS3	52.42	56.04	50.02	41.25	93.82	54.18	48.65	36.88

Note: "-" indicates no water stress experiment was taken up at 30-45 DAS during summer 2017 variation for the traits related to WUE was reported in numerous reports depending upon the material used for their study by Mashamba *et al.* (2016), Srivalli *et al.* (2016) and Bhavya *et al.* (2017).

Higher estimates of heritability and low GAM for SCMR at 30-45 DAS was observed during summer 2018. High heritability and low GAM were recorded for SCMR at 45-65 DAS across the seasons. High heritability and moderate GAM were recorded for SCMR at 65-85 DAS indicating less influence of environment and wider scope for selection of this trait. Selection for drought tolerance based on SCMR could be effective only at later stages of stress imposition compared to the early stages. Similar kind of results of high heritability and moderate GAM was reported for SCMR by John *et al.* (2011). Srivalli *et al.* (2016) and Mashamba *et al.* (2016).

High heritability and moderate to high GAM was observed during summer 2018 for SLA at 30-45 DAS as well as at 45-65 DAS indicating the additive gene effects and selection for this trait could be effective in the early stages. The results were in accordance with the reports of high heritability and high GAM for SLA by Mahesh and Hasan (2019) and Savitha (2012). Low to moderate heritability along with low GAM were recorded for SLA at 65-85 DAS during summer 2017 and 2018 indicating the influence of environmental effects on the expression of SLA at this stage. Similar results of moderate $h^2_{(b)}$ and low GAM was reported by John *et al.* (2011). The heritability and GAM for pod yield per plant were moderate to high in well watered and water stress conditions indicating the prevalence for selection. Differential heritability and GAM estimates for physiological traits and pod yield in the present study are because of varied stages and duration of drought stress imposition. The mechanisms of physiological responses to early season drought might be different from those for long duration drought, which have been reported previously (Mashamba *et al.,* 2016). Kalariya *et al.* (2015) reported that broad sense heritability of drought tolerance traits varied among groundnut crosses, traits and stages depending on levels of genetic variation for the trait and stage of imposition of drought stress.

In the study, SLA with high heritability, moderate GAM under well-watered and water stress at 30-45 DAS indicated that SLA can be potentially used as selection tool as it is least influenced by the environment. SCMR at 65-85 DAS under well watered and water stress conditions showed high heritability, moderate GAM and hence, SCMR could be used as selection criteria along with other yield parameters for identifying superior genotypes for water stress. Thus, it is suggested to record SCMR at 65-85 DAS as a rapid technique for screening a large number of stable peanut breeding materials for water stress tolerance. This technique will help breeders to identify probable superior breeding materials based on SCMR before harvest which could be confirmed later based on pod yield under water stress conditions.

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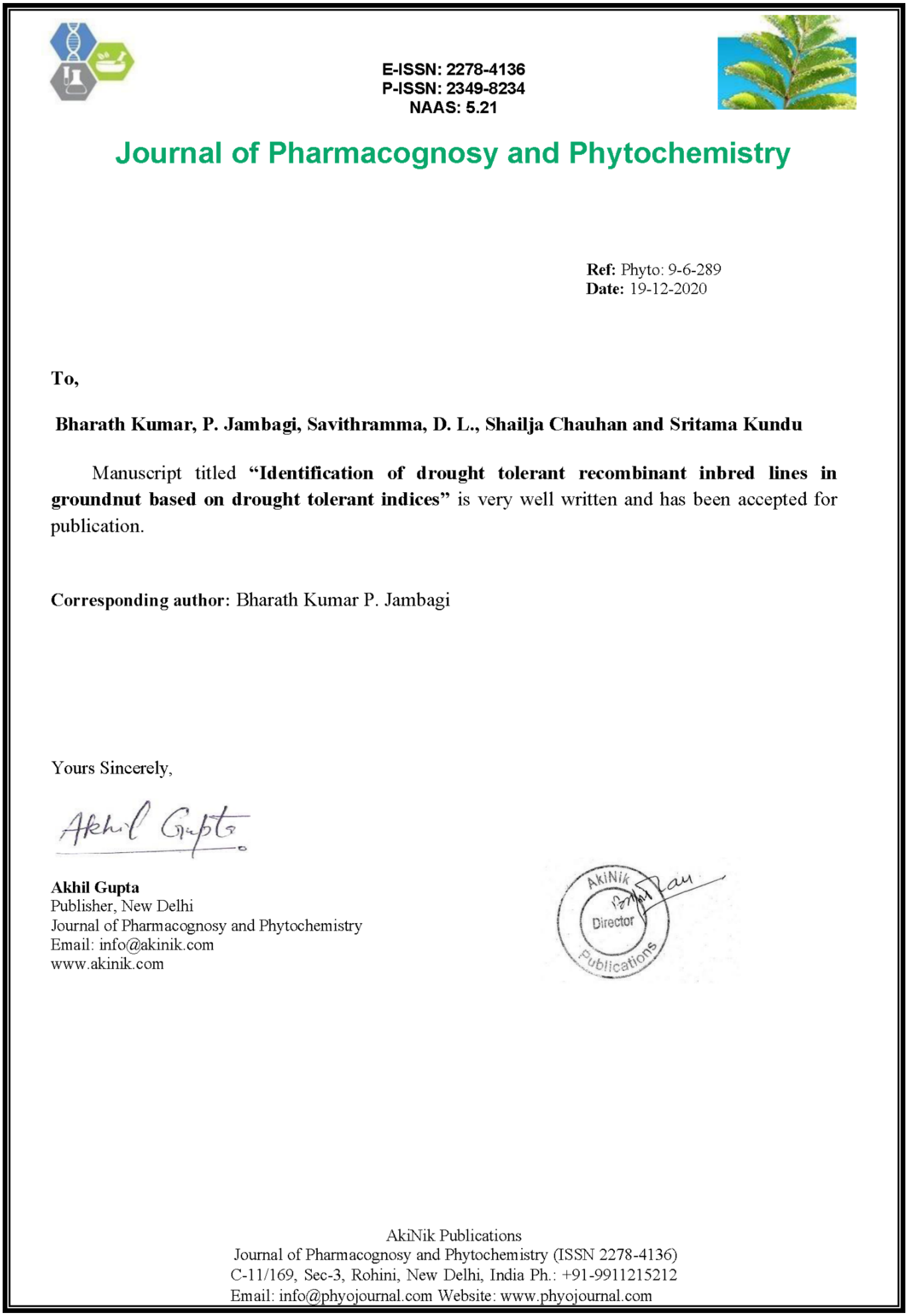

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To,

Bharath Kumar, P. Jambagi, Savithramma, D. L., Shailja Chauhan and Sritama Kundu

Manuscript titled **"Identification of drought tolerant recombinant inbred lines in groundnut based on drought tolerant indices"** is very well written and has been accepted for publication.

Corresponding author: Bharath Kumar P. Jambagi

Yours Sincerely,

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Identification of drought tolerant recombinant inbred lines in groundnut based on drought tolerant indices

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Abstract

The improvement for drought tolerance requires reliable assessment of variability for drought tolerance among genotypes. In order to assess drought stress in groundnut, a mapping population of 147 recombinant inbred lines (RILs) derived from the cross NRCG 12568 × NRCG 12326 were evaluated under moisture stress and non-stress field environments. The stress was imposed by withholding irrigation from 30-45 DAS (flowering and peg initiation stage) at University of Agricultural Sciences, Bengaluru, India during summer 2018 in augmented design along with parents and checks (GKVK 5 and TMV 2) to investigate relationships and repeatability among fifteen drought tolerance indices. Based on the criteria of both correlation and discriminating ability of the indices, only four indices namely stress tolerant index (STI), mean productivity (MP), harmonic mean productivity (HMP) and geometric mean productivity (GMP) showed highly significant and positive correlation with pod yield under well-watered and water stress conditions. Therefore, these indices assist in screening of RILs for performance under various soil moisture levels. Principal component analysis (PCA) indicated that first and second components justified 85.60% of variations among drought tolerance criteria under water stress. The results of PCA were interpreted through biplot analysis and revealed STI, GMP, MP, and HMP are effective for identifying drought tolerant genotypes with high pod yield. Based on rank sum method, involving the four indices distinguished RIL 541, RIL 245, RIL 158 and RIL 175 as the most drought tolerant RILs. The identified lines can be evaluated in varied rainfed environments to exploit their drought tolerance and yield potential. The validated lines can be utilized as improved cultivars for rainfed or drought prone environments under changing climate.

Keywords: Moisture stress, Drought tolerant indices, Principal component analysis,

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Introduction

Breeding for drought tolerance is cumbersome due to lack of fast, reproducible screening techniques and the inability to routinely create defined and repeatable water stress conditions where large populations can be evaluated efficiently (Ramirez and Kelly, 1998). Although a large number of different traits during vegetative and reproductive growth phases have been employed to characterize the physiological and genetic basis of drought tolerance, it is still difficult to identify drought tolerant genotypes (Foolad, 2005). It is of great importance to choose the selection criteria applied to distinguish desirable genotypes. Drought tolerance indices calculated on the basis of plant performance (in terms of yield, dry matter yield, and/or other quantitative traits) in stress and non-stress environments, to assess the response under limited irrigation. However, numerous indices that have been proposed by different authors for selection by classifying the genotypes in different manners.

Loss of yield is the main concern of plant breeders and hence emphasize on yield performance under moisture stress conditions. But variation in yield potential could arise from factors related to adaptation rather than to drought tolerance *per se*. Thus, drought indices which provide a measure of drought tolerance based on loss of yield under drought-conditions in comparison to normal conditions have been used for screening drought-tolerant genotypes (Mitra, 2001). These indices are either based on drought tolerance or susceptibility of genotypes (Fernandez, 1992). Drought susceptibility of a genotype is often measured as a function of the reduction in yield under drought stress (Blum, 1988) whilst the values are confounded with differential yield potential of genotypes (Ramirez and Kelly, 1998).

Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the differences in yield between the stress (Ys) and non-stress (Yp) environments and mean productivity (MP) as the average yield of Ys and Yp. Fischer and Maurer (1978) proposed a stress susceptibility index (SSI) of the cultivar. Fernandez (1992) defined a new advanced index (STI= stress tolerance index), which can be used to identify genotypes that produce high yield under both stress and non-stress conditions. Other yield based estimates of drought resistance are geometric mean (GM), mean productivity (MP) and TOL. The geometric mean is often used by breeders interested in relative performance since drought stress can vary in severity in field environment over years (Ramirez and Kelly, 1998).

The optimal selection criterion should distinguish genotypes that express uniform superiority in both stress and non-stress environments from the genotypes that are favorable only in one environment. Among the stress tolerance indicators, a larger value of TOL and SSI represent relatively more sensitivity to stress, thus a smaller value of TOL and SSI are favored. Selection based on these two criteria favors genotypes with low yield potential under non-stress conditions and high yield under stress conditions. On the other hand, selection based on STI and GMP will be resulted in genotypes with higher stress tolerance and yield potential will be selected (Fernandez, 1992).

The main task of plant breeder is to understand the association of indices with drought tolerance in cultivars under environmental stress conditions for exploiting the genetic variations to improve the stress tolerant cultivars. Several researchers across the crop groups have used these indices to quantify the responses and degree of tolerance of the genotypes to abiotic stresses. To quote a few, Safavi *et al.* (2015) in sunflower, Uday *et al.* (2016) in chickpea, and Bennani *et al.* (2016) and Bennani *et al.* (2017) in bread wheat have used different combinations of the indices to quantify the degree of tolerance of genotypes to abiotic stress. To differentiate the degree of drought tolerance between different genotypes, several drought tolerant indices (DTIs) have been suggested. The present study attempted to assess the selection criteria for identifying drought tolerant RILs using fifteen drought tolerant indices based on pod yield plant per plant under water stress and non-stress field conditions.

Material and methods

The experiment was conducted at University of Agricultural Sciences, Bangalore, in medium red sandy loam soil. The material comprised of 147 RILs derived from the cross NRCG 12568 × NRCG 12326 along with two check varieties (GKVK 5 and TMV 2). The experiment was taken up in Augmented design (Federer, 1956) during *summer* 2018, under control and stress condition. Water stress was imposed at 30 days after sowing (DAS) to all the RILs by withholding irrigation in stress plot for fifteen days while the control plot was given regular irrigation twice a week. To understand the responsiveness of the RILs in water stress at flowering to 50 *per cent* flowering period. Harvesting was carried out at about 120 DAS. The maturity of pods was assessed by the *per cent* blackening of the internal inner parenchyma (Miller and Burns, 1971). Comparison of RILs before and after release of stress in normal and water stress condition are shown in Fig 1.

Five randomly chosen plants in each RIL were labeled and used for recording pod yield plant^{-1}, yield related, physiological and agronomical traits. Fifteen drought tolerant indices, *viz*., tolerance index (TOL), mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HAM), stress tolerance index (STI), relative drought index (RDI), abiotic tolerance index (ATI), stress susceptibility percentage index (SSPI), stress non-stress production index (SNPI), yield index (YI), yield stability index (YSI), modified stress tolerance index calculated by pod yield plant^{-1} under normal condition (K_1STI), modified stress tolerance index calculated by pod yield plant^{-1} under stress condition (K_2STI), drought resistance index (DI) and stress susceptibility index (SSI) were calculated based on pod yield plant per plant under stress (Y_S) and control (Y_P) condition to screen for drought tolerance.

Correlation analysis was performed by SPSS *ver*. 16. Principal component analysis, based on the rank correlation matrix and biplot analysis were computed using computer software Microsoft Excel along with XLSTAT 2012, Copyright Addinsoft 1995-2012 (http:/ /www.xlstat.com) as followed by Iqbal *et al.* (2014). Based on the value of each DTI of all the groundnut RILs, a rank for each RIL was classified and rank sum (RS) was calculated [RS= Rank mean (Rm) + Standard deviation of rank (SDR) as conducted by Farshadfar *et al.* (2012)]. The above mentioned procedure was implemented to identify best tolerant genotypes.

Results and Discussion

Improvement in groundnut for drought tolerance requires reliable assessment of drought tolerance variability among genotypes/RILs. Water stress during crop growth period at certain interval considerably affects pod yield of the RILs. The RILs varied widely for pod yield plant per plant under moisture stress relative to that under well watered condition (Fig.1). However, pod yield in water stress differentiated RIL 245 and RIL 439, as they out-performed even during early stages of the crop growth in comparison with the performance of the same RIL in well watered conditions. Higher magnitude of result was expected for pod yield per plant against RILs in well watered conditions. Few RILs in the population performed better even under stress conditions, while few couldn’t cope up with the stress limits. This genic action is majorly due to exposure of the RILs to intermittent moisture stress. The relative adaptability of RILs reflects their degree of moisture stress tolerance.

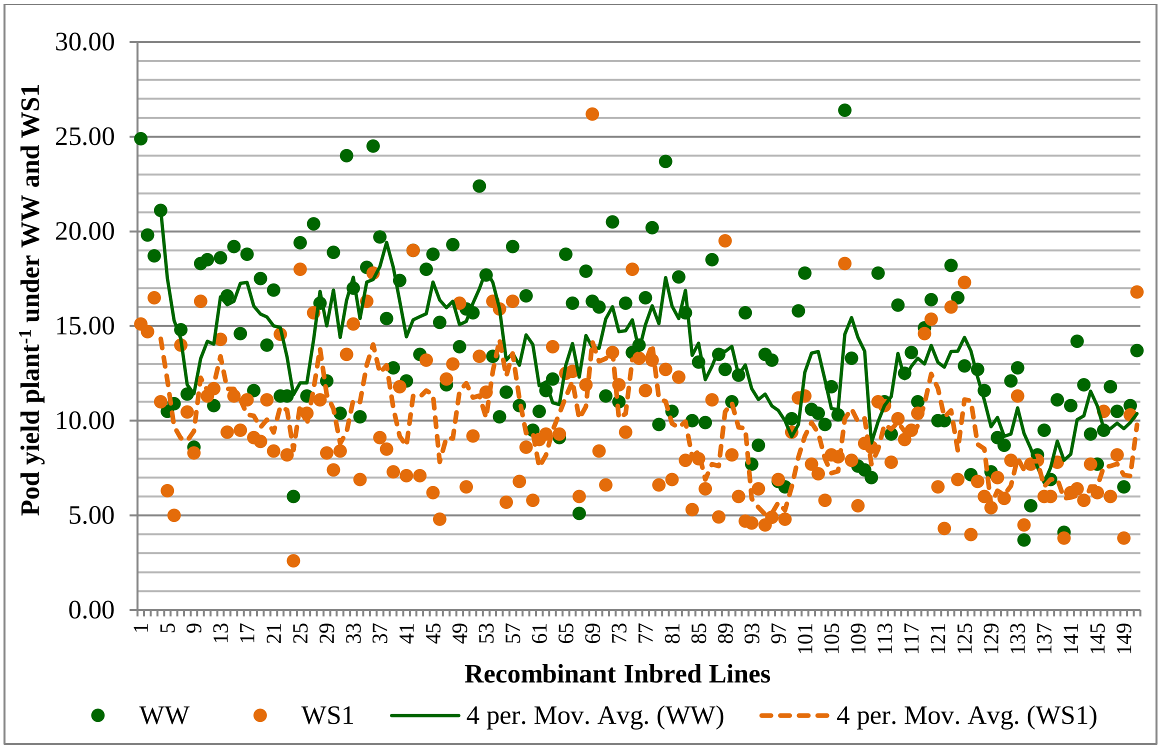

Fig 1. Responses of the RILs for pod yield plant^{-1} under well watered and water stress condition in groundnut during summer 2018

Loss of yield is the main concern of plant breeders and they hence emphasize on yield performance under stress conditions. In the absence of special mechanisms to quantify the tolerance, the quantification of drought tolerance should be based on yield in both stress and non-stress environments, that can lead to selection of high yielding genotypes under stress condition. Since, the response of selection under non-stress condition is maximal and heritability of the yield under these conditions is high (Shirinzadeh *et al.*, 2010 and Geravandi *et al.*, 2011). Thus, drought indices which provide a measure of drought based on yield loss under drought conditions in comparison to normal conditions have been used for screening drought-tolerant genotypes (Mitra, 2001). These indices are based on drought resistance or susceptibility of genotypes (Fernandez, 1992).

Good discrimination of genotypes by the DTI's helps in effective selection of most desirable RILs for moisture stress environment. The indices that discriminate the RILs for responses to moisture stress environment assume importance in this context. In the present study, only eight of the 15 indices namely TOL, STI, ATI, SSPI, K_1STI, K_2STI, SNPI and SSI showed better ability to discriminate the RILs for their responses to moisture stress environments than other indices as indicated from high magnitude of estimates in both standardized range (SR) and Phenotypic coefficient of variation (PCV) under water stress (Table 1). It is desirable to preferentially use these indices for screening the genotypes for responses to moisture stress environment in groundnut. Bennani *et al.* (2016) and Bennani *et al.* (2017) have also suggested one of these five indices, *i.e.*, STI for discriminating bread wheat genotypes for their responses to drought stress. However, they used significant mean squares attributable to indices as criteria to identify those with better discriminating ability.

Table 1. Estimates of measures of variability for various drought tolerant indices based on pod yield plant^{-1} under moisture stress environment

Indices	Pod yield plant^{-1} (g)	
	Standardized Range	PCV (%)
Tolerance index (TOL)	3.81	62.30
Mean productivity (MP)	1.80	32.72
Geometric mean productivity (GMP)	1.88	33.61
Harmonic mean productivity (HMP)	2.07	35.52
Stress tolerance indices (STI)	3.42	70.09
Relative drought index (RDI)	1.75	78.37
Abiotic tolerance index (ATI)	3.03	78.58
Stress susceptibility percentage index (SSPI)	3.81	62.30
Yield index (YI)	2.62	44.90
Yield stability index (YSI)	1.75	78.37
Modified STI (K_1STI)	2.18	63.68
Modified STI (K_2STI)	2.26	70.09
Drought resistant index (DI)	1.78	64.43
Stress non-stress production index (SNPI)	11.61	58.43
Stress susceptibility index (SSI)	3.46	55.02

Correlation and Rank sum method

Suitable stress tolerant indices are indicators, which have relatively high significant positive correlation with yield in both non-stress and stressed condition (Kandalkar, 2014 and Subhani *et al.*, 2015). Correlation analysis between pod yield per plant and DTIs can be a good criterion for screening the best RILs and indices used. Y_S was significantly and positively correlated with MP, GMP, HMP, STI, RDI, YI, YSI,

K_2STI and DI and negatively correlated with TOL, ATI, SSPI, SNPI and SSI. Y_P was significant and positively correlated with all drought indices, except RDI, YSI and DI. The drought tolerant indices, MP, GMP, HMP and STI were significantly positively correlated with both Y_P and Y_S (Table 2) indicating that these indices are more effective in identifying high yielding RILs under different water regimes. Farshadfar and Elyasi (2012) reported that the most suitable indices for screening drought tolerant genotypes in bread wheat were GMP, MP, STI, K_1STI, K_2STI, YI, DRI (drought response index), DI, SNPI, RDI and YSI. Thi *et al*. (2015) and Abejide *et al.* (2017) also identified similar findings in tomato and bambara groundnut, respectively, that MP, GMP, HMP and STI are good indicators or parameters to be used for screening and selection of drought tolerant genotypes under well watered and water stress condition.

To determine the most desirable drought tolerant RIL, rank mean, SDR and RS for each RIL were calculated based on rank of three DTI's (MP, GMP and STI) which are correlated with pod yield under control and stress condition. The best tolerant genotypes were identified as having low rank mean, SDR and least in RS (Farshadfar *et al.*, 2012). The RILs with low rank values among all DTIs denoted as drought tolerant. In consideration to all indices, RIL 126, RIL 249, RIL 133, RIL 145, RIL 129, RIL 539, RIL 248, RIL 233, RIL 258 and RIL 139 recorded the lower rank mean and standard deviation of ranks in well watered and water stress condition, Hence rank sum method effectively combines all the three indices into one to select water stress tolerant RIL (Table 3). The same procedures have been used for screening quantitative indicators of drought tolerance in wheat (Mohammadi *et al*., 2012), groundnut (Savita, 2017; Eradasappa, 2018), tomato (Thi, 2016; Suresh, 2018) and Dolichos (Balaraju, 2018).

Principal component and biplot analysis

In order to further investigate the inter-relationships and repeatability among drought selection indices, as well as to distinguish tolerant RIL, principal component (PC) analysis was performed and corresponding biplot has been drawn which shows overview of inter-relationship among drought tolerant indices using XLSTAT *ver.* 2017. The relationships among different indices are graphically displayed in a biplot of PCA1 and PCA2 (Fig. 2). The PCA1 and PCA2 axes which justify 85.60 *per cent* of total variation, mainly distinguishes the indices in four groups. Of which, group 1 consisted of major indices MP, GMP, HMP, STI, YI and K_2STI are strongly correlated with yield under both conditions indicating that these criteria are suitable for identification of drought tolerant accessions.

Table 2. Correlation coefficients of the RIL population for drought tolerant indices and pod yield plant per plant under normal and moisture stress condition in groundnut

Variables	**TOL**	**MP**	**GMP**	**HMP**	**STI**	**RDI**	**ATI**	**SSPI**	**YI**	**YSI**	**K_1STI**	**K_2STI**	**DI**	**SNPI**	**SSI**	**Y_p**	**Ys**
TOL	1.00	0.10	0.02	-0.05	0.04	-0.78*	0.73*	0.98*	-0.63*	-0.78*	0.68*	-0.60*	-0.72*	0.53*	0.78*	0.71*	-0.63*
MP		1.00	0.97*	0.92*	0.95*	-0.02	-0.05	0.10	0.71*	-0.02	0.72*	0.69*	0.22*	-0.03	0.02	0.77*	0.71*
GMP			1.00	0.99*	0.97*	-0.05	0.01	0.02	0.75*	-0.05	0.61*	0.71*	0.16*	-0.04	0.05	0.70*	0.75*
HMP				1.00	0.95*	-0.07	0.05	-0.05	0.76*	-0.07	0.51*	0.70*	0.12	-0.05	0.07	0.62*	0.76*
STI					1.00	-0.05	0.01	0.04	0.72*	-0.05	0.64*	0.72*	0.16	-0.04	0.05	0.70*	0.72*
RDI						1.00	-0.88*	-0.78*	0.54*	0.96*	-0.36*	0.52*	0.93*	-0.39*	-0.90*	-0.51*	0.54*
ATI							1.00	0.73*	-0.56*	-0.88*	0.34*	-0.62*	-0.96*	0.43*	0.88*	0.43*	-0.56*
SSPI								1.00	-0.63*	-0.78*	0.68*	-0.60*	-0.72*	0.53*	0.78*	0.71*	-0.63*
YI									1.00	0.54*	0.08	0.96*	0.68*	-0.40*	-0.54*	0.10	0.90*
YSI										1.00	-0.36*	0.52*	0.93*	-0.39*	-0.90*	-0.51*	0.54*
K_1STI											1.00	0.09	-0.21*	0.27*	0.36*	0.94*	0.08
K_2STI												1.00	0.69*	-0.43*	-0.52*	0.10	0.96*
DI													1.00	-0.39*	-0.93*	-0.31*	0.68*
SNPI														1.00	0.39	0.31*	-0.40*
SSI															1.00	0.51*	-0.54*
Y_p																1.00	0.10
Y_s																	1.00

*Significance at P=0.05 Y_p- yield under non stress Y_s- Yield under stress

Table 3. **Schematic description of rank sum (RS) method to identify tolerant RILs in groundnut based on hypothetical magnitude of three indices**

RILs	MP	Rank	GMP	Rank	STI	Rank	Rank Mean (RM)	Standard deviation of ranks (SDR)	Rank sum (RS) (RM +SDR)
126	22.82	1	22.67	1	0.69	1	1.00	0.00	1.00
249	20.16	3	19.90	2	0.53	2	2.33	0.58	2.91
133	20.73	2	19.24	4	0.50	4	3.33	1.15	4.49
145	19.54	5	19.54	3	0.52	3	3.67	1.15	4.82
129	19.72	4	18.72	5	0.47	5	4.67	0.58	5.24
539	17.93	6	17.89	6	0.43	6	6.00	0.00	6.00
248	17.42	9	17.41	7	0.41	7	7.67	1.15	8.82
233	17.32	10	17.31	8	0.41	8	8.67	1.15	9.82
258	17.45	8	16.94	10	0.39	10	9.33	1.15	10.49
139	17.14	11	16.92	11	0.39	11	11.00	0.00	11.00
231	17.10	12	17.08	9	0.39	9	10.00	1.73	11.73
128	16.61	13	16.54	12	0.37	12	12.33	0.58	12.91
529	16.54	14	16.53	13	0.37	13	13.33	0.58	13.91
237	16.49	15	16.29	14	0.36	14	14.33	0.58	14.91
541	17.52	7	16.02	15	0.35	15	12.33	4.62	16.95
631	15.69	18	15.47	16	0.32	16	16.67	1.15	17.82
201	15.89	17	15.38	18	0.32	18	17.67	0.58	18.24
245	15.60	19	15.41	17	0.32	17	17.67	1.15	18.82
108	15.49	20	15.07	19	0.31	19	19.33	0.58	19.91

MP- Mean Productivity GMP- Geometric Mean Productivity STI- Stress Tolerance Index

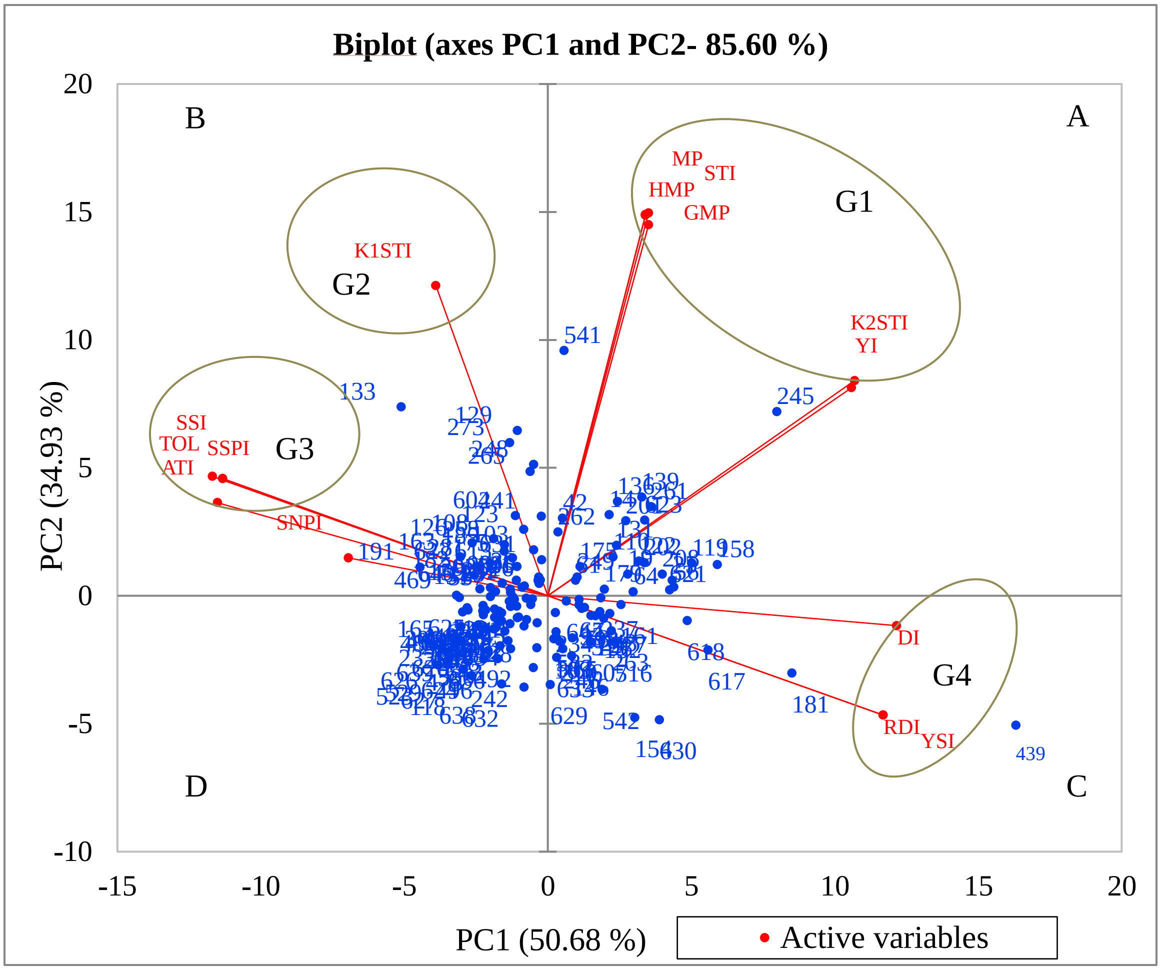

Fig 2. Biplot analysis based on first (PC1) and second (PC2) principal components

The numbers, *viz.*, 133,... indicating code of RILs as shown in Table 3; **G-group of DTIs**: G1, 2, 3 and 4= Group 1, 2, 3 and 4 respectively.

In general, indices in the same group distinguish drought tolerant accessions in the same manner. Farshadfar *et al.*, (2001) believe that the most suitable indices for selection of drought tolerant cultivars is an indicator which has relatively high correlation with grain yield in both stress and non-stress condition. Higher score of PC1 were in accordance with the higher rank of drought tolerance, whereas low score for PC2 showed drought sensitive genotypes. Therefore, first component was named as drought tolerant.

Relationship between RILs and tolerance to drought was used as a biplot for identification of drought tolerant RILs. The higher scores for PC1 and lower scores for PC2 were in accordance with the higher rank of drought tolerance. RILs were categorized into four groups (A, B, C and D) based on their performance in well-watered and water-stressed environments: Group A - accessions expressing uniform superiority in well-watered and water-stressed conditions, Group B - accessions that perform favourably only in well-watered conditions, Group C - accessions that gives relatively higher yield only in water-stressed condition and Group D - accessions perform poorly in both the conditions. Biplot analysis (Fig. 2) showed that RILs *viz*., RIL 541, RIL 245, RIL 126, RIL 139, RIL 262, RIL 131, RIL 42, RIL 529 and RIL 128 as most drought tolerant (Group A). Zare (2012) used same method for identification of drought tolerant genotypes in barley. Similarly in safflower by Bahrami *et al.* (2014), in Tomato by Thi (2016) and Suresh (2018) and in groundnut by Savita (2017). Principal component analysis provided grouping of RIL population that is similar to grouping on the basis of three- principal component analysis provided grouping of tomato populations that is similar to grouping on the basis of three- dimensional graph. Since the method allows simultaneous evaluation of the RILs and the interpretation of inter-relationships among the indices, it may be recommended as a method of choice for data analysis in further studies on drought tolerance in groundnut.

Conclusion

The groundnut population included in this study differed significantly in terms of pod yield plant per plant determined at the stage of intensive vegetative growth in conditions of optimal and limited irrigation, as well as in terms of the calculated drought tolerance indices. Grouping of RILs in terms of drought tolerance was similar when carried out via PCA which additionally allowed the interpretation of the relationships among the indices. In conclusion, we identified four drought tolerant indices *viz*., MP, GMP, HMP and STI positively significant correlated with pod yield planr^{-1}under stress and control condition indicating that these indices were more effective in identifying high yielding accessions under different irrigated conditions. Based on biplot diagram and ranking method calculated from suitable drought indices, RIL 541, RIL 245, RIL 158 and RIL 175 were identified as the most drought tolerant RILs which could be used for developing high yielding hybrids or developing pure line varieties. Further, evaluation of identified promising genotypes in varied rainfed environments is required to exploit the drought potential of these lines for climate smart agriculture.

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