IDENTIFICATION OF QTL GOVERNING WATER USE EFFICIENCY AND ROOT TRAITS IN A RECOMBINANT INBRED POPULATION OF GROUNDNUT

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In partial fulfillment of the requirements
For the award of the Degree of

MASTER OF SCIENCE (Agriculture)

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BANGALORE **JULY, 2013**

AFFECTIONATELY DEDICATED

to

My

Beloved Parents

&

Chairman

DEPARTMENT OF CROP PHYSIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
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CERTIFICATE

This is to certify that the thesis entitled **"IDENTIFICATION OF QTL GOVERNING WATER USE EFFICIENCY AND ROOT TRAITS IN A RECOMBINANT INBRED POPULATION OF GROUNDNUT"** submitted in partial fulfilment of the requirement for the degree of **MASTER OF SCIENCE (Agriculture)** in **CROP PHYSIOLOGY** to the University of Agricultural Sciences, Bangalore is a bonafide record of research work done by **Mr. PRINCE CHOYAL, ID. No. PALB 1188** 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, associate ship, fellowship or other similar titles.

Bangalore **(M. S. SHESHSHAYEE)**

July, 2013 (Major advisor)

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IDENTIFICATION OF QTL GOVERNING WUE AND ROOT TRAITS IN A RECOMBINANT INBRED POPULATION OF GROUNDNUT

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ABSTRACT

Cultivated groundnut (*Arachis hypogaea* L.), an important dietary energy source, is grown as rainfed crop in the semi- arid regions of the world. Water availability is hence the most important constraint. Water mining associated with roots and efficiency of water use for biomass production are considered as the most important physiological traits that when pyramided can boost productivity under rainfed conditions. Pyramiding complex traits requires the adoption of focused molecular breeding strategy. Toward identifying markers flanking QTL governing these traits, a mapping population comprising of 268 recombinant inbred lines developed by crossing TAG 24 and GPBD 4 were phenotyped for root traits, WUE and associated physiological traits. A significant variability in all these traits was observed.

Transposon based markers (5) were added to the existing 188 SSR marker map and a QTL analysis was done by both SMA and CIM strategies using QTL cartographer. Along with several makers linked, markers GM 1357 for root length and GM1717 and pPGSseq18A05b for root biomass were found to be associated by both SMA and CIM. Likewise Marker GM 2724b and GM 1573 were found to be associated for total leaf area by both strategies. Similarly, $QTL_{TDM\text{-}12\text{-}1}$ and $QTL_{TDM\text{-}9\text{-}1}$ were identified for total dry matter. In addition, five QTLs were identified for mineral ash content by CIM. These QTLs would be useful in breeding for improving drought tolerance in groundnut.

Student (PRINCE CHOYAL)

Chairperson (M.S.SHESHSHAYEE)

ಪ್ರಬಂದದ ಸಾರಾಂಶ

ಶೀರ್ಷಿಕೆ: ಬೇರುಗಳ ವೈವಿಧ್ಯತೆ ಹಾಗು ನೀರು ಬಳಕೆಯ ಸಾಮರ್ಥ್ಯತೆ ಗುಣಗಳಿಗೆ ಹೊಂದುವ ಕ್ಯು.ಟಿ.ಎಲ್. ಗಳ ಪತ್ತೆ ಮತ್ತು ವಿಮರ್ಷೆ

ನೆಲಗಡಲೆ ದೈನಂದಿನ ಬದುಕಿನಲ್ಲಿ ಬಳಸುವ ಪೌಷ್ಠಿಕ ಪದಾರ್ಥಗಳನ್ನು ಪೂರೈಸುವ ಒಂದು ಅತಿಮುಖ್ಯವಾದ ದ್ವಿದಳಧಾನ್ಯವಾಗಿದೆ. ಈ ಬೆಳೆಯನ್ನು ಹೆಚ್ಚಾಗಿ ಮಳೆಯಾಶ್ರಿತ ಪ್ರದೇಶಗಳಲ್ಲಿ ಬೆಳೆಯಲಾಗುತ್ತಿದ್ದು, ನಿತ್ಯವು ಬರ ಪರಿಸ್ಥಿತಿಗೆ ಒಳಗಾಗುತ್ತದೆ. ಇದರಿಂದ ಇಳುವರಿಯ ಮೇಲೆ ಹೆಚ್ಚು ಪರಿಣಾಮ ಬೀರುತ್ತದೆ. ನೀರಿನ ಅಭಾವವಿರುವ ಸಂದರ್ಭಗಳಲ್ಲಿ ಕೆಲವು ಮುಖ್ಯವಾದ ಸಸ್ಯ ಶರೀರ ಕ್ರಿಯೆಗಳಿಗೆ ಸಂಭಂಧಪಟ್ಟ ಗುಣಗಳ ಅಭಿವ್ಯೆಕ್ತಿಯನ್ನು ಹೆಚ್ಚಿಸಿದಲ್ಲಿ, ಇಳುವರಿಯನ್ನು ಕಾಪಾಡಿಕೊಳ್ಳಬಹುದೆಂದು ತಿಳಿದುಬಂದಿದೆ. ಭೋಮಿಯ ಆಳದಿಂದ ನೀರು ಬಗೆಯುವ ಬೆರುಗಳಿರಿವುದು ಮತ್ತು, ಹೀಗೆ ಸಂಪಾದಿಸಿದ ನೀರನ್ನು ಪ್ರಮಾಣಿಕವಾಗಿ ಬಳಸಿಕೊಳ್ಳುವ ಎಲೆಗಳಿರುವುದು ಅತಿ ಮುಖ್ಯವೆಂದು ಕಂಡುಬಂದಿದೆ. ಎಲೆಗಳ ಕೋಷಗಳಲ್ಲಿ ಉತ್ತಮವಾದ ಧಾರಣಾಶಕ್ತಿ ಇರುವುದು (cellular tolerance) ಅತಿ ಮುಖ್ಯ. ಈ ಕಠಿಣವಾದ ಗುಣಗಳನ್ನು ಒಟ್ಟುಗೋಡಿಸಲು ಆಧುನಿಕ ಮೊಲಿಕ್ಯುಲಾರ್ ತಳಿ ಅಭಿವೃದ್ಧಿ ತಂತ್ರಗಳನ್ನು ಬಳಸಬೇಕಾಗುತ್ತದೆ.

ಈ ನಿಟ್ಟಿನಲ್ಲಿ ಬರನಿರೋಧಕ ಶರೀರ ಕ್ರಿಯೆಗಳನ್ನು ಡಿ.ಎನ್.ಏ ಗುರುತುಕಾರಕಗಳೊಂದಿಗೆ ಬೆಸೆದು, ಅನಂತರ ಕೇವಲ ಗುರುತುಕಾರಕಗಳನ್ನು ಬಳಸಿ, ತಳಿ ಅಭಿವೃದ್ಧಿಪಡಿಸುವುದು ಉತ್ತಮವಾದ ತಂತ್ರಜ್ಞಾನವಾಗಿದೆ (Molecular marker assisted selection). ರೀಕಾಂಬಿನೆಂಟ್-ಇನ್ಬ್ರೆಡ್ ತಳಿಗಳನ್ನು ಬೇರಿನ ಉದ್ದ ಹಾಗು ನೀರು ಬಳಕೆಯ ಸಾಮರ್ಥವನ್ನು ಆಧಿನಿಕ ತಂತ್ರಜ್ಞಾನಗಳನ್ನು ಬಳಸಿ ಅಧ್ಯಯನ ಮಾಡಲಾಯಿತು. ನಮ್ಮಲ್ಲಿದ್ದ SSR ಗುರುತುಕಾರಕಗಳ ಜೊತೆಗೆ Transposon ಆಧಾರಿತ ಗುರುತುಕಾರಕಗಳನ್ನು ಒಗ್ಗೂಡಿಸಿ ನೂತನ Linkage ನಕ್ಷೆಯನ್ನು ಬರೆಯಲಾಯಿತು.

ಗುರುತುಕಾರಕಗಳ ವಿಂಗಡನೆಯೊಂದಿಗೆ ತಳಿಗಳ ಬರನಿರೋಧಕ ಶರೀರ ಕ್ರಿಯೆಗಳಲ್ಲಿನ ವೈವಿಧ್ಯತೆಯನ್ನು ಅಧ್ಯಯಿಸಿ, QTL ಗಳನ್ನು ಕಂಡುಹಿಡಿಯಲಾಯಿತು. ಜಿ.ಎಂ-೧೩೫೭ ಬೇರಿನ ಆಳಕ್ಕೂ, ಜಿ.ಎಂ-೧೭೧೭ ಹಾಗು ಪಿ.ಪಿ.ಜಿ.ಎಸ್.ಎಸ್.ಇ.ಕ್ಯು-೧೮.ಎ.೦೫.ಬಿ ಗಳು ಬೇರಿನ ಒಣ ದ್ರವ್ಯರಾಶಿಗಳೊಂದಿಗೆ ಜೊತೆಗೂಡಿವೆ ಎಂಬುದು ತಿಳಿದು ಬಂದಿದೆ.

ನೆಲಗಡಲೆಯಲ್ಲಿ ಬೇರಿನ ಉದ್ದ ಹಾಗು ನೀರು ಬಳಕೆಯ ಸಾಮರ್ಥ್ಯಗಳಿಗೆ ಹೊಂದಿಕೊಂಡ ಗುರುತುಕಾರಕಗಳನ್ನೊಳಗೊಂಡ ಈ ಪ್ರಯೋಗ ಮೊದಲನೆಯದಾಗಿದೆ. ಇನ್ನೂ ಹೆಚ್ಚಿನ ಗುರುತು ಕಾರಕಗಳನ್ನು ಬಳಸಿ linkage ನಕ್ಷೆಯನ್ನು ತಯಾರಿಸಿದ್ದಲ್ಲಿ, ಪ್ರಮುಖವಾದ QTL ಗಳ ಪತ್ತೆಗೆ ನಾಂದಿಯಾಗಬಹುದೆಂದು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ತಿಳಿದುಬಂದಿದೆ.

ವಿಧ್ಯಾರ್ಥಿಯ ಸಹಿ
(ಪ್ರಿನ್ಸ್. ಚೋಯಲ್)

ಮುಖ್ಯ ಸಲಹೆಗಾರರ ಸಹಿ
(ಡಾ. ಎಂ. ಎಸ್. ಶೇಷಶಾಯಿ.)

ಎಮ್.ಎಸ್.ಸಿ ಪದವಿಯ ಪ್ರಬಂದದ ಸಾರಂಶ

ಬೆಳೆ ಶರೀರ ಕ್ರಿಯಾಶಾಸ್ತ್ರ ವಿಭಾಗ

ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ, ಬೆಂಗಳೂರು-೫೬೦೦೬೫

ಪ್ರಬಂದದ ಹೆಸರು: **ಬೇರುಗಳ ವೈವಿಧ್ಯತೆ ಹಾಗು ನೀರು ಬಳಕೆಯ ಸಾಮರ್ಥ್ಯತೆ ಗುಣಗಳಿಗೆ ಹೊಂದುವ ಕ್ಯು.ಟಿ.ಎಲ್. ಗಳ ಪತ್ತೆ ಮತ್ತು ವಿಮರ್ಷೆ**

ವಿದ್ಯಾರ್ಥಿಯ ಹೆಸರು: ಪ್ರಿನ್ಸ್. ಚೋಯಲ್

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ತೇರ್ಗಡೆ ಹೊಂದಿದ ವರ್ಷ: ೨೦೧೩

ಮುಖ್ಯ ವಿಷಯ: ಬೆಳೆ ಶರೀರ ಕ್ರಿಯಾಶಾಸ್ತ್ರ ವಿಭಾಗ

ಪ್ರಬಂದದ ಒಟ್ಟು ಪುಟಗಳ ಸಂಖ್ಯೆ: ೯೮

ಸಾರಾಂಶದ ಒಟ್ಟು ಪದಗಳ ಸಂಖ್ಯೆ: ೨೫೦

ಪ್ರಬಂದದ ಒಟ್ಟು ಕೋಷ್ಟಕ ಪಟ್ಟಿಗಳ ಸಂಖ್ಯೆ: ೧೬

ಪ್ರಬಂದದ ಒಟ್ಟು ನಕ್ಷೆಗಳ ಸಂಖ್ಯೆ: ೬

ಪ್ರಬಂದದ ಒಟ್ಟು ಚಿತ್ರಗಳ ಸಂಖ್ಯೆ: ೧೧

ವಿಭಾಗದ ಮುಖ್ಯಸ್ಥರ ಸಹಿ ಮತ್ತು ವಿಳಾಸ:

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<u>ಪ್ರಮಾಣ ಪತ್ರ</u>

ನಾನು ಈ ಮೂಲಕ ಪ್ರಮಾಣೀಕರಿಸುವುದೇನೆಂದರೆ, ಈ ಸಂಶೋಧನ ಪ್ರಬಂಧದ ಯಾವುದೇ ಒಂದು ಭಾಗದ ನಕಲನ್ನು ಯಾವುದೇ ವಿಜ್ಞಾನಿಗೆ ಪರಾಮರ್ಶೆಗಾಗಿ ಗ್ರಂಥಾಲಯದ ಮಾಹಿತಿ ಕೇಂದ್ರದಿಂದ ಎರವಲು ಪಡೆಯಲು ನನ್ನಿಂದ ಯಾವುದೇ ಅಭ್ಯಂತರವಿರುವುದಿಲ್ಲ.

ವಿದ್ಯಾರ್ಥಿಯ ಸಹಿ
ದಿನಾಂಕ:
ಸ್ಥಳ: ಬೆಂಗಳೂರು

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ABBREVIATION

CID	Carbon Isotope Discrimination
CIM	Composite Interval Mapping
GA	Genetic Advancement as % of mean
GCV	Genotypic Co-efficient of Variance
HI	Harvest Index
H^2	Heritability
IRMS	Isotopic Ratio Mass Spectrometer
LA	Leaf Area
LWT	Leaf Weight
MASH	Mineral Ash
MTR	Mean Transpiration Rate
PCV	Phenotypic Coefficient of Variance
QTL	Quantitative Trait Loci
RH	Relative Humidity
RIL	Recombinant Inbred Line
RLD	Root Length Density
RL	Root Length
RS	Root to shoot ratio
RuBisCO	Ribulose 1, 5- Bis Phosphate Carboxylase/Oxygenase
RWC	Relative Water Content
RWT	Root Weight
SCMR-	SPAD Chlorophyll Meter Reading
SHT	Shoot Height
SLA	Specific Leaf Area
SMA	Single Marker Analysis
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
SWT	Shoot Weight
TDM	Total dry matter
TE	Transpiration Efficiency
TLA	Total Leaf Area
TMASH	Total Mineral Ash
VPD	Vapour Pressure Deficit
WUE	Water Use Efficiency
‰	Parts per thousand
$\Delta^{13}C$	Discrimination against ^{13}C

Introduction

I. INTRODUCTION

Groundnut (*Arachis hypogaea*.L) is one of the important oil seed crop of the world, which is presently cultivated throughout tropical, subtropical and warm temperate regions of the world. The cultivated groundnut (*Arachis hypogaea* L.), an annual herb belonging to the family Fabaceae (Leguminosae), is classified into two subspecies, subsp. *Fastigiata* and subsp. *hypogaea*. The subsp. *fastigiata* contains four botanical varieties, var, *vulgaris*, *fastigiata*, *peruviana*, and *aequatoriana*. The subsp. *hypogaea* contains two varieties, var. *hypogaea* and *hirsuta*. Groundnut is an allotetraploid (2n = 2x = 40) with "AA" and "BB" genomes. All species, except the cultivated species (*A. hypogaea* and *A. monticola*) in Section *Arachis*, and certain species in Section *Rhizomatosae*, are diploid (2n = 2x = 20). The diploid progenitors, *A.duranensis* and *A.ipaensis*, contributed "AA" and "BB" genomes, respectively, to the cultivated groundnut (Kochert *et al.,* 1996). It has a genome size of 2800 Mb (Gautami *et al.,* 2009). Southern Bolivia and Northern Argentina are thought to be centre of origin of this crop (Kochert *et al.,* 1996).

Peanut or groundnut is widely used as a food and cash crop around the world. It is mainly grown by resource-poor farmers in Africa and Asia to produce edible oil (48–50%) and for human consumption. The crop is grown in about 24 million hectares worldwide with a total production of 38 million tons in 2010 (FAOSTAT, 2010) and productivity of 1.60 million tons per hectare (AGROSTAT 2009/10). India shares 22 percent of the world groundnut production. In India, it is grown in about 6.41 million hectares with a production of 7.3 million tons with productivity of 1460 kg per hectare (2009-10 Indiastat.com).

In the last decade (2000–2010), the global groundnut production increased marginally. The global annual increase in production was 0.4% which was due to both, an annual increase in yield by 0.1% and in area by 0.3%. The projected demand of groundnut in Asia alone by 2020 is expected to be 1.6 times more than the level of production in 2000 (Birthal *et al*, 2010). If the projected demands have to be met, the productivity and production of the crop has to increase at a much higher growth rate than the present one. Asia and Africa account for 95% of global groundnut area where it is cultivated under rainfed conditions with low inputs by resource poor farmers.

Mechanisms of drought adaptation in groundnut are limited to drought escape and drought avoidance (Zhang *et al.* 2001). In drought escape, plants take advantage of developmental flexibility to match its phenology to the length of the cropping period (early flowering to escape late season drought). The principle of drought avoidance is to either increase water absorption ability (from rooting differences) or decreasing their water loss (shoot/leaf morphological traits or physiological traits).

The simple growth model propounded by John Passioura, states that total biomass production is a function of total water transpired and the efficiency with which water transpired is used for biomass production (Passioura, 1986). The latter term is often referred to as Water Use Efficiency (WUE), which is the ratio of the biomass produced per unit water transpired over a specified period. Water use efficiency can be considered as a drought avoidance trait, which deals with using soil water more efficiently for biomass production, therefore to ‘‘avoid’’ drought. Improving these traits has been shown to be quite relevant in crop improvement. Maximized use of stored soil water, increased biomass productivity per unit water use and highest conversion of biomass into economic yield under limited-water conditions are the ultimate goals of any drought research (Krishnamurthy *et al.,* 2007).

Groundnut producing regions represent typically the semi-arid tropics (SAT) environment which is characterized by short and erratic rainfall and then long periods with virtually no rain. Improving WUE (Rebetzke *et al.,* 2002) and root biomass (Li *et al.,* 2005) have been shown to be associated with higher biomass production under water limited conditions. Therefore it has been hypothesized that improving water use efficiency (WUE) would be the best strategy to cope with episodes of intermittent drought. Similarly, growth and productivity under drought conditions is dependent on the ability of plants to harness water from deeper soil profiles associated with better root system and the subsequent efficient use of water for biomass production. However under irrigated conditions, increased WUE was not always useful (Richards *et al.,* 2002; Sheshshayee *et al.,* 2003). Thus, it appears that improving WUE and root traits is a potential option to improve productivity under drought stress conditions.

Conventional breeding for developing drought-tolerant crop varieties is time-consuming. Tolerance to drought is not a simple response, but is mostly conditioned by many genes and has been shown to interact with environment, and thus the networks

involved in drought tolerance are quite complex in nature. Therefore, selection based on the phenotype would be difficult for such traits (Collins *et al.* 2008).

Recent advances in the area of crop genomics offer tools to assist breeding (Varshney *et al.,* 2005, 2006). The identification of genomic regions associated with drought tolerance would enable breeders to develop improved cultivars with increased drought tolerance using marker-assisted selection (MAS) (Ribaut *et al.,* 1996). To identify the genomic regions suitable for marker-assisted breeding strategies, it is important to establish accurate phenotyping methods, develop highly saturated molecular marker-based genetic linkage maps, and then identify QTLs (quantitative trait loci) associated with traits of interest. Such QTLs would then simply speed up the process of introgression of beneficial traits into preferred varieties, especially for complex traits such as drought.

Several studies were conducted in the past that reported identification of QTLs for drought tolerance or related traits. For instance, in soybean, 5 QTLs were identified for WUE (Mian *et al.* 1998). In case of wheat, Dashti *et al.* (2007) identified five QTLs for drought tolerance. However 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 drought tolerance related traits (Gautami *et al.*, 2011; Ravi *et al.*, 2011; Varshney *et al.*, 2009c), resistance to foliar disease (Khedikar *et al.*, 2010; Sujay *et al.*, 2011) and nutritional quality traits (Sarvamangala *et al.*, 2011).

Although QTLs for a few drought tolerance traits are discovered, but QTLs for root traits and water use efficiency is not identified yet. Since these two traits, water use efficiency (Rebetzke *et al.,* 2002), and root traits (Li *et al.,* 2005) can improve to drought tolerance in crop plants under water stress, it is important to identify the QTLs for these traits. Breeding for these traits using QTLs can enhance the drought tolerance in the groundnut. From this context, the major emphasis of this study was to discover QTLs for root traits and water use efficiency in RIL population of groundnut. The specific objectives of the study were:

- Phenotyping the recombinant inbred line population of groundnut for variability in drought tolerance traits i.e. WUE and root traits.
- Genotyping the RIL population using TRANSPOSON based markers.
- QTL mapping by composite interval mapping using SSR and TE markers genotyping data.

Review of Literature

II. REVIEW OF LITERATURE

Groundnut (*Arachis hypogea*) is an important legume cash crop, where the seeds contain high amounts of edible oil (43-55%) and protein (25-28%) on a dry seed basis (Savage and Keenam, 1994). Among the seven annual edible oilseeds, groundnut holds key position by contributing 35 per cent of the total oilseed area and 46 per cent of the total oilseed production in the country.

Several biotic and abiotic stress factors have been the major factors responsible for the appallingly low yield in India. Furthermore, about 70% of agricultural area is exposed to drought. This whooping area because of the constraint caused by water scarcity contributes to a meager 38% to the agriculture production globally (Dilley *et al.,* 2005).

Recent years have seen a growing scarcity of water worldwide and is estimated that by 2050, as much as two-third of the global production will live in water scarce areas (Wallace, 2000). So, drought is the major abiotic constraint affecting peanut productivity and quality worldwide.

Two-thirds of the global production occurs in rain-fed regions of the semi-arid tropics where rainfall is generally erratic and insufficient, causing unpredictable drought stress, the most important constraint for peanut production (Wright *et al.,* 1994; Reddy *et al.,* 2003). Even peanut grown under irrigated conditions may experience drought because of limited water supply or because irrigation water is applied in amounts at frequencies less than optimal for plant growth.

Improving water access and management are practically difficult since water is a scarce resource. Therefore, to develop drought tolerant strategies is an important aspect in alleviating the problem and offers the best long-term solution.

2.1 Plant performance under drought stress

It has been showed that drought stress has adverse effect on water relations (Babu & Rao, 1983), photosynthesis (Bhagsari *et al*., 1976), mineral nutrition, metabolism, growth and yield of groundnut (Suther & Patel, 1992). Apart from this, drought conditions also influence the growth of weeds, agronomic management and,

nature and intensity of insects, pests and diseases (Wheatley *et al*., 1989). Parameters like relative water content (RWC), leaf water potential, stomatal resistance, transpiration rate, leaf temperature and canopy temperature influences water relations in peanut during drought.

Stressed plants have lower RWC than non-stressed plants. Babu and Rao, (1983) have been reported that relative water content (RWC) of non-stressed plants range from 85 to 90%, while in drought stressed plants, it may be as low as 30%. Erickson and Ketring, (1985) showed that groundnut leaves exhibit large diurnal variation with high values in the morning when solar radiation and vapour pressure deficits are low, followed by low values around midday and gradual increase after midday.

Subramaniam and Maheswari, (1990) showed that leaf water potential, photosynthetic rate and transpiration rate decreased progressively with increased duration of water stress indicating that plants were postponing tissue dehydration under mild stress. During the stress period stomatal conductance decreased almost steadily indicating that stomatal conductance (g_s) was more sensitive than transpiration during the initial stress period.

Stirling *et al*., (1989) found that under water stressed conditions the leaves exhibited marked diurnal variation in leaf turgor, while pegs showed less variation and maintained much higher turgor levels largely because of their lower solute potentials. Marked osmotic adjustment occurred in growing leaves but not in mature ones, allowing them to maintain higher turgor during periods of severe water stress. This adjustment was rapidly lost when stress was released (Ali Ahmad & Basha, 1998).

Azam Ali (1984) reported that older leaves were showed greater stomatal resistance than that of younger leaves and the leaves also become thicker under moderate drought stress (Reddy and Rao, 1968). The developing leaves of peanut have an unusual thick layer of cells devoid of chloroplasts with lower epidermis below the sponge parenchyma. Cells of this layer are considered to be as water storage cells (Reddy and Rao, 1968).

During moisture stress condition, the opposing leaflets of tetra -foliate leaf come together and orient themselves parallel to incident solar radiation, in an effort to reduce solar radiation load on the leaf. Black *et al* (1985) has been showed that leaf expansion is more sensitive to soil water deficit than stomatal closure. Drought stress reduces the leaf area by slowing leaf expansion and reducing the supply of carbohydrates. Reddy and Rao (1968) showed that severe drought stress significantly decreased the levels of chlorophyll *a*, *b* and total chlorophyll. This decreased in chlorophyll was attributed to the inhibition of chlorophyll synthesis as well as to accelerated turnover of chlorophyll that is already present.

Periodic water stress results into the anatomical changes such as a decrease in cell size and intercellular spaces, cell walls thinking and greater development of epidermal tissue. Due to the moisture stress, nitrogen fixation in leguminous plants is reduced because of the reduction in leghaemoglobin in nodules, specific nodule activity. Even dry weight of nodules also significantly reduced in drought stressed plants. Drought stress also delays the nodule formation in leguminous crops (Reddi & Reddy, 1995). Kulkarni *et al*., (1988) reported that even the N, P and K uptake of peanut is also reduced under drought stress.

2.1.1 Shoot growth

Water deficits reduce both the number of leaves per plant and in size of individual leaf. Because of decreasing soil water potential, leaf longevity and leaf area duration is also reduced. Leaf area expansion depends on leaf turgor, temperature and assimilates supply for growth, which are all affected by drought stress.

Water stress alters the leaf and stem morphology. Continuous water deficit results in smaller and fewer leaves, which have smaller and more compact cells and greater specific leaf weight (Chung *et al*. 1997). Main axis and cotyledonary branches are shorter for water stressed peanut plants. Soil moisture deficit reduces internodal length more drastically than node number.

2.1.2 Root growth

Roots grow rapidly during germination and seedling stages and within 5 or 6 days after sowing, the tap root may grow 10–16 cm deep and develop a number of lateral roots (Yarbrough 1949). Groundnut roots grow rapidly, consuming a considerable portion of the early-produced assimilates. By 80 days after sowing more than 80% of the total root system is established in long duration varieties (150 days).

Ketring and Reid (1993) reported that root length density has been significantly increased at 10 cm depth until 80 days. At 40–45 days, roots had penetrated to a depth of 120 cm and spread laterally at least 46 cm. Gregory and Reddy (1982) investigated that the total root length of cultivar Robout 33-1 followed a sigmoid growth curve and peaked at 68 days after sowing.

It has been reported that water stress stimulates the growth of roots into deeper soil (Lenka and Mishra, 1973). Allen *et al*. (1976) concluded from measured soil water extraction that during water deficit, roots in lower depths continue to grow deeper even though vegetative growth appears to stop. They further stated that peanut roots extracted soil water to depths of at least 180 cm in fine sandy soil. Simmonds and Ong (1987) showed that the cultivar Robut 33-1 more rapidly extracted water from deeper layers when grown at high vapour pressure deficits than when grown in more humid air.

Devries *et al*. (1989) investigated that cultivar Florunner had higher root length density in deeper layers (60–150 cm) during drought periods. Florunner exhibited greater capacity for deep rooting at 55 days after sowing than that of soybean or cowpea, especially when grown under moisture stress condition. All these traits contribute to groundnut's ability to avoid drought stress. Pandey *et al*. 1984) showed that groundnut had greater root length density deeper in the soil than other legumes when grown under drought stress. Sabale and Khuse (1989) reported the highest root lengths when available soil moisture was 80–85% field capacity. They also reported that application of antitranspirants did not influence either root length or root volume. Fertilizer phosphorus had favourable influence only on root volume but not on root length.

Meisner and Karnok (1991) observed groundnut root growth under 30-day drought stress periods beginning 20, 50, 80 and 110 days after sowing by using two non-destructive methods, a rhizotron and minirhizotron . Root growth was significantly reduced by drought stress during 20–50 days after sowing compared with irrigated control in the rhizotron study, however, such differences were not observed in peanut plants grown in minirhizotron (Meisner and Karnok 1991). All other stress periods had the influence on root growth.

Meisner and Karnok (1992) observed root growth on rhizotron glass every week and found that groundnut root system, regardless of moisture stress, did not exhibit signs of senescence. Root colour and florescence of the root system did not change throughout the season at all depths indicating viability of roots.

The ability of peanut to maintain a viable root system during moisture stress may contribute to the crop's drought resistance (Sanders *et al*. 1993). Greater carbon partitioning to the root system before pod set and a root system that maintains itself for a long period should be an advantage over plants whose roots are continuasally dying and regrowing during reproductive development.

2.1.3 Flowering

Boote & Ketring, (1990) reported that initiation of flowering was not delayed by drought stress . The rate of flower production was reduced by water stress during flowering but the total number of flowers per plant was not affected due to an increase in the duration of flowering (Meisner & Karnok, 1992).

Janamatti *et al*., (1986) observed that significant burst in flowering on alleviation of stress is a unique feature in the pattern of flowering under moisture stress, particularly when drought is imposed just prior to reproductive development. When stress was imposed during 30–45 days after sowing the first flush of flowers produced up to 45 days did not form pegs during that time, however, flowers produced after re-watering compensated for this loss (Gowda & Hegde, 1986).

2.1.4 Pod formation

Jogloy *et al.,* (1996) showed that groundnut plants experiencing water stress during pegging and pod development and after that having adequate amount of water would result in a drastic reduction of crop yield, and the magnitude of reduction would depend on the peanut cultivars.

Not only the yield of groundnut but also the quality of products decreases under drought stress (Rucker *et al*., 1995). Boote & Ketring, (1990) showed that peg elongation, which is turgor dependent, is delayed due to drought stress. Pegs failed to penetrate effectively into air-dry soil, especially in crusted soils. Often, within 4 days of withholding water, the soil surface becomes too dry to peg penetration.

Skelton & Shear (1971) showed that adequate root zone moisture could keep pegs alive until pegging zone moisture content is sufficient to allow penetration and initiation of pod development. Once pegs penetrate the soil, adequate moisture and darkness are needed for pod development. Adequate pod zone moisture is critical for development of pegs into pods and adequate soil moisture in the root zone cannot compensate for lack of pod zone water for the initial 30 days of peg development. Dry pegging zone soil delayed pod and seed development. Soil water deficits in the pegging and root zone decreased pod and seed growth rates by approximately 30% and decreased weight per seed from 563 to 428 mg.

Peg initiation growth during water stress demonstrated the ability to suspend development during the period of soil water deficit and to re-initiate pod development after the drought stress was relieved (Sexton *et al.*, 1988). It has frequently been reported that under moisture stress, pegging and seed set responses of various groundnut cultivars varied substantially, this leads to a large reduction in pod yield, and the reduction percentage also varies among groundnut cultivars (Haris *et al*., 1988, Nageswara Rao *et al*., 1998).

2.1.5 Aflatoxin content

Under adequate moisture conditions invasion of the fungus elicits phytoalexin production by the plant, which suppress fungal growth and avoid subsequent aflatoxin contamination (Wotton and Strange, 1987).

When groundnuts are subjected to drought stress however, two factors are directly affected. Firstly, increase in soil temperatures as the peanut canopy recedes (Cole *et al*., 1985) and secondly, kernel water activity decreases as a result of reducing the plant water status in drying soil. Reduction in water activity results in decrease in phytoalexin production, creating favourable conditions for growth of *A. Flavus* which leads to aflatoxin production (Cole *et al*., 1989). Therefore the traits which contribute to drought tolerance can be useful to minimize the contamination of aflotoxin.

Arunyanark and Jogloy (2009) has been showed the association between drought tolerance trait (SLA and Root length density) and aflotoxin contamination in peanut grown under long term drought. They had reported that elevated soil temperatures and reduced soil moisture, favoured aflatoxin production. They have shown that drought tolerance traits (SLA and RLD) could be contributing to resistance to aflatoxin contamination suggesting that a combination of SLA, RLD and kernel colonization could be used as selection criteria in selecting parents for aflatoxin resistance.

2.2 Importance of roots for drought tolerance

The genetic improvement of root traits by conventional method is rather slow due to difficulty in measuring root dynamics and below ground environment. In fact, it has been shown the relevance of root biomass especially root length on biomass production in several systems (Sinclair and Muchow, 2001). People have also shown the much-awaited reward when they have incorporated root traits like root length, root biomass and root pulling force in their breeding for improved root system to achieve better productivity.

Root traits associated with drought tolerance are important for drought resistant mechanisms of plants. Root characteristics such as root length density, rooting depth and root distribution have been established as constituting factors of drought resistance (Matsui and Singh, 2003). Rucker *et al.* (1995) reported that a large root system may improve a plant's ability to continue growth during drought stress. Benjamin and Nielsen (2006) and Songsri *et al.* (2008) reported individually that the ability of a plant to change its root distribution in the deeper soil water profile is an important mechanism for drought avoidance.

Meisner and Karnok (1992) found that root growth was reduced during water stress, but, after re-watering, there was a trend for root growth to recover in the peanut cultivar. The information on the ability of drought resistant peanut genotypes to alter their root systems contributing to high yield under water stress might reveal avoidance mechanisms and could result in the development of improved breeding strategies for drought resistance in peanut (Songsri *et al.,* 2008). Under stress, root length seems to be increased in plants. In fact, Pace *et al*. (1999) have reported that, drought-stressed seedlings showed some increase in root length but a reduced diameter.

On the other hand, Prior *et al.* (1995) showed that, inadequate soil moisture reduced root elongation. In another study, Plaut *et al.* (1996) have showed that the soil moisture deficit reduced root length and density. In fact, in one of the studies, it was shown that, sunflower with deep and extensive root system can extract water from up to 270 cm (Gimenz and Fereres, 1986; Rachidi *et al.,* 1993). Thus, root system has a practical relevance under water limited conditions.

Drought tolerance may be enhanced by improving the ability of the crop to extract water from the soil (Wright *et al.,* 1994). Deep rooting, root length density (RLD) and root distribution have been identified as drought adaptive traits (Turner 1986; Ludlow and Muchow, 1990; Matsui and Singh, 2003; Taiz and Zeiger, 2006) which can be used as selection criteria for drought tolerance. Variation among genotypes for shifting root distribution downwards in response to drought has been found in cowpea (Matsui and Singh, 2003), white clover (Annicchiarico and Piano, 2004) and chickpea (Yusuf Ali *et al*., 2005; Benjamin and Nielsen, 2006).

In contrast, Benjamin and Nielsen (2006) found that, water deficit did not affect root distribution in soybean. In this regard, Rucker *et al.* (1995) found that, some genotypes with large root systems under non-stress conditions gave high yield under drought conditions and they suggested that, these genotypes possessed drought avoidance traits. In crop plants, selection for drought tolerance in the past has primarily been based on biomass production and pod yield under drought conditions.

2.3 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.

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 X Water Use Efficiency X 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 contexts by hydrologists, agronomists, and physiologists. Physiologically, Water Use Efficiency (WUE) is defined at either single leaf level or at whole plant level and or at canopy level. Existence of genetic variability both 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

A) At canopy level, under field conditions using crop growth and yield model. This technique is more employed in perennial tree species.

B) At whole plant (canopy) level in small pots or big containers (field mini-lysimeter) by adopting gravimetric technique.

C) At a single leaf level by adopting the gas exchange approach.

In container experiments, genetic variability in 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, Nageshwara 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) and reported a significant variability in WUE between genotypes and moisture regimes. Wright *et al.* (1994) studied four groundnut genotypes under two drought regimes in a mini-lysimeter, involving 35 genotypes of groundnut showed the significant genotypic difference in WUE exists among different genotypes.

Similar study was conducted by Roy Stephen (1995) who reported a range of WUE between 2.92 to 4.07 g dry matter/kg of water under 100% field capacity and

between 3.19 to 5.46 g dry matter/kg of water under 50 % field capacity. Groundnut plants grown in small canopies in field and in mini lysimeter showed same ranking for WUE in two environments indicating water use efficiency was not affected by the contrast in environment (Wright *et al.,* 1988). In another experiment, Prasad *et al.* (1992) tested 14 bunch groundnut genotypes, which were selected for higher WUE in a container studies, under limited water inputs in field with a long duration water stress and showed similar ranking in field and container studies also.

2.3.1 Significance of WUE as a drought tolerant trait

WUE a popular term, which is intuitively taken for, granted as being important for conditions of limited water supply. High WUE implies good returns on a given amount of water used or moderate water use for a given amount of product.

C_4 species typically have a WUE of 4 to 5.5 g. kg^{-1} compared to the C_3 species (1.5 - 3.0 g. kg^{-1}). Perhaps because of this trait, the C_4 species have significantly higher productivity compared to the C3 species under arid and semi-arid tropics where water availability is the major constraint. These observations suggest that improving WUE of our crop species have relevance. There are several approaches available to assess the genetic variability in Water Use efficiency. They are gravimetric approach, gas exchange studies and Carbon isotope discrimination.

2.3.2 Gravimetric approach

Gravimetric measurements can give reliable estimations of WUE, as they allow accurate measurement of T and dry matter production including roots (Udayakumar *et al*., 1998).There was a significant positive correlation ($r= 0.96$, $p<0.01$) between the WUE values measured in the container and the field experiment for three of the four groundnut genotypes (Hebbar *et al*., 1994). In another study Boominathan (2001) showed considerable variability in WUE ranging from 2.49 -5.41g/kg among rice genotypes adopting gravimetric approach.

Several other authors have also shown genetic variability in WUE using this approach (Impa, 2002) where WUE is estimated as the ratio of dry matter produced to the amount of water lost through transpiration over a period of time. The measurement

of WUE is cumbersome and labour intensive because of the practical difficulties associated with the measurement of transpiration and root dry matter.

2.3.3 Carbon isotopes discrimination

There are two naturally occurring stable isotopes of carbon, ^{12}C and ^{13}C. Most of the carbon is ^{12}C (98.9%) with 1.1% being ^{13}C. The isotopes are unevenly distributed among and within different compound and this isotopic distribution can reveal information about the physical, chemical and metabolic processes involved in carbon transformations.

The overall abundance of ^{13}C relative to ^{12}C in plant tissue is commonly less than in the carbon of atmospheric CO_2, indicating that carbon isotope discrimination occurs during the incorporation of CO_2 into plant biomass. Because the isotopes are stable and non-radioactive in nature, the information is inherent in the ratio of abundance of carbon isotopes, presented by convention as $^{13}C/^{12}C$, is invariant as long as carbon is not lost.

Plants discriminate against the heavy isotope of carbon (^{13}C) during photosynthesis resulting in the depletion of the ^{13}C content in the biomass (O' Leary, 1981). This deviation of the carbon isotopic ratio ($^{13}C/^{12}C$) of biomass from that of air, called discrimination ($\Delta^{13}C$) is related to the ratio of the partial pressures of CO_2 inside the leaf to that in ambient air (Pi/Pa) (O' Leary, 1981; Farquhar *et al.*, 1989a; Hubick and Farquhar, 1989) as follows;

$$\Delta^{13}C = \delta_{air} - [a + (b - a)\, Pi/\, Pa]$$

where, a and b are fractionation against ^{13}C ($\delta^{13}C$) during diffusion through stomata and carboxylation by RuBisCO respectively. Since WUE is also related to the CO_2 partial pressures, at a given VPD, a strong inter-relationship between $\Delta^{13}C$ and WUE is expected (Hubick and Farquhar, 1989).

The $\Delta^{13}C$ in plant samples is generally determined using a sophisticated analytical instrument called Isotope Ratio Mass Spectrometer (IRMS) specially designed for high precision measurements of the ratio R, defined as:

$$R = {}^{13}CO_2/{}^{12}CO_2$$

The plant material is converted to CO_2 by combustion to determine the isotope composition. In general R is low in organic sample. The atmosphere has a relatively higher fractionation value of around –7.8 per mil (‰), which is in comparison with a standard PDB (Pee Dee Belemnite, from North Carolina, USA). The R in this standard is 0.0124 and in many of the plant material it is approximately 0.012, suggesting a very minor changes in the R value, and hence R in a sample can be compared with that of standard and expressed as ^{13}C in units per mil or parts per thousand (‰)

$$\delta^{13}C = R_{sample} - R_{standard}/R_{standard} * 1000$$

Since the organic sample has less R-value than the standard, $\delta^{13}C$ of organic material is more negative, i.e., less ^{13}C content hence more discrimination and vice versa (O'Leary, 1984).

Importance and biochemical basis of $\Delta^{13}C$ and the relationship of $\Delta^{13}C$ with WUE have extensively been studied (Farquhar *et al.,*1982; Hubick *et al.,* 1988; Condon *et al.,* 1990; Read *et al.,* 1991). The range of $\Delta^{13}C$ values across the crop types, having different photosynthetic pathways, is given below:

Range of $\delta^{13}C$ composition

1. Atmosphere (Air) = -6.4 to –7.0‰
2. C_3 plants = -22 to –44‰
3. C_4 plants = -9 to –19‰
4. CAM plants = -11‰ (approximate)

2.3.4. Carbon isotope discrimination ($\Delta^{13}C$) at different steps during photosynthesis

The fractionation of carbon isotope during photosynthesis involves several distinct biochemical and physical processes. These processes have different tendencies to discriminate between ^{12}C and ^{13}C, and the overall discrimination of a particular plant will be a function of the mechanism it uses for CO_2 fixation and the relative balance of the processes that participate in photosynthesis. During photosynthesis, CO_2 must diffuse

from the atmosphere to the chloroplast stroma. Since $^{12}CO_2$ diffuses faster than $^{13}CO_2$, several fractionation processes occurs along this diffusion path, so that the CO_2 available at the sites of carboxylation is always significantly depleted in ^{13}C compared to the atmosphere (Table 3) (Farquhar *et al.*, 1989a; Bragnoli and Farquhar, 1998).

Table 1: Fractionation of carbon isotope during photosynthesis.

Process	Discrimination (‰)	Reference
Diffusion of CO_2 in air through the stomatal pore	4.4	Craig, (1953)
Diffusion of CO_2 in air through the boundary layer to the stomata	2.9	Farquhar, (1983)
Diffusion of dissolved CO_2 through water	0.7	O'Leary, (1984)
Fixation of gaseous CO_2 by RuBisCO	29	Guy and Hoering, (1987)

The principal components of photosynthesis that influence discrimination are diffusion of CO_2 through the stomata and the carboxylation process mediated by RuBisCO (O'Leary, 1988, 1993). Irrespective of the photosynthetic sub component-determining discrimination, $\Delta^{13}C$ is related to Pi as following relationship suggests:

$$\Delta = \{a+(b-a)Pi/Pa - d\}$$

where, a, b are constants for the discrimination against $^{13}CO_2$ during diffusion of CO_2 into the leaf and carboxylation, respectively and d is a component contributed from respiration, diffusion of dissolved CO_2 and Pi & Pa are the intercellular and ambient CO_2 partial pressures.

2.3.5. $\Delta^{13}C$ and WUE – relationship

Plants discriminate against the heavy isotope of carbon ($\Delta^{13}C$) during the process of photosynthesis. However the extent of discrimination depends on the Pi and hence ^{13}C content in the plant samples has been emerged as a potential technique to quantify Pi and WUE. Several reports confirm the close relationship between Pi and $\Delta^{13}C$, therefore $\Delta^{13}C$ could be a time-integrated estimate of Pi.

Since, Pi/Pa ratios predominantly determine the variations in WUE and $\Delta^{13}C$, therefore, a strong relationship between $\Delta^{13}C$ and WUE can be expected and explained by the following equation (Farquhar *et al.,* 1989b).

$$WUE = \{(1 - \theta)(b - d - \Delta)\}/1.6V(b - a)$$

where, θ is the proportion of fixed CO_2 lost in respiration, V is the leaf-air vapor pressure gradient.

An inverse relationship between A/g_s and $\Delta^{13}C$ (Meinzer *et al.,* 1990; Richards and Tieszen, 1993) and a positive relationship between Pi/Pa and $\Delta^{13}C$ signify that Pi determines the variability in $\Delta^{13}C$ (Hubick *et al.,* 1988; Gutterrez and Meinzer, 1994).

Although WUE and $\Delta^{13}C$ are related through the ratio of Pi/Pa, as well as with A/g_s (Condon *et al.,* 1990), because of diurnal and seasonal fluctuations in `A' and g_s, these parameters will not give an integrated estimate of WUE over a period of time (Hall *et al.,* 1993 and Udayakumar and Prasad, 1994). From, this context, $\Delta^{13}C$ is a dependable parameter as it is a reflection of time integrated estimate of carbon gain per unit transpiration, especially in C_3 plants. $\Delta^{13}C$ in whole plant dry matter appears to be reliable indicator of plant WUE in pot grown sunflower and negative relationship was obtained between these two traits in structural carbon both in well watered and drought conditions (Johnson *et al.,* 1993). In wheat, as in other C_3 species, genetic variability in $\Delta^{13}C$ is reflected in variation in WUE at both the leaf and at the whole-plant level (Condon and Richards, 1993).

Such a relationship between $\Delta^{13}C$ and WUE in several crop species as depicted (Table 4) was not altered even when plants were subjected to abiotic stresses. Maintenance of the relative ranking of genotypes in control and stress implies that for WUE and $\Delta^{13}C$, genotype and environment interaction is low and the broad sense heritability is high (Hubick *et al.,* 1988; and Wright *et al.,* 1993). Due to these distinct advantages, $\Delta^{13}C$ appear to be a very reliable parameter for the identification of variability in WUE. This led to the initiation of several breeding programs to improve WUE using carbon isotope discrimination technique (Hall *et al.,* 1993; White, 1993).

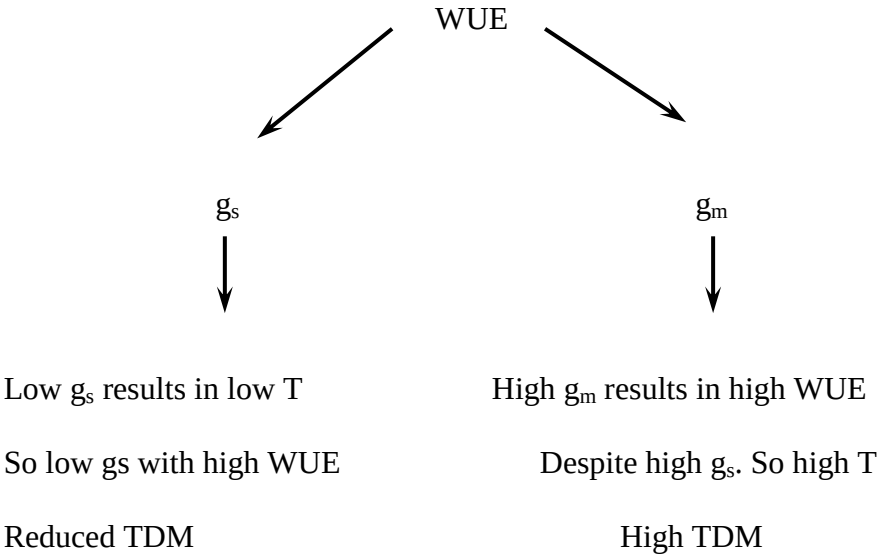
Table 2: Relationship between $\Delta^{13}C$ and WUE in crop species

Species	Relationship			Reference
	r	**p <**	**n**	
Cereals and millets				
Wheat	-0.75	0.01	12	Farquhar and Richards, (1984)
Wheat well watered plant Stressed plant	 -0.74 -0.75	 0.01 0.01	 16 16	Condon *et al.,* (1990)
Rice	-0.83	0.00001	34	Boominathan, (2001)
Rice	-0.47	0.01	39	Nadaradjan *et al.,* (2005)
Rice	-0.815	0.05	6	Impa *et al.,* (2005)
Pulses				
Cowpea	-0.93	0.05	5	Ismail and Hall, (1992)
Soybean Irrigated Water stressed	 -0.68 -0.80	 - -	 - -	White *et al.,* (1996)
Navy bean	-0.80	0.05	-	Wright,(1996)
Chickpea	-0.83	0.001	12	Gangadhara, (1995)
Cowpea	-0.68	0.02	11	Bindumadhava, (2000)
Oilseeds				
Peanut	-0.81	0.01	34	Wright, (1996)
Peanut	-0.64	0.05	8	Roy Stephen, (1995)
Peanut	-0.69	0.01	17	Shashidhar, (2002)
Others				
Kentucky Bluegrass	-0.55	0.01	11	Ebdon *et al.,* (1998)
Crested wheat grass	-0.87	0.05	14	Read *et al.,* (1991)

2.3.6. WUE is a quantitative trait

A major gene trait is digital in most cases, the character is either expressed or not. But many traits, particularly those of significance for crop physiology are analogue and are observable in segregating population as a more or less continuous range of behaviour between extremes which may even lies outside the mean range of the parents. WUE has such a quantitative mode of inheritance. The genes that contribute to these complex phenotypes will usually be several in number (polygene) and may be linked only in the physiological but not the genetic sense. This trait has high G x E interactions.

WUE is predominantly regulated by the stomatal diffusive factor (g_s) and /or mesophyll efficiency (g_m) that regulated carbon assimilation. Therefore, the variability in WUE brought about by intrinsic differences in g_m is desirable. In such cases WUE and T will be less dependent on each other. In capacity types, the mesophyll factors associated with A will determine the variability in WUE. So, in these types, WUE is independent of g_s and hence T will not be associated with WUE. Selection for high WUE from these types will result in high CGR (Hubick and Gibson, 1993). WUE as well as yield levels can be enhanced only when capacity types selected and are used in breeding program.



Inter relationship between WUE, T and mesophyll conductance in determining biomass production

2.3.7. Gas exchange studies

Physiological WUE can be evaluated by measuring CO2 fixation and transpiration rate. These measurements are usually performed on a single leaf over a limited period of time. Biomass produced is a function of photosynthetic rate. Hence WUE at a single leaf level is the ratio of carbon assimilation rate (A) to transpiration (T). Transpiration rate is determined by the intrinsic stomatal conductance and the existing leaf to air vapour pressure difference (V). If the plants being studied are grown under a very similar environmental condition, it can be expected that the leaf to air vapor pressure will be similar and hence, the major factor that determines transpiration would be the intrinsic stomatal conductance (gs). Therefore,

$$WUE = A/ g_s$$

Though not strong and statistically significant, a positive association between WUE measured by gravimetry and A/g_s was noticed which suggests that A/g_s could be a useful indicator of variation in whole plant WUE (Boominathan, 2001). Poor relationship between intrinsic WUE (A/g_s) and WUE measured gravimetrically was found in Cowpea suggesting that instantaneous measure of WUE, though dynamic but not stable, hence do not correlate with season long WUE (Bindumadhava, 2000).

2.3.8. Traits contributing to WUE

WUE and its related traits demonstrated in peanut that it is negatively correlated with leaf carbon isotopic discrimination (Δ) in a range of crop species including peanut (Hubick *et al*., 1986; Wright *et al*., 1988, 1994), and it is negatively correlated with SLA over a wide range of genotypes and environments under normal growing conditions (Wright *et al*., 1994; Nageswara Rao and Wright, 1994).

A strong and positive relationship between SCMR and WUE was also reported (Sheshshayee *et al*., 2006). SCMR and SLA are negatively correlated (Nageswara Rao *et al*., 2001; Upadhyaya, 2005) and genetic variation for SCMR has also been reported (Upadhyaya, 2005).

Songsri *et al* , (2008) demonstrated that root dry weight and SLA are important traits related to WUE under moderate (2/3 AW) and severe (1/3 AW) drought,

respectively. Root dry weight and SLA should be useful selection criteria for high WUE under moderate (2/3 AW) and severe (1/3AW) drought conditions respectively.

2.4 Total mineral ash content

Integrative physiological criteria, such as carbon isotope discrimination (Δ) and mineral ash content have been found to be very useful, under drought conditions, to elucidate the association between yield gains and variation of photosynthesis-related traits and orientate future breeding efforts.

Monneveux, *et al*, (2004) suggested that ash content in leaves could be also used as predictive criteria for yield not only under drought, but also under irrigated conditions, particularly when evaporative demand is high.

Transpiration efficiency (TE) is the weight of dry matter produced per unit of transpiration. If mineral nutrients are taken up in proportion to transpiration, then the concentration of minerals in dry matter may be predictive of TE. The mineral or ash content of vegetative tissues was found to be positively related to the transpiration ratio (1/TE) or Δ in C_3 species (Masle *et al*. 1992). For grasses, single minerals, such as silicon (Si) and potassium (K), have been evaluated as surrogates of Δ but they consistently showed lower correlations with Δ or 1/TE compared to total mineral or ash content (Walker and Lance 1991, Masle *et al*. 1992, Mayland *et al*. 1993, Merah *et al*. 1999)

According to Masle *et al.*, (1992) if mineral nutrient uptake is proportional to transpiration, then a relationship of mineral concentration with TE would be expected as follows:

$$m = z/M = Z/E \bullet E/M = xR$$

Where, m = mineral concentration in the dry matter, Z = mineral mass, M = dry mass, E = water transpired, and R = transpiration ratio (I/TE). If no recycling of minerals from tissue to the xylem stream occurred, then x would represent the average mineral concentration in the transpiration stream during the period represented by m and R, and if x were constant, the relationship of m to R would be linear.

Mineral uptake is not a function of transpiration when atmospheric conditions are the cause of variation in transpiration (Masle *et al*., 1992) and thus *x* varies with environment. However, genotypic variation in *x* may be small enough when compared under the same environment to allow prediction of TE from *m.*

Masle *et al*. (1992) have reported the existence of a positive linear relationship between total mineral concentration in vegetative tissues and either the transpiration ratio (1/TE) or Δ. The passive mineral transport driven by transpiration would be an important factor responsible for such association (Masle *et al*., 1992; Mayland *et al*., 1993). Low-TE (i.e. high Δ) genotypes may maintain a higher stomatal conductance and sustain, in turn, a higher transpiration (Richards, 1996). Such genotypes may therefore, accumulate more minerals in their tissues than high-TE genotypes.

It has been reported that other factors different from a passive mineral transport might also be involved, since the relation between Δ and mineral concentration has been found to differ under varying environmental conditions, even disappearing in severe drought trials (Masle *et al*., 1992; Mayland *et al*., 1993).

Furthermore, Febrero *et al*. (1994) proposed the, total mineral ash concentration, in mature kernels but not in green tissues, as a complementary criterion (in addition to Δ) to assess genotypic differences in grain yield of barley grown in rainfed Mediterranean environments. They reported a negative association between yield and ash concentration in kernels only under drought conditions. However, for instance, ash concentration in mature kernels could indicate the importance of retranslocation processes during grain filling.

2.5. Breeding for drought tolerance

Crop improvement in terms of production, desirable traits and resistance to drought stress is a pre-requisite in modern day agriculture. Conventional breeding for developing drought-tolerant crop varieties is time-consuming and labour intensive due to the quantitative nature of drought tolerance trait and difficulties in selection for drought tolerance (Ribaut *et al*., 1997). Combining high levels of resistance into higher yielding cultivars with acceptable market traits continues to be difficult (Holbrook & Stalker, 2003).

Breeding programs, aimed at incorporating resistance genes from wild *Arachis* relatives have proved largely unsuccessful due to genetic incompatibility. Due to limitations of conventional groundnut breeding either because of limited gene pool or the restricted range of organism between which genes can be transferred, new *omics* techniques in addition to conventional methods are needed to develop groundnut cultivars with resistance to drought stress.

2.5.1. Genomic approach

Groundnut is a polyploid with a large genome size, complete sequencing will be too expensive and labour intensive to perform with current resources. Research with molecular aspects of the groundnut genome began in the 1980s when protein and isozyme variation in *A.hypogaea* was determined to be of little use for characterizing variation within the cultivated peanut. Although large numbers of polymorphisms were detected among other species in the genus (Lu & Pickersgill, 1993; Stalker *et al*., 1994), the number of markers was too small to be routinely used in breeding programs.

2.5.2. Molecular markers

Improvement of drought tolerance is an important area of research for peanut breeding programs. Recent advances in the area of crop genomics offer tools to assist in breeding (Varshney *et al*., 2005, 2006). The identification of genomic regions associated with drought tolerance would enable breeders to develop improved cultivars with increased drought tolerance using marker-assisted selection (Ribaut *et al*., 1996).

To make selection on large populations of progeny for breeding work, the accessions must be grown out and tested for traits. This is time consuming and subject to environmental variability. The scarcity of DNA polymorphism in cultivated peanut posses a considerable obstacle in genetic mapping of peanut. The Texas Peanut Breeding and Genetics Program is working on a long-term program to integrate modern physiological and molecular methods with plant breeding, to develop peanut varieties that can be grown efficiently under reduced water inputs and high heat stress. There are RFLP (Restricted Fragment Length Polymorphism) maps of wild type x cultivar crosses but the polymorphisms are too low for a cultivated x cultivated species cross; therefore, new markers are needed (Burow *et al*., 2001).

Restricted Fragment Length Polymorphism markers also have disadvantages of using radioisotope, and results take longer to obtain than the use of PCR-based methods. Burow *et al*., (2001) study focused on finding traits useful in selecting genotypes for drought and heat tolerance. Heat stress was determined by fluorescence from cultivars grown in a high thermal stress greenhouse environment. Selections were made for drought and heat tolerance and crosses were made for further progeny evaluation. Further, they suggested that the research would entail sequencing cDNA in mapped RFLP clones to start the development of molecular markers in peanut.

A considerable number of SSR sequences have been identified from peanut genome by several research groups (Hopkins *et al*., 1999; He *et al*., 2003; Ferguson *et al*., 2004; Moretzsohn *et al*., 2005; Proite *et al*., 2007; Cuc *et al*., 2008). SSR markers developed from these repeat sequences offer promising genetic and genomic tools in peanut research. Genetic diversity of peanut germplasm has been studied in Valencia (Krishna *et al*., 2004), mini-core collection (Barkley *et al*., 2007), and in Chinese (Tang *et al*., 2007) and Japanese peanut germplasm collections (Naito *et al*., 2008) using SSR markers.

Genetic linkage maps with SSR markers have been constructed for diploid AA genome (Moretzsohn *et al*., 2005), BB genome (Moretzsohn *et al*., 2009), tetraploid AABB genome derived from a cross of cultivated with amphidiploids (Fonceka *et al*., 2009), and tetraploid AABB genome in the cultivated peanut (Hong *et al*., 2008, Varshney *et al*., 2009; Hong *et al*., 2010). Although an exceedingly large number of SSRs have been identified, the polymorphic SSR markers may not be sufficient for the construction of a saturated linkage map in the cultivated groundnut, provide enough meaningful markers for marker-assisted selection in peanut breeding programs, or sufficient coverage of important domains of the peanut genome for functional genomics research.

In contrast to the multiple morphological variations being observed among different accessions of cultivated peanut, extremely low levels of molecular polymorphism were observed between different genotypes (Kochert *et al*. 1991; Subramanian *et al*. 2000; Milla *et al*. 2005). Ferguson *et al*. 2004 showed that SSR can detect more polymorphism in peanut than RFLP, RAPD, and AFLP , indicating that

SSR markers have great potential for genetic studies of the cultivated peanut (Jiang *et al*. 2007).

2.5.3 Transposon markers

Miniature inverted repeat transposable elements (MITEs) are the predominant TEs among plant genomes (Naito *et al*., 2006; Shan *et al*., 2005; Wessler *et al*., 1995). Transposition preference for low copy genic regions underscores the role of MITEs in modulating gene expression (Wessler, 1998; Wessler, 2001; Zhang *et al*., 2000) and aiding crop evolution (Naito *et al*., 2006; Shan *et al*., 2005). In peanut, several copies of MITE have been identified using Southern hybridization. One of them when induced by diethyl sulfonate (DES) caused high oleate mutation (Patel *et al*., 2004). This MITE (later named *AhMITE1I*) was studed for its site of integration in TMV 2, a Spanish cultivar by recovering a 151 bp 5'-flanking sequence tag (FST1) (Bhat *et al*., 2008). Homology search indicated that a small region (23/24bp) of the FST1 corresponded to a genomic sequence of *A. batizocoi* (GenBank Acc. No.DX508954) of the methylation filtered library (LibID703) (Bhat *et al*., 2008). So the genomic location of *AhMITE1* as identified by the FST1 was referred to as FST1-linked site.

Presence or absence of *AhMITE1* at this pre-determined site (FST1-linked site) in various genotypes/mutants was checked by developing an *AhMITE1*-specifc PCR (Gowda *et al*., 2009). The forward primer (5' GGGAGAAGAAAAGGATGAGA 3') was designed based on the *AhMITE1* flanking sequence tag (FST1) recovered from TMV 2 genotype of peanut (Bhat *et al*., 2008), whereas the reverse primer (5' TCTCATGAAGATGCTTTGGT 3') was specific to *AhMITE1* (Patel *et al*., 2004). Amplification of a 242 base pair (bp) product indicated the presence of *AhMITE1* at FST1-linked site in the genome.

The transposition of *AhMITE1* was associated with the high-frequency origin of late leaf spot (LLS) resistant mutants (Gowda *et al*., 2009). Also, the stress-induced transposition of *AhMITE1* had bearing on mutational and evolutionary origin of botanical types.

Recently, distribution and frequency of excision of a group of the miniature inverted-repeat transposable elements (MITEs) were determined for the groundnut genome. Copy number of *AhMITE1* was very high in the genomes of *A. hypogaea*, *A.*

magna and *A. monticola*, but not in *A. duranensis*. A total of 504 *AhMITE1*s were identified from the MITE-enriched genomic libraries of *A. hypogaea*. PCR analyses were performed using primer pairs designed against both flanking sequences of each *AhMITE1*. These analyses detected polymorphisms at 169 out of 411 insertional loci in diverse groundnut lines. In subsequent analyses of 60 gamma-irradiated mutant lines, four *AhMITE1* excisions showed footprint mutations at the 109 loci tested. This study characterized *AhMITE1*s in groundnut and implicated their use as DNA markers and mutagens for the genetics, genomics and breeding of groundnut and its relatives (Shirasawa *et al*., 2011). A new set of TE markers were also developed by in silico analysis (Shirasawa *et al*., 2012).

2.5.4 Linkage map and QTL Mapping

Conventional breeding for developing drought-tolerant crop varieties is time-consuming and labour intensive due to the quantitative nature of drought tolerance and difficulties in selection for drought tolerance (Ribaut *et al*. 1997). The identification of genomic regions associated with drought tolerance would enable breeders to develop improved cultivars with increased drought tolerance using marker-assisted selection (MAS) (Ribaut *et al*. 1996).

To identify the genomic regions suitable for marker-assisted breeding strategies, it is important to establish accurate phenotyping methods, develop highly saturated molecular marker-based genetic linkage maps, and then identify QTLs (quantitative trait loci) associated with traits of interest.

Developing molecular maps for peanut is critical for identifying linkage relationships, since its complex genome structure is not well understood and defined. Furthermore a comprehensive molecular map for peanut genome is essential for the comparative mapping using the genomic information from other model legumes, and should be valuable to utilize the genetic resources within the cultivated peanuts and related species.

Several studies were conducted in the past that reported identification of QTLs for drought tolerance or related traits. For instance, in soybean, 5 QTLs were identified for WUE in an F2 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.

Hong *et al.*, (2008) has been constructed a genetic linkage map based on SSR markers in groundnut. They were used 142 recombinant inbred lines (RILs) derived from a cross between Yueyou 13 and Zhenzhuhei as mapping population in peanut (*Arachis hypogaea* L.). A total 652 pairs of genomic-SSR primer and 392 pairs of EST-SSR primer were used to detect the polymorphisms between the two parents. 141 SSR primer pairs, 127 genomic-SSR and 14 EST-SSR ones, which detect the polymorphism in parents, were selected to analyze the RILs population. Thus, a linkage genetic map which consists of 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. This was the first report of construction of a comprehensive genetic map with SSR markers in peanut (*Arachis hypogaea* L.)

Varshney *et al*. (2009) based on (TAG 24 × ICGV 86031) RIL mapping population, a framework linkage map has developed earlier for cultivated groundnut having 135 SSR loci. Shirasawa K., *et al* (2012) developed a high-density genetic linkage map, SKF2, of a total length of 2,166.4 cM consisting of 1,114 marker loci. The resultant linkage maps possess the highest number of marker loci in cultivated peanut as well as Arachis spp. This was the first time in silico polymorphism analysis has been used in peanut. They were also detected a total of 23 significant QTLs for the 15 agronomic traits.

Three mapping populations, namely, TAG 24 × ICGV 86031, ICGS 44 ×ICGS 76 and ICGS 76 × CSMG 84-1, developed at ICRISAT targeted for mapping drought tolerance. These populations were phenotyped for transpiration, transpiration efficiency, biomass, specific leaf area, pod weight, total dry matter, SPAD chlorophyll meter reading, total dry weight, shoot dry weight and harvest index traits for multiple seasons. After screening 3,215 markers, genetic maps were developed for all the three mapping populations that comprise 82 (ICGS 44 × ICGS 76) to 191 (TAG 24 × ICGV 86031) marker loci. Detailed analysis using the phenotyping data and genotyping as mentioned identified 153 main effect and 25 epistatic QTLs for drought-tolerance-related traits (Varshney *et al*. 2009c; Gautami *et al*. 2011; Ravi *et al*. 2011).

In addition to drought, markers associated with late leaf spot (LLS) and rust , two main foliar diseases of groundnut, have identified for these two diseases. In this regard, two RIL populations, namely, TAG 24 × GPBD 4 and TG 26 × GPBD 4 comprising 268 and 146 lines, respectively, were extensively phenotyped for rust and LLS resistance for 7–8 seasons (2004–2010). After screening a total of 3,097 SSR markers among parental genotypes of these two populations, a total of 209 polymorphic markers were identified for each of the two populations at ICRISAT. Genetic linkage maps were constructed for TAG 24 × GPBD 4 (188 loci) and TG 26 × GPBD 4 (181 loci). By using genotyping and phenotyping data on these populations, a total of 28 QTLs for LLS and 13 QTLs for rust explaining 10.07 to 67.8% and 2.54 to 82.96% phenotypic variation, respectively, were detected (Khedikar *et al*. 2010; Sujay *et al*. 2011).

Furthermore, a total of seven QTLs for protein content (2.54 – 9.78%), eight QTLs for oil content (1.5.10.2%) and six common QTLs for oleic and linoleic acid (3.3–9.7%) Sarvamangala *et al,* (2011), identified in TG26 × GPBD4 population.

Materials and Methods

III. MATERIALS AND METHODS

Water is the overriding abiotic factor affecting crop productivity worldwide (Boyer, 1982). Drought stress is the major constraint to groundnut (*Arachys hypogea*. L.) production and yield stability in rainfed ecosystems. However germplasm displays a diverse range of genetically complex mechanisms of drought resistance, *viz*., Drought escape (short duration), drought avoidance (e.g. deep rooting) and drought tolerance (e.g. osmotic adjustment).

Among several traits that impart drought tolerance, water use efficiency (WUE) and the ability to exploit water from deeper soils are the most relevant traits. As per Passioura's growth and yield model, WUE is a yield-determining factor. Improving WUE would reduce the water requirement for a specific yield potential and thus can save considerable amount of irrigation water. Further, deep root systems help plants maintain better water status under drought. Thus, WUE and deep root systems have significant implications for enhanced biomass and yield.

Root traits, particularly rooting depth and biomass are expected to play an important role in avoidance of drought in receding soil moisture conditions by improving water availability to the plant through more efficient extraction of available soil moisture. While root systems acquire the majority of the essential elements required for crop growth, the role that root trait selection could play in crop breeding programs has not been fully explored.

But all these traits are complex in nature. Therefore locating the parts of the genome which contributes to these drought adaptive traits by the use of specific molecular tool promises to increase our understanding of drought resistance and to produce markers for the crop improvement by marker assisted breeding (Price and Courtois, 1999). The major goal of this investigation is to phenotype the RIL population for root traits and water use efficiency and than by using already existing genotypic data and linkage map, find out major QTLs governing the root traits and water use efficiency.

3.1 Phenotyping for root traits, WUE and associated physiological parameters

Mapping population

A RIL mapping population of F14 generations was used which was developed at University of Agricultural Sciences, Dharwad, India from the crosses between TAG 24 and GPBD 4 using the single-seed descent (SSD) method. The parents showed contrasting phenotype for root traits.

Root structure

The root structure of dimension 5 ft tall, 10 ft wide, 60 ft long was prepared using cement bricks. A wall is built in the centre for dividing the structures into two halves of 5 ft wide each Soil had filled in these structures and compacted to mimic the real field condition.

The RIL population was sown in randomized block design (RBD). Seeds were treated with seed protectant before sowing. Ten seeds of each RIL were sown in a row with 25×10 cm inter and intra-row spacing, respectively. The parental genotypes (TAG 24and GPBD 4) of the RIL population were also sown after every 20 rows as control. (Plate1).

Genetic variability in biometric traits

When plants were 80 days old, the brick walls were dismantled and soil was washed off carefully using a jet of water as shown in Plate 2. The entire root was then carefully removed and root length, root volume and shoot length was determined and each samples were collected separately and oven dried at 80^{o}C for one week. Finally weights of oven dried samples were recorded. Observations recorded and different analyses performed in this experiment are described below.

a. Shoot length: The shoots were separated from the plants and the shoot length was recorded using a graduated scale

b. Root length: The roots were separated from the plants and the root length was recorded using a graduated scale (Plate 3).

Plate 1: Groundnut RIL population grown in root structures

Plate 2: Depicting the washing of roots at the time of harvest to study the root traits

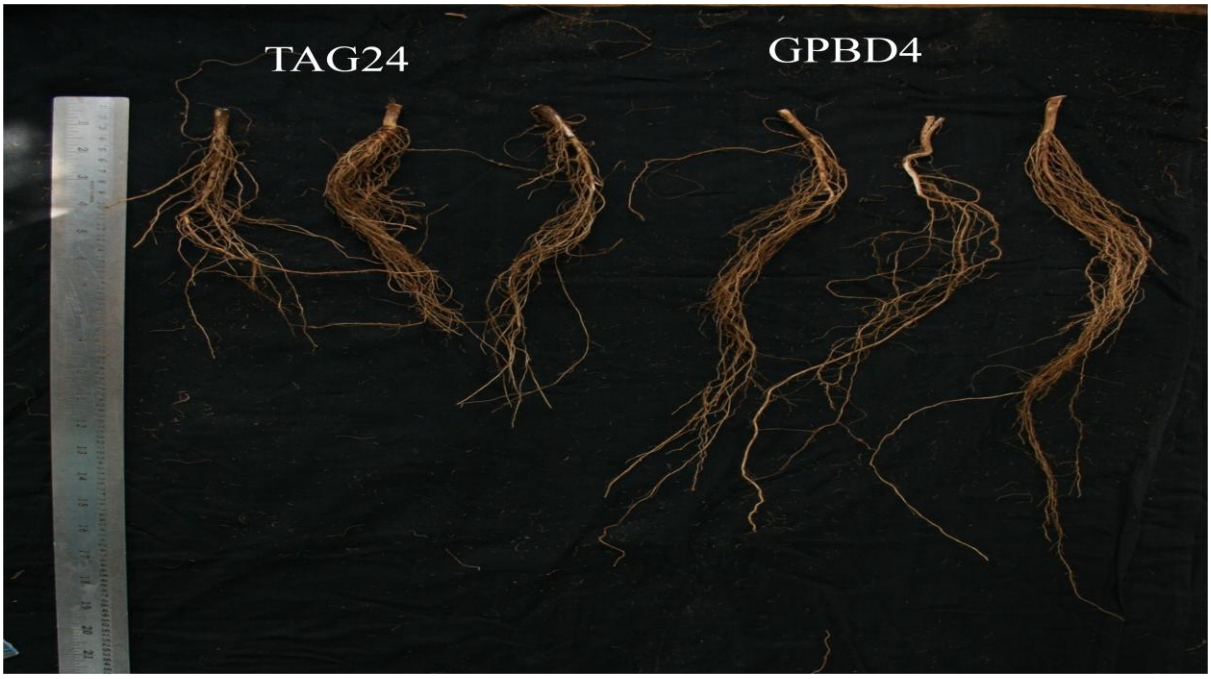


Plate 3: Genetic variability in root length between two parents of RIL population grown in root structure

Plate 4: Isotopic Ratio Mass Spectrometer (IRMS) used for the determination of Carbon Isotope Discrimination

c. Total Leaf Area: All the leaves were collected separately and oven dried at 70^{o}C for at least 48 hrs to determine the leaf dry weight. Immediately after drying, the leaves were weighed and the total leaf area was computed by multiplying leaf weight with SLA, as follows.

Total leaf area (cm^2pl^{-1}) = total leaf dry weight (gpl^{-1}) x SLA cm^2g^{-1}

d. Shoot weight: All shoots were collected separately and oven dried at 70^{o}C for 48 h to determine the dry weight for other biometric analysis.

f. Root weight: Roots were washed from the structure, then oven-dried at 80^{o}C for 48 h and dry weights were recorded.

g. Total Biomass (TBM): The biomass accumulated during the experimental period was computed by summing up leaf, stem and root dry weights.

3.1.2.4. Determination of genetic variability in WUE based on Carbon Istope Discrimination ($\Delta^{13}C$)

The leaves harvested for the measurement of SLA were powered using a ballmill and used for mass spectrometric analysis.

The $\Delta^{13}C$ of the leaf samples was determined using Continuous-Flow Isotope Ratio Mass Spectrometer (IRMS) at the National Facility for stable isotopes studies in

Biological Sciences, Department of Crop Physiology, GKVK, UAS, Bangalore shown in Plate 4. The details of the methodology are described below.

Mass spectrometric analysis

Carbon isotopic discrimination is measured as the deviation of the molar ratio between ^{13}C and ^{12}C from an international standard – PDB (Pee Dee Belmnite). Carbon isotopes are fractionated due to diffusion through stomata denoted as "a" and at the carboxylation site by RuBisCO denoted as "b". The total isotope discrimination is the deviations of these fractions from the isotopic ratio in air. Therefore the discrimination ($\Delta^{13}C$) was computed as follows:

$$\mathbf{\Delta^{13}C = \delta a - [a + (b - a)\, Ci/\, Ca]}$$

Where, a = 4.4 ‰, b = 29 ‰, Ci and Ca respectively are the CO_2 concentration inside the leaf and in the ambient air.

Alternatively, $\Delta^{13}C$ was also computed as the deviation of the isotopic composition of the plant organic sample from that of the ambient air, as follows:

$$\mathbf{\Delta^{13}C\ (‰) = (\delta a - \delta p\ ) / (1 + \delta p/1000)}$$

Where, δ_a is the isotopic composition of atmospheric air and it was considered as – 8

δ_p is the isotopic composition of the leaf samples

The IRMS installed at our center works on the continuous flow basis interfaced with an elemental analyzer (CN1112, CarloErba (CE), Italy) for sequential combustion of biomass samples to generate CO_2 gas.

Finely powdered leaf samples were accurately weighed in the range of 0.8 to 1.2 mg into silver capsules. The crimped capsules with the sample were placed sequentially in the carousel of the Autosampler. The samples were dropped at precise times along with an injection of pure O_2 into the oxidation reactor.

The Combustion (oxidation) reactor contains chromium oxide and silvered cobaltous-cobaltic-oxide in a quartz column heated to 1080^{o}C. The biomass is completely oxidized to produce CO_2, N_2O and H_2O. These gases were swept into the reduction furnace using helium carrier gas (purity 99.99 %). The reduction furnace contains reduced copper in quartz tubes heated to 680^{o}C. In this reaction, the N_2O is reduced to N_2 and the excess O_2 is absorbed. The resultant gases are then flushed through scrubbers to trap water. The pure CO_2 and N_2 gases are then passed through a GC column (5^{o}A molecular sieve). The N_2 gas elutes faster through the GC and hence gases can be effectively separated before introduction into the ion source of the IRMS.

At the ion source, CO_2 is ionized by electron impacts ionization to produce molecular radicals (CO_2). These CO_2 radicals are accelerated under the influence of high voltage potential (3000 volts) through a strong magnetic field. When accelerated radicals pass through a strong magnetic field, the radicals are deflected with the radius of deflection being proportional to the molecular mass of the radicals. Faraday cups collect these deflecting $^{12}CO_2$ and $^{13}CO_2$ and the signal is amplified and transmitted to the computer.

3.1.3. Statistical analysis

The data obtained from above experiments were analyzed using statistical software packages like MSTATC and MS EXCEL etc.

3.1.3.1 Analysis of Variance

The genotypic variability for WUE and associated physiological traits were assessed using Analysis of Variance as per Fisher's method. The level of significance was tested at

0.05 and 0.01 probability level in 'F' test. The genotypic means were compared with the critical difference values. This analysis was performed using MSTATC.

3.1.3.2 Correlation analysis

Correlation coefficients were computed to find the association among the traits using the formula by Sunder raj *et al*., (1972).

$$r_{(x, y)} = \frac{\text{Cov } (x/y)}{\sqrt{V(x) * V(y)}}$$

where, r (x, y) = correlation coefficient between x and y

V (x) = variance of x

V (y) = variance of y

Significance of coefficient of correlation were tested at (n-2) degree of freedom " t" table from Fisher's and Yates table at 5% and 1% significance. All these analysis were done using the software "STATISTICA".

3.2 Genetic studies

3.2.1 Phenotypic and genotypic coefficient of variations

The phenotypic and genotypic coefficients of variations were computed as per Burton and Dewane (1953).

$$PCV\ \% = (P/\overline{X}) \times 100$$

$$GCV\ \%= (G/\overline{X}) \times 100$$

Where,

P = Phenotypic standard deviation

G = Genotypic standard deviation

X = Grand mean of the character

PCV = Phenotypic coefficient of variation

GCV = Genotypic coefficient of variation

GCV and PCV were classified (Robinson *et al*., 1949) as given below.

0 –10 % - low

10- 20% - moderate

20% and above – high

3.2.2 Heritability

Broad sense heritability was estimated using the formula (Hanson *et al*., 1956).

$$H\ \% = (V(g) / V(p)) \times 100$$

Where,

H = broad sense heritability

V (g) = Genotypic variance

V (p) = Phenotypic variance

Heritability was categorized into following three classes (Robinson *et al*, 1949).

0-30 % - low heritability

30-60% - medium heritability

60% and above- high heritability

3.2.3 Genetic advance

Genetic advance was calculated by the formula given by Johnson *et al*, 1955

$$GA = H \times \sigma p \times k$$

Where,

H is the broad sense heritability

σ P = phenotypic standard deviation

k = selection differential which is 2.06 at 5% intensity of selection (Lush, 1949).

3.2.3 Genetic advance as per cent mean

$$\text{GA as per cent mean} = (\text{GA} / \overline{X}) \times 100$$

Where GA = Genetic advance

Genetic advance as per cent means (Johnson *et al*., 1955) as given below:

0-10% - low
10-30% - moderate

20% and above – high

3.3 Measurement of mineral ash content

Leaf samples were oven dried (70˚C for three days) and ground into fine powder using a pestel and mortar. The procedure involves ashing of the samples by complete combustion in a muffle furnace. After recording the empty weight of a silica crucible (W_{EC}), 1 gm of finely powdered leaf sample is placed in the crucible and the crucible is kept in the muffle furnace at 650˚C for 4 hours, until the sample is completely oxidised.

The crucible is cooled for a sufficient time and the weight of the crucible plus ash (W_{CA}) is recorded. The difference between the initial and final sample weight indicates the mineral ash content, which can be expressed as percentage or as mg kg^{-1}**.** The ash weight (WA) is calculated as

$$\mathbf{W_A = W_{CA} - W_{EC}}$$

3.3 Genotyping of RIL population using transposon based markers.

3.3.1 DNA samples.

As the DNA samples for all the 268 RIL population plus parents were already available (Sujay *et al*., 2011). So we directly used these DNA aliquot for the genotyping to all 268 RIL mapping population.

3.3.2. PCR amplification

The reaction mixture was set up in sterile 2.0 mL microfuge tubes. The reaction mixture volume per one reaction is as follows.

10x Taq Polymerase buffer	1.5µL
2 mM dNTPs	1.5µL
$MgCl_2$ (2mM)	0.3µL
Forward primer (5pMole/µL)	1.5µL
Reverse primer (5pMole/µL)	1.5µL
Taq Polymerase (1U)	0.3µL
Template DNA (20-25ng)	2.0 µL
Sterile water	6.4 µL
Total volume	**15.0 µL**

Standardization of annealing temperature for transposon primers

The annealing temperature standardized by using gradient PCR technique. In this method different annealing temperature (Tm +/- 5˚C) were set to each block and amplification is carried as per below mentioned reaction conditions. After standardization of annealing temperature the progeny and parents were amplified using the same protocol.

Primer amplification condition

Initial denaturation 5 min at

Denaturation 1min at

Annealing 1 min at 55˚C (primer specific) } 35 cycles (step 2 to 4)

Extension 2 min at 72˚C

Final extension 8 min at 72˚C

This takes about 2:30 hrs for PCR amplification and then analyzed the PCR amplified products by gel electrophoresis by using 3% agarose to detect polymorphism.

Preparation of agarose gel and Elecctrophorosis

3.0g of agarose was weighed and taken in a clean 250mL conical flask. To this 100 mL of 1x TBE buffer was added. [TBE buffer (0.89M Tris base, 0.02 MEDTA, 0.89M Boric acid) pH = 8].

Agarose was dissolved by heating. After agarose completely melts, it was cooled to 60^0C and 3.5 µL of ethidium bromide was added (10mg/mL) and mixed.

The ends of gel casting trays were sealed with tapes. Agarose was poured, comb was inserted and allowed to solidify. After solidification the tape on either side was removed and the gel was immersed in electrophoresis tank containing 1x TBE buffer. Then the comb was removed.

To 5 µL of DNA samples 3µL of 6x loading buffer was added, mixed well and loaded into the well. 3µL of standard uncut Lambda DNA was used as marker. (Loading buffer or tracking dye 6x – 40% sucrose, 0.025% bromophenol blue, 0.25% xylene cyanol)

Electrophoresis was carried out at 50V for 2 to 3 hours until the bromophenol blue dye migrated two-thirds of the gel. The gel tray was removed and the gel was observed under UV transilluminator and documented using Herolab Gel Doc system Belgium.

Genotyping of the RIL population

Genotyping data with 188 SSR markers were already existing(Sujay, et al., 2011). In addition, a set of 24 transposon based markers (Shirasawa, et al., 2011) were used to screen parental genotypes of the mapping populations. Subsequently, polymorphic markers were identified and used to genotype the RILs of the populations.

Scoring of bands

The bands showing polymorphism between the parents were taken for analysis. The parent 1(P1) (TAG 24) type of bands scored as 'A', parent 2 (P2) (GPBD 4) type of band scored as 'B' and missing band scored as '0' in the population.

Construction of genetic maps

Genotyping data for 188 SSR polymorphic loci were already available (Sujay, *et al.,* 2011). The genetic linkage map with these SSR markers was made available for the present study. In addition to these, genotyping data obtained for the polymorphic marker loci for seven transposon markers on the mapping population were used for linkage map construction using Mapmaker/EXP v.3.0 (Lander et al. 1987; Lincoln et al. 1992).

Quantitative trait loci (QTL) analysis

For identification of QTL, the composite interval mapping (CIM) approach (Zeng 1994) was employed using WinQTL Cartographer,version 2.5. CIM was performed using Model 6, scanning intervals of 2.0 cM between markers and putative QTL with a window size of 10.0 cM. The number of marker cofactors for the background control was set by forward–backward stepwise regression. Automatically ''Locate QTLs'' option was used with a minimum of 5 cM between QTL to define a QTL region and, if the peak's distance was less than 5.0 cM, then the highest peak was considered to locate QTL.

Single marker analysis (SMA) was also performed using WinQTL Cartographer to confirm markers linked to the trait.

Results

IV RESULTS

Groundnut is an important commercial crop exploited for its oil content and also as a source of proteins and carbohydrates. It is generally grown under rainfed conditions in India where water availability severely constraints productivity. It is well documented that the annual average productivity of India is lower than most other groundnut growing countries. Therefore understanding the drought tolerance deserves the greatest emphasis in addressing groundnut improvement programmes to attain food and nutrient security.

Drought, which is a complex phenomenon, requires a highly focused and concerted approach for crop improvement based on the physiological strategies involved in adoption for drought, thereby deriving breeding technique for improving groundnut productivity under water limited conditions.

Plant breeding efforts significantly contributed towards improving crop productivity. Because of a narrow genetic variability in yield, a high G X E interaction and low heritability in yield *per-se,*a further improvement in crop productivity is possible only through introgressing relevant physiological traits. Among several such traits, the ability to harness water from deeper soil profiles, efficient use of water for biomass production, water conservation strategies associated with stomatal control are perhaps the most relevant traits that deserve exploitation. However, these are complex multigene regulated traits and hence breeding for them would be difficult. Hence, identifying QTL regions conditioning these traits is expected to strongly augment the breeding efforts for crop improvement.

Towards this end, a mapping population consisting of Recombinant Inbred Lines (RILs) developed from the cross between TAG-24 and GPBD-4 were characterized for phenotypic variations in several physiological traits and genetic polymorphism using transposon based markers.

The results of these experiments leading to the identification of QTLs governing the variability in WUE, root traits and related parameters are described in this chapter.

4.1 Phenotyping for root traits, WUE and associated physiological traits

Difference in WUE, root traits and associated physiological traits between the parents

GPBD4 showed higher root length and root biomass compared to TAG24. So it could mine more water from the deeper soil layer. Because of that it could transpire more water, therefore accumulate high biomass.

GPBD4 showed higher leaf weight, shoot weight, shoot length, SLA, total leaf area, compared to TAG24, therefore high dry matter production. TAG24 was having higher mineral ash (per gram of leaf weight) content compared to GPBD 4. But, because of having higher leaf weight and total biomass, GPBD4 showed higher total mineral ash content compare to TAG24.

The two parents did not differ significantly for Carbon Isotope Discrimination ($\Delta^{13}C$), often regarded as a time averaged surrogate for WUE which were 18.76 ‰ for TAG24 and 18.31‰ for GPBD4 (Table 3).

4.1.1 Phenotypic Evaluation of the mapping population

Phenotypic variations for biometric traits in the RIL population of groundnut

Analysis of variance was carried out to see the significance level of genetic variability for root traits, WUE and various associated physiological traits among the RIL population derived from the cross of TAG24×GPBD4 (Table 4).

Leaf traits: All the leaf traits like, total leaf area, total leaf weight and SLA showed significant variability among the RILs. The total leaf area varied from 657.6$cm^2.plant^{-1}$ to 3453.2 $cm^2.plant^{-1}$ with a mean of 1664.6 $cm^2.plant^{-1}$. The total leaf weight also showed a significant variability ranging from 3.05 $g.plant^{-1}$ to 15.4 $g.plant^{-1}$ with a mean value of 8.23 $g.plant^{-1}$. The ratio of leaf area to leaf weight referred to as Specific Leaf Area (SLA) varied between 138.40cm2 g^{-1} to 277.76cm^2g^{-1} with a mean value of 203.75 cm^2 g^{-1} representing significant genetic variability (Table 5).

Table 3: Genetic variability in various physiological traits between the parents.

	$\Delta^{13}C$	LWT	MASH	RL	RS	RWT	SHT	SLA	SWT	TDM	TLA	TMASH
TAG24	18.76	7	0.1202	29.9	0.07	0.91	28.03	200.55	10.35	18.54	1673.28	1.94
GPBD4	18.31	8.3	0.1033	38.17	0.06	1.3	31.77	219.17	12.11	22.75	2179.15	2.14
Significance	NS	*	*	**	NS	**	NS	*	NS	*	**	*

Note: NS = Non significant, S* = Significant at 5%, S** = Significant at 1%

LWT - Leaf weight, MASH - Mineral ash (unit leaf weight basis) , RL - Root length, SWT- Shoot weight, TMASH- Total mineral ash, RWT- Root weight, RS – Root to shoot ratio, TDM- Total dry matter, SHT- Shoot length, SLA- Specific leaf area, TLA- Total leaf area

Table 4: ANOVA (MSS) for various physiological traits in TAG 24×GPBD 4 mapping population

SOV	$\Delta^{13}C$	LWT	MASH	RL	RS	RWT	SHT	SLA	SWT	TDM	TLA	TMASH
Genotypes*	2.1712	14.65	0.00073	109.2	0.0009	0.208	75.08	2021.8	20.727	64.49	7.19E+05	0.955
res/error	0.05	0.13	0	0.37	0	0.02	0.31	39.18	0.16	0.28	431.53	0.03
LSD	1.39	1.95	0.01	4.11	0.01	0.17	2.53	20.7	1.48	6.72	204.68	0.28

LWT - Leaf weight, MASH - Mineral ash (unit leaf weight basis) , RL - Root length, SWT- Shoot weight, TMASH- Total mineral ash, RWT- Root weight, RS – Root to shoot ratio, TDM- Total dry matter, SHT- Shoot length, SLA- Specific leaf area, TLA- Total leaf area

Note: *- All trait mean sum of square of genotypes were significant at 0.01%

Table 5: Genetic variability for the root traits, water use efficiency and associated physiological traits in TAG24×GPBD4 RIL population of groundnut

TRAIT	MEAN	MIN	MAX	LSD	SD
$\Delta^{13}C$	18.86	16.68	22.96	1.39	0.85
LWT	8.23	3.05	15.4	1.95	2.22
TLA	1664.55	657.55	3453.23	204.68	489.5
RL	34.26	19.25	55.33	4.11	6.06
RS	0.06	0.03	0.12	0.01	0.01
RWT	1.06	0.46	2.07	0.17	0.26
SHT	28.58	14.5	47	2.53	5.02
SLA	203.75	138.4	277.76	20.7	25.96
SWT	9.33	3.35	17.73	1.48	1.81
TDM	19.14	8.4	36.38	6.72	4.65
MASH	0.11	0.08	0.18	0.01	0.02
TMASH	2.13	1.04	4.38	0.28	0.57

LWT - Leaf weight, MASH - Mineral ash (unit leaf weight basis) , RL - Root length, SWT- Shoot weight, TMASH- Total mineral ash, RWT- Root weight, RS – Root to shoot ratio, TDM- Total dry matter, SHT- Shoot length, SLA- Specific leaf area, TLA- Total leaf area

Note: SD =Standard deviation, LSD=Least Square Deviation

Shoot traits: Parameters related to shoot growth such as plant height and shoot weight were recorded on 80 DAS. Shoot weight varied from 3.35g plant^{-1} to a maximum of 17.73 g plant^{-1} with a mean value of 9.33g.palnt^{-1}. The mean plant height of RILs was 28.58 cm, ranging from 14.50 cm to 47.00 cm representing a significant variability.

Root traits: Water acquisition from deeper soil profile is a function of canopy leaf area and the ability of root traits to harness water from the soil. One of the major objectives of the investigation was to access genotypic variability in several parameters associated with the roots. The RILs had the mean root length of 34.26cm where as the mean for root weight was 1.06g plant^{-1}.The lowest root length was 19.2cm and the longest root was 55.3cm, depicting a large variability in the root length among the RILs. The lowest root weight observed was 0.46gm and the maximum root weight was 2.07 g plant^{-1} showing a large variability in the RIL population for root biomass (Table).

Total Biomass: The total biomass accumulated among the RILs showed a significantly large variability with a minimum of 8.40 g.plant^{-1} to a maximum dry matter of 36.38 g.plant^{-1}. The mean value for total biomass accumulated was 19.14 g plant^{-1}(Table 4).

4.1.2 Stable Isotope studies

Plants discriminate against stable isotope of carbon ($\Delta^{13}C$) during photosynthesis. This discrimination has been shown to occur during the diffusion of CO_2 through the stomata and during the primary carboxylation in the chloroplast. Since these processes also influence variation in WUE, a strong inverse relationship between $\Delta^{13}C$ and WUE is expected. This technique is widely being used as a time averaged surrogate for WUE.

$\Delta^{13}C$ was determined in the dried leaf sample using IRMS. The maximum value of $\Delta^{13}C$ measured was 22.96‰, while the minimum value observed was 16.68‰ showing a significantly high genetic variability among the RIL population for $\Delta^{13}C$, therefore variability in WUE as $\Delta^{13}C$ and WUE are inversely related. The mean value for $\Delta^{13}C$ was 18.86‰ (Table 4).

4.1.3 Study of genetic variability in RIL population for Mineral Ash Content

During the water absorption by the roots to fulfil the demand for transpiration, roots also absorb the mineral nutrients in addition to water. Therefore more

transpiration leads to more absorption of water from soil, thereby more accumulation of mineral nutrients. Transpiration efficiency (TE) is the weight of dry matter produced per unit of transpiration. Therefore, the mineral ash content per unit leaf biomass as well as to total mineral ash content was determined in the 268 recombinant inbred line of groundnut.

The mineral ash content per gram of leaf weight varied from a minimum value of 0.08 $g.g^{-1}$ leaf weight to a maximum value of 0.18 $g.g^{-1}$ leaf with a mean value of 0.11$g.g^{-1}$leaf. The total mineral ash content is the amount of minerals accumulated in total biomass. The difference for total mineral ash content among RIL population varied from a minimum of 1.04 g. $plant^{-1}$ to a maximum of 4.38 $g.plant^{-1}$ with a mean value of 2.13 $g.plant^{-1}$ showing a significantly high genetic variability among RIL population (Table 4).

4.2 Physiological interpretation of biometric traits

Several biometric traits were recorded in accordance with Passioura yield model. Total biomass is a function of water transpired and efficiency with which plant use water fir biomass production. Two parameters recorded as a reflection of water use, namely- Total mineral ash and root traits and water use efficiency was measured using $\Delta^{13}C$ as a surrogate. These parameters determine the variability in total dry matter to various extents.

A thorough analysis was made to access the dependence of TDM on these traits. Total leaf area was by far the strongest determinant of the TDM. The relationship illustrated in Fig.1D, emphasized the dependence of TDM on total leaf area among 268 RILs. Further a strong correlation between total mineral ash and TDM (Fig.1A) was also noticed. Mineral ash content increased as a function of transpiration; hence reiterate the relevance of water use in producing biomass. However, there was no deserevable relationship between $\Delta^{13}C$ and TDM (Fig. 1C), which enhance the scepticism about the use of WUE in determining biomass in groundnut. It is apparent that a trait that has larger variance would contribute more to TDM. Thus leaf area followed by total mineral ash significantly contributed to TDM compare to $\Delta^{13}C$. A strong correlation between total mineral ash and root weight was observed (Fig.1B).

To analyse these further, genotype with similar leaf area were selected. Among these similar leaf area types, the relationship between $\Delta^{13}C$ and TDM was significantly increased (Fig.2C). Since genotypes with similar leaf area were identified, contribution of leaf area to TDM decreased compared to all genotypes (Fig. 2A). However, the regression between TDM and mineral ash was positive and significant (Fig.2B).

Further, a few RILs with relatively higher leaf area among the similar leaf area group were selected to see the association of various parameters to TDM. Since similar leaf area types were selected, they differed in biomass; there is no regression between leaf area and TDM (Fig. 3C). However, the regression between $\Delta^{13}C$ and TDM significantly increased (Fig. 3A). This suggests that increased WUE can contribute to growth rate only when leaf area is optimized. Total mineral ash continues to show a positive relationship with TDM illustrating the relevance of water used by the crop (Fig. 3B).

To examine this further, the RIL with highest leaf area were selected. This category of RILs recorded a mean leaf area of 28.7cm^2.$plant^{-1}$ with an average biomass of 28.7g.$plant^{-1}$(Table 6). It was observed that among these high leaf area types, total leaf area was significantly associated with TDM (Fig. 4C). However, $\Delta^{13}C$ also showed a significant regression with TDM (Fig.4B). Further emphasizing the fact that chloroplast capacity contributes significantly to TDM when canopy cover is maximized. Mineral ash also showed significant correlation with total dry matter (Fig.4A).

4.3 Genetic Analysis for various physiological traits

4.3.1 Estimation of GCV, PCV, GA and h^2 for various physiological traits

The chief difference between quantitative and qualitative traits lies in the degree to which they are affected by the environment. Qualitative characters are little or not at all affected by the environment while quantitative characters are considerably affected. The environmental factors bring about these differential expressions of the phenotype through its effect on various genes governing the trait. When the number of genes controlling a trait increases, the environmental modulation also increases leading to a continuous variability in the trait.

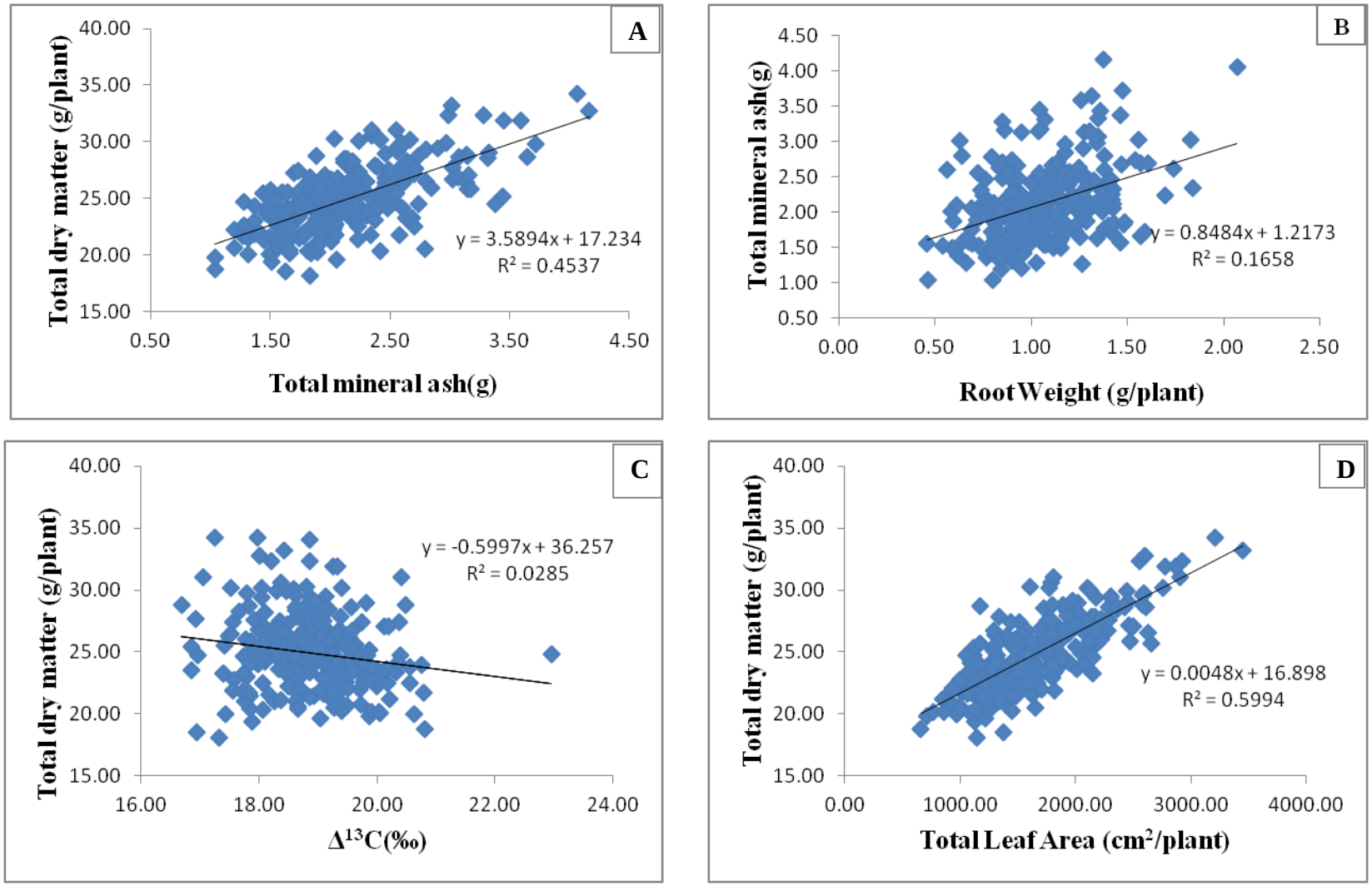


Fig 1: Dependence of total dry matter (TDM) on (A) total mineral ash, (B) $\Delta^{13}C$ and (C) total leaf area, among entire population

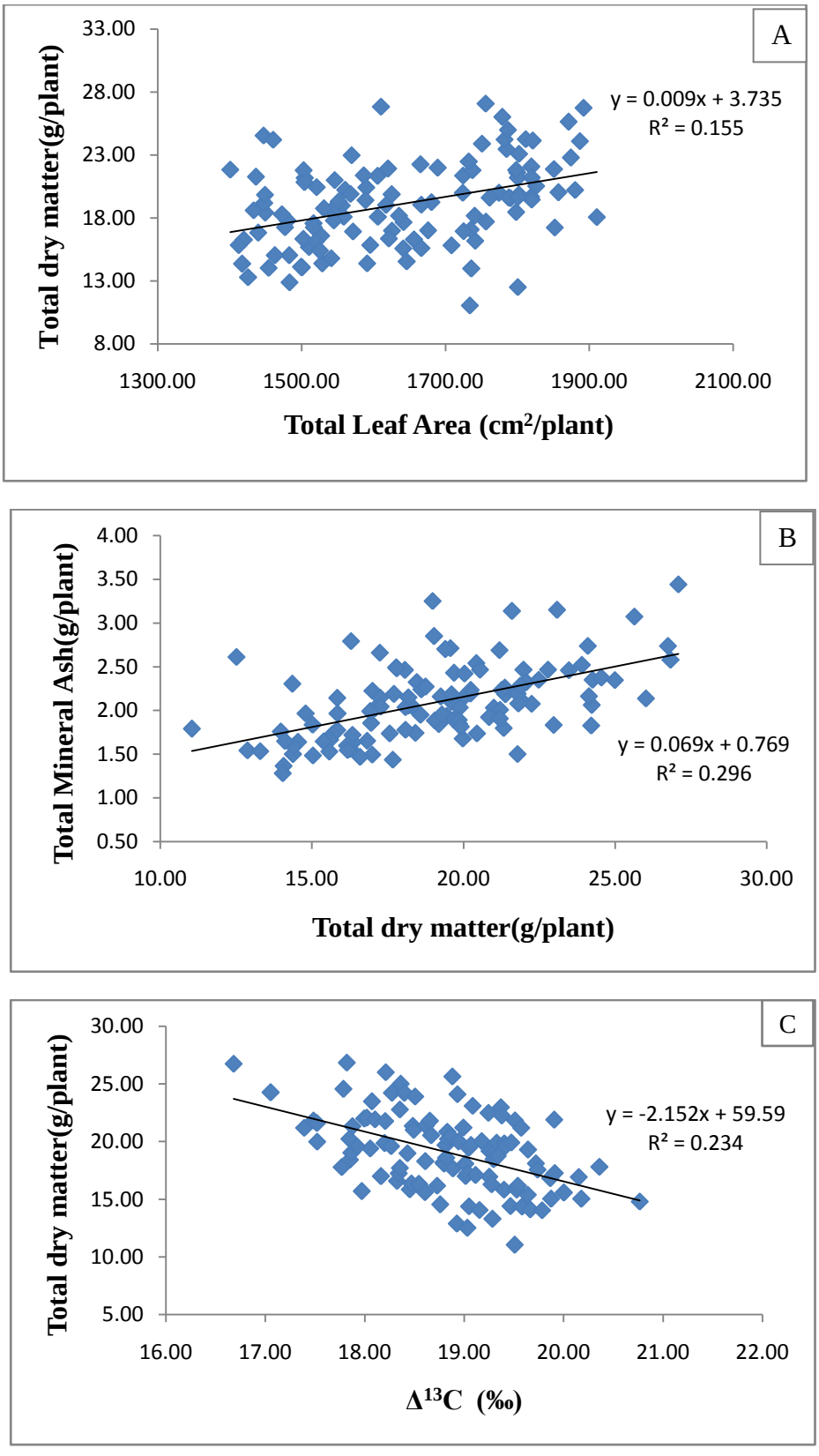


Figure 2: Dependence of TDM on (A) total Leaf Area, (B) mineral ash and (C) $\Delta^{13}C$ among the similar leaf area type RILs selected from entire population.

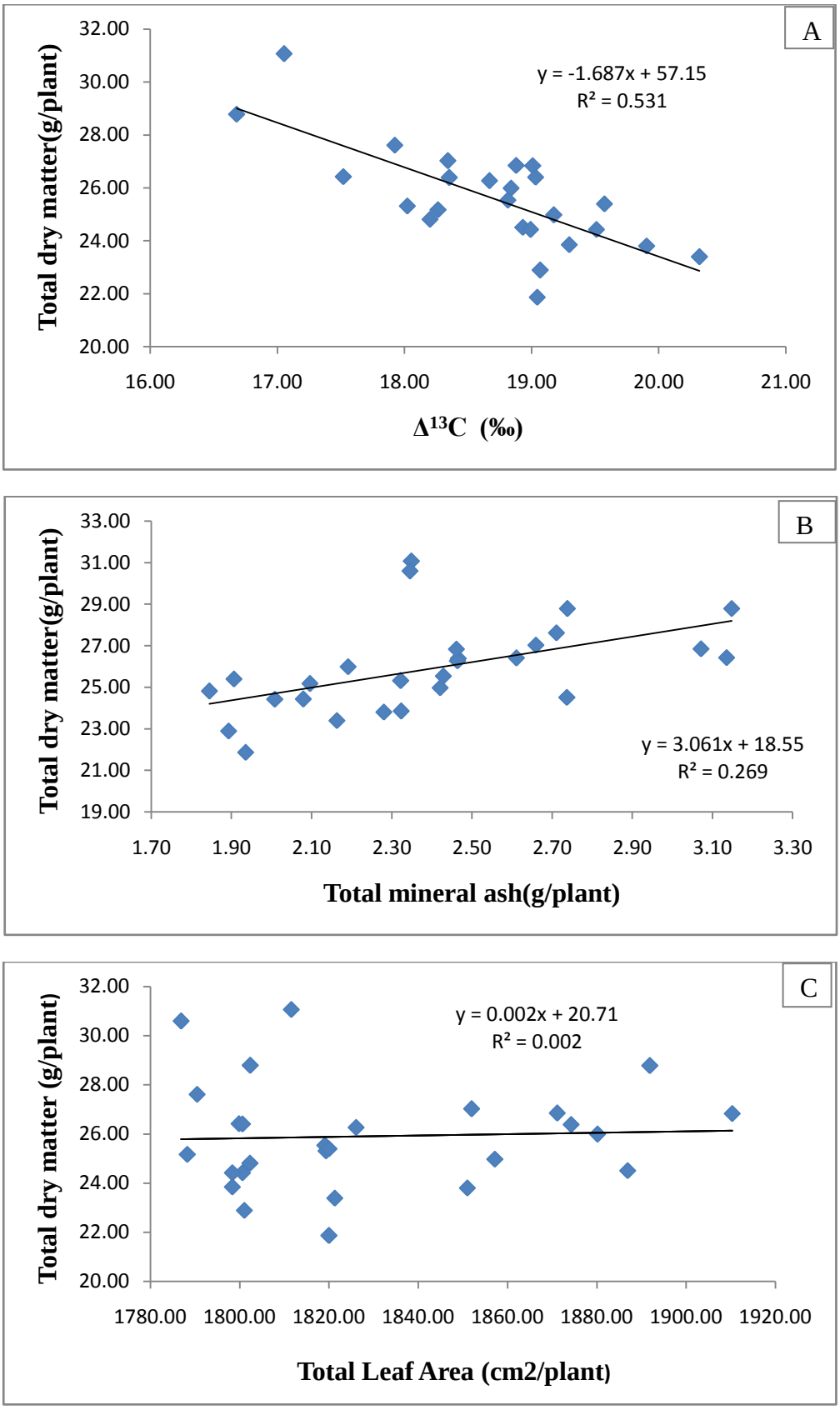


Figure 3: Dependence of TDM on (A) $\Delta^{13}C$, (B) total leaf area and (C) mineral ash, among the higher leaf area type RILs selected from similar leaf area type RILs.

Table 6: Different physiological parameters for recombinant inbred lines with highest leaf area selected from entire population

RIL	TLA	$\Delta^{13}C$	ASH	TDM	RIL	TLA	$\Delta^{13}C$	ASH	TDM
RIL-170	2186.0	19.3	1.8	25.7	**RIL-252**	2174.5	18.0	2.8	29.4
RIL-243	2100.9	19.5	1.9	25.4	**RIL-118**	2628.3	18.4	2.8	26.5
RIL-168	2104.1	20.1	2.0	27.1	**RIL-132**	2304.3	18.9	2.9	29.4
RIL-133	2224.7	18.6	2.0	26.0	**RIL-72**	2134.6	18.3	3.0	29.9
RIL-20	2359.4	19.2	2.1	28.5	**RIL-40**	2554.2	18.2	3.0	32.3
RIL-58	3083.7	18.6	2.2	28.3	**RIL-163**	3453.2	18.4	3.0	33.2
RIL-54	2654.3	18.6	2.2	25.7	**RIL-158**	2190.3	19.6	3.0	26.7
RIL-154	2111.6	18.6	2.2	30.1	**RIL-166**	2615.1	18.6	3.1	28.7
RIL-88	2142.7	18.7	2.4	27.8	**RIL-11**	2474.1	19.2	3.2	25.8
RIL-143	2102.3	18.1	2.4	30.2	**RIL-10**	2498.8	20.2	3.2	27.1
RIL-188	2280.4	18.4	2.5	26.7	**RIL-8**	2125.7	18.7	3.2	25.9
RIL-106	2108.6	19.1	2.5	27.5	**RIL-193**	2927.0	18.9	3.3	32.3
RIL-169	2587.6	18.6	2.6	29.5	**RIL-121**	2287.1	18.6	3.3	28.6
RIL-167	2244.0	18.8	2.6	26.8	**RIL-86**	2459.3	19.0	3.3	29.0
RIL-190	2306.7	19.1	2.6	29.5	**RIL-82**	2866.1	19.3	3.5	31.9
RIL-84	2166.5	18.6	2.6	27.8	**RIL-122**	2781.1	19.3	3.6	31.9
RIL-110	2442.0	18.5	2.6	29.9	**RIL-68**	2365.3	19.6	3.7	28.6
RIL-123	2292.6	19.0	2.6	27.6	**RIL-180**	2590.1	17.8	3.7	29.8
RIL-114	2762.7	19.4	2.7	30.2	**RIL-149**	3145.2	18.0	4.1	34.2
RIL-242	2464.5	19.0	2.7	27.1	**RIL-116**	2603.4	18.0	4.2	32.7

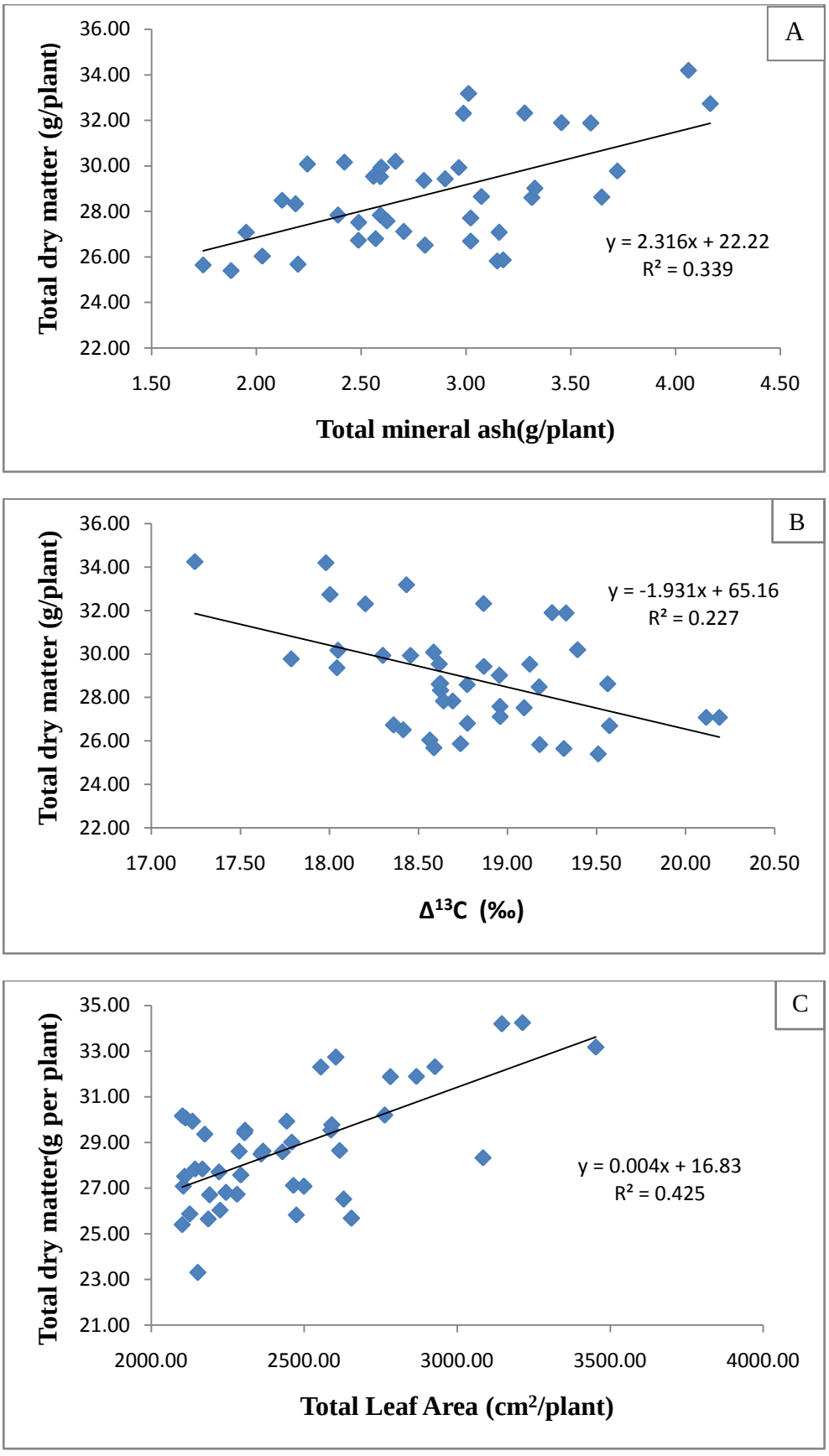


Figure 4: Dependence of TDM on (A) mineral ash, (B) $\Delta^{13}C$ and(C) total leaf area among the highest leaf area type RILs selected from population

The distribution of the variation among the mean for each trait was computed to ascertain the quantitative behaviour of the traits. Water use efficiency measured by $\Delta^{13}C$, root traits like root length and root weight, canopy leaf area, total biomass etc. showed a typical normal distribution for these parameters are illustrates in figure 5, and the skewness and kurtosis values are given in Table 7.

All parameters except SLA, showed leptokurtic distribution. $\Delta^{13}C$, MASH, TMASH and root length density had sharper peaks with relatively low skewness (Table 7). Shoot weight and root weight had lower kurtosis value indicating flatter peaks with heavy tails, which is a characteristic feature of traits governed by larger no of genes. Total biomass and total leaf area also had moderately broad peaks as evidenced by kurtosis values. These results clearly demonstrated that the traits that have relevance for drought adaptation are controlled by large number of genes and have such traits display quantitative inheritance.

In crop improvement programme we select plants based on their phenotype. The effectiveness of selection would largely depend on the proportion of phenotype due to the genotype. Therefore to distinguish the extent of genotype and environment effects on the phenotype, the genetic parameters like genotypic coefficient of variation (GCV), and phenotypic coefficient of variation (PCV) calculated for root traits, WUE and associated physiological traits. Success in introgressing a trait in crop improvement programme depends on the availability of exploitable genetic variability of the trait. Based on the genotypic and phenotypic coefficient of variation, the heritability and genetic advancement parameters were computed.

4.3.2 Genetic parameters for biometric traits

Heritability denotes the phenotypic variance that is due to genotype. All the traits studied showed a remarkably high heritability ranging from 86.29% for mineral ash content to as high as 99.94% for total leaf area. All the parameters showed the heritability of more than 90%. (Table 7).

$\Delta^{13}C$, root length, plant height and SLA showed a low GCV ranging from 4.46% for $\Delta^{13}C$ to 17.58% for root length whereas all other parameters showed a

Table 7: Genetic parameters for physiological traits among the RIL population of TAG 24 × GPBD 4

TRAIT	GCV	PCV	H^2	GAM	KURTOSIS	SKEWNESS
Δ13C	4.46	4.51	97.61	9.07	1.465	0.279
LWT	26.74	26.86	99.08	54.83	0.198	0.53
MASH	40.36	43.45	86.29	77.23	3.486	1.396
RL	17.58	17.61	99.66	36.15	0.36	0.215
RS	87.11	92.07	89.51	169.77	0.233	0.569
RWT	23.86	24.84	92.27	47.21	0.352	0.434
SHT	17.47	17.51	99.59	35.92	0.684	0.29
SLA	12.62	12.74	98.06	25.74	-0.081	0.1025
SWT	28.07	28.18	99.23	57.6	0.193	0.483
TDM	24.17	24.22	99.56	49.68	0.568	0.605
TLA	29.4	29.41	99.94	60.54	0.79	0.818
TMASH	26.01	26.49	96.4	52.61	1.395	0.997

Note: GCV- Genetic Coefficient of Variation (%)
PCV - Phenotypic Coefficient of Variation (%)
H^2 - Broad sense heritability (%)
GAM - Genetic Advancement as % of mean.

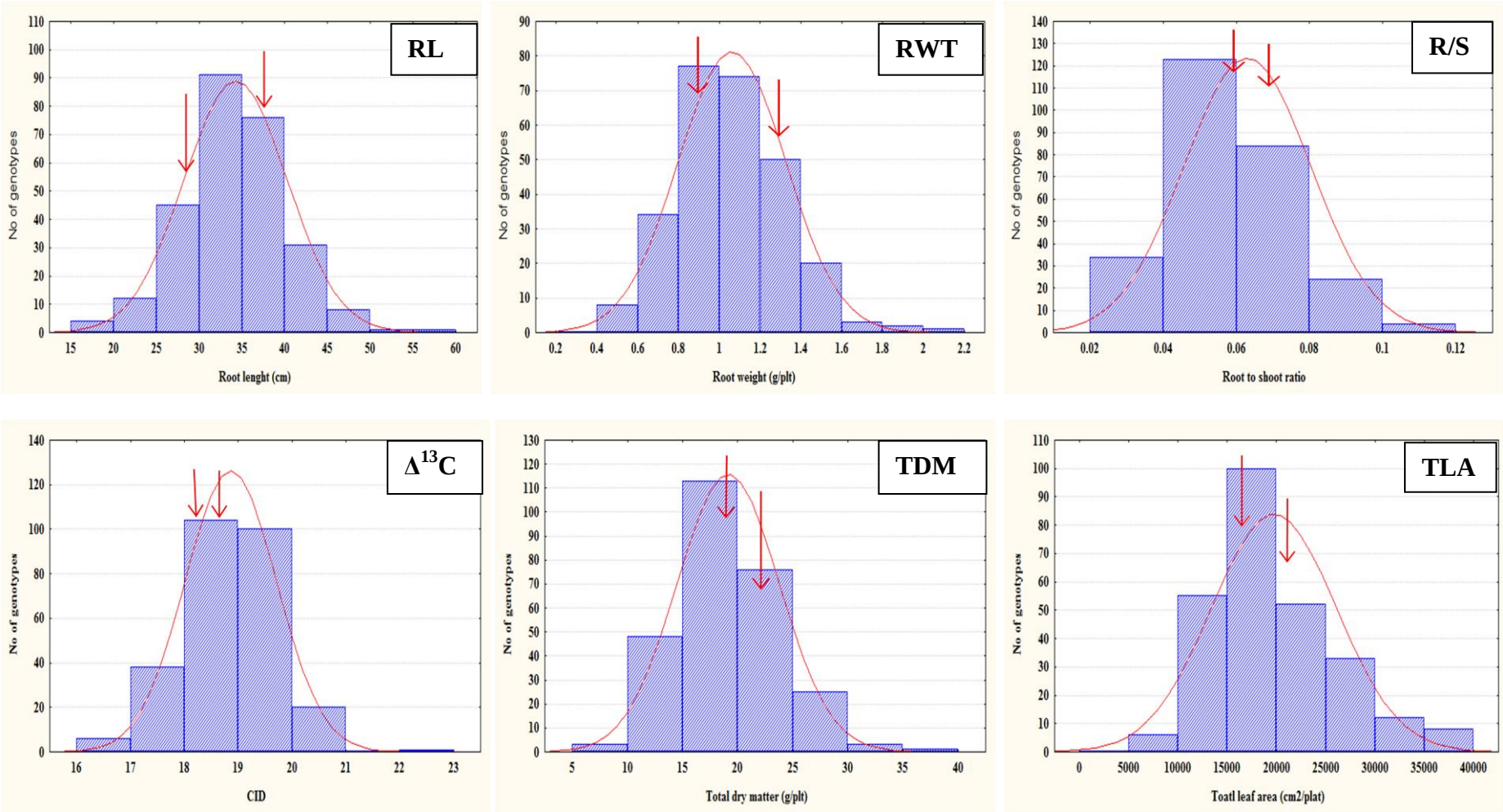


Fig. 5A: Figure showing the continuous variations for various physiological traits among RILa of Groundnut.

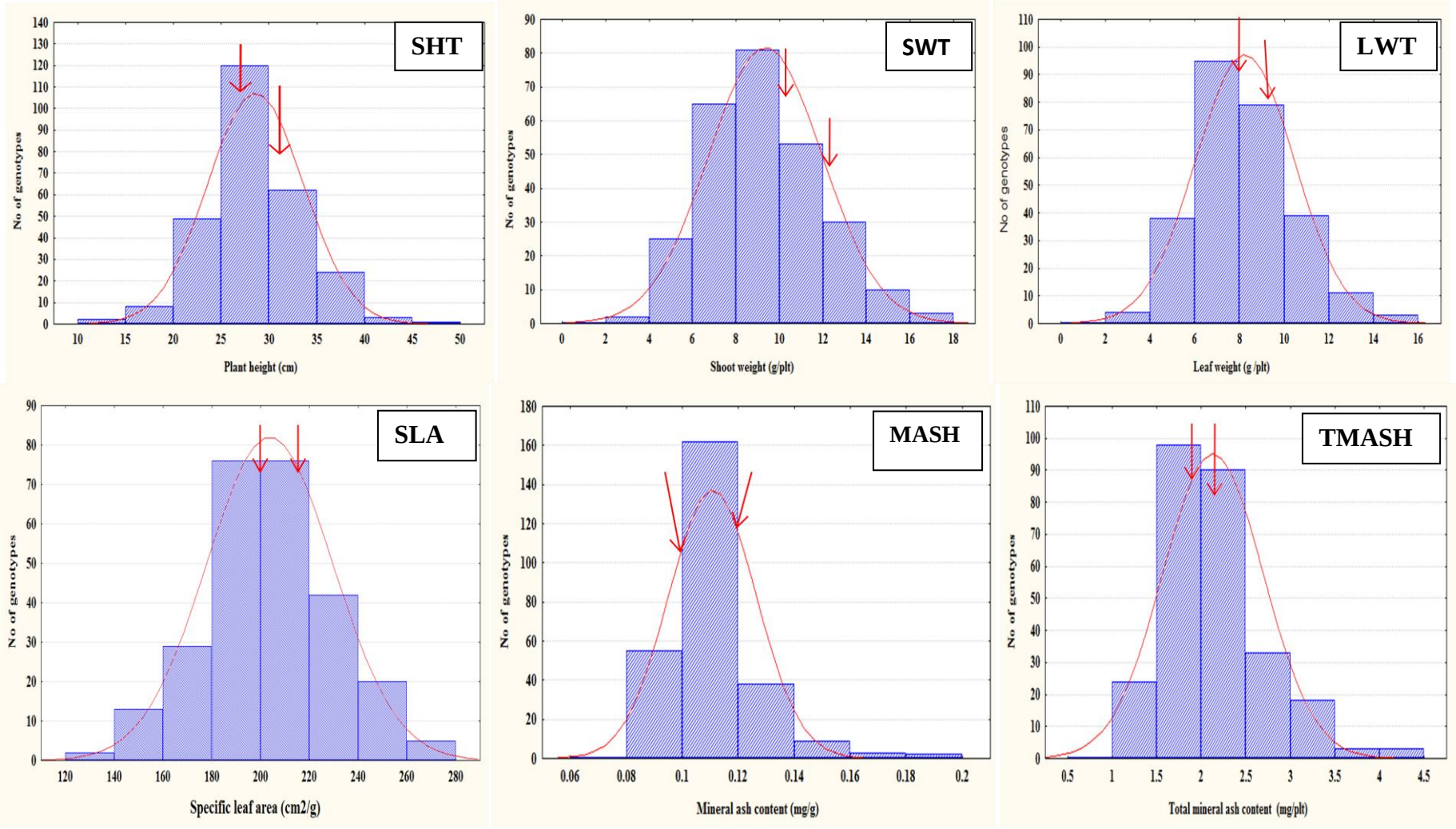


Fig. 5B: Figure showing the continuous variations for various physiological traits among RILs of Groundnut.

moderately high GCV ranging from 23.86% for root weight to 40.36% for mineral ash content (unit weight basis). Root to shoot ratio show remarkably high GCV value of 87.11% (Table 7).

The PCV value varied in proportion to GCV for all the traits, i.e. all the parameters showed same range of variability for GCV and PCV. $\Delta^{13}C$, root length, plant height and SLA showed a low PCV ranging from 4.51% for $\Delta^{13}C$ to 17.61% for root length whereas all other parameters showed a moderately high PCV ranging from 24.84% for root weight to 43.45% for mineral ash content (unit weight basis). Root to shoot ratio show remarkably high GCV value of 92.07%.

Genetic advancement as percent of mean(GA) was low for $\Delta^{13}C$ while all other parameters showed a high GA ranging from 25.74% in SLA to 77.23% in mineral ash content (MASH).

4.4 Molecular characterization

4.4.1 Transposon based marker analysis:

The genomic DNA of two parents TAG24 and GPBD 4 were screened for polymorphism using 24 transposon based markers. Out of 24, 7 markers showed polymorphism between the parents (Plate 5), which were selected for progeny screening.

By using these seven polymorphic primers, the RIL population were screened and the pattern of segregation among the RIL population for these markers is showed in Plate 6. These genotypic data were used to put these marker loci on existing linkage map which was already constructed (Sujay, et al, 2011) using 188 SSR marker loci (Fig. 6)

Out of seven, five markers linked to chromosome number 2, 4 and 8. One on chromosome number 2, one on chromosome number 8 and three on chromosome number 4. These transposon based markers were located on the telomeric regions of the chromosome. Since they are transposable elements and the telomeric regions are rich in the transposon, therefore they mainly positioned at the edge of chromosomes (Fig.7).

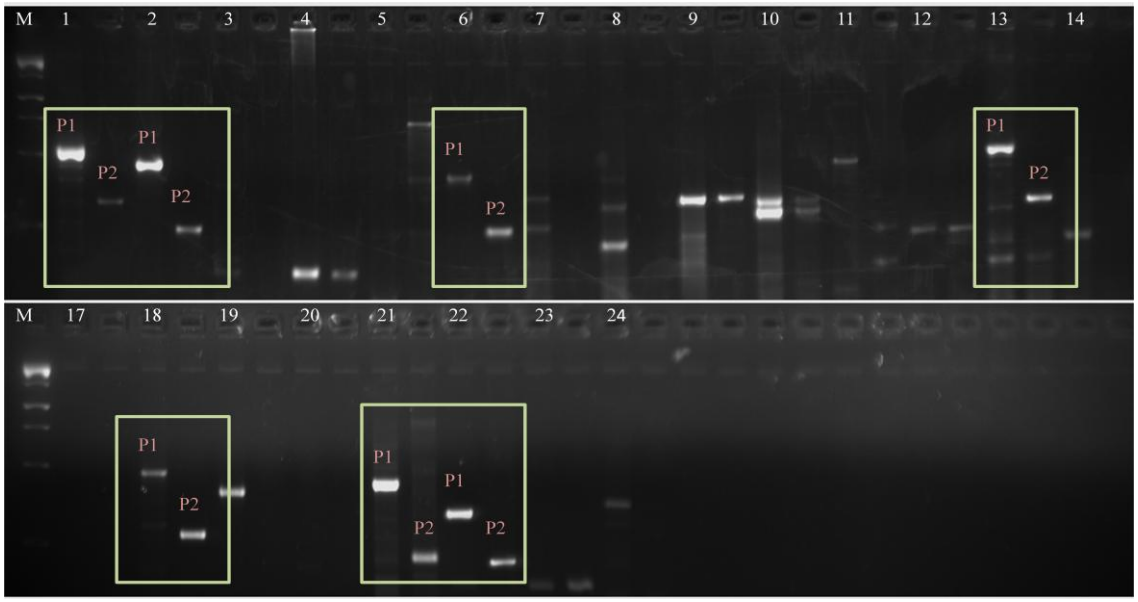


Plate 5: Transposon based marker profile showing the parental polymorphism

Note : M- indicates the Ladder (100 bp), P1 & P2 indicates TAG24 and GPBD4

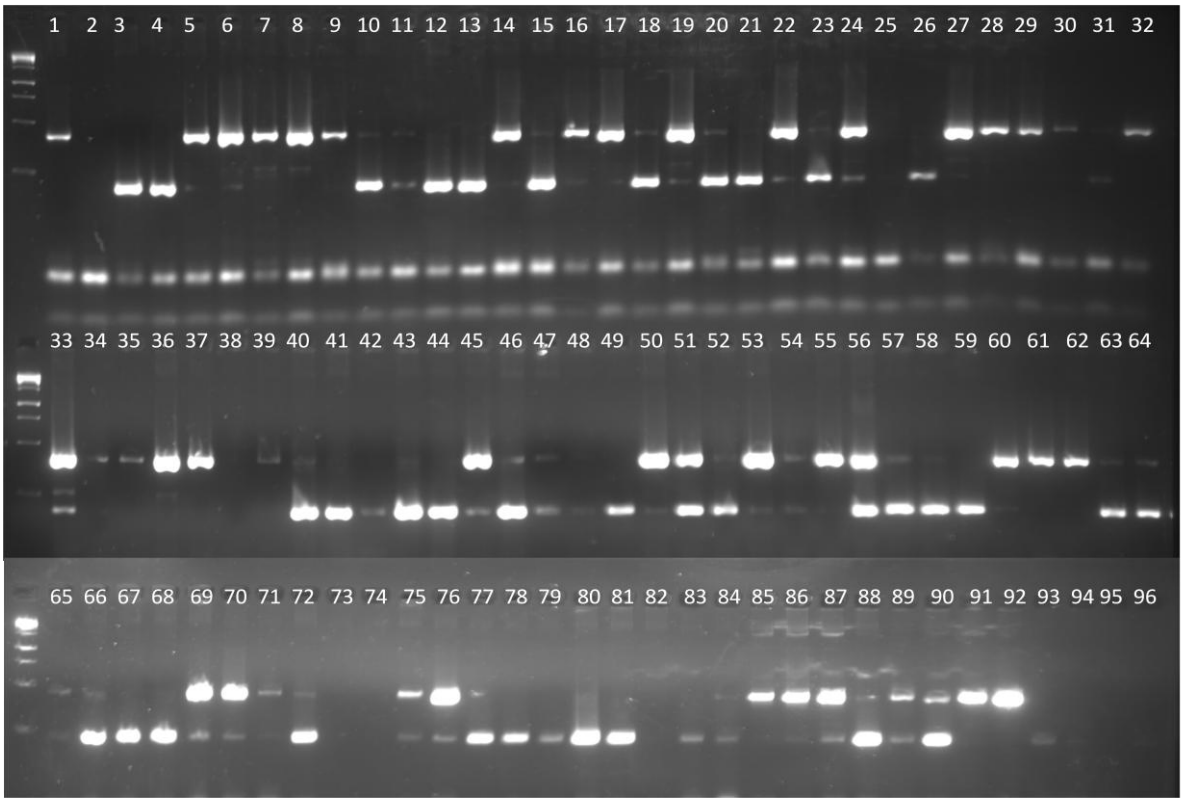


Plate 6: A representative Transposon based marker profile of the marker AhTE222 for RILs of TAG24×GPBD4.

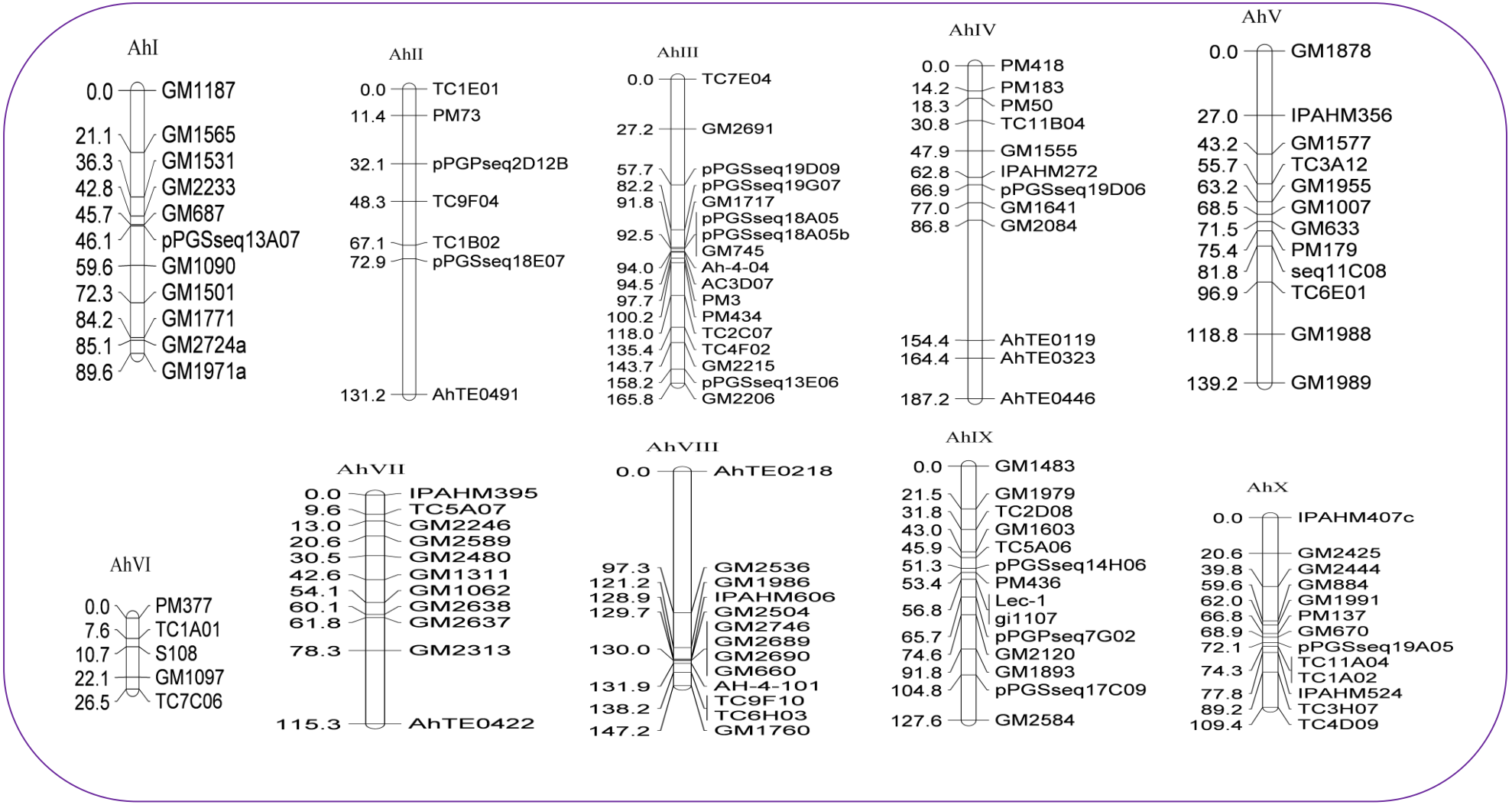


Figure 6A: Molecular Genetic Linkage Map of Groundnut RIL population derieved from cross b/w TAG24×GPBD4

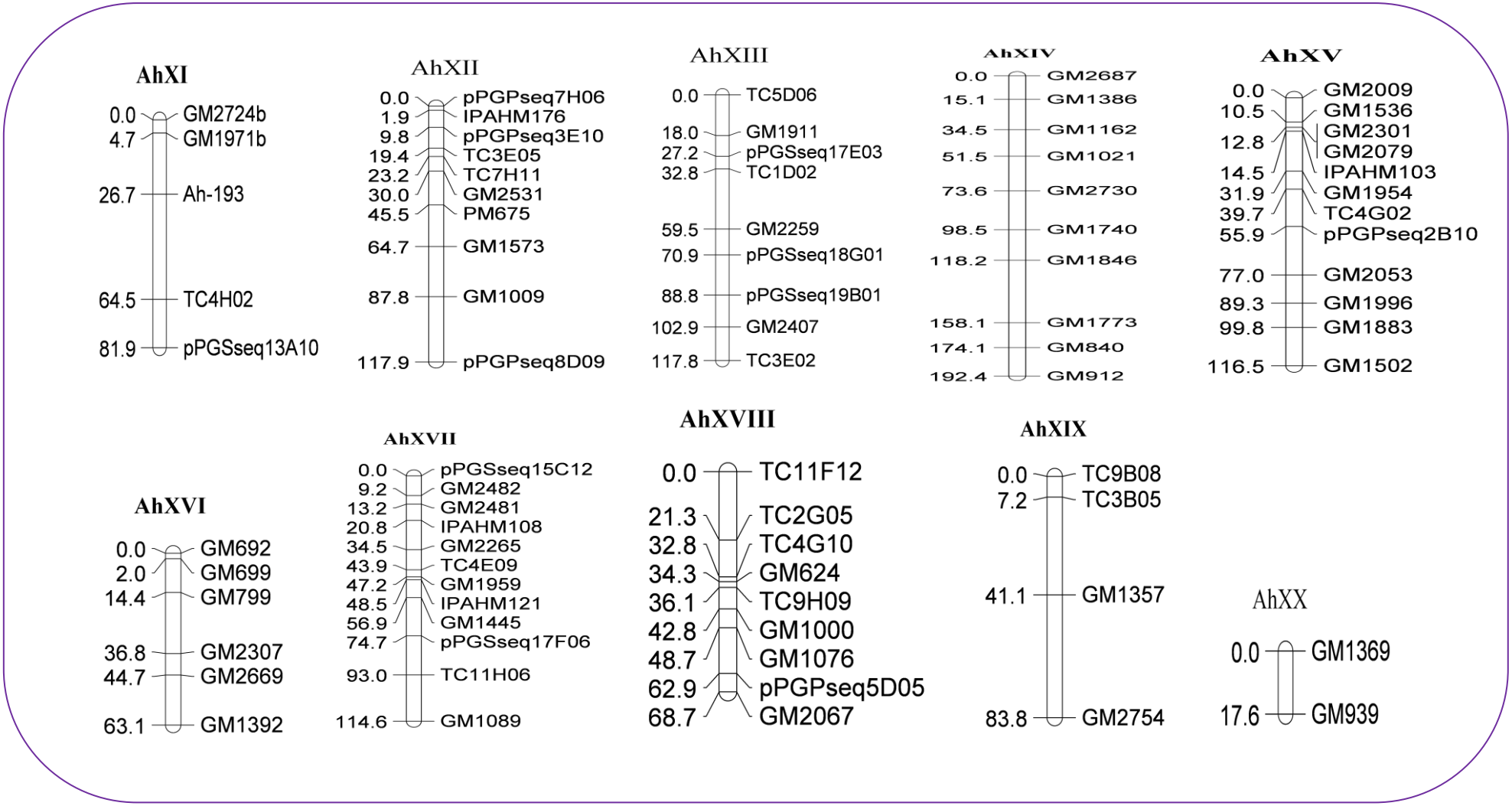


Figure 6B: Molecular Genetic Linkage Map of Groundnut RIL population derieved from cross b/w TAG24×GPBD4

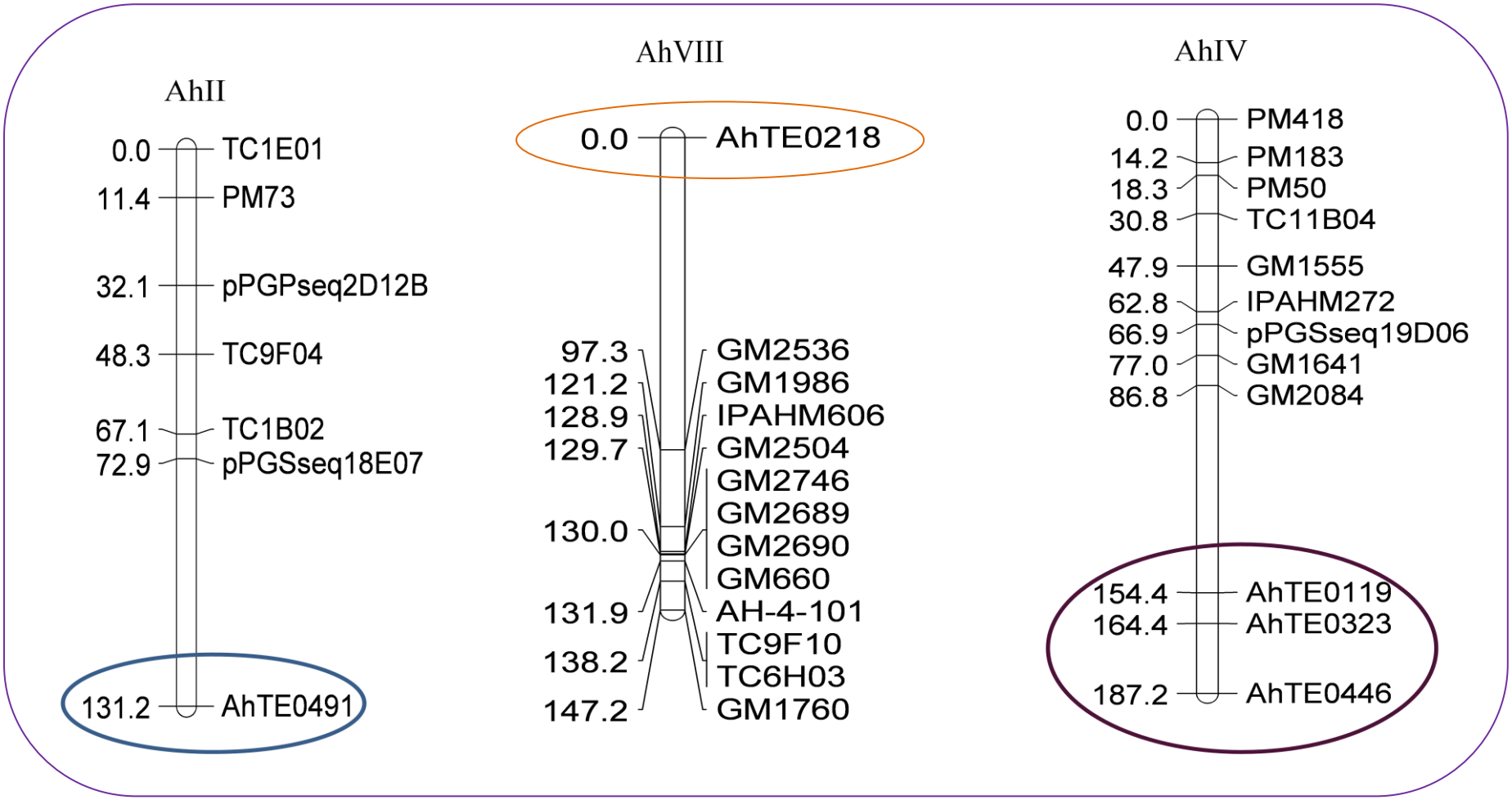


Figure 7: Relative Positive of Five Transposon Based Markers on Chromosome.

4.4.2 Association of Markers / QTLs for root traits, WUE and associated physiological traits

The RILs had been extensively characterized for molecular diversity and segregation of polymorphic markers alleles by an earlier study (Sujay, et al, 2011). Genetic analysis data comprised of 188 SSR markers mapped onto 20 linkage group of the allotetraploid groundnut. The genotyping data was kindly spared by the authors and was used for the mapping of QTLs in the groundnut for the important and relevant traits for improved drought adaptation**.**

4.4.2.1 Single Marker analysis (SMA)

Single marker analysis revealed that a large number of markers were associated with various traits under study. The markers linked with traits with 0.1% or more probability was only considered.

A total nine markers showed significant association with root length which are located on chromosome number 9, 14 and 19, while five markers, located on chromosome number three showed significant association with root biomass (Table 8).

Chromosome number five, ten, twelve and twenty with a total five marker showed association with leaf weight, while for total leaf area four markers on chromosome number nine, eleven and twelve were linked (Table 9).

Single marker analysis revealed that two markers located on chromosome number six showed association with Δ13C with a significant level of 5%. For total dry matter, three markers located on chromosome number five showed significant association (Table 10).

A total twelve markers were linked for mineral ash content were located on five different chromosome like three, six, eight , thirteen and nineteen (Table 11). Twelve markers located on chromosome number five, seven and twelve were associated with shoot length. Five markers on chromosome number five, eight markers on chromosome nine and one marker on chromosome number twelve showed a significant association with shoot weight (Table 12). Whenever two or more tendomly mapped markers showed strong association with single marker analysis were considered a single novel QTL.

Table 8: Marker linked to root traits in RILs through Single Marker Analysis

Trait	Chromosome	Marker no.	Marker name	P
Root length	9	6	pPGSseq14H06	0.008**
	9	7	PM436	0.007**
	9	8	Lec-1	0.003**
	9	9	gi1107	0.003**
	9	10	pPGSseq7G02	0.005**
	9	12	GM2120	0.002**
	9	13	pPGSseq17C09	0.004**
	14	2	GM1386	0.008**
	19	9	GM1357	0.003**
Root weight	3	5	GM1717	0.002**
	3	7	pPGSseq18A05b	0.005**
	3	8	GM745	0.003**
	3	9	Ah-4-04	0.007**
-	3	10	AC3D07	0.009**

Table 9: Marker linked to leaf traits in RILs through Single Marker Analysis

Trait	Chromosome	Marker no.	Marker name	P
Leaf wt	1	1	GM1187	0.008**
	10	9	TC11A04	0.007**
	10	10	TC1A02	0.007**
	12	8	GM1573	0.000***
	20	2	GM939	0.010**
Total leaf area	9	6	pPGSseq14H06	0.004**
	9	7	PM436	0.006**
	11	1	GM2724b	0.002**
	12	8	GM1573	0.008**

Table 10: Marker linked to $\Delta^{13}C$ and total biomass in RILs through Single Marker Analysis

Trait	Chromosome	Marker no.	Marker name	P
Δ13C	6	2	TC1A01	0.014*
	6	3	S108	0.028*
Total Biomass	9	2	GM1979	0.009**
	9	6	pPGSseq14H06	0.008**
	10	9	TC11A04	0.003**
	10	10	TC1A02	0.003**
	12	8	GM1573	0.001**

Table 11: Marker linked to total mineral ash content in RILs through Single Marker Analysis

Trait	Chromosome	Marker no.	Marker name	P
ASH	3	1	TC7E04	0.000***
	3	3	pPGSseq19D09	0.000***
	6	1	PM377	0.002**
	8	4	PIPAHM606	0.008**
	8	5	GM2504	0.004**
	8	7	GM2689	0.004**
	8	8	GM2690	0.003**
	8	9	GM660	0.003**
	13	5	GM2259	0.003**
	13	6	pPGSseq18G01	0.001**
	13	8	GM2407	0.007**
	19	3	GM1357	0.009**

Table 12: Marker linked to shoot traits in RIL population through Single Marker Analysis

Trait	Chromosome	Marker no	Marker name	P
Shoot length	5	6	GM1007	0.005**
	5	7	GM633	0.010**
	5	10	TC6E01	0.008**
	7	2	TC5A07	0.002**
	7	4	GM2480	0.005**
	7	7	GM1062	0.003**
	9	6	pPGSseq14H06	0.002**
	9	7	PM436	0.001**
	9	8	Lec-1	0.001**
	9	9	gi1107	0.001**
	9	10	pPGSseq7G02	0.004**
	12	1	pPGSseq7H06	0.004**
Shoot wt	5	6	GM1007	0.001***
	5	7	GM633	0.004**
	5	8	PM179	0.005**
	5	9	seq11C08	0.004**
	5	10	TC6E01	0.002**
	9	5	TC5A06	0.007**
	9	6	pPGSseq14H06	0.000***
	9	7	PM436	0.000***
	9	8	Lec-1	0.000****
	9	9	gi1107	0.000****
	9	10	pPGSseq7G02	0.000***
	9	12	GM2120	0.009**
	9	13	pPGSseq17C09	0.006**
	12	7	PM675	0.007**

4.4.2.2 Composite Interval Mapping by QTL CARTOGRAPHER

Identifying DNA markers flanking genomic regions that determine variations in a particular trait is pivotal in crop improvement through molecular breeding. Most of the component physiological traits that determine crop growth and productivity are quantitatively inebriated, and hence DNA markers flanking the quantitative trait loci (QTL) would be of great relevance.

QTL mapping involve construction of linkage maps and identifying relationship between traits and polymorphic markers. Commonly used approach for QTL mapping are single marker t- test, simple linear regression, interval mapping and composite interval mapping. These approaches enable us to estimate the locations, numbers and magnitude of phenotypic effects of individual determinants that contribute to the inheritance of traits of interest.

Composite interval mapping (CIM) approach increases the resolution of QTL location by using markers other than the markers flanking the segment, to control residual noise. This approach uses multiple markers as factor in analysis, and is an expansion of the basic interval mapping technique. Additional marker usage allows much greater power in the detection of QTLs and in estimates of position.

The QTLs were mapped onto the genetic linkage map through composite interval mapping by using the software WinQTL Cartographer, version 2.5. Graphical representation of QTLs identified by composite interval mapping revealed in figure 8. By using the specific location of these QTLs, they were added located on existing linkage map to develop a QTL map. The QTL map developed is illustrated in Figure 9. A total of 25 QTLs were identified conditioning various physiological and biometric traits among the population of 268 recombinant inbred lines and were spread across thirteen chromosomes (Fig. 9).

QTL for Root Traits

The QTLs associated with root length and root weight are given in Table 13. Three QTLs namely $QTL_{RL\text{-}19\text{-}1}$, $QTL_{RL\text{-}16\text{-}1}$ and $QTL_{RL\text{-}12\text{-}1}$ were found to be conditioning root length on linkage group 19, 16 and 12 respectively. These QTLs had LOD of 2.78, 2.18

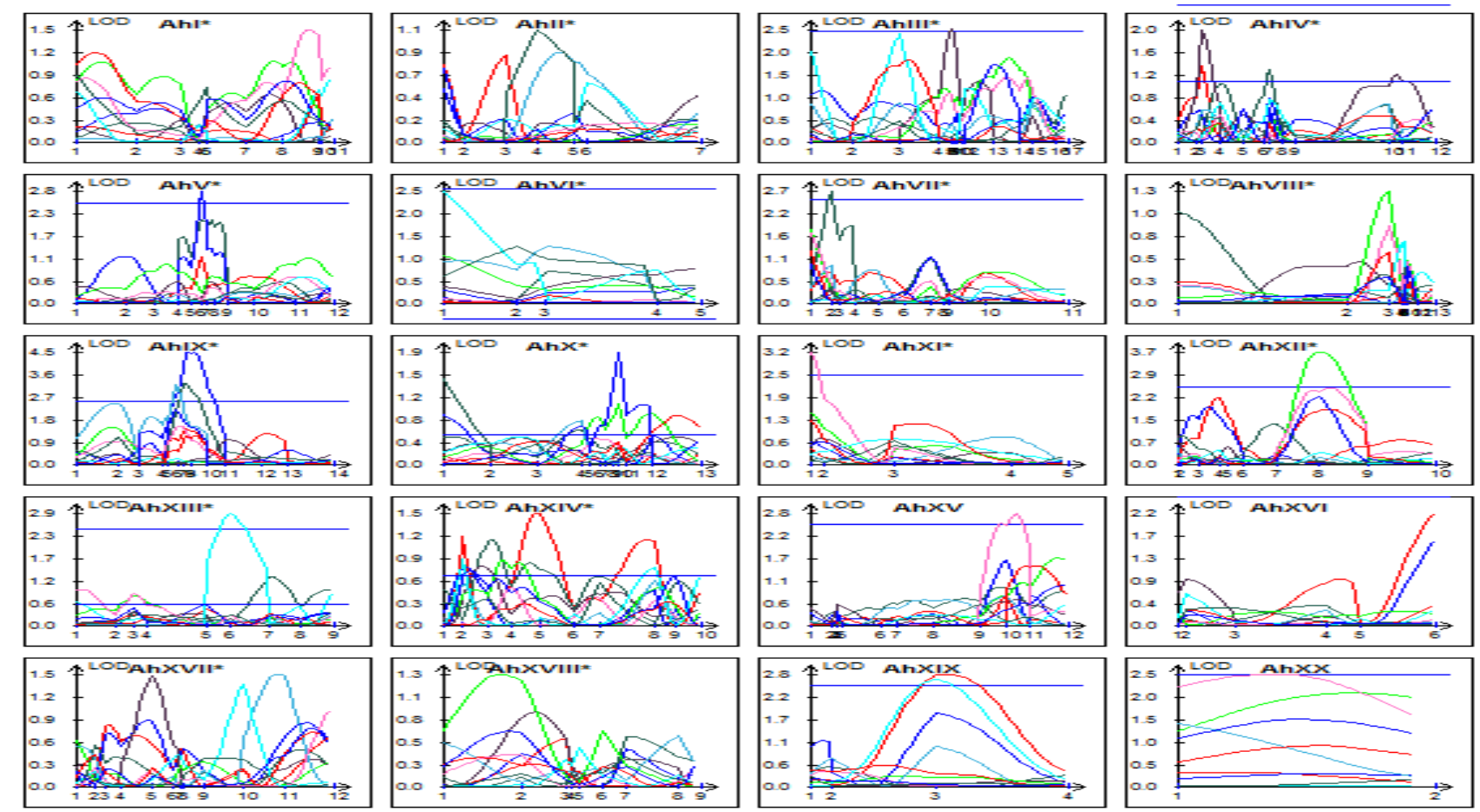


Figure 8: Graphical representation of QTLs of all the traits on all the chromosomes by CIM

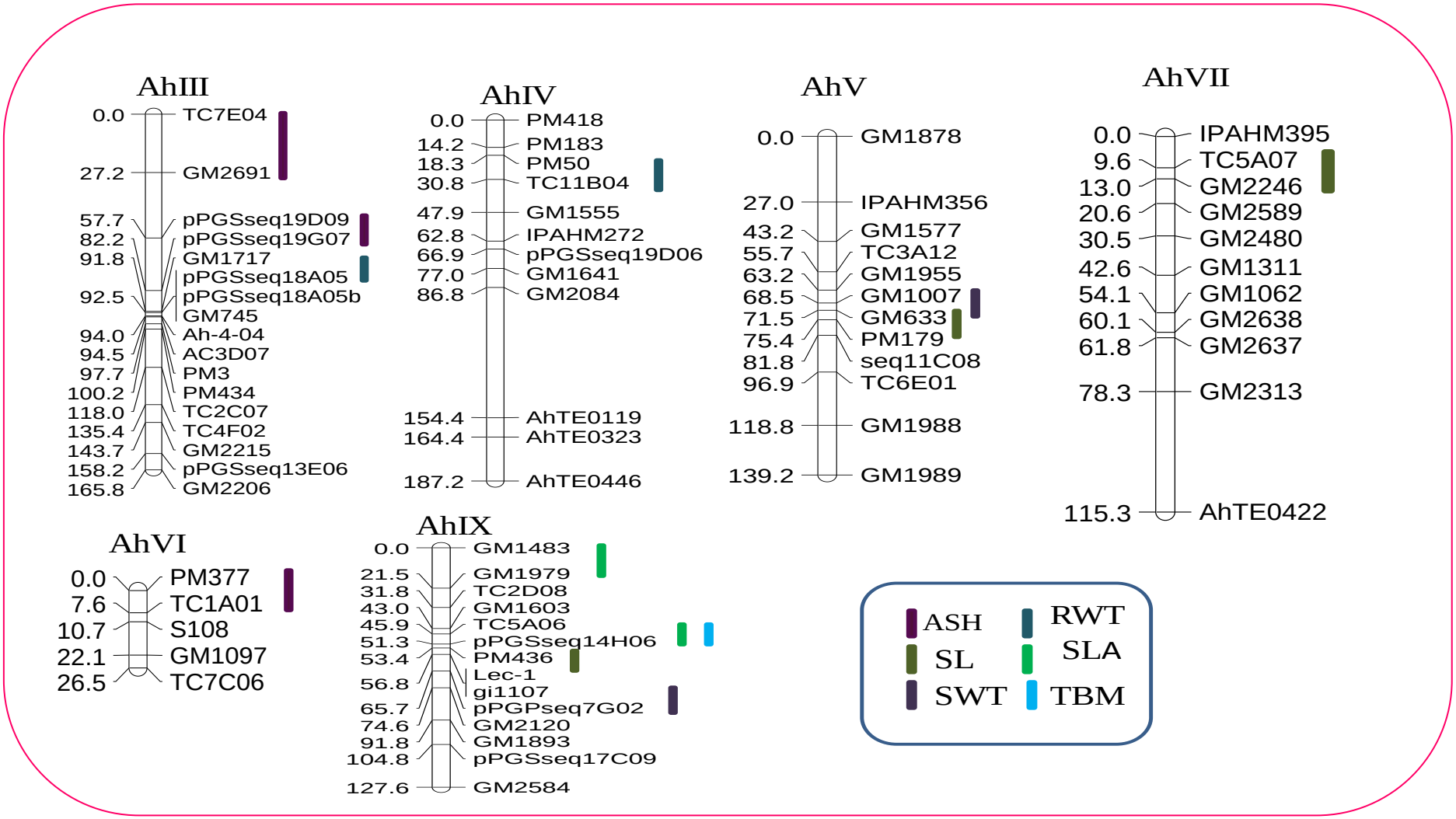


Figure 9A: QTL Map showing the Location of QTLs for Root traits and associated physiological traits in TAG 24 x GPBD 4 RILs

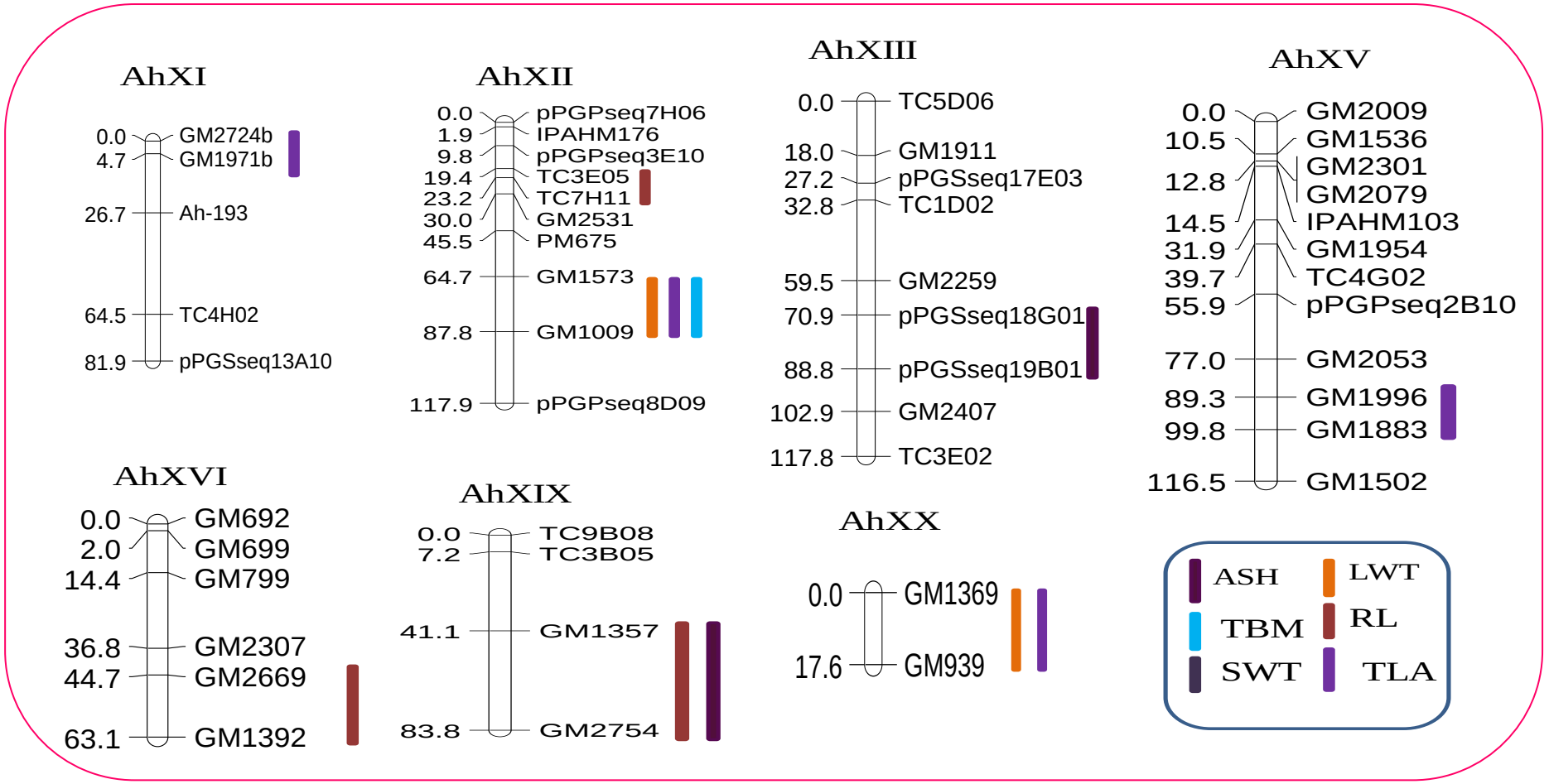


Figure 9B: QTL Map showing the Location of QTLs for Root traits and associated physiological traits in TAG 24 x GPBD 4 RILs

and 2.16 respectively and explained 6.89%, 5.00% and 3.34 % of the total phenotypic variance. $QTL_{RWT-3-1}$ and $QTL_{RWT-4-1}$ were associated with root weight. $QTL_{RWT-3-1}$ was located on linkage group 3 having a LOD value of 2.54 with 4.03% explained phenotypic variance, while $QTL_{RWT-4-1}$ presented on linkage group 4 showed a LOD value of 2.04 with 3.25% phenotypic variance explained.

QTLs for Leaf Traits

The QTLs associated with leaf traits are given in Table 15. On linkage group 12 and 20, two QTLs were found for leaf weight with LOD value of 3.67 and 2.03 respectively.$QTL_{SLA-9-1}$ and $QTL_{SLA-9-2}$ were the QTLs associated with Specific leaf area, both were identified on linkage group 9 with a LOD value of 3.22 and 2.25 respectively (Table15).

QTLs for total leaf area were detected on linkage group 11, 12, 15 and 20 with a LOD value of 3.17, 2.49, 2.79 and 2.50 respectively (Table 15). Two QTLs for total dry matter with LOD value of 2.21 and 2.16 on linkage group of 12 and 9, respectively (Table 15).

$QTL_{SL-5-1,}$ QTL_{SL-7-1} and QTL_{SL-9-1} were associated with shoot length with LOD value of 3.20, 2.72, and 2.08 respectively. While for Shoot weight two QTLs were detected on linkage group 5 and 9 with a comparatively high LOD value of 2.82 and 4.47(Table 14).

QTLs for Mineral Ash content

A total of five QTLs were identified for mineral ash content. $QTL_{ASH-3-1}$ and $QTL_{ASH-3-2}$ located on linkage group 3, while $QTL_{ASH-13-1,}$ $QTL_{ASH-19-1}$ and $QTL_{ASH-6-1}$ found to located on chromosome number 13,19 and 6 respectively (Table 16).

Table 13: QTLs associated with root traits like, root length (cm) and root weight by CIM.

Trait	Linkge	QTL name	Marker Interval	Position	LOD	R^2 (%)	Additive
Root length	AhXIX	$QTL_{RL-19-1}$	GM1357-GM2754	43.11	2.78	6.89	1.69
Root length	AhXVI	$QTL_{RL-16-1}$	GM2669-GM1392	62.71	2.18	5.00	1.44
Root length	AhXII	$QTL_{RL-12-1}$	TC3E05-TC7H11	19.41	2.16	3.34	-1.19
Root Weight	AhIII	$QTL_{RWT-3-1}$	GM1717-pPGSseq18A05	91.81	2.54	4.03	0.055
Root Weight	AhIV	$QTL_{RWT-4-1}$	PM50-TC11B04	18.31	2.04	3.25	0.050

Table 14: QTLs associated with shoot traits like, shoot length (cm) and shoot weight (g) by CIM.

Trait	Linkge	QTL name	Marker Interval	Position	LOD	R^2 (%)	Additive
Shoot length	AhIX	QTL_{SL-9-1}	PM436-Lec-1	53.41	3.20	5.15	-1.21
Shoot length	AhVII	QTL_{SL-7-1}	TC5A07-GM2246	9.61	2.72	4.19	1.10
Shoot length	AhV	QTL_{SL-5-1}	GM633-PM179	73.51	2.08	3.41	-1.00
Shoot Weight	AhIX	$QTL_{SWT-9-1}$	gi1107-pPGPseq7G02	58.81	4.47	7.51	-1.26
Shoot Weight	AhV	$QTL_{SWT-5-1}$	GM1007-GM633	68.51	2.82	4.33	-0.96

Table 15: QTLs associated with Leaf traits like, Leaf weight (g), Specific leaf area (SLA), total leaf area (TLA) and total dry matter (TDM) by CIM.

Trait	Linkge	QTL name	Marker Interval	Position	LOD	R^2	Additive effect
Leaf Weight	AhXII	$QTL_{LWT-12-1}$	GM1573-GM1009	64.71	3.67	8.00	-0.64
Leaf Weight	AhXX	$QTL_{LWT-20-1}$	GM1369-GM939	12.01	2.09	6.35	-0.59
SLA	AhIX	$QTL_{SLA-9-1}$	TC5A06-pPGSseq14H06	49.91	3.22	6.45	-8.83
SLA	AhIX	$QTL_{SLA-9-2}$	GM1483-GM1979	18.01	2.25	6.80	8.38
TLA	AhXI	$QTL_{TLA-11-1}$	GM2724b-GM1971b	0.01	3.17	6.71	-130.62
TLA	AhXV	$QTL_{TLA-15-1}$	GM1996-GM1883	93.31	2.79	7.18	135.12
TLA	AhXX	$QTL_{TLA-20-1}$	GM1369-GM939	6.01	2.50	7.69	-144.09
TLA	AhXII	$QTL_{TLA-12-1}$	GM1573-GM1009	68.71	2.49	6.91	-131.81
TDM	AhXII	$QTL_{TDM-12-1}$	GM1573-GM1009	64.71	2.21	4.64	-1.04
TDM	AhIX	$QTL_{TDM-9-1}$	TC5A06-pPGSseq14H06	49.91	2.16	3.86	-0.95

Table 16: QTLs associated with mineral ash content by CIM

Trait	Linkge	QTL name	Marker Interval	Position	LOD	R^2 (%)	Additive
Ash	AhXIII	$QTL_{ASH-13-1}$	seq18G01-seq19B01	70.91	2.91	4.29	-0.004
Ash	AhXIX	$QTL_{ASH-19-1}$	GM1357-GM2754	41.11	2.63	6.32	0.004
Ash	AhXVI	$QTL_{ASH-6-1}$	PM377-TC1A01	0.01	2.47	3.59	-0.003
Ash	AhIII	$QTL_{ASH-3-1}$	pPGSseq19D09-pPGSseq19G07	57.71	2.43	3.74	0.004
Ash	AhIII	$QTL_{ASH-3-2}$	TC7E04-GM2691	0.01	2.03	5.40	0.004

Discussion

V. DISCUSSION

Groundnuts (*Arachis hypogaea* L.) are an important grain legume grown and consumed predominantly in the arid and semi-arid tropical regions of the world. They are an excellent source of high quality edible oil (ca 50 percent), easily digestible protein (ca 25 percent) and carbohydrates (ca 20 percent).

Presently, groundnuts are cultivated on about 26.5 million ha globally, with an average annual production of 35.7 million tons. Although productivity of over 6 ton.ha^{-1} has been recorded under high-input conditions, the average global productivity ranges around 1.4-2.0ton.ha^{-1} (Badigannavar, 2007, Weiss 2000). Around 70 percent of groundnut cultivation is under rain-fed conditions where water limitation is the most significant constraint for achieving potential productivity (Smartt, 1984). The inevitable climate change scenario predicts that, besides global warming, there will be significant changes in monsoon cycles (Intergovernmental Panel on Climate Change (IPCC), 2007). This will lead to severe water shortages, especially in tropical countries, with rain-fed agriculture being the worst affected sector. Hence, the emphasis in research needs to be on improving groundnut productivity under water limited conditions.

Breeding to improve crop productivity under water-limited conditions is a formidable challenge, owing mainly to the complexity of drought stress and an equally complex crop response. Although significant progress has been achieved in breeding for drought tolerance through selection for higher absolute yields under stress, its application in larger breeding programmes catering for a wider range of environments is becoming increasingly limited (Araus et al, 2002; Branch and Hildebrand, 1989; Cooper and Hammer, 1996). Hence, introgression of a few important and relevant drought tolerance traits into a single genetic background through a 'trait-based' breeding approach is being suggested as the most plausible strategy.

It has been recognised that several of the traits (Chart 1) that have relevance in improving drought tolerance need to be combined to achieve a comprehensive improvement in productivity under water-limited conditions. These traits are quantitatively inherited and bringing them together would be quite difficult through

conventional breeding approaches. Crop breeding programmes are increasingly relying on a DNA MAS strategy as a powerful complementary approach. However, this approach depends almost totally on the accurate phenotyping of a set of mapping populations or diverse unrelated germplasm accessions, and genetic characterisation of the population with appropriate marker systems leading to the identification of QTLs controlling the traits through linkage and/or association analysis. Therefore, success in identifying robust molecular markers depends on the availability and development of genomic resources to be employed while identifying QTLs.

The ambitious goal of 'trait introgression' into a single genetic background warrants the adoption of modern genomic techniques, and a well focused marker-assisted breeding (MAB) strategy needs to be evolved. Trait introgression into a single genetic background requires identification of suitable trait donor parental lines, with a robust marker assisted selection (MAS) strategy. Hence, identification of such donor parents and robust QTLs governing the traits is essential.

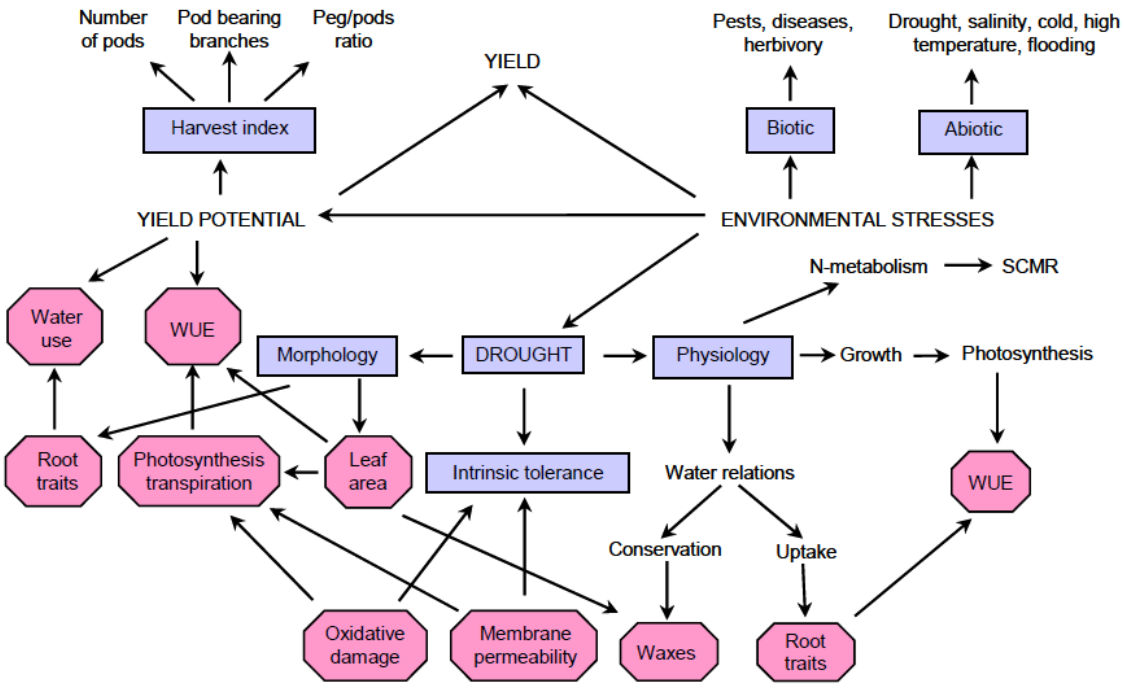


Chart 1: Various traits that influence growth and productivity of Groundnut in water-limited environments

Plants have naturally evolved several mechanisms to cope with drought stress which range from morphological features to phenological processes such as leaf expansion, parahelionastic movement, development of pubescence, accumulation of epicuticular waxes to rapid phenological development, sensitive stomata etc. most of these traits, though are relevant for water conservation, have only survival significance and are often counterproductive.

Therefore, any trait to have relevance in breeding programme should be associated with superior growth rates (Udayakumar and Prasad, 1994). From this context, superior water acquisition from deeper profiles of soil and a better use of water for biomass production are often considered as most important physiological traits that have relevance in drought tolerance.

Simple growth model such as Passioura (1986), provide a good framework for attempts to improve crop productivity under water limited conditions through trait based breeding (Reynolds and Tuberosa, 2008). Although the relevance of these traits in crop improvement was realized, progress in achieving improvement in these traits was limited owing mainly to the lack of proper screening techniques. This lacuna was almost completely overcome with the discovery that plants discriminate against heavy isotope of carbon during photosynthesis (O' Leary 1988) and its link with water use efficiency (Farquhar and Richard, 1984; Farquhar *et al*, 1989; Impa, 2005).

This discovery provided a very strong impetus for breeding programme and several attempts of selection for superior WUE through Δ^{13} were indeed successful (Richard *et al*, 2002; Wright, 1996; Sheshshayee *et al*, 2003).

The best utility of the traits is possible only when they are pyramided together in a single genetic background. Hence to increase the productivity of the crop under water limited condition. These two traits should be breed together to increase the availability of water to plants (Sheshshayee *et al*, 2003) and there will be the efficient use of this available water use moisture stress condition.

As the drought tolerance traits are polygenic in nature and their introgression through conventional breeding into a single genetic background is difficult. Therefore to attain success, molecular breeding approach which requires both the genetic and genomic resources is needed.

Identifying molecular markers linked with traits, several mapping population have been developed and characterised for molecular diversity. Using RIL mapping population (TAG24×ICGV 86031) a framework linkage map was developed earlier for cultivated groundnut having 135 SSR loci (Varshney *et al*, 2009). A high density genetic linkage map of a total length of 2,166.4 cM consisting of 1,114 marker loci has been developed (Shirasawa, 2012). This linkage map possesses the highest number of marker loci in cultivated peanut as well as *Arachis* spp. Another linkage map containing 188 SSR marker loci, using RIL mapping population (TAG24×GPBD4), has been developed (Sujay, *et al* 2011).

Despite these resources, phenotyping for the traits relevant for drought tolerance is still not done. The two parents TAG24 and GPBD4 was initially studied for mapping certain disease resistance in groundnut. However, GPBD4 is a well known genotype well suited for rainfed conditions. Hence this genotype has also been known to be a fairly good drought tolerant line. Initial experiment carried out at our centre revealed that the parental lines differed significantly in root traits (Fig.10C & 10D) but not that remarkably for Δ^{13} (Fig. 10B). Therefore a programme for phenotyping of RIL population for root traits, Water use efficiency and associated physiological traits was initiated. These parents were also significantly varied in terms of biomass production with 24.54 g.plant^{-1} in TAG 24 to 35.77 g.plant^{-1} in GPBD 4. Since there was a significantly high variability between these two parents for root traits, therefore the RIL population.

The RIL population is derived from the crossing of TAG24 and GPBD 4. These two parents were contrasting in the root traits i.e. root length and root biomass. TAG24 is a low root type while GPBD4 is a high root type (Fig.10C). These two parents were not significantly different for Δ^{13}C value (Fig.10B). The delta value for TAG24 ranges from 16.968‰ to 17.366‰ with a mean value of 17.20‰, while GPBD4 also show somewhat similar same value ranging from 17.143 to 17.928‰ with a mean value of 17.62‰. Although these parents differ in maximum and minimum values for delta, but this is not the case of mean value.

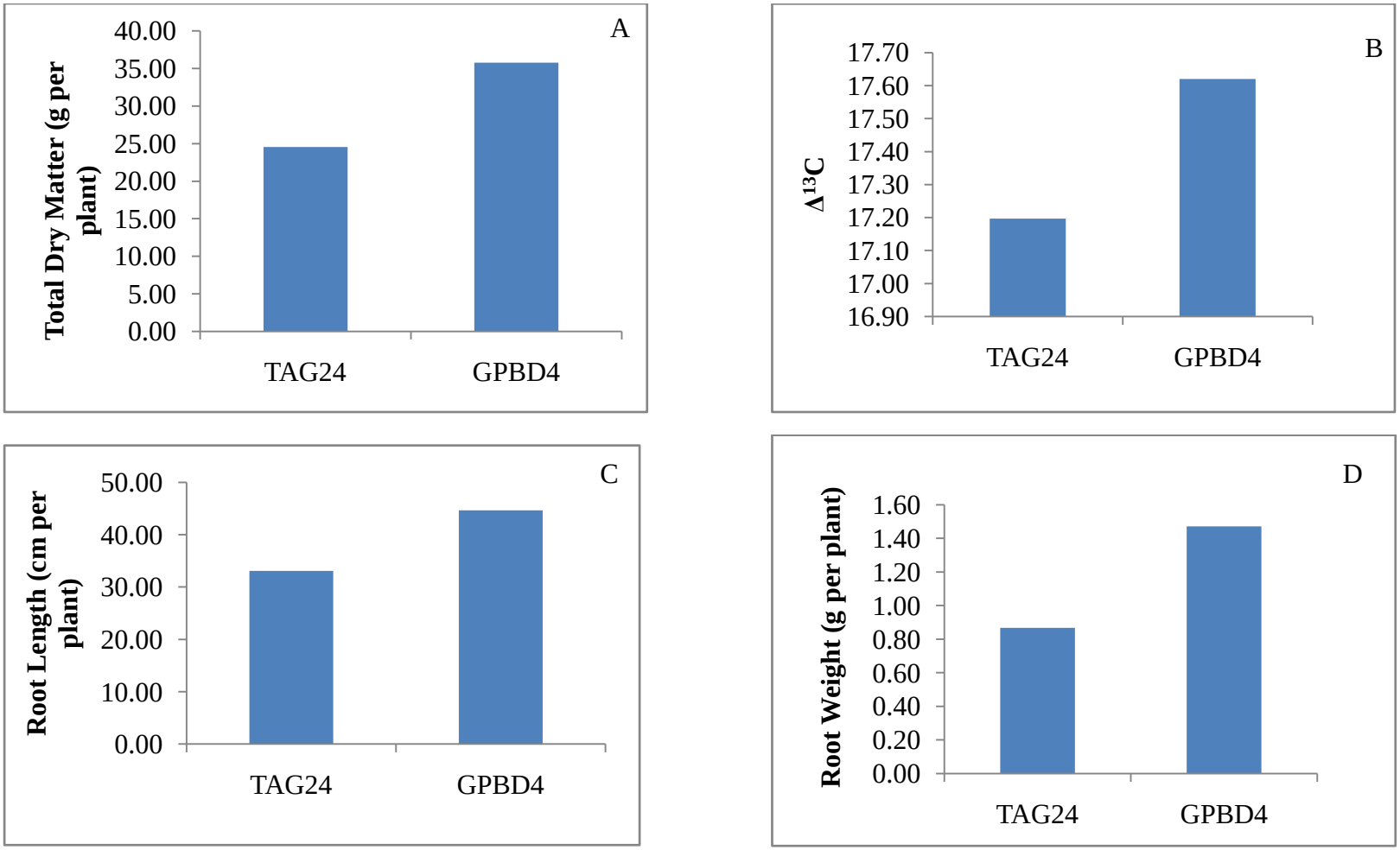


Figure 10: Genetic variability for root traits, $\Delta^{13}C$ and total biomass among TAG24 and GPBD 4 in earlier experiments.

These two parents also significantly varied in terms of biomass production, which ranged from 24.54g plant^{-1} in case of TAG 24 to 35.77g.plant^{-1} in GPBD 4. Since there was a significantly high variability between these two parents for root traits, therefore the RIL population should segregate for the traits. Therefore based on this previously available information for these two parents, the RIL population of size 268, derived from TAG 24 and GPBD 4 were used to identify the QTLs for root traits and WUE.

Considering the importance of groundnut, significant efforts have been made to improve productivity under water limited conditions. Recent experiment conducted at our centre (Sheshshayee *et al*, 2003; Sheshshayee *et al*, 2006) as well as elsewhere (Wright *et al*, 1994, Rao *et al*, 1993) clearly emphasised the complexity of enhancing productivity under water limited condition. It is apparent that several diverse traits linked to be pyramided onto a single elite genetic background in order to enhance yield under water limited conditions. From this context, water mining associated with deep root system and efficient use of water for biomass production emerged as the most potential trait that deserve exploitation.

Further, it is well known that these traits are quite complex, polygenic and exhibit quantitative inheritance pattern. Pyramiding such traits through conventional breeding is formidable challenge. Recent advancement in genome research with significant large number of DNA markers such as SSR have developed (Varshney *et al*, 2009c) a focused molecular breeding programme is expected to strongly augment the efforts in genetic enhancement of groundnut crop, improve drought adaptation and productivity. However, despite the availability of genetic resources progress in molecular breeding has been limited in groundnut.

The polyploidy nature of species and the lack of high throughput phenotype strategy are often considered as most important bottleneck in achieving success in groundnut improvement. In present investigation we characterized in available Recombinant Inbred Line population for variability in relevant drought adaptive traits. A throughput screening for variability in relevant drought adaptive traits. A through screening for $\Delta^{13}C$ as surrogate for WUE, and root traits was made by growing the population comprising of 268 RILs.

5.1 Water Use Efficiency

Water use efficiency is one of the drought tolerance traits such that can contribute to productivity when water resources are scarce. The yield model proposed by Passioura (1986) (*i.e.,* Grain yield = Transpiration X Water Use Efficiency X Harvest Index) emphasises that water use efficiency is an important parameter influencing the biomass production. Water Use Efficiency (WUE) is defined at either single leaf level or at whole plant level and or at canopy level. There are several approaches available to assess the genetic variability for Water Use efficiency. They are gravimetric approach, gas exchange studies and Carbon isotope discrimination.

The measurement of WUE and transpiration through Gravimetric approach is cumbersome and labour intensive. Measurement of water use efficiency through gas exchange approach though is rapid, but showes a poor relationship with WUE measured gravimetrically suggesting that the instantaneous measure are dynamic and hence do not account for divers and season variation (Bindumadhava, 2000).

In this context, $\Delta^{13}C$ has emerged as a dependable parameter to study WUE as it reflects time integrated estimate of carbon gain per unit transpiration, especially in C_3 plants. $\Delta^{13}C$ in whole plant dry matter appears to be a reliable indicator of plant WUE in pot grown plants and negative relationship was obtained between these two traits in structural carbon both in well watered and drought conditions.

Theory explaining the relationship between $\Delta^{13}C$ and WUE in well developed and validated in several pot and field grown plants (Farquhar et al, 1989), including groundnut (Nageshwara Rao *et al*, 1993; Sheshshayee *et al*, 2006). This approach has been adapted to assess genetic variability in WUE in several crop species (Farquar and Richard, 1984; Ismail and Hall, 1992; Wright, 1996)

Maintenance of the relative ranking of genotypes in control and stress implies that for WUE and $\Delta^{13}C$, genotype and environment interaction is low and the broad sense heritability is high (Hubick *et al,* 1988; and Wright *et al,* 1993). Due to these distinct advantages, $\Delta^{13}C$ appears to be a very reliable parameter for WUE.

Though WUE is an important component of yield model of Passioura(1986), no deservable relationship of WUE (estimated by $\Delta^{13}C$) and TBM was not often

noticed. This fact was one of the important reason that distracted crop scientists from breeding for improving WUE.

A recent analysis was made to understand the physiological control of growth traits by WUE in rice (Sheshshayee *et al*, 2012). A similar analysis was made in groundnut. RILs with similar leaf area were selected and the relationship between $\Delta^{13}C$ and TDM was examined. The results illustrated in fig. 3 & fig. 4, clearly demonstrated that WUE is still an important parameter influencing growth rate only after optimizing canopy architecture. This warrants identification of QTL for canopy leaf area besides the other drought adaptive traits.

5.2 Root traits

Ability of the plant to explore water source and extracting water from deeper profiles of soil thus has great relevance in maintaining water relation as well as carbon assimilation. Deep rooted plants have been shown to be better productive under water limited conditions (Li *et al*, 2005). Such a termed was recently noticed also in a C_4 crop like finger millet at our centre.

Several of the root related traits described above have been shown to be related with improved growth under stress. Hence, improving these component traits has significance in sustaining productivity under water limited conditions. After having achieved considerable understanding of root growth and development both at the whole plant level and at the molecular level, strategic approaches for crop improvement can be formulated.

Though deep rooted plants produced more grains under low water availability, these plants had the risk of exhausting soil water early. Hence, Condon *et al*, (1993); Richards *et al*, (2002) ; Sheshshayee *et al*, (2003) have emphasized that soil factors also need to considered before attempting to improve root traits.

Individual root characteristics, such as thickness, depth of rooting, and the ability to penetrate compacted soils have been associated with drought avoidance (O'Toole and Chang, 1979; Yoshida and Hasegawa, 1982). Significant genetic variability in some of these root traits have been demonstrated and implicated for improved drought tolerance in crop plants (Sinclair and Muchow, 2001). Biomass

accumulation in plants is always a function of total water used (Passioura, 1986). Plants with deep root system hence have the ability to supply water to support a higher transpiration demand, thereby enhancing total biomass (Li *et al.*, 2005). In their simulation experiments, Sinclair and Muchow (2001) demonstrated an increase in biomass and yield when root growth was better. These studies emphasized the relevance of breeding to improve root traits to achieve better productivity under water limited conditions.

The root structure represents as near natural condition for real root trait phenotyping. Since the plant population is maintained as that in the main field, the plants would experience the inter-plant completion which might have an important effect on the phenotypic expression of root growth. Thus, the measurements of the root traits from plants grown in such root structures would be very accurate. The investigation revealed significant genetic variability in this trait. A large number of transgressive segregant for roots and $\Delta^{13}C$ and various other traits were found.

Water use efficiency and root traits among the RILs revealed significant variability. However, there was no significant relationship between total biomass and $\Delta^{13}C$. This lack of relationship between these parameters was one of the major factors for the lack of the interest among breeders in exploiting variability in WUE. Similar inferences were also drawn based on such data in other species like rice (Sheshshayee *et al*., 2012). A constitutive and integral trait like leaf area is generally known to have greater variability and hence, has greater influence in determining the difference in total dry matter. Relevance of WUE was evident in genotype with similar leaf area was compared. A significant inverse relationship between Δ13C and total dry matter implied that increase WUE still has relevance when canopy cover is optimized or maximized.

Similarly, root traits and WUE also seemed to play a significant role in determining the variations in total biomass. RILs were classified based on root traits and WUE, into four categories. The genotypes that fell into category of low $\Delta^{13}C$ and high root recorded the highest total dry matter. In contrast, the group categorized by high $\Delta^{13}C$ and low root showed the least biomass (Fig. 11). These results emphatically indicate the relevance of WUE in determining growth rates of crop.

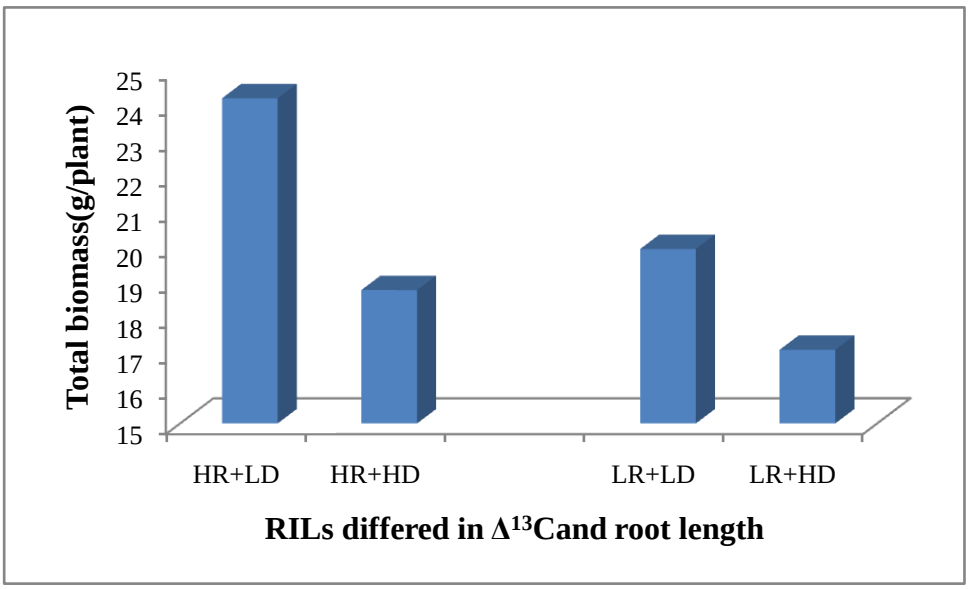


Figure 11: Relevance of root traits and $\Delta^{13}C$ on biomass production.

Several transgressive segregants which had better roots and better WUE then both the parents hence have been identified. These lines would form strong basis for further experimentation towards understanding the genetic basis of the observed variation in these traits and as parents for further trait based breeding programmes.

The markers that had emerged have closely linked with root traits and WUE in groundnut can augment such breeding efforts. However, more robust phenotyping in couple of seasons and increased marker density on the existing linkage map would strongly enhance the efficiency of molecular breeding efforts.

5.3 Molecular characterization

All the traits studied showed quantitative inheritance as illustrated by normal distribution around mean (Figure 5). The large number of transgressive segregants is a strong indication of the recombination of large numbers of alleles among the two parents used for developing the mapping population. This mapping population was characterized for molecular diversity and a genetic linkage map comprised of 188 SSR marker loci was reported earlier (Sujay *et al,* 2011).

The genotyping data was enriched with additional markers that were designed to target the transposon region of groundnut genome.

5.3.1 Transposon based marker

Transposon based marker represents a robust co-dominant marker system that can strongly distinguish the functional variation among the recombinants. Though we designed 24 transposon markers (Shirasawa *et al,* 2011) of which seven were polymorphic. Along with available segregation of SSR marker loci, seven transposon markers were included to construct the genetic linkage map. The transposon markers which are mostly regulatory in function mapped more towards the telomeric regions of chromosome which explain hyper variability.

5.3.2 QTL Mapping

QTL mapping is a process of implicating statistical hypothesis tests and parameters estimations for the models using observations on the traits and genetic markers

in certain genetic designs. The use of QTL mapping in agricultural research is the possibility of using the markers flanking the QTL for trait introgression through molecular breeding and identification of candidate genes through map based cloning.

Therefore, an attempt was made to identify QTLs conditioning relevant traits such as WUE, root traits and other related physiological parameters in groundnut. Using the phenotyping data and available genetic information, marker trait linkage analysed by both single marker analysis and composite interval mapping (CIM).

5.3.2.1 Association of QTLs through Composite Interval Mapping

In the present study, many QTLs for root traits and associated physiological traits identified by CIM analysis by using WinQTL Cartographer, version 2.5. A total 25 QTLs were identified for different physiological traits. The LOD values for the QTLs ranged from 2.04 to a maximum of 4.47 (Table 12-15).

QTLs for all the traits identified but no QTL were detected for $\Delta^{13}C$. Though both the parents did not have significant variability for $\Delta^{13}C$, but still the mapping population showed a large variability for Δ13C ranging from 16.68‰ to as high as 22.96‰.

Since WUE is a complex trait that is a ratio of photosynthesis (biomass accumulation) to total water transpired. So several other traits contributes for the water use efficiency, therefore one possibility for not to found a QTL for $\Delta^{13}C$ might be that the several other genomic regions that contributes for the traits is not covered by the markers. Therefore to identify QTLs such a complex trait, a more number of markers are needed to integrate. Similar kind of inferences for drought tolerance in groundnut was recently drowned by Ravi *et al*, (2011).

Even though several QTLs were identified for all the traits the majority of the identified QTLs did not reveal a high phenotypic variance. The phenotypic variance explained for different QTLs ranges from 3.25% to a maximum of 8.00%. However, the traits are highly polygenic in nature and because of large population size, QTLs with lower phenotypic variation is expected. Based on QTL mapping studies in other species, it can be generalized that higher phenotypic variation for the given trait in the mapping

population and higher marker density genotyping data is the pre- requisites to identify the major QTL explaining higher phenotypic variation.

However, these markers can still form the basis for the identification novel recombinants that can be used for further crop improvement.

Summary

VI. SUMMARY

Groundnut (*Arachis hypogaea* L.) is an important grain legume grown and consumed predominantly in the arid and semi-arid tropical regions of the world. It is generally grown under rainfed conditions where water availability is less, so improving groundnut productivity under water-limited conditions is a formidable challenge, owing mainly to the complexity of drought stress and an equally complex crop response in adoption for drought.

Tolerance to drought is not a simple response, but is mostly conditioned by many genes and has been shown to interact with environment, and thus the networks involved in drought tolerance are quite complex in nature. Hence, selection based on the phenotype would be difficult for such traits (Collins *et al.* 2008). Therefore, a DNA based molecular marker assisted approach would strongly complement the conventional breeding method to introgress these relevant traits on to agronomically elite genetic background.

Although QTLs for some the drought tolerance traits has been discovered, but that is not the case for root traits and water use efficiency. Since these two traits, water use efficiency (Rebetzke *et al.*, 2002), root traits (Li *et al.*, 2005) can improve to drought tolerance in crop plants under water stress, it is important to identify the QTLs for these traits.

With this background a investigation carried out using a subset of 268 RILs of groundnut developed by crossing TAG24 × GPBD4 to identify QTLs conditioning these traits. The RIL population were sown in root structure and were grown till 80 DAS. After 80 days plants were harvested and different biometric measurement were taken.

Significant genetic variability was noticed in physiological parameters like root length, root biomass, mineral ash content, specific leaf area, total leaf area, etc (Table 5). This variability was normally distributed around the mean (Fig.5), conferring their quantitative inheritance. WUE was determined by carbon isotope discrimination using IRMS. $\Delta^{13}C$ were also norma1ly distributed with a significant variability.

Regression analysis of several parameters with biomass revealed the overriding influence of total leaf area (Fig.1). Further strong correlation between total mineral ash and total biomass was also observed. However there was no deserevable relationship between $\Delta^{13}C$ and total biomass, which enhance the scepticism about the use of WUE in determining biomass in groundnut. It is apparent that a trait that has larger variance would contribute more to TDM. Thus leaf area followed by total mineral ash significantly contributes more to TDM.

To ascertain the influence of other constituent physiological traits, RILs with similar leaf area type were selected. Among these similar leaf area types, the relationship between $\Delta^{13}C$ and TDM was significantly increased. Since RILs with similar leaf area were selected, contribution of total leaf area to TDM decreased compare to all genotypes.

Further RILs with high leaf area among the similar leaf area type were selected. The regression between $\Delta^{13}C$ and TDM significantly increased, suggesting that increased water use efficiency can contribute to growth rate only when leaf area is optimized.

With the major objective to identify QTLs conditioning WUE, root traits and other physiological traits, a existing genetic linkage map of 188 SSR marker loci (Sujay *et al*, 2011) was used. In addition to this, a set of 24 transposon based markers used to screen the parents for polymorphism. Seven, out of 24 showed polymorphism which further used to screen the RIL population, and further these markers used to add on existing linkage map.

DNA markers associated with the traits were identified by both single marker analysis and composite interval mapping. SIM revealed a numbers of markers for almost all the the recorded. Through Composite Interval Mapping a total 25 QTLs were identified for all the traits except $\Delta^{13}C$.

Even though several QTLs were identified for almost all the traits, the majority of the identified QTLs did not reveal a high phenotypic variance. Based on QTL mapping studies in other species, it can be generalized that higher phenotypic variation for the given trait in the mapping population and reasonable marker density genotyping

data are the pre-requisites to identify the major QTLs explaining high phenotypic variation.

Future Plan of Work

There is a need to add more markers on the existing linkage map to saturate it and it is essential to validate the identified QTLs for future breeding programme.

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Appendices

Appendix 1: TE marker list and respective nucleotide sequence

Marker Name	Nucleotide Sequence
AhTE0119F	AAGCTAGCGATGGCGATAAC
AhTE0119R	TTTATGCCCGCAAACTTTTT
AhTE0130F	TGGCTTGTCTCACTTATCCTCA
AhTE0130R	TGAAAAATTCATTAACTTAACACCTT
AhTE0164F	AACCACCACCATTCTTTTAAGG
AhTE0164R	TCGTCTCCTCTAAGGTTTGCTT
AhTE0190F	AAATTTATCTGTTTCGATCGAGTG
AhTE0190R	TCGAACCGACTCTGGTTTTAAT
AhTE0202F	GAGAGCGGTAATTTTTATGATTTG
AhTE0202R	TACTTTTGCGACTTTGTCTCCA
AhTE0218F	ATGGAGTTTCAGCAAATGAGGT
AhTE0218R	GAAAATCAAAACTGGCTGCAT
AhTE0222F	ATTCACACCCAAAACAAAACAT
AhTE0222R	ACGAGGATCGATCGTTATACAT
AhTE0248F	CAGCCTTAACCCTAGCACTTTTT
AhTE0248R	TTAGAATTGTAGGTGGTGGGTTG
AhTE0268F	GAAGGCCTATCTCATTTTGTTTG
AhTE0268R	CGCCGTTACCTAGGTTTTAGAAT
AhTE0278F	CTTTTCTTTTGTAATGAATCTGTTTTT
AhTE0278R	GCAATGCTAATATGCTAAATCGTT
AhTE0300F	GGTTGTGTAATCCAATTTCCACT
AhTE0300R	TCGTCCCATTCATAGTTCTTTTG
AhTE0303F	AAGATGCATACCTTTGGTTTTCT
AhTE0303R	CTGCACTTAAGCCAACTTCTCAT
AhTE0323F	TGGGGAGGGAGAGAGAAAAT
AhTE0323R	CCCCCATACATTCAATTCCA
AhTE0343F	CGATCGCTACTTGCTACCAC
AhTE0343R	GGACATCAATCAAGAGGCGT
AhTE0360F	GGATATGATGCCCATAGCTGA
AhTE0360R	TGCTGACTACTTGCAATGCC
AhTE0372F	GCAATTTGGCATAGCCTCTC
AhTE0372R	CGTATTGACAAGGGTTCCGT
AhTE0398F	TCAGCCCAACAGAATACACAA
AhTE0398R	GGACTTTGTTGGGGTGGTAG

Marker Name	Nucleotide Sequence

AhTE0422F	TGGCGTAATCTTTTAAGAACCAA
AhTE0422R	AGAATTAATGTCATCAAACGAATG
AhTE0426F	CAACCCATGATTTGTGAATTAAG
AhTE0426R	TGACTACAATGTTTGGTCATTTTG
AhTE0437F	TGGCTTTTGGGTGTGTATGA
AhTE0437R	GCCACGAGAGAATCCAAAAA
AhTE0446F	GTGCCACGAGGTACACGATA
AhTE0446R	AGACACACACCACACGCATT
AhTE0491F	ATTATAACAGTATACAATTTCTAT
AhTE0491R	GAAGTTTTATTTGGTTCAGAATT
AhTE0496F	TTGTGTGTTTGAGACATGGATTT
AhTE0496R	TCCCGTAATTTCGTACAACTATTTA
AhTE0501F	GAAGCAGGGAACACCCACTA
AhTE0501R	TCTCATGCCCTTAATTAACTACAAA

Appendix 2: Details of transposon markers used in study

Sl. No.	Marker Name	Tm.	Amplicon size (INSERTION)	TAG 24	GPBD4
1	AhTE0119	51.75	537	537	332
2	AhTE0130	50.95	466	466	261
3	AhTE0164	51.75	378	378	173
4	AhTE0190	51.9	381	381	176
5	AhTE0202	51.7	437	437	232
6	AhTE0218	51.65	343	343	138
7	AhTE0222	50.1	419	214	419
8	AhTE0248	52.9	471	266	471
9	AhTE0268	52.4	550	550	345
10	AhTE0278	51.7	511	511	306
11	AhTE0300	52.2	472	472	~500
12	AhTE0303	51.7	452	247	452
13	AhTE0323	51.85	501	501	296
14	AhTE0343	50.75	418	418	213
15	AhTE0360	51.5	374	374	169
16	AhTE0372	51.55	464	464	259
17	AhTE0398	50.75	505	505	300
18	AhTE0422	51.35	452	452	247
19	AhTE0426	51.05	383	383	178
20	AhTE0437	51.5	408	408	203
21	AhTE0446	50.8	428	428	223
22	AhTE0491	41.95	385	385	180
23	AhTE0496	51.45	385	385	180
24	AhTE0501	51.55	360	360	155