Leveraging genomic mapping and QTL analysis to enhance drought tolerance of cultivated peanut (*Arachis hypogaea* L.)

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ABSTRACT

Peanut (Arachis hypogaea L.) is second major legume crop grown after soybean in the United States, and its productivity is often limited by drought stress. Drought negatively impacts the yield and quality of peanut. Drought stress in peanut causes an annual loss of approximately $520 million in the United States. Improving peanut yield under water deficit conditions is crucial for peanut growers to maintain their profitability in the market. To achieve this, it is essential to either breed or adopt already available drought tolerant cultivars that can produce higher yield under water deficit conditions. Therefore, the objectives of this research were to (1) evaluate five commercially available virginia and runner type peanut cultivars for pod yield stability using multilocation trials by studying G × E interaction across 13 environments including year, location, and irrigation regime. Linn and Binns, AMMI, Shukla, Wricke's, Finlay and Wilkinson stability models were used to determine pod yield stability. Bailey and Sullivan showed higher stability and adaptability across all stability indices whereas Wynne and TUFRunner presented high mean productivity with lesser stability across environments reflecting specific adaptation to just a few environments. Bailey and Sullivan are recommended for sustainable production across the growing region of Virginia and Carolinas. The second objective (2) was identification of drought tolerance related quantitative trait loci (QTL) and genetic markers to facilitate the development of drought tolerant cultivars. Three diverse recombinant inbred line (RIL) populations, derived from crossing lines N05006 × N04074FCT (Pop-1), line N05006 × Phillips, an old virginia-type cultivar (Pop-2), and lines N08086olJCT × PI 585005 (Pop-3) were phenotyped for the Normalized Difference Vegetation Index (NDVI), Canopy Temperature Depression (CTD), SPAD-meter relative chlorophyll content of the leaves (SPAD) and wilting for QTL mapping. Mapping identified 27 minor QTL on eight chromosomes for all physiological characteristics, i.e NDVI, CTD, SPAD and wilting, with logarithmic of odds values ranging from 2.5 to 38.5 and the phenotypic variance explained by these traits from 1.04 to 11.46 %. There were 4 loci on chromosome 2 associated with NDVI in Pop-1 and Pop-3, explaining 1.8 to 10.38% of the phenotypic variation. These genomic regions may be important resources in peanut breeding programs to improve drought tolerance. Further research is needed to increase the marker density in order to fine map the identified QTL and validate markers linked with these regions.

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GENERAL AUDIENCE ABSTRACT

Peanut is a multi million-dollar industry in the United States, but water limitations have a detrimental impact on yield, quality, and grower income. Drought along with aflatoxin contamination are two major challenges faced by U.S peanut industry. Annual losses to peanut caused by drought are around $520 million in the United States. Irrigation can alleviate water shortage in drought prone regions, but around 65% of peanut production in U.S is under rainfed condition, meaning that only rainfall can satisfy peanut crop water requirements. The most feasible and economical solution to peanut growers under these circumstances is to adopt drought tolerant varieties. In this research, our goal was to facilitate breeding drought tolerant cultivars through identification of molecular markers associated with drought tolerance and to identify already available drought tolerant peanut cultivars that could be a game changer for the producers. Therefore, the objectives of my research were to (1) evaluate in multiple environments five commercially available virginia and runner type peanut cultivars for pod yield stability and grade factors. In this study, we considered 13 environments, including 4 years, 4 locations, and 2 water regime. Statistical tools including Linn and Binns, AMMI, Shukla, Wricke's, and Finlay and Wilkinson were used to determine pod yield stability. These stability indices showed that Bailey and Sullivan are more stable and adaptable across different locations in terms of yield, whereas Wynne and TUFRunner presented high mean yield with lesser stability showing specific adaptation to only few environments. Based on stability analysis, Bailey and Sullivan are recommended for sustainable production across different growing region of Virginia and Carolinas. The second objective (2) was to identify drought tolerance related genomic regions using three mapping populations. Phenotyping and genotyping of three diverse recombinant inbred line (RIL) populations, derived from crossing lines N05006 × N04074FCT (Pop-1), lines N05006 × Phillips, an old virginia-type cultivar (Pop-2), and lines N08086olJCT × PI 585005 (Pop-3) were done to find quantitative trait loci (QTL) for drought related traits. These population were phenotyped for the Normalized Difference Vegetation Index (NDVI), Canopy Temperature Depression (CTD), SPAD-meter relative chlorophyll content of the leaves (SPAD) and wilting for QTL mapping. These surrogate traits are related to trait of interest for drought tolerance. NDVI is effective in predicting biomass and yield. Similarly, CTD is associated with transpiration efficiency and carbon dioxide assimilation. Mapping identified 27 minor QTL on eight chromosomes for all physiological characteristics, i.e NDVI, CTD, SPAD and wilting with logarithmic of odds values range from 2.5 to 38.5 and the phenotypic variance explained by these traits ranging from 1.04 to 11.46 %. There were 4 loci on chromosome 2 associated with NDVI in Pop-1 and Pop-3, explaining 1.8 to 10.38% of the phenotypic variation. These genomic regions may be important resources in peanut breeding programs to improve drought tolerance. Further research is needed to increase the marker density in order to fine map the identified QTL and validate markers linked with these regions.

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DEDICATION

To my Parents, Farmers, and Healthcare workers of COVID-19 pandemic

TABLE OF CONTENTS

CHAPTER 1: Multilocation Evaluation of Virginia and Runner Type Peanut Cultivars for Yield and Grade Factors in Virginia-Carolina Region

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ABSTRACT

Peanut farm profitability is driven by yield, price, and costs. Irrigation adds up to the cost of production. The average annual precipitation received in the Virginia-Carolina (VC) region is around 1300 mm; however, unpredictable distribution of this rainfall can result in significant periods of water deficit and subsequent reduction in yield and dollar return. Development of new peanut cultivars with high yield and acceptable levels of yield stability across various water availability scenarios is an important component of the peanut breeding program in Virginia and Carolinas, and simultaneous use of runner cultivars developed in the dryer southeastern region has been proposed as a practical solution to limited irrigation availability in the VC region. Still, the identification and adequate utilization of available commercial cultivars with the best combination of yield, drought tolerance, and dollar return value is more immediately beneficial to the peanut industry, yet this assessment has not yet been done to date. Multilocation trials provide reliable guidance for selecting the best cultivars, determining yield stability and patterns of response across environments. The objective of this study was to evaluate the magnitude of genotype by environment interaction (G × E) and to find stable, high-yielding cultivars. Additive main effect and multiplicative interaction (AMMI) model was used to study the effect of G × E in the trials. Wricke's ecovalence, Shukla's stability, and Finlay-Wilkinson variance agreed on ranking Sullivan and Bailey as the most stable and adaptable cultivars across the testing region, whereas Wynne exhibited specific adaptability to some environments. This study concludes that yield stability analysis provides a better understanding of which commercial cultivars are better suited to production across the VC region in terms of meeting peanut industry standards for yield, grading quality, and gross income.

Key Words: *Arachis hypogaea,* adaptability, mega-environment, stability

Introduction

The U.S. produced $1.5 billion worth of peanut (*A. hypogaea* L.) in 2021, of which 15% was contributed by Virginia-Carolina (VC) region (USDA-NASS 2021). The VC region is dominated by the globally recognized, large-seeded virginia-type peanut in the United States. In contrast, runner-type peanut is primarily grown in the lower southeastern production areas of the U.S. Peanut production requires proper soil and climatic conditions, cultivar selection, weed and pest control, and adequate irrigation or rainfall. To get maximum peanut yield, each requirement must be effectively managed (Lamb et al., 1997). The majority (95%) of peanut production in the VC region is rainfed, which leads to occasionally erratic episodes of water deficit. Yield and quality of peanut under rainfed conditions are significantly reduced under drought conditions—including periodic drought (Reddy et al., 2003). The duration and intensity of periodic drought vary by year and location. Optimally wet soils recorded yields over 6,000 kg/ha, but in excessively wet or dry fields, yields were less than 4,000 kg/ha. Peanut cultivation cost per acre is about $900 to $950 varying across different growing regions of the VC region (Peanut Information, NC, 2022). Therefore, the economic feasibility of peanut cultivation, based on the cost per hectare, can be achieved if peanut yield is greater than 4500 kg/ha, which can be attained by minimizing the effects of biotic and abiotic factors on yield and quality (Balota et al., 2020). However, dollar returns on peanut are dependent on both yield and quality, thus maximizing production does not always equate to maximum economic returns. Thus, successful peanut breeding programs focus on delivering cultivars with guaranteed superior yield and quality performance across a wide range of environmental conditions. The virginia-type peanut is the predominant market type grown in the VC region because of the desirability of its large pod and kernel size, but occasionally runners are grown. For kernel size, virginia-type peanuts are almost twice the size of runners for which growers can receive premiums. Importantly however, the cost of production has considerable differences between virginia market type and runner type. The total estimated cost of production for virginia-type peanuts requires an additional $231.30 per acre in inputs compared to runner-type peanuts (Jordan, 2020). For virginia-type, high yield and grade factors, including high extra-large kernel (ELK) content is achieved at the cost of higher requirements of calcium than runner types (Gaines et al., 1991). In addition to higher calcium requirements, large size virginia-type peanut requires more soil moisture for germination than runner type (Pallas Jr et al., 1977); which implies an increased cost from extra seeds to compensate for germination failures, especially in dry years, or supplemental costs incurred to irrigate for good plant stand establishment. Drought conditions in peanut also enhance the growth of mold Aspergillus *flavus* which causes aflatoxin contamination in seeds. Aflatoxin contaminated peanuts not only reduce the market value of crop but also cause human diseases (Williams et al., 2004). Breeding for improving drought tolerance in peanut might be a successful approach to the reduction of aflatoxin contamination suggested by Guo et al., 2008. For example, studies have shown a strong correlation that peanut varieties with drought tolerance attributes were more resistant to aflatoxin contamination (Holbrook et al., 2000; Arunyanark et al., 2009). The sandy nature of the soils in the VC region often leads to rapid depletion of soil moisture and it is well established that virginia-types are less productive than runner types on poorly drained soil (Batten, 1943). The virginia market type requires around 135 to 155 DAP (Days After Planting) to reach maturity, while runners need over 155 DAP in the VC region (Balota et al., 2018). The late maturity of runner types in the VC region results in freeze damage and a subsequent yield penalty. All these factors account for yearly variations in yield performance of genotypes due to environmental variations and G × E. The G × E complicates the selection of genotypes for target traits, as genotypes differ in their response across environments. In this scenario, stability analyses provide a reasonable solution for evaluating the relative performance of cultivars over multiple environments. Interpreting G × E effects in multi-environment trials assists in the selection of stable genotypes for a wide range of environments (Vaezi et al., 2018). The aim of this study was to identify cultivars that maintain high yield across a wide range of environmental conditions. Specific objectives were 1) to compare yield and grade performance of virginia and runner-type peanut cultivars across different growing environments in the VC region, and 2) to investigate the G × E and yield stability of five virginia and runner-type peanut cultivars across 13 environments.

Materials and Methods

A total of five peanut cultivars, three virginia-type (Bailey, Sullivan, and Wynne) and two runner-type (FloRun 107, TUFRunner 297) were evaluated for four years (2016, 2017, 2018, and 2020) at four locations in Virginia and North Carolina under two levels of water regime, i.e., rainfed and irrigated (rainfed plus irrigation) (Table 1). The trials were conducted at Tidewater Agricultural Research and Extension Center (TAREC) in Suffolk, VA (36°68′ N, 76°77′ W, 25 m elevation), the Upper Coastal Plain Research Station (UCPRS) near Rocky Mount, NC (35° 57 N, 77° 48' W, 33 m elevation), and two farmers' fields in Capron (36° 42' N, 77° 12' W, 34 m elevation) and Dinwiddie (37°

04' N, 77° 37' W, 78 m elevation), VA. Soils for the four locations were Eunola-Kenansville soil (fine-loamy, siliceous, thermic Aquic Hapludults) at Suffolk, Goldsboro sandy loam soil (fine-loamy, siliceous, thermic Aquic Paleudalts) at Rocky Mount, Nansemond soil (fine-loam, Aquic Hapludults) at Capron, and Helena sandy loam soil (fine, semiactive, thermic Aquic Hapludults) at Dinwiddie. The trials were sown in randomized complete block designs within each water regime with four replications. Cultural practices were performed based on the recommendations of Virginia Peanut Production Guide (Balota et al., 2020). Air temperature, relative humidity (RH), and rainfall were continuously monitored next to the plots with a weather station (Watchdog Temperature/RH Station, Model 2450, Spectrum Technologies Inc., Plainfield, IL). In addition, climate data for precipitation and temperature for all years were collected from PRISM climate group from the nearest weather station to the field trials. Based on peanut growth stage and weekly precipitation requirement, plots were irrigated using a lateral pull boom cart sprinkler irrigation system (E1025 Reel Rain, Amadas Ind., Suffolk, VA) shown in Fig.1. Plots were dug from mid-September to early October at the harvest maturity (Boote, 1982) with a KMC two-row digger. Pods were combined after a few days of windrow drying with a Hobbs peanut combine (Model 325A). Pod yield was calculated from plot weight and adjusted to 7% seed moisture and foreign material (FM) percentage. Yield samples were cleaned and adjusted to 10% moisture. Grade characteristics include extra-large kernels (ELK), i.e., kernels not passing a 25.4 mm (1 in) × 8.5 mm (21.5/64 in) screen; sound mature kernels (SMK) were percent whole kernels not passing a 25.4 × 6 mm screen for virginia and 19.05 × 6.4 mm slotted screen for runner type peanut. The damage kernels (DK) content represents the decayed, molded, sprouted, and discolored kernels due to insects and weather damage; sound splits (SS) were halved and broken kernels; and the other kernels (OK) were those passing through a 25.4 mm × 5.9 mm (15/64 in) screen determined by subjecting a 250 g pod sample to the Federal- state Inspection procedure (USDA, 2020). The sum of SMK, DK, SS, and OK constituted the total sound mature kernels (TSMK). The crop value was determined from yield and calculated price per pound from these grade standards using the USDA Agricultural Marketing Service approach (USDA, 2019). Yield and grade data from individual trials were collected from all environments.

Statistical analysis

Data from yield was subjected to analysis of variance (ANOVA) using a fitted mixed model to assess the magnitude of G × E where genotype (G) was considered as a fixed effect and growing environment (E) as a random effect. Boxplots for grading factors (SMK, SS, and TSMK) were created to explore the heterogeneity of genetic variance using '*statgenGXE*' package (Van Rossum, 2021). The means for each grading factor and market type were compared with independent t-tests and a significance level of 0.05 was used to judge the significance (R Core Team, 2021). There is no one biometrical model that can adequately explain the stability performance of genotypes across environments (Farshadfar et al., 2013). Therefore, different statistical approaches and models were used to avoid the limitations of any single model. In this study, analysis of yield stability was done using Wricke's Ecovalence(wi), Shukla's stability variance (σ^2_i), Finlay and Wilkinson's joint regression analysis, Lin and Binn's superiority measures (Pi) and AMMI. Wricke's ecovalence (Wi) stability coefficient shows the contribution of each genotype to the G × E, squared and summed across environments, in an unweighted analysis of the G × E means. The formula for this model is: $Wi = \Sigma\ (Yij - Y.\ j - Yi + Y\ldots)$; Where: Yij = Mean of genotype i in environment j, $Y.j$ = Mean yield of genotype across environments, Yi = environment mean, $Y\ldots$ = Overall mean (Alberts, 2004) (Wricke, 1962**).** Wricke ecovalence evaluates the yield dynamic stability of the cultivar which is desirable for breeding in water-limited conditions with inconsistent rainfall patterns. In Wricke's ecovalence, cultivars with the lowest values have smaller deviations from the mean across environments and contributed least to the G × E interaction and are, therefore, more stable. Shukla's stability variance (σ^2_i) is defined as the variance around the genotype's phenotypic mean across all environments (Shukla, 1972). It measures each genotype's contribution to the overall G × E and error term. Superiority measure was proposed by Lin and Binns (1988). The cultivar-superiority measure is a function of the sum of the squared differences between a cultivar's mean and the best cultivar's mean, where the sum is across environments. The formula for this model is as follows: $Pi = \Sigma\ (Xij - Mj)\ 2\ /(2n)$; Where: Pi is the superiority measure of the ith cultivar, Xij is the yield of the ith cultivar grown in the jth environment, and Mj is the maximum yield value among all cultivars in the jth environment, n is the number of locations. Finlay and Wilkinson's joint regression coefficient is the response of genotype to the environmental index that is derived from the average performance of all genotypes in each environment (Finlay and Wilkinson, 1963). The model fitted in Finlay – Wilkinson analysis is$Yij = \mu + Gi + \beta iEj + \epsilon ij$; Where Yij is phenotypic value of genotype i in the environment j, $\boxed{\mu}$ is the general mean, Gi is genotypic effect, βi is sensitivity parameters, Ej is environment effect and ϵij is residual. According to Finlay and Wilkinson, regression coefficients approximating to 1.0 indicate average stability; however, it must always be associated and interpreted with the mean yield to determine adaptability. An AMMI stability model is one of the most widely used tools in multiple-environment trials to understand complex G × E and increase the accuracy to improve recommendations, selection, and genetic gains (Gauch, 2013). AMMI first performs an analysis of variance (AOV) to partition the variance into genotype, environment, and genotype x environment interaction effects, and then it applies principal components analysis (PCA) to GxE. Here, an AMMI model was used to estimate the response variable for the i^{th} cultivar in the j^{th} environment given as follows:

$\boxed{Yij = \mu + \alpha i + \tau j + \sum_{k=1}^{p} \;.\; \lambda k \; \alpha ik \; tjk + \varepsilon ij}$; Where: Yij is the yield of the i^{th} genotype in the j^{th} environment; $\boxed{\mu}$ is the grand mean; $\boxed{\alpha i \text{ and } \tau j}$ are the genotype and environment deviations from grand mean respectively; $\boxed{\lambda k}$ is the eigenvalue of the PCA analysis axis; $\boxed{\varepsilon ij}$ is the residual.

The AMMI biplot graphs showed the dispersion of genotypes, environments, and interactions between them. Biplots in AMMI1 identify genotypes that are adapted to a particular environment or broadly adapted. The biplot origin represents the overall phenotypic mean and yield. The position of genotype and environment from the origin provides insight into the G × E interaction. Genotypes near the origin are insensitive to environmental interaction hence broadly adapted. All statistical analyses involving G × E, AMMI model, and stability indices were performed in R (R Core Team, 2021) using the package ‘statgenGXE’ (Van Rossum, 2021) and ‘metan’ (Olivoto, 2020). Figures were produced using the package ggplot2 (Wickham, 2009).

Results and Discussion

The environments included in the present study differed in soil type and weather conditions, especially rainfall. The degree and distribution of rainfall varied during the growing seasons at Suffolk location in 2017, 2018, and 2020. Rainfall was uniform throughout the growing season in 2017, but in 2018 it was reduced in the latter part of the season, whereas in 2020 the month of July had a rainfall deficit (Table 3). Insufficient rainfall in July affected peg and pod development by prolonging the vegetative phase of peanuts. In the Rocky Mount location, MIN and MAX temperature in 2018 was higher than in 2017 (Table 3). The increase in average temperature was predicted to decrease the gross income from peanut (Ramsey et al., 2020). Indeed, a slight decrease in gross income was recorded in 2018 compared to 2017 at Rocky Mount (Fig. 2). All years met the sufficient heat unit requirement for peanut maturity and growth (Pallas and Samish, 1974).

The distribution of five virginia- and runner-peanut cultivar means for yield, gross income, SMK, SS, and TSMK in 13 environments for water regime and market type are summarized through boxplots in Figures 2 to 6. Genotype yield for each trial (Fig. 3) showed that irrigated plots produced more yield than rainfed plots. This agrees with findings by others (Geethalakshmi and Lourduraj, 1998; Nautiyal et al., 2002; Ramakrishna et al., 2006). Overall, there was no significant difference in pod yield between virginia type (5619 ±823 kg/ha) and runner type (5426 ±921 kg/ha; $p = 0.07$) cultivars. The virginia type performed better than the runner-type specifically in the environments Rkm17FR (E05), Rkm17RfIR (E06), and Suf20RfIR (E13) as shown in Figure 3. Gross income was greater under the irrigated regime as compared to rainfed.

Virginia type (2191 ±378 \$/ha) cultivars provided higher gross returns then runner types (2079 ±407; $p = 0.02$) (Fig. 2) in the study. The sound mature kernels (SMK) percent for runner types (66.6 ±4.82) was significantly higher than virginia type (65.0 ±5.31, $p = 0.02$) (Fig. 4). A similar trend was noticed in Sound splits (SS) for runner types (3.53 ± 2.1) and virginia types (2.80 ±1.64, $p = 0.004$) (Fig. 5). Sound splits were linearly proportional with the SMK content for the runners, in agreement with recent data published by Anco et al., (2021). Virginia (71±3.07) and runner (75 ±2.66) cultivars are significantly different from each other for the TSMK content (Fig. 6). The TSMK percent for runners was higher than virginia type ($p = 0.0001$).

A combined analysis of variance using a fitted mixed model was performed to assess the magnitude of G × E. The results of the combined ANOVA across 13 environments for the five Virginia and runner peanut cultivars showed that genotype main effect (G), environment main effect (E), and G × E were all highly significant ($p < 0.001$) for pod yield (Table 4). A highly significant G × E indicates the need for stability analysis to evaluate the impact of each genotype on G × E, which may shape differences among genotypes in each environment. Based on the yield ecovalence rank, Sullivan can be regarded as the most stable followed by FloRun 297 and Bailey (Table 5). Wynne ranked last in yield (Table 5). Bailey ranked top in pod yield (5796 kg/ha) coupled with third rank (1360238) in ecovalence stability (Table 5). Shukla provides a measure of the consistency of the genotype in a static manner i.e., static stability. The stability variance is a linear combination of the ecovalence, and therefore both Wi and σ^2_i are equivalent for ranking purposes (Wricke and Weber, 1980). Shukla's stability ranking as well as the mean yield are given in Table 5. The most stable cultivars indicated by this stability measure were Sullivan and Bailey whereas Wynne was least stable across all environments. Runner type cultivar TUFRunner 297 ranked intermediate for stability with the third rank in Shukla's stability ranking. Genotypes with the smallest values of Lin and Binn's superiority tend to be more stable and closer to the best genotype in each environment. From this analysis, the most stable cultivar ranked first in Lin and Binn's superiority (98452) with the mean pod yield was Bailey (5796 kg/ha), followed by cultivar TUFRunner 297 (161207) and Sullivan (179910). Among all, the least stable ranked in Lin and Binns superiority and mean pod yield was Wynne (5344 kg/ha (Table 7). Finlay -Wilkinson (FW) ranking analysis (Table 5) showed that Sullivan was most stable among all cultivars and adapted to most of the environments, followed by Bailey which had the highest mean yield. Wynne was the least stable and adapted to only a few specific environments. Figure 7 reveals a similar pattern, showing that Sullivan and Bailey are more stable and performed better in poor environments. Wynne yielded low in most of the environments and indicated less adapted by stability indices but performed better in Rkm17RfIR (E06) and Cap17RfIR (E02) reflecting its narrow adaptation to those environments. The other cultivars that lie between Bailey and Wynne in Fig. 7 are of average stability according to Finlay and Wilkinson's model. The mean squares from AMMI analysis of variance (Table 6) show significant variation among the genotypes, environments, and their interaction for pod yield. The G × E is highly significant and partitioned into four interaction principal component analysis axes (IPCA). The PC1 and PC2 scores were significant and explained 46.9% and 22.27% of variability relating to G × E, respectively. Biplots (Figs. 8 and 9; Gabriel, 1971) provide a graphical representation of genotypes and environment from AMMI analyses and reveal three mega-environments. The first biplot (Fig. 8; AMMI1) includes mean pod yield and PC1, whereas AMMI2 (Fig. 2) includes PC1 Vs PC2. According to Fig. 8, genotypes that were stable and can be grown sustainably in multiple locations are G01 and G03, corresponding to Bailey and Sullivan. FloRun107(G02) and TUFRunner 297(G04) were more productive cultivars in terms of yield for most of the environments, whereas Wynne showed specific adaptability to environment E06 (representing irrigated regime in 2017 at Rocky Mount) similarly with TUFRunner 297 in E04 (representing irrigated regime in 2016 at Dinwiddie) (Fig. 8). AMMI 2 biplot is used for generating mega-environments. In the AMMI biplot, there were three mega-environments including, Megaenv1 as Cap17Rf, Cap17RfIR, Rkm17Rf, Rkm18Rf, Rkm18RfIR, Suf18Rf, Suf18RfIR, Suf20Rf, and Suf20RfIR with high yielding cultivar as Bailey; Megaenv2 with Dwd16Rf, Dwd16RfIR, and Suf17R with TUFRunner297; and Megaenv3 with Rkm17RfIR by Wynne as best cultivar (Fig. 9).

Summary and Conclusions

The objective of this study was to evaluate five virginia and runner peanut cultivars for pod yield stability by studying G × E interaction over four years across 13 environments between 2016 and 2020. Pod yield and grading factors were determined, and mean pod yield was used to determine yield stability using different stability models, including Linn and Bins, Shukla, Wricke’s, Finlay and Wilkinson, and AMMI. Sullivan and Bailey displayed higher adaptability and stability across multiple stability indices. Therefore, they could be recommended for sustainable production across multiple environments in the VC region. Wynne and TUFRunner presented high mean productivity. However, they were unstable and had specific adaptation to limited environmental conditions. Environment E12 (representing rainfed condition in 2020 at Suffolk) gave the lowest mean yield and can be considered as unfavorable for peanut production, whereas environment E09 (representing the rainfed condition in 2017 at Suffolk) had the highest mean yield and was indicated as the most favorable environment for peanut production in this study. This study showed that the peanut market types had broad and specific responses to the environments under investigation.

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Figures and Tables

Figure 1. Lateral pull boom cart sprinkler irrigation system for the irrigated regime.

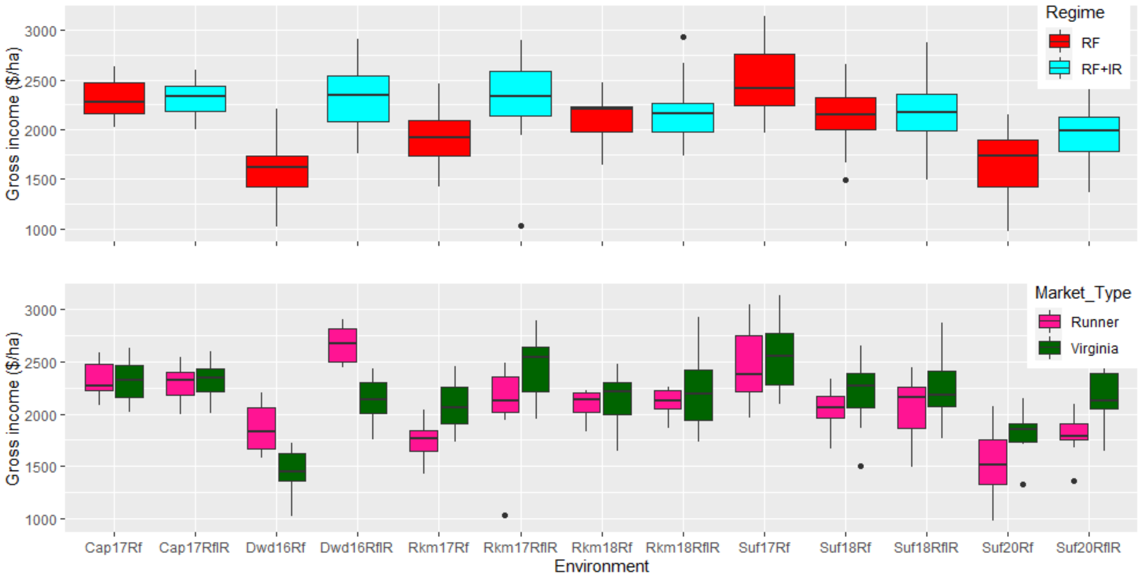

Figure 2. Boxplot for the effect of water regime and market type on gross income($/ha) of 5 virginia and runner peanut cultivars in 13 environments. Each plot indicates total range, interquartile range (box) and median (line). Environment names are coded and the first three letters indicate location; Cap, Capron, VA; Din, Dinwiddie, VA; RM, Rocky Mount, NC; Suf, Suffolk, VA; two digits indicate year of the trial; 16 for 2016, 17 for 2017, 18 for 2018, 20 for 2020 and the last letters Rf and RfIR for water regime: Rf, rainfed, RfIR, rainfed plus irrigated. The upper part of the figure shows the effect of water regime on gross income and the lower part shows the gross income of runner and virginia cultivar in 13 environments.

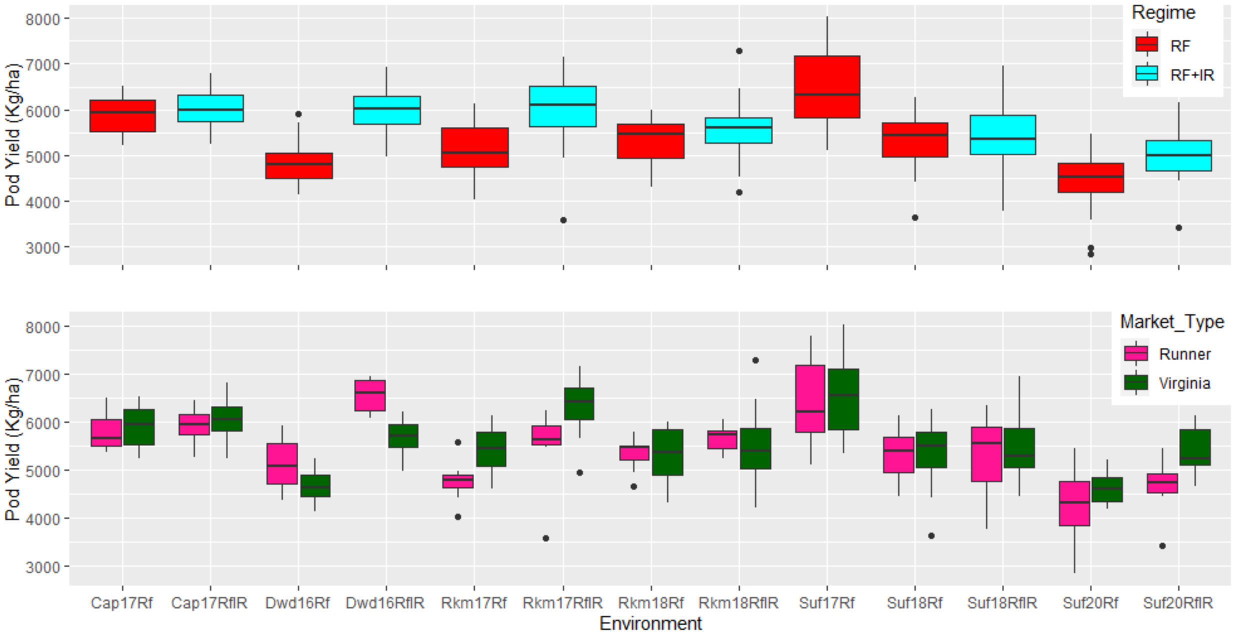

Figure 3. Boxplot for the effect of water regime and market type on yield of 5 virginia and runner peanut cultivars in 13 environments. Each plot indicates total range, interquartile range (box) and median (line). Environment names are coded and the first three letters indicate location; Cap, Capron, VA; Din, Dinwiddie, VA; RM, Rocky Mount, NC; Suf, Suffolk, VA; two digits indicate year of the trial; 16 for 2016, 17 for 2017, 18 for 2018, 20 for 2020 and the last letters Rf and RfIR for water regime: Rf, rainfed, RfIR, rainfed plus irrigated. The upper part of the figure shows the effect of water regime on yield and the lower part shows the yield performance of runner and virginia cultivar in 13 environments.

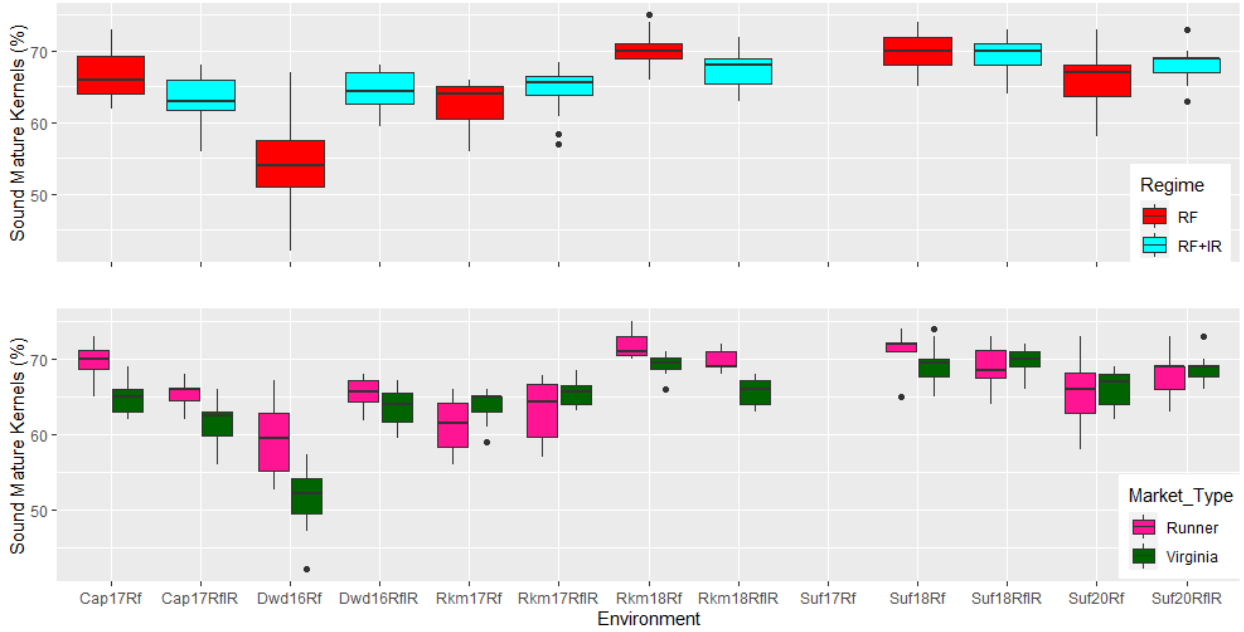

Figure 4. Boxplot for the effect of water regime and market type on Sound Mature Kernels (SMK) of 5 virginia and runner peanut cultivars in 13 environments. Each plot indicates total range, interquartile range (box) and median (line). Environment names are coded and the first three letters indicate location; Cap, Capron, VA; Din, Dinwiddie, VA; RM, Rocky Mount, NC; Suf, Suffolk, VA; two digits indicate year of the trial; 16 for 2016, 17 for 2017, 18 for 2018, 20 for 2020 and the last letters Rf and RfIR for water regime: Rf, rainfed, RfIR, rainfed plus irrigated. The upper part of the figure shows the effect of water regime on Sound Mature Kernels (SMK) and the lower part shows the Sound Mature Kernels (SMK) of runner and virginia cultivar in 13 environments.

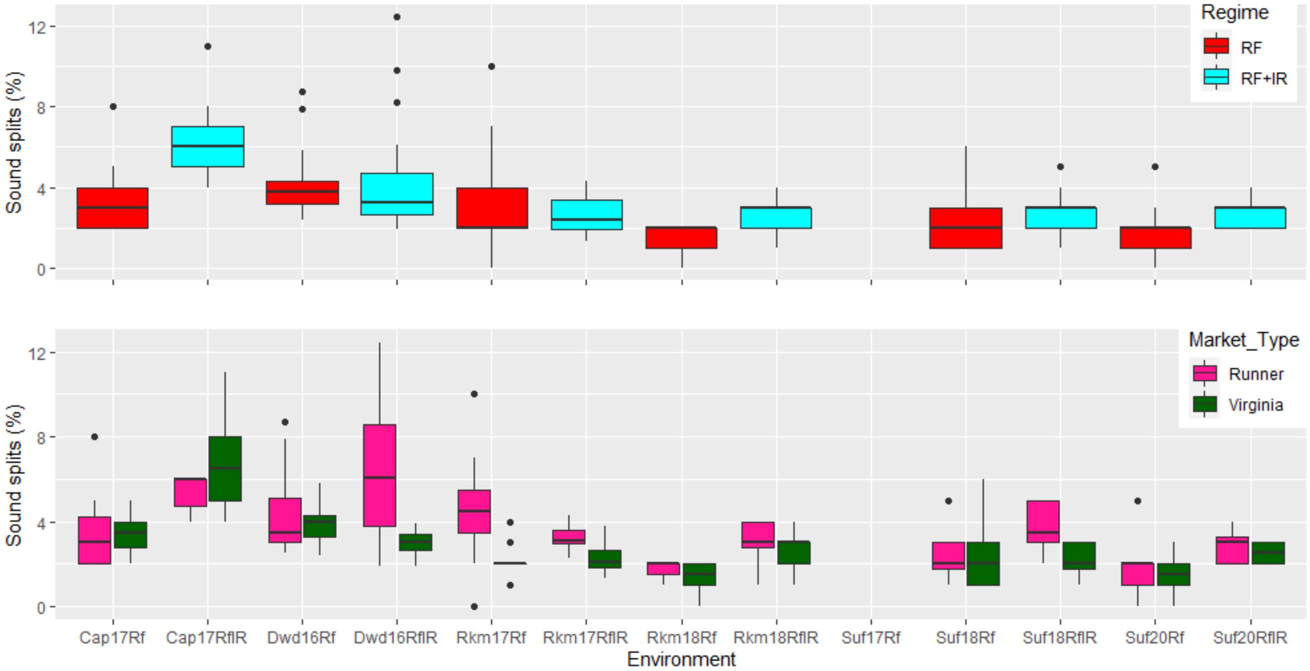

Figure 5. Boxplot for the effect of water regime and market type on Sound Splits (SS) of 5 virginia and runner peanut cultivars in 13 environments. Each plot indicates total range, interquartile range (box) and median (line). Environment names are coded and the first three letters indicate location; Cap, Capron, VA; Din, Dinwiddie, VA; RM, Rocky Mount, NC; Suf, Suffolk, VA; two digits indicate year of the trial; 16 for 2016, 17 for 2017, 18 for 2018, 20 for 2020 and the last letters Rf and RfIR for water regime: Rf, rainfed, RfIR, rainfed plus irrigated. The upper part of the figure shows the effect of water regime on Sound Splits (SS) and the lower part shows the Sound Splits (SS) of runner and virginia cultivar in 13 environments.

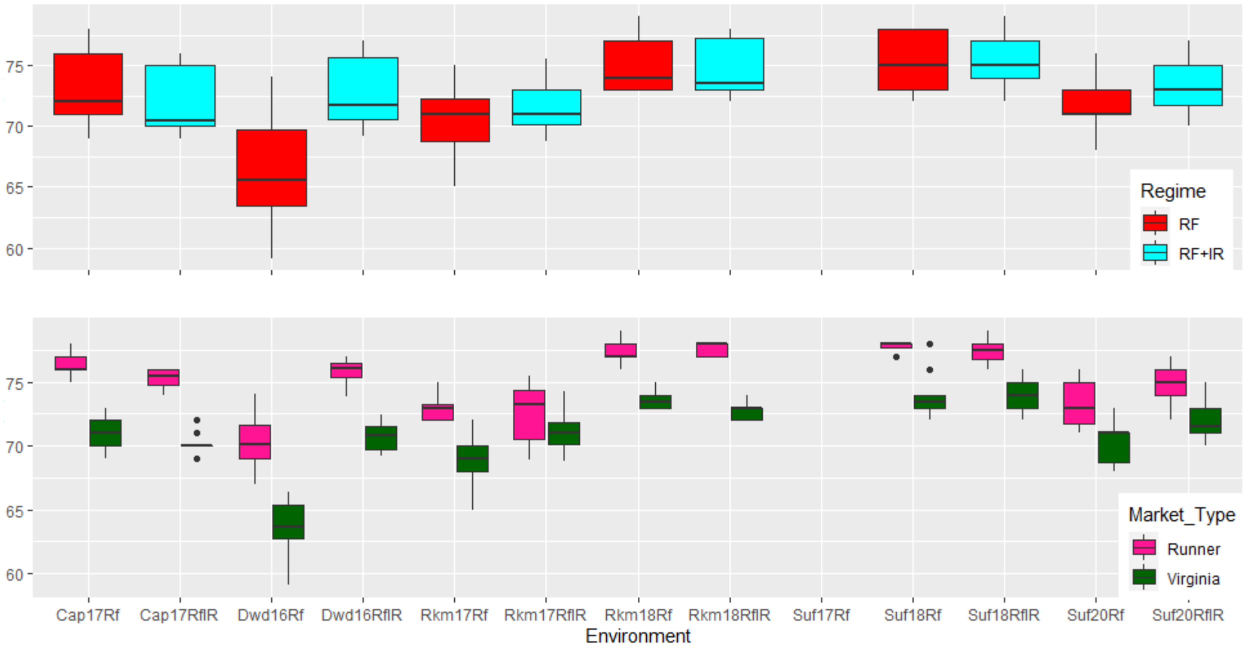

Figure 6. Boxplot for the effect of water regime and market type on Total Sound Mature Kernels (TSMK) of 5 virginia and runner peanut cultivars in 13 environments. Each plot indicates total range, interquartile range (box) and median (line). Environment names are coded and the first three letters indicate location; Cap, Capron, VA; Din, Dinwiddie, VA; RM, Rocky Mount, NC; Suf, Suffolk, VA; two digits indicate year of the trial; 16 for 2016, 17 for 2017, 18 for 2018, 20 for 2020 and the last letters Rf and RfIR for water regime: Rf, rainfed, RfIR, rainfed plus irrigated. The upper part of the figure shows the effect of water regime on Total Sound Mature Kernels (TSMK) and the lower part shows the Total Sound Mature Kernels (TSMK) of runner and virginia cultivar in 13 environments.

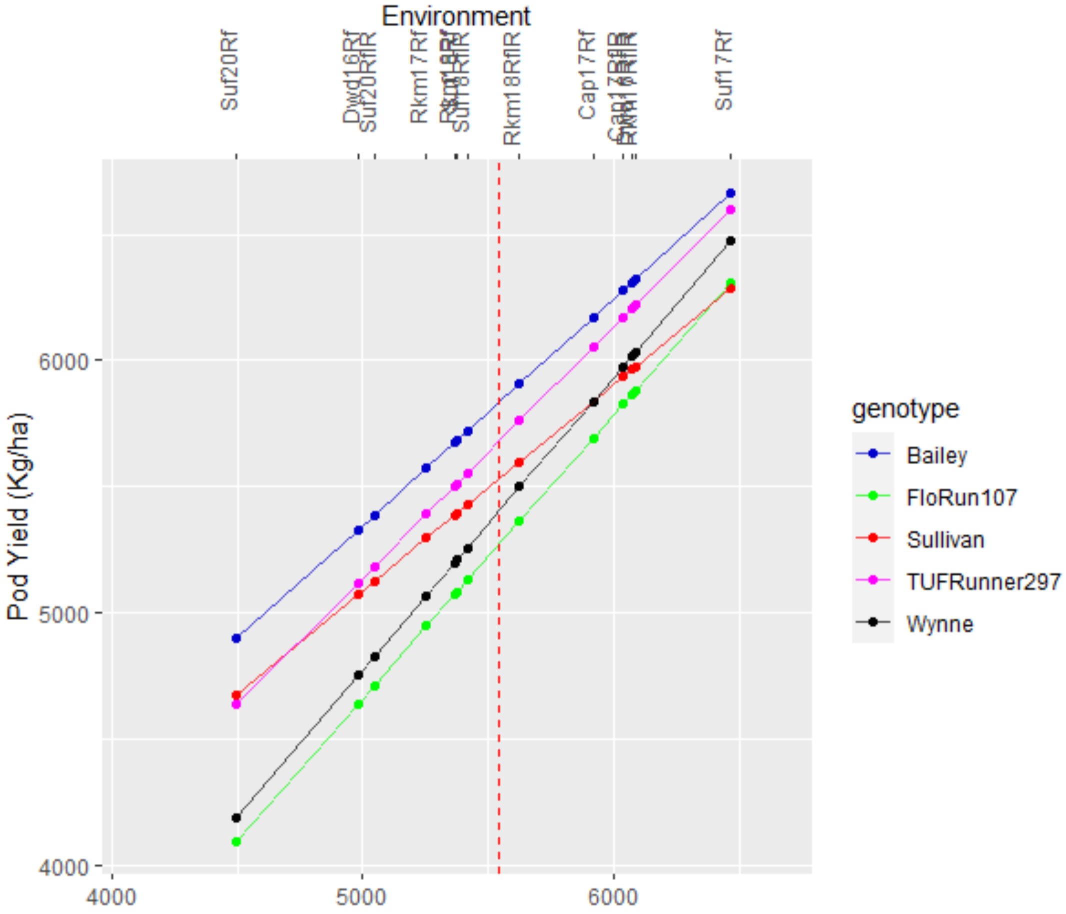

Figure 7. Plot of the performance of five cultivars on estimated environment values. Each color represents a different cultivar. Lines are fitted values of genotype by environment combination. The horizontal axis displays the estimated environmental effects and the vertical axis represents the yield performance.

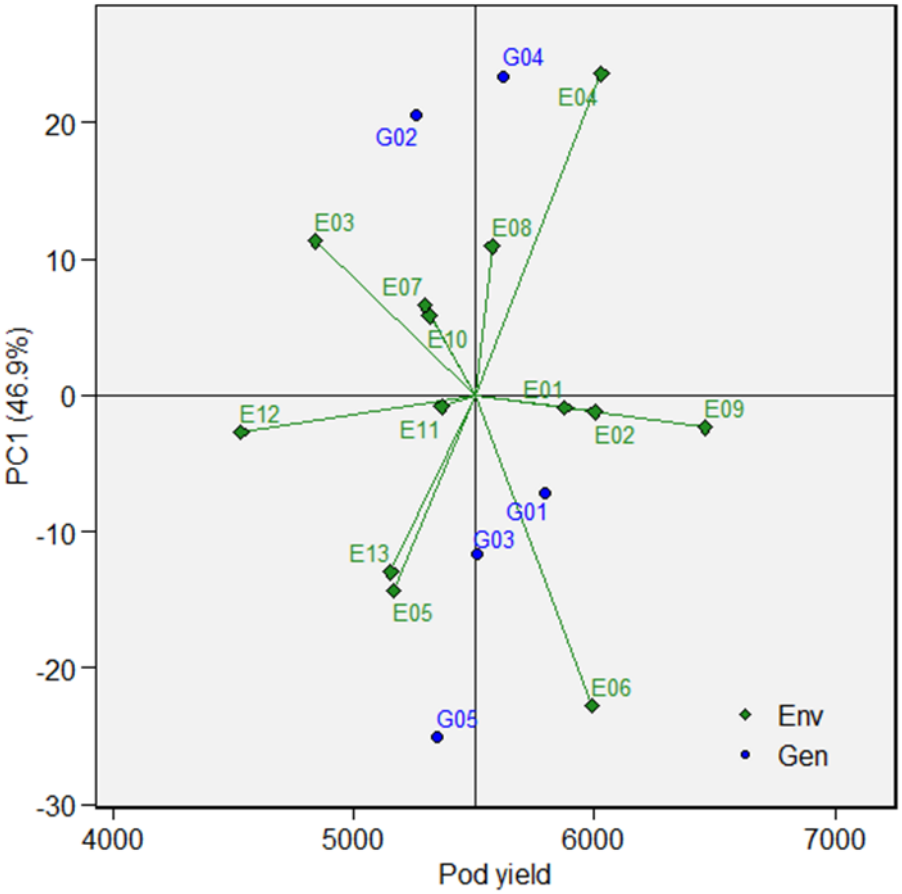

Figure 8. AMMI1 biplot (mean Vs PC1) for pod yield (kg/ha) with five cultivars (G) and thirteen environments (E).

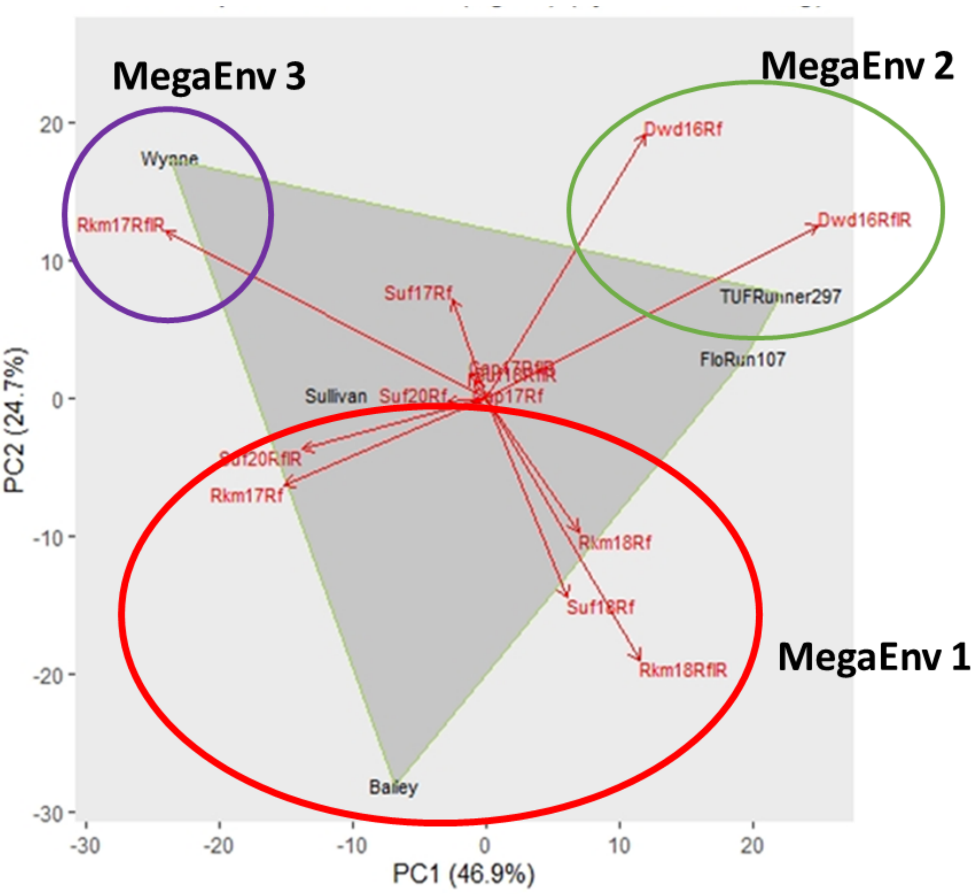

Figure 9. AMMI2 biplot (PC1 Vs PC2) for pod yield(kg/ha) with five cultivars (G) and thirteen environments (E).

Table 1. Description of virginia and runner cultivars tested from 2016 to 2020, and the reasons for inclusion in the testing.

Name	**Genotype**	**Type**	**Use**	**Year /Location of use in test**	**Reason**	**References**
FloRun '107'	G02	Runner	Cultivar	2016, Din; 2017, Cap, RM & Suf; 2020: Suf	High yield	Tillman and Gorbet, 2010
TUFRunner '297'	G04	Runner	Cultivar	2016, Din; 2017 Cap, RM & Suf; 2018 RM & Suf; 2020: Suf	High oleic/high yield	Tillman, 2017
Bailey	G01	Virginia	Cultivar	2016, Din; 2017 Cap, RM & Suf; 2018 RM & Suf; 2020: Suf	Widely grown/High yield	Isleib et al., 2011
Sullivan	G03	Virginia	Cultivar	2016, Din; 2017 Cap, RM & Suf; 2018 RM & Suf; 2020: Suf	High oleic/TSWV resistant	Isleib et al., 2013
Wynne	G05	Virginia	Cultivar	2016, Din; 2017 Cap, RM & Suf; 2018 RM & Suf; 2020: Suf	High oleic/large kernels	Isleib et al., 2013

*Location abbreviation: Cap, Capron, VA; Din, Dinwiddie, VA; RM, Rocky Mount, NC; Suf, Suffolk, VA

Table 2. Description of 13 peanut growing environments with location, water regime, and soil type in tests from 2016-2020.

Season	**Environment**	**ENV**	**Location**	**Regime**	**Soil type**
2016	Dwd16RfIR	E04	Dinwiddie, VA	Rainfed+Irrigated	Helena sandy loam
	Dwd16Rf	E03	Dinwiddie, VA	Rainfed	Helena sandy loam
2017	Cap17RfIR	E02	Capron, VA	Rainfed+Irrigated	Nansemond
	Cap17Rf	E01	Capron, VA	Rainfed	Nansemond
	Rkm17RfIR	E06	Rocky Mount, NC	Rainfed+Irrigated	Goldsboro sandy loam
	Rkm17Rf	E05	Rocky Mount, NC	Rainfed	Goldsboro sandy loam
	Suf17Rf	E09	Suffolk, VA	Rainfed	Eunola- Kenansville
2018	Rkm18RfIR	E08	Rocky Mount, NC	Rainfed+Irrigated	Goldsboro sandy loam
	Rkm18Rf	E07	Rocky Mount, NC	Rainfed	Goldsboro sandy loam
	Suf18RfIR	E11	Suffolk, VA	Rainfed+Irrigated	Eunola- Kenansville
	Suf18Rf	E10	Suffolk, VA	Rainfed	Eunola- Kenansville
2020	Suf20RfIR	E13	Suffolk, VA	Rainfed+Irrigated	Eunola- Kenansville
	Suf20Rf	E12	Suffolk, VA	Rainfed	Eunola- Kenansville

Table 3. Weather information from seven environments (locations and years) recorded during the peanut growing season. GDD13.3 is the growing degree days using a base temperature of 13.3 °C.

Location	**Dinwiddie (VA)**	**Capron (VA)**	**Rocky Mount (NC)**		**Suffolk (VA)**		
Year	**2016**	**2017**	**2017**	**2018**	**2017**	**2018**	**2020**
MAX temp (°C)							
May	23	25	26	28	26	28	25
June	29	29	30	31	32	31	29
July	32	32	32	31	33	31	34
Aug	32	29	30	31	31	32	32
Sep	28	27	28	31	28	31	26
Oct	23	25	25	30	27	25	24
MIN temp (°C)							
May	13	14	15	18	14	18	16
June	17	18	19	21	21	19	19
July	21	21	22	21	22	20	22
Aug	20	20	21	21	19	21	22
Sep	19	16	17	21	16	20	17
Oct	14	14	12	18	16	13	11
Monthly cumulative GDD13.3 (°C)							
May	141	188	219	291	195	285	144
June	298	308	321	366	318	357	300
July	401	414	425	388	415	371	442
Aug	401	342	365	393	354	402	417
Sep	303	251	268	368	268	363	258
Oct	51	65	70	108	74	106	39
Rainfall (mm)							
May	219	137	125	165	119	104	74
June	128	105	122	74	89	104	97
July	123	133	150	180	61	208	53
Aug	51	204	175	94	185	150	216
Sep	159	40	76	130	94	112	259
Oct	120	6	86	3	58	58	20

Table 4. Combined analysis of variance for pod yield in the study of 5 virginia and runner peanut cultivars in 13 environments.

Source	**df**	**SS**	**MS**	**F-Value**	**P**
Environment (E)	12	95555434	7962953	26.57969	***
Rep (E)	39	19263173	493927.5	1.64869	0.015
Genotype (G)	4	16411368	4102842	13.69496	***
G × E	48	29139325	607069.3	2.026348	***
Residuals	182	54524984	299587.8		
CV (%)	9.88				

df, degree of freedom; G × E, genotype by environment interaction; SS, sum of square; MS, Mean square; P, level of probability. Significant at $p \leq 0.001$(***); $p \leq 0.01$(**) and $p \leq 0.05$(*)

Table 5. Mean yield and average stability ranking of 5 virginia and runner peanut cultivars tested across 13 environments.

Genotype	GEN	Yield(kg/ha)	Lin & Binn's	Shukla's	Wricke's	FW	Average Rank
Sullivan	G03	5511 (3)	(3)	(1)	(1)	(1)	1.5
Bailey	G01	5796 (1)	(1)	(2)	(3)	(2)	2.0
TUFRunner297	G04	5622 (2)	(2)	(3)	(4)	(3)	3.0
FloRun107	G02	5262 (5)	(4)	(4)	(2)	(4)	3.5
Wynne	G05	5344 (4)	(5)	(5)	(5)	(5)	5.0

Figures between bracket denote rank

Table 6. Analysis of variance of additive main effects and multiplicative interaction (AMMI) for yield on the genotype and environment interactions.

Source	**DF**	**SS**	**MS**	**F-Value**	**Prob.>F**	**Variation explained (%)**
Environment (E)	12	95555434	7962953	26.57969	***	
Rep (E)	39	19263173	493927.5	1.64869	0.015	
Genotype (G)	4	16411368	4102842	13.69496	***	
G × E	48	29139325	607069.3	2.026348	***	
PC1	15	12765739	851049.3	2.84	***	46.9
PC2	13	6723011	517154.7	1.73	*	24.7
PC3	11	5845645	531422.3	1.77		21.5
PC4	9	1885576	209508.5	0.7		6.9
Residuals	182	54524984	299587.8			
Total	333					

PC1, PC2, PC3, and PC4 are the principal component axes 1,2,3 and 4 respectively.
Significant at $p \leq 0.001$(***); $p \leq 0.01$(**) and $p \leq 0.05$(*)
$p \leq 0.05$(*)

Table 7. Genotype Environment code for G x E

ENV	Environment	GEN	Cultivar
E01	Cap17Rf	G01	Bailey
E02	Cap17RfIR	G02	FloRun107
E03	Dwd16Rf	G03	Sullivan
E04	Dwd16RfIR	G04	TUFRunner297
E05	Rkm17Rf	G05	Wynne
E06	Rkm17RfIR		
E07	Rkm18Rf		
E08	Rkm18RfIR		
E09	Suf17Rf		
E10	Suf18Rf		
E11	Suf18RfIR		
E12	Suf20Rf		
E13	Suf20RfIR		

Chapter 2: Phenotyping of RIL population to drought tolerance in virginia-type peanut in Virginia Carolina region

Introduction

Peanut (*Arachis hypogaea* L.), planted on 20 M ha worldwide, ranks second as the most economically important legume after soybean (FAOSTAT, 2020). In the United States, peanuts are grown on 619,000 ha across eleven states and three geographical regions: Southeast (Alabama, Florida, Georgia, and Mississippi), the Southwest (Oklahoma, New Mexico, and Texas), and Virginia-Carolina (VC; North Carolina, South Carolina, and Virginia; USDA-NASS, 2020). In 2021, the U.S. produced $1.5 billion worth of peanuts of which 15% was contributed by the VC growing region (USDA-NASS 2021). Peanut is generally grown as a rainfed crop in both tropical and subtropical agro-climatic regions of the world, which makes the crop more vulnerable to both biotic and abiotic stresses. Among abiotic stressors, drought is one of the major limiting factors in peanut production in the U.S. and globally (Bray, 1993). Model-based weather forecasts indicate greater drought and heat stress during the 21st Century (Teixeira et al., 2013; Gourdji et al., 2013; Li et al., 2015), which may have significant impacts on peanut production. For example, it has been well documented that drought can result in significant economic losses of the peanut crop (Reddy et al., 2003; Wright and Nageswara Rao, 1994). The VC region has over 90% rainfed production with abundant precipitation ranging between 965 and 1270 mm each year. However, the uneven distribution of rainfall significantly limits peanut yield and quality (Balota, 2020). Similarly, extreme temperature predictions suggest yield reductions of up to 11% for each degree Celsius increase in temperature (Ramsey et al, 2020).

Being grown in a hot and humid climate of that predominates most of the US regions, and the VC region in particular, peanut is prone to many diseases including southern stem rot (caused by *Sclerotium rolfsii Sacc.*), early leaf spot (caused by *Cercospora arachidicola*), Sclerotinia blight (caused by *Sclerotinia minor* Jagger), late leaf spot (caused by *Cercosporidium personatum* (Berk and Curt) Deighton), Cylindrocladium black rot (caused by *Cylindrocladium parasiticum*) and tomato spotted wilt (caused by tomato spotted wilt virus genus Tospovirus; family Bunyaviridae; Shew, 2019). Low moisture stressed peanuts were prone to *Aspergillus flavus* mold contamination, known to produce carcinogenic aflatoxin (Wilson and Stansell, 1983; Sanders et al., 1993; Luis et al., 2020). The peanut researchers found a positive relationship of aflatoxin contamination with drought stress (Dickens et al., 1973; Holbrook et al., 2000; McDonald and Harkness, 1967; Pettit et al., 1971; Wilson and Stansell, 1983).

The effect of drought stress on peanut differs at different growth stages (Holbrook et al., 2020). Moderate drought stress during the pre-flowering stage had the least detrimental impact on yield and quality. In contrast, mid-season drought (flowering to pod development stage) and late-season drought (pod filling stage) resulted in yield losses, and the late-season drought, in particular, resulted in the most significant yield decrease from delayed pegging and pod development (Stansell et al., 1976; Pahalwan and Tripathi, 1984; Smartt, 1994; Prasad et al., 1999). The critically high-water demanding growth stages at pegging and pod filling usually lasts from mid-June through end-August in the VC region. Unfortunately, increasingly recurrent drought events in the VC region are threatening peanut production, demanding solutions for improved tolerance. One of the possible solutions to drought under rainfed conditions would be irrigation, but the extent to which that is feasible is economically limited (Christiansen, 1982). Integrated approaches including targeted trait breeding and conventional breeding based on genetic variation are used in peanut breeding programs for the development of improved cultivars that meet the requirements of growers (Janila et al., 2013).

The development of drought-tolerant peanut cultivars through selection on markers or genes associated with tolerance traits has been viewed as a solution for developing adapted cultivars (Holbrook et al., 2000). The classical approach of crop improvement using artificial selection solely based on the phenotypic expression of the desired species has been carried out for centuries (Stuber et al., 1999). Breeding for drought tolerance employs several strategies, with the simplest one being to select for high yield under water-limited conditions (Branch and Kvien, 1992). Peanut breeding efforts in the past have followed an empirical approach focused almost exclusively on pod yield. However, yield is sensitive to environmental conditions and as such limits this approach to the identification of drought-tolerant genotypes in a narrow range of environments. Selecting for cultivars that are widely adapted across environments has been slow, and remains challenging (Nigam et al., 2005). Conventional breeding for drought resistance has resulted in several drought tolerant cultivars across different crops over the last century with higher yields and abiotic stress tolerances (Ashraf, 2010). However, this approach is resource intensive requiring substantial labor inputs and time. Higher throughput phenotyping and selection criteria can reduce these inputs and result in more quickly developing adapted cultivars. For example, selection for early maturity types is best for the regions where drought occurs primarily at the end of the growing season, Virginia-type peanut cultivar “Streeton” is a good example of a drought avoidant cultivar (Cruickshank, 2000). In addition to maintain yield, it showed 40% lower preharvest aflatoxin contamination than “NC-7”. Blum (2009) strongly argued for selecting drought-tolerant genotypes based on water use efficiency (WUE) because the high transpiration efficiency is often due to reduced water use, hence reducing yield rather than enhancing biomass. He suggested that Effective use of water (EUW), rather than WUE, should be the target for yield improvement under water limited conditions; EUW signifies the maximum capture of soil moisture for transpiration by reducing non-stomatal transpiration and minimum loss of water by soil evaporation. It is vital for drought breeding programs to address the complexity of drought stress by understanding the physiological and genetic effects which are generally confounded with drought traits that are polygenic in nature (Barnabas et al., 2008; Fleury et al., 2010). Crop improvement requires precise quantification and partitioning of these combined effects under drought conditions. Higher yield and drought-tolerant cultivars can be developed by understanding the complexity of drought traits through physiology (Reynolds and Trethowan 2007; Sinclair, 2011), genomics, and molecular biology (Ashraf, 2010; Varshney et al., 2012). Simple physiological traits such as specific leaf area (SLA) or SPAD-meter relative chlorophyll content are used for screening genetic variation and drought-associated traits for empirical selection or trait-associated molecular markers (Dang et al., 2013). Water deficient cells have low cellular turgor pressure causing the leaf to lose structural integrity resulting in folding, rolling, or dropping down of leaves (Blum, 2011). These symptoms constitute a visual expression of wilting that can be used as a proxy for low moisture stress in plants. Studies have shown that reduced leaf water potential due to low moisture stress is directly related to wilting severity (Engelbrecht et al., 2007). In peanut, wilting was negatively associated with carbon assimilation rate and yield under drought (Sarkar, 2021). Wilting is a clear visual symptom of low moisture stress; therefore, quantifying wilting is an important step toward developing cultivars with tolerance to low soil moisture. Thus, visual rating is an important tool for quickly quantifying moisture stress in plants across large populations (Engelbrecht et al., 2007; Hamidou et al., 2012; Luis et al., 2016; Balota and Oakes, 2017; Zhou et al, 2020). The selection of drought-resistant cultivars increasingly relies on the use of yield-related proxies selected either directly (Reynolds and Tuberosa, 2008) or via marker-assisted selection once the quantitative trait loci (QTL) underpinning the variability of the relevant trait are identified (Langridge and Reynolds, 2015; Maccaferri et al., 2016).

Developing drought-tolerant cultivars would help alleviate the effects of irregular rainfall events on yield and grade, reduce the risk of aflatoxin contamination, and enhance profitability with no additional production costs (Harris et al., 1988; Mathew et al., 1988). Using a combination of traits, instead of relying on a single trait, will lead to identifying genotypes with stable drought tolerance across environments and aid in understanding the mechanisms of drought tolerance in peanuts. The efficiency of breeding programs is enhanced by the advancement in precision physiological phenotyping along with combining molecular and genetic approaches (Mir et al., 2012; Kosová et al., 2014; Choudhary et al., 2018). For higher yield, indirect selection rather than direct selection for underlying physiological traits contributing to yield improvement is more efficient and beneficial. In addition, it is an advantage if the physiological trait of interest for selection under stressed environment has higher heritability than yield itself, which confers greater success for developing drought tolerant cultivars. The present study aimed to evaluate and identify drought tolerant peanut genotypes using normalized difference vegetation index (NDVI), canopy temperature depression (CTD), SPAD, canopy height (HT), plant width (WD), wilting, CO_2 assimilation rate, and pod yield in a RIL population of 337 lines.

Materials and Methods

Plant material and experimental site

A recombinant inbred line (RIL) population of 337 individual peanut genotypes(lines) generated from a cross between 'N08086olJCT' and 'PI 585005 (ICGV 86015)' was grown at the Virginia Tech' s Tidewater Agricultural Research and Extension center (TAREC), in Suffolk, VA (latitude 36.66 N, longitude 76.73 W) and Peanut Belt Research Station, at Lewiston-Woodville, NC (latitude 36.13 N, longitude 77.17 W) between 2018 and 2021. PI 585005 is a drought tolerant spanish-type line (Devi et al., 2010) from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India, whereas N08086olJCT a virginia-type, large-seeded, high yielding, and less drought-tolerant line from the North Carolina State University. These parents were selected based upon their contrasting traits for drought tolerance.

Experimental design under Rainfed Condition

Parents and RILs were phenotyped across two years (2018 and 2019) under rainfed conditions at both locations. RILs were planted in two-row plots of 3.05 m long × 0.9 m wide at Suffolk, VA and single row plots at Lewiston, NC using a randomized complete block design (RCBD) with two replications. The seeding rate was 13 seeds m^{-2} with a 2.13 m alley between each replication.

Induced drought experiment

From the total of 337 RILs, a selected subset of 52 RILs lines was used in a rainout shelter experiment. In this experiment, cultivar 'Bailey' and germplasm 'GP-NC WS 17' were used as checks. Bailey is a high-yielding commercial cultivar whereas GP-NC WS 17 is a drought-tolerant line with a stay green characteristic (Tallury et al., 2014). The study was conducted for two growing seasons (2020 – 2021) at TAREC, Suffolk, VA. The subset (n=52) was planted in single-row plots of 1.52 m long × 0.9 m wide at a rate of 13 seeds m^{-2} in a RCBD with two checks in three replications. All plots were irrigated before planting to provide uniform germination. Irrigation treatments were designed as two regimes: Irrigated (full irrigation) and drought (rain-shelter). Irrigated plots received at least 600 mm of total water, rainfall and irrigation, throughout the growing season (Putnam et al., 2014; Rowland at al., 2012). A lateral pull boom cart sprinkler irrigation system (E1025 Reel Rain, Amadas Ind., Suffolk, VA) was used. Rain shelter plots were subjected to drought stress starting at 60 days after planting (DAP) when water was completely withheld, plots were covered with the rainout shelters for 6 weeks, after which the shelters were removed and plots received rainfall until harvest. Except for the irrigation, agronomic practices were performed for all plots as recommended by the Virginia Peanut Production Guide (Balota, 2020). Daily weather including air temperature and relative humidity (RH) was recorded using an on-site weather station (WatchDog 2000 Series Weather Station) starting from May 1 until end-September. Soil moisture data were collected every other week starting after 4 weeks after planting (WAP) at 10 cm, 20 cm, 30 cm, and 40 cm depth under irrigated and rain shelters plots where ‘Bailey’ and ‘’ were grown. Soil moisture information was extracted using a Delta-T HH2 moisture meter (Delta-T Devices Ltd, Cambridge, UK).

Phenotyping of RIL populations

Normalized difference vegetation index (NDVI) was measured for each plot on progressive weeks starting after 5 weeks after planting (WAP) at different growth stages using a handheld GreenSeeker Crop Sensor (Trimble Ag., Sunnyvale, CA) until physiological maturity. The measurements were taken at a height of 50 cm above and perpendicular on the canopy on sunny and wind free days by scanning the hand-held GreenSeeker® over both rows of each plot, from end to end of each row, and the average of both rows NDVI were recorded. The NDVI values were derived from the formula of red:near-infrared ratio [NDVI =

(NIR−RED)/(NIR+RED), where RED and NIR are the amount of red and near-infrared light respectively (Rouse et al., 1974).

Canopy temperature depression (CTD) of each plot was measured with a hand-held AGRI-THERM II™ infra-red thermometer (Agri-Therm Model 6110L, Everest Interscience Inc., Tucson, AZ). CTD was measured twice over a random spot on each row, at approximately 50 cm above the canopy and 45-degree angle. CTD values from both rows were averaged to calculate plot CTD. CTD was measured on sunny days with minimal wind. CTD was measured starting from 5 WAP until physiological maturity throughout the growing season. The “diff” option was selected to get the CTD value calculated by subtracting the canopy temperature (T_c) from the ambient air temperature (T_a) i.e. CTD = ($T_c - T_a$).

Plant height (HT) was manually measured weekly between 4 WAP and 8 WAP. Two plants were randomly selected within each row per plot and height of each plant was determined from the base of the main stem to the newest leaf. The canopy height of each plot was obtained by averaging the value of plant height from two rows. Similarly, lateral branch or plant width (WD) was randomly taken from each row and the two rows of a plot were averaged for lateral growth of the plot.

Leaf wilting was visually scored using a 0-5 rating scale. A score of 0 represented a potentially healthy plant with no visible wilting or leaf drooping symptoms; 1 represented some terminal and newer leaves fold but overall the plant looked healthy; 2 represented upper leaves almost all folded with visible signs of wilting, lower and older leaves started to fold; 3 represented wilting and drooping of all leaves on the plant, low-moisture effect on older leaves was prominent, and bare ground started to become visible; 4 represented all leaves were wilted and some started to change color due to chlorophyll degradation, bare ground was prominently visible, some leaves dried and crisped; 5 represented all leaves were severely wilted and color of all leaves was light green to yellow, bare ground was fully visible, more than 50% of leaves were crisp and dry, plant was almost physiologically dead (Sarkar et al., 2021). Leaf wilting was measured at different growth stages and drought severities starting from 6 WAP until physiological maturity.

The LI6800 portable photosynthesis system (LI-COR Biosciences, Lincoln, NE) was used for the measurement of CO_2 assimilation rate. Two LICOR readings were taken per plot from the newest fully developed leaf on the main stem. The chamber environment was set at 2000 µmols of light, 50% relative humidity, 400 ppm of CO_2, 500 µmol s^{-1} flow rate, and 10000 rpm mixing fan speed. The chamber temperature was maintained at ambient temperature. The leaf was clipped for 60-90 seconds in the chamber until the readings stabilized.

Plots were dug from mid-September to early October at physiological maturity (Boote, 1982) with a KMC two-row digger. Pods were combined after a few days of windrow drying with a Hobbs peanut combine (Model 325A). Pod yield was calculated from plot weight and adjusted to 7% seed moisture.

Statistical analysis

Phenotypic evaluation of RIL population (n = 337) under rainfed condition for different traits were performed using general linear model procedure (GLM) in R (R Core Team, 2021) using the package ‘agricolae’ (Mendiburu, 2015). For selected subset (n=52), we fit linear mixed model in which RILs were considered as fixed effects whereas year and replication were random effects using the ‘lmer’ package in R (Kuznetsova et al., 2017). Pearson’s correlation was performed separately for irrigated and drought conditions for different traits and yield using package ‘corrplot’ in R studio (Wei et al., 2017). All the data were analyzed in R 4.1.2 (R Core Team 2021) and visualized in R Studio (RStudio Team 2021).

Results

Population wide phenotypic variation among RILs under rainfed condition

Analysis of variance (ANOVA) showed significant variation among RILs at $p \leq 0.01\%$ for pod yield, NDVI and CTD at the full pod stage, and wilting at the beginning of peg development (Table 1). The NDVI values ranged from 0.20 to 0.93 whereas for CTD and wilting values ranged from -6.0 to 0.6 and 1.5 to 3.6 respectively under rainfed conditions (Table 1).

Experimentally induced phenotypic variation among a selected subset of RILs under irrigated and drought conditions

A linear mixed model was applied to the phenotypic data using 52 RILs and the two checks, cultivar Bailey and germplasm GP-NC WS 17. Genotypes were treated as fixed effects, whereas replications nested within years were fitted as random effects. Principal component analysis (PCA) was carried out to create a PCA biplot of phenotypic measurements. Pearson’s correlation matrix was used to measure correlation among phenotypes.

Analysis of variance of 52 RIL for 8 phenotypic traits, evaluated under drought and irrigated conditions, is presented in Tables 2 and 3, respectively. Under drought-stressed conditions, the ANOVA revealed significant differences at $p<0.05$ among lines for NDVI, CTD, SPAD, and at $p<0.001$ for plant height. Under irrigated conditions, there were no significant differences for CTD, wilting, SPAD, and photosynthesis; but there were significant differences for NDVI, HT, WD, and yield. Under irrigation, line #42 performed significantly better than Bailey for pod yield when Bailey was used as the check. Under drought, lines #33, #4, #71, and

#76 performed better using GP-NC WS 17 as the check (Table 3 and Figure 2a & 2b). Under both, drought and irrigated conditions, RIL that performed significantly ($p<0.005$) better from both checks for NDVI were #11, #136, #154, #188, #220, #35, #38, #44, #56, #60 (Table 4). For CTD, RIL #131, #146, and #215 were significantly better than Bailey under irrigated conditions, whereas there was not a single line under drought to perform better than GP-NC WS 17 check (Table 4). For leaf wilting we identified line #69 performing significantly better than 'Bailey' under irrigated conditions. For plant height, under both irrigation regimes, lines # 110 #135, #154, #165, #166, #188, #201, #220, #280, #31, #1, #44, #69, #8, and #92 were higher than respective checks. (Table 4). The RILs performing better ($p<0.1$) for SPAD under irrigated conditions were #42 and #154 however, we did not notice any differences among lines under drought conditions (Table 4). In contrast to SPAD, we found lines (#2, #56, 59, #77, and #90) significantly ($p<0.01$) different for CO_2 assimilation rate under drought (Table 4). In terms of yield, the lines which performed better than checks under both water regimes were # 31, #35, #56, #60 and #73 (Figure 9).

Association among physiological traits for drought and irrigated conditions

The correlation matrix between NDVI, CTD, and wilting at different growth stages or time points are shown in Figure 1. Correlations were weaker under rainfed conditions for NDVI with pod yield compared to NDVI with CTD. The association was significant and NDVI was inversely related to CTD, i.e. the higher NDVI, the cooler canopies. Pearson correlation among the traits for drought and irrigated conditions is summarized in Figures 3 and 4, respectively. The correlation under drought revealed that CTD and wilting were negatively correlated with pod yield ($r = -0.42$, $p<0.005$) whereas NDVI and HT were positively correlated ($r = 0.65$, $p<0.005$) with each other (Figure 3). Under irrigated conditions, NDVI and HT were negatively correlated with CTD (r = -0.52, p<0.005). Peanut WD exhibited significant correlation with NDVI (r = 0.5, p<0.005).

The first three PCs with Eigenvalues greater than one accounted for 63.50% of the total phenotypic variability exhibited by the studied traits under drought-stressed conditions (Figure 5) whereas, under irrigated conditions, the first four PCs with eigenvalues greater than one account for 76.37% of the total phenotypic variability (Figure 6). The main contributing traits to the PC1 and PC2 under drought conditions were NDVI, CTD, plant height, and yield (Figure 7). Under irrigated conditions, NDVI, CTD, and plant height contributed mostly to the PC1 and PC2 (Figure 8).

Discussion

Crop growth during the growing season and yield are dictated by the cumulative rates of photosynthesis and transpiration, however, both reduced by drought stress. Previous studies reported that an increased CO_2 assimilation under water limited conditions enhanced peanut growth and pod yield (Ketring et al., 1982; Osuji et al., 2011; Ratnakumar et al., 2013). Both parents (N08086olJCT x ICGV 86015) were contrasting for transpiration traits under field and controlled conditions. ICGV 86015 is an early maturing, drought tolerant Spanish line (Nigam et al, 1995) with high transpiration efficiency (TE) under rapid drying of soil moisture (Devi et al., 2010). Screening of 337 lines in replications under field conditions for direct measurements of photosynthesis, transpiration, and biomass is expensive and time consuming. Therefore, NDVI, CTD, and SPAD, which are closely related to photosynthesis and transpiration, were, instead, used to phenotype the RIL population for drought tolerance. NDVI is calculated from light reflectance by leaves in the red and near-infrared bands of the electromagnetic spectrum. Because both chlorophyll *a* and *b* absorb red light, higher NDVI values denote healthier leaves, i.e. healthy leaves absorb more in red and less infra-red, therefore reflect less in red and more in the near-infrared wavebands that the unhealthy leaves (Holm et al., 1987, Weier and Herring, 2000). In diverse crops, CTD has been recognized as a key trait for assessing/comparison of genotypic response to low water use, high temperature, and other environmental stresses (Balota et al., 2012). The current study evaluated the genetic variation present among the 337 RIL and a subset of lines (n=52) for NDVI, CTD and wilting for drought tolerance. There was significant variation among the evaluated RILs for traits such as NDVI under the rainfed conditions, however, the variation for other traits such as CTD, Wilting, pod yield was not significant. In case of the subsets (n=52), analysis of variance under drought stress revealed significant differences for NDVI, CTD, SPAD and HT, whereas under irrigated conditions NDVI, HT, WD and yield show significant differences evaluated across two years. This indicates genetic variability for these traits among the tested RILs.

Developing high yielding genotypes requires knowledge of the existing variability and degree of association between yield and the relative contribution of other yield contributing traits (Zongo et al., 2017). The correlation of NDVI and pod under rainfed condition was low whereas under drought conditions the correlation was high, ranging from 0.17 to 0.35. This might be due to greater ability of NDVI to detect biomass differences between RILs under drought conditions, where the canopy is not fully covered. These results agree with previous studies in wheat (Gutierrez et al., 2010; Lopes and Reynolds, 2012). Higher CTD (positive) values under drought might be due to decreased transpiration and increased respiration resulting from stomatal closure. There were no significant differences reported for wilting under both conditions. However, CTD showed differences under drought conditions and CTD was correlated with wilting. This shows that RILs with cooler canopies transpired more efficiently by using soil moisture and wilted less.

These findings support previous studies in phenotyping drought tolerance genotypes in peanuts (Balota and Oakes, 2017). The negative and strong correlations between CTD and wilting with pod yield revealed the significance of these traits in determining yield under drought conditions. SPAD showed significant variation among the selected subset of RILs under drought stress suggesting their sensitivity to detect differences among lines in response to drought tolerance. SPAD has been used as a surrogate trait for selecting for WUE in peanut (Upadhyaya, 2005). Canopy height (HT) was highly significant and correlated with yield under irrigated and drought conditions. Reduction of canopy height under water deficit conditions was attributed to decrease in internodal length and was suggested to change the dry matter partitioning and light interception (Bell, Wright, & Harch, 1993; Collino, Dardanelli, Sereno, & Racca, 2001; Pandey, Herrera, Villegas, & Pendleton, 1984).

In conclusion, we found that certain RILs from the N08086olJCT x ICGV 86015 cross showed desirable recombination of traits related to yield improvement under water deficit conditions. The selected RILs maintained high NDVI, low CTD and wilting under water stress conditions to enhance yield. These RILs can be used to find genomic regions that are desirable for drought tolerance.

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Figures and Tables

	NDVI_1	NDVI_2	NDVI_3	NDVI_4	CTD_1	CTD_2	Wilting_1	Wilting_2	Yield
NDVI_1	1.00	0.71	0.71	0.68	-0.55	-0.22	0.04	-0.06	0.00
NDVI_2	0.71	1.00	0.64	0.61	-0.38	-0.19	-0.03	-0.02	-0.01
NDVI_3	0.71	0.64	1.00	0.88	-0.50	-0.29	-0.01	0.03	0.03
NDVI_4	0.68	0.61	0.88	1.00	-0.44	-0.26	0.02	0.02	0.01
CTD_1	-0.55	-0.38	-0.50	-0.44	1.00	0.14	-0.12	-0.01	-0.07
CTD_2	-0.22	-0.19	-0.29	-0.26	0.14	1.00	0.22	0.03	0.13
Wilting_1	0.04	-0.03	-0.01	0.02	-0.12	0.22	1.00	-0.10	-0.04
Wilting_2	-0.06	-0.02	0.03	0.02	-0.01	0.03	-0.10	1.00	0.06
Yield	0.00	-0.01	0.03	0.01	-0.07	0.13	-0.04	0.06	1.00

Figure 1. Pearson correlation coefficients among relevant phenotypic traits for drought tolerance on 337 RILs under rainfed field conditions.

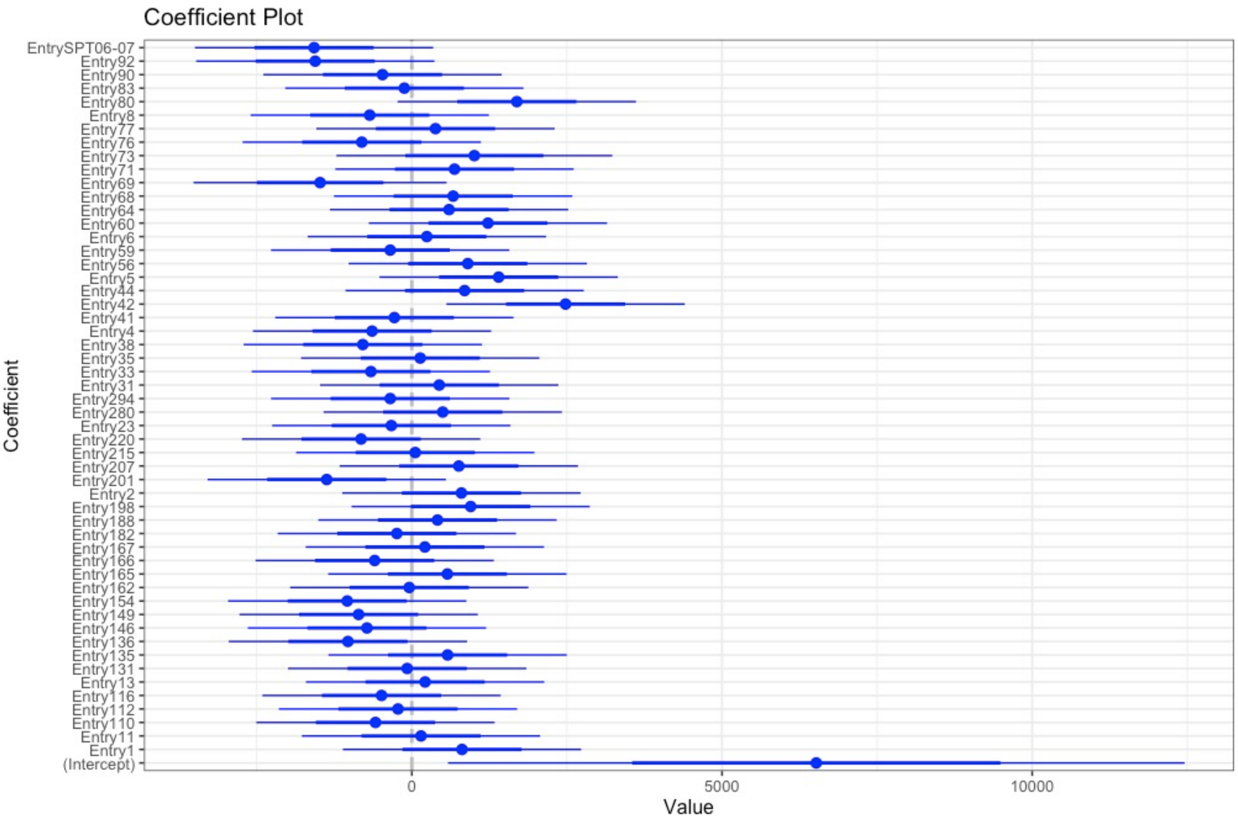


Figure 2a. Coefplot depicting pod yield for 52 RILs under irrigated conditions using Bailey as a check for comparison.

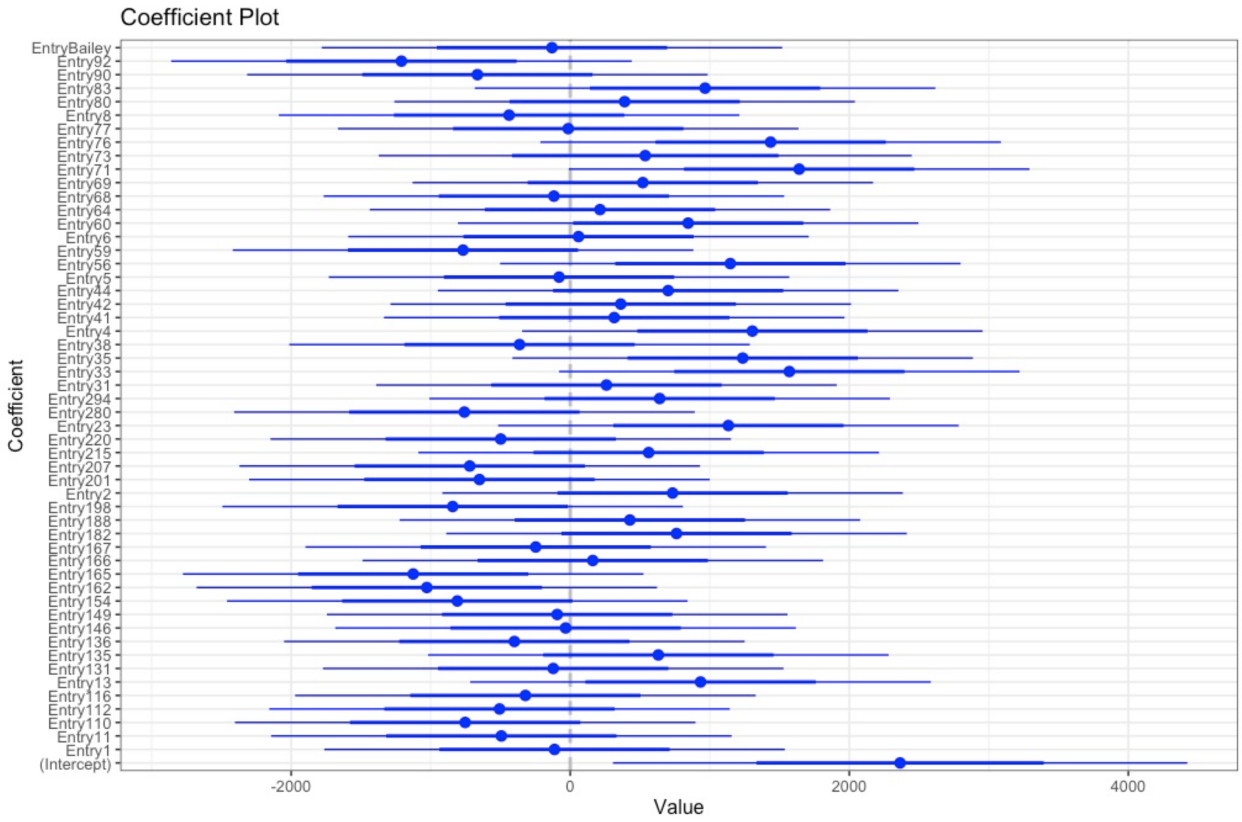
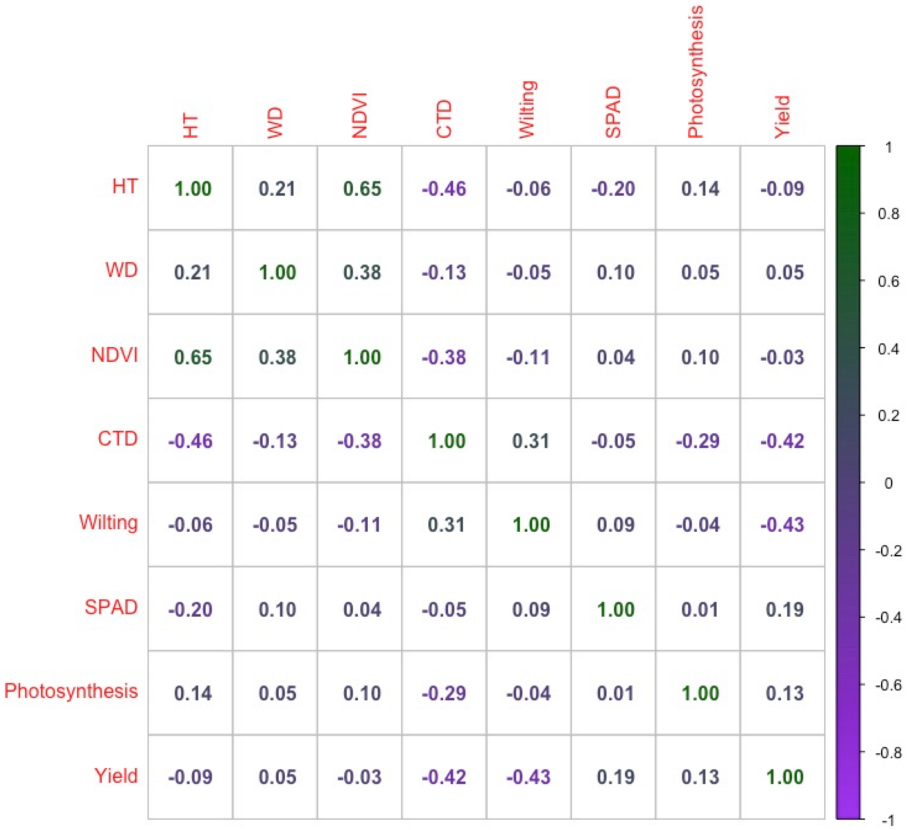
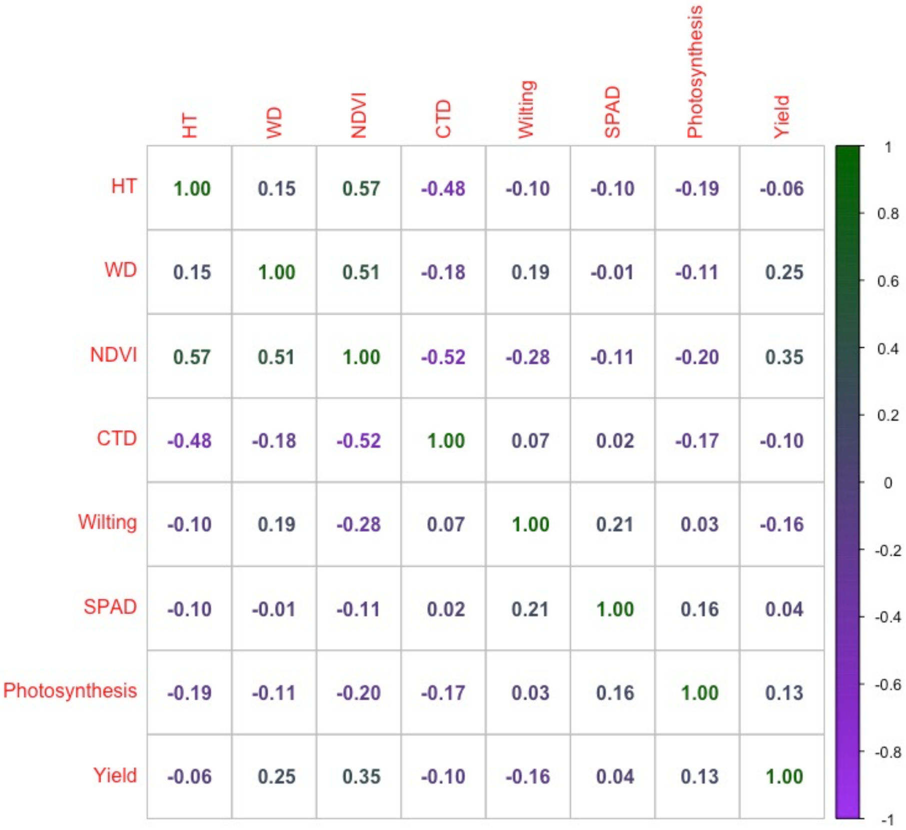


Figure 2b. Coefplot depicting pod yield for 52 RILs under drought conditions using GP-NC WS 17 as a check for comparison.



	HT	WD	NDVI	CTD	Wilting	SPAD	Photosynthesis	Yield
HT	1.00	0.21	0.65	-0.46	-0.06	-0.20	0.14	-0.09
WD	0.21	1.00	0.38	-0.13	-0.05	0.10	0.05	0.05
NDVI	0.65	0.38	1.00	-0.38	-0.11	0.04	0.10	-0.03
CTD	-0.46	-0.13	-0.38	1.00	0.31	-0.05	-0.29	-0.42
Wilting	-0.06	-0.05	-0.11	0.31	1.00	0.09	-0.04	-0.43
SPAD	-0.20	0.10	0.04	-0.05	0.09	1.00	0.01	0.19
Photosynthesis	0.14	0.05	0.10	-0.29	-0.04	0.01	1.00	0.13
Yield	-0.09	0.05	-0.03	-0.42	-0.43	0.19	0.13	1.00

Figure 3. Pearson's correlation matrix of phenotypic traits on 52 RILs measured under drought (rain shelters) conditions in this study.



	HT	WD	NDVI	CTD	Wilting	SPAD	Photosynthesis	Yield
HT	1.00	0.15	0.57	-0.48	-0.10	-0.10	-0.19	-0.06
WD	0.15	1.00	0.51	-0.18	0.19	-0.01	-0.11	0.25
NDVI	0.57	0.51	1.00	-0.52	-0.28	-0.11	-0.20	0.35
CTD	-0.48	-0.18	-0.52	1.00	0.07	0.02	-0.17	-0.10
Wilting	-0.10	0.19	-0.28	0.07	1.00	0.21	0.03	-0.16
SPAD	-0.10	-0.01	-0.11	0.02	0.21	1.00	0.16	0.04
Photosynthesis	-0.19	-0.11	-0.20	-0.17	0.03	0.16	1.00	0.13
Yield	-0.06	0.25	0.35	-0.10	-0.16	0.04	0.13	1.00

Figure 4. Pearson's correlation matrix phenotypic traits measured under irrigated conditions in this study.

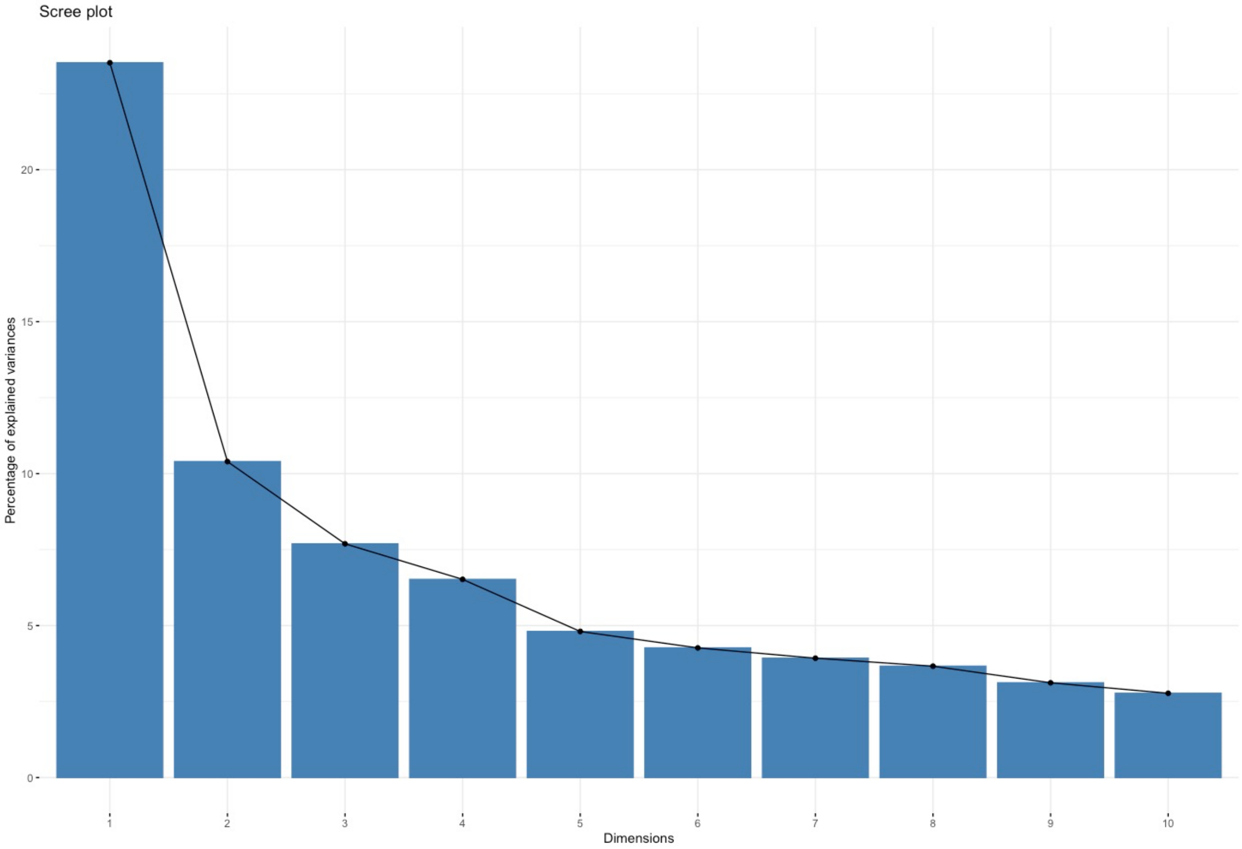


Figure 5. Scree plot of eigenvalues representing the percentage of variance explained by each principal component under drought-stress (rain shelters) conditions.

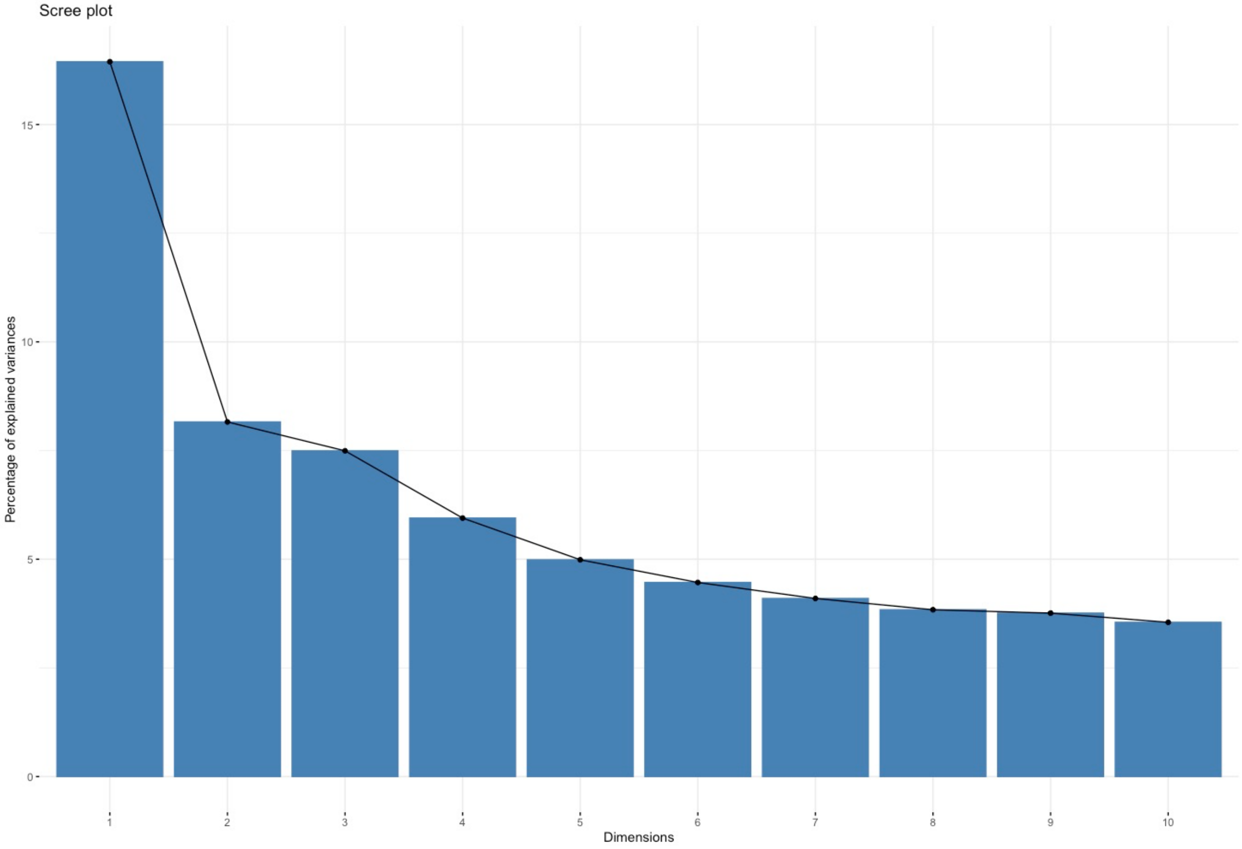


Figure 6. Scree plot of eigenvalues representing the percentage of variance explained by each principal component under irrigated conditions.

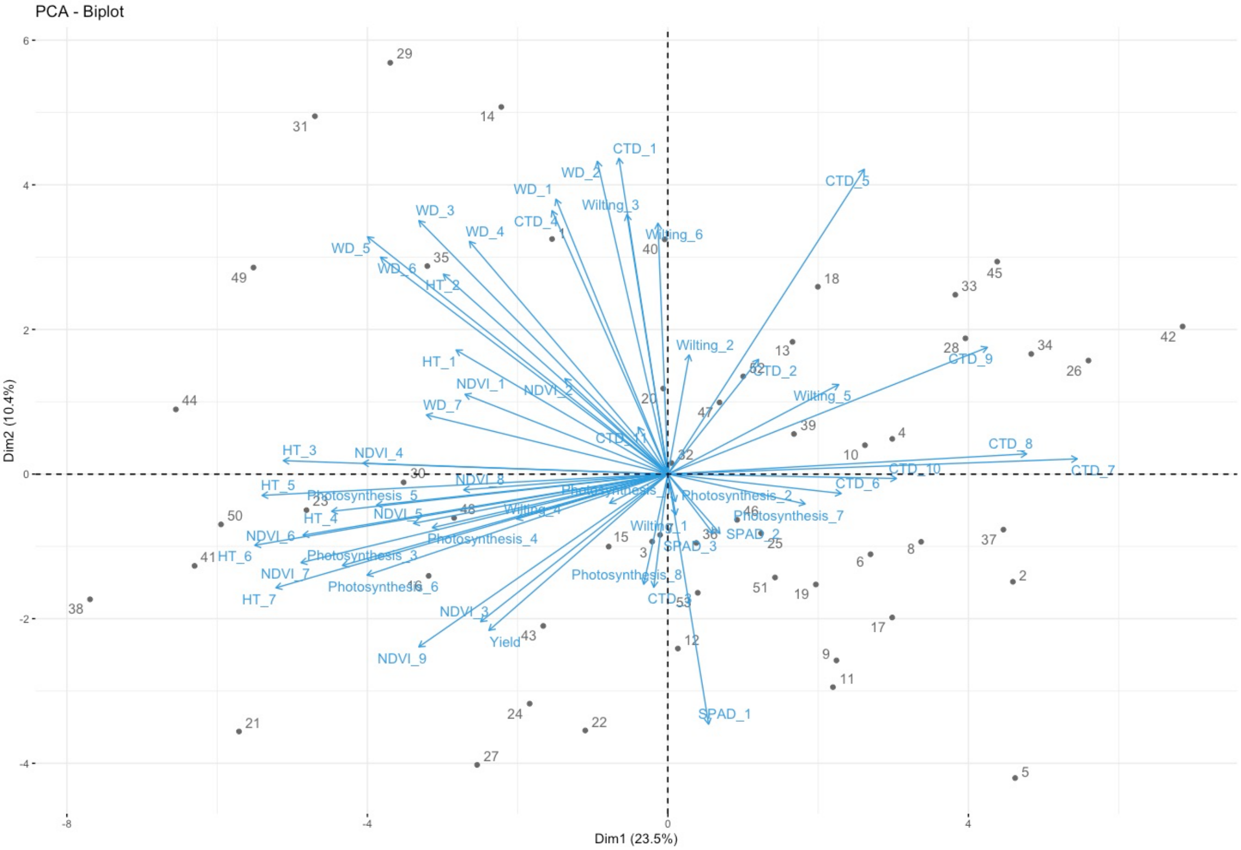


Figure 7. Principal component biplot of various phenotypic traits of 52 RILs evaluated under drought (rain shelters) conditions. Traits farther from the center have higher variance, and the higher the angle between traits the lower the correlation.

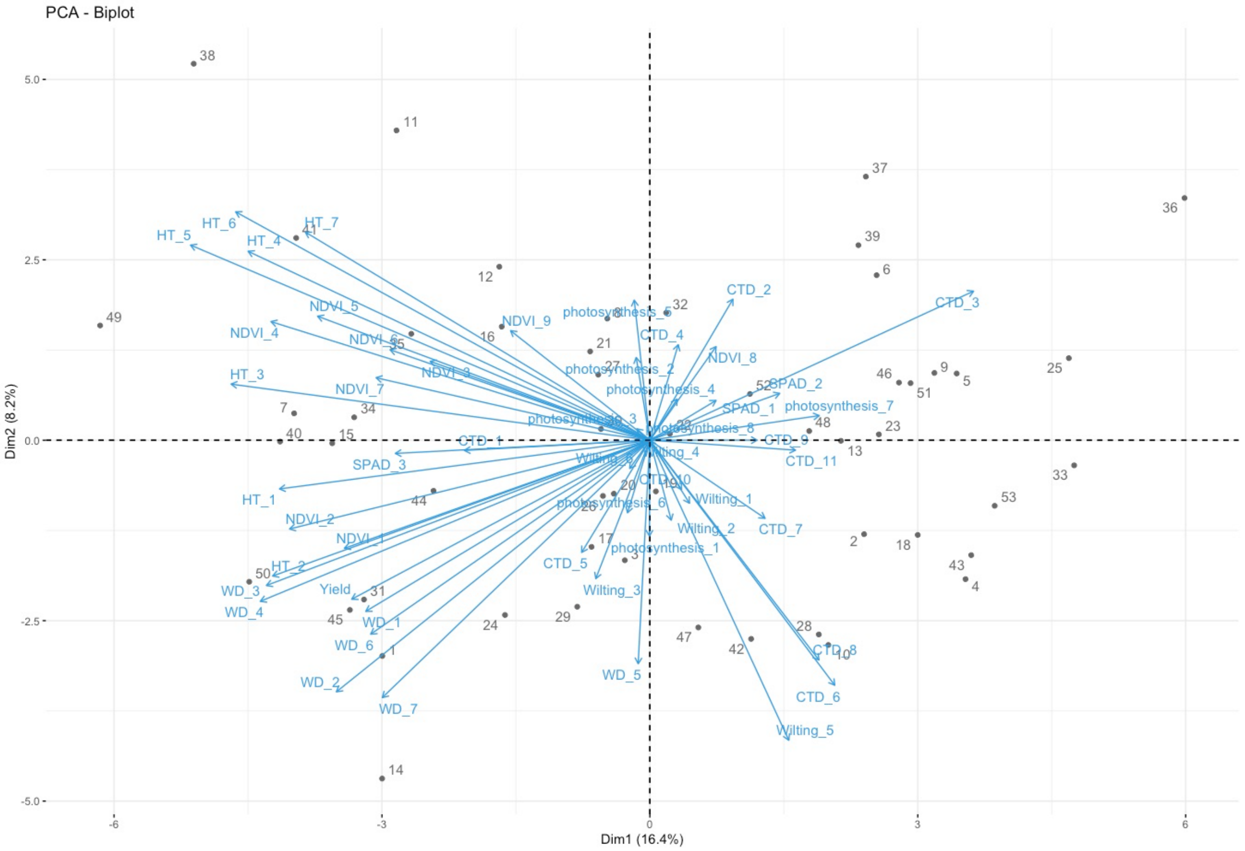


Figure 8. Principal component biplot of various phenotypic traits of 52 RILs evaluated under irrigated conditions. Traits farther from the center have higher variance, and the higher the angle between traits the lower the correlation.

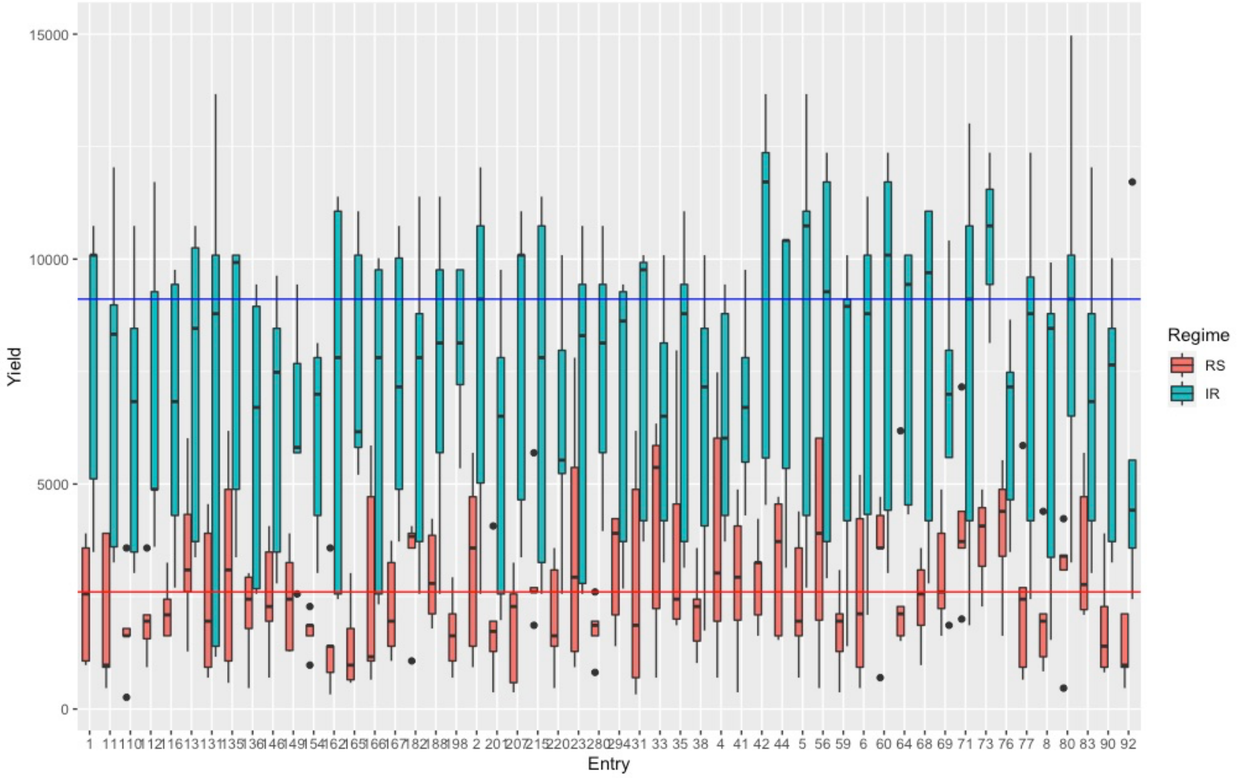


Figure 9. Boxplot indicating variation of pod yield under irrigated and drought conditions for the years 2020 and 2021 for the selected subset (n=52) lines. Red horizontal line represents the mean yield of GP-NC WS 17 as check under drought regime. Blue line represents the mean yield of Bailey as check under irrigated regime.

Table 1. Mean squares, minimum, maximum, and mean of NDVI, CTD, wilting, and pod yield of 337 RILs that were tested under rainfed conditions in 2019.

SOV	**DF**	**NDVI**	**CTD**	**Wilting**	**Yield**
RILs	336	0.02 **	0.89 *	0.14**	3874631.03 **
Rep	1	0.02 ns	54.17 *	0.01 ns	663610350.1**
Residuals	335	0.01	0.74	0.1	2908793.7
Min.		0.20	-6.0	1.5	317
Max.		0.93	0.6	3.6	9944
Mean		0.83	-3.1	2.7	5167

SOV=Source of variation, DF =degrees of freedom, ns = non-significant, Significant at $p \leq 0.001$(***); $p \leq 0.01$(**) and $p \leq 0.05$(*)

Table 2. Analysis of variance showing means square values of 52 RILs for 8 phenotypic traits across two years evaluated under drought (rain shelter) conditions.

Trait	df	SS	MS	F-value	P-value
NDVI	53	0.12733	0.0024025	1.786	0.002167 **
CTD	53	38.489	0.7262	1.8667	0.001041 **
Wilting	53	684.32	12.912	1.288	0.1085
SPAD	53	1784.8	33.675	1.6547	0.006784 **
HT	53	390.03	7.3591	4.3304	1.483e-14 ***
WD	53	833.57	15.728	1.5911	0.01146 *
Photosynthesis	53	1357.7	25.616	1.0206	0.4455
Yield	53	142673427	2691951	1.5812	0.01257 *

df, degree of freedom; SS, sum of square; MS, Mean square; P, level of probability. Significant at $p \leq 0.001$(***); $p \leq 0.01$(**) and $p \leq 0.05$(*)

Table 3. Analysis of variance showing means square values due to 52 RILs for 8 phenotypic traits across two years evaluated under irrigated conditions.

Trait	df	SS	MS	F-Value	P- value
NDVI	53	0.13984	0.0026385	2.1862	4.828e-05 ***
CTD	53	14.706	0.27748	1.3627	0.06608
Wilting	53	53.627	1.0118	1.2619	0.1279
SPAD	53	1375.8	25.959	0.9519	0.5721
HT	53	463.17	8.7391	6.3976	2.2e-16 ***
WD	53	590.74	11.146	5.1786	2.2e-16 ***
Photosynthesis	53	1225.4	23.12	1.0174	0.4512
Yield	53	185934317	3508195	1.5208	0.02046 *

df, degree of freedom; SS, sum of square; MS, Mean square; P, level of probability. Significant at $p \leq 0.001$(***); $p \leq 0.01$(**) and $p \leq 0.05$(*)

Table 4. Regression Coefficient estimates for RILs along with checks under drought and irrigated conditions for NDVI, CTD, HT and SPAD

RILLines	**NDVI^z**		**CTD^y**		**HT^x**		**SPAD^w**	
	Drought	**Irrigated**	**Drought**	**Irrigated**	**Drought**	**Irrigated**	**Drought**	**Irrigated**
Line1	0.030	0.038*	0.256		2.640***		-1.400	
	(0.023)	(0.022)	(0.394)		(0.824)		(2.853)	
Line11	0.072***	0.042*	-0.098	-0.262	4.220***	2.640***	0.800	2.190
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line110	0.058**	-0.016	-0.004	0.170	4.060***	2.200***	-3.660	2.470
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line112	0.072***	0.026	0.346	-0.150	3.360***	0.400	0.320	-4.030
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line16	0.028	0.022	0.218	-0.052	2.880***	0.420	-3.440	-2.610
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line13	0.018	0.032	0.026	-0.194	1.580*	0.740	-3.760	1.180
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line131	0.014	0.020	0.288	0.544*	1.240	-0.560	-2.680	-3.550
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line135	0.020	0.042*	0.154	-0.092	2.560***	2.200***	-0.900	0.930
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line136	0.056**	0.040*	0.112	-0.138	4.260***	3.060***	-2.260	-1.230
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line146	0.016	-0.006	0.020	0.614**	1.200	-0.380	3.720	-1.790
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line149	0.006	0.004	0.414	0.130	2.060**	0.600	-2.480	-2.570

	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line154	0.056**	0.044**	-0.716*	0.112	4.800***	3.580***	-3.400	-6.390*
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line162	0.042*	0.018	0.624	0.426	0.660	-0.840	-4.360	-3.930
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line165	0.032	0.026	-0.072	0.098	2.860***	2.700***	-3.480	-0.270
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line166	0.060***	0.028	-0.280	0.096	4.520***	1.800**	-1.120	-3.750
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line167	-0.008	0.002	0.386	0.144	0.820	-0.920	-1.220	-1.890
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line182	0.056**	0.008	-0.088	-0.110	1.420*	-0.260	2.680	-2.810
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line188	0.078***	0.044**	-0.076	-0.326	4.000***	2.260***	-5.180*	-5.110
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line198	0.012	0.036	0.638	0.064	0.960	0.100	1.600	-1.850
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line2	0.048**	0.014	0.322	0.000	1.800**	-0.500	4.980*	-2.590
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line201	0.040*	0.006	0.140	0.256	3.260***	1.740**	-0.940	-6.250*
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line207	0.054**	0.018	0.202	0.344	1.420*	-0.700	0.780	-3.750
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line215	0.040*	-0.008	-0.406	0.664**	2.620***	0.820	-2.960	-1.090
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line220	0.056**	0.064***	-0.106	-0.018	4.600***	3.140***	1.820	-4.790
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)

Line23	0.026	0.034	-0.254	0.386	1.780**	0.900	3.040	-1.490
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line280	0.086***	0.056**	-0.772*	-0.144	5.660***	3.340***	-4.060	-2.310
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line294	0.006	-0.010	-0.080	0.320	2.220***	0.540	-1.040	-4.810
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line31	0.018	-0.018	-0.540	0.262	1.080	-0.660	1.680	-0.610
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line33	0.042*	0.016	0.202	-0.148	3.240***	2.100***	-0.640	-3.490
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line35	0.058**	0.046**	-0.218	-0.108	3.040***	2.460***	-1.620	-2.150
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line38	0.062***	0.038*	-0.382	0.156	2.480***	0.980	-0.640	-2.350
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line4	0.052**	0.024	-0.456	-0.082	2.120**	0.740	3.480	3.170
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line41	0.068***	0.030	0.192	0.268	3.000***	2.280***	1.040	-1.810
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line42	0.048**	0.066***	-0.422	-0.212	2.320***	1.200	-0.640	-6.250*
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line44	0.070***	0.052**	-0.828**	-0.138	3.560***	2.060***	1.620	0.630
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line5	0.046**	0.018	-0.020	-0.084	2.020**	-0.040	4.120	-3.710
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line56	0.040*	0.048**	-0.194	0.368	1.760**	0.300	-1.180	2.690
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line59	0.042*	0.016	0.292	0.278	2.140***	0.720	-3.020	-1.170

	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line6	0.036	-0.008	0.278	0.070	1.640**	0.560	0.400	-2.430
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line60	0.060***	0.048**	-0.122	0.114	2.280***	0.740	-3.420	-1.810
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line64	0.074***	0.036	-0.108	0.160	3.380***	1.560**	-0.720	0.090
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line68	0.072***	0.024	-0.398	-0.190	3.440***	1.800**	4.300	-3.270
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line69	0.018	0.018	-0.448	0.052	3.580***	2.547***	1.020	-3.542
	(0.023)	(0.023)	(0.394)	(0.303)	(0.824)	(0.785)	(2.853)	(3.505)
Line71	0.036	0.024	-0.866**	0.266	2.600***	-0.180	-5.320*	0.930
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line73	0.093***	0.040	-0.245	0.419	4.135***	2.071**	3.062	-4.531
	(0.027)	(0.025)	(0.457)	(0.330)	(0.954)	(0.856)	(3.302)	(3.822)
Line76	0.046**	-0.008	-0.494	0.368	2.280***	-0.400	1.220	-2.550
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line77	0.056**	0.020	-0.196	0.136	2.680***	-0.180	-1.380	-0.510
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line8	0.052**	0.030	-0.234	0.038	3.320***	2.160***	1.240	-2.550
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line80	0.026	0.022	0.730*	0.322	1.420*	0.720	-2.120	-0.050
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line83	0.046**	-0.004	-0.310	0.302	4.280***	1.140	0.320	1.970
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line90	0.048**	0.026	0.746*	0.232	0.780	-1.180	1.780	-2.450
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)

Line92	0.054**	0.034	0.396	0.224	3.720***	1.980***	-3.320	-3.850
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Bailey	0.040*		-0.190	0.468	2.360***	-0.320	-3.840	-2.070
	(0.023)		(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
		-0.058***		0.490*		-2.380***		-0.730
		(0.022)		(0.285)		(0.739)		(3.303)
Constant	0.588***	0.627***	-0.773	-1.290***	6.382***	8.980***	40.182***	42.462***
	(0.018)	(0.020)	(0.930)	(0.311)	(0.842)	(0.750)	(2.918)	(2.782)

^{z} Normalized difference vegetation index, ^{y}Canopy temperature depression (CTD), ^{x}Canopy height, ^{w}SPAD Chlorophyll Meter Readings

Chapter 3: Genetic Mapping and QTL Analysis for Drought Tolerance in Recombinant Inbred Populations of Cultivated Peanut (*Arachis hypogaea* L.)

Abstract

Peanut (*Arachis hypogaea L.*) is an important cash and food crop grown predominantly in semi-arid tropical regions of the world where drought is a major limiting factor on productivity. Improving drought tolerance could be an effective way to improve the yield and quality of peanut in drought prone regions. An important step in this goal is the identification of drought tolerance related quantitative trait loci (QTL) and genetic markers, to facilitate the development of drought tolerant cultivars. This study aimed to identify QTL associated with drought tolerance in virginia type peanut across three diverse recombinant inbred line (RIL) populations, namely N05006 × N04074FCT (Pop-1), N05006 × Phillips (Pop-2) and N08086olJCT × PI 585005 (Pop-3). Phenotyping included, normalized difference vegetation index (NDVI), canopy temperature depression (CTD), pod yield and visual ratings of wilting. After screening parents for all three populations with 1596 SNP markers, three genetic maps were developed with 239 (Pop-1), 225 (Pop-2), and 1132 (Pop-3) SNP loci distributed over 20 linkage groups belonging to 20 peanut chromosomes. These three populations segregate for traits related to drought, a total of 27 minor QTL were identified for NDVI, CTD, SPAD and wilting with log-odds ratio values ranging from 2.5 - 38.5 and the proportion of phenotypic variance explained by these genomic regions ranged from 1.04 – 11.46 %. These small effect QTL and associated genomic markers can be employed in plant breeding programs that using genomic approaches like marker-assisted recurrent selection and genomic selection to develop elite peanut varieties that exhibit drought tolerance in the Virginia-Carolina region.

Keywords: Peanut, recombinant inbred line, QTL analysis, drought tolerance, molecular breeding

Introduction

Cultivated peanut (*Arachis hypogaea* L.) is an allotetraploid (2n = 4x = 40) legume with two different genomes 'AABB' (Stalker 1992; Raina and Mukai 1999); grown as an important oil and food crop in more than 100 countries worldwide (FAOSTAT, 2020). In the USA, peanut is grown annually at approximately 619,000 ha in 11 states with an average production of 4400 kg ha^{-1} (USDA-NASS, 2019). Over two-thirds of global peanut production occurs seasonally under rainfed regions where drought is the major constraint for crop production (Smartt, 1994; Rucker et al., 1995; Songsri et al., 2008; Balota et al., 2012). Drought significantly impacts the yield and quality of peanut. At cellular level drought affects stability of membrane lipids and photosynthetic activity (Lauriano et al., 2000) and yield in peanut (Suther and Patel, 1992). Yield losses due to drought are dependent on crop growth stage, intensity, and duration of its occurrence (Nigam et al. 2005). Pod yield reduction occurs in peanut as drought hampers the critical stage of flowering and pod development which requires maximum water for both high quality and yield (Stansell and Pallas, 1985; Sterling et al., 1989). Drought conditions reduce peanut quality by predisposing pods to aflatoxin contamination (Blankenship et al., 1984; Cole et al., 1988). The severity of aflatoxin contamination increases with increasing drought stress (Hamidou *et al*, 2014).

In the Virginia-Carolina (VC) region peanuts are often grown in sandy soils as peg formation and pod development is easy in sandy soil. However, sandy soils are low in water-holding capacity, hence water deficit can develop quickly. Therefore, peanut without irrigation may be frequently subjected to periodic drought stress. Drought results in the reduction of transpiration and photosynthesis, which results in a reduction of biomass accumulation and yield (Tardieu & Tuberosa, 2010; Sinclair et al., 2010). The estimated losses per year to the U.S. peanut industry due to drought stress is $50 million (USDA-ARS, 2009). The majority of this loss occurs due to extended drought as well as episodic drought during critical developmental growth stages in non-irrigated (rainfed) fields, which is common in peanut production. For instance, in the VC region only 15% of farmers have access to irrigation. Yield loss due to the impact of drought can be mitigated by developing cultivars that are better adapted and tolerant to drought. Therefore, acquisition of genetic information on drought tolerance of peanut is of great importance.

Plants under moisture limited conditions demonstrate various mechanisms like escape, avoidance and tolerance to complete their life cycle. Plants also respond through a combination of multiple adaptation strategies during drought stress to survive (Ludlow, 1989). Previous efforts to develop peanut varieties with drought tolerance have focused on drought avoidance and drought escape (Zhang et al., 2001). The main limitation for selecting drought avoidance adapted phenotypes is because they exhibit morphological plasticity under water limited conditions. Phenotyping traits like root architecture at field levels is logistically limiting, and impacts selection criteria (Richards, 2008). In particular, our understanding of the traits that afford discriminating power that capture plastic phenotypes is poor (Sanad et al., 2019). Therefore, selection based on phenotypes would be difficult for such traits (Collins et al., 2008). In addition, conventional breeding for developing drought tolerant crop varieties is time-consuming and labor-intensive (Ribaut et al. 1997). Crop tolerance to drought is not a simple response but it is mostly conditioned by many genes and their interaction with the environment. Identifying genes for many agronomic traits is challenging because they are underscored by many genes and their expression is dependent on interactions with the growing environment. Asharaf (2010) suggested that one of the reasons for limited progress towards improving crop drought tolerance could be because of the multiplicity of the genes involved in encoding for yield. However, recent advances in crop genomics offer tools like molecular mapping of QTL, association mapping and introgression of genomic regions using marker-assisted selection (MAS) to assist in breeding for abiotic stress tolerance (Varshney et al., 2005, 2006).

The development of peanut cultivars with improved performance under drought stress relies on underlying genotypic variability (Stansell and Pallas, 1985; Greenberg et al., 1992; Puangbut et al., 2009; Devi et al., 2010; Dinh et al., 2013). For instance, the identification of genetic variation in peanut for physiological traits like photosynthetic rate and transpiration, which are correlated with drought tolerance, offers opportunities to develop high yielding drought tolerant cultivars (Pimratch et al., 2008; Balota et al., 2012). Here, identifying genomic regions through dense molecular genetic maps can help accelerate peanut genetic research and enable breeders to identify genomic markers and regions for developing improved cultivars with increased drought tolerance (Ribaut et al. 1996; Li et al., 2013; Zhang et al., 2017). 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 QTL (quantitative trait loci) associated with drought. Mapping QTL using bi-parental populations is an important approach for dissecting complex traits and identifying genomic regions associated with quantitative phenotypic traits. Prior work in diverse crops has revealed several QTL associated with drought tolerance or related traits. For instance, in soybean, 5 QTL were identified for water use efficiency (WUE) in an F2 population with 14–20% phenotypic variation explained (PVE) (Mian et al. 1998). In wheat, Dashti et al. (2007)

identified five QTL for drought tolerance that explained 13–34% of the variance in phenoytpe. In another study, 47 QTL for different plant stress indicators in rice with 5–59% PVE were identified. In peanut, a total of 117 small main effect QTL with 3.48 -33.36 % PVE and 23 epistatic QTL with 1.7 -8.34 % PVE were identified for drought related traits including transpiration efficiency (TE), specific leaf area (SLA), SPAD chlorophyll meter reading, canopy conductance, dry weight, total dry matter (Ravi et al., 2011). In a similar study, Gautami et al. (2011) pooled markers across several studies and used a consensus map to identify 153 main effect QTL and 25 epistatic QTL for drought tolerance in peanut. Several studies in peanut have identified genes involved in drought tolerance by sequencing transcriptomes of drought-stressed versus non-stressed plants (Sun et al., 2013; Holbrook et al., 2016). Several hundred genes have been identified from gene-expression studies that are either induced or repressed by drought conditions (Sahi et al., 2006).

Low marker density has been one of the major limiting factors in the genomic study of drought tolerance in peanut, therein reducing the accuracy and efficiency of QTL mapping. However, the rapid development of so-called Next Generation Sequencing (NGS) technologies have paved the way for the development of sequence-based SNP markers that combines the advantages of time and cost effectiveness, resulting in improved genome-wide marker coverage. This has resulted in increased genetic map resolution and more comparable genomic and genetic maps among mapping populations. Genotyping-by-sequencing (GBS) is a cost-effective method of using NGS to produce a large number of genetic markers for genotyping hundreds of individuals across a reduced representation of the whole genome. The power of the GBS for high throughput low-cost genotyping helps in the development of high-density linkage maps and QTL mapping in various crops including those with polyploid genomes. Identification of high-density

SNPs through GBS to construct linkage maps has significant value to different applications of plant breeding (He et al., 2014). SNP markers have been used to discover QTL in many crops, including rice, maize, wheat, soybean, canola, barley, sugar beet, and cowpea (Mammadov et al., 2012). The efficiency of QTL mapping largely depends upon the recombination events and density of markers used for genetic map. Increasing marker density for a given trait can increase the resolution of genetic maps, therein increasing the precision of QTL mapping (Davey et al., 2011). In peanut, a number of efforts have been made to dissect complex traits using via QTL mapping (Ravi et al., 2011). However, because of the large polyploid genome, marker density is typically low (tens of SSRs) and thus provided limited information about the number and location of QTL controlling the traits. For better precision of QTL mapping, high-density linkage maps based on high-throughput markers like SNPs (single nucleotide polymorphism) is necessary. High-density linkage maps provide an accurate position of QTL, increase the precision of effect estimates for QTL detected through high marker density, and may lead to the identification of candidate genes underlying the complex traits. This approach is advancing the development of enhanced stress tolerance in crop plants (Jaganathan, 2005). In peanut, mapping QTL in RIL populations has further advanced the identification of genomic regions associated with the complex traits of drought tolerance/avoidance (Ravi et al., 2011; Gautami et al., 2011).

In this study, we used three RIL mapping populations constructed from crosses between five parental lines with contrasting drought associated phenotypes. The advantage of using these RILs is that they have more recombination events resulting in finer mapping of QTL over other mapping populations. In addition, the RIL population is a near permanent genetic resource as the genetic composition of RILs are not altered over time, which allows them to replicate the experiments across multiple locations. The study RIL populations were phenotyped for drought-surrogate traits like Normalized difference vegetation index (NDVI), Canopy temperature depression (CTD), wilting for drought tolerance. These surrogate traits are regarded as highly affected traits due to drought stress (Luis et al., 2016; Balota et al., 2017; Sarkar et al., 2021). GBS derived SNPs from our RIL populations were used to identify QTL associated with drought tolerant traits.

Materials and Methods

Mapping Populations

Three recombinant inbred line (RIL) mapping population, viz. Pop-1(N05006 × N04074FCT), Pop-2(N05006 × Phillips) and Pop-3(N08086olJCT × PI 585005) were phenotyped and genotyped. Pop-1 and 2 parents (N05006 and N04074FCT) are advanced breeding lines which were selected based on their differential response to reduced soil moisture in the field. Pop-3 was constructed from a cross between, PI 585005, a drought-tolerant Spanish-type line from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and N08086olJCT, a virginia-type, large-seeded, high yielding, and less drought-tolerant line from North Carolina State University (NCSU). Pop-1, 2 and Pop-3 consists of 129, 142 and 232 RILs, respectively, which were used in the study for developing the genetic maps and QTL analysis.

Experimental Design and Phenotyping

All three mapping populations (Pop 1, 2 & 3) were phenotyped at Virginia Tech Tidewater Agricultural Research and Extension center (TAREC), Suffolk, VA in a randomized complete block design (RCBD) with two replications as described in chapter 2. Cultural practices were followed as per recommendation by Virginia Peanut Production Guide (Balota et al., 2020). Phenotypic evaluations were done by NDVI, CTD and wilting. The NDVI was measured for each plot on progressive weeks starting after 5 weeks after planting (WAP) at different growth stages using a handheld GreenSeeker Crop Sensor (Trimble Ag., Sunnyvale, CA) until physiological maturity. The NDVI values were derived from the formula of red:near-infrared ratio [NDVI = (NIR−RED)/(NIR+RED), where RED and NIR are the amount of red and near-infrared light respectively (Rouse et al., 1974). The measurements were taken at a height of 50 cm above and perpendicular on the canopy on sunny and wind free days by scanning the hand-held GreenSeeker® over both rows of each plot, from end to end of each row, and the average of both rows NDVI were recorded. Canopy temperature depression (CTD) values were measured using "diff" option in a hand-held AGRI-THERM II™ infra-red thermometer (Agri-Therm Model 6110L, Everest Interscience Inc., Tucson, AZ). The CTD values were calculated by subtracting the canopy temperature (T_c) from the ambient air temperature (T_a) i.e., CTD = ($T_c - T_a$). CTD was measured starting from 5 WAP until physiological maturity throughout the growing season on sunny days with minimal wind. CTD was measured twice over a random spot on each row, at approximately 50 cm above the canopy and 45-degree angle and values from both rows were averaged to calculate plot value. Visual scoring of leaf wilting was done using a 0-5 rating scale. A score of 0 represented a potentially healthy plant with no visible wilting or leaf drooping symptoms; 1 represented some terminal and newer leaves fold but overall the plant looked healthy; 2 represented upper leaves almost all folded with visible signs of wilting, lower and older leaves started to fold; 3 represented wilting and drooping of all leaves on the plant, low-moisture effect on older leaves was prominent, and bare ground started to become visible; 4 represented all leaves were wilted and some started to change color due to chlorophyll degradation, bare ground was prominently visible, some leaves dried and crisped; 5 represented all leaves were severely wilted and color of all leaves was light green to yellow, bare ground was fully visible, more than 50% of leaves were crisp and dry, plant was almost physiologically dead (Sarkar et al., 2021). Leaf wilting was measured at different growth stages and drought severities starting from 6 WAP until physiological maturity.

DNA Extraction, Library Construction and Sequencing

DNA was isolated from from young leaves of peanut seedlings from RILs and parents using a modified cetyltrimethyl-ammonium bromide (CTAB) method (Porebski et al., 1997; Saghai-Maroof et al., 1984; Appendix A). DNA quantification was initially performed by 2% agarose gel and NanoDrop spectrophotometery, followed by fluorometry using a Qubit 3.0 fluorometer (Invitrogen Life technologies). Genotyping-by-sequencing (GBS) was used for constructing libraries of reduced representation for Illumina NGS platform described by Elshire et al. (2011) and modified as in Soorni et al. (2015), the full protocol can be found in Appendix B. In brief, GBS libraries were constructed from RILs and parents using 10 ng DNA, digested using *ApeKI* endonuclease restriction enzyme. Barcoded adaptors were ligated on to these fragments and then these were pooled and amplified via PCR to form a library, and sequenced using the Illumina NovaSeq 6000 platform (Illumina Inc, San Diego, CA, USA) at the Duke University Center for Genomics and Bioinformatics. In addition, DNAseq libraries were prepared for each of the parental lines and resequenced on the Illumina NovaSeq 6000 platform at the Duke University Center for Genomics and Bioinformatics.

Mapping reads and variant identification

Raw GBS reads were demultiplexed according to the sample barcodes and adapter sequences were removed using *GBSX v1.3* (Herten et al. 2015) by a custom designed Linux script (Appendix C). Mismatched reads with any barcode or that lack appropriate enzyme cut sites were discarded. All sequence reads (GBS and resequencing) were quality checked using FastQC v.11.6 (Andrews, 2010). Quality filtered reads were aligned to the reference genome of cv. Tifrunner version 2.0 (Bertioli et al., 2019) to facilitate SNP calling among all samples. Short-read alignment was done using a BWA v.0.7.12 tool based on Burrows-Wheeler transform (Li and Durbin, 2009). SAMtools v.1.11-GCC-10.20 (Li et al., 2009) was then used to convert the alignment from sequence alignment/map (SAM) format to binary alignment/map (BAM) format, sort, remove duplicates from the sequence alignments, and index the files. The package bamaddrg of BAMtools (Barnett et al. 2011) was used to add read group information to the alignment files for sample tracking. Variant calling was conducted using Freebayes v1.3.2-40-gcce27fc (Garrison and Marth, 2012) with the following options. Freebayes uses the Bayesian method to model any sequencing error and identifies SNPs based on the most likely combination of sample genotypes at each position in the reference genome (Garrison and Marth, 2012). The output file from Freebayes is in the Variant Call Format (VCF). A custom bash shell script (Appendix C) was used to process the RIL population and parent vcf files separately. These VCF files from all the three populations contain each identified variant with their positions and scores. After initial variant calling vcf files were processed for homozygous markers in parent and RIL files, low frequency and low quality markers removed, this was completed using vcffilter v0.2 and bcftools v.1.3.1 (Danecek et al., 2011). Finally the set of intersecting markers between the RIL populations and associated parents were generated using bcftools v.1.3.1.

Genetic map construction and QTL mapping

The filtered SNP data with polymorphic SNP markers were used to construct linkage maps for each of the three populations using QTL IciMapping v.4.1 software (Meng et al., 2015). The mapping data were formatted according to the instruction of IciMapping software protocol.

First, the BIN module of QTL IciMapping v4.1(Meng et al., 2015) was used to remove completely linked (distorted) and redundant markers. The marker ordering was calculated by the 'nnTwoOpt' algorithm and recombinant frequencies were calculated with a minimum logarithm of odds (LOD) threshold of 3. Rippling was performed using SARF (sum of adjacent recombination frequencies) with a window size of 5 (Meng et al., 2015). Linkage groups (LGs) based on the LOD scores and the maximum likelihood algorithm were applied for determining loci orders using Kosambi's mapping function (Kosambi, 1944). The mean values of phenotypic traits of each RIL from two replicates were used for statistical analysis. Analysis of variance (AOV) was performed in R with Rstudio version 2022.02.1 (RStudio, Inc., USA, 2022). To identify QTL, analysis was performed using phenotypic data of Pop-1 & 2 (NDVI, CTD, SPAD and wilting) and Pop-3 (NDVI, CTD and wilting) along with the genotypic data with inclusive composite interval mapping of additive (ICIM-ADD) method. The probability used in stepwise regression for QTL was 0.001. To claim a significant QTL, a LOD threshold of 2.5 was set for additive QTL. Significant LOD thresholds were determined for each dataset by 1000 permutations with type I probability of 0.05 (Doerge, 2020). Graphics for linkage map and different QTL were generated through the MAP function of QTL IciMapping.

Results and Discussion

Phenotypic Evaluation

The mapping population Pop-1 'N05006 x N04074FCT' showed a wide range of phenotypic variation for traits evaluated in the study (Table 1). Analysis of variance showed significant variation among RILs of Pop-1 for pod yield, NDVI and SPAD (Table 1). Values for NDVI ranged from 0.53 to 0.90, whereas for SPAD the values ranged from 26.97 to 48.53

(Table 1). The greenness index and plant health measured by SPAD and NDVI values showed a strong positive correlation with leaf chlorophyll concentration (Araus et al., 2018). Thus, a decrease in the values of NDVI and SPAD leads to reduction of photosynthetic carbon assimilation which in turn reduces pod yield (Balota et al., 2012; Luis et al., 2016). In Pop-2 ‘N05006 x Phillips’ analysis of variance showed significant variation among lines for CTD, SPAD and wilting (Table 2). The values for CTD ranged from -6.9 to 5.5; SPAD ranged from 31.50 to 50.60 and wilting from 0 to 2 (Table 2). In peanut, CTD and visual symptoms of low moisture stress, quantified by wilting, is recommended for screening and development of drought tolerant cultivars (Luis et al., 2016; Balota and Oakes, 2017; Sarkar et al., 2021). Pop-3 ‘N08086olJCT x PI 585005’ only showed significant variation between lines for NDVI with values ranging from 0.25 to 0.92 (Table 3). However, there are previous studies which assessed phenotypic traits such as specific leaf area (SLA) for identifying and selecting peanuts with high water use efficiency (Wright et al. 1994). Other phenotypic evaluations for drought tolerance in peanut included SPAD chlorophyll meter readings (Nageswara Rao et al., 2001), as a rapid and non-destructive screening for large breeding populations. Similarly, present study used a combination of different trait evaluations which included NDVI, SPAD, CTD, wilting and pod yield to evaluate RIL population to capture the genetic variation among different traits for drought tolerance. Multiple trait evaluations for screening RILs for drought tolerance in peanuts provide more reliable method of identification of genotypes for quantifying complex traits such as drought.

QTL analysis

Pop-1, Pop-2 and Pop-3 comprised 239, 225 and 1132, respectively, filtered polymorphic SNP markers that were identified from both parents for each population (Table 4). Further, these polymorphic markers were binned (p<0.001 and missing data with >20%) to eliminate redundant markers. Once redundant markers were removed there were 177 (Pop-1), 142 (Pop-2) and 976 (Pop-3) SNPs remaining for construction of a genetic linkage map using the MAP function in IciMapping v4.1. Genetic linkage maps were constructed for all the three mapping populations. The Pop-1 linkage map constructed for the 129 RILs showed 177 markers spanning around the 19 linkage groups (Figure 1 A&B). The total genetic length covered by the Pop-1 linkage map was 1243.7 cM (Table 4). Similarly, Pop-2 has 225 markers present on 19 linkage groups with a total length of 1209.822 cM and Pop-3 with 1132 markers spanning around 20 linkage groups with a total length 7785.7 cM (Table 4).

These linkage maps were then used, along with phenotypic data for NDVI, CTD, SPAD, and wilting, to identify QTL associated with each of these traits. In Pop-1, a QTL for NDVI trait was identified on chromosome 2 with LOD score of 3.0648 and PVE % of 10.38 located between the SNP markers M11 and M14. Another QTL for SPAD was identified between SNP marker M10 and M3 on chromosome 2 with a LOD score of 2.97 and PVE% of 10.10. Similar results were reported in marker trait association studies in peanut for NDVI trait (Shaibu et al., 2019). In Pop-2, two minor effect QTL for NDVI were flanked between SNP markers M198 and M201 on chromosome 16 with a LOD score of 2.64 and PVE% 4.39. A QTL for CTD was identified on chromosome 4 with LOD score of 4.13 and PVE% 14.61 flanked between marker M65 and M88. For SPAD, two QTL were found on chromosome 4 and 16 with LOD 3.03 and phenotypic variance explained in percentage was up to 13.23. Out of six QTL for wilting were on four different chromosomes (2, 12, 16 and 17) with LOD score ranging from 2.5 to 38.58; two QTL on chromosome 2 flanked between markers M11-M27 and M27-M9 with maximum LOD score of 38.377 explained PVE% 4.80. In Pop-3, three out of 16 QTL for NDVI are identified on chromosome 2 flanked between SNP markers (M592-M1082 and M1082-M567) with high LOD score of 11.8 and cumulative PVE% 4.08. Two QTL for CTD were located on chromosome 17 flanked between (M711-M709 and M709-M708) with LOD score of 5.19. Across Pop-1 and Pop-3, QTL for NDVI are identified on chromosome 2 which might reflect presence of hotspots region for NDVI on chromosome 2. The total number of QTL covered by each chromosome in Pop-1, Pop-2 and Pop-3 are summarized in Tables 5, 6, 7 and 8.

In summary, three linkage maps were generated from three diverse RIL populations of virginia type peanut including with minor QTL for drought tolerance related traits like NDVI, CTD, SPAD and wilting. In the future, consensus map could be developed from these three populations to get a high-density genetic map in the peanut. This consensus map helps us to remove the large gaps present in the individual maps of all populations, except for the linkage group showing poor coverage in those genomic regions. For the drought related complex traits which are governed by many genes, QTL with low phenotypic variance can be identified and utilized with other advanced breeding approaches like genomic selection (GS) or marker assisted recurrent selection (MARS).

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Figures and Tables

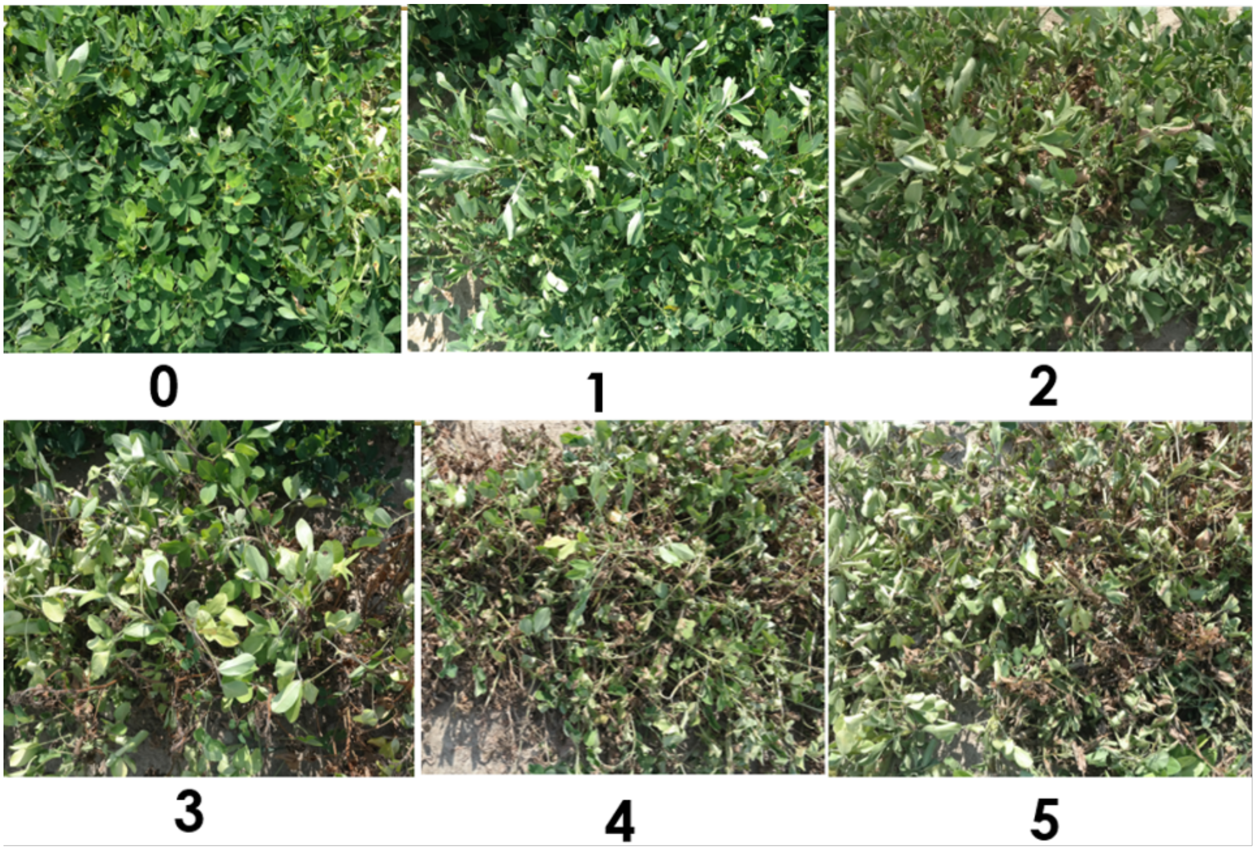

Figure 1. Wilting scale used for rating of peanut. The scale ranges from 0 to 5, 0 is a potentially healthy plant with no observable wilting, and 5, where all leaves have severely wilted and their color is light green to yellow, bare ground is fully visible, more than 50% of leaves are crisp and dry, the plant is almost physiologically dead (Sarkar et al., 2021).

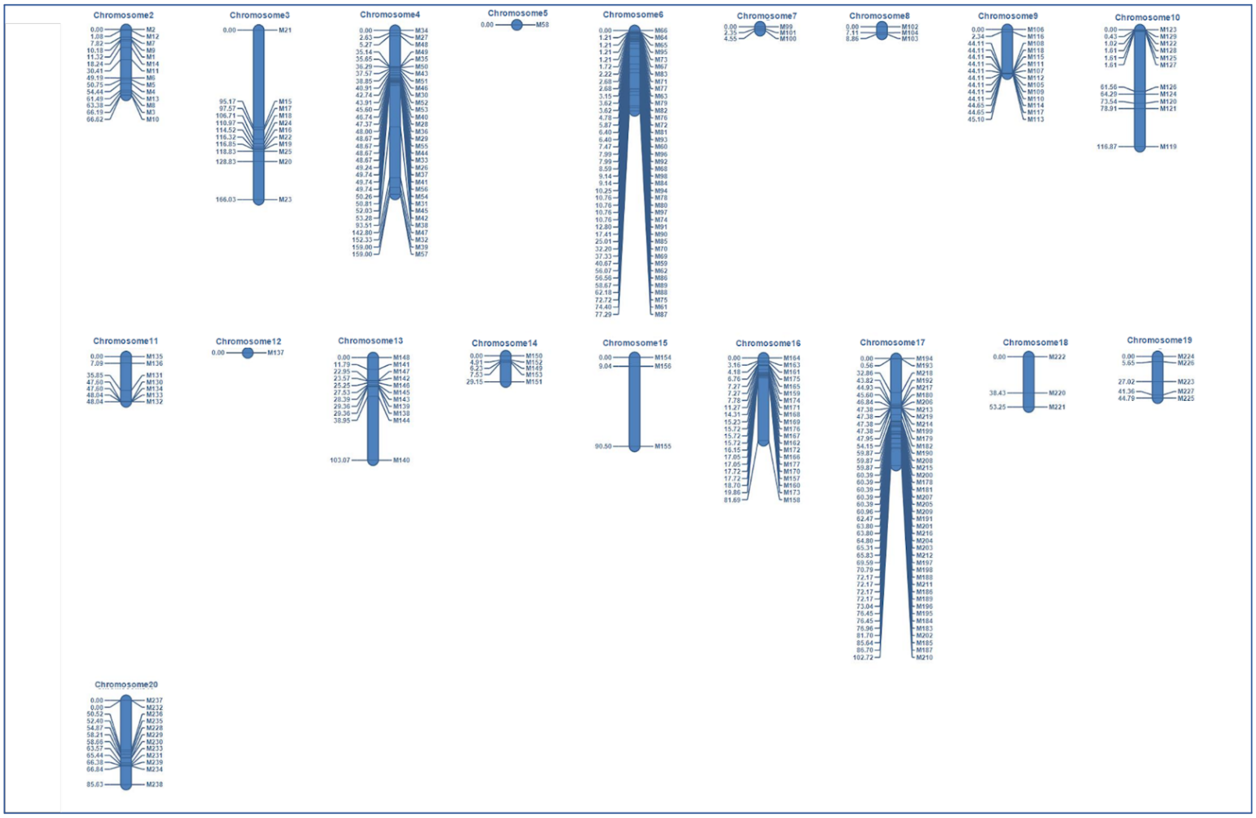

Figure 2 A. Genetic linkage map constructed for the 129 RIL population derived from the cross of N05006 × N04074FCT (Pop-1). The 976 SNP markers are shown at the right side of the 19 linkage groups.

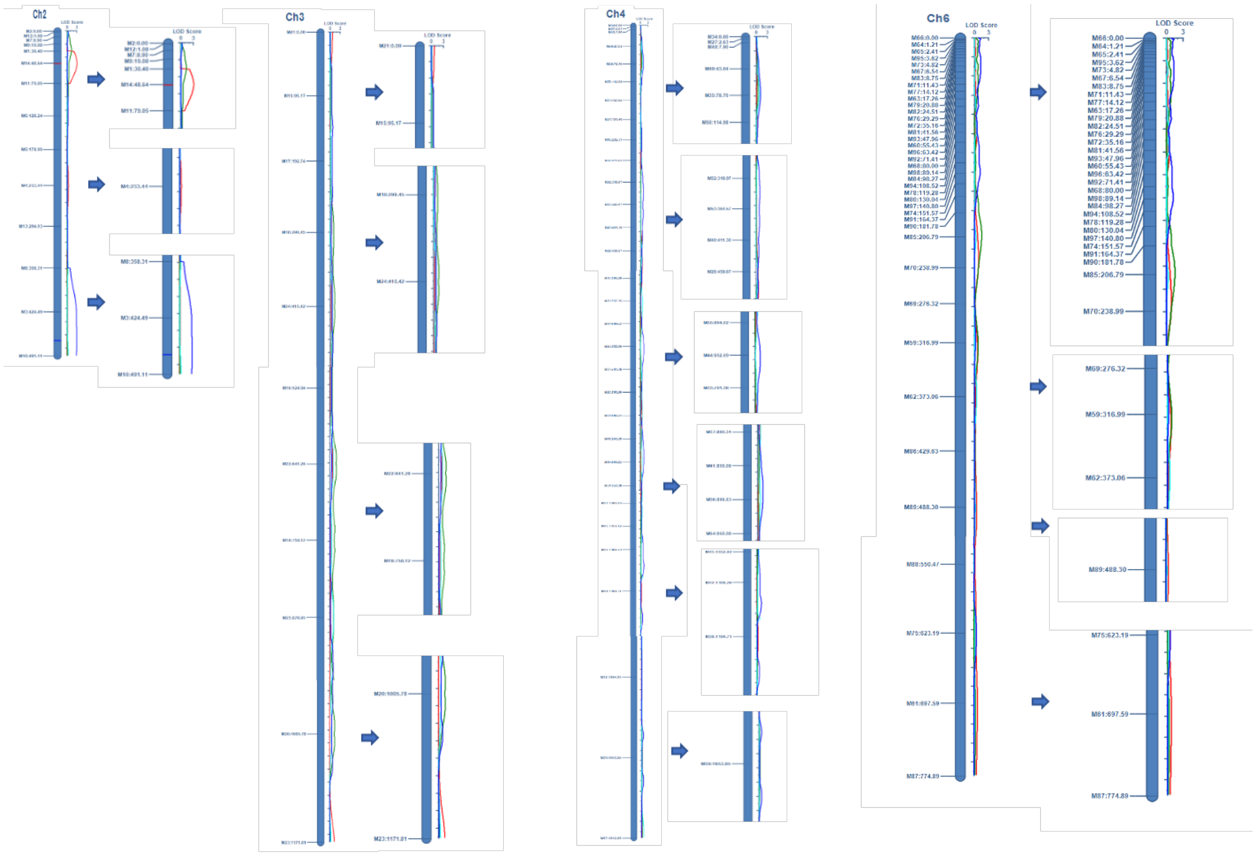

Figure 2 B. QTL positions for NDVI and SPAD on chromosome 2, 3 and 6 for Pop-1 ‘N05006 × N04074FCT’. The vertical bar indicates the LOD score values from 0 to 3 along the chromosomes, the corresponding red peak is an indicative of NDVI and blue represents SPAD QTL. The solid blue arrow shows the magnified position of QTL position between flanking markers.

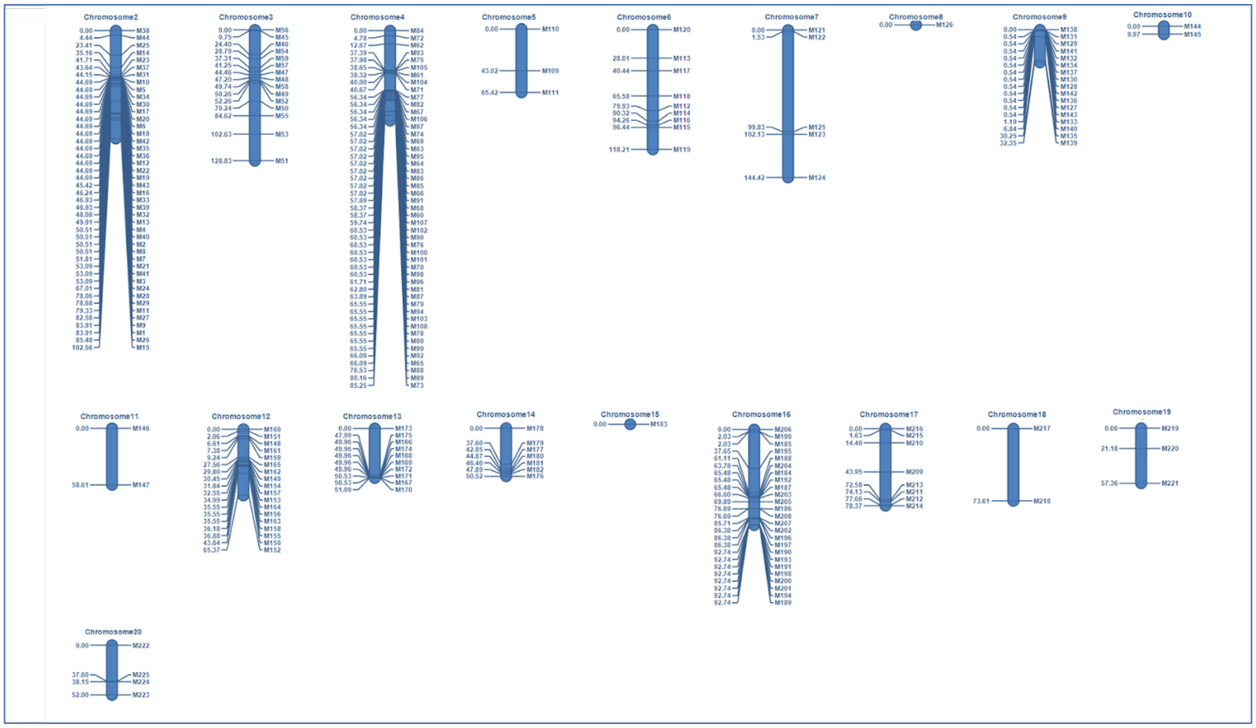

Figure 3A. Genetic linkage map constructed for the 109 RIL population derived from the cross of N05006 × Phillips (Pop-2). The 976 SNP markers are shown at the right side of the 19 linkage groups.

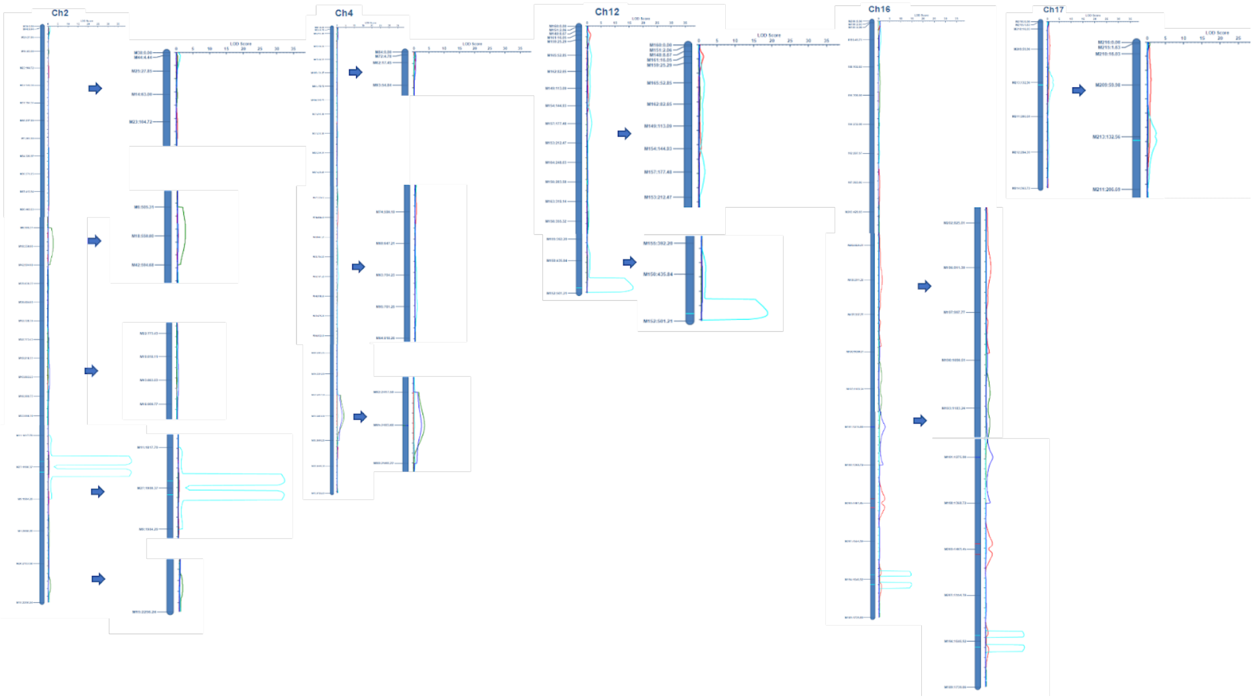

Figure 3 B. QTL positions for NDVI, CTD, wilting and SPAD on chromosome 2, 4, 12, 16 and 17 for Pop-2 ‘N05006 x Phillips’. The vertical bar indicates the LOD score values from 0 to 35 along the chromosomes, the corresponding red peak is an indicative of NDVI, green represents CTD, blue represents SPAD and sky-blue represents wilting QTL. The solid blue arrow shows the magnified position of QTL position between flanking markers.

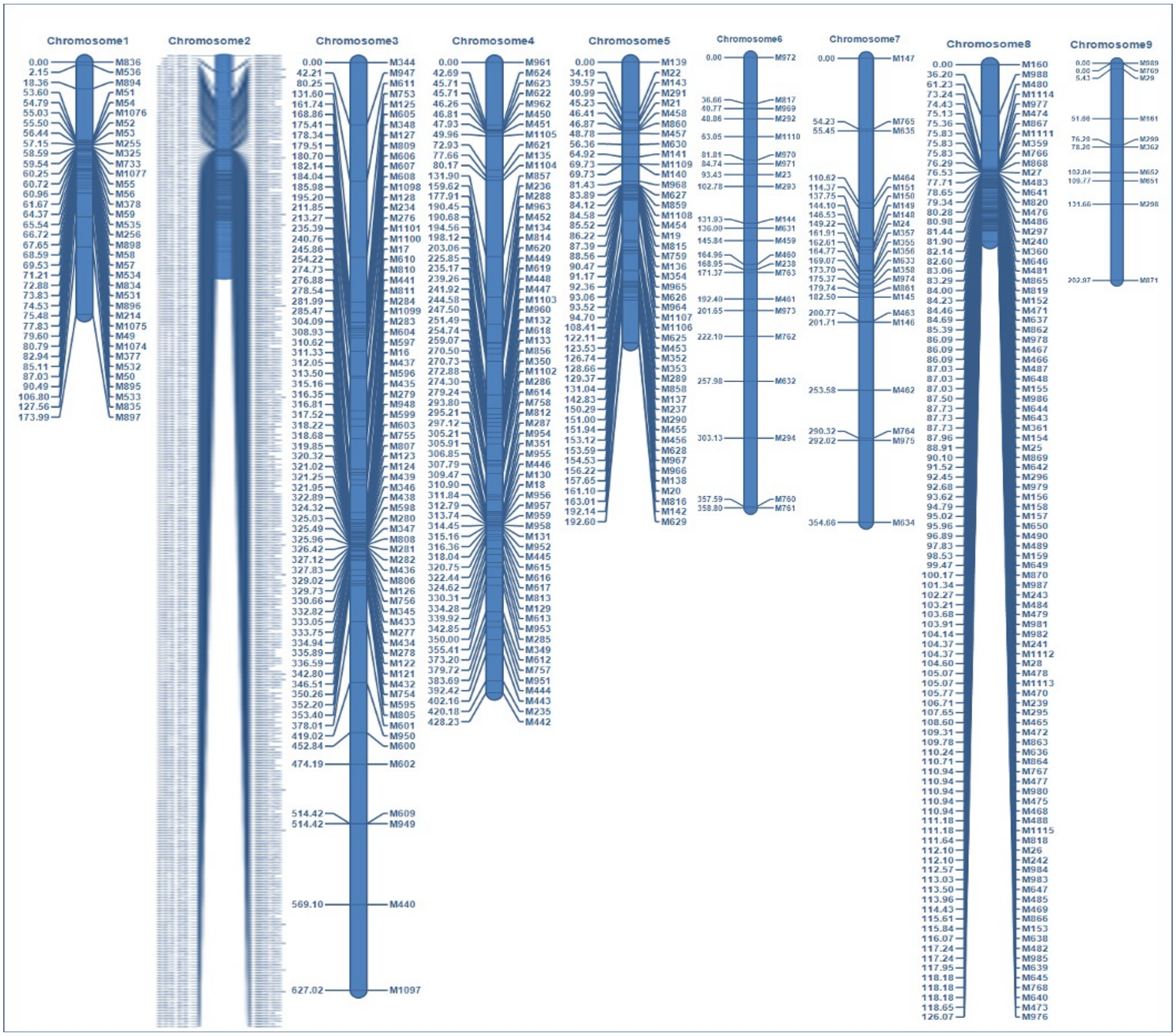

Figure 4A. Genetic linkage map constructed for 218 RIL population derived from a cross of 'N08086olJCT × PI 585005' (Pop-3). The 976 SNP markers are shown at the right side of the 9 out of 20 linkage groups.

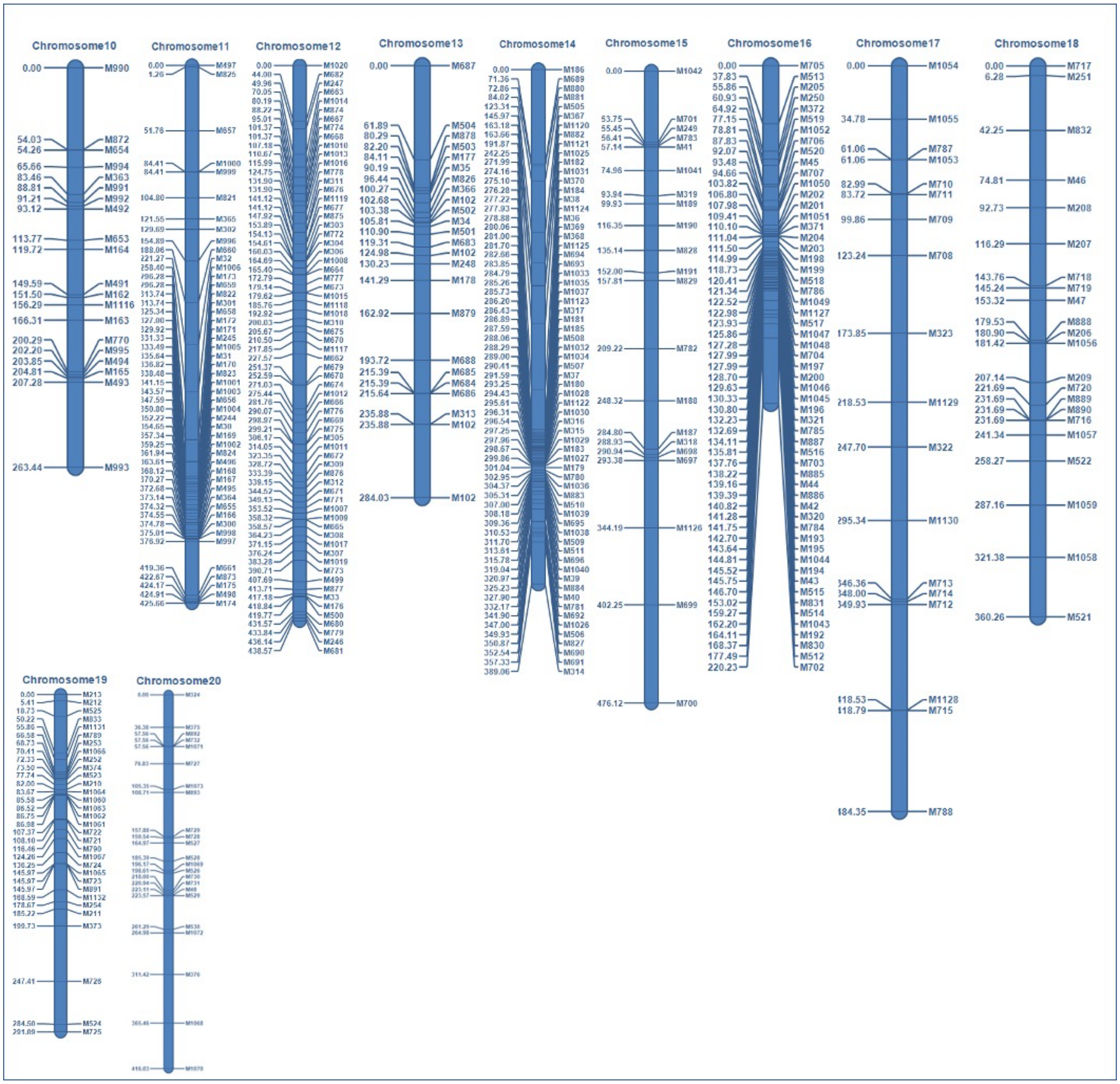

Figure 4B. Genetic linkage map constructed for the 218 RIL population derived from the cross of N08086olJCT × PI 585005 (Pop-3). The 976 SNP markers are shown at the right side of 11 out of 20 linkage groups.

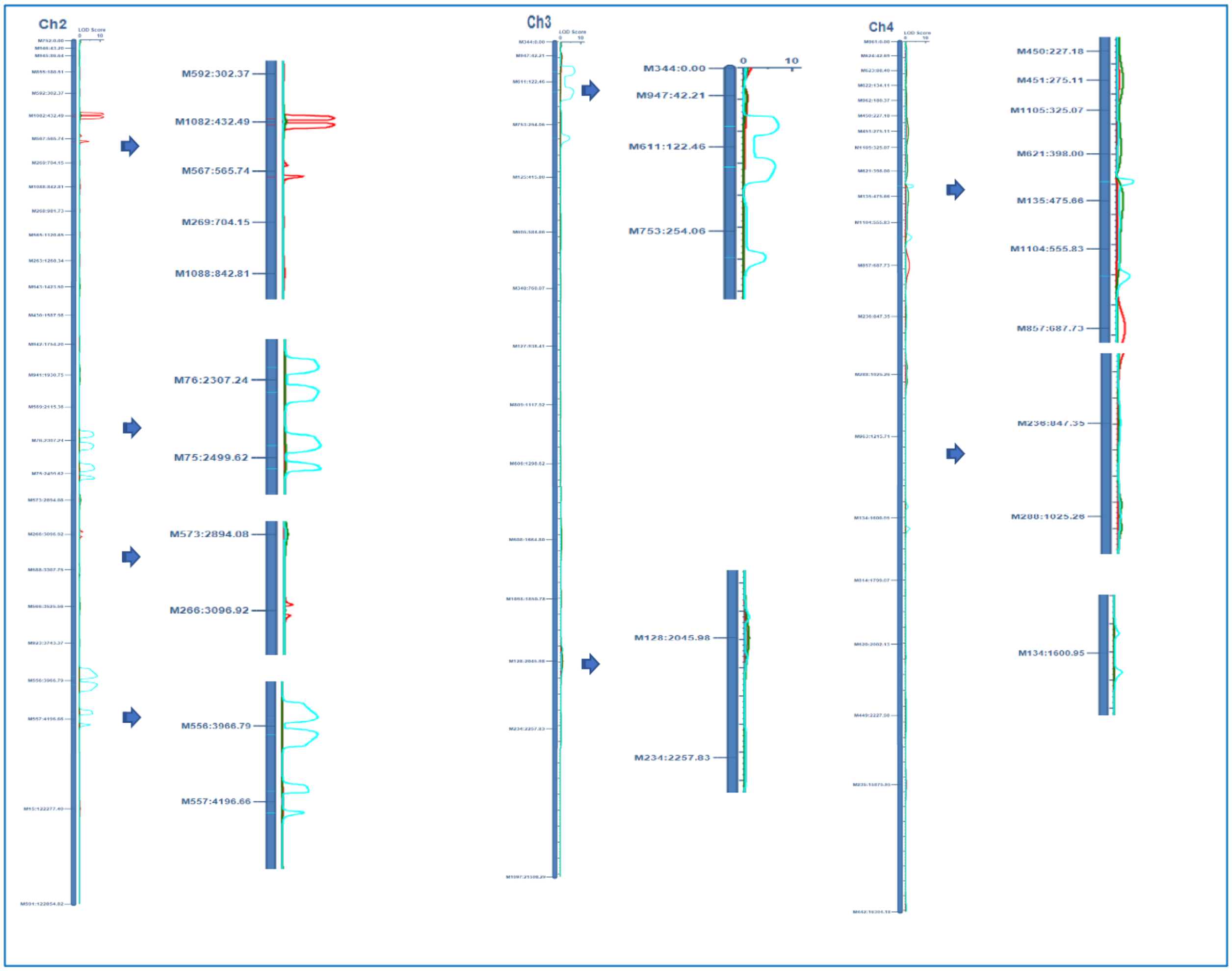

Figure 5A. QTL positions for NDVI, CTD, wilting and SPAD on chromosome 2, 3 and 4 for Pop-3 'N08086olJCT × PI 585005'. The vertical bar indicates the LOD score values from 0 to 10 along the chromosomes, the corresponding red peak is an indicative of NDVI, green represents CTD, blue represents SPAD and sky-blue represents wilting QTL. The solid blue arrow shows the magnified position of QTL position between flanking markers.

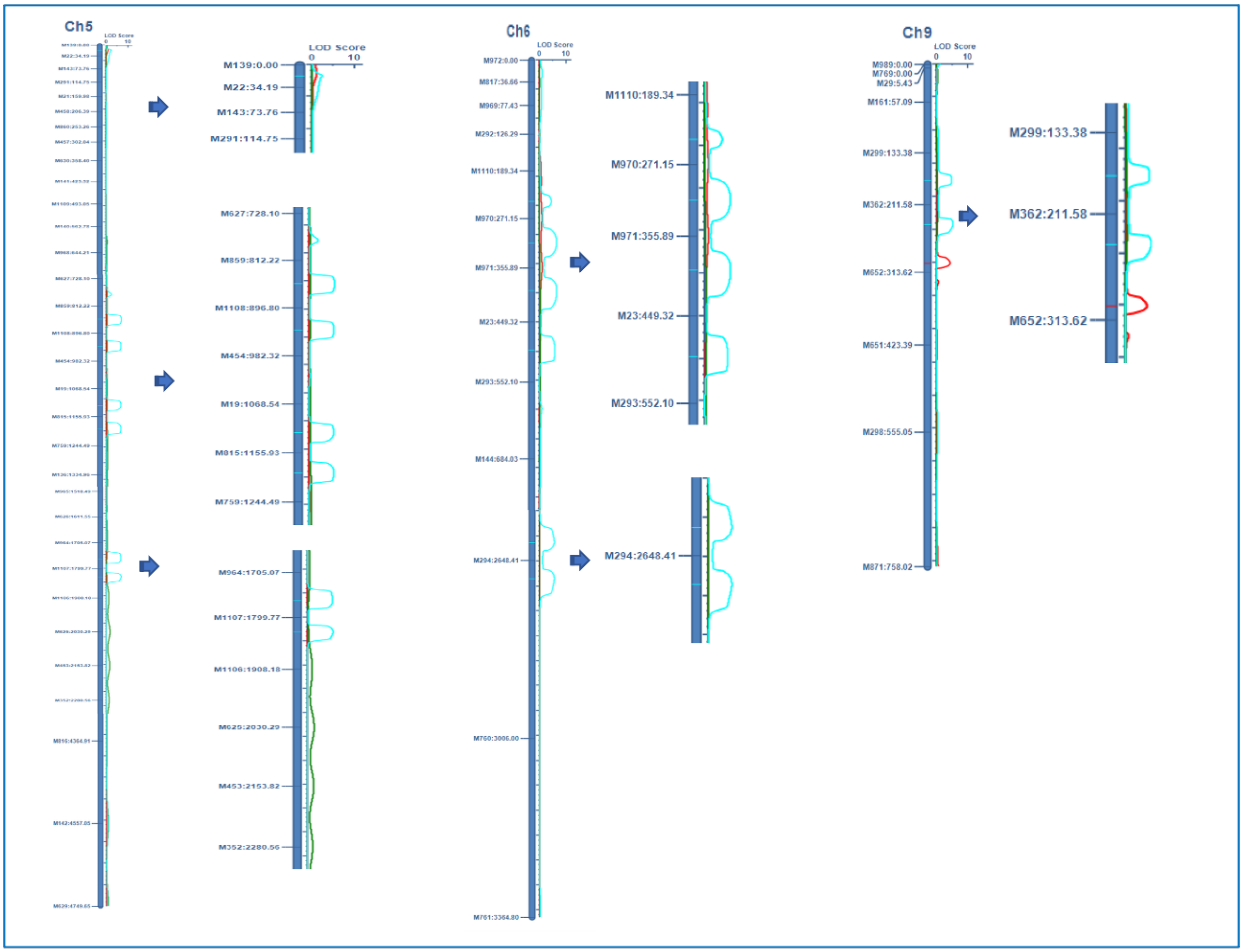

Figure 5 B. QTL positions for NDVI, CTD, wilting and SPAD on chromosome 5, 6 and 9 for Pop-3 ‘N08086olJCT × PI 585005’. The vertical bar indicates the LOD score values from 0 to 10 along the chromosomes, the corresponding red peak is an indicative of NDVI, green represents CTD, blue represents SPAD and sky-blue represents wilting QTL. The solid blue arrow shows the magnified position of QTL position between flanking markers.

Table 1. Analysis of variance (ANOVA) of NDVI, CTD, wilting, SPAD, and pod yield of 129 RILs of Pop-1 with mean squares, minimum, maximum, and mean tested under rainfed conditions in 2018.

SOV	**DF**	**NDVI**	**CTD**	**Wilting**	**SPAD**	**Yield**
RILs	128	0.01 **	1.25ns	0.11ns	24.3 **	1504211.85 *
Rep	1	0.02 ns	251.08 *	0.66*	0.153	1936358804.1**
Residuals	127	0.01	3.06	0.14	13.76	1084884.29
Min.		0.53	-6.7	0.00	26.97	2033
Max.		0.90	0.00	1.5	48.53	8221
Mean		0.80	-3.8	0.12	40.93	4531

SOV=Source of variation, DF =degrees of freedom, ns = non-significant, Significant at $p \leq 0.001$(***); $p \leq 0.01$(**) and $p \leq 0.05$(*)

Table 2. Analysis of variance (ANOVA) of NDVI, CTD, wilting, SPAD, and pod yield of 109 RILs of Pop-2 with mean squares, minimum, maximum, and mean tested under rainfed conditions in 2018.

SOV	**DF**	**NDVI**	**CTD**	**Wilting**	**SPAD**	**Yield**
RILs	108	0.01ns	7.37*	0.33**	14.79**	1099751.45ns
Rep	1	0.85**	29.25 *	0.04ns	0.06ns	6018501.3*
Residuals	107	0.02	2.13	0.11	8.78	916223.49
Min.		0.36	-6.9	0.00	31.50	2115
Max.		0.93	5.5	2.0	50.60	6587
Mean		0.76	-2.81	0.2	39.40	4409

SOV=Source of variation, DF =degrees of freedom, ns = non-significant, Significant at $p \leq 0.001$(***); $p \leq 0.01$(**) and $p \leq 0.05$(*)

Table 3. Analysis of variance (ANOVA) of NDVI, CTD, wilting, and pod yield of 232 RILs of Pop-3 with mean squares, minimum, maximum, and mean tested under rainfed conditions in 2019.

SOV	**DF**	**NDVI**	**CTD**	**Wilting**	**Yield**
RILs	231	0.02 **	0.92 ns	0.14**	3823240.94 *
Rep	1	0.0 ns	33 *	0.05 ns	450842284.33**
Residuals	230	0.01	0.77	0.1	2723294.33
Min.		0.25	-5.9	1.5	366.2
Max.		0.92	-0.2	3.5	9341.7
Mean		0.83	-3.13	2.7	5133.1

SOV=Source of variation, DF =degrees of freedom, ns = non-significant, Significant at $p \leq 0.001$(***); $p \leq 0.01$(**) and $p \leq 0.05$(*)

Table 4. Summary of genetic map constructed using single nucleotide polymorphism (SNP) markers for all the three populations

	Pop-1 (129 RILs)		**Pop-2 (142 RILs)**		**Pop-3 (232 RILs))**	
Chrom.ID	Markers	Length(cM)	Markers	Length(cM)	Markers	Length(cM)
Ch 1					36	190.98
Ch 2	14	66.91	44	102.71	362	585.45
Ch 3	11	166.03	15	140.94	72	721.16
Ch 4	32	159.09	49	91.71	65	477.52
Ch 5	1	0.00	3	65.42	46	193.02
Ch 6	40	77.93	9	113.65	22	392.27
Ch 7	3	4.55	5	83.28	23	398.45
Ch 8	3	8.86	1	0.00	98	128.53
Ch 9	14	45.10	17	32.94	10	200.83
Ch10	11	102.38	2	9.97	20	306.29
Ch 11	7	48.04	2	58.61	48	519.20
Ch 12	1	0.00	18	72.25	67	493.44
Ch 13	11	103.13	10	51.52	24	324.81
Ch14	5	29.15	7	50.52	66	410.33
Ch 15	3	53.84	1	0.00	21	500.26
Ch16	21	81.72	25	92.77	57	219.78
Ch 17	42	103.61	8	67.78	18	558.70
Ch 18	3	53.25	2	73.61	22	423.71
Ch 19	5	44.79	3	57.36	32	292.45
Ch 20	12	95.37	4	44.78	23	448.55
Total	**239**	**1243.77**	**225**	**1209.82**	**1132**	**7785.75**

Table 5. Chromosome ID and their QTL loci related to NDVI, CTD, SPAD and wilting for three diverse mapping population of peanut for twenty chromosomes

	QTL											
	Pop-1				**Pop-2**				**Pop-3**			
Chrom.ID	**NDVI**	**CTD**	**SPAD**	**Wilting**	**NDVI**	**CTD**	**SPAD**	**Wilting**	**NDVI**	**CTD**	**Wilting**	**Total**
Ch 1												
Ch 2	1		1					2	3		10	**17**
Ch 3											8	**8**
Ch 4						1	1				3	**5**
Ch 5											8	**8**
Ch 6											6	**6**
Ch 7											2	**2**
Ch 8									3			**3**
Ch 9									1		2	**3**
Ch10												
Ch 11											52	**52**
Ch 12								1	2		32	**35**
Ch 13											5	**5**
Ch14									2		69	**71**
Ch 15											3	**3**
Ch16					2		1	2	1		24	**30**
Ch 17								1		2	4	**7**
Ch 18											10	**10**
Ch 19											2	**2**
Ch 20											1	**1**
Total	**1**		**1**		**2**	**1**	**2**	**6**	**12**	**2**	**241**	

Table 6. Trait, chromosome position and distribution of QTL related to NDVI and SPAD at different chromosomes of Pop-1 ‘N05006 x N04074FCT’

Trait	Chromosome	Position	Left Marker	Right Marker	LOD	PVE (%)	Add	Left CI	Right CI
NDVI	2	49	M14	M11	3.07	10.38	-0.0149	31.5	66.5
SPAD	2	468	M3	M10	2.98	10.10	-1.727	433.5	491

Table 7. Trait, chromosome position and distribution of QTL related to NDVI, CTD, SPAD and wilting (WLT) at different chromosomes of Pop-2 'N05006 x Phillips'

Trait	chromosome	Position	Left Marker	Right Marker	LOD	PVE (%)	Add	Left CI	Right CI
NDVI	16	1451	M198	M200	2.65	4.39	0.0583	1436.5	1457.5
NDVI	16	1472	M200	M201	2.58	4.40	0.0581	1464.5	1484.5
CTD	4	2484	M65	M88	4.14	14.62	-0.3925	2452.5	2509.5
WLT	2	1886	M11	M27	38.59	4.80	-0.5769	1873.5	1892.5
WLT	2	1914	M27	M9	38.38	4.80	-0.5769	1908.5	1925.5
WLT	12	491	M150	M152	17.11	3.68	-0.5051	482.5	495.5
WLT	16	1635	M201	M194	14.63	3.68	-0.5051	1626.5	1639.5
WLT	16	1659	M194	M189	14.77	3.68	-0.5051	1654.5	1668.5
WLT	17	138	M213	M211	2.58	1.04	0.1992	133.5	152.5
SPAD	4	2484	M65	M88	3.04	13.23	-1.006	2442.5	2515.5
SPAD	16	1276	M191	M198	2.84	12.06	-1.8807	1263.5	1288.5

Table 8. Trait, chromosome position and distribution of QTL related to NDVI, CTD and wilting (WLT) at different chromosomes of Pop-3 ‘N08086olJCT × PI 585005’

Trait	Chromosome	Position	Left Marker	Right Marker	LOD	PVE (%)	Add	Left CI	Right CI
NDVI	2	424	M592	M1082	11.88	2.05	0.1078	418.5	427.5
NDVI	2	441	M1082	M567	11.87	2.05	0.1078	437.5	446.5
NDVI	2	581	M567	M269	4.63	1.83	0.0871	579.5	583.5
NDVI	8	6942	M468	M488	6.78	0.52	-0.036	6941.5	6949.5
NDVI	8	7053	M488	M1115	7.10	0.54	-0.0366	7045.5	7059.5
NDVI	8	7163	M488	M1115	6.84	0.54	-0.0364	7156.5	7163.5
NDVI	9	300	M362	M652	4.28	2.03	0.0977	293.5	304.5
NDVI	12	4038	M310	M675	6.20	1.95	0.0945	4035.5	4039.5
NDVI	12	4065	M675	M670	4.93	1.73	0.0847	4063.5	4066.5
NDVI	14	488	M505	M367	9.94	2.07	-0.1087	482.5	491.5
NDVI	14	507	M367	M1120	9.91	2.07	-0.1086	503.5	512.5
NDVI	16	37	M705	M513	3.53	0.27	0.0258	17.5	53.5
CTD	17	419	M711	M709	5.20	0.00	2.5368	412.5	421.5
CTD	17	428	M709	M708	5.21	0.00	2.5441	425.5	435.5
WLT	2	2277	M569	M76	7.09	0.25	0.2351	2259.5	2286.5
WLT	2	2338	M76	M75	7.09	0.25	0.2351	2327.5	2356.5
WLT	2	2470	M76	M75	7.52	0.25	0.2358	2453.5	2479.5
WLT	2	2528	M75	M572	7.51	0.25	0.2353	2519.5	2531.5
WLT	2	3941	M923	M556	8.81	0.25	0.2369	3927.5	3949.5
WLT	2	3993	M556	M557	8.81	0.25	0.2368	3984.5	4006.5
WLT	2	4164	M556	M557	6.53	0.25	0.2358	4147.5	4169.5
WLT	2	4231	M557	M564	5.32	0.22	0.2161	4227.5	4234.5
WLT	2	16641	M551	M842	5.72	0.23	0.2223	16636.5	16645.5
WLT	2	16708	M842	M544	4.90	0.21	0.2125	16704.5	16712.5
WLT	3	91	M947	M611	7.18	0.25	0.2346	77.5	100.5
WLT	3	154	M611	M753	6.50	0.25	0.2333	143.5	170.5

WLT	3	296	M753	M125	4.50	0.24	-0.224	288.5	304.5
WLT	3	3410	M17	M610	4.32	0.22	-0.2144	3404.5	3414.5
WLT	3	3485	M610	M810	3.90	0.21	-0.2088	3480.5	3490.5
WLT	3	18314	M601	M950	2.99	0.20	0.2038	18307.5	18319.5
WLT	3	18780	M950	M600	7.46	0.25	0.2357	18762.5	18789.5
WLT	3	18839	M600	M602	7.46	0.25	0.2356	18829.5	18855.5
WLT	4	444	M621	M135	3.96	0.24	-0.2285	440.5	448.5
WLT	4	602	M1104	M857	3.09	0.21	-0.2066	594.5	608.5
WLT	4	11507	M617	M813	2.67	0.17	-0.1846	11503.5	11512.5
WLT	5	17	M139	M22	2.51	0.18	-0.2098	14.5	37.5
WLT	5	854	M859	M1108	6.93	0.25	-0.2363	840.5	869.5
WLT	5	937	M1108	M454	6.77	0.25	-0.236	923.5	951.5
WLT	5	1120	M19	M815	6.71	0.25	-0.2351	1105.5	1133.5
WLT	5	1192	M815	M759	6.75	0.25	-0.2351	1178.5	1207.5
WLT	5	1470	M354	M965	6.77	0.25	-0.2342	1456.5	1485.5
WLT	5	1765	M964	M1107	7.22	0.25	-0.2353	1744.5	1778.5
WLT	5	1830	M1107	M1106	7.31	0.25	-0.2353	1820.5	1846.5
WLT	6	242	M1110	M970	4.46	0.24	-0.2285	232.5	250.5
WLT	6	313	M970	M971	6.54	0.24	-0.2307	296.5	330.5
WLT	6	395	M971	M23	6.60	0.25	-0.2312	379.5	418.5
WLT	6	497	M23	M293	5.87	0.25	-0.2326	476.5	515.5
WLT	6	2612	M632	M294	5.77	0.25	0.2319	2586.5	2624.5
WLT	6	2685	M294	M760	5.77	0.25	0.2319	2672.5	2711.5
WLT	7	2905	M146	M462	6.40	0.25	0.2331	2884.5	2915.5
WLT	7	2971	M462	M764	6.40	0.25	0.2331	2960.5	2990.5
WLT	9	175	M299	M362	4.85	0.24	-0.2306	166.5	182.5
WLT	9	241	M362	M652	5.19	0.24	-0.2311	232.5	252.5
WLT	11	1662	M1006	M173	6.17	0.25	-0.2341	1644.5	1673.5
WLT	11	1731	M173	M659	6.17	0.25	-0.2341	1719.5	1748.5

WLT	11	1960	M173	M659	6.37	0.25	-0.2332	1938.5	1970.5
WLT	11	2026	M659	M822	6.37	0.25	-0.2333	2015.5	2048.5
WLT	11	2280	M659	M822	8.37	0.25	-0.2362	2265.5	2288.5
WLT	11	2334	M822	M301	8.37	0.25	-0.2362	2324.5	2345.5
WLT	11	2594	M822	M301	8.35	0.25	-0.2363	2586.5	2602.5
WLT	11	2647	M301	M658	8.35	0.25	-0.2363	2638.5	2662.5
WLT	11	2918	M301	M658	7.90	0.25	-0.235	2901.5	2926.5
WLT	11	2974	M658	M172	7.91	0.25	-0.2349	2965.5	2989.5
WLT	11	3243	M658	M172	7.31	0.25	-0.2339	3225.5	3252.5
WLT	11	3303	M172	M171	7.31	0.25	-0.2339	3293.5	3320.5
WLT	11	3570	M172	M171	6.58	0.25	-0.2345	3550.5	3580.5
WLT	11	3635	M171	M245	6.58	0.25	-0.2346	3624.5	3653.5
WLT	11	3900	M171	M245	6.13	0.25	-0.2341	3889.5	3906.5
WLT	11	3969	M245	M1005	6.13	0.25	-0.2341	3961.5	3978.5
WLT	11	4236	M245	M1005	6.73	0.25	-0.2352	4218.5	4245.5
WLT	11	4300	M1005	M31	6.73	0.25	-0.2352	4289.5	4315.5
WLT	11	4905	M31	M170	5.73	0.24	-0.2272	4899.5	4909.5
WLT	11	4975	M170	M823	5.91	0.24	-0.2321	4969.5	4983.5
WLT	11	5246	M170	M823	6.50	0.25	-0.2348	5228.5	5256.5
WLT	11	5311	M823	M1001	6.50	0.25	-0.2348	5300.5	5329.5
WLT	11	5586	M823	M1001	6.34	0.25	-0.2343	5575.5	5592.5
WLT	11	5653	M1001	M1003	6.33	0.25	-0.2329	5647.5	5661.5
WLT	11	5930	M1001	M1003	6.34	0.25	-0.2329	5922.5	5935.5
WLT	11	5996	M1003	M656	4.73	0.21	-0.2099	5992.5	5999.5
WLT	11	6980	M1004	M244	6.15	0.25	-0.2342	6969.5	6988.5
WLT	11	7048	M244	M30	6.15	0.25	-0.2341	7039.5	7058.5
WLT	11	7692	M30	M169	6.33	0.25	-0.2339	7683.5	7698.5
WLT	11	7759	M169	M1002	6.33	0.25	-0.2342	7752.5	7769.5
WLT	11	8414	M1002	M824	6.36	0.25	-0.2346	8401.5	8422.5

WLT	11	8481	M824	M496	6.36	0.25	-0.2345	8471.5	8494.5
WLT	11	8779	M824	M496	6.83	0.25	-0.2346	8759.5	8789.5
WLT	11	8842	M496	M168	6.83	0.25	-0.2347	8832.5	8861.5
WLT	11	9146	M496	M168	6.45	0.25	-0.2344	9133.5	9153.5
WLT	11	9212	M168	M167	6.45	0.25	-0.2344	9203.5	9224.5
WLT	11	9520	M168	M167	7.57	0.25	-0.2352	9503.5	9529.5
WLT	11	9578	M167	M495	7.57	0.25	-0.2352	9568.5	9594.5
WLT	11	9895	M167	M495	8.65	0.25	-0.2356	9881.5	9903.5
WLT	11	9948	M495	M364	8.65	0.25	-0.2357	9939.5	9962.5
WLT	11	10266	M495	M364	7.78	0.25	-0.2354	10250.5	10275.5
WLT	11	10324	M364	M655	7.78	0.25	-0.2354	10314.5	10339.5
WLT	11	10642	M364	M655	8.50	0.25	-0.2349	10628.5	10651.5
WLT	11	10696	M655	M166	8.50	0.25	-0.2349	10687.5	10710.5
WLT	11	11018	M655	M166	8.81	0.25	-0.2349	11004.5	11026.5
WLT	11	11070	M166	M300	8.81	0.25	-0.2349	11061.5	11083.5
WLT	11	11392	M166	M300	8.50	0.25	-0.2349	11377.5	11400.5
WLT	11	11445	M300	M998	8.50	0.25	-0.2349	11436.5	11459.5
WLT	11	11766	M300	M998	8.19	0.25	-0.2349	11751.5	11774.5
WLT	11	11821	M998	M997	8.19	0.25	-0.2349	11812.5	11835.5
WLT	11	12138	M998	M997	6.72	0.25	-0.2339	12118.5	12148.5
WLT	11	12202	M997	M661	6.72	0.25	-0.2339	12192.5	12222.5
WLT	12	710	M668	M1010	5.20	0.20	0.2128	707.5	712.5
WLT	12	818	M1010	M1013	8.40	0.25	0.2353	789.5	828.5
WLT	12	877	M1013	M1016	8.29	0.25	0.2354	867.5	899.5
WLT	12	1754	M677	M875	8.02	0.25	0.2359	1738.5	1763.5
WLT	12	1811	M875	M303	8.06	0.25	0.2359	1801.5	1827.5
WLT	12	3237	M673	M1015	7.32	0.25	0.2357	3232.5	3246.5
WLT	12	3297	M1015	M1118	7.33	0.25	0.2365	3287.5	3311.5
WLT	12	4025	M310	M675	8.69	0.25	0.2362	4011.5	4033.5

WLT	12	4077	M675	M670	8.69	0.25	0.2362	4068.5	4091.5
WLT	12	4229	M675	M670	6.59	0.25	0.2351	4208.5	4240.5
WLT	12	5459	M678	M674	10.11	0.25	0.2377	5447.5	5466.5
WLT	12	5505	M674	M1012	10.11	0.25	0.2377	5497.5	5517.5
WLT	12	5734	M674	M1012	10.09	0.25	0.236	5722.5	5742.5
WLT	12	5781	M1012	M666	10.09	0.25	0.236	5773.5	5792.5
WLT	12	6012	M1012	M666	8.09	0.25	0.2357	5996.5	6020.5
WLT	12	6067	M666	M776	8.09	0.25	0.2357	6058.5	6082.5
WLT	12	11345	M308	M1017	7.96	0.24	0.2349	11342.5	11353.5
WLT	12	11398	M1017	M307	7.92	0.24	0.2345	11390.5	11399.5
WLT	12	12896	M773	M499	6.21	0.25	-0.2332	12874.5	12907.5
WLT	12	12964	M499	M877	6.21	0.25	-0.2332	12953.5	12979.5
WLT	12	13314	M499	M877	7.44	0.25	-0.2333	13297.5	13323.5
WLT	12	13373	M877	M33	7.44	0.25	-0.2333	13363.5	13390.5
WLT	12	13731	M877	M33	7.43	0.25	-0.2332	13714.5	13740.5
WLT	12	13790	M33	M176	7.43	0.25	-0.2332	13780.5	13807.5
WLT	12	14151	M33	M176	7.80	0.25	-0.2329	14135.5	14160.5
WLT	12	14208	M176	M500	7.80	0.25	-0.233	14198.5	14224.5
WLT	12	14569	M176	M500	7.26	0.25	-0.2329	14551.5	14579.5
WLT	12	14630	M500	M680	7.26	0.25	-0.2329	14619.5	14647.5
WLT	12	15424	M680	M779	4.12	0.22	-0.2145	15418.5	15428.5
WLT	12	15506	M779	M246	4.76	0.24	-0.2251	15500.5	15513.5
WLT	12	15862	M779	M246	5.35	0.25	-0.2318	15842.5	15874.5
WLT	12	15940	M246	M681	5.35	0.25	-0.2318	15927.5	15958.5
WLT	13	1223	M683	M1023	5.65	0.24	-0.2322	1214.5	1234.5
WLT	13	1362	M1023	M248	7.34	0.25	-0.236	1340.5	1372.5
WLT	13	1423	M248	M178	7.38	0.25	-0.236	1413.5	1442.5
WLT	13	1927	M688	M685	5.77	0.25	0.2338	1919.5	1939.5
WLT	13	2811	M313	M1021	5.30	0.23	0.2245	2806.5	2816.5

WLT	14	469	M505	M367	7.89	0.25	-0.2358	452.5	478.5
WLT	14	526	M367	M1120	7.83	0.25	-0.2357	516.5	537.5
WLT	14	1774	M182	M1031	7.01	0.24	-0.2312	1768.5	1778.5
WLT	14	1835	M1031	M370	7.09	0.25	-0.235	1829.5	1845.5
WLT	14	2048	M1031	M370	6.71	0.25	-0.2344	2037.5	2055.5
WLT	14	2112	M370	M184	6.71	0.25	-0.2345	2104.5	2123.5
WLT	14	2325	M370	M184	6.97	0.25	-0.2344	2306.5	2335.5
WLT	14	2387	M184	M38	6.97	0.25	-0.2344	2376.5	2401.5
WLT	14	3718	M369	M368	5.10	0.21	-0.2127	3714.5	3721.5
WLT	14	3784	M368	M1125	5.92	0.23	-0.2221	3780.5	3788.5
WLT	14	4000	M368	M1125	6.59	0.25	-0.2339	3989.5	4009.5
WLT	14	4065	M1125	M694	6.59	0.25	-0.2339	4055.5	4078.5
WLT	14	4567	M694	M693	6.73	0.25	-0.2341	4548.5	4577.5
WLT	14	4631	M693	M1033	6.73	0.25	-0.2341	4620.5	4650.5
WLT	14	4853	M693	M1033	6.88	0.25	-0.2347	4834.5	4862.5
WLT	14	4916	M1033	M1035	6.88	0.25	-0.2347	4905.5	4932.5
WLT	14	5136	M1033	M1035	5.15	0.21	-0.2145	5132.5	5139.5
WLT	14	5203	M1035	M1037	5.15	0.21	-0.2144	5199.5	5206.5
WLT	14	5423	M1035	M1037	6.71	0.25	-0.2344	5412.5	5430.5
WLT	14	5487	M1037	M1123	6.71	0.25	-0.2343	5479.5	5496.5
WLT	14	5709	M1037	M1123	3.98	0.18	-0.1991	5704.5	5711.5
WLT	14	5774	M1123	M317	4.01	0.19	-0.1993	5770.5	5777.5
WLT	14	5996	M1123	M317	6.71	0.25	-0.2343	5986.5	6003.5
WLT	14	6060	M317	M181	6.71	0.25	-0.2344	6051.5	6071.5
WLT	14	6284	M317	M181	7.21	0.25	-0.2345	6266.5	6294.5
WLT	14	6345	M181	M185	7.21	0.25	-0.2346	6335.5	6362.5
WLT	14	6570	M181	M185	6.71	0.25	-0.2345	6557.5	6577.5
WLT	14	6634	M185	M508	6.71	0.25	-0.2345	6626.5	6646.5
WLT	14	6860	M185	M508	7.11	0.25	-0.235	6842.5	6869.5

WLT	14	6921	M508	M1032	7.11	0.25	-0.235	6910.5	6938.5
WLT	14	7147	M508	M1032	6.95	0.25	-0.2346	7132.5	7156.5
WLT	14	7210	M1032	M1034	6.95	0.25	-0.2346	7200.5	7223.5
WLT	14	7436	M1032	M1034	6.71	0.25	-0.2344	7421.5	7444.5
WLT	14	7500	M1034	M507	6.71	0.25	-0.2344	7490.5	7513.5
WLT	14	7726	M1034	M507	6.64	0.25	-0.2347	7710.5	7735.5
WLT	14	7790	M507	M37	6.64	0.25	-0.2347	7780.5	7803.5
WLT	14	8311	M37	M180	6.87	0.25	-0.2349	8300.5	8319.5
WLT	14	8374	M180	M1028	6.87	0.25	-0.2349	8365.5	8384.5
WLT	14	10387	M1029	M183	6.50	0.25	-0.2341	10374.5	10397.5
WLT	14	10452	M183	M1027	6.50	0.25	-0.2341	10441.5	10467.5
WLT	14	10687	M183	M1027	6.72	0.25	-0.2343	10672.5	10697.5
WLT	14	10751	M1027	M179	6.72	0.25	-0.2343	10741.5	10767.5
WLT	14	10991	M1027	M179	7.49	0.25	-0.2344	10974.5	11000.5
WLT	14	11050	M179	M780	7.49	0.25	-0.2344	11040.5	11066.5
WLT	14	11294	M179	M780	7.49	0.25	-0.2344	11277.5	11303.5
WLT	14	11353	M780	M1036	7.49	0.25	-0.2344	11343.5	11369.5
WLT	14	11598	M780	M1036	7.50	0.25	-0.2345	11581.5	11607.5
WLT	14	11657	M1036	M883	7.50	0.25	-0.2345	11647.5	11673.5
WLT	14	11901	M1036	M883	6.71	0.25	-0.2334	11892.5	11906.5
WLT	14	11965	M883	M510	6.64	0.24	-0.2306	11960.5	11971.5
WLT	14	12208	M883	M510	6.59	0.25	-0.2337	12191.5	12218.5
WLT	14	12273	M510	M1039	6.59	0.25	-0.2337	12261.5	12289.5
WLT	14	12516	M510	M1039	6.66	0.25	-0.2336	12501.5	12526.5
WLT	14	12581	M1039	M695	6.66	0.25	-0.2336	12570.5	12595.5
WLT	14	12826	M1039	M695	6.79	0.25	-0.2341	12814.5	12833.5
WLT	14	12889	M695	M1038	6.79	0.25	-0.2338	12882.5	12898.5
WLT	14	13136	M695	M1038	6.43	0.24	-0.2313	13129.5	13140.5
WLT	14	13201	M1038	M509	6.43	0.24	-0.2312	13195.5	13207.5

WLT	14	13448	M1038	M509	6.71	0.25	-0.2344	13436.5	13456.5
WLT	14	13512	M509	M511	6.71	0.25	-0.2344	13502.5	13528.5
WLT	14	13766	M509	M511	8.34	0.25	-0.2348	13751.5	13775.5
WLT	14	13821	M511	M696	8.34	0.25	-0.2348	13811.5	13835.5
WLT	14	14075	M511	M696	6.00	0.23	-0.2248	14070.5	14078.5
WLT	14	15701	M40	M781	6.24	0.24	-0.2323	15693.5	15706.5
WLT	14	15768	M781	M692	6.24	0.25	-0.2331	15761.5	15776.5
WLT	14	16388	M692	M1026	5.97	0.25	-0.232	16370.5	16400.5
WLT	14	16459	M1026	M506	5.97	0.25	-0.232	16446.5	16478.5
WLT	14	16740	M1026	M506	6.40	0.25	-0.2333	16722.5	16750.5
WLT	14	16807	M506	M827	6.40	0.25	-0.2333	16796.5	16820.5
WLT	15	255	M41	M1041	7.42	0.25	-0.2332	244.5	267.5
WLT	15	1479	M782	M188	6.76	0.25	-0.2364	1464.5	1487.5
WLT	15	1542	M188	M187	6.76	0.25	-0.2364	1532.5	1557.5
WLT	16	333	M519	M1052	6.32	0.25	-0.2331	321.5	345.5
WLT	16	424	M1052	M706	7.31	0.25	-0.2343	403.5	439.5
WLT	16	815	M707	M1050	7.51	0.25	-0.2344	798.5	826.5
WLT	16	880	M1050	M202	7.42	0.25	-0.2344	868.5	915.5
WLT	16	1134	M201	M1051	6.32	0.25	-0.2322	1122.5	1142.5
WLT	16	1210	M1051	M371	6.35	0.25	-0.2332	1199.5	1245.5
WLT	16	1322	M371	M204	2.75	0.17	-0.1866	1316.5	1331.5
WLT	16	1470	M204	M203	6.89	0.25	-0.234	1443.5	1480.5
WLT	16	1538	M203	M198	7.06	0.25	-0.2341	1527.5	1567.5
WLT	16	3343	M1045	M196	6.49	0.24	-0.2289	3335.5	3347.5
WLT	16	3412	M196	M321	6.66	0.25	-0.2328	3406.5	3423.5
WLT	16	3474	M196	M321	6.45	0.25	-0.2325	3463.5	3480.5
WLT	16	3545	M321	M785	6.18	0.24	-0.2268	3540.5	3551.5
WLT	16	4432	M44	M886	5.99	0.23	-0.2251	4425.5	4436.5
WLT	16	4502	M886	M42	6.25	0.24	-0.2301	4497.5	4510.5

WLT	16	4571	M886	M42	4.47	0.20	-0.2081	4566.5	4575.5
WLT	16	6037	M831	M514	5.99	0.25	-0.233	6023.5	6046.5
WLT	16	6108	M514	M1043	5.99	0.25	-0.233	6098.5	6120.5
WLT	16	6362	M1043	M192	5.64	0.24	-0.2293	6354.5	6368.5
WLT	16	6436	M192	M830	5.72	0.25	-0.2324	6426.5	6449.5
WLT	16	6536	M192	M830	7.17	0.25	-0.233	6517.5	6546.5
WLT	16	6598	M830	M512	7.19	0.25	-0.233	6587.5	6616.5
WLT	16	6712	M830	M512	6.60	0.25	-0.2321	6689.5	6722.5
WLT	16	6777	M512	M702	6.58	0.25	-0.2321	6766.5	6797.5
WLT	17	399	M711	M709	9.01	0.25	-0.2373	387.5	406.5
WLT	17	442	M709	M708	7.94	0.22	-0.2337	437.5	443.5
WLT	17	586	M708	M323	5.47	0.24	-0.2261	581.5	593.5
WLT	17	3809	M715	M788	5.29	0.24	-0.2306	3784.5	3822.5
WLT	18	88	M832	M46	6.83	0.24	-0.2325	76.5	98.5
WLT	18	169	M46	M208	6.87	0.25	-0.2334	150.5	188.5
WLT	18	302	M208	M207	8.03	0.25	-0.2345	277.5	312.5
WLT	18	361	M207	M718	7.72	0.25	-0.2344	351.5	377.5
WLT	18	739	M719	M47	3.19	0.18	-0.1971	734.5	743.5
WLT	18	809	M47	M888	2.92	0.18	-0.1929	805.5	814.5
WLT	18	1716	M209	M720	7.58	0.25	0.2341	1699.5	1725.5
WLT	18	1774	M720	M889	7.58	0.25	0.2341	1764.5	1791.5
WLT	18	2655	M716	M1057	8.58	0.25	0.2364	2641.5	2663.5
WLT	18	2708	M1057	M522	8.58	0.25	0.2364	2699.5	2722.5
WLT	19	1440	M790	M1067	7.04	0.24	0.232	1435.5	1450.5
WLT	19	1494	M790	M1067	7.19	0.25	0.236	1475.5	1498.5
WLT	20	347	M727	M1073	5.46	0.23	0.2227	342.5	352.5

Appendix

A. DNA Extraction

1. Prepare a CTAB master mix by combining in trough 500 μL of CTAB* Buffer + 1μL of β-mercaptoethanol per sample
2. Preheat the extraction buffer (CTAB) in a water bath at 65°C for about 15 minutes.
3. Identify the samples and label the tubes.
4. Pour liquid nitrogen on the top of leaves in mortar and pestle, grind the tissue to a fine powder.
5. Transfer the ground material into 2 ml centrifuge tubes and incubate in the water bath at 65°C for 1 hour.
6. Add 1.5ul RNase A (10 ug/ul) and incubate at 37ºC for 15 minutes.
7. Centrifuge the tubes at 10,000 rpm for 10 minutes at 4°C and collect the supernatant in 1.5 ml tube
8. To the supernatant add equal volume of chloroform: isoamyl alcohol (24:1) and mix by inversion for 15 minutes.
9. Centrifuge the tubes at 10,000 rpm for 10 minutes at 4°C and collect the supernatant in a 1.5 ml centrifuge tube.
10. Again, add equal volume of chloroform: isoamyl alcohol (24:1) to the supernatant and mix by inversion for 15 minutes.
11. Centrifuge the tubes at 10,000 rpm for 10 minutes at 4°C and collect the supernatant.
12. To the supernatant add twice the volume of chilled isopropanol to precipitate the DNA and incubate it at −20°C for 30 minutes. (Overnight is recommended for difficult samples)
13. Centrifuge the tubes at 10,000 rpm for 10 minutes at 4°C and collect the pellet.
14. Wash the pellet 2–3 times with first 100% ethanol and then with 70% and air dry the pellet in room temperature for 2 hours
15. Add 50–75 μl of distilled water to dissolve the DNA
16. Store DNA at −20°C

B. GBS Library Construction

1. DNA extraction using modified CTAB method
2. DNA extraction followed by DNA cleanup with “Monarch PCR and DNA Cleanup Kit
3. Use only high-quality DNA with 260/230 and 260/280 ratios higher than 1.8
4. Digestion with APEKI restriction enzymes
5. Ligation

a) Aliquot 10 µl of 10 ng/µl of DNA from each sample into 0.2 ml PCR tubes
b) Transfer 10 µl of master mix into each tube with DNA sample
c) Incubate for 2 hours at 75°C (Lid on at 80°C)
d) Hold at 4°C

6. Pooling and cleanup

a) Aliquot 6 µl of each **working adapter stock** into the digested samples
b) Incubate for 2 hours at 22°C ~ room temperature
c) Incubate for 30 minutes at 65°C
d) Hold at 4°C

7. PCR amplification

C. Bash script

```
#Parents


bcftools filter -e 'QUAL<20 || INFO/DP<10 ||  F_MISSING > 0.00  ' pop1_parents.c3.af2.vcf -o
pop1_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.vcf
bcftools filter -e 'QUAL<20 || INFO/DP<10 ||  F_MISSING > 0.00  ' pop2_parents.c3.af2.vcf -o
pop2_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.vcf
bcftools filter -e 'QUAL<20 || INFO/DP<10 ||  F_MISSING > 0.00  ' pop3_parents.c3.af2.vcf -o
pop3_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.vcf

bcftools view -m2 -M2 -v snps pop1_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.vcf \
--output-file pop1_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.vcf

bcftools view -m2 -M2 -v snps pop2_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.vcf \
--output-file pop2_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.vcf

bcftools view -m2 -M2 -v snps pop3_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.vcf \
--output-file pop3_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.vcf

### Filtering homozygous sites
vcffilter -g 'GT = 0/0/0/0 | GT = 1/1/1/1' pop1_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.vcf >
pop1_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf
vcffilter -g 'GT = 0/0/0/0 | GT = 1/1/1/1' pop2_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.vcf >
pop2_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf
vcffilter -g 'GT = 0/0/0/0 | GT = 1/1/1/1' pop3_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.vcf >
pop3_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf


#populations

bcftools filter -e 'QUAL<20 || INFO/DP<10 ||  F_MISSING > 0.3 ' \
pop2.c3.af2.noduds.output.vcf \
-o pop2.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.vcf
```

```
bcftools view -m2 -M2 -v snps pop2.filtered.DP10.QUAL20.strandbias.miss7.vcf \
--output-file pop2.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf

bcftools filter -e 'QUAL<20 || INFO/DP<10 ||  F_MISSING > 0.3' || GT \
pop2.c3.af2.noduds.output.vcf \
-o pop2.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.vcf

bcftools view -m2 -M2 -v snps pop1.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.vcf \
--output-file pop1.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf

bcftools filter -e 'QUAL<20 || F_MISSING > 0.3 || F_PASS(DP=10 & GT!=".") >0.7' \
pop3.c3.af2.output.vcf \
-o pop3.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.vcf

bcftools view -m2 -M2 -v snps pop3.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.vcf \
--output-file pop3.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf


bgzip pop1_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf
bgzip pop2_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf
bgzip pop3_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf
bgzip pop1.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf
bgzip pop2.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf
bgzip pop3.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf



#Indexing with tabix
tabix pop1_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf.gz
tabix pop2_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf.gz
tabix pop3_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf.gz
tabix pop1.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf.gz
tabix pop2.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf.gz
tabix pop3.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf.gz
```

```
### BCFtools generating intersected vcf file
#population 1
bcftools isec /projects/dhaak_lab/peanut_gbs/06_parents/03_variants/pop1_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf.gz pop1.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf.gz -p markers
bcftools merge /projects/dhaak_lab/peanut_gbs/06_parents/03_variants/pop1_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf.gz pop1.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf.gz -o pop1_RIL_parents_merged.vcf

bgzip pop1_RIL_parents_merged.vcf
bgzip markers/0002.vcf
bcftools index markers/0002.vcf.gz
bcftools index pop1_RIL_parents_merged.vcf.gz
bcftools filter --targets-file markers/0002.vcf.gz pop1_RIL_parents_merged.vcf.gz -o pop1_RIL_parents_merged_intersected.vcf --threads 20

#population 2
bcftools isec /projects/dhaak_lab/peanut_gbs/06_parents/03_variants/pop2_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf.gz pop2.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf.gz -p markers
bcftools merge /projects/dhaak_lab/peanut_gbs/06_parents/03_variants/pop2_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf.gz pop2.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf.gz -o pop2_RIL_parents_merged.vcf

bgzip pop2_RIL_parents_merged.vcf
bgzip markers/0002.vcf
bcftools index markers/0002.vcf.gz
bcftools index pop2_RIL_parents_merged.vcf.gz
bcftools filter --targets-file markers/0002.vcf.gz pop2_RIL_parents_merged.vcf.gz -o pop2_RIL_parents_merged_intersected.vcf --threads 20

#population 3
bcftools isec /projects/dhaak_lab/peanut_gbs/06_parents/03_variants/pop3_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf.gz pop3.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf.gz -p markers
```

```
bcftools merge /projects/dhaak_lab/peanut_gbs/06_parents/03_variants/pop3_parents.c3.af2.filtered.DP10.QUAL20.strandbias.nomiss.SNPs.hom.vcf.gz pop3.c3.af2.filtered.DP10.QUAL20.strandbias.miss7.SNPs.vcf.gz -o pop3_RIL_parents_merged.vcf

bgzip pop3_RIL_parents_merged.vcf
bgzip markers/0002.vcf
bcftools index markers/0002.vcf.gz
bcftools index pop3_RIL_parents_merged.vcf.gz
bcftools filter --targets-file markers/0002.vcf.gz pop3_RIL_parents_merged.vcf.gz -o pop3_RIL_parents_merged_intersected.vcf --threads 20
```