Identification of QTLs for rust resistance in an interspecific population derived from *A. hypogaea* x (*A. magna* x *A. stenosperma*)^{4x}

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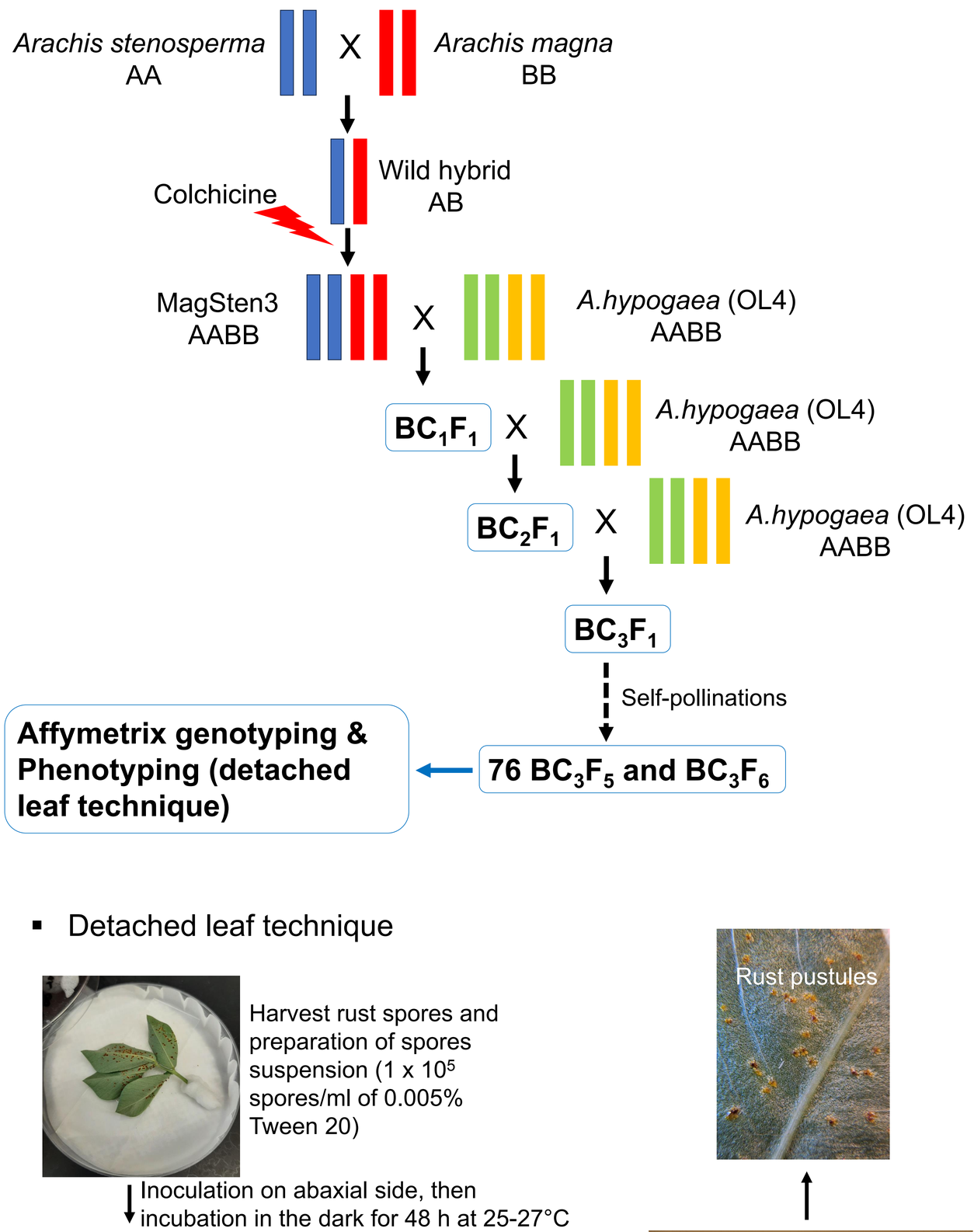
Introduction

- Leaf rust, caused by *Puccinia arachidis* Speg., is one of the most important foliar diseases in peanut.
- Previous studies have shown that the wild species *Arachis magna* is an important source of rust-resistant alleles.
- The objective of this study is to identify chromosome regions involved in rust resistance by evaluating an interspecific population derived form a cross between *Arachis hypogaea* and (*Arachis magna* x *Arachis stenosperma*)^{4x}.

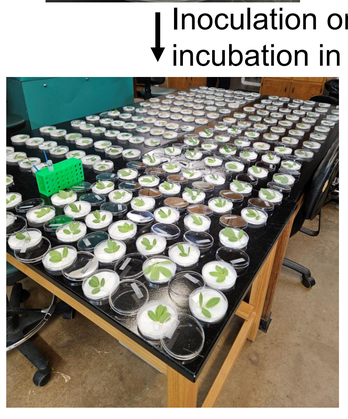
Material and Methods



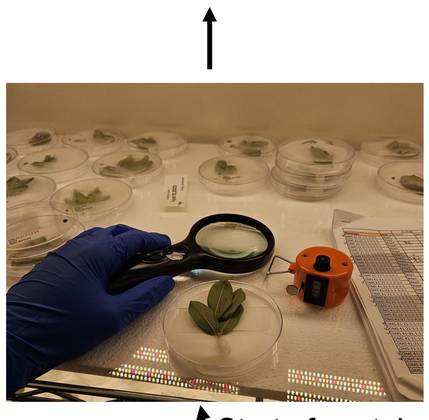
- Detached leaf technique

Harvest rust spores and preparation of spores suspension (1 x 10^5 spores/ml of 0.005% Tween 20)

Inoculation on abaxial side, then incubation in the dark for 48 h at 25-27°C



Incubation of inoculated leaves with a photoperiod of 12/12 at 25°C



Start of pustules counting after ~10 days of incubation

Results and Discussion

Identification of QTLs and candidate genes for rust resistance

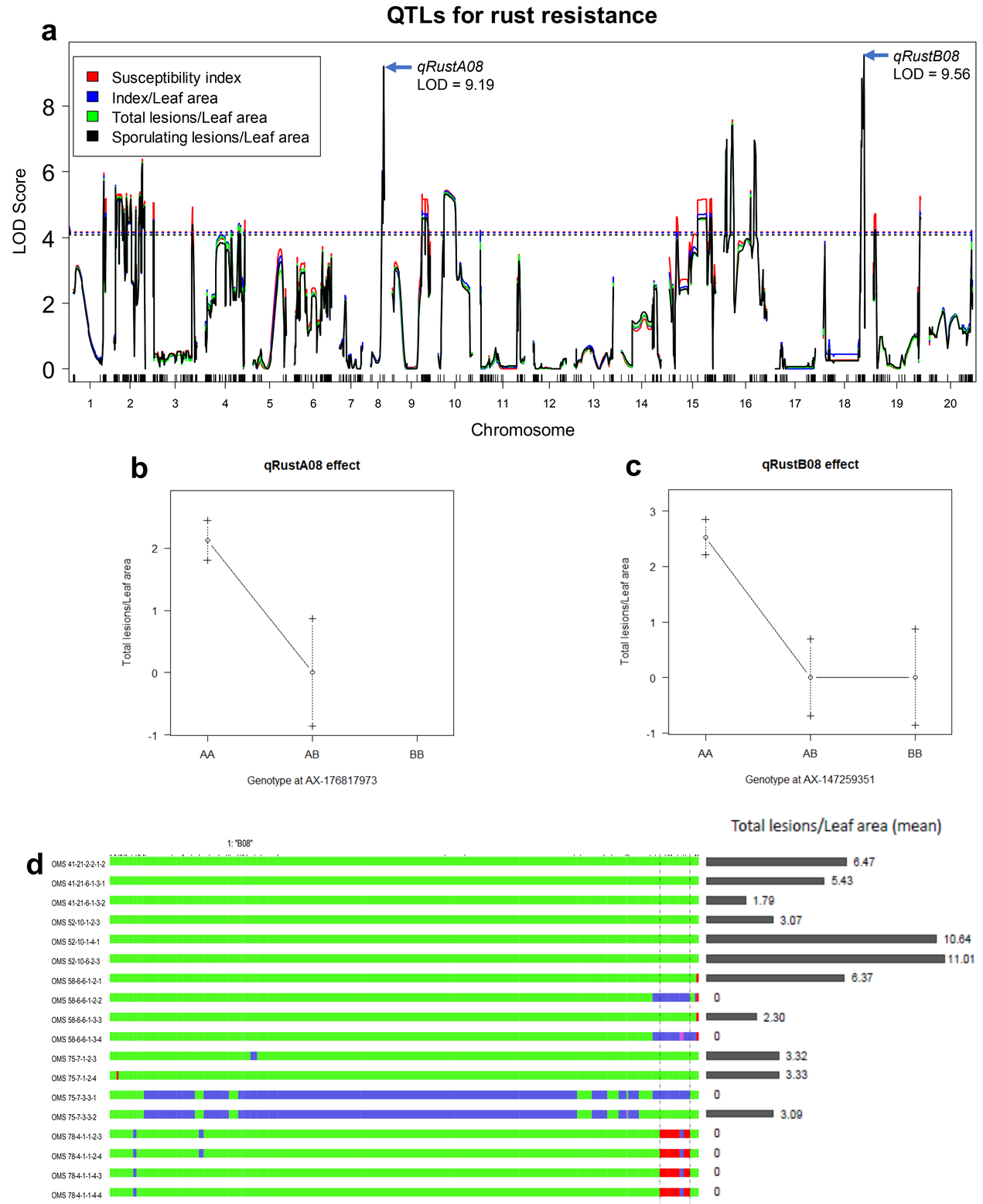


Figure 1: a. Identification of QTLs associated with rust resistance. **b.** Effect of *qRustA08*. **c.** Effect of *qRustB08*. **d.** Genotyping/phenotyping association of selected lines on chromosome B08. ***A. hypogaea*** **alleles**; **wild alleles**; **heterozygous** and **missing data**.

- **13 QTLs** for rust resistance were identified using 76 BC_3 lines (Figure1a).
- QTLs on chr8 (A08) and chr18 (B08) have the highest LOD score (Figure 1a).
- The identified QTLs could explain 3.6 – 14.67% of PVE. ***qRustB08*** is a main effect QTL (PVE = 14.67%) and at this QTL, heterozygous and wild alleles confer rust resistance (Figure 1c,d).
- ***qRustB08*** is located at the end of chromosome B08 (based on the *A. ipaensis* reference genome), between markers AX-147259163 and AX-176792136, which delimitated an interval of 4.4 Mb (121.56 – 125.96 Mb) (Figure 1d).
- This interval contains 265 predicted genes. Among these genes, 10 code for terpene synthases, which have been shown to be involved in resistance to fungi.
- ***qRustB08*** coincides with previously reported QTLs for rust resistance derived from *A. magna* in diploid and tetraploid populations (Leal-Bertioli *et al.* 2015; Moretzsohn *et al.* 2023).

Ongoing works

- Development of KASP markers associated with the QTL.
- Genotyping of the next generation of 34 selected lines to validate the QTL.
- Fine-mapping of the QTL associated with rust resistance and identification of the causative gene(s).

Acknowledgements

ADVANCES IN ARACHIS THROUGH GENOMICS & BIOTECHNOLOGY

Transcriptome analysis identified important genes and pathways for heat tolerance in peanut (*Arachis hypogaea* L.)

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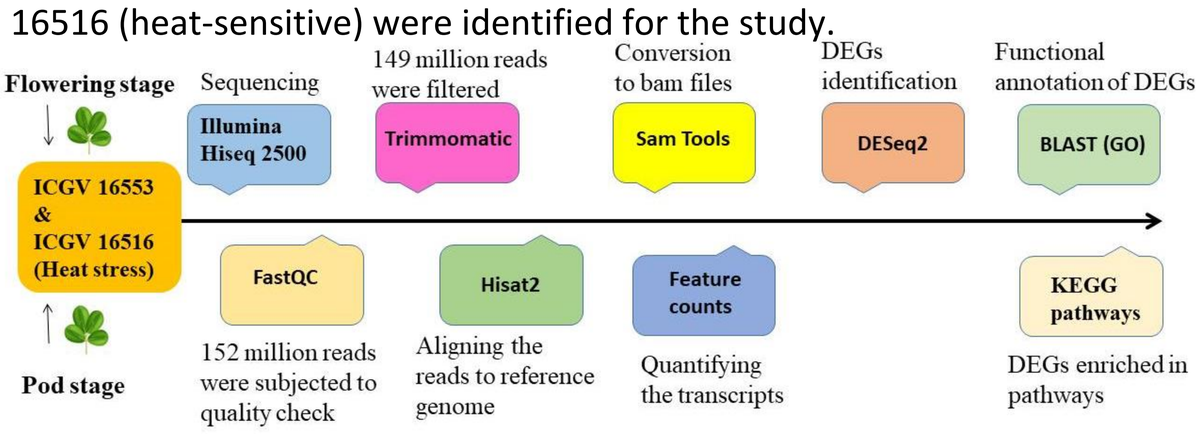
Background

Tolerance to heat stress is important for future peanut production. A prediction study estimated -2.3% to 43.2% change in pod yield across the peanut growing regions in India as a consequence of high-temperature stress (Kadiyala et al. 2021). This study was conducted to elucidate the regulatory mechanisms and identify heat-stress responsive genes through comparative transcriptomic analysis in peanut.

Methodology

Plant material: Based on multi-season and multi-location agronomic data analysis, two genotypes, ICGV 16553 (heat-tolerant) and ICGV 16516 (heat-sensitive) were identified for the study.

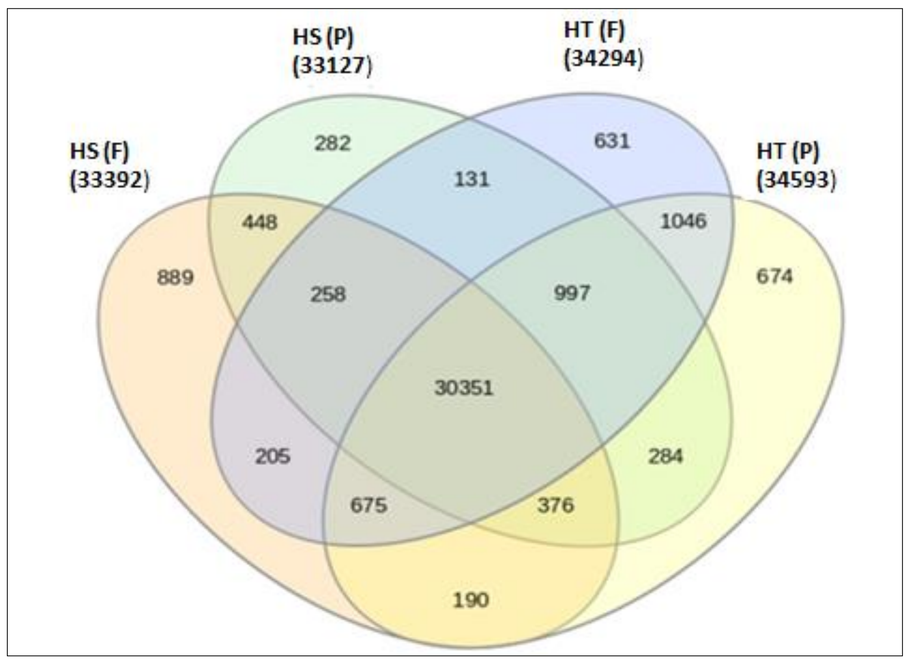


RNA Seq work flow from sampling to enriched DEGs in pathways

Total antioxidant activity (TAA), total phenol content (TPC) and sugars were estimated at flowering and pod stage.

Results

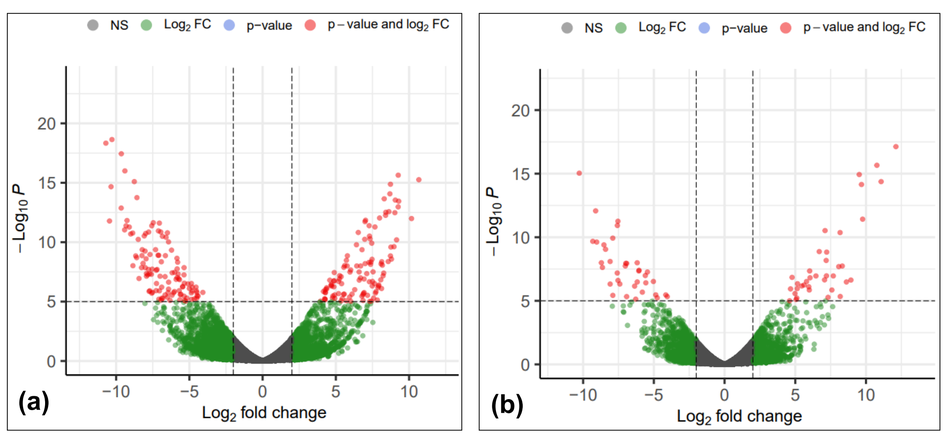
A total of 2,467 differentially expressed genes (DEGs) were identified between heat-tolerant and sensitive genotypes.



Venn diagram of different genes expressed at flowering and pod stage under heat stress

Note: HS (F)- Heat-susceptible line at flowering stage; HS (P)- Heat-susceptible line at pod stage; HT (F)- Heat-tolerant line at flowering stage; HT (P)- Heat-tolerant line at pod stage

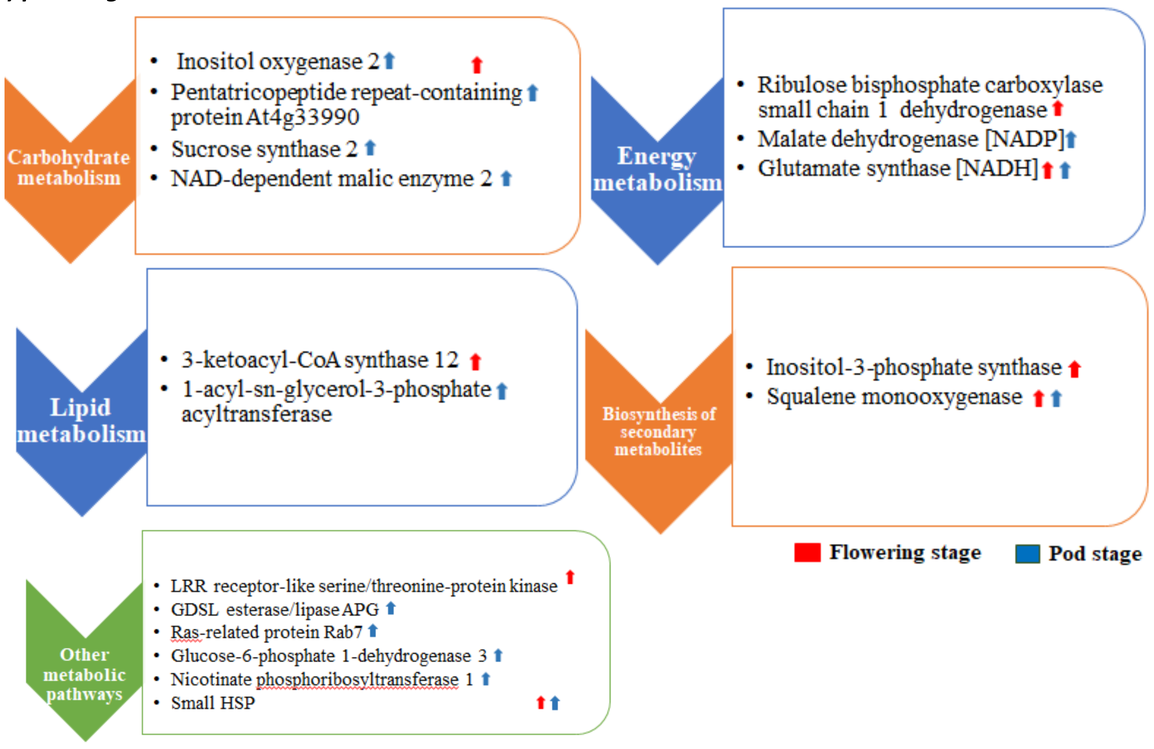
Flowering stage : 1631 DEGs (975 up-regulated, 656 down-regulated)
Pod stage: 836 DEGs (477 up-regulated, 359 down-regulated)



Comparison of differentially expressed genes (DEGs) in leaves between HT and HS genotypes at (a) flowering stage and (b) pod stage

- 106 DEGs were enriched in 39 pathways.
- Several metabolic pathways, such as glycerophospholipid metabolism, inositol phosphate metabolism, starch and sucrose metabolism, thiamine and purine metabolism, were enhanced during heat stress in ICGV 16553.

Pie chart denoting the distribution of pathways to the identified DEGs at (a) flowering stage and (b) pod stage.



DEGs identified under heat stress in peanut

The expression patterns of sixteen selected genes involved in heat tolerance were further validated using qRT-PCR.

Conclusion

(A) DEGs identified in heat-tolerant line (ICGV 16553) encode:

1. Heat shock proteins,
2. Sucrose synthase,
3. Transcriptional genes, and
4. Antioxidants

(B) Total antioxidant activity (TAA)and total phenol content (TPC):

- At flowering stage, increased TAA (by 74%), TPC (by 34%) was observed in heat-tolerant line as a response to heat stress .
- On contrary, at pod development stage, the TAA and TPC was reduced by ~45% in both heat-sensitive and tolerant lines under heat stress.

(C) Sucrose metabolism:

- During pod development stage, efficient sucrose loading into phloem and transport to sink tissues under heat stress was observed in heat-tolerant line.
- Pod sucrose content was highly reduced in heat sensitive line (by 90%), while the reduction was by 56% in ICGV 16653 indicating active sucrose metabolism in heat tolerant line.

References:

Kadiyala et al. 2021 https://doi.org/10.1016/j.scitotenv.2021.145996

A multifaceted screening study to assess stem rot disease resistance in peanut (*Arachis hypogaea* L.)

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Background

Owing to the changing cropping systems and climatic conditions, the incidence of stem rot of groundnut has been rising since the past decade. *Sclerotium rolfsii,* the causal agent, causes up-to 80% pod loss under severe conditions. Host-plant resistance strategies serve as the most feasible option to manage this destructive disease. The current study was taken up to assess the genetic variability among the groundnut genotypes and to identify stem-rot resistant genotypes.

Material and methods

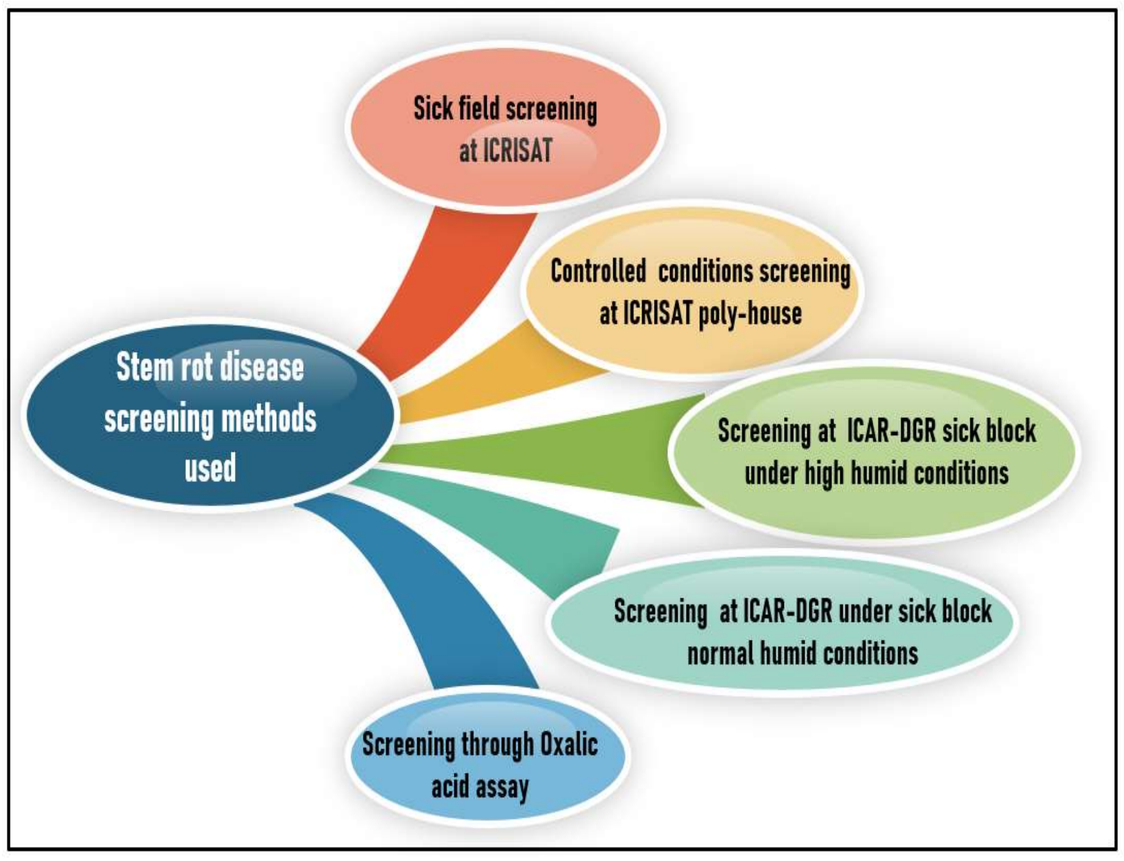
Plant material

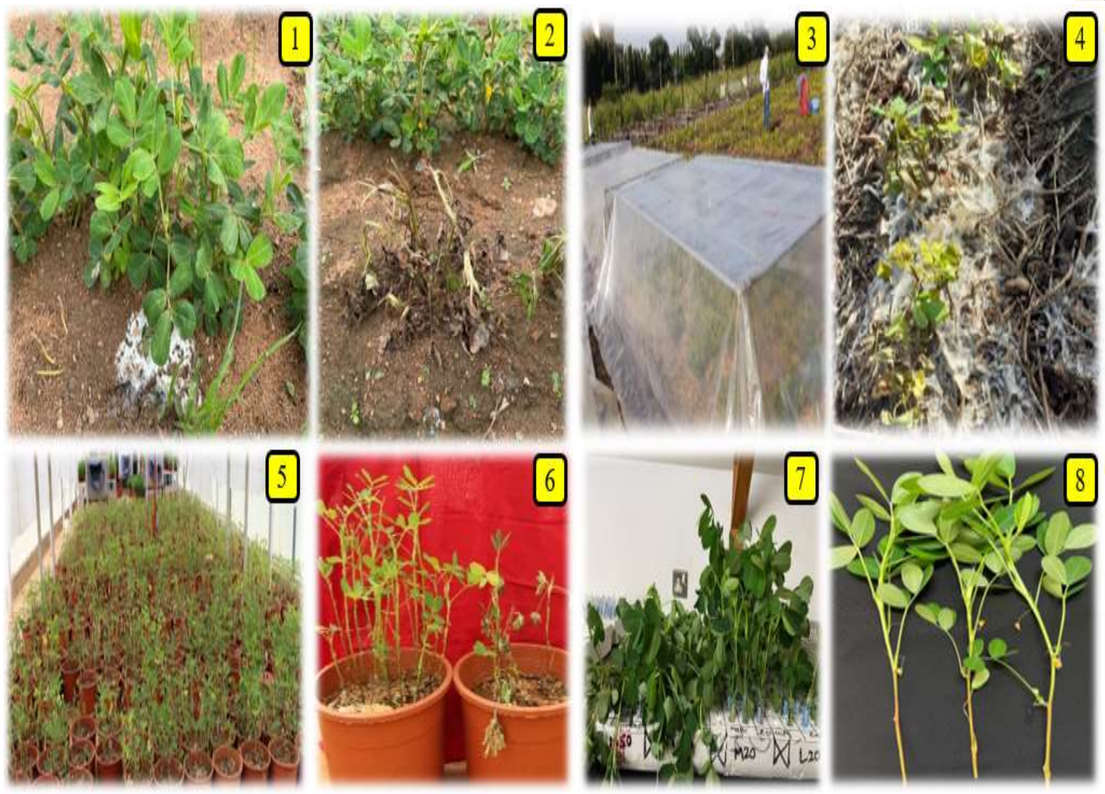
160 groundnut genotypes of diverse origin (interspecific derivatives of wild *Arachis* species, advanced breeding lines, pre breeding and ICRISAT mini-core lines)

Pathogen

The stem rot pathogen, *S. rolfsii,* used in the study was obtained from the groundnut pathology laboratory culture collection at ICRISAT. The isolate was cultured on potato dextrose agar (PDA) medium at a temperature of 25±2°C and mass multiplied on sorghum grains.

Methods





Stem rot disease screening methods: 1 and 2 indicating the sick field screening at ICRISAT; 3 and 4 showing the screening block experiments at DGR; 5,6 depicting the poly-house experiment at ICRISAT; 7, 8 screening through oxalic acid assay.

Results

- ICRISAT sick field screening showed a good differentiating ability with percent mortality (PM) varying from 13% to 80%.
- At ICAR-DGR, under normal humidity conditions, PM varied from 8-58% indicating moderate disease pressure.
- Under pot method at ICRISAT and high humidity conditions at ICAR-DGR, variability for PM was 48-67% and 51-80%, respectively, indicating that all the lines succumbed to disease under high disease conditions.
- Based on sick field screening at ICRISAT, 10 lines were resistant (PM 13-19%) and 40 lines were moderately resistant (PM 20-29%). Of these 50 lines, 43 were bred using inter-specific derivatives of wild *Arachis* species.
- The identified ten resistant genotypes from ICRISAT sick field screening are, ICGR 161954, ICGR 161940, ICGV 181045, ICGV 11447, ICGR 162036, ICGR 162035, ICGR 161932, NRCGCS-224, VG 1007 and ICGR 161951.
- Oxalic acid (a principal pathogenic metabolite) assay on 29 selected resistant and susceptible genotypes revealed that the lines ICGR 161939, ICGR 162044 and ICGR 162032 recorded low wilting disease score (1-2) and less lesion length (1-3cm). These lines have shown moderate resistance under ICRISAT sick field conditions with their PM ranging from 23-28%.

Conclusions & Future perspectives

- Severe disease pressure is not ideal to screen host-resistance for stem rot.
- The PM is a good indicator for host-resistance and the method with good discriminating ability (screening at ICRISAT sick field) is used to identify host-resistance.
- Most of the resistance comes from the interspecific derivatives of wild *Arachis* species with AA genome.
- Resistance to stem rot genes/alleles from these derivatives of wild accessions can be easily transferred into cultivated species using traditional breeding procedures, or resistant QTLs could be mapped and transferred into cultivated groundnut.

Molecular characterization of thermotolerance in groundnut (*Arachis hypogaea* L.) through expression analysis

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Introduction

- Groundnut production in arid and semi-arid regions faces numerous challenges, despite its adaptability to various environments.
- Understanding the molecular basis of heat stress tolerance is crucial, as the differential gene expression at various growth stages leads to a greater adaptation to high temperatures (Sita *et al*., 2017).
- This study was conducted to gain insights into the molecular changes and to provide a better understanding of the regulatory roles of stress responsive genes involved in heat tolerance.

Methodology

(i) Experimental material

- Sampling of leaves from heat tolerant (HT) and susceptible (HS) genotypes, identified from two different methods of screening.

Temperature Induction Response (TIR) experiment	• Induced & Non-induced treatments at seedling stage
Field screening experiment	• At flowering & pod development stages

- Further validation of selected differentially expressed genes (DEGs) was done in identified HT and HS RIL lines of JL 24 × 55-437 RIL population.

(ii) qRT-PCR analysis

- From a comparative transcriptomic study on heat tolerance in groundnut (Rachana *et al.* unpublished data), sixteen heat stress responsive genes were selected based on their role in abiotic stress tolerance, to study their role at seedling, flowering, and pod development stages (Table 1).
- Through qRT-PCR, threshold cycle (Ct) values were determined, and fold changes (FC) were calculated using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008).
- Further validation of selected differentially expressed genes (DEGs) was done in identified HT RIL lines of JL 24 × 55-437 RIL population.

S. No.	Gene description	Gene ID
1	Ribulose bisphosphate carboxylase small chain 1, chloroplastic	I56F9F
2	Inositol-3-phosphate synthase	HNK90J
3	Probable LRR receptor-like serine/threonine-protein kinase At4g26540	K82E85
4	GDSL esterase/lipase APG	S7XZ9Q
5	Inositol oxygenase 2-like	ELH53L
6	Malate dehydrogenase [NADP], chloroplastic	1WBZ5F
7	NAD-dependent malic enzyme 2, mitochondrial isoform X2	I325BF
8	Pentatricopeptide repeat-containing protein At4g33990-like	T3W1R1
9	Sucrose synthase 2	G9F7ZA
10	1-acyl-sn-glycerol-3-phosphate acyltransferase-like	MV6CSW
11	ras-related protein Rab7	8JE5KI
12	AAA-type ATPase	MKNY28
13	Glucose-6-phosphate 1-dehydrogenase 3, chloroplastic-like isoform X2	SV13L6
14	Nicotinate phosphoribosyl transferase 1 isoform X1	WH2MYH
15	Glutamate synthase [NADH], amyloplastic-like isoform X1	HI2JIU
16	ATP-dependent zinc metalloprotease FTSH 12, chloroplastic	HH3FWD

Table 1: List of sixteen heat stress responsive genes selected for expression analysis

Conclusion & Future prospectives

- The differentially expressed genes (DEGs) play an essential role in the growth and development of plants, and thus, they may contribute to heat tolerance.
- Molecular markers can be developed for the identified DEGs and can be used in the breeding program for heat tolerance.
- The molecular information could serve as a foundation for further research aimed at developing heat tolerant varieties, ultimately contributing to food security in the face of climate change.

References

- Schmittgen, T.D. and Livak, K.J., 2008. https://doi.org/10.1038/nprot.2008.73
- Sita *et al.,* 2017. https://doi.org/10.3389/fpls.2017.01658

Results

- Expression analysis at the seedling stage revealed the upregulation of 14 genes in the HT genotype compared to the HS genotype in both induced and non-induced treatments (Fig. 1).
- The relative expression of genes was notably higher in induced plants, followed by non-induced plants of HT genotype.
- In contrast, the relative expression was comparatively lower in the HS genotype of both induced and non-induced treatments.
- The expression analysis at the seedling stage not only identified key genes associated with heat stress tolerance but also provided information on their response to different heat treatments in TIR experiment.

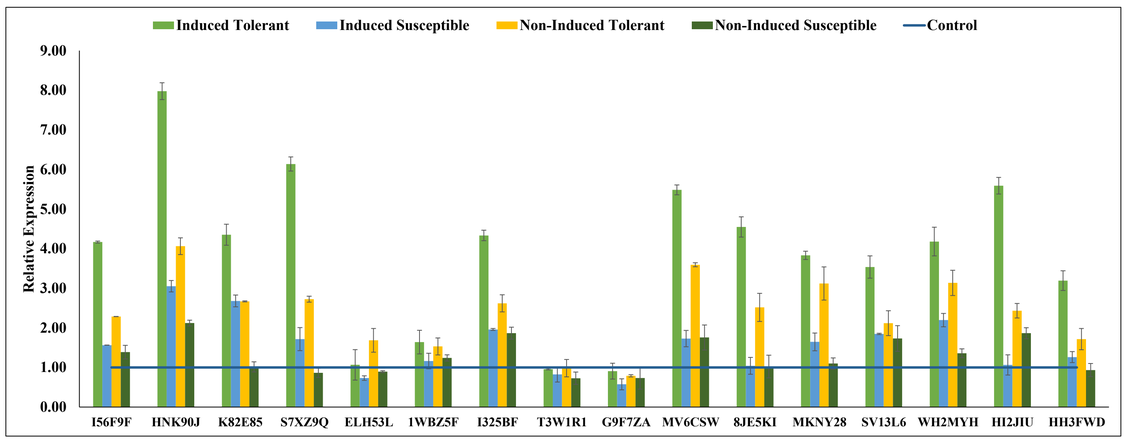

Fig. 1. Expression patterns of heat stress responsive genes in Induced and Non-Induced HT genotypes, compared to HS genotypes at seedling stage in TIR experiment

- Six genes exhibited upregulation during the flowering stage and nine genes during the pod-development stage in the HT genotype compared to HS genotype under field conditions (Fig. 2).
- The sucrose synthase 2 gene (G9F7ZA) exhibited increased expression in the HT genotype during the pod-development stage (7.46-fold) compared to flowering stage (1.99-fold).
- Under heat stress, these results indicate differential response of genotypes at different growth stages *i.e,* flowering and pod-development.

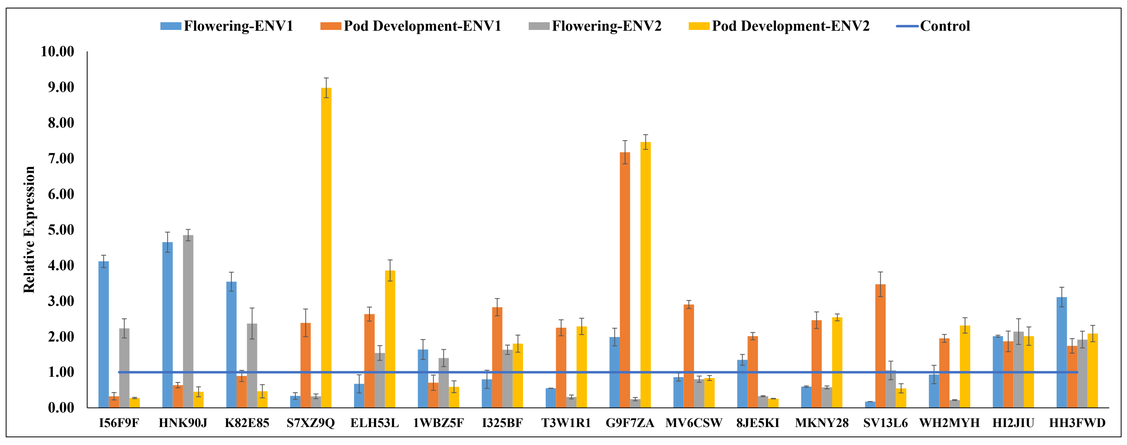

Fig. 2. Expression patterns of heat stress responsive genes at flowering and pod-development stages in HT genotype, compared with HS genotype under field conditions (ENV1 & 2)

- Five DEGs were selected for validation in the identified HT RIL lines – HI2JIU; HH3FWD; S7XZ9Q; I325BF and 1WBZ5F.
- Out of the five selected DEG's for validation, malate dehydrogenase [NADP], chloroplastic gene (1WBZ5F) showed differential regulation in the identified HT RIL lines, compared to the HS RIL lines (Fig. 3).
- Malate dehydrogenase [NADP], chloroplastic gene (1WBZ5F) has been involved in photosynthesis and removal of oxidative stress (ROS), contributing for heat tolerance.

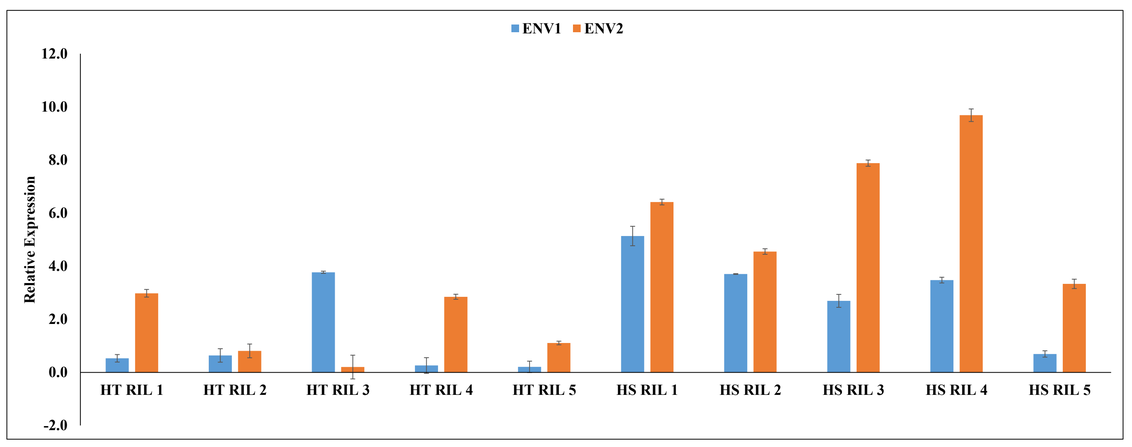

Fig. 3. Expression pattern of malate dehydrogenase [NADP], chloroplastic gene (1WBZ5F) in HT RIL lines, compared with HS RIL lines in JL 24 × 55-437.

ADVANCES IN ARACHIS THROUGH GENOMICS & BIOTECHNOLOGY

DISTRIBUTION OF ALLELES AND GENETIC MARKER DEVELOPMENT ASSOCIATED WITH GENETIC RESISTANCE FOR SCLEROTINIA BLIGHT AND PEANUT SMUT IN A DIVERSE COLLECTION OF PEANUT LANDRACES

AUTHORS

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INTRODUCTION

Sclerotinia blight caused by *Sclerotinia minor* is a prominent soilborne disease with a global impact on peanut cultivation. Concurrently, peanut smut caused by *Thecaphora frezzii* has emerged as a significant challenge to the production in Argentina and threatens the worldwide production. Both diseases led to yield losses exceeding 30% of grain production in Argentina. The development of resistant cultivars is considered the most efficient, sustainable, and environmentally friendly approach to control these diseases. This study evaluated the resistance to both diseases in 134 accessions of Latin American landraces belonging to different botanical varieties and its association with SNPs genomic markers

OBJECTIVE

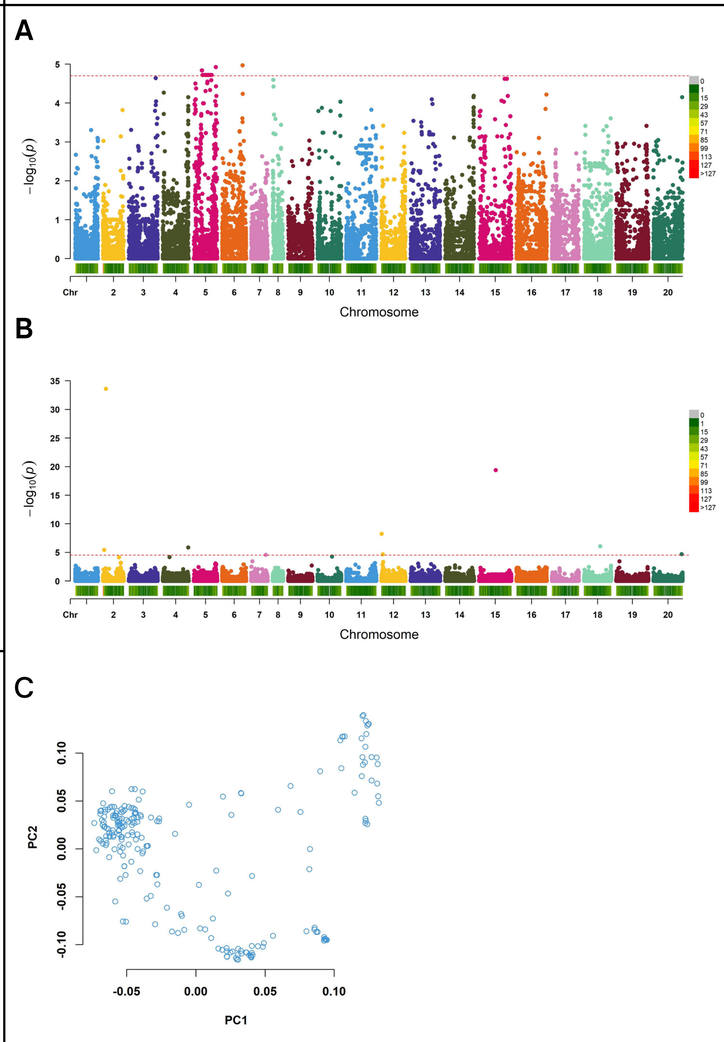
To achieve the knowledge and characterise multiple resistance sources for peanut smut and sclerotinia blight in Latin American landraces

METHODOLOGY

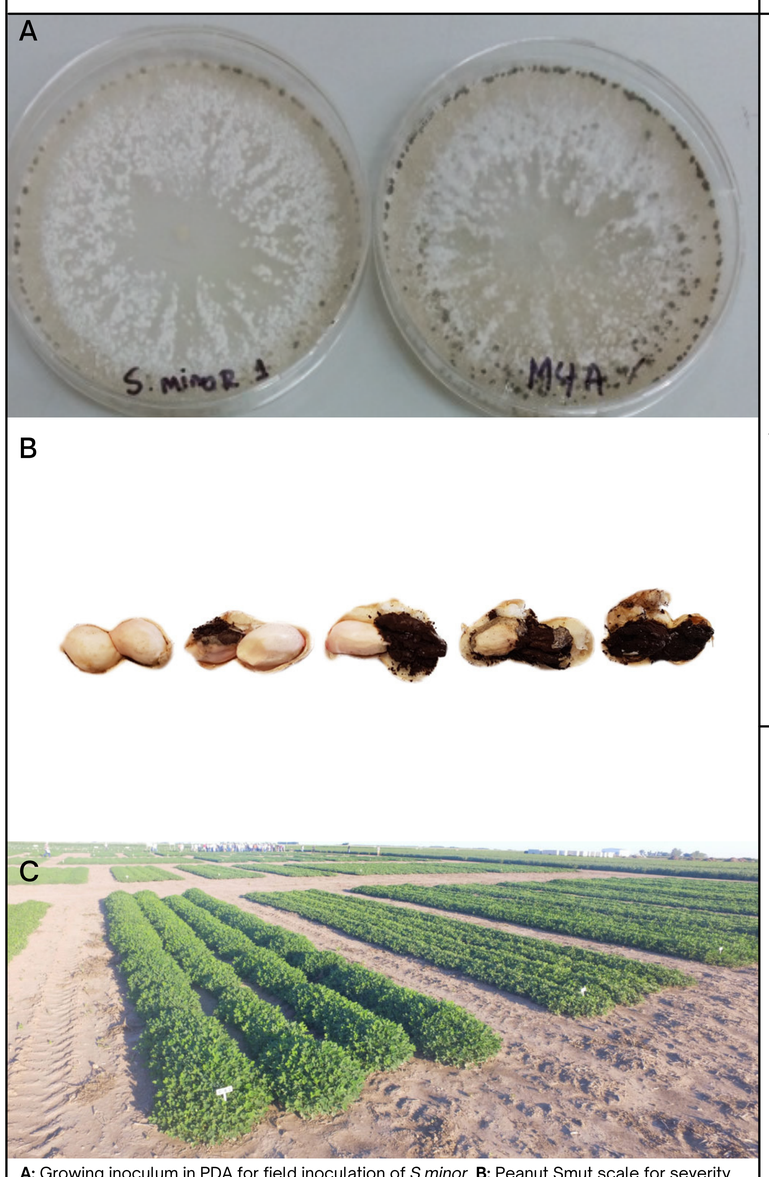
The phenotypic evaluations took place in field trials under intense inoculum pressure at Criadero El Carmen in General Cabrera, Córdoba, Argentina (Latitude 32° 48' 48.63"S; Longitude 63° 52' 12.84"W) during 2019, 2020, 2022, and 2023 for sclerotinia blight and in 2019 and 2020 for peanut smut.
The DNA accessions samples were genotyped with the Axiom Arachis2 SNP 48K array platform.
A GWAS was performed using rMVP R package
Finally a Pearson correlation analysis was performed to test the correlation between botanical varieties and frequency of accessions with lower incidence and severity to both diseases

FINDINGS

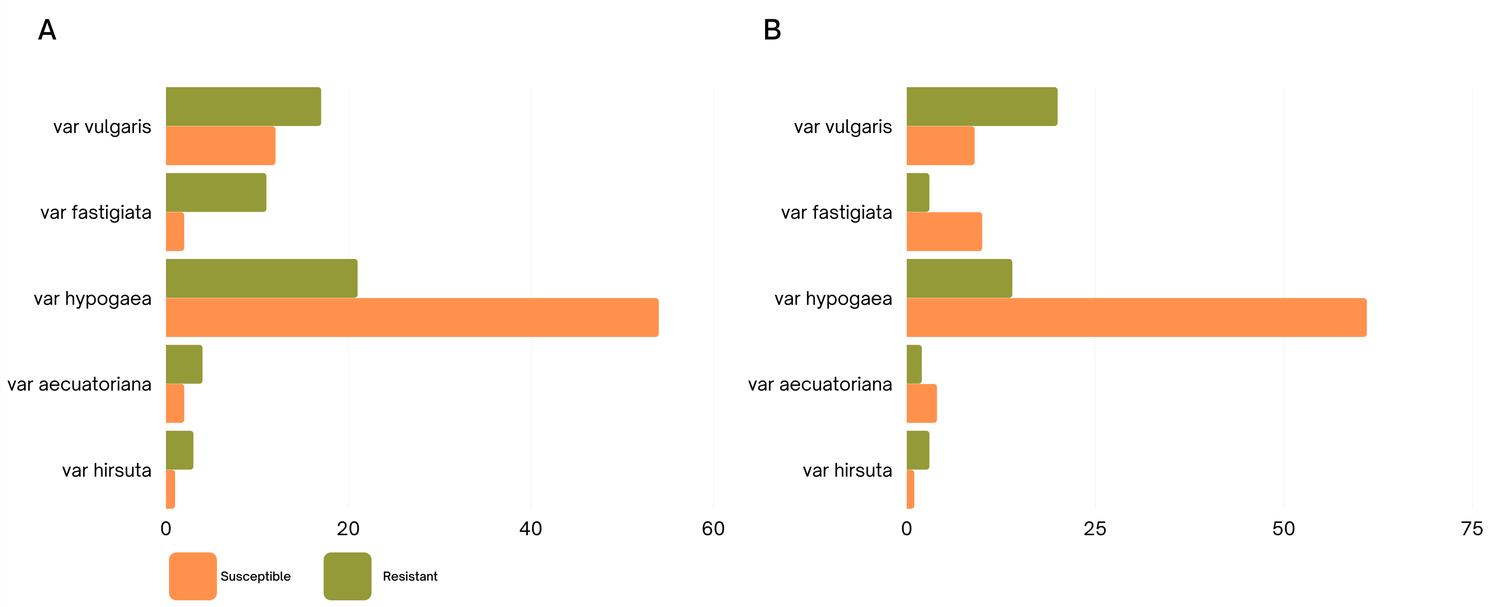
GWAS analysis identified 2 major QTLs associated with peanut smut resistance and 2 with sclerotinia blight resistance. The analysis of the allelic variants among the botanical varieties of peanut showed that those of the spp. *fastigiata* (mainly those of the *vulgaris* variety) presented the highest frequency of accessions with lower incidence and severity to both diseases. Moreover, 16% of the accessions have co-occurrence of resistance for the two diseases.



A: Manhattan plot depicting GWAS analysis for Sclerotinia blight. **B:** Manhattan plot depicting GWAS analysis for Peanut smut. **C:** PCA analysis for population structure modeling .



A: Growing inoculum in PDA for field inoculation of *S.minor*. **B:** Peanut Smut scale for severity evaluation. **C:** Field trial for incidence Sclerotinia blight and Peanut Smut severity evaluation.



A: Resistance occurence for Sclerotinia blight. **B:** Resistance occurence for Peanut smut.

CONCLUSION

This genetic knowledge founded the development of functional genetic markers used to develop elite varieties that addresses to the current challenges facing the peanut industry in Argentina.

Investigating Physiological Traits Responsible of Drought in F2 Biparental crossing

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Background

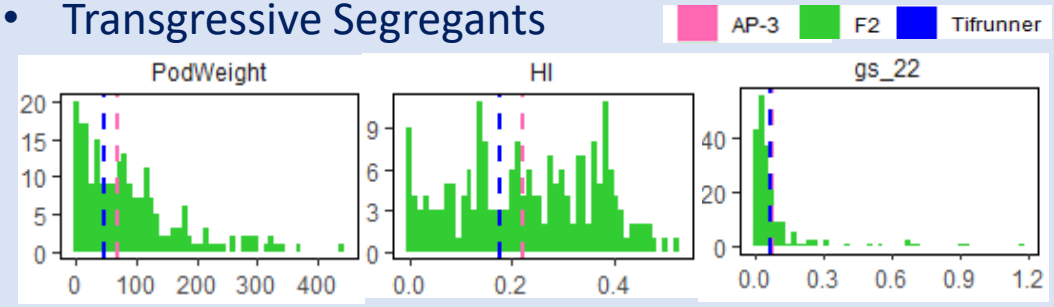
- Development of drought tolerant cultivars could be the solution for food security in more frequent extreme climate.
- Drought tolerance is a complex and polygenic trait resulting from the contribution of many factors.
- Direct selection for yield is difficult due to low heritability and G×E in drought conditions.
- However, measurable phenotype, like physiological characters, is essential for a more accurate breeding program accounting into environmental effects.
- To further locate the candidate drought tolerant genes, QTL mapping will be developed with phenotypic BLUP of F2 population due to its genetic diversity allowing to identify broad QTLs.

Material & Method

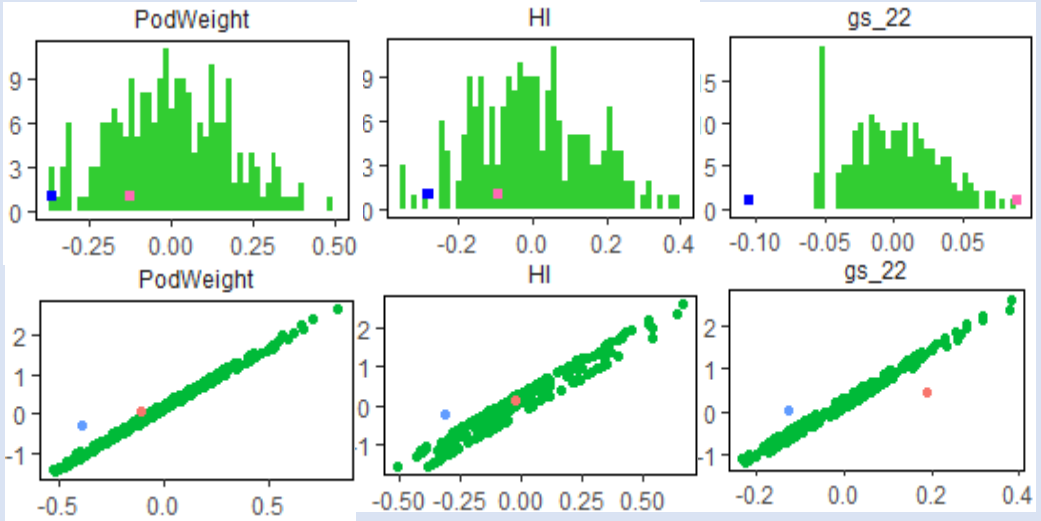
- Parental lines: AP-3 × TifRunner
- **I**ncomplete **R**andomized **A**ugmented **D**esign :
  256 plots in total = [55 F2 + (4 TifRunner + 5 AP-3)]* 4 Blocks
- Planting date: 3/May/2022
- Drought started: 65 DAP; lasted for 5 weeks
- Data collection:
  - Yield
  - HI
  - shoot biomass
  - Photosynthesis
  - Stomata conductance,
  - SPAD

Results

- Transgressive Segregants



- BLUP Distribution and Correlation



- Broad Sense Heritability

Yield	Shoot	HI	A_14	gs_14	A_22	A_28
0.53	0.09	0.43	0.75	0.44	0.72	0.61

Future work

Correlation between physiological traits and yield. QTL mapping with BLUP

Development and evaluation of the utility of GenoBaits Peanut 40K for a peanut MAGIC population

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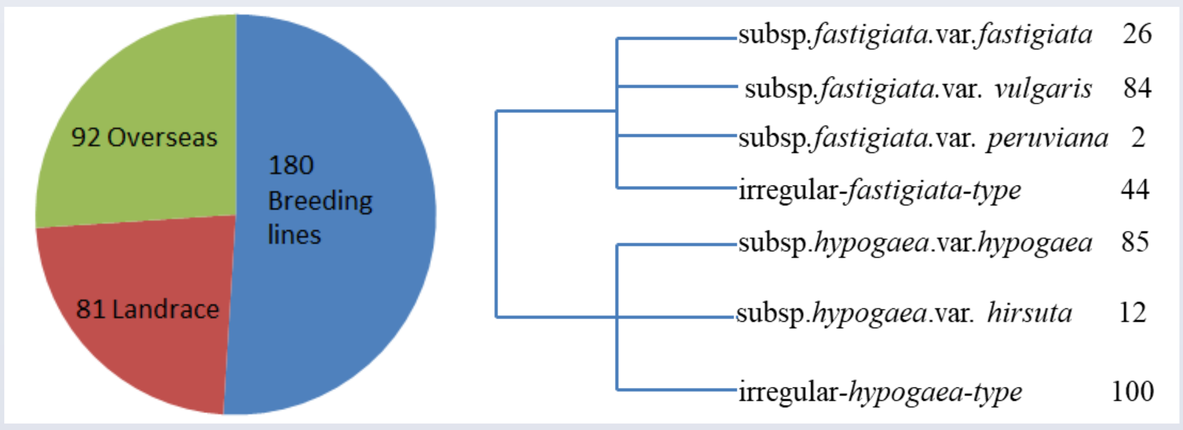
Abstract

Population and genotype data are essential for genetic mapping. The multi-parent advanced generation intercross (MAGIC) population is a permanent mapping population used for precisely mapping quantitative trait loci. Moreover, genotyping-by-target sequencing (GBTS) is a robust high-throughput genotyping technology characterized by its low cost, flexibility, and limited requirements for information management and support. In this study, an 8-way MAGIC population was constructed using eight elite founder lines. In addition, GenoBaits Peanut 40K was developed and utilized for the constructed MAGIC population. A subset (297 lines) of the MAGIC population at the S2 stage was genotyped using GenoBaits Peanut 40K. Furthermore, these lines and the eight parents were analyzed in terms of pod length, width, area, and perimeter. A total of 27 single nucleotide polymorphisms (SNPs) were revealed to be significantly associated with peanut pod size-related traits according to a genome-wide association study. The GenoBaits Peanut 40K provided herein and the constructed MAGIC population will be applicable for future research to identify the key genes responsible for important peanut traits.

Materials and Methods

SNP selection and array design for GenoBaits Peanut 40K



A diverse set comprising 353 peanut germplasms that underwent a whole-genome re-sequencing (20×) analysis was used to select SNPs. Approximately 0.93 million high-quality SNPs and insertions/deletions (*Arachis hypogaea* cv. Tifrunner version 1) were identified after the quality control and filtering: missing rate > 0.05 (any alleles with fewer than five supporting reads were marked as missing), minor allele frequency (MAF) < 0.01, and number of heterozygous alleles > 10 (Zheng et al. 2022). The SNP sites were selected according to the following criteria: (1) unique for each of the eight founder lines used as the parents of the MAGIC population (e.g., the genotype of one parent was A:A, whereas the genotype of the other seven parents was G:G); (2) evenly distributed across 20 chromosomes (as much as possible).

Developing MAGIC populations in peanut

A half-allele mating system was used for the three stages required for the construction of the MAGIC population. At the first stage, 28 bi-parental crosses were conducted by inter-mating the eight founder lines. The resulting 28 F_1 lines were inter-crossed for the 4-way cross (i.e., all 210 of the possible crosses). The combinations were set so that no parent was represented more than once in the 4-way cross. The 210 4-way F1 lines were inter-crossed for the 8-way cross (i.e., all 315 possible crosses were completed in the same manner).

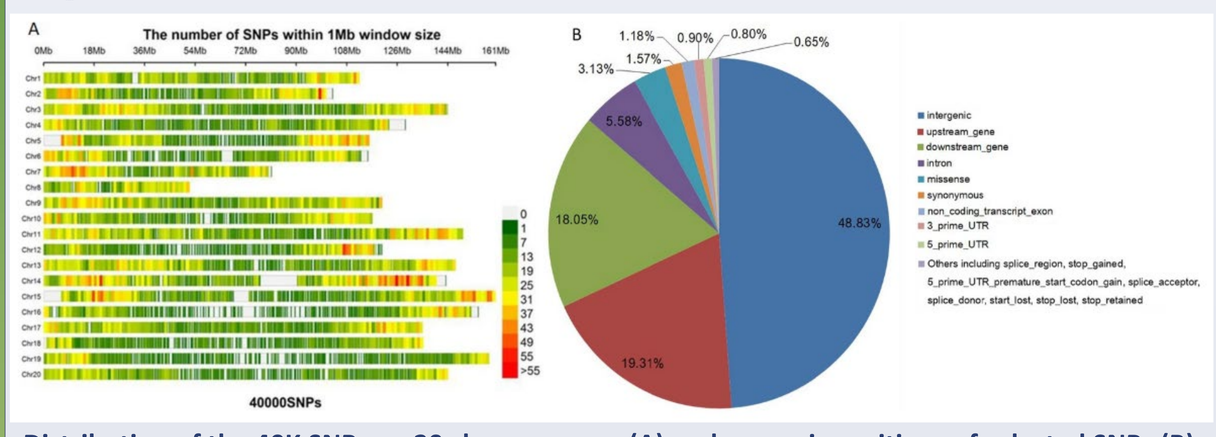
Table 1 Characteristics of the eight founder lines used in developing the MAGIC population

ID	Germplasm/Variety	Variant	Type	Origin	Characteristics
N730	Yuanza9102 (A)	irregular-*fastigiata*-type	Breeding line	Henan, China	Distant hybridization variety with high oil content, bacterial wilt resistance and wide adaption
N709	Zhonghua6 (B)	subsp.*fastigiata*.var.*vulgaris*	Breeding line	Hubei, China	Early maturity variety with small seed size, bacterial wilt resistance and pale green leaves
N734	Yuhua15 (C)	irregular-*hypogaea*-type	Breeding line	Henan, China	High yielding variety with good combining ability and high oil content, progenitor of many breeding lines
N743	Weihua8 (D)	irregular-*hypogaea*-type	Breeding line	Shandong, China	High yielding variety with moderate pod size and thin pod shell
N745	Yueyou20 (E)	subsp.*fastigiata*.var.*vulgaris*	Breeding line	Guangdong, China	High resistance to leaves diseases, thick shell and deep pod mesh on peanut shell
N744	Fuhuasheng (F)	irregular-*hypogaea*-type	Landrace	Shandong, China	One of the main progenitors of Chinese peanut varieties, in the pedigrees of most varieties of China with deep pod waist
N741	Silihong (G)	subsp.*fastigiata*.var.*fastigiata*	Landrace	Liaoning, China	Multi-seeds in one pod with red seed coat and low number of branches
N739	NC94022 (H)	subsp.*hypogaea*.var.*hypogaea*	Breeding line	America	Late maturity with prostrate growth habit, small size seed and light pod mesh

Results

The GenoBaits Peanut 40K

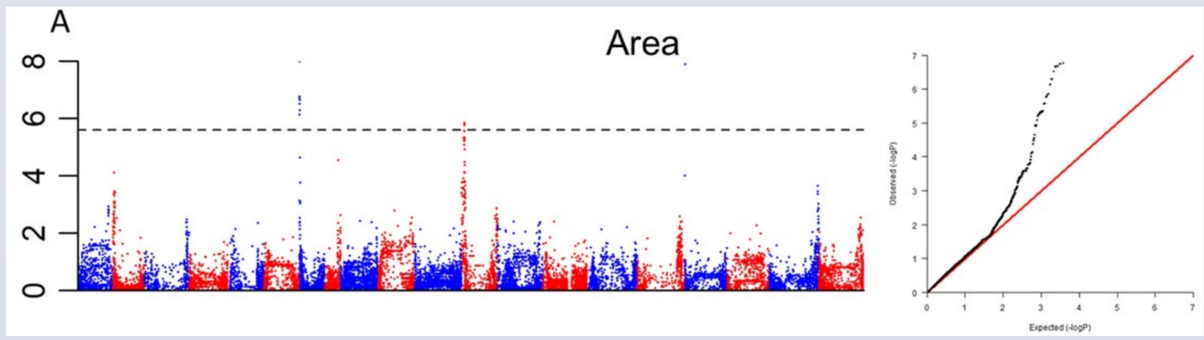
The 40,000 SNPs were evenly distributed across the 20 peanut chromosomes. The number of SNPs per chromosome ranged from 1070 (Arahy.08) to 3029 (Arahy.14), with an average of one SNP per 63,457 bases. In terms of their genomic positions, 48.83% of the SNPs were located in intergenic regions, but the SNPs were also present in the following locations: upstream_gene (19.31%), downstream_gene (18.05%), intron (5.58%), missense (3.13%), synonymous (1.57%), non_coding_transcript_extron (1.18%), 3_prime_UTR (0.90%), 5_prime_UTR (0.80%), and others (0.65%) including splice_region, stop_gained, splice_accepter, splice_donor, 5_prime_UTR_premature_start_codon_gain, start_lost, stop_lost and stop_retained.



Distribution of the 40K SNPs on 20 chromosomes (A) and genomic positions of selected SNPs (B)

Genome-wide association study for pod size-related traits

A total of 18,816 filtered SNPs were screened for SNPs significantly associated with the peanut pod area, perimeter, length, and width according to the MLM model. The Q file for K = 9 generated during the population structure analysis was used as the covariate (Q) in the MLM model. Kinship (K) was calculated using TASSEL v5.0. A total of 27 SNPs significantly associated with at least two of the four pod size-related traits were identified at the threshold of 5.50 [−log(0.05/18,186)]. Of these SNPs, 10 were on chromosome 7, 16 were on chromosome 12, and one was on chromosome 17.



Manhattan and QQ plots for peanut pod area

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USDA Peanut Germplasm Collection and Its Value for Peanut Improvement

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Introduction

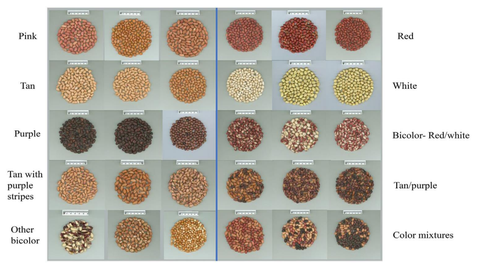
- The USDA peanut germplasm collection is considered a global treasure.
- It was accumulated from 102 countries with 9,139 *A. hypogaea* and 539 *Arachis* wild species accessions in the collection.
- Seeds are stored at -18^{0}C with a distribution sample kept at 4^{0}C plus 25% RH.
- From 2018-2022, seeds of 13,946 accessions of both *A. hypogaea* and wild species were distributed to researchers in 24 US states and 19 countries.
- The collection displays significant genetic diversity that is essential for peanut improvement.

Objective

- Characterization and evaluation of the USDA germplasm collection for agronomically useful traits.

Methods

- Over many years and locations, the germplasm collection was characterized for several phenotypic traits including morphological descriptors of pods and seeds, using the USDA Peanut Descriptors (6).
- Additionally, in collaborations with researchers, the collection was evaluated for useful traits such as maturity, pathogen/pest resistances and seed quality traits.



Characterization and evaluation of the germplasm collection

Maturity

- 698 early maturing PIs (<125 days)
- 1798 medium maturing PIs (145 days)

Pathogen/pest resistance PIs

Leaf spot resistance:
- 859 PIs were highly resistant

Peanut smut resistance:
- 8 PIs were highly resistant

TSWV tolerance:
- 1031 PIs expressed field tolerance

Thrips feeding:
- 24 PIs were highly resistant

Spider mites:
- 248 PIs were tolerant

Leafhoppers:
- 257 PIs were tolerant

Southern corn root worm:
- 163 PIs with 1-10% pods damaged

Seed quality traits

Seed coat color:
- Variation for seed coat color was observed with 2552 Tan, 2442 Pink, 2221 Red, 678 Purple, 148 White and 425 Multicolor types.

Seed weight:
- 100-seed weight ranged from 22g in PI 270904 to 160 g in PI 221068 with a mean of 49g.

Oil content:
- Total oil content ranged from 37% in PI 487337 (var. *vulgaris*) to 59% in PI 668529 (var. *hypogaea*) with a mean of 49%.

Oleic and Linoleic acids:
- Significant variation in oleic and linoleic acids was observed with means of 45% and 33%, respectively.
- Oleic acid ranged from 31% to 82% whereas linoleic acid ranged from 2% to 47%.

Summary

The USDA Peanut Germplasm Collection likely contains every trait that is needed for peanut improvement.

Large genetic variation for economically important traits was observed in the peanut germplasm collection (8)

PI 203396 provided resistance to TSWV and leaf spots (1 and 4)

33 peanut cultivars released in the US between 1984 and 2017 contained PI 203396 as an ancestor (3)

PI 475871 provides TSWV resistance (1)

PI 478819 is Sclerotinia blight resistant (1)

A. glandulifera and *A. correntina* accessions provided Sclerotinia blight resistance (1)

Eight *A. hypogaea* PIs were highly resistant to peanut smut (2)

Root-knot nematode and leaf spot resistance was developed from. *A. cardenasii* (GKP 10017). *A. diogoi,* (GK 10602) provided TSWV and leaf spot resistance (1)

New high-oleic acid mutants identified, PI 342664 and PI 342666 (7)

The PIs contributed 20.6% of the ancestry of cultivars (5)

Economic impact of disease resistant cultivars using germplasm resources is more than $200 million annually (4)

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Multiple strategies revealed in alternative splicing provide a refine annotated genome in allopolyploid peanut

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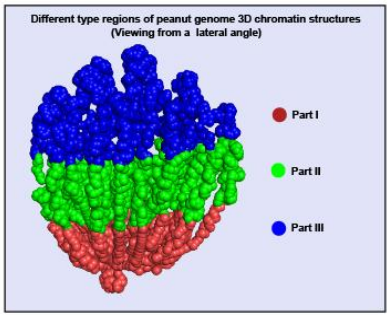
Abstract

Alternative splicing (AS), an important post-transcriptional regulation mechanism in eukaryotes, could significantly increase transcript diversity and contribute to controlling gene expression and many other complicated developmental processes. Here we use multi-omics to analysis the peanut AS events. Using long-read isoform sequencing, 146,464 full-length non-chimeric transcripts were obtained, resulting in corrected annotation of 1,782 genes and identification of 4,653 new loci. 271,776 unique splice junctions were identified and 82.49% of them were supported by transcriptome data. There is a suppressed effect of 6mA on AS and gene expression. By analysis of chromatin structures, the genes locating in TAD boundaries, proximal chromosomal telomere region, inter or intra chromosomal loops have more isoforms, higher expression, lower 6mA and TEs occupancy than the other ones, indicating that chromatin interaction, 6mA and TEs play an important role in AS and gene expression. These results greatly refine peanut genome annotations and showed AS in higher organisms are associated with multiple strategies for their regulation.

Keywords: Peanut, Alternative splicing, Chromatin structures, Iso-seq, 6mA

A



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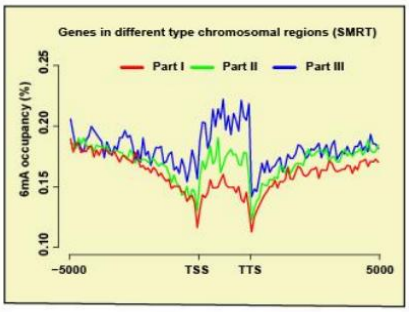

Characterization of chromosomal 3D structures in peanut

(A) The 3D structure of peanut chromosomes was divided into three roughly equal parts: the proximal telomere region (part I), the intermediate region (part II) and the proximal centromere region (part III). (B) Comparison of SMRT sequencing identified 6mA occupancy among gene body, 5kb upstream of the TSS and downstream of the TTS regions among three parts chromosomal regions in peanut.