NCARS/NCCES CODE: NC-42
ON CAMPUS RESEARCH
REPORT PERIOD: 1/1/2023-12/31/2023
ANNUAL

PROGRESS REPORT
TO
NORTH CAROLINA PEANUT GROWER'S ASSOCIATION, INC.

TITLE: **Marker Development through Next-generation Sequencing (NGS) for Late Generation Selection**

LEADER(S): **Jeffrey C. Dunne & Ryan J. Andres**

DEPARTMENT(S): **Crop and Soil Science**

REPORT:

A total of 265 advanced breeding lines, germplasm lines and introgression materials were evaluated for two years across 2-3 research stations in North Carolina, the Peanut Belt Research Station (PBRS) in Lewiston-Woodville, NC, the Upper Coastal Plains Research Station (UCPRS) in Rocky Mount, NC and the Border Belt Research Station (BBTRS) in Whiteville, NC for leaf spot evaluations, yield and seed grade quality in order to identify regions of interest for marker-assisted selection. Following a Genome-wide Association Study (GWAS) of more than 6,000 SNP markers generated from previous work on this project, 16 PACE markers were designed and validated to track four primary regions of interest on chromosomes 4, 6, 12, and 14. These four were prioritized as they consist of numerous significant SNPs within reasonably sized haplotype blocks, generally only found in highly leaf spot resistant lines. An additional 17 PACE markers were designed and validated to track additional regions of interest on Chr 3, 4, 5, 7, 11, 13, 14, 16, 19, and 20. All of these 17 markers were significantly associated with leaf spot in the GWAS, but tend to exist as singly significant markers, and thus their effects were considered more suspect. These markers were used in the selection of novel breeding lines coming through the greenhouse selection program. In addition to leaf spot resistance, 16 SNP peaks on eight different chromosomes were identified for pod width and pod area. Six of the eight identified chromosomes have been previously reported to contain quantitative trait loci (QTL) associated with pod, seed or yield related traits. From the identification of these QTL, PACE markers were designed on chromosomes 2, 5, 6, 15, 16 and 19, conferring selection for pod size and area. Lastly, a second set of genotypes was subjected to whole-genome sequencing in order to bias marker design and use towards advanced breeding materials within the NCSU peanut breeding program. Previously designed markers were heavily influenced by germplasm materials selected for their response to major diseases in the region; however, this reduced the number of usable genome-wide markers representing advanced breeding materials selected for yield and other agronomically important traits to the program. Therefore, 96 additional lines (Table 1) were sequenced, which generated more than >5,000,000 markers that will be further filtered in order to develop a new mid-range genotyping panel for continued use on breeding lines evaluated in the advanced testing program.

Table 1. List of 96 genotypes subjected to whole-genome sequencing (10x) for single nucleotide polymorphism (SNP) discovery.

Genotype	Genotype	Genotype	Genotype
Comrade	N18018	N19047	N09042olF
NC20	N18026	N19048	N12006ol
Walton	N18033	N19049	N13056olSm
N10066	N18041	N19050	N14035olSmT
N13031	N18042	N21001	N14040olLSmT
N14001	N18043	N21003	N14043olLSmT
N14002	N18045	N21004	N15052ol
N14004	N18049	N21006	N15068olLSmT
N14007	N18050	N21007	Wilson
N14009	N18055	N21008	N13003olF
N14039	N19002	N21009	N05044FCSm
N15017	N19003	N21011	N91026E
N15039	N19006	N21014	N05008
N15053	N19007	N21016	N92020
N15060	N19008	N21017	NC8C
N15066	N19013	N21019	N04041
N16005	N19014	N21020	N00087ol
N16011	N19016	N21021	NC2
N16032	N19019	N21022	N00088ol
N17044	N19020	N21024	IpaCorr18
N17047	N19021	N21033	GreSten8
N18002	N19022	N21042	BatSten5
N18011	N19043	N21043	ValSten1
N18012	N19045	Phillips	N16034

IMPACT STATEMENT

The identification of the regions controlling both leaf spot resistance and pod size/area segregating within the NCSU peanut breeding program has incredible implications towards improved efficiency in marker-assisted selection approaches (see NC-43 report). While selecting through rapid generation in the greenhouse, increasing the frequency of these favorable alleles/genes in the peanut breeding population would ensure all materials released would carry the same level of resistance to each other, while selecting for superior agronomically performing genotypes for cultivar release. Similarly, resistance to diseases or insects; tolerance to drought; or improvement of flavor quality, tend to extend outside the diversity that exists exclusively within Virginia-type germplasm and previously released varieties. These wider crosses make it challenging to recover the Virginia-type pod and seed size. The identification of SNP-based marker assays for selecting on pod size allows the program to cross other peanut market-types while ensuring the large pod and seed sizes of the Virginia-type peanut. Lastly, identifying genome-wide markers that differentiate advanced breeding materials coming through the peanut breeding pipeline will allow for the selection of the genomes with higher yields, earlier maturity, and improved drought stress tolerance.

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PROGRESS REPORT
TO
NORTH CAROLINA PEANUT GROWER'S ASSOCIATION

TITLE: **Marker-Assisted Selection in Virginia-type Peanut for Multiple Disease Resistance**

LEADER(S): **Jeffrey C. Dunne & Ryan J. Andres**

DEPARTMENT(S): **Crop and Soil Sciences**

REPORT:

Using the markers generated through the Genome-wide Association Study (GWAS) though the work conducted on the Next-Generation Sequencing (NGS) project (see NC-42 report) has provided an efficient way to select for improved leaf spot resistance, while recovering the Virginia-type pod size. Paired with the speed breeding methodology, crosses with Bailey II x SPT 10-12 (X21022) and Bailey II x IAC 322 (X21023) have been selected in the greenhouse to the F_5 generation. Selections were imposed on the *A. cardenasii* blocks on chromosomes 7, 10 and 13 for X21022 lines and chromosomes 2, 8, and 13 for X21023 lines yielding 35 and 47 single plant selections that are fixed for each introgression block, respectively. In addition, the other leaf spot regions of interested (17 markers identified in the GWAS) have been used in the selection of stacked introgression materials from *A. cardenasii* x *A. diogoi* lines. In the summer of 2023, 576 F_2 seedlings out of four different 3-way crosses were genotyped with the 16 prioritized PACE markers and 121 F_2 seedlings selected and advanced based on retention of all four primary QTL of interest. $F_{2:3}$ seed has been collected from these plants and will be genotyped, selected, and advanced based on the same criteria over the winter of 2023-2024. The 17 additional markers will not be used for selection in these populations. However, once material reaches the $F_{4:5}$ generation, each will be genotyped with these 17 markers to determine the presences/absence of the resistance alleles. Once paired with the resulting phenotypic information, this will enable validation/rejection of these more suspicious markers.

Arachis hypogaea ‘Bailey II’ was exposed to differing light intervals in order to examine whether photo-intensity has an effect on peanut pod set, seed count, and post-harvest germination percentage. Data is being utilized for application in a peanut speed breeding program, which aims to decrease time between generations, allowing for faster cultivar development and release. In a controlled environment (CE) trial using a randomized complete blocked design with four replications and two subplots, peanuts were evaluated at 0, 200, 400, and 600 µmol//m /s of additional light provided by two LG LED panels over each treatment. Four harvest intervals (70, 80, 90, and 100 days after planting), were employed in each treatment to see whether photo-intensity influences maturity. Results of the CE study was used in the development of an applied application in working conditions. As supplemental light intensity increased, above ground biomass and flower, peg, pod and seed count increased; however, conditions governing seed weight did not follow this linear trend. Average seed weight was highest in the 400, 200, 0, and 0 µmol treatments in the 70-, 80-, 90-, and 100-day harvests, respectively. In this experiment, the lowest threshold for radical emergence was approximately 0.03g while leaf emergence was detected in seeds as little as 0.039g in weight. In early harvests, intermediate intensities (200

and 400) saw the greatest percentage of seeds root and form visible leaves. At 70 days, proximately 50% of seeds from the 200 and 400 µmol treatments developed leaves after 14 days, while less than 40% developed leaves from the lowest ad highest intensity (0 and 400 µmol). By the 100-day treatment, low-intensity treatments (0 and 200 µmol) had, on average, a higher germination rate of approximately 70% compared to around 60% for 400 and 600 µmol, however, there was significantly more seeds in the higher light treatments. This research will enable better usage of time and space to develop germplasm for breeding and improvement in the NCSU Peanut Breeding Program.

Finally, an applied trial for speed breeding in the greenhouse was initiated to validate the recommendations from the controlled study previously mentioned. Arachis hypogaea 'Bailey II' was exposed to LED lighting and harvested at intervals of 60, 70, 80, and 90 days after planting at a photoperiod of 20 hours in order to examine speed breeding practicality for a peanut breeding program. Data is being utilized for application in a peanut speed breeding program, which aims to decrease time between generations, allowing for faster cultivar development and release. A winter and spring cycle were examined for determination of growing cycle length when light and temperatures are limited. This work is ongoing but preliminary results suggest that 80- to 90-day harvest times can be achieved across different growing season.

IMPACT STATEMENT

The development of materials with all of the introgressions from IAC 322 and SPT 10-12, including the high oleic trait may lead to novel cultivar releases to both early and late leaf spot along with the agronomic performance of Bailey and Bailey II; the importance of these two cultivars to the Virginia-Carolina region cannot be overstated. These lines, in addition to other cultivars (Wynne, Sullivan, Emery, etc.) have occupied nearly 92% of all acreage in North Carolina and 85% of acreage grown in the Virginia-Carolina region. In addition, multi-species introgression would provide an invaluable resource to the peanut breeding program. The strongest, most durable resistance would result from pyramiding or stacking resistance loci from both A. cardenasii and A. diogoi within the same cultivar. However, to do so would involve making crosses with numerous germplasm lines, followed by a lengthy backcrossing and selection program to recover necessary agronomic qualities. If resistance loci could be stacked within a smaller number of germplasm lines, deploying this elevated resistance in new cultivars would be expedited. Molecular markers delineating the introgression blocks would further hasten the breeding process by allowing leaf spot resistance selection to occur in early generations and off-season environments. This would obviate the need for extensive, replicated testing in later generations to determine leaf spot resistance, allowing the breeder to concentrate resources on selecting for high yield. Markers also offer the potential to reduce linkage drag by rapidly eliminating unwanted introgression fragments. Lastly, the speed at which these advanced breeding lines can be developed will increase due to the rapid cycling in the greenhouse through speed breeding. The speed breeding methods that are currently being developed at NCSU can provide new advanced breeding lines in less than two years, where current efforts utilizing a winter nursery program or a single generation per year at the Peanut Belt Research Station in Lewiston-Woodville, NC can provide materials in 4 to 7 years, respectively. This will allow the NCSU peanut breeding program the ability to adapt to changing climates in the face of disease or other newly identified biotic or abiotic stressors to the Virginia-Carolinas region.

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PROGRESS REPORT
TO
NORTH CAROLINA PEANUT GROWER'S ASSOCIATION

TITLE: **Advances in Peanut Phenotyping for Genetic Improvement**

LEADER(S): **Jeffrey C. Dunne & Ryan J. Andres**

DEPARTMENT(S): **Crop & Soil Sciences**

REPORT:

Leaf Spot Evaluations:

A total of 265 advanced breeding lines, germplasm lines and introgression materials were evaluated for two years across two research stations in North Carolina, the Peanut Belt Research Station (PBRS) in Lewiston-Woodville, NC, and the Upper Coastal Plains Research Station (UCPRS) in Rocky Mount, NC for leaf spot resistance. Each replicated trial was evaluated using a visual screening method (1-9 scale), a low-throughput screening method capturing lesion counts and defoliation using harvested leaf tissue and aerial drone imaging and a high-throughput method utilizing a 10-band multispectral sensor for extracting various plant vegetative indices (Table 1) to correlate to the visual scores. Each method correlated highly with the visual scores (>0.75) and a moderate to high heritability estimate (>0.41), suggesting these methods could be used to support breeding decisions on selections and the oligo-to-polygenic inheritance of the trait (Table 1). The results of the GWAS have led to the selections on the multi-species introgression populations mentioned previously. One additional outcome of the high-throughput drone imaging platform was the identification of a waypoint design for imaging on a plot-level basis (Figure 1). The images captured can routinely estimate multiple estimates simultaneously with higher resolution when compared to composite orthomosaic images of the entire field from an extreme elevation (Figure 2).

Pod Size Evaluations:

Size characterization of peanut pods and seeds is a crucial aspect of determining the market value of the crop. Currently, this task is performed using mechanical methods such as rollers and vibratory shakers, which are labor intensive, time-consuming, and prone to errors. To address these issues with pod size, a computer vision-based solution was developed to detect and quantify the size characteristics of individual pods in a grade sample. In order to gather pod data on a grade sample, a computer vision algorithm was trained on a set of peanut pod images that encompasses a wide range of genotypes and backgrounds. The images were labeled, and a Mask-RCNN model was trained for image segmentation of individual pods (Figure 3). The output from this model was then used to extract features of each pod in the image used in downstream data analysis. A trial containing 25 genotypes, replicated three times was designed to verify the correlation between this new image-based method and the current mechanical grading method. Results show that this computer vision-based method provides consistent data when compared to the current mechanical grading method (Table 2). This proposed method is also able to capture more detailed pod information to provide distributions and individual pod characteristics instead of the current system’s classification method. Furthermore, the proposed method can be

expanded for feature extraction from pod images in various applications along with seed size and quality characteristics.

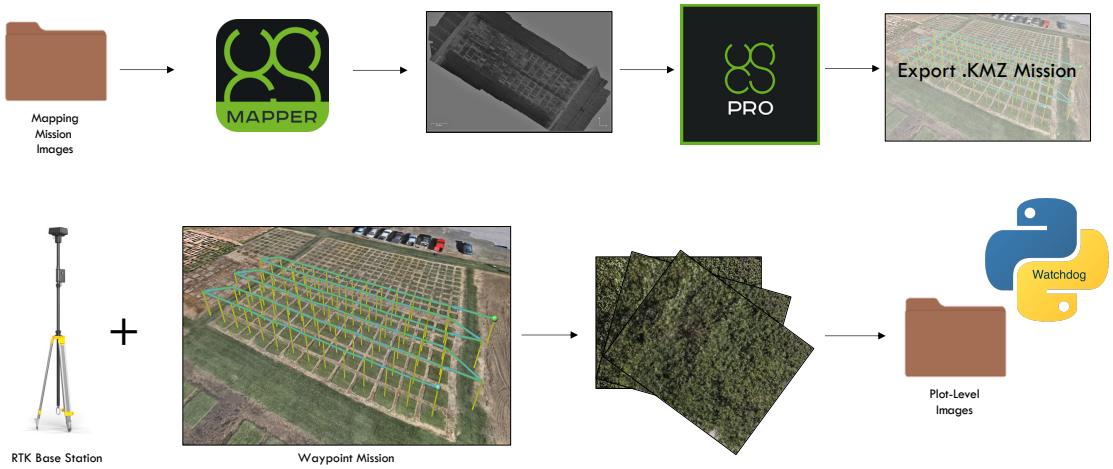

Figure 1. Proposed waypoint mission pipeline for developing waypoint designs for plot level resolution of leaf spot severity using a DJI Matrice 300 RTK, DJI Base Station and UgCS Software integration for downstream data analysis on raw plot-level images of lesion and leaf defoliation percentages.

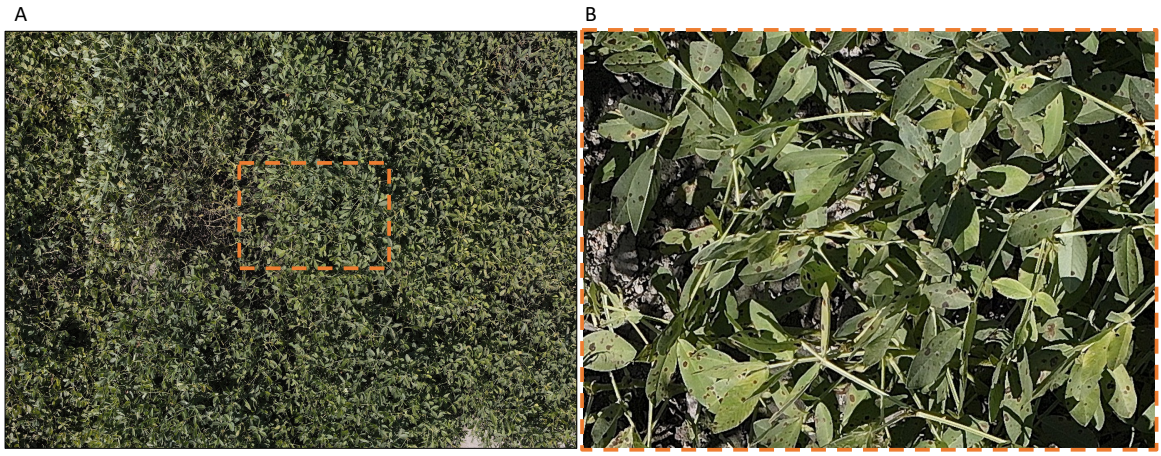

Figure 2. Waypoint designed, plot level images of leaf spot infested peanut plots in an advanced selection nursery (A) and the resolution associated with plot level imaging using a Zenmuse P1 RGB sensor mounted on a DJI Matrice 300 RTK drone for leaf spot severity characterization (B).

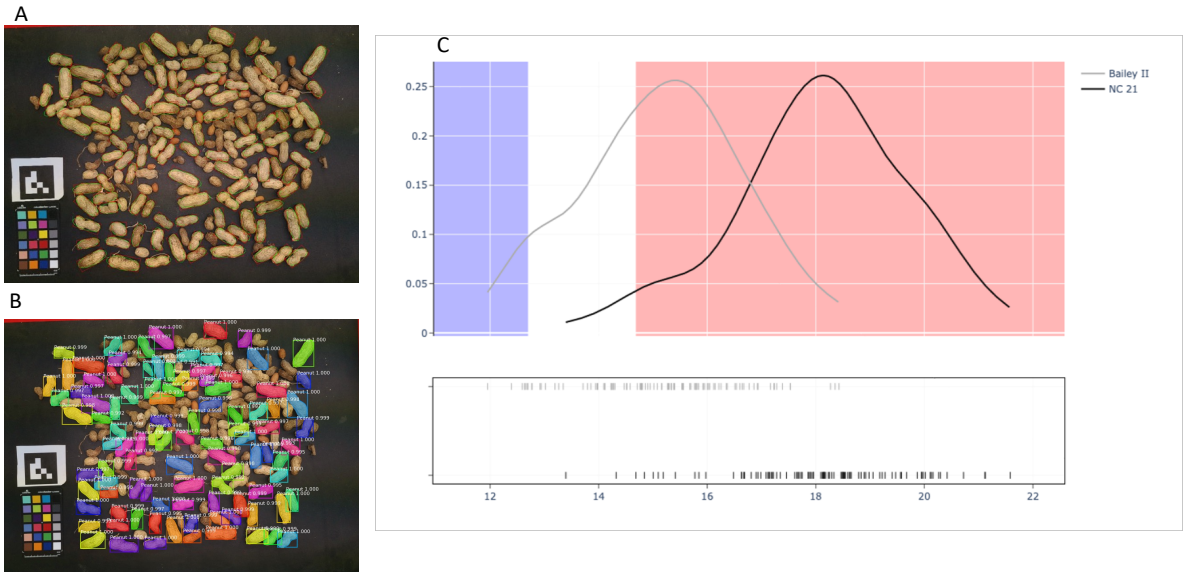

Figure 3. Segmentation of peanut pods (A) and the resulting peanut pod masks (B) used to extract peanut pod characteristics to compare peanut grade samples, specifically pod sizes among peanut cultivars (C) in the NCSU peanut breeding program. In C, the background color determines the fraction of pods falling into Jumbo (Red), Fancy (White) and No. 1 (Blue) pod size categories

Table 1. Correlation coefficients and broad-sense heritability estimates of feature scores compared to visual leaf spot evaluations and the genetic control for the trait, respectively

Feature	Correlation Coefficient	Broad Sense Heritability
Visual Score (1-9)	1	0.8276
Visible Atmospherically Resistant Index	-0.812089	0.6659
Defoliation Score [RGB]	0.789940	0.6237
Triangle Vegetation Index	-0.780999	0.4454
Red Band [842]	-0.779900	0.4077
Difference Vegetation Index	-0.778447	0.4213
Modified Triangular Vegetation Index [1]	-0.778236	0.4270
Leaf Area Index	-0.775914	0.4412
Soil-Adjusted Vegetation Index	-0.775759	0.4478
Normalized Vegetation Index	-0.755071	0.4929

Table 2. Pearson's correlations among 25 genotypes with three replications for Jumbo, Fancy and No. 1 pod size classifications.

Pod Classification	Rho value
Jumbo	93% (P-value = $2.2e^{-16}$)
Fancy	89% (P-value = $2.2e^{-16}$)
No. 1	89% (P-value = $2.2e^{-16}$)

IMPACT STATEMENT

The ability to identify regions in the genome controlling both leaf spot resistance and pod size/area segregating within the NCSU peanut breeding program can only happen through consistent and precise phenotypic data collected in the field among segregating peanut breeding lines. Historically, these measurements have been made visually on leaf spot severity (1-9 scale) and through mechanical equipment; however, both methods can be subjective, temporally sensitive, and/or prone to errors. These image-based methods for leaf spot and grade sample evaluations improve the efficiency and quality of data used for mapping these important traits to regions of interest in the peanut genome. When these are known, effective marker-assisted selection methods can rapidly increase beneficial alleles/genes within the peanut breeding program, providing superior varieties for release in the Virginia-Carolina's region.