# Genome Wide Association Study (GWAS) on Root-Knot Nematode Resistance in Cultivated Peanut

by

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## ABSTRACT

The peanut root-knot nematode, *Meloidogyne arenaria*, is one of the major soil-borne pests for peanut (*Arachis hypogaea* L.). It causes economic losses in the production of peanut in the southeastern region, especially in Alabama, Georgia, Florida, and in Texas as well. Losses due to root-knot nematodes can reach up to 50% at dense infested fields without using nematicides. The use of nematode resistant cultivars is the most convenient economical way of biological control method for producers. The identification of resistant peanut germplasm to nematode diseases is a fundamental task for breeding nematode resistant cultivar. The objectives of this research are to evaluate 161 accessions of peanut germplasm in the greenhouse for resistance and to identify SNP markers associated with root-knot nematode resistance via genome-wide association study (GWAS). Randomized complete block design with three replications for each genotype is performed for phenotyping by using greenhouse inoculation techniques. The genetic diversity panel used in this experiment was genotyped by Affymetrix version 2.0 SNP assay. Forty-six quantitative trait loci (QTLs) located on twelve different chromosomes underlying root-knot nematode resistance were determined with phenotypic variation explained (PVE) between 7.8% and 17% by GWAS. Out of 46 QTLs, 957 candidate genes detected including 520 genes on A sub-genome and 437 genes o on B sub-genome. Specifically, 26 candidate genes related to LRR encoding gene were found on chromosomes A01, A04, A05, B07, B08, and B10. The associated markers could be applied in breeding programs for marker assisted selection.

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| | |
|---|---|
| ANOVA | analysis of variance |
| BLAST | basic local alignment search tool |
| BM | biomass |
| cc | cubic centimeter |
| DNA | deoxyribonucleic acid |
| Eggs/g RFW | eggs per gram of the root fresh weight |
| GLM | general linear model |
| GWAS | genome wide association study |
| NBS-LRR | nucleotide binding site-leucine-rich repeat |
| PCA | principal component analysis |
| PH | plant height |
| QTL | quantitative trait locus |
| RFW | root fresh weight |
| RKN | root-knot nematode |
| SAS | statistical analysis software |
| SFW | shoot fresh weight |
| SNP | single nucleotide polymorphism |
| SSR | simple sequence repeat |
| USDA | United States Department of Agriculture |

# CHAPTER ONE

# LITERATURE REVIEW

## The Origin and Early History of The Peanut

The peanut (*Arachis hypogaea* L.) likely originated in South America near what is now present-day Brazil and Peru, and where almost 15 wild peanut species are found, before being spread worldwide by European traders (Acquaah, 2012). Since then it has been grown extensively in the tropical and subtropical regions of Asia, Africa, and North America (Hammons et al., 2016). Although some commercial peanut farms were present in the United States during the 1700s and 1800s, the peanut was primarily used as animal feedstock during that time; it was not commonly grown for human consumption until the 1900s (Tillman & Stalker, 2009). In the early 1900s, George Washington Carver encouraged peanut production in the United States, suggesting that it be planted in rotation with cotton (National Peanut Board, 2017). While cotton depletes nitrogen levels in the soil, the peanut, which is a legume, has the ability to fix nitrogen and thereby replenish those levels (Tallury, 2017). Increased demand for high-protein food sources during World War I saw a surge in the consumption of peanut products like peanut butter and peanut-based candies (National Peanut Board, 2017).

## The Characteristics of Peanut

The peanut is a legume within the botanical family Fabaceae. The peanut is a leading oilseed crop, whose contents include 36% to 54% oil, 16% to 36% protein, and 10% to 20%

carbohydrates. The peanut is also a good source of several vitamins (E, K, and B1) and minerals (Ca, Mg, P, and K) (Tillman & Stalker, 2009).

The peanut is self-pollinated, and propagated commercially via its seed, with planting dates occurring anytime from late April until June. There are around 80 peanut species (Tallury, 2017); however, all cultivated peanut species are allotetraploid (AABB; 2n=40), while all wild species are diploid (2n=20) except *A. monticola* (2n=40). *Arachis hypogaea* is an allotetraploid (AABB; 2n= 40) that has originated from the hybridization of two ancient diploid species followed by a natural duplication of chromosomes. These two ancient diploid species, *A. Duranensis*, and *A. ipaensis*, are the progenitors of A-genome and B-genome, respectively (do Nascimento et al., 2018).

Cultivated peanuts are categorized into two subspecies, *hypogaea* and *fastigiata*, which are further divided into six botanical varieties depending on their morphology (e.g. leaf color and branching patterns) and growth habits. More specifically, while the subspecies *hypogaea* has two botanical varieties, *hypogaea* and *hirsuta*, the subspecies *fastigiata* has four botanical varieties, *fastigiata*, *vulgaris*, *aequatoriana*, and *peruviana*. Of these subspecies, four are market types grown in the U.S—namely, Runner (subsp. *hypogaea* var. *hypogaea)*, Virginia (subsp. *hypogaea* var. *hypogaea)*, Valencia (subsp. *fastigiata* var. *fastigiata)*, and Spanish (subsp. *fastigiata* var. *vulgaris)* (Tallury, 2017; Vishwakarma et al., 2017). The Runner variety is the type most commonly used in peanut butter, and accounts for 80% of total U.S. peanut production, while the Virginia variety is primarily used to make gourmet snacks, and accounts for about 15% of total U.S. peanut production (National Peanut Board, 2017).

## Economic Importance

Total global peanut production in 2017-2018 was almost 45 million metric tons. The major peanut-producing countries in 2018 were China, India, the United States, Nigeria, and Sudan (U.S. Department of Agriculture [USDA], 2018). Peanuts have a variety of commercial uses including as animal feeds like peanut hay, in foodstuffs like roasted peanuts, and in industrial products like cosmetics. As such, the peanut constitutes an excellent cash crop for U.S. domestic and international trade (National Peanut Board, 2017). The United States is the world's fourth largest peanut producer with roughly 3.5 million metric tons, with exports totaling roughly 250,000 metric tons per year (USDA, 2017). Within the United States, Georgia is the largest peanut producing state, accounting for over 50% of the country's total annual production. Alabama, which is the United States' second largest peanut producing state, accounts for only 14%. More than 80% of U.S. peanut exports are to Canada, Mexico, Europe, and Japan. Worldwide peanut exports reached $690 million dollars in 2016. China and Argentina are important peanut exporters; however, India and Vietnam are also major players when crop quality and demand are high (USDA, 2017).

## Major Plant-parasitic Nematodes Associated with The Peanut

Plant-parasitic nematodes are microscopic, bilateral, and unsegmented worm-like animals that live in water, soil, and as the parasites of plants and animals. Plant-parasitic nematodes include only 10% of all nematode species but result in 14% of annual crop losses worldwide (Agrios, 2005). As one of the most important soilborne diseases affecting peanuts, plant-parasitic nematodes are a major threat to peanut production. The plant-parasitic nematode species that are economically significant threats to peanut production include *Meloidogyne spp.* (three species), *Pratylenchus brachyurus*, *Belonolaimus longicaudatus*, *Criconemoides ornatus*, *Aphelenchoides arachidis*, *Scutellonema cavanessi*, *Tylenchorynchus brevilineatus*, and *Ditylenchus africanus* (Dickson & Waele, 2005).

**Overview of Root-knot Nematodes**

Root-knot nematodes (*Meloidogyne spp.)* (RKN) were first described in 1855 by Joseph Berkeley after he noticed damage that had occurred to cucumbers. Root-knot nematodes include nearly 100 different species of plant-parasitic roundworms (Mitkowski & Abawi, 2003). Some of the most noteworthy species of root-knot nematodes are *M. arenaria*, *M. incognita*, *M. javanica,* and *M. hapla* (Jones et al., 2013). However, peanut is a non-host of *M. incognita* (Davis & Webster, 2005). Three of them, *M. areneria*, *M. javanica*, and *M. hapla*, are present in the peanut producing regions of North, Central, and South America as well as Africa, Asia, Europe, and Australia. While *M. hapla* is common in temperate regions, *M. arenaria* and *M. javanica* occur mostly in warmer areas (Dong et al., 2008). Though plant-parasitic nematodes as a whole account for 14% of annual crop losses worldwide, 5% of those losses are attributable to root-knot nematodes alone (Sasser et al. 1983).

In the United States, the most damaging nematode species for peanut production is *M. arenaria* (Neal) Chitwood race 1. *M. arenaria* was first identified by Chitwood from a diseased peanut plant. Two host races of *M. arenaria* had been defined in 1978; while race 1 reproduces on peanuts, race 2 requires a different host (Dickson, 1985). *M. arenaria* race 3 were identified but it was found that could not reproduce on peanut Florunner (Robertson et al, 2009).

*M. arenaria* can be found throughout much of the southern United States including in Alabama, Florida, Georgia, Texas, and South Carolina. Its presence in these regions causes an estimated 3-15% decrease in peanut yields each year (Dong et al., 2007). In fact, RKN are so pervasive in Florida, and peanut fields with heavy infested RKN have more than 75% yield losses (Rich & Tillman, 2009).

## Life Cycle of the Root-knot Nematode

There are six stages in the life cycle of the root-knot nematode, and these include the egg stage, four juvenile steps, and adulthood. The root-knot nematode disease cycle in peanut begins when an egg hatches into a juvenile; the disease cycle progresses to the infective stage when the juvenile 2 (J2) penetrates the plant's roots with its stylet by repeatedly puncturing the surface cells. Then, the juvenile migrates to a place near the vascular tissue where it will remain to feed. After two or three days, the nematode enlarges and becomes sedentary—that is, unable to move. Root-knot nematodes must molt four times before entering the adult stage (Figure 1). The mature female nematode is pear-shaped, which facilitates the swelling necessary to produce eggs, while males are vermiform and can move freely. Notably, however, root-knot nematodes do not need males to reproduce since they are parthenogenetic. At 27°C, the entire RKN life cycle lasts 25 days, but under different environmental conditions it can last anywhere from 3 to 6 weeks (Williamson and Hussey, 1996; Agrios, 2005).

## Development of Resistant Peanut Cultivars

In the early 1970s, a field in Central Texas had extensive root-knot nematode stress. As a result, the wild peanut species present were analyzed for genes conferring resistance to different root-knot nematode species (Acquaah, 2012). Genes for resistance to root-knot nematode were determined in three wild peanut species: *A. batizocoi*, *A. cardenasii*, and *A. diogoi* (Simpson, 1990). Moreover, Garcia et al. (1996) identified two important resistance genes (R-genes) against *M. arenaria* race 1 from the cross of 4x (*A. hypogaea* x *A. cardenasii*)- GA 6 and PI 261942. The first found gene, *Mae*, restricts nematode egg number, while the second gene, *Mag*, inhibits RKN galling. The TxAG-6 germplasm line was created to transmit nematode R-genes from wild diploid peanut species into cultivated tetraploid species (Nagy et al., 2010).

The COAN and NemaTAM cultivars were generated by backcrossing from a hybrid between TxAG-6 and Florunner as these varieties have a high level of RKN resistance. These cultivars carry *Rma*, a resistance gene for the root-knot nematode, and have an equally high-level of root-knot nematode reproduction (Nagy et al., 2010). However, COAN had one major flaw: the resulting plant was too small, and this restricted its seed production. Although COAN yields under severe nematode pressure were 150–200% better than susceptible cultivars, overall COAN yields were still too low to be profitable for growers. However, NemaTAM crop yields average of 30% higher than those of COAN (Acquaah, 2012).

Tifguard is a runner-type peanut cultivar that was released by the USDA Agricultural Research Service (USDA-ARS) and the Georgia Agricultural Experiment Stations in 2007. This cultivar has a resistance not only to the root-knot nematode *M. arenaria* (Neal) Chitwood race 1, but also against tomato spotted wilt virus (TSWV). More specifically, Tifguard was produced from the hybridization of TSWV resistant C-99R and the RKN resistant COAN (Holbrook et al., 2008). In addition, another cultivar, TifNV-High-O/L, was produced by hybridizing RKN-resistant Tifguard with Florida-07, a high-oleic cultivar. The desired characteristics of the final cultivar—RKN resistance and a high oleic to linoleic fatty acid ratio (O/L)—were selected with the aid of marker-assisted selection (Holbrook et al., 2017).

**Symptoms of Root-knot Nematode Infestation**

The root-knot nematode *M. arenaria* (Neal) Chitwood race 1 is one of the world's major soilborne pests. It is found in tropical, subtropical, and warm temperate soils. *M. arenaria* primarily damages the plant root system and obstructs nutrient transport (Dufour et al., 1998). In addition, environmental stressors such as drought, flooding, nutrient deficiencies, and soil compactness will worsen the aboveground damage observed in RKN-infested plants. In dry

weather conditions, plants with severe infections are noticeably stunted and exhibit a yellowing of their foliage (Kenneth & Curtis, 1973).

RKN-infected plants have irregular swellings, or galls, on their pods and roots. These galls include one or more sedentary, adult female RKNs. The total number of galls present reflects the density of the nematodes and the timing of the infection. However, each gall is less than 1mm in size, making them difficult to identify. (Grabau & Dickson, 2018).

**Methods for Managing the Root-knot Nematode**

There are several different disease management methods for dealing with plant-parasitic nematodes—namely, biological, cultural, and chemical controls. The most convenient biological control method is the use of nematode-resistant plants. Moreover, this approach is affordable for producers and growers (Lambert & Bekal, 2002). RKN-resistant peanut cultivars include TifGP-2, Tifguard, Georgia14N, TifNV-High O/L, NR 0812, and NR 0817 (Hajihassani et al., 2018).

The primary cultural control method is crop rotation, which acts by decreasing nematode population density. For example, cotton is affected by *M. incognita*, while peanuts are not a host for this nematode species. Therefore, rotating cotton and peanut crops helps lower the density of the *M. incognita* population (Hajihassani et al., 2018). According to Star et al. (2002), two-year rotations between peanut crops and either bahiagrass or velvet grass are also effective against RKN (Starr et al., 2002).

In general, plant-parasitic nematodes can survive in patchy clusters throughout a field. However, their distribution may change depending on soil texture, plant growth, and the exact nematode species in question. By sampling according to a systematic grid, researchers can determine where nematodes are located within a field. If the nematode population reaches the economic threshold level, nematicides should be used (Hajihassani et al., 2018). Studies have

shown that the nematicides aldicarb (granular) and 1,3-dichloropropene (a fumigant) are successful against RKN (Starr et al., 2002). Ultimately, controlling RKN populations requires a combination of all of these strategies as part of an integrated pest management plan (Escobar & Fenoll, 2015).

**Genome Wide Association Studies**

Traditional breeding methods have been very successful at developing new cultivars throughout the 10,000-year history of plant domestication. Moreover, since the middle of the 1990s, the use of traditional pre-genomic breeding methods has yielded improvements to modern cultivars that allow for a dramatic increase in staple crop yields. These days, genomic tools and other new plant breeding technologies make it possible to study the genotypes associated with desirable phenotypes. Developments in next generation sequencing (NGS) and bioinformatics have facilitated the mass sequencing of genomes and transcriptomes as well as the identification of new regulatory sequences, molecular markers, and their loci (Pérez-de-Castro et al., 2012).

The detection of a quantitative trait locus (QTL) depends on a linkage analysis; however, it is restricted by the number of recombination possible per generation that are needed to improve the mapping population (Brachi et al., 2011) Linkage disequilibrium mapping, also known as association mapping, is a new and effective way to map complex traits. Relying on statistics, this method can detect the strength of the linkage between a marker locus and trait. Nowadays, association mapping may be categorized into two approaches. The first is candidate gene association, which requires comprehension of biochemistry and trait genetics. The second is genome wide association study (GWAS), which is also called a whole-genome scan (Pérez-de-Castro et al., 2012).

The first successful GWAS occurred in 2002 and involved the identification of the susceptibility gene for myocardial infarctions (Ikegawa, 2012). Over the past fifteen years, genome wide association studies have continued to evolve into powerful tools for investigating the genetic architecture of common diseases and for improving agriculture. For example, Genome wide association studies can help researchers understand the genetics of disease resistance in both wild and cultivated plants (Bartoli & Roux, 2017). Moreover, methods are now available to allow for GWAS on large samples within reasonable timeframes. This allows for the quick discovery of thousands of single nucleotide polymorphisms (SNPs) (Zhao et al., 2016). Unlike genome wide association studies in humans, genome wide association studies in plants have been successful, particularly with respect to rice and maize (Brachi et al., 2011).

In order to conduct an association mapping of seed quality traits in peanuts, Wang et al. (2011) evaluated 94 *A. hypogaea* mini-core collection germplasm accessions with 81 simple sequence repeat (SSR) markers and two functional SNP markers made from fatty acid desaturase 2. The authors concluded that the peanut mini-core set is appropriate for association mapping studies. Later, Pandey et al. (2014) used GWAS to investigate 300 genotypes for 36 traits including disease resistance, oil content and quality, drought tolerance, yield components, and overall yield. More recently, GWAS on peanuts tested 158 genotypes for 11 important agronomic traits. In doing so, the study explored the complex genetic relationship between agronomic traits and domestication processes in peanuts (Zhang et al., 2017).

**Objectives**

This research aims to screen 161 accessions for RKN resistance in peanuts and to define the SNP markers responsible for resistance. More specifically, this project will:

1) Evaluate 161 accessions for RKN resistance in peanut germplasm maintained in a greenhouse.

2) Identify SNP markers associated with RKN resistance via a genome-wide association study.

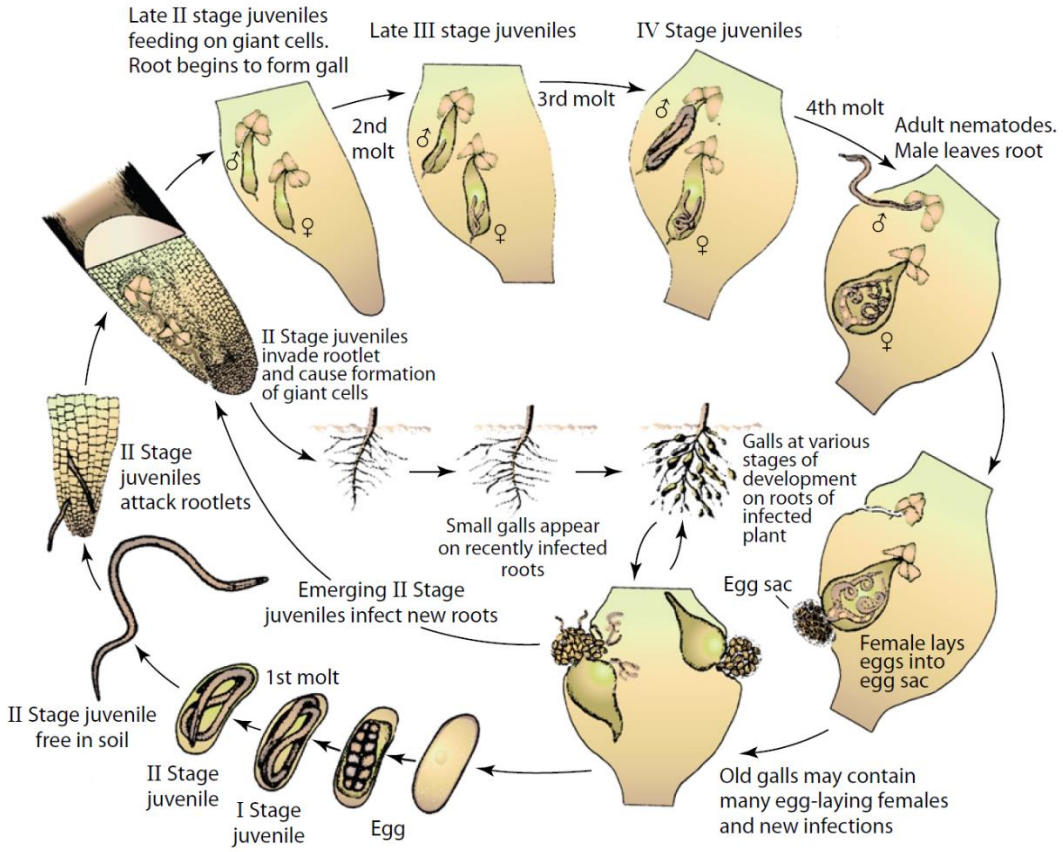



**Figure 1:** Disease cycle of root-knot caused by nematodes of the genus *Meloidogyne* (Agrios, 2005; reprinted with permission from Elsevier)

## REFERENCES


Acquaah, G. (2012). *Principles of plant genetics and breeding.* 2nd ed. Hoboken, NJ: Wiley.

Agrios, G. (2005). *Plant Pathology*. 5th Edition, Elsevier Academic Press, Amsterdam.

Bartoli, C., & Roux, F. (2017). Genome-Wide Association Studies in Plant Pathosystems: Toward an Ecological Genomics Approach. *Frontiers in plant science* 8: 763.

Brachi, B., Morris, G.P., & Borevitz, J.O. (2011). Genome-wide association studies in plants: The missing heritability is in the field. *Genome Biology* 12(10), 232.

Davis, R., & Webster, T. (2005). PLANT PATHOLOGY AND NEMATOLOGY Relative Host Status of Selected Weeds and Crops for Meloidogyne incognita and *Rotylenchulus reniformis*. *Journal of Cotton Science* 9, 41–46.

Dickson D.W., & Waele, D.D. (2005). Nematode parasites of peanut nematode parasites of cotton and other tropical fibre crops. In: Luc M, Sikora RA, Bridge J (eds) *Plant parasitic nematodes intropical and subtropical agriculture*. (2nd ed., pp. 393-436).CAB International, Wallingford.

Dickson, D. W. (1985). Nematode Diseases of Peanut. *Nematology Circular*,121.

do Nascimento, E.F.M.B., dos Santos, B.V., Marques, L.O.C., Guimarães, P.M., Brasileiro, A.C.M., Leal-Bertioli, S.C.M., Bertioli, D.J., & Araujo, A.C.G. (2018). The genome structure of Arachis hypogaea (Linnaeus, 1753) and an induced Arachis allotetraploid revealed by molecular cytogenetics. *Comparative Cytogenetics* 12(1), 111–140.

Dong, W. B., Holbrook, C.C., Timper, P., Brenneman, T.B., Chu, Y., & Ozias-Akins, P. (2008). Resistance in peanut cultivars and breeding lines to three root-knot nematode species. *Plant Disease* 92, 631-638.

Dong, W., Holbrook, C.C., Timper, P., Brenneman, T.B., & Mullinix, B.G. (2007). Comparison of Methods for Assessing Resistance to *Meloidogyne arenaria* in Peanut. *Journal of Nematology* 39(2), 169–175.

Dufour, R., Earles, R., Kuepper, G., & Greer, L. (1998). Alternative Nematode Control. Elsevier Academic Press Is an Imprint of Elsevier 1, 1-26.

Escobar, C., & Fenoll, C. (2015). *Advances in botanical research*. London: Academic Press 76, 1-24.

Garcia, G.M., Stalker, H.T., Shroeder, E., & Kochert, G. (1996). Identification of RAPD, SCAR, and RFLP markers tightly linked to nematode resistance genes introgressed from Arachis cardenasii into Arachis hypogaea. *Genome* 39, 836–845.

Grabau, Z.J., & D.W. Dickson. (2018). Management of Plant-Parasitic Nematodes in Florida Peanut Production1. UF/IFAS Extension ENY069.

Hajihassani A., Lawrence K.S., & Jagdale G.B. (2018) Plant Parasitic Nematodes in Georgia and Alabama. In: Subbotin S., Chitambar J. (eds) Plant Parasitic Nematodes in Sustainable Agriculture of North America. Sustainability in Plant and Crop Protection. *Springer* 2, 357-385.

Holbrook, C. C., Ozias-Akins, P., Chu, Y., Culbreath, A.K., Kvien, C.K., & Brenneman, T.B. (2017). Registration of 'TifNV-High O/L' Peanut. *J. Plant. Reg* 11, 228-230.

Holbrook, C. C., Timper P., Culbreath, A.K., & Kvien, C.K. (2008). Registration of 'Tifguard' peanut. *Journal of Plant Registrations* 2, 92-94.

Ikegawa, S. 2012. A Short History of the Genome-Wide Association Study: Where We Were and Where We Are Going. *Genomics & Informatics* 10(4), 220.

Jones, J. T., Haegeman, A., Danchin, E.G., Gaur, H.S., Helder, J., Jones, M.G., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J.E., Wesemael, W.M., & Perry, R.N. (2013). Top 10 plant-parasitic nematodes. *Molecular Plant Pathology* 14, 946-961.

Kenneth, H.G., & Curtis, R.J. (1973). Peanuts--culture and uses: Chapter 13-Peanut diseases. Stillwater, OK: American Peanut Research and Education Association.

Lambert, K. & Bekal, S. (2002). Introduction to Plant-Parasitic Nematodes. The Plant Health Instructor.

Mitkowski, N.A., & Abawi G.S. (2003). Root-knot nematodes. The Plant Health Instructor.

Nagy, E., Chu, Y., Guo, Y. Khanal, S., Tang, S., Li, Y., Dong, W., Timper, P., Taylor, C., Ozias-Akins, P., Holbrook, C.C, Beilinson, V., & Nielsen, N., Stalker, H., & Knapp, S. (2010). Recombination is suppressed in an alien introgression in peanut harboring Rma, a dominant root-knot nematode resistance gene. *Molecular Breeding* 26, 357-370.

National Peanut Board. Peanut types. [Online]. (Verified 17Nov.2017).Available at http://nationalpeanutboard.org/peanut-info/peanut-types.htm

Pandey, M. K., Upadhyaya, H.D., Rathore, A., Vadez, V., Sheshshayee, M.S., Sriswathi, M., Govil, M., Kumar, A., Gowda, M.V., Sharma, S., Hamidou, F., Kumar, V.A., Khera, P., Bhat, R.S., Khan, A.W., Singh, S., Li, H., Monyo, E., Nadaf, H.L., Mukri, G., Jackson, S.A., Guo, B., Liang, X., & Varshney, R.K. (2014). Genomewide association studies for 50 agronomic traits in peanut using the 'reference set' comprising 300 genotypes from 48 countries of the semi-arid tropics of the world. *PloS one* 9(8), e105228.

Pérez-de-Castro, A. M., Vilanova, S., Cañizares, J., Pascual, L., Blanca, J.M., Díez, M.J., Prohens, J., & Picó, B. (2012). Application of genomic tools in plant breeding. Current genomics. 13(3): 179-95.

Rich, J., & Tillman, B. (2009). Root-Knot Nematode Resistance in Peanut1. UF/IFAS Extension ENY057.

Robertson, L., Díez-Rojo, M. A., López-Pérez, J. A., Piedra Buena, A., Escuer, M., López Cepero, J., Martínez, C., & Bello, A. (2009). New host races of Meloidogyne arenaria, M. incognita, and M. javanica from horticultural regions of Spain. *Plant Dis* 93, 180-184.

Sasser, J.N., Eisenback, J.D., Carter, C.C., & Triantaphyllou, A.C. (1983). The International meloidogyne project-Its goals and accomplishments. [Online] Available https://www.researchgate.net/profile/Jonathan_Eisenback/publication/234837889_The_International_Meloidogyne_ProjectIts_Goals_and_Accomplishments/links/0fcfd51277c243929f000000/The-International-Meloidogyne-Project-Its-Goals-and-Accomplishments.pdf

Simpson, C.E. (1990). Pathways for introgression of pest resistance into Arachis hypogaea L. *Peanut Science* 18, 22–26.

Hammons, R.O., Herman, D., Stalker, H.T. Origin and Early History of the Peanut. In: Peanuts: Genetics, Processing, and Utilization. Stalker, H. T., Wilson R. F. (Eds). (2016). Peanuts Genetics, Processing, and Utilization. American Oil Chemists' Society Press, 1-22.

Starr, J.L., Morgan, E., & Simpson, C.E. (2002). Management of the peanut root-knot nematode, Meloidogyne arenaria, with host resistance. [Online] Plant Health Progress. doi: 10.1094/PHP-2002-1121-01-HM.

Tallury, S.P. 2017. Peanut (Arachis hypogaea L.): Origin and botanical descriptions. In: Varshney, R. K., Pandey, M.K., & Puppala, N. The Peanut Genome (pp. 27-31). Springer International Publishing, Cham.

Tillman B.L., & Stalker, H.T. (2009). Peanut. In: Vollmann J., Rajcan, I. (eds). *Oil Crops*. Handbook of Plant Breeding. (vol 4, pp. 287-315). Springer, New York, NY

US Department of Agriculture (USDA) - National Agricultural Statistics Service (NASS). (Online). Available at https://quickstats.nass.usda.gov/results/22E5CD7F-2BA4-3718B2C4C5F5DF6B2A0E?pivot=short_desc (Verified 17Nov.2017)

US Department of Agriculture (USDA). (2018). Foreign Agricultural Service. World Agricultural Production. *Circular Series* WAP, 8-18

Vishwakarma, M. K., Kale, S.M., Sriswathi, M., Naresh, T., Shasidhar, Y., Garg, V., & Varshney, R.K. (2017). Genome-wide discovery and deployment of insertions and deletions markers provided greater insights on species, genomes, and sections relationships in the genus *Arachis*. *Frontiers in Plant Science* 8, 2064. http://doi.org/10.3389/fpls.2017.02064

Wang, M.L., Sukumaran, S., Barkley, N.A., Chen, Z., Chen, C.Y., Guo, B., Pittman, R.N., Stalker, H.T., Holbrook, C.C., Pederson, G.A., & Yu, J. (2011). Population structure and marker–trait association analysis of the US peanut *(Arachis hypogaea L.)* mini-core collection. *Theoretical and Applied Genetics 123(8),* 1307-1317.

Williamson, V. M., & Hussey S.R. (1996). Nematode pathogenesis and resistance in plants. *Plant Cell* 8, 1735–1745.

Zhang, X., Zhang, J. He, X., Wang, Y., Ma, X., & Yin, D. (2017). Genome-Wide Association Study of Major Agronomic Traits Related to Domestication in Peanut. *Frontiers in Plant Science* 8, 1611.

Zhao, Y., Zhang, C., Chen, H., Yuan, M., Nipper, R., Prakash, C.S., & He, G. (2016). QTL mapping for bacterial wilt resistance in peanut (*Arachis hypogaea L.*). *Molecular Breeding* 36, 13.

# CHAPTER TWO

## INTRODUCTION

The peanut is a globally important crop, both for smallholders as well as large commercial producers. Widely grown in tropical and subtropical regions, the peanut can be classified as either a grain legume or an oil crop. Worldwide, annual peanut production reaches around 46 million tons (Bilello, 2016). In the United States, specifically, peanuts are the 12th most valuable cash crop, boasting a total farm value of more than $1 billion (National Peanut Board, 2018).

Nematode damage is one of the most important factors affecting peanut production. A recent study identified root-knot nematodes (*Meloidogyne spp*.), in particular, as the most economically destructive genus of plant-parasitic nematodes. Root-knot nematodes include nearly 100 species. Of these, *Meloidogyne arenaria* is one of the most problematic for agricultural crop production (Jonesh et al., 2013). Moreover, *M. arenaria* race 1 is the primary root-knot nematode species that infects the peanut plant (Sasser et al., 1983).

The roots of plants infected with nematodes typically exhibit galls filled with *M. arenaria* females and their egg masses (Sasser et al., 1983). Root galls containing RKN eggs inhibit plant nutrient absorption, which results in slowed growth, stunting, and yellowish leaves. RKN-infected pegs weaken, eventually breaking during harvest, and this results in yield losses. By remaining in the soil, detached pods further reduce crop yields. Moreover, pod infections caused by root-knot nematodes result in low quality harvests (Starr et al., 2002).

Root-knot nematodes can be effectively controlled with Termik 15G applying mid-season and can be provided an increase in yield of about 130% in infested RKN field (Kemerait & Davis, 2003). Another viable method is crop rotation with cotton, velvet bean, or bahiagrass (Bridge & Starr, 2007).

Plants that possess RKN-resistant genes are able to limit nematode reproduction; consequently, nematode population density is lower in those cultivars. More specifically, resistant cultivars are able to decrease the number of galls that develop on their roots (Williamson, 1999). There was no resistant peanut cultivar for RKN until 2001. The first RKN-resistant peanut cultivar, COAN, was released in 2001; however, the second such a resistant cultivar, NemaTAM, was released shortly after in 2002. Unfortunately, both COAN and NemaTAM have low yield potential compared to parent Florunner planted in noninfected fields (Dong et al., 2008). As such, COAN and NemaTAM are rarely used in agricultural applications. However, in 2014 the USDA-ARS and the Georgia Agricultural Experiment Stations released TifNV-High-O/L, a newer cultivar that exhibits substantial resistance to both *M. arenaria* (Neal) Chitwood race 1 and TSWV. TifNV-High-O/L presented notably higher yields compared with extensively preferable susceptible Georgia-06G in nematode infested fields (Holbrook et al., 2017).

Still, there is continuous evolutionary pressure on disease-causing nematodes to overcome the genetic resistance of cultivars like COAN, NemaTAM, Tifguard. Indeed, if a plant has only one RKN-resistant gene, then RKNs will eventually evolve the ability to subvert that resistance, thereby leaving the plant susceptible to infection. In order to increase the longevity of RKN-resistance, breeders must continue to search for new resistance genes to combine them in multi-resistant variety.

Developing new cultivars with nematode resistance requires reliable and effective screening techniques that identify resistant progeny within segregated breeding populations. Breeding lines can be evaluated in naturally infested fields; however, seasonal restrictions and soil non-uniformity mean that standardized comparisons are not possible. Thus, field-based screening may not be ideal. The screening method used to identify RKN-resistant breeding lines should be capable of readily and reliably evaluating thousands of genotypes. Greenhouses are key tools in this pursuit because they allow screening to occur throughout the year. In addition, greenhouse-based screening allows for important standardizations like sterilized soil and a uniform inoculum level (Boerma & Hussey, 1992). Data obtained from greenhouse-based testing can be analyzed with GWAS. As GWAS can directly use available genotype and phenotype data, researchers save time and resources. Moreover, GWAS can help identify the genes that confer RKN-resistance to different cultivars. This research aims to screen 161 accessions for RKN resistance in peanuts and to define the SNP markers responsible for resistance.

## MATERIALS AND METHODS

This experiment consisted of 161 accessions including 124 from the U.S. peanut mini-core collection and 37 from commercial cultivars and breeding lines. These accessions covered six botanical varieties: *fastigiata*, *hypogaea*, *peruviana*, *vulgaris*, *aequatoriana*, and *hirsute* (Figure 4). TifNV-High-O/L was selected as the resistant control. Tested accessions were classified into three groups based on the number of eggs per gram of root fresh weight (Eggs/g RFW): resistant, moderately resistant, and susceptible.

### Phenotyping in Greenhouse

The 161 peanut accessions were germinated on germination paper for 4 days, and then one peanut seed was transplanted into one cone-tainer (150cc) each containing soil (33.3%) and sandy mixture (66.3%) on June 15th, 2018. One day after planting, the plants were inoculated with 1 ml of nematode suspension; each suspension contained 3000 eggs. Plants were watered regularly to keep up soil moisture. A randomized complete block design with three replications for each sample was utilized for this research at the Plant Science Research Center and maintained 27°C ± 1. One month after planting, plant height (PH), shoot fresh weight (SFW), and root fresh weight (RFW) were measured. For the dried plant weight, shoot fresh plant were stored with 70 ° C for 2- 3 days or until the consistent weight was obtained, and then they were measured.

**Extraction of Nematode Eggs from Plant Roots**

Washed peanut roots were blotted, weighed, and then placed into beakers. Roots were covered with 0.625% NaOCI solution and stirred with a motorized stirrer for 4 minutes (Hussey & Barker, 1973). In order to collect eggs, roots were rinsed in a 75 μm pore sieve nested within a 25 μm pore sieve. The liquid remaining in the 25 μm pore sieve was poured very slowly into a clean cup. Sucrose solution (454g sugar/1L water) was added to the sample collected in the cup. The contents of the cup were mixed until homogenous, and then transferred into new tubes. These were placed into a centrifuge at 1400 rpm for 1 minute. After centrifuging, the liquid that separated to the top of each tube was again passed through the mesh sieves (75 μm pore sieve nested within 25 μm pore sieve). The liquid remaining in the 25 μm pore sieve was poured very slowly into a clean cup. Finally, the number of nematode eggs collected within the liquid were counted under the Nikon TS100 inverted microscope.

**DNA Extraction, Genotyping and Quality Control**

Plant samples were taken from grown plants in the greenhouse and protected at -80 °C for DNA extraction. The modified CTAB method was used for DNA extraction (Porebski et al., 1997). Purified DNA was dissolved in TE buffer for the next analysis. The ND 2000 was used to measure the quantity and quality of DNA.

GeneSeek (Lincoln, Nebraska, USA) conducted the genotyping by using SNP array (Affymetrix). The call rate for a given SNP is the proportion of individuals that do not lack corresponding SNP information. Samples of low quality or with a low call rate were excluded (< 0.95). Following filtering, SNPs were retained at a <0.95 minor allele frequency <0.05, depending on the Mendelian law. STRUCTURE 2.2.3 was used to identify the optimal value of K.

**Statistical Analysis**

All data were performed by using SAS 9.4 PROC GLIMMIX (SAS Institute Inc., Cary, NC), and LS-means were compared between accessions and replications using Tukey-Kramer's method with a significance level of $P \leq 0.05$. Dependent variables were plant height (PH), root fresh weight (RFW), shoot fresh weight (SFW), the number of *M. arenaria* eggs per gram of the root fresh weight (Eggs/g RFW), and biomass (BM). Independent variable was genotypes. A log transformation was applied to Eggs/g RFW the normal assumption. The LS-means estimates for the lognormal distribution function were back transformed to the original data by using PROC MEANS. The ANOVA table and associated P-values was created separately for each trait.

**Genome-Wide Association Analysis**

116 peanut genotypes were used in association analyses using TASSEL 5.0 software. The general linear model (GLM) comprises the principal component analysis (PCA) model. PCA is a potential approach that can be used in GWA studies and raise the power of QTL detection. The threshold of significance level between traits and SNPs was determined as $P$ <0.001, (for example, $-\log_{10}(p) = 3.0$) (Zhang et al., 2015; Zhang et al., 2016; Li et al., 2017). The regions approximately 1 Mb upstream and downstream of peak SNPs were checked for candidate genes associated with the traits of interest (database at https://peanutbase.org). BLAST was used to find the gene positions on the physical map.

The GWAS results were visualized with Manhattan and quantile-quantile (Q-Q) plots that were generated using R package qqman (Figure 3). In Figure 3, the alternating orange and blue dots represent SNPs mapped to different chromosomes. Dots above the red horizontal line are SNPs with $P$-value < 0.001.

## RESULTS AND DISCUSSION

A total of 161 accessions were screened for RKN in the greenhouse. There was a statistically significant difference in the mean of PH, SFW, RFW, Eggs/g RFW, and BM between genotypes and replications ($P \leq 0.05$). At $P \leq 0.01$, there was no difference in RFW among replications and in Eggs/g RFW among genotypes (Table 1). Means that have more than 2500 Eggs/g RFW are significantly different from each other (Table 6); however, mean of PH (Table 6), RFW, SFW, and BM are significantly different from each in own accessions of its group (Tukey–Kramer test, $P > 0.05$) (Table 7). Mean of all traits were shown with frequency distribution in Figure 2.

A plant's resistance or susceptibility to plant-parasitic nematodes can be measured by nematode reproductive success (Cook and Evans, 1987). Thus, for the classification of resistance, the ability to produce nematodes was considered, and TifNV-High-O/L was determined as resistant control. More specifically, accessions were classified as resistant (R), moderately resistant (MR), and susceptible (S) based on Eggs/g RFW (Table 2). Compared to the resistant control, eleven accessions could be classified resistant or moderately resistant to *M. arenaria*. The number of Eggs/g RFW varied between 95 and 7129, with genotype PI 370331 having the least and genotype PI 494034 having the most. Genotypes PI 370331, Lot4-37Line-2, PI390428, PI497648, PI268868, PI295309, and PI407667 were classified as R because their number of Eggs/g RFW were fewer than that of TifNV-High O/L (except for Fla-07 with 190 Eggs/g RFW). In addition, genotypes PI 461434, AU-17, and PI 493938 were classified as MR because their

number of Eggs/g RFW were around 150% of that in TifNV-High-O/L. Genotypes with a higher number of Eggs/g RFW than this were categorized as S. All genotypes whose number of Egg/g RFW $\leq$ 807 are listed in Table 2.

In order to identify genetic loci related to resistance, five traits (PH, SFW, RFW, Eggs/g RFW, and BM) were examined. In total, 46 QTLs associated with four traits reached the corrected *P*-value ($p < 0.001$, $-\log_{10}(p) = 3.0$). No QTL was significantly related to SFW (Table 3). Forty-six quantitative trait loci (QTLs) located on twelve different chromosomes underlying root-knot nematode resistance were determined with phenotypic variation explained (PVE) between 7.8% and 17% by GWAS from greenhouse data (Table 4). Distribution of 46 QTLs on 12 different chromosomes illustrate that 27 QTLs were on the A sub-genome while 19 QTLs took part in the B sub-genome (Table 3). However, B07 contained the highest quantity with 11 QTLs, and the next one is A07 with 9 QTLs. Besides this, there were no QTL on several chromosomes, namely, A02, A03, A10, B01, B02, B03, B04, and B05. Overall, the A sub-genome had more resistant regions than the B sub-genome. Pandey et al. (2017) similarly found that the A sub-genome hosts a large number of resistant genes. That is, out of 42 total QTLs, 34 were located on the A sub-genome, while 8 were located on the B sub-genome (Pandey et al., 2017). Likewise, Bertioli et al. (2016) found more the nucleotide-binding (NB) and leucine-rich repeat (LRR) encoding disease resistant genes on the A sub-genome (397) than on the B sub-genome (345).

Out of 46 QTLs, 957 candidate genes detected including 520 genes on A sub-genome and 437 genes o on B sub-genome. B07 contained the greatest number of genes (210 genes, 21.94%), and also A07 and A08 included 124 and 126 genes corresponding to 12.95 and 13.16%, respectively. Specifically, 26 out of 957 candidate genes related to LRR encoding gene were found on chromosomes A01, A04, A05, B07, B08, and B10 (Table 5). Moreover, 80.76% of these LRR

encoding genes took part in chromosome B07 at location 2810620 surrounding 1 Mb. Most disease resistance in plants is conferred by genes of the nucleotide binding site-leucine-rich repeat (NBS-LRR) class. LRR domains are found in various protein groups, including among process regulators that both control development and plant defense (Knepper and Day, 2010). QTL for resistance to *M. arenaria* (Neal) Chitwood 1 was found on chromosome A02 of *Arachis stenosperma* V10309. This included a cluster of 38 NB-LRR–encoding genes covering 6.1 Mb. Another source of nematode resistance, which has been widely used in the United States arises from the introgression of the A-genome species *Arachis cardenasii* (Bertioli et al., 2016). More specifically, a gene called *Rma* is assumed to be a dominant gene related to RKN introduced into *Arachis hypogaea* from TxAG-6 such as the superfamily of NBS-LRR encoding R genes. The R gene in wild peanut was located on chromosome A09 and B09. Moreover, *Mag* and *Mae*, which are also genes that confer resistance to RKN, are associated with *Rma* (Nagy et al., 2010).

# CONCLUSION

Root-knot nematodes are some of the most economically destructive pathogens in peanut production areas of the Southern USA. The identification of genes that confer resistance to RKNs will guide researchers in future screening and mapping experiments of the peanut genome, moreover, help to eliminate this disease. As a result of the evaluation of peanut germplasm in the greenhouse experiment, in the total, eleven accessions were identified as resistant or moderately resistant to RKN compared with resistant control TifNV-High-O/L. These genotypes may be beneficial to future breeding efforts aimed at RKN prevention. The results of GWAS, the R gene located on the A09 and B09 chromosomes, a dominant root-knot nematode resistance gene was not found to be among the tested QTLs. Nonetheless, this study identified 26 candidate genes related to LRR-encoding gene on chromosomes A01, A04, A05, B07, B08, and B10. This thesis research will extend the knowledge on the sources of resistance to root-knot nematode in peanut as well as give a lead for improvement of resistant peanut cultivar.

**Table 1**: Analysis of variance of plant height, shoot fresh weight, root fresh weight, eggs/g root fresh weight and biomass

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Plant Height** | | | | | |
| Genotype | 160 | 10351.6413 | 64.69776 | 3.26 | <.0001 |
| Replication | 2 | 495.31388 | 247.65694 | 12.49 | <.0001 |
| **Shoot Fresh Weight** | | | | | |
| Genotype | 160 | 3029.03393 | 18.931462 | 1.73 | <.0001 |
| Replication | 2 | 290.661515 | 145.330757 | 13.29 | <.0001 |
| **Root Fresh Weight** | | | | | |
| Genotype | 160 | 812.175423 | 5.0760964 | 1.83 | <.0001 |
| Replication | 2 | 21.1558918 | 10.5779459 | 3.81 | 0.0231 |
| **Eggs/g RFW** | | | | | |
| Genotype | 160 | 794337449 | 4964609.1 | 1.36 | 0.0106 |
| Replication | 2 | 255031899 | 127515949 | 35.03 | <.0001 |
| **Biomass** | | | | | |
| Genotype | 160 | 123.883551 | 0.7742722 | 1.5 | 0.0013 |
| Replication | 2 | 34.9169084 | 17.4584542 | 33.79 | <.0001 |

**Table 2**: Resistance classification for peanut genotypes tested in the greenhouse.

| PI# | Egg/g RFW | RL | PI # | Egg/g RFW | RL | PI# | Egg/g RFW | RL | PI# | Egg/g RFW | RL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PI 370331 | 95 | R | PI 476025 | 603 | S | Valencia | 491 | S | PI 338338 | 631 | S |
| Lot4-37 Line-2 | 111 | R | PI 482120 | 325 | S | Grif 12579 | 496 | S | Florunner | 644 | S |
| PI 390428 | 123 | R | PI 290620 | 328 | S | PI 475863 | 506 | S | PI 270998 | 655 | S |
| PI 497648 | 127 | R | PI 259658 | 334 | S | Grif 12545 | 509 | S | PI 494018 | 666 | S |
| PI 268868 | 133 | R | PI 196705 | 335 | S | NM Val | 513 | S | PI 290536 | 672 | S |
| PI 295309 | 139 | R | PI 270905 | 345 | S | Grif 14051 | 516 | S | PI 502111 | 674 | S |
| PI 407667 | 149 | R | PI 157542 | 352 | S | PI 576634 | 527 | S | PI 493717 | 674 | S |
| **TifNV-High-O/L** | **174** | **R** | C99R | 372 | S | PI 290560 | 527 | S | PI 240560 | 687 | S |
| Fla-07 | 190 | R | PI 576636 | 403 | S | C76-16 | 530 | S | PI 481795 | 692 | S |
| PI 461434 | 229 | MR | PI 331297 | 403 | S | PI 155107 | 542 | S | PI 576614 | 695 | S |
| AU-17 | 239 | MR | PI 482189 | 409 | S | PI 200441 | 545 | S | EXP27-1516 | 705 | S |
| PI 493938 | 243 | MR | PI 158854 | 431 | S | G06G | 563 | S | PI 298854 | 708 | S |
| PI 471954 | 262 | S | PI 493880 | 454 | S | PI 442768 | 575 | S | PI 343384 | 714 | S |
| PI 290594 | 276 | S | PI 355271 | 459 | S | PI 152146 | 579 | S | Lot5-101/Line-8 | 730 | S |
| PI 162655 | 288 | S | PI 313129 | 462 | S | PI 268696 | 581 | S | PI 162857 | 738 | S |
| PI 372305 | 292 | S | PI 268847 | 468 | S | PI 478819 | 581 | S | PI 648241 | 740 | S |
| PI 259851 | 304 | S | PI 372271 | 475 | S | PI 648242 | 583 | S | CG7-A | 746 | S |
| PI 268755 | 309 | S | PI 355268 | 477 | S | PI 295250 | 595 | S | PI 496448 | 776 | S |
| FL-279 | 311 | S | PI 296550 | 477 | S | PI 288146 | 604 | S | SPT06-6 | 788 | S |
| VC-2 (1) | 319 | S | NC-3033 | 482 | S | PI 496401 | 612 | S | PI 290566 | 805 | S |
| PI 648250 | 320 | S | PI 259748 | 488 | S | PI 268586 | 627 | S | Ga Green | 807 | S |

RL: Resistant Level

R: Resistant, < eggs/g RFW of TifNV-High O/L and
eggs/g RFW up to "TifNV-High O / L + 10% of TifNV-High O / L" ≥ eggs/g RFW

MR: Moderately resistant, eggs/g RFW up to "TifNV-High O / L + 50% of TifNV-High O / L"

S: Susceptible, eggs/g root > 50% of TifNV-High O / L

**Table 3:** Distribution of QTLs in twelve chromosomes identified

| Trait | Marker | Chromosome | Position | P-Value | - $\log_{10}$ (*P* value) | PVE (%) |
|---|---|---|---|---|---|---|
| BM | AX-176809414 | A05 | 1.04E+08 | 4.84E-05 | 4.31 | 16.62 |
| BM | AX-176796238 | A05 | 1.04E+08 | 1.39E-05 | 4.85 | 15.89 |
| BM | AX-176794905 | A05 | 1.04E+08 | 4.12E-04 | 3.38 | 13.24 |
| BM | AX-176812683 | A07 | 18777607 | 8.32E-04 | 3.07 | 12.13 |
| Eggs/g RFW | AX-147219410 | A04 | 9307072 | 4.50E-05 | 4.34 | 14.93 |
| Eggs/g RFW | AX-176821623 | B06 | 2198106 | 8.23E-04 | 3.08 | 10.88 |
| Eggs/g RFW | AX-176822589 | B07 | 2810620 | 6.42E-04 | 3.19 | 8.90 |
| Eggs/g RFW | AX-177644370 | B08 | 1.24E+08 | 3.08E-04 | 3.51 | 10.26 |
| PH | AX-176805890 | A01 | 1580925 | 3.25E-04 | 3.48 | 11.17 |
| PH | AX-176796691 | A05 | 86185395 | 3.03E-04 | 3.51 | 9.29 |
| PH | AX-147225431 | A06 | 63883081 | 9.88E-04 | 3.00 | 7.80 |
| PH | AX-177637748 | A07 | 56104900 | 1.18E-04 | 3.92 | 12.54 |
| PH | AX-176822013 | A07 | 59068844 | 1.18E-04 | 3.92 | 12.54 |
| PH | AX-176821798 | A07 | 63657369 | 1.18E-04 | 3.92 | 12.54 |
| PH | AX-176812653 | A07 | 64217536 | 1.18E-04 | 3.92 | 12.54 |
| PH | AX-177639255 | A07 | 58292539 | 3.73E-04 | 3.42 | 11.06 |
| PH | AX-177639847 | A07 | 68894297 | 5.35E-04 | 3.27 | 10.59 |
| PH | AX-177637603 | A07 | 24206696 | 5.53E-04 | 3.25 | 10.54 |
| PH | AX-177637650 | A07 | 58745504 | 6.31E-04 | 3.19 | 8.37 |
| PH | AX-177637432 | A08 | 4160623 | 3.24E-06 | 5.48 | 16.96 |
| PH | AX-176822307 | A08 | 2405445 | 1.77E-05 | 4.75 | 14.91 |
| PH | AX-177638264 | A08 | 2401835 | 1.18E-04 | 3.92 | 12.54 |
| PH | AX-176821485 | A08 | 2924597 | 1.18E-04 | 3.92 | 12.54 |
| PH | AX-177637391 | A08 | 4130418 | 1.18E-04 | 3.92 | 12.54 |
| PH | AX-177637155 | A08 | 4160628 | 1.18E-04 | 3.92 | 12.54 |
| PH | AX-176794999 | A09 | 81732757 | 3.89E-04 | 3.41 | 8.90 |
| PH | AX-176810505 | A09 | 1.02E+08 | 3.89E-04 | 3.41 | 8.90 |
| PH | AX-176820309 | B06 | 50767609 | 9.88E-04 | 3.00 | 7.80 |
| PH | AX-147255972 | B07 | 99843248 | 5.14E-06 | 5.28 | 16.50 |
| PH | AX-177637764 | B07 | 51500083 | 1.17E-04 | 3.93 | 12.68 |
| PH | AX-147255754 | B07 | 57047298 | 1.18E-04 | 3.92 | 12.54 |
| PH | AX-176823112 | B07 | 1E+08 | 1.18E-04 | 3.92 | 12.54 |
| PH | AX-147255998 | B07 | 1.02E+08 | 1.18E-04 | 3.92 | 12.54 |
| PH | AX-147256091 | B07 | 1.06E+08 | 1.18E-04 | 3.92 | 12.54 |
| PH | AX-177637951 | B07 | 68777401 | 8.54E-04 | 3.06 | 9.97 |
| PH | AX-147256234 | B07 | 1.12E+08 | 9.88E-04 | 3.00 | 9.78 |
| PH | AX-177638959 | B07 | 88051816 | 9.28E-04 | 3.03 | 7.93 |
| PH | AX-177638466 | B07 | 24656861 | 9.52E-04 | 3.02 | 7.86 |
| PH | AX-177644247 | B08 | 9988581 | 7.04E-04 | 3.15 | 8.14 |
| PH | AX-177643492 | B09 | 1.39E+08 | 8.15E-04 | 3.08 | 8.12 |
| PH | AX-177639393 | B10 | 1.1E+08 | 4.08E-04 | 3.38 | 8.89 |
| PH | AX-177643787 | B10 | 95718840 | 9.88E-04 | 3.00 | 7.80 |

**Table 3**: Continued.

| Trait | Marker | Chromosome | Position | P-Value | - $\log_{10}$ (*P* value) | PVE (%) |
|---|---|---|---|---|---|---|
| RFW | AX-176794905 | A05 | 1.04E+08 | 2.22E-04 | 3.65 | 13.78 |
| RFW | AX-176806726 | A05 | 1.04E+08 | 7.47E-04 | 3.12 | 9.52 |
| RFW | AX-147223670 | A05 | 1.03E+08 | 7.51E-04 | 3.12 | 9.49 |

**Table 4:** Total number of QTLs associated with traits.

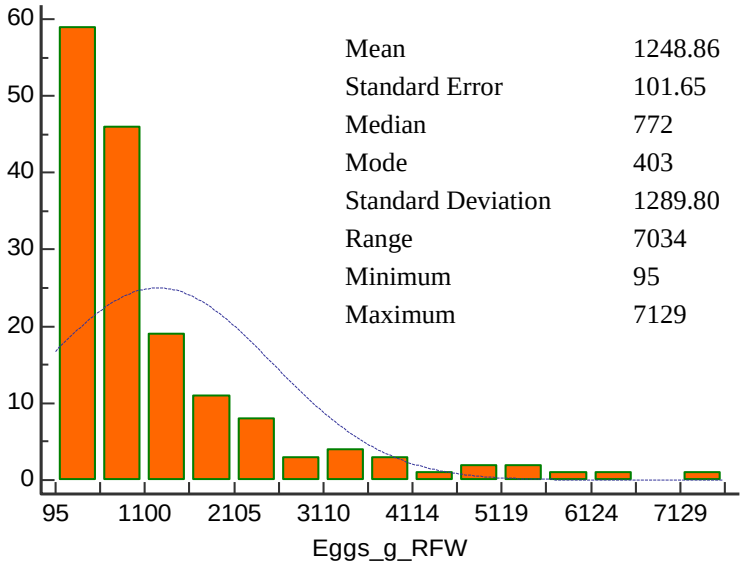
| Traits | Determined QTLs | - $\log_{10}$ (*P* value) | PVE (%) |
|---|---|---|---|
| BM | 4 | 4.85-3.07 | 16.62-12.13 |
| Eggs/g | 4 | 4.34-3.08 | 14.93-8.90 |
| PH | 35 | 5.48-3.00 | 16.96-7.81 |
| RFW | 3 | 3.65-3.12 | 13.78-9.49 |
| Total | 46 | 3.00-5.48 | 7.80-16.96 |

**Table 5:** 26 significant SNPs and candidate genes including LRR encoding genes associated with RKN.

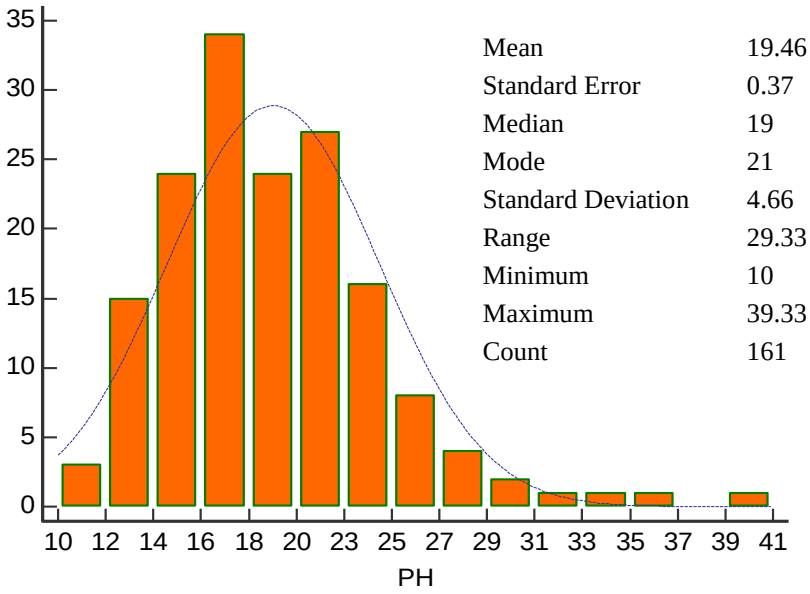
| Trait | SNP Location (bp) | Candidate Gene | Gene Location (bp) |
|---|---|---|---|
| **Egg/g RFW** | A04-9307072 | Arahy.CT5ZD3 | 9616353-9621745 |
| **Egg/g RFW** | B07-2810620 | Arahy.18C3F6 | 2756450-2763400 |
| **Egg/g RFW** | B07-2810620 | Arahy.21TZH2 | 2812840-2814981 |
| **Egg/g RFW** | B07-2810620 | Arahy.24K8LL | 2750311-2753514 |
| **Egg/g RFW** | B07-2810620 | Arahy.2ZUN3L | 2637378-2640386 |
| **Egg/g RFW** | B07-2810620 | Arahy.6DS8WC | 2669806-2670378 |
| **Egg/g RFW** | B07-2810620 | Arahy.6R79T7 | 2670436-2672808 |
| **Egg/g RFW** | B07-2810620 | Arahy.BBM6FL | 2909926-2919339 |
| **Egg/g RFW** | B07-2810620 | Arahy.DKBS4X | 2807891-2812032 |
| **Egg/g RFW** | B07-2810620 | Arahy.F3E022 | 2710902-2713877 |
| **Egg/g RFW** | B07-2810620 | Arahy.FXNF4I | 2359004-2369234 |
| **Egg/g RFW** | B07-2810620 | Arahy.HW8B30 | 2344577-2347990 |
| **Egg/g RFW** | B07-2810620 | Arahy.L85DV8 | 2719236-2722201 |
| **Egg/g RFW** | B07-2810620 | Arahy.R600FJ | 2692253-2695219 |
| **Egg/g RFW** | B07-2810620 | Arahy.S3Z82H | 2794965-2798706 |
| **Egg/g RFW** | B07-2810620 | Arahy.TTKZ9K | 2728271-2733731 |
| **Egg/g RFW** | B07-2810620 | Arahy.U2RA4L | 2347581-2350586 |
| **Egg/g RFW** | B07-2810620 | Arahy.V485GG | 2644198-2647224 |
| **Egg/g RFW** | B07-2810620 | Arahy.WH0DJX | 2811829-2812563 |
| **Egg/g RFW** | B07-2810620 | Arahy.S3Z82H | 2654758-2657706 |
| **Egg/g RFW** | B07-2810620 | Arahy.XTV6UZ | 2622342-2633564 |
| **Egg/g RFW** | B07-2810620 | Arahy.ZU31CU | 2687768-2691573 |
| **PH** | A01-1580925 | Arahy.ZKQR71 | 1679221-1680513 |
| **PH** | B08-9988581 | Arahy.VNIH7N | 10098971-10104661 |
| **PH** | B10-110263083 | Arahy.JVX2H3 | 109849739-109860797 |
| **RFW** | A05-103711123 | Arahy.IGT6GQ | 104068602-104072237 |
| **BM** | A05-104023520 | Arahy.IGT6GQ | 104068602-104072237 |

**Figure 2:** Frequency distribution of mean of all traits. **A**: Frequency distribution for eggs per gram of the root fresh weight. **B**: Frequency distribution for plant height. **C**: Frequency distribution for root fresh weight. **D**: Frequency distribution for shoot fresh weight. **E**: Frequency distribution for biomass.

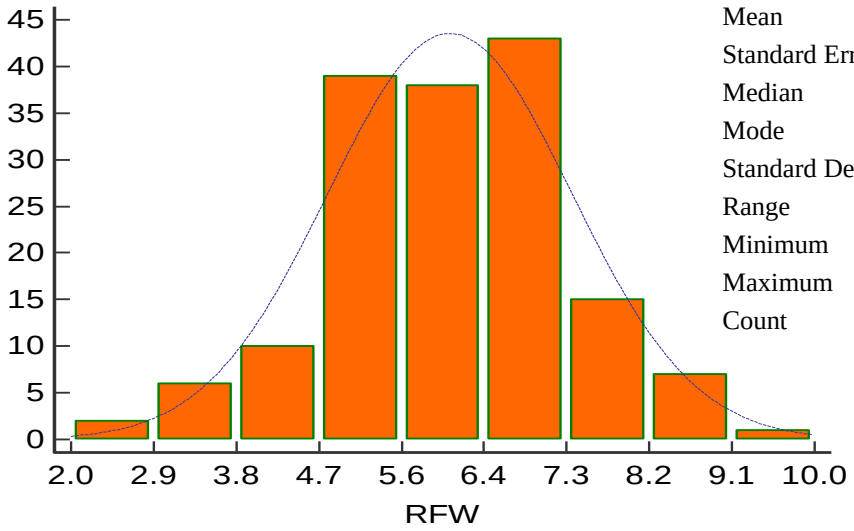
**A**




**B**

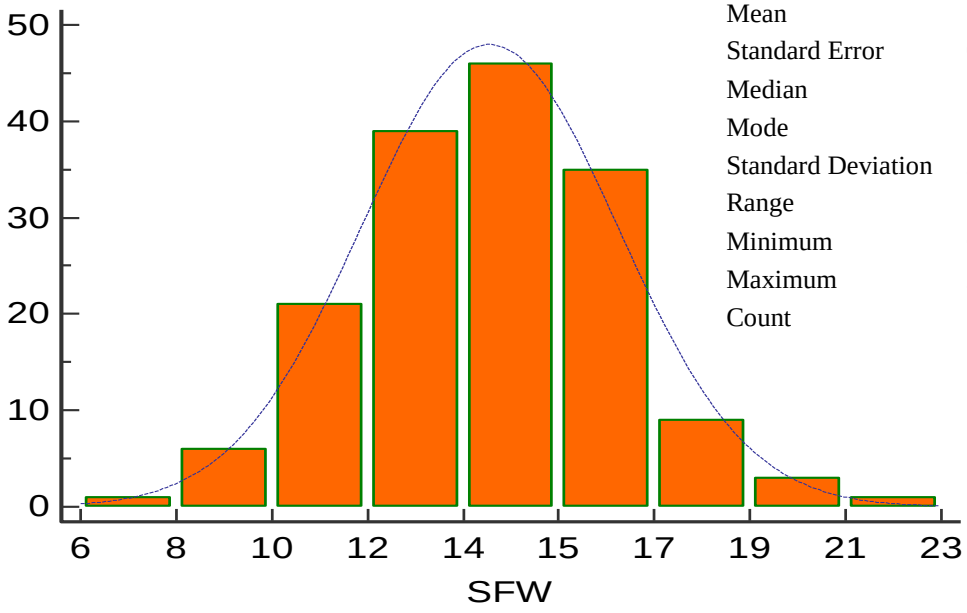


**C**




| | |
|---|---|
| Mean | 6.075 |
| Standard Error | 0.103 |
| Median | 6.200 |
| Mode | 6.447 |
| Standard Deviation | 1.312 |
| Range | 7.383 |
| Minimum | 2.207 |
| Maximum | 9.590 |
| Count | 161 |

**D**




| | |
|---|---|
| Mean | 14.08 |
| Standard Error | 0.199 |
| Median | 13.96 |
| Mode | 16.14 |
| Standard Deviation | 2.530 |
| Range | 15.50 |
| Minimum | 6.897 |
| Maximum | 22.39 |
| Count | 161 |

**E**

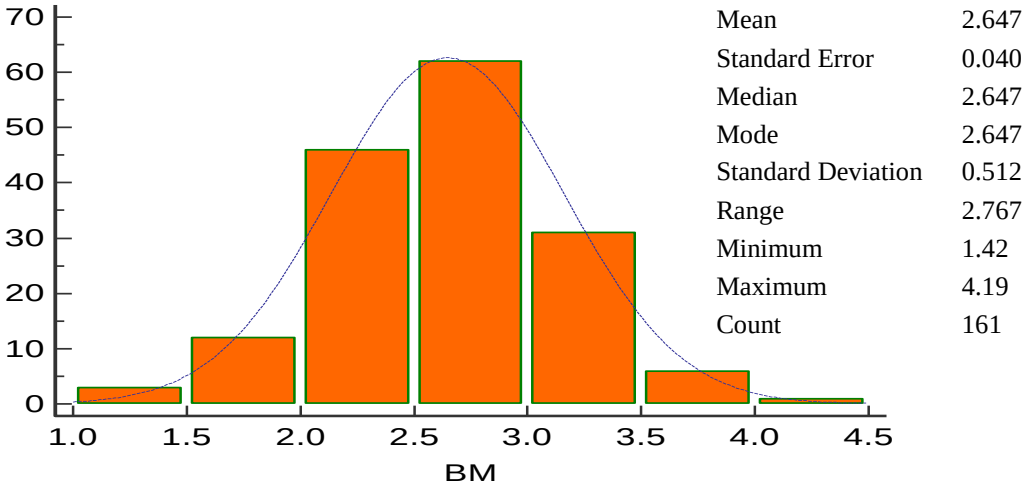
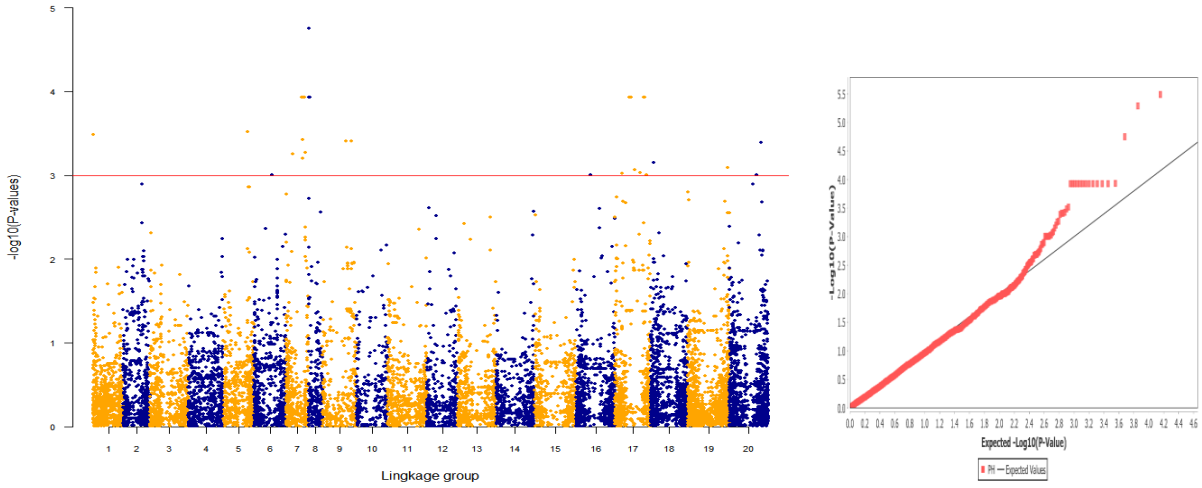
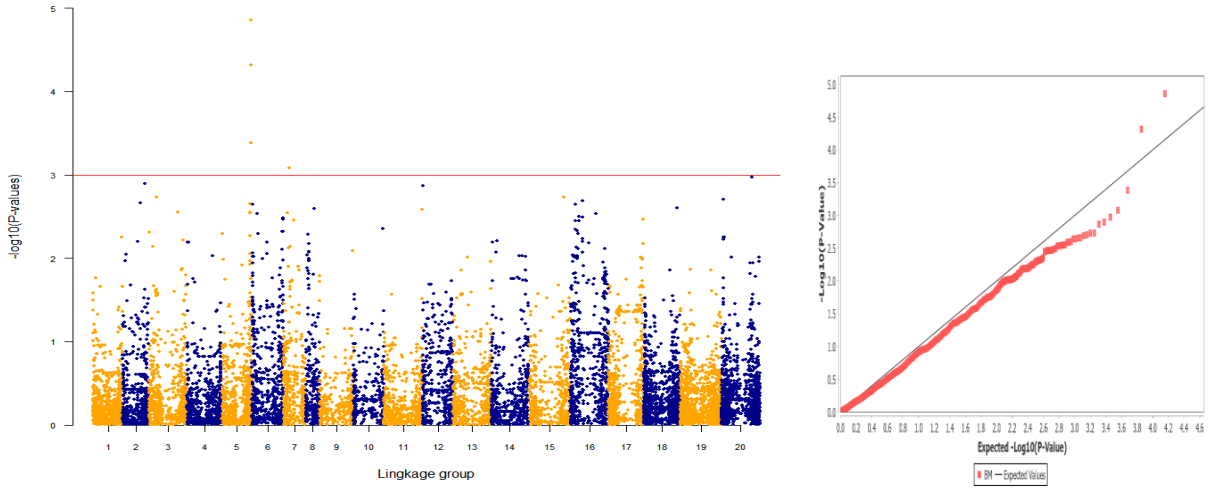



**Figure 3:** Manhattan plots of genome-wide association for RKN resistance. The red line demonstrates the genome-wide significant threshold: $-\log_{10}$(P value) = 3.0. **A:** P-values by linkage group and Q-Q plots for plant height. **B:** P-values by linkage group and Q-Q plots for biomass. **C:** P-values by linkage group and Q-Q plots for eggs per gram of the root fresh weight. **D:** P-values by linkage group and Q-Q plots for root fresh weight. **E:** P-values by linkage group and Q-Q plots for shoot fresh weight.

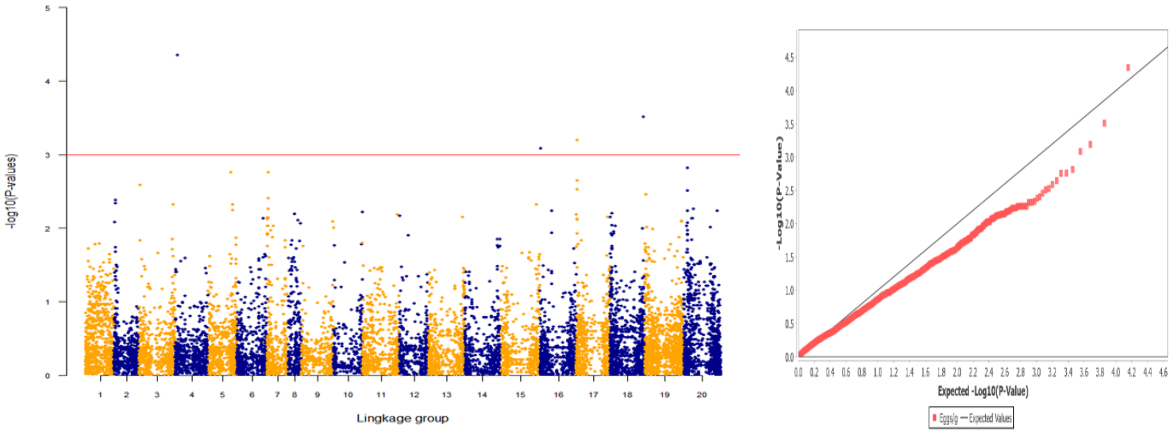
**A**



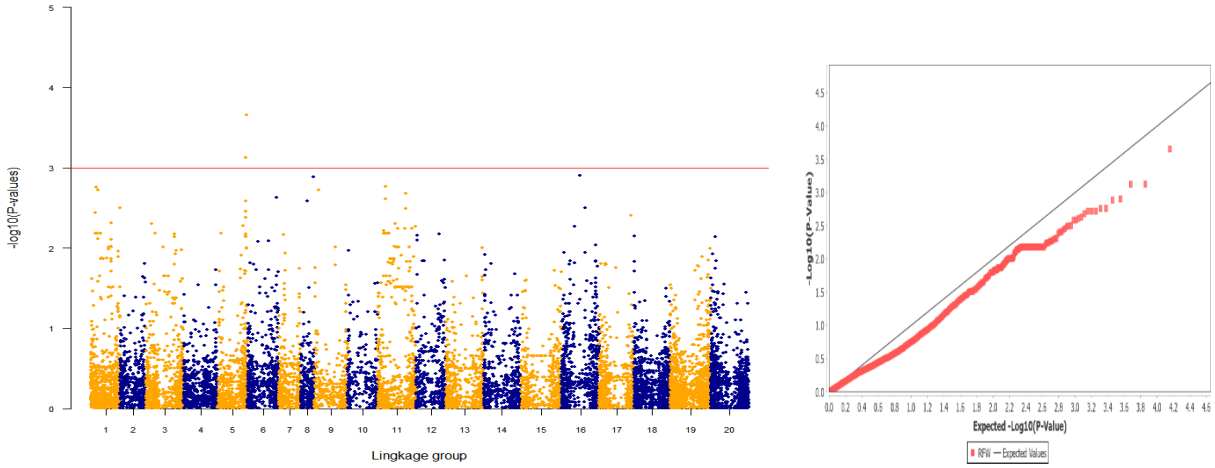
**B**


-log10(P-values)
Lingkage group
-Log10(P-Value)
Expected -Log10(P-Value)
BM — Expected Values

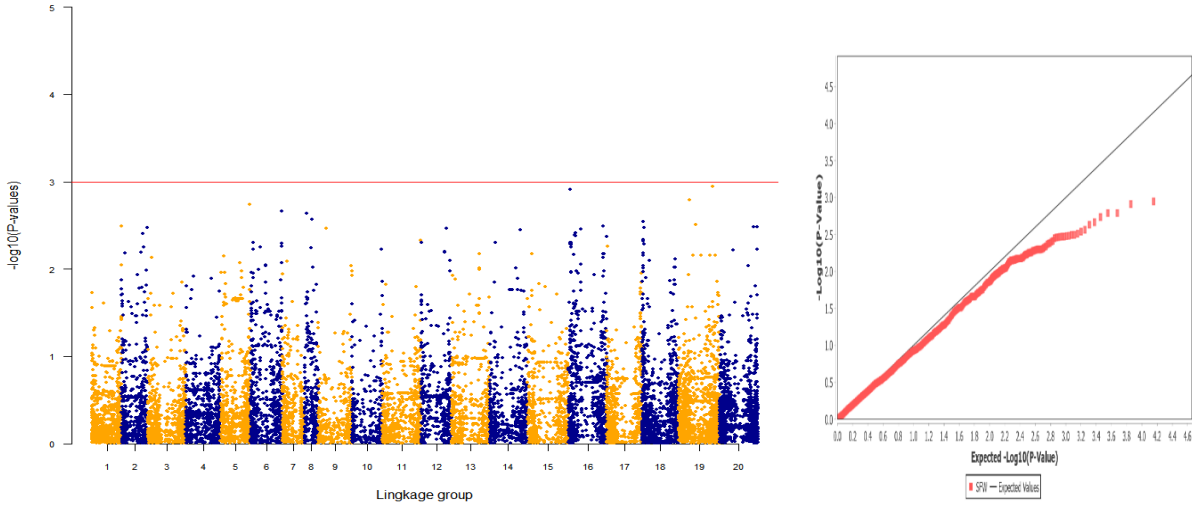
**C**


-log10(P-values)
Lingkage group
-Log10(P-Value)
Expected -Log10(P-Value)
Egg/g — Expected Values

**D**


-log10(p-values)
Lingkage group
-Log10(P-Value)
Expected -Log10(P-Value)
RPW
Expected Values

**E**


-log10(p-values)
Lingkage group
-Log10(P-Value)
Expected -Log10(P-Value)
SPW
Expected Values

## REFERENCES


Bertioli, D. J., Cannon, S. B., Froenicke, L., Huang, G., Farmer, A. D., Cannon, E. K. S., et al. (2016). The genome sequences of *Arachis duranensis* and *Arachis ipaensis*, the diploid ancestors of cultivated peanut. *Nature Genetics*. 48, 438–446.

Bilello, S. (2016). 21st Centruy Homestead*: Nitrogen-fixing crops*. Place of publication not identified: LULU COM.

Boerma, H. R., & Hussey, R. S. (1992). Breeding plants for resistance to nematodes. *Journal of Nematology*, 24:242-252.

Bridge, J., & Starr, J. L. (2007). *Plant nematodes of agricultural importance a color handbook*. Boston, MA: Academic Press.

Cook, R., & Evans, K. (1987). Resistance and tolerance. Pp. 179–231 in R. H. Brown and B. R. Kerry, eds. *Principles and practice of nematode control in crops*. Marrickville, NSW, Australia: Academic Press.

Dong, W. B., Holbrook, C. C., Timper, P., Brenneman, T. B., Chu, Y., & Ozias-Akins, P. (2008). Resistance in peanut cultivars and breeding lines to three root-knot nematode species. *Plant Disease.* 92, 631-638.

Holbrook, C. C., Ozias-Akins, P., Chu, Y., Culbreath, A.K., Kvien, C.K., & Brenneman, T.B. (2017). Registration of 'TifNV-High O/L' Peanut. *Journal of Plant Registrations*, 11, 228-230.

Hussey, R. S., & Barker, K. B. (1973). A comparison of methods of collecting inoculate of *Meloidogyne spp*., including a new technique. Plant Disease Reporter 57:1025-1028.

Jones, J. T., A. Haegeman, E.G. Danchin, H.S. Gaur, J. Helder, M.G. Jones., T. Kikuchi, R. Manzanilla-López, J.E. Palomares-Rius, W.M. Wesemael, & R.N. Perry. (2013). Top 10 plant-parasitic nematodes. *Molecular Plant Pathology* 14, 946-961.

Kemerait, R.C., & Davis, R.F. 2003. Evaluation of nematicides to reduce losses to root-knot nematode in peanut [abstract]. Fungicide and Nematicide Tests. 58 Report No. NO14.

Knepper, C., & Day, B. (2010). From perception to activation: the molecular-genetic and biochemical landscape of disease resistance signaling in plants. *The arabidopsis book*, *8*, e012. doi:10.1199/tab.0124

Li, H., Zhang, L., Hu, J., Zhang, F., Chen, B., Xu, K., Gao, G., Li, H., Zhang, T., Li, Z. and Wu, X. (2017). Genome-Wide Association Mapping Reveals the Genetic Control Underlying Branch Angle in Rapeseed (*Brassica napus L.*). *Frontiers in Plant Science*, 8, 1054.

Nagy, E., Chu, Y.Y., Guo, S., Khanal, & Tang, S. (2010). Recombination is suppressed in an alien introgression in peanut harboring *Rma*, a dominant root-knot nematode resistance gene. *Molecular Breeding* 26, 357-370.

National Peanut Board. Peanut types. [Online]. (Verified 15Nov.2018). Available at https://www.nationalpeanutboard.org/peanut-info/history-peanuts-peanut-butter.htm

Pandey, M. K., Wang, H., Khera, P., Vishwakarma, M. K., Kale, S. M., Culbreath, A. K., … Guo, B. (2017). Genetic Dissection of Novel QTLs for Resistance to Leaf Spots and Tomato Spotted Wilt Virus in Peanut (*Arachis hypogaea* L.). *Frontiers in plant science*, *8*, 25.

Porebski, S., Bailey, G. & Baum, B.R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter* 15, 8-15.

Sasser, J.N., Eisenback, J.D, Carter, C.C., & Triantaphyllou, A.C. (1983). The International meloidogyne project-Its goals and accomplishments. *Ann. Rev. Phytopathol* 21, 271-88.

Starr, J.L., Morgan, E., & Simpson, C.E. (2002). Management of the peanut root-knot nematode, *Meloidogyne arenaria*, with host resistance. [Online] Plant Health Progress. doi: 10.1094/PHP 2002-1121-01-HM.

Williamson, V. M. (1999). Plant nematode resistance genes. *Plant Biology* 2, 327-331.

Zhang, H., Li, C., Davis, E.L., Wang, J., Griffin, J.D., Kofsky, J., & Song, B.H. (2016) Genome-Wide Association Study of Resistance to Soybean Cyst Nematode (*Heterodera glycines*) HG Type 2.5.7 in Wild Soybean (*Glycine soja*). *Frontiers Plant Science* 7, 1214.

Zhang, J.P., Song, Q.J., Cregan, P.B., Nelson, R.L., Wang, X.Z., Wu, J.X., & Jiang G.L. (2015). Genome-wide association study for flowering time, maturity dates and plant height in early maturing soybean (*Glycinemax*) germplasm. *BioMed Central* (*BMC) Genomics* 16, 217.

## APPENDIX

**Table 6**: Tukey-Kramer's results for eggs per gram of the root fresh weight and plant height.

| PI # | Eggs/g RFW | Pr > \|t\| | | PH (cm) | Pr >\|t\| | |
|---|---|---|---|---|---|---|
| PI 494034 | 7129 | <.0001 | A | 27.67 | <.0001 | ABCDEFGH |
| PI 476636 | 6521 | <.0001 | AB | 18.00 | <.0001 | BCDEFGHI |
| PI 259617 | 5661 | <.0001 | ABC | 20.33 | <.0001 | BCDEFGHI |
| PI 356004 | 5290 | <.0001 | ABCD | 31.33 | <.0001 | ABCD |
| PI 292950 | 5225 | <.0001 | ABCDE | 14.50 | <.0001 | EFGHI |
| PI 403813 | 4917 | 0.0002 | ABCDEF | 39.33 | <.0001 | A |
| Lot5-83 Line-5 | 4873 | 0.0002 | ABCDEF | 19.00 | <.0001 | BCDEFGHI |
| Olin | 4599 | 0.0004 | ABCDEF | 20.00 | <.0001 | BCDEFGHI |
| PI 476432 | 4062 | 0.0015 | BCDEFG | 26.33 | <.0001 | ABCDEFGHI |
| PI 648245 | 3979 | 0.0019 | BCDEFG | 15.00 | <.0001 | DEFGHI |
| PI 493581 | 3787 | 0.0029 | BCDEFGH | 35.33 | <.0001 | AB |
| SunOleic 93R | 3487 | 0.0059 | BCDEFGHI | 18.33 | <.0001 | BCDEFGHI |
| PI 337399 | 3314 | 0.0086 | CDEFGHIJ | 23.33 | <.0001 | ABCDEFGHI |
| Flavorunner 458 | 3265 | 0.0096 | CDEFGHIJ | 16.00 | <.0001 | DEFGHI |
| PI 501272 | 3233 | 0.0103 | CDEFGHIJ | 22.00 | <.0001 | BCDEFGHI |
| PI 271019 | 2760 | 0.0272 | CDEFGHIJ | 17.67 | <.0001 | BCDEFGHI |
| PI 497318 | 2693 | 0.031 | CDEFGHIJ | 21.00 | <.0001 | BCDEFGHI |
| PI 295730 | 2673 | 0.0321 | CDEFGHIJ | 17.67 | <.0001 | BCDEFGHI |
| PI 274198 | 2529 | 0.0422 | CDEFGHIJ | 16.00 | <.0001 | DEFGHI |
| PI 461427 | 2410 | 0.0524 | CDEFGHIJ | 21.00 | <.0001 | BCDEFGHI |
| AU16-28 | 2407 | 0.0527 | CDEFGHIJ | 16.00 | <.0001 | DEFGHI |
| PI 493547 | 2369 | 0.0564 | DEFGHIJ | 19.67 | <.0001 | BCDEFGHI |
| PI 475918 | 2365 | 0.0568 | DEFGHIJ | 20.67 | <.0001 | BCDEFGHI |
| PI 493631 | 2160 | 0.0812 | EFGHIJ | 15.67 | <.0001 | DEFGHI |
| PI 274194 | 2156 | 0.0816 | FGHIJ | 14.00 | <.0001 | FGHI |
| PI 196622 | 2113 | 0.0878 | FGHIJ | 18.00 | <.0001 | BCDEFGHI |
| PI 371521 | 2039 | 0.0991 | FGHIJ | 12.00 | <.0001 | GHI |
| Lot 5-100 Line-7 | 2016 | 0.1029 | FGHIJ | 27.00 | <.0001 | ABCDEFGH |
| PI 493329 | 2012 | 0.1036 | FGHIJ | 23.00 | <.0001 | ABCDEFGHI |
| PI 323268 | 1909 | 0.1034 | FGHIJ | 20.51 | <.0001 | BCDEFGHI |
| PI 478850 | 1859 | 0.1318 | FGHIJ | 15.67 | <.0001 | DEFGHI |
| PI 493729 | 1796 | 0.1452 | FGHIJ | 34.67 | <.0001 | ABC |
| PI 262038 | 1790 | 0.1464 | FGHIJ | 28.33 | <.0001 | ABCDEFG |
| PI 494795 | 1729 | 0.2342 | FGHIJ | 17.62 | <.0001 | BCDEFGHI |
| PI 274193 | 1723 | 0.162 | FGHIJ | 19.33 | <.0001 | BCDEFGHI |
| PI 159786 | 1662 | 0.1771 | FGHIJ | 20.67 | <.0001 | BCDEFGHI |
| Lot5-73 Line-1 | 1641 | 0.1825 | FGHIJ | 14.00 | <.0001 | FGHI |
| PI 331314 | 1580 | 0.1991 | FGHIJ | 17.33 | <.0001 | BCDEFGHI |

Table: Continued.

| PI # | Eggs/g RFW | Pr > \|t\| | | PH (cm) | Pr > \|t\| | |
|---|---|---|---|---|---|---|
| PI 337406 | 1516 | 0.2176 | GHIJ | 18.67 | <.0001 | BCDEFGHI |
| PI 497517 | 1482 | 0.2278 | GHIJ | 30.67 | <.0001 | ABCDE |
| PI 468250 | 1451 | 0.2375 | GHIJ | 22.67 | <.0001 | BCDEFGHI |
| PI 337293 | 1448 | 0.2385 | GHIJ | 17.67 | <.0001 | BCDEFGHI |
| PI 339960 | 1411 | 0.2507 | GHIJ | 24.67 | <.0001 | ABCDEFGHI |
| PI 268806 | 1393 | 0.2567 | GHIJ | 18.33 | <.0001 | BCDEFGHI |
| PI 296558 | 1310 | 0.2856 | GHIJ | 24.00 | <.0001 | ABCDEFGHI |
| PI 196670 | 1301 | 0.2892 | GHIJ | 20.33 | <.0001 | BCDEFGHI |
| PI 325943 | 1296 | 0.291 | GHIJ | 30.00 | <.0001 | ABCDEF |
| NC-7 | 1295 | 0.2913 | GHIJ | 17.67 | <.0001 | BCDEFGHI |
| PI 270786 | 1259 | 0.3046 | GHIJ | 20.33 | <.0001 | BCDEFGHI |
| PI 504614 | 1254 | 0.3068 | GHIJ | 24.33 | <.0001 | ABCDEFGHI |
| PI 319768 | 1251 | 0.3076 | GHIJ | 24.67 | <.0001 | ABCDEFGHI |
| PI 269037 | 1218 | 0.3207 | GHIJ | 21.67 | <.0001 | BCDEFGHI |
| PI 493356 | 1216 | 0.3212 | GHIJ | 21.67 | <.0001 | BCDEFGHI |
| Lot5-63 Line-6 | 1130 | 0.3565 | GHIJ | 16.67 | <.0001 | CDEFGHI |
| Ap-4 | 1118 | 0.3616 | GHIJ | 23.00 | <.0001 | ABCDEFGHI |
| GA Greener | 1110 | 0.3651 | GHIJ | 16.33 | <.0001 | DEFGHI |
| PI 502120 | 1076 | 0.3798 | GHIJ | 20.00 | <.0001 | BCDEFGHI |
| Lot5-80 Line-3 | 1065 | 0.3845 | GHIJ | 14.33 | <.0001 | EFGHI |
| PI 429420 | 1056 | 0.2946 | GHIJ | 26.51 | <.0001 | ABCDEFGHI |
| PI 196635 | 1052 | 0.3903 | GHIJ | 18.00 | <.0001 | BCDEFGHI |
| PI 497395 | 1047 | 0.3928 | GHIJ | 21.00 | <.0001 | BCDEFGHI |
| PI 576637 | 1036 | 0.3978 | GHIJ | 26.33 | <.0001 | ABCDEFGHI |
| N0808201 | 1029 | 0.4009 | GHIJ | 17.33 | <.0001 | BCDEFGHI |
| PI 288210 | 1021 | 0.4044 | GHIJ | 19.33 | <.0001 | BCDEFGHI |
| PI 343398 | 993 | 0.4173 | HIJ | 15.00 | <.0001 | DEFGHI |
| Ga HI O/L | 990 | 0.4189 | HIJ | 18.67 | <.0001 | BCDEFGHI |
| PI 497639 | 988 | 0.4195 | HIJ | 20.67 | <.0001 | BCDEFGHI |
| PI 471952 | 978 | 0.4243 | HIJ | 14.67 | <.0001 | EFGHI |
| PI 270907 | 903 | 0.4607 | HIJ | 20.67 | <.0001 | BCDEFGHI |
| PI 259836 | 868 | 0.4781 | HIJ | 18.33 | <.0001 | BCDEFGHI |
| PI 268996 | 847 | 0.4887 | HIJ | 22.00 | <.0001 | BCDEFGHI |
| PI 648249 | 841 | 0.4919 | HIJ | 15.33 | <.0001 | DEFGHI |
| AT 3085RO | 840 | 0.4924 | HIJ | 17.33 | <.0001 | BCDEFGHI |
| Lot4-8 Line-4 | 824 | 0.5003 | HIJ | 14.33 | <.0001 | EFGHI |
| PI 502040 | 811 | 0.5074 | HIJ | 17.00 | <.0001 | CDEFGHI |
| Ga Green | 807 | 0.5094 | HIJ | 17.00 | <.0001 | CDEFGHI |
| PI 290566 | 805 | 0.5103 | HIJ | 20.00 | <.0001 | BCDEFGHI |
| SPT06-6 | 788 | 0.5193 | HIJ | 18.33 | <.0001 | BCDEFGHI |
| PI 496448 | 776 | 0.5257 | HIJ | 22.00 | <.0001 | BCDEFGHI |
| PI 274195 | 774 | 0.5268 | HIJ | 10.00 | 0.0001 | I |
| Tifrunner | 772 | 0.528 | HIJ | 11.67 | <.0001 | HI |

Table: continued.

| PI # | Eggs/g RFW | Pr > \|t\| | | PH (cm) | Pr > \|t\| | |
|---|---|---|---|---|---|---|
| CG7-A | 746 | 0.5415 | HIJ | 16.00 | <.0001 | DEFGHI |
| PI 648241 | 740 | 0.5449 | HIJ | 14.33 | <.0001 | EFGHI |
| PI 162857 | 738 | 0.5462 | HIJ | 20.67 | <.0001 | BCDEFGHI |
| Lot5-101 Line-8 | 730 | 0.5502 | HIJ | 17.33 | <.0001 | BCDEFGHI |
| PI 343384 | 714 | 0.5589 | HIJ | 12.33 | <.0001 | GHI |
| PI 298854 | 708 | 0.5626 | HIJ | 16.67 | <.0001 | CDEFGHI |
| EXP27-1516 | 705 | 0.5644 | HIJ | 18.33 | <.0001 | BCDEFGHI |
| PI 576614 | 695 | 0.5695 | HIJ | 15.00 | <.0001 | DEFGHI |
| PI 481795 | 692 | 0.5712 | HIJ | 13.67 | <.0001 | FGHI |
| PI 240560 | 687 | 0.574 | HIJ | 22.67 | <.0001 | BCDEFGHI |
| PI 493717 | 674 | 0.5813 | HIJ | 14.00 | <.0001 | FGHI |
| PI 502111 | 674 | 0.5816 | HIJ | 17.67 | <.0001 | BCDEFGHI |
| PI 290536 | 672 | 0.5826 | HIJ | 22.33 | <.0001 | BCDEFGHI |
| PI 494018 | 666 | 0.5858 | HIJ | 22.00 | <.0001 | BCDEFGHI |
| PI 270998 | 655 | 0.5918 | HIJ | 13.67 | <.0001 | FGHI |
| Florunner | 644 | 0.5984 | HIJ | 20.00 | <.0001 | BCDEFGHI |
| PI 338338 | 631 | 0.6054 | HIJ | 15.00 | <.0001 | DEFGHI |
| PI 268586 | 627 | 0.6079 | HIJ | 24.67 | <.0001 | ABCDEFGHI |
| PI 496401 | 612 | 0.6167 | HIJ | 15.33 | <.0001 | DEFGHI |
| PI 288146 | 604 | 0.6211 | IJ | 22.33 | <.0001 | BCDEFGHI |
| PI 476025 | 603 | 0.6218 | IJ | 17.67 | <.0001 | BCDEFGHI |
| PI 295250 | 595 | 0.6261 | IJ | 20.00 | <.0001 | BCDEFGHI |
| PI 648242 | 583 | 0.6333 | IJ | 23.00 | <.0001 | ABCDEFGHI |
| PI 478819 | 581 | 0.6343 | IJ | 19.33 | <.0001 | BCDEFGHI |
| PI 268696 | 581 | 0.6346 | IJ | 23.67 | <.0001 | ABCDEFGHI |
| PI 152146 | 579 | 0.6358 | IJ | 19.33 | <.0001 | BCDEFGHI |
| PI 442768 | 575 | 0.638 | IJ | 21.67 | <.0001 | BCDEFGHI |
| G06G | 563 | 0.4778 | IJ | 17.01 | <.0001 | BCDEFGHI |
| PI 200441 | 545 | 0.6552 | IJ | 15.00 | <.0001 | DEFGHI |
| PI 155107 | 542 | 0.6575 | IJ | 16.33 | <.0001 | DEFGHI |
| C76-16 | 530 | 0.6645 | IJ | 20.33 | <.0001 | BCDEFGHI |
| PI 290560 | 527 | 0.666 | IJ | 15.33 | <.0001 | DEFGHI |
| PI 576634 | 527 | 0.6664 | IJ | 21.00 | <.0001 | BCDEFGHI |
| Grif 14051 | 516 | 0.6726 | IJ | 19.00 | <.0001 | BCDEFGHI |
| NM Val | 513 | 0.6745 | IJ | 22.00 | <.0001 | BCDEFGHI |
| Grif 12545 | 509 | 0.6766 | IJ | 18.33 | <.0001 | BCDEFGHI |
| PI 475863 | 506 | 0.6788 | IJ | 23.00 | <.0001 | ABCDEFGHI |
| Grif 12579 | 496 | 0.685 | IJ | 22.00 | <.0001 | BCDEFGHI |
| Valencia | 491 | 0.688 | IJ | 20.33 | <.0001 | BCDEFGHI |
| PI 259748 | 488 | 0.6893 | IJ | 27.33 | <.0001 | ABCDEFGH |
| NC-3033 | 482 | 0.693 | IJ | 14.00 | <.0001 | FGHI |
| PI 296550 | 477 | 0.6964 | IJ | 13.67 | <.0001 | FGHI |
| PI 355268 | 477 | 0.6964 | IJ | 22.67 | <.0001 | BCDEFGHI |

**Table:** Continued.

| PI # | Eggs/g RFW | Pr > \|t\| | | PH (cm) | Pr > \|t\| | |
|---|---|---|---|---|---|---|
| PI 372271 | 475 | 0.6971 | IJ | 19.67 | <.0001 | BCDEFGHI |
| PI 268847 | 468 | 0.7014 | IJ | 13.67 | <.0001 | FGHI |
| PI 313129 | 462 | 0.7053 | IJ | 13.67 | <.0001 | FGHI |
| PI 355271 | 459 | 0.7068 | IJ | 16.67 | <.0001 | CDEFGHI |
| PI 493880 | 454 | 0.7099 | IJ | 15.33 | <.0001 | DEFGHI |
| PI 158854 | 431 | 0.7243 | IJ | 15.67 | <.0001 | DEFGHI |
| PI 482189 | 409 | 0.7378 | J | 20.67 | <.0001 | BCDEFGHI |
| PI 331297 | 403 | 0.7415 | J | 19.00 | <.0001 | BCDEFGHI |
| PI 576636 | 403 | 0.7416 | J | 21.00 | <.0001 | BCDEFGHI |
| C99R | 372 | 0.7605 | J | 16.67 | <.0001 | CDEFGHI |
| PI 157542 | 352 | 0.7731 | J | 14.00 | <.0001 | FGHI |
| PI 270905 | 345 | 0.7775 | J | 21.67 | <.0001 | BCDEFGHI |
| PI 196705 | 335 | 0.7841 | J | 23.33 | <.0001 | ABCDEFGHI |
| PI 259658 | 334 | 0.7847 | J | 21.00 | <.0001 | BCDEFGHI |
| PI 290620 | 328 | 0.7882 | J | 23.33 | <.0001 | ABCDEFGHI |
| PI 482120 | 325 | 0.7901 | J | 18.33 | <.0001 | BCDEFGHI |
| PI 648250 | 320 | 0.7931 | J | 14.00 | <.0001 | FGHI |
| VC-2 (1) | 319 | 0.7941 | J | 25.67 | <.0001 | ABCDEFGHI |
| FL-279 | 311 | 0.799 | J | 16.67 | <.0001 | CDEFGHI |
| PI 268755 | 309 | 0.8004 | J | 24.00 | <.0001 | ABCDEFGHI |
| PI 259851 | 304 | 0.8033 | J | 21.67 | <.0001 | BCDEFGHI |
| PI 372305 | 292 | 0.8109 | J | 14.67 | <.0001 | EFGHI |
| PI 162655 | 288 | 0.8133 | J | 16.67 | <.0001 | CDEFGHI |
| PI 290594 | 276 | 0.8214 | J | 22.67 | <.0001 | BCDEFGHI |
| PI 471954 | 262 | 0.8304 | J | 15.67 | <.0001 | DEFGHI |
| PI 493938 | 243 | 0.8423 | J | 25.00 | <.0001 | ABCDEFGHI |
| AU-17 | 239 | 0.8449 | J | 20.00 | <.0001 | BCDEFGHI |
| PI 461434 | 229 | 0.8515 | J | 23.67 | <.0001 | ABCDEFGHI |
| Fla-07 | 190 | 0.8766 | J | 21.00 | <.0001 | BCDEFGHI |
| TifNV-High O/L | 174 | 0.8868 | J | 17.67 | <.0001 | BCDEFGHI |
| PI 407667 | 149 | 0.903 | J | 20.00 | <.0001 | BCDEFGHI |
| PI 295309 | 139 | 0.6758 | J | 18.51 | <.0001 | BCDEFGHI |
| PI 268868 | 133 | 0.9132 | J | 14.00 | <.0001 | FGHI |
| PI 497648 | 127 | 0.9173 | J | 16.17 | <.0001 | DEFGHI |
| PI 390428 | 123 | 0.9196 | J | 12.33 | <.0001 | GHI |
| Lot4-37 Line-2 | 111 | 0.9278 | J | 13.33 | <.0001 | GHI |
| PI 370331 | 95 | 0.9854 | J | 16.62 | <.0001 | CDEFGHI |

Significant differences indicated by Tukey by $P< 0.05$.
Means followed by the same letter are not significantly different.
Statistical analysis of Eggs/g RFW was performed on log transformed data, but the means presented are untransformed.

**Table 7:** Tukey-Kramer's results for shoot fresh weight, root fresh weight and biomass.

| PI # | SFW (g) | Pr > \|t\| | | RFW (g) | Pr > \|t\| | | BM (g) | Pr > \|t\| | |
|---|---|---|---|---|---|---|---|---|---|
| PI 494034 | 11.37 | <.0001 | ABC | 5.87 | <.0001 | ABC | 2.48 | <.0001 | AB |
| PI 476636 | 11.27 | <.0001 | ABC | 3.61 | 0.0002 | ABC | 1.83 | <.0001 | AB |
| PI 259617 | 12.76 | <.0001 | ABC | 5.03 | <.0001 | ABC | 2.71 | <.0001 | AB |
| PI 356004 | 16.34 | <.0001 | ABC | 6.39 | <.0001 | ABC | 2.78 | <.0001 | AB |
| PI 292950 | 11.95 | <.0001 | ABC | 5.02 | <.0001 | ABC | 3.18 | <.0001 | AB |
| PI 403813 | 11.09 | <.0001 | ABC | 3.50 | 0.0003 | ABC | 2.06 | <.0001 | AB |
| Lot5-85 Line-5 | 13.76 | <.0001 | ABC | 2.21 | 0.0224 | BC | 2.08 | <.0001 | AB |
| Olin | 11.83 | <.0001 | ABC | 2.67 | 0.0058 | BC | 2.23 | <.0001 | AB |
| PI 476432 | 14.83 | <.0001 | ABC | 5.45 | <.0001 | ABC | 2.47 | <.0001 | AB |
| PI 648245 | 6.90 | 0.0004 | C | 4.78 | <.0001 | ABC | 1.42 | 0.0007 | B |
| PI 493581 | 16.09 | <.0001 | ABC | 5.81 | <.0001 | ABC | 2.57 | <.0001 | AB |
| SunOleic 93R | 12.70 | <.0001 | ABC | 6.86 | <.0001 | ABC | 2.60 | <.0001 | AB |
| PI 337399 | 13.81 | <.0001 | ABC | 4.54 | <.0001 | ABC | 2.88 | <.0001 | AB |
| Flavorunner 458 | 15.05 | <.0001 | ABC | 6.29 | <.0001 | ABC | 2.65 | <.0001 | AB |
| PI 501272 | 10.60 | <.0001 | ABC | 3.06 | <.0001 | BC | 1.50 | 0.0004 | B |
| PI 271019 | 10.31 | <.0001 | ABC | 2.92 | 0.0026 | BC | 1.79 | <.0001 | AB |
| PI 497318 | 16.14 | <.0001 | ABC | 5.85 | <.0001 | ABC | 2.84 | <.0001 | AB |
| PI 295730 | 14.10 | <.0001 | ABC | 5.70 | <.0001 | ABC | 2.26 | <.0001 | AB |
| PI 274198 | 14.61 | <.0001 | ABC | 4.39 | <.0001 | ABC | 3.07 | <.0001 | AB |
| PI 461427 | 12.27 | <.0001 | ABC | 7.04 | <.0001 | ABC | 2.78 | <.0001 | AB |
| AU16-28 | 13.38 | <.0001 | ABC | 4.87 | <.0001 | ABC | 2.17 | <.0001 | AB |
| PI 493547 | 16.20 | <.0001 | ABC | 5.96 | <.0001 | ABC | 3.07 | <.0001 | AB |
| PI 475918 | 14.09 | <.0001 | ABC | 7.32 | <.0001 | ABC | 2.94 | <.0001 | AB |
| PI 493631 | 13.11 | <.0001 | ABC | 4.86 | <.0001 | ABC | 2.59 | <.0001 | AB |
| PI 274194 | 10.70 | <.0001 | ABC | 5.84 | <.0001 | ABC | 2.30 | <.0001 | AB |
| PI 196622 | 13.43 | <.0001 | ABC | 4.75 | <.0001 | ABC | 2.40 | <.0001 | AB |
| PI 371521 | 13.00 | <.0001 | ABC | 6.21 | <.0001 | ABC | 2.08 | <.0001 | AB |
| Lot5-100 Line-7 | 14.89 | <.0001 | ABC | 6.57 | <.0001 | ABC | 3.34 | <.0001 | AB |
| PI 493329 | 13.42 | <.0001 | ABC | 4.76 | <.0001 | ABC | 2.76 | <.0001 | AB |
| PI 323268 | 15.39 | <.0001 | ABC | 6.21 | <.0001 | ABC | 2.64 | <.0001 | AB |
| PI 478850 | 14.33 | <.0001 | ABC | 6.43 | <.0001 | ABC | 2.48 | <.0001 | AB |
| PI 493729 | 16.31 | <.0001 | ABC | 4.86 | <.0001 | ABC | 2.66 | <.0001 | AB |
| PI 262038 | 15.53 | <.0001 | ABC | 6.40 | <.0001 | ABC | 2.95 | <.0001 | AB |
| PI 494795 | 14.24 | <.0001 | ABC | 4.89 | <.0001 | ABC | 2.16 | <.0001 | AB |
| PI 274193 | 15.00 | <.0001 | ABC | 5.39 | <.0001 | ABC | 2.76 | <.0001 | AB |
| PI 159786 | 14.08 | <.0001 | ABC | 4.83 | <.0001 | ABC | 2.00 | <.0001 | AB |
| Lot5-73 Line-1 | 9.44 | <.0001 | BC | 3.97 | <.0001 | ABC | 1.49 | 0.0004 | B |
| PI 331314 | 14.50 | <.0001 | ABC | 6.14 | <.0001 | ABC | 2.94 | <.0001 | AB |
| PI 337406 | 12.64 | <.0001 | ABC | 5.56 | <.0001 | ABC | 2.47 | <.0001 | AB |
| PI 497517 | 21.09 | <.0001 | AB | 8.55 | <.0001 | ABC | 3.79 | <.0001 | AB |
| PI 468250 | 14.21 | <.0001 | ABC | 4.80 | <.0001 | ABC | 2.64 | <.0001 | AB |
| PI 337293 | 13.45 | <.0001 | ABC | 4.14 | <.0001 | ABC | 2.76 | <.0001 | AB |
| PI 339960 | 15.87 | <.0001 | ABC | 4.92 | <.0001 | ABC | 2.94 | <.0001 | AB |

**Table:** Continued.

| PI # | SFW (g) | Pr > \|t\| | | RFW (g) | Pr > \|t\| | | BM (g) | Pr > \|t\| | |
|---|---|---|---|---|---|---|---|---|---|
| PI 268806 | 10.13 | <.0001 | ABC | 5.31 | <.0001 | ABC | 2.09 | <.0001 | AB |
| PI 296558 | 22.39 | <.0001 | A | 6.76 | <.0001 | ABC | 3.88 | <.0001 | AB |
| PI 196670 | 15.17 | <.0001 | ABC | 6.29 | <.0001 | ABC | 2.91 | <.0001 | AB |
| PI 325943 | 11.07 | <.0001 | ABC | 4.33 | <.0001 | ABC | 1.98 | <.0001 | AB |
| NC-7 | 16.61 | <.0001 | ABC | 6.83 | <.0001 | ABC | 3.00 | <.0001 | AB |
| PI 270786 | 15.86 | <.0001 | ABC | 6.64 | <.0001 | ABC | 2.80 | <.0001 | AB |
| PI 504614 | 9.72 | <.0001 | BC | 6.09 | <.0001 | ABC | 1.84 | <.0001 | AB |
| PI 319768 | 13.37 | <.0001 | ABC | 5.82 | <.0001 | ABC | 2.70 | <.0001 | AB |
| PI 269037 | 13.67 | <.0001 | ABC | 5.05 | <.0001 | ABC | 2.42 | <.0001 | AB |
| PI 493356 | 11.99 | <.0001 | ABC | 6.21 | <.0001 | ABC | 2.53 | <.0001 | AB |
| Lot5-63 Line-6 | 14.39 | <.0001 | ABC | 7.15 | <.0001 | ABC | 2.41 | <.0001 | AB |
| Ap-4 | 13.96 | <.0001 | ABC | 4.01 | <.0001 | ABC | 2.34 | <.0001 | AB |
| GA Greener | 12.68 | <.0001 | ABC | 7.22 | <.0001 | ABC | 2.19 | <.0001 | AB |
| PI 502120 | 15.43 | <.0001 | ABC | 7.67 | <.0001 | ABC | 3.26 | <.0001 | AB |
| Lot5-80Line-3 | 11.45 | <.0001 | ABC | 4.91 | <.0001 | ABC | 2.31 | <.0001 | AB |
| PI 429420 | 14.13 | <.0001 | ABC | 7.02 | <.0001 | ABC | 2.57 | <.0001 | AB |
| PI 196635 | 15.23 | <.0001 | ABC | 6.55 | <.0001 | ABC | 2.92 | <.0001 | AB |
| PI 497395 | 17.26 | <.0001 | ABC | 7.71 | <.0001 | ABC | 3.36 | <.0001 | AB |
| PI 576637 | 20.60 | <.0001 | AB | 7.21 | <.0001 | ABC | 4.19 | <.0001 | A |
| N0808201 | 13.29 | <.0001 | ABC | 4.84 | <.0001 | ABC | 2.69 | <.0001 | AB |
| PI 288210 | 13.92 | <.0001 | ABC | 6.32 | <.0001 | ABC | 2.46 | <.0001 | AB |
| PI 343398 | 14.97 | <.0001 | ABC | 6.93 | <.0001 | ABC | 2.85 | <.0001 | AB |
| Ga HI O/L | 15.81 | <.0001 | ABC | 5.25 | <.0001 | ABC | 2.65 | <.0001 | AB |
| PI 497639 | 15.85 | <.0001 | ABC | 6.36 | <.0001 | ABC | 3.11 | <.0001 | AB |
| PI 471952 | 10.39 | <.0001 | ABC | 7.58 | <.0001 | ABC | 2.63 | <.0001 | AB |
| PI 270907 | 11.58 | <.0001 | ABC | 5.41 | <.0001 | ABC | 1.60 | 0.0001 | B |
| PI 259836 | 11.12 | <.0001 | ABC | 6.45 | <.0001 | ABC | 2.14 | <.0001 | AB |
| PI 268996 | 16.14 | <.0001 | ABC | 6.27 | <.0001 | ABC | 2.82 | <.0001 | AB |
| PI 648249 | 11.91 | <.0001 | ABC | 7.00 | <.0001 | ABC | 2.44 | <.0001 | AB |
| AT 3085RO | 16.85 | <.0001 | ABC | 8.47 | <.0001 | ABC | 3.40 | <.0001 | AB |
| Lot4-8 Line-4 | 13.03 | <.0001 | ABC | 5.28 | <.0001 | ABC | 2.54 | <.0001 | AB |
| PI 502040 | 13.92 | <.0001 | ABC | 5.85 | <.0001 | ABC | 2.41 | <.0001 | AB |
| Ga Green | 16.75 | <.0001 | ABC | 5.93 | <.0001 | ABC | 2.98 | <.0001 | AB |
| PI 290566 | 14.03 | <.0001 | ABC | 5.84 | <.0001 | ABC | 2.50 | <.0001 | AB |
| SPT06-6 | 13.01 | <.0001 | ABC | 5.18 | <.0001 | ABC | 2.29 | <.0001 | AB |
| PI 496448 | 14.82 | <.0001 | ABC | 6.49 | <.0001 | ABC | 2.50 | <.0001 | AB |
| PI 274195 | 13.95 | <.0001 | ABC | 5.45 | <.0001 | ABC | 3.18 | <.0001 | AB |
| Tifrunner | 8.90 | <.0001 | BC | 4.14 | <.0001 | ABC | 1.74 | <.0001 | AB |
| CG7-A | 13.62 | <.0001 | ABC | 7.35 | <.0001 | ABC | 2.23 | <.0001 | AB |
| PI 648241 | 11.62 | <.0001 | ABC | 7.03 | <.0001 | ABC | 2.08 | <.0001 | AB |
| PI 162857 | 18.38 | <.0001 | ABC | 7.02 | <.0001 | ABC | 3.35 | <.0001 | AB |
| Lot5-101 Line-8 | 14.70 | <.0001 | ABC | 6.71 | <.0001 | ABC | 2.64 | <.0001 | AB |
| PI 343384 | 11.15 | <.0001 | ABC | 5.05 | <.0001 | ABC | 1.81 | <.0001 | AB |

**Table:** Continued.

| PI # | SFW (g) | Pr > \|t\| | | RFW (g) | Pr > \|t\| | | BM (g) | Pr > \|t\| | |
|---|---|---|---|---|---|---|---|---|---|
| PI 298854 | 13.37 | <.0001 | ABC | 5.22 | <.0001 | ABC | 2.74 | <.0001 | AB |
| EXP27-1516 | 12.78 | <.0001 | ABC | 6.53 | <.0001 | ABC | 2.20 | <.0001 | AB |
| PI 576614 | 14.92 | <.0001 | ABC | 7.21 | <.0001 | ABC | 2.65 | <.0001 | AB |
| PI 481795 | 11.24 | <.0001 | ABC | 4.55 | <.0001 | ABC | 2.26 | <.0001 | AB |
| PI 240560 | 11.71 | <.0001 | ABC | 6.45 | <.0001 | ABC | 2.31 | <.0001 | AB |
| PI 493717 | 12.41 | <.0001 | ABC | 7.06 | <.0001 | ABC | 2.21 | <.0001 | AB |
| PI 502111 | 13.00 | <.0001 | ABC | 7.69 | <.0001 | ABC | 2.41 | <.0001 | AB |
| PI 290536 | 12.26 | <.0001 | ABC | 5.19 | <.0001 | ABC | 1.94 | <.0001 | AB |
| PI 494018 | 16.65 | <.0001 | ABC | 6.83 | <.0001 | ABC | 3.13 | <.0001 | AB |
| PI 270998 | 11.73 | <.0001 | ABC | 5.70 | <.0001 | ABC | 2.45 | <.0001 | AB |
| Florunner | 16.03 | <.0001 | ABC | 7.09 | <.0001 | ABC | 2.95 | <.0001 | AB |
| PI 338338 | 9.01 | <.0001 | BC | 6.23 | <.0001 | ABC | 2.14 | <.0001 | AB |
| PI 268586 | 15.52 | <.0001 | ABC | 6.72 | <.0001 | ABC | 3.02 | <.0001 | AB |
| PI 496401 | 12.11 | <.0001 | ABC | 3.77 | <.0001 | ABC | 1.47 | 0.0005 | B |
| PI 288146 | 13.43 | <.0001 | ABC | 8.14 | <.0001 | ABC | 2.65 | <.0001 | AB |
| PI 476025 | 16.68 | <.0001 | ABC | 7.21 | <.0001 | ABC | 3.17 | <.0001 | AB |
| PI 295250 | 15.20 | <.0001 | ABC | 6.49 | <.0001 | ABC | 2.98 | <.0001 | AB |
| PI 648242 | 11.72 | <.0001 | ABC | 5.70 | <.0001 | ABC | 2.06 | <.0001 | AB |
| PI 478819 | 11.18 | <.0001 | ABC | 5.94 | <.0001 | ABC | 1.83 | <.0001 | AB |
| PI 268696 | 12.49 | <.0001 | ABC | 4.86 | <.0001 | ABC | 2.46 | <.0001 | AB |
| PI 152146 | 12.77 | <.0001 | ABC | 5.49 | <.0001 | ABC | 2.33 | <.0001 | AB |
| PI 442768 | 16.78 | <.0001 | ABC | 6.73 | <.0001 | ABC | 3.38 | <.0001 | AB |
| G06G | 17.40 | <.0001 | ABC | 8.06 | <.0001 | ABC | 3.32 | <.0001 | AB |
| PI 200441 | 13.30 | <.0001 | ABC | 4.82 | <.0001 | ABC | 2.77 | <.0001 | AB |
| PI 155107 | 16.58 | <.0001 | ABC | 8.30 | <.0001 | ABC | 3.18 | <.0001 | AB |
| C76-16 | 17.71 | <.0001 | ABC | 5.26 | <.0001 | ABC | 3.28 | <.0001 | AB |
| PI 290560 | 11.32 | <.0001 | ABC | 5.90 | <.0001 | ABC | 2.06 | <.0001 | AB |
| PI 576634 | 15.02 | <.0001 | ABC | 6.38 | <.0001 | ABC | 2.80 | <.0001 | AB |
| Grif 14051 | 15.06 | <.0001 | ABC | 6.57 | <.0001 | ABC | 3.00 | <.0001 | AB |
| NM Val | 18.66 | <.0001 | ABC | 7.53 | <.0001 | ABC | 3.59 | <.0001 | AB |
| Grif 12545 | 13.96 | <.0001 | ABC | 8.33 | <.0001 | ABC | 2.54 | <.0001 | AB |
| PI 475863 | 17.66 | <.0001 | ABC | 4.45 | <.0001 | ABC | 3.16 | <.0001 | AB |
| Grif 12579 | 14.46 | <.0001 | ABC | 7.18 | <.0001 | ABC | 2.66 | <.0001 | AB |
| Valencia | 18.02 | <.0001 | ABC | 4.89 | <.0001 | ABC | 3.41 | <.0001 | AB |
| PI 259748 | 14.89 | <.0001 | ABC | 6.20 | <.0001 | ABC | 2.92 | <.0001 | AB |
| NC-3033 | 16.04 | <.0001 | ABC | 6.88 | <.0001 | ABC | 3.20 | <.0001 | AB |
| PI 296550 | 15.63 | <.0001 | ABC | 6.88 | <.0001 | ABC | 2.92 | <.0001 | AB |
| PI 355268 | 17.89 | <.0001 | ABC | 5.04 | <.0001 | ABC | 3.61 | <.0001 | AB |
| PI 372271 | 16.59 | <.0001 | ABC | 6.02 | <.0001 | ABC | 2.93 | <.0001 | AB |
| PI 268847 | 8.99 | <.0001 | BC | 7.31 | <.0001 | ABC | 2.18 | <.0001 | AB |
| PI 313129 | 14.11 | <.0001 | ABC | 7.72 | <.0001 | ABC | 2.56 | <.0001 | AB |
| PI 355271 | 10.29 | <.0001 | ABC | 5.19 | <.0001 | ABC | 1.84 | <.0001 | AB |
| PI 493880 | 13.00 | <.0001 | ABC | 6.20 | <.0001 | ABC | 2.53 | <.0001 | AB |

**Table:** Continued.

| PI # | SFW (g) | Pr > \|t\| | | RFW (g) | Pr > \|t\| | | BM (g) | Pr > \|t\| | |
|---|---|---|---|---|---|---|---|---|---|
| PI 158854 | 16.03 | <.0001 | ABC | 7.02 | <.0001 | ABC | 3.06 | <.0001 | AB |
| PI 482189 | 11.45 | <.0001 | ABC | 5.09 | <.0001 | ABC | 2.14 | <.0001 | AB |
| PI 331297 | 14.12 | <.0001 | ABC | 6.82 | <.0001 | ABC | 2.99 | <.0001 | AB |
| PI 576636 | 15.77 | <.0001 | ABC | 7.34 | <.0001 | ABC | 2.88 | <.0001 | AB |
| C99R | 9.49 | <.0001 | BC | 6.55 | <.0001 | ABC | 2.68 | <.0001 | AB |
| PI 157542 | 17.30 | <.0001 | ABC | 6.20 | <.0001 | ABC | 3.16 | <.0001 | AB |
| PI 270905 | 13.19 | <.0001 | ABC | 7.65 | <.0001 | ABC | 2.53 | <.0001 | AB |
| PI 196705 | 16.24 | <.0001 | ABC | 4.61 | <.0001 | ABC | 2.90 | <.0001 | AB |
| PI 259658 | 17.03 | <.0001 | ABC | 5.53 | <.0001 | ABC | 2.56 | <.0001 | AB |
| PI 290620 | 13.61 | <.0001 | ABC | 4.87 | <.0001 | ABC | 2.41 | <.0001 | AB |
| PI 482120 | 10.37 | <.0001 | ABC | 9.05 | <.0001 | AB | 2.02 | <.0001 | AB |
| PI 648250 | 10.80 | <.0001 | ABC | 7.43 | <.0001 | ABC | 2.12 | <.0001 | AB |
| VC-2 (1) | 16.49 | <.0001 | ABC | 5.76 | <.0001 | ABC | 3.08 | <.0001 | AB |
| FL-279 | 15.96 | <.0001 | ABC | 7.15 | <.0001 | ABC | 3.13 | <.0001 | AB |
| PI 268755 | 15.60 | <.0001 | ABC | 7.36 | <.0001 | ABC | 3.21 | <.0001 | AB |
| PI 259851 | 16.66 | <.0001 | ABC | 6.01 | <.0001 | ABC | 2.90 | <.0001 | AB |
| PI 372305 | 11.71 | <.0001 | ABC | 9.00 | <.0001 | AB | 2.03 | <.0001 | AB |
| PI 162655 | 14.65 | <.0001 | ABC | 8.21 | <.0001 | ABC | 2.83 | <.0001 | AB |
| PI 290594 | 16.55 | <.0001 | ABC | 3.47 | 0.0004 | ABC | 3.14 | <.0001 | AB |
| PI 471954 | 18.68 | <.0001 | ABC | 6.45 | <.0001 | ABC | 3.23 | <.0001 | AB |
| PI 493938 | 13.67 | <.0001 | ABC | 5.47 | <.0001 | ABC | 2.94 | <.0001 | AB |
| AU-17 | 13.72 | <.0001 | ABC | 6.86 | <.0001 | ABC | 2.80 | <.0001 | AB |
| PI 461434 | 18.22 | <.0001 | ABC | 9.59 | <.0001 | A | 3.62 | <.0001 | AB |
| Fla-07 | 17.25 | <.0001 | ABC | 6.08 | <.0001 | ABC | 2.98 | <.0001 | AB |
| TifNV-High O/L | 11.84 | <.0001 | ABC | 6.77 | <.0001 | ABC | 1.97 | <.0001 | AB |
| PI 407667 | 13.89 | <.0001 | ABC | 7.13 | <.0001 | ABC | 3.02 | <.0001 | AB |
| PI 295309 | 12.38 | <.0001 | ABC | 5.26 | <.0001 | ABC | 2.28 | <.0001 | AB |
| PI 268868 | 14.96 | <.0001 | ABC | 8.19 | <.0001 | ABC | 3.39 | <.0001 | AB |
| PI 497648 | 12.23 | <.0001 | ABC | 7.33 | <.0001 | ABC | 2.91 | <.0001 | AB |
| PI 390428 | 13.60 | <.0001 | ABC | 5.17 | <.0001 | ABC | 2.59 | <.0001 | AB |
| Lot4-37 Line-2 | 13.24 | <.0001 | ABC | 6.02 | <.0001 | ABC | 2.46 | <.0001 | AB |
| PI 370331 | 19.25 | <.0001 | ABC | 8.62 | <.0001 | ABC | 3.82 | <.0001 | AB |

Significant differences indicated by Tukey by $P< 0.05$.
Means followed by the same letter are not significantly different.
Statistical analysis of Eggs/g RFW was performed on log transformed data, but the means presented are untransformed.

**Figure 4:** Population structure analysis. The y-axis is the subgroup membership, and x-axis is the genotypes. G1-G4 indicate for subpopulations.

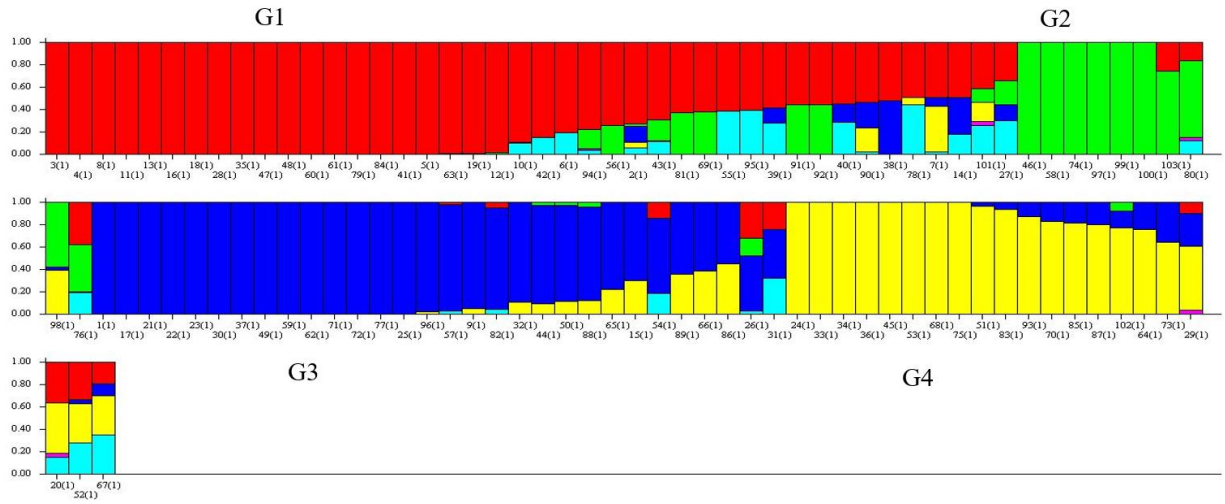



**Figure 5:** Principal component analysis based on Chord distance.

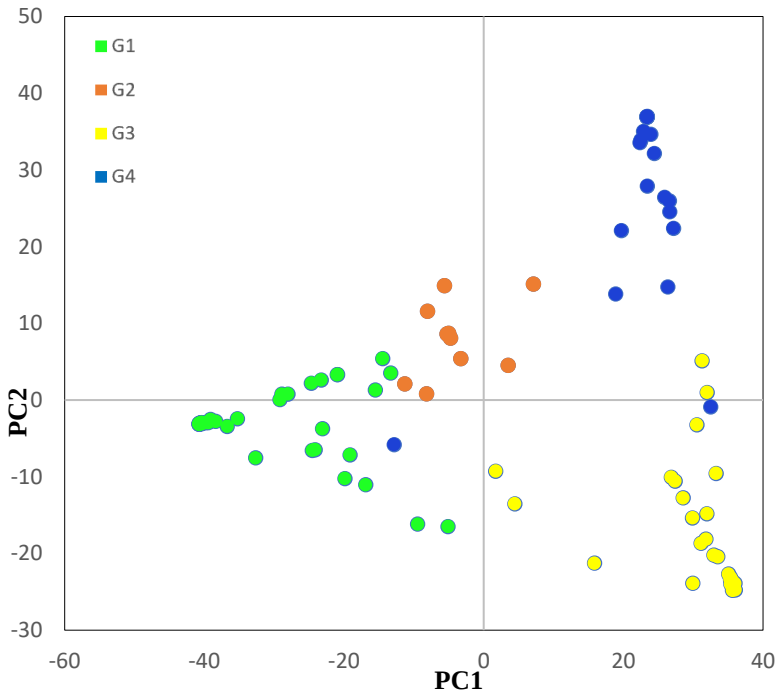
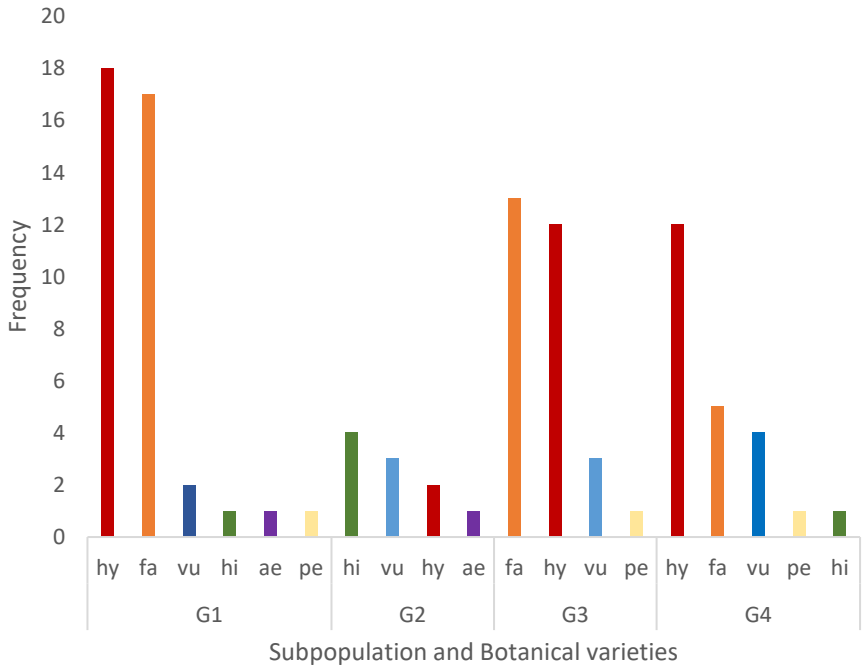


**Figure 6:** Distribution of botanical variety within each subpopulation.




hy: var. *hypogaea*

fa: var. *fastigiate*

vu: var. *vulgaris*

hi: var. *hirsute*

ae: var. *aequatoriana*

pe: var. *peruviana*

**Figure 7:** Screening of resistance to root-knot nematode in the greenhouse.