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ARTICLE

Genetic Difference among Two Old Landrace Peanut Cultivars

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ABSTRACT

In the U.S. early peanut (*Arachis hypogaea* L) breeding programs began by making pure-line selections within mixed seed stock such as farmer saved seed and progressed toward hybridization-based programs. Some of the early imported seed stock came from Africa into the U.S. and became known as 'Carolina African Runner', 'Southeastern Runner', 'North Carolina Runner', 'Wilmington Runner', and 'Virginia Runner' depending upon where they were grown. The objective of this study was to compare two of these old landrace cultivars to determine possible genetic differences. The pure-line selections 'Southeastern Runner 56-15' and 'North Carolina Runner No. 4' were compared by genomic sequencing to 'Tifrunner' and 'Bailey II'. Results from this study clearly shows these two landrace peanut cultivars were only 3% different or 97% genetically similar. However, both North Carolina Runner No. 4 and Southeastern Runner 56-15 were found to be 39.2% and 38.3% different from Bailey II, and are 37.5% and 35.5% different from Tifrunner, respectively. Results from this study suggest that these two older landrace cultivars are likely the same.

INTRODUCTION

Runner market-type peanuts *(Arachis hypoagea* L.) were previously grown in Georgia before the Civil War (1861-1865) for hog feed known as "hogging off" (Branch, 1993). Since 1919, virginia-type peanuts have been grown in Georgia (Bailey, 1951). However, during this time these virginia-types became relatively small to medium in seed size and had different shapes from typical U.S. virginia market types. Consequently, these smaller virginia-types were marketed in trade channels as 'Southeastern Runners' (Bailey, 1951).

Higgins (1951) reported that peanuts from Africa were imported into the U.S. and became known as 'Carolina African Runner', 'Southeastern Runner', 'North Carolina Runner', 'Wilmington Runner', and 'Virginia Runner'. It seems that these imported runner-type seeds were named according to the region or state where they were grown and thus may be similar. Woodruff (1973) even suggested that the old Southeastern Runner included the North Carolina Runner. None of these old landraces resulted from hybridization but instead were plant introductions that became badly mixed. Consequently, plant selections from within these mixtures resulted in newly selected pure-line cultivars (Boswell, 1937).

Southeastern Runner 56-15 was developed through such pure-line selection from a sample of the old landrace Southeastern Runner obtained from a grower in 1931 and was released in 1947 (Hammons, 1970). Southeastern Runner 56-15 is a late maturing (ca. 150+ DAP in south Georgia) runner-type cultivar with a runner growth habit and pink testa color (Higgins and Bailey, 1955). At the time of release, it was higher yielding than the widely-grown 'Dixie Runner' cultivar from Florida (Carver and Hull, 1950). Another old landrace cultivar 'North Carolina Runner' as previously mentioned is possibly similar to African Runner and Southeastern Runner. Interest in 'Carolina African Runner' was recently reported to have

distinctively good roasted flavor (Anonymous, 2016). The NC State peanut breeding program had a few seed in cold storage of North Carolina Runner No. 4. So, the objective of this study was to compare these two old landrace cultivars to determine how different each were to one another based upon DNA sequence polymorphisms.

MATERIALS AND METHODS

During 2024, the two runner-type cultivars, Southeastern Runner 56-15 and North Carolina Runner No. 4, were planted in April at the Gibbs Research Farm near the University of Georgia, Coastal Plain Experiment Station, Tifton, GA. A randomized complete block field design with only two replications was used due to lack of seed. Twenty seed were planted per 30.5 cm of row withing each of two rows, 6.1 m long x 1.8 m wide. The soil type was a Tifton loamy sand (fine-loamy, siliceous, thermic, Plinthic Kandidult). Recommended cultural practices with irrigation were followed throughout the growing season.

Each cultivar was individually dug near optimum maturity based upon the hull-scrape method from adjacent border plants (Williams and Drexler, 1981). After harvesting (digging and picking), peanut pods from each plot were dried with forced warm air to approximately 6% seed moisture content. Pod samples were then cleaned, and 1000g grade samples were presized and shelled on federal-state inspection service (FSIS) equipment accordingly for runner-type peanut (USDA-AMS, 2019). Seed samples of Southeastern Runner 56-15 and North Carolina Runner No. 4 were sent back to North Carolina for genomic sequencing at N. C. State University in Raleigh, NC. Two other cultivars, a normal-oleic runner-type 'Tifrunner' (Holbrook and Culbreath, 2007) and a high-oleic virginia-type 'Bailey II' which was tested in the Uniform Peanut Performance Tests as breeding line N12008olCLSmT were included for comparison. Bailey II was derived by backcrossing to 'Bailey' (Isleib *et al.*, 2011) as the recurrent parent (Branch *et al.*, 2018). Both cultivars were also included as current checks that were developed by hybridization. Tifrunner was the peanut cultivar used in the original genomics study by Bertioli *et al.*, (2019).

For genomic sequencing of the four cultivars, young leaf tissue was flash frozen in liquid nitrogen from a single, greenhouse-grown seedling of each line. DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Germantown, MD) and quantified using Promega QuantiFluor (Madison, WI). Sequencing libraries were prepared by the N. C. State University Genomic Sciences Laboratory and sequenced on a single NextSeq2000 P4 150bp paired end XLEAP flow cell. WGS data were cleaned by fastp v. 0.20.1 (Chen *et al.*, 2018). Cleaned reads were globally aligned against the Bailey II reference genome with Bowtie 2 (Langmead and Salzberg, 2012). SAMtools (Li *et al.*, 2009) was used to remove discordant alignments, remove reads with map quality below 12 and mark optical duplicates. Freebayes (Garrison and Marth, 2012) was used for joint calling with no requirements for minimum mapping quality. The resulting set of variant calls were filtered with VCFtools (Danecek *et al.*, 2011) and BCFtools (Danecek *et al.*, 2021) by keeping only biallelic SNPs with an average read depth between 5 and 200, excluding any site with any missing data and removing sites without polymorphism. The resulting marker set was loaded into Tassel 5.0 (Bradbury *et al.*, 2007) and analyzed using the 'Distance Matrix' to determine genomic differences between all lines. The 'Distance Matrix' calculates genomic differences based upon DNA sequence polymorphisms.

RESULTS AND DISCUSSION

The U.S. Dept of Agriculture began the earliest peanut breeding program about 1918 in Virginia, and the following state experiment stations began in Florida during 1920 (Gorbet,1999), North Carolina during 1929, and Georgia during 1931 (Gregory *et al.*, 1951). These early breeding programs started by making pure-line selections within mixed seed stocks of supposedly different old landrace cultivars and progressed toward hybridization-based breeding programs involving genetically diverse parental lines to increase pod yields, grade, disease and insect resistance, etc.

Table 1. Genomic distance matrix percentages based upon DNA sequence polymorphisms comparing two old landrace selected peanut cultivars (Southern Runner 56-15 and North Carolina Runner No. 4) versus two hybrid developed check cultivars (Tifrunner and Bailey II).

	Bailey II	N. C. Runner 4	SE Runner 56-15	Tifrunner
Bailey II	**0.000**	**0.392**	**0.383**	**0.495**
N. C. Runner 4	**0.392**	**0.000**	**0.030**	**0.375**
SE Runner 56-15	**0.383**	**0.030**	**0.000**	**0.355**
Tifrunner	**0.495**	**0.375**	**0.355**	**0.000**

Brown *et al.* (2021) evaluated genetic diversity of the Georgia peanut breeding program since 1931. Results from this study showed that these early pure-line selections generally clustered together in an unrooted phylogenetic tree which

included a total of 32 peanut cultivars developed by the University of Georgia. However, the genotyping-by-sequencing (GBS) method grouped the early pure-line selection (Southeastern Runner 56-15) with three other pure-line selected cultivars (Virginia Runner G29, Virginia Bunch G2, and Virginia Bunch 67) and one hybrid-developed cultivar (Georgia 119-20). The Georgia 119-20 cultivar is a virginia market-type resulting from a cross between 'Jumbo Runner' with a bunch selection from a cross of North Carolina Runner with 'Dixie Giant' (Bailey, 1951).

During the 2024 growing season at Tifton, GA both Southeastern Runner 56-15 and North Carolina Runner No. 4 had 91% and 100% tomato spotted wilt virus (TSWV) disease incidence, respectively. Thus, each of these two old landraces were found to be very susceptible to TSWV. The sound mature kernel weight averaged 42g 100^{-1} and 44g 100^{-1} for Southeastern Runner 56-15 and North Carolina Runner No. 4, respectively. Also, both old landrace peanut cultivars have similar spreading runner growth habit and a late maturity approximately 150+ days after planting in south Georgia.

The question still remains about the genetic difference or similarity between these two old landrace cultivars Southeastern Runner 56-15 versus North Carolina Runner No. 4. Molecular results from this current study clearly show that these two peanut cultivars are only 3% genetically different based upon approximately 444,000 SNP markers (Table 1). Whereas, both North Carolina Runner No. 4 and Southeastern Runner 56-15 are 39.2% and 38.3% different from Bailey II and are 37.5% and 35.5% different from Tifrunner, respectively. These results should not be unexpected based upon the narrow genetic base available during the time of these early state peanut breeding programs. In conclusion, this study suggests that these two older cultivars are likely the same. Similar to the Brown *et al* (2021) genetic diversity study, Southeastern Runner 56-15 and the older Georgia runner cultivar had within line genomic distance between 0-5.3%

LITERATURE CITED

Anonymous. 2016. Heirloom peanut makes comeback. Pp: 16-17 In: The Peanut Grower Magazine. One Grower Publishing, LLC.

Bailey W. K. 1951. Virginia type peanuts in Georgia. Univ. of Georgia Expt. Sta. Bulletin 267: 22 pp.

Bertioli D. J., J. Jenkins, J. Clevenger, O. Dudchenko, D. Gao., G. Seijo, S.C.M. Leal – Bertioli, et al. 2019. The genome sequence of segmental allotetraploid peanut *Arachis hypogaea*. Nature Genetics. 51: 877-884.

Boswell V. R. 1937. Improvement of vegetable crops – appendix. pp. 340-378. In: Yearbook of Agriculture. U.S.D.A. U.S. Gov. Printing Off., Washington, DC.

Bradbury P.J., Z. Zhang, D.E. Kroon, T.M. Casstevens, Y. Ramdoss, and E.S. Buckler. 2007. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics. 23(19): pp.2633-2635.

Branch W. D. 1993. Peanut Research In: M. H. Bass (Ed.) The UGA Coastal Plain Experiment Station…The First 75 Years. Lang Printing Co., Tifton, GA.

Branch W. D., M. Balota, T. G. Isleib, D. J. Anco, J. P. Bostick, C. Y. Chen, B. L. Tillman, M. D. Burow, M. R. Baring, and K. D. Chamberlin. 2018. Uniform Peanut Performance Tests – 2017. Univ. of Georgia, Coastal Plain Expt. Station Res-Prog. Report No. 4-17. pgs. 1-25.

Brown N., W. D. Branch, M. Johnson, and J. Wallace. 2021. Genetic diversity assessment of Georgia peanut cultivars developed during ninety years of breeding. Plant Genome. 14:e20141.

Carver W. A. and F. H. Hull. 1950. Dixie Runner Peanuts. Univ. of Florida, Agric. Expt. Stn. Circular S-16:3p.

Chen S., Y. Zhou, Y. Chen, and J. Gu. 2018. Fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 34 (17): i884–i890. doi: 10.1093/bioinformatics/bty560.

Danecek P., A. Auton, G. Abecasis, C. A. Albers, E. Banks, and M. A. DePristo, et al. 2011. The variant call format and VCFtools. Bioinformatics. 27 (15): 2156–2158. doi: 10.1093/bioinformatics/btr330.

Danecek P., J. K. Bonfield, J. Liddle, J. Marshall, V. Ohan, M. O. Pollard, et al. 2021. Twelve years of SAMtools and BCFtools. GigaScience. 10 (2): giab008. doi: 10.1093/gigascience/giab008.

Garrison E. and G. Marth 2012. Haplotype-based variant detection from short-read sequencing. Available at: http://arxiv.org/abs/1207.3907.

Gorbet D.W. 1999. University of Florida Peanut Breading Program. Florida Proc. Soil and Crop Sci. Soc. 58:2-7.

Gregory W. C., B. W. Smith, and J. A. Yarbrough. 1951. Chap III Morphology, Genetics and Breeding, pp. 28-88. In The Peanut-The Unpredictable Legume: A Symposium. The Nat. Fertilizer Assoc. Washington DC.

Hammons R. O. 1970. Registration of Southern Runner 56-15 peanuts. Crop Sci. 10: 727.

Higgins B. B. 1951. Chap. II Origin and early history of the peanut. pp: 18-27 In The Peanut – The Unpredictable Legume A Symposium. The Nat. Fertilizer Assoc., Washington DC.

Higgins B. B. and W. K. Bailey. 1955. New varieties and selected strains of peanuts. Georgia Agr. Expt. Sta. Bulletin N. S. 11: 35pp.

Holbrook C. C., and A. K. Culbreath. 2007. Registration of 'Tifrunner' peanut. J. Plant Reg. 1: 124.

Isleib T. G., S. R. Milla-Lewis, H. E. Pattee, S. C. Copeland, M. C. Zuleta, B. B. Shew, J. E. Hollowell, T. H. Sanders,

L. O. Dean, K. W. Hendrix, M. Balota, and J. W. Chapin. 2011. Registration of 'Bailey' peanut. J. Plant Reg. 5:27-39.

Langmead B. and S. L. Salzberg. 2012. Fast gapped-read alignment with bowtie 2. Nat. Methods. 9 (4): 357–359. doi: 10.1038/nmeth.1923.

Li H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, et al. 2009. The sequence Alignment/Map format and SAMtools. Bioinformatics. 25 (16), 2078–2079. doi: 10.1093/bioinformatics/btp352.

USDA-AMS. 2019. Farmer's stock inspection instructions. USDA-Agricultural Marketing Service, Fruit and Vegetable Division. Washington, DC.

Williams E. J. and J. S. Drexler. 1981. A non-destructive method for determining peanut pod maturity. Peanut Sci. 8:134-141.

Woodruff J. G. 1973. Chap. 3-The culture of peanuts. pp 44-92 In Peanuts: Production Processing Products 2nd Ed. The AVI Publishing Co. Westport, CT.