THE CROP JOURNAL XX (2018) XXX–XXX

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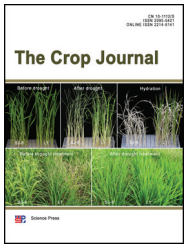

Population structure and association mapping to detect QTL controlling tomato spotted wilt virus resistance in cultivated peanuts

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ARTICLE INFO

Article history:
Received 5 February 2018
Received in revised form 9 April 2018
Accepted 29 April 2018
Available online xxxx

Keywords:
Association mapping
SSR markers
Tomato spotted wilt virus
Peanuts

ABSTRACT

Tomato spotted wilt (TSW) is a serious virus disease of peanut in the United States. Breeding for TSWV resistance would be facilitated by the implementation of marker-assisted selection in breeding programs; however, genes associated with resistance have not been identified. Association mapping is a type of genetic mapping that can exploit relationships between markers and traits in many lineages. The objectives of this study were to examine genetic diversity and population structure in the U.S. peanut mini-core collection using simple sequence repeat (SSR) markers, and to conduct association mapping between SSR markers and TSWV resistance in cultivated peanuts. One hundred and thirty-three SSR markers were used for genotyping 104 accessions. Four subpopulations, generally corresponding to botanical varieties, were classified by population structure analysis. Association mapping analysis indicated that five markers: pPGPseq5D5, GM1135, GM1991, TC23C08, and TC24C06, were consistently associated with TSW resistance by the Q, PCA, Q+K, and PCA+K models. These markers together explained 36.4% of the phenotypic variance. Moreover, pPGPseq5D5 and GM1991 were associated with both visual symptoms of TSWV and ELISA values with a high R^2. The potential of these markers for use in a marker-assisted selection program to breed peanut for resistance to TSWV is discussed.

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Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.
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https://doi.org/10.1016/j.cj.2018.04.001

Please cite this article as: J. Li, et al., Population structure and association mapping to detect QTL controlling tomato spotted wilt virus resistance in cultivated peanuts, The Crop Journal (2018), https://doi.org/10.1016/j.cj.2018.04.001

1. Introduction

Peanut (*Arachis hypogaea* L.) is a species in the family Fabaceae, the legume family, that is grown in tropical, subtropical, and temperate areas of the world. Peanut is an oil crop that contains 50% oil on average. Peanut is believed to have originated in South America, the most likely center of origin being Brazil and Peru, where >10 wild species were found [1]. The cultivated peanut is an allotetraploid ($2n = 4x = 40$) that originated through hybridization of two ancient diploid species, probably *A. duranensis* (A genome) and *A. ipaensis* (B genome) [2]. It contains two subspecies: *A. hypogaea* ssp. *hypogaea* and *A. hypogaea* ssp. *fastigiata*. The ssp. *hypogaea* consists of the botanical varieties *hypogaea* and *hirsuta*, whereas the ssp. *fastigiata* consists of the botanical varieties *fastigiata*, *vulgaris*, *peruviana*, and *aequatoriana* [3].

Tomato spotted wilt virus (TSWV) is a species of the *Tospovirus* genus, which is transmitted in nature exclusively by thrips [4]. The virus has caused severe damage on peanuts in U.S. since 1984, and was estimated to have cost at least USD 44 million in 1995 in Georgia alone [5]. Economic losses caused by TSWV in peanut have been reduced with the development of resistant varieties [5]. No cultivar is completely resistant to TSW; resistant cultivar can become infected and may exhibit minor symptoms under high pathogen pressure. In addition, TSWV can evolve and cause more severe symptoms in resistant cultivars over time. The genes responsible for resistance and the mechanism of resistance, however, remain unknown, limiting the ability to select for resistance in newly developed peanut cultivars. Breeding resistant cultivars is the optimal method of avoiding severe TSW-incited yield loss.

Simple sequence repeat (SSR) markers have been the most widely used DNA marker in plant breeding programs because of their high degree of polymorphism, high abundance, codominant inheritance, easy use, ready transferability, and relatively low cost [6, 7]. The advantage of SSRs over other markers includes their facility of use to track desirable traits in large-scale breeding programs and to serve as anchor points for map-based gene cloning [8]. Association mapping based on linkage disequilibrium (LD) provides an effective way to map quantitative trait loci, given that ancestral recombination events that occurred in natural populations generate a potentially large number of alleles per locus to associate markers and traits. In peanut, the first attempt at association mapping was reported for seed quality traits using SSR and single nucleotide polymorphism (SNP) markers in 2011 [9]. More recently, a genome-wide association study [10] that included 300 peanut accessions identified 524 significant associations for 36 traits. These could be used in improving biotic and abiotic stress resistance, seed quality, and yield.

To date, no research on SSR marker association for resistance to TSW in peanut has been reported. To improve peanut breeding programs that include breeding for resistance to TSW, it is vital to conduct association mapping of SSR markers with TSWV resistance in peanuts. The objectives of this study were (1) to examine TSWV resistance in the peanut mini-core collection under natural conditions in field and greenhouse studies using artificial mechanical transmission; (2) to characterize the genetic diversity and population structure in the mini-core collection using SSR markers; (3) to identify, by association mapping, SSR markers associated with TSWV.

2. Materials and methods

2.1. Plant material and DNA extraction

A total of 118 accessions were included in the experiment, of which 104 were from the U.S. peanut mini-core collection. These accessions included six botanical varieties: *fastigiata*, *hypogaea*, *peruviana*, *vulgaris*, *aequatoriana*, and *hirsuta*. DNA from seeds of each accession was extracted following Dang and Chen [11]. DNA samples were dissolved and diluted in 0.1× TE (1 mmol L^{-1} Tris, 0.1 mmol L^{-1} EDTA, pH 8.0) to a final concentration of 10 ng μL^{-1} for use in PCR, and a Nano-Drop 2000c spectrophotometer (Vernon Hills, IL, USA) was used to evaluate the quality and concentration of DNA samples.

2.2. Phenotyping in greenhouse

The 118 peanut accessions were grown in the greenhouse at 25–30 °C and 60%–90% relative humidity. The variety 'Georgia Green' was used as a control. Nine seeds per accession were sown in a plastic seedling tray (7.87 cm × 7.87 cm × 5.92 cm per cell) containing all-purpose professional growing mix consisting of Canadian sphagnum peat moss, coarse perlite, vermiculite, and dolomitic limestone (Sun Gro Horticulture, Agawam, MA, USA). Peanut plants at the two- to three-leaf stage (7 to 9 days after planting [DAP]) were dusted with carborundum. To prepare inoculum, infected tobacco leaves were collected and ground into a sap in fresh cold 0.01 mol L^{-1} potassium phosphate buffer at pH 7.0, including 0.2% sodium sulfite and 0.01 mol L^{-1} 2-mercaptoethanol, using a chilled pestle and mortar at the ratio of 1:6 of tissue/buffer. Celite 545 (Fisher Scientific, Fair Lawn, NJ, USA) and Carborundum 320 grit (Fisher Scientific) were added to the sap at a concentration of 1% and 2%, respectively. The sap was kept on ice until the inoculation was completed. Inoculum (1 mL per plant) was applied by rubbing both surfaces of the leaf with a cotton swab. After inoculation, the sap and carborundum were rinsed off the seedlings with distilled water. Inoculated plants were observed daily for symptom development. Plants were considered to have localized infection when chlorotic rings or concentric rings developed only on inoculated leaves without symptoms on new leaves. The plants were considered to be systemically infected when the symptoms developed on newly emerging leaves. The plants were monitored in the greenhouse for 40 days after inoculation. The proportion of infected plants with visual symptoms was recorded at 40 days post inoculation (DPI).

At 40 DPI, 0.2 g of roots was collected from every plant to confirm infection by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using TSWV-specific antiserum (AgDia, Inc. Elkhart, IN, USA). Each ELISA plate contained a negative control with buffer only, two negative controls from a healthy peanut plant, and a TSWV positive control supplied by AgDia. A plant was considered to be infected with TSWV if the ELISA value after subtraction of the

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