SNP marker-based genetic mapping of rust resistance gene in the vegetable cowpea landrace ZN016

Xinyi Wu, Baogen Wang, Xiaohua Wu, Zhongfu Lu, Guojing Li and Pei Xu*

Institute of Vegetables, State Key Lab. Breeding Base for Sustainable Control of Plant Pest and Disease, Zhejiang Academy of Agricultural Sciences, Hangzhou, 310 021, People’s Republic of China

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ABSTRACT
Cowpea rust, caused by Uromyces vignae, is one of the most destructive foliar diseases in cowpea [V. unguiculata. (L.) Walp]. Deployment of rust-resistant cultivars is the most environment-friendly and effective way to curb this disease. For this purpose, a fundamental step is to map the rust resistance genes in elite germplasm lines. In the current study, one major and two minor QTLs conferring rust resistance were detected in a 119-line recombinant inbred line (RIL) population derived from a cross between the rust resistant line “ZN016” and a susceptible cultivar “Zhijiang 282”, using the state-of-the-art cowpea iSelect Consortium Array. The major QTL, designated as Ruv1, was mapped to a 12.48 cM interval between the SNP markers 2_01772 and 2_03292 on LG09, which explained 34.8% of the phenotypic variation. The minor QTLs, designated as Ruv2 and Ruv3, were mapped to a 7.01 cM interval on LG7 and a 6.19 cM interval on LG8, which accounted for 13.4% and 11.9% of the phenotypic variation, respectively. This study demonstrates the usefulness of the Consortium SNP Array on complex trait mapping and solidifies the basis for marker-assisted breeding of new cowpea varieties with improved rust resistance.

Key words: Genetic mapping, Rust resistance, SNP marker, Vegetable cowpea.

INTRODUCTION
The vegetable cowpea (V. unguiculata L. Walp. ssp. sesquipedalis), commonly known as asparagus bean or yardlong bean, is a unique group of cowpea [Vigna. unguiculata (L.) Walp.] used as legume food in the world. Grown primarily in the humid warm east/southeast Asian regions, frequently suffers from diseases, which causes severe loss of pod and grain yields (Singh and Allen, 1979; Wang, 2004). Cowpea rust is a major disease caused by Uromyces vignae, is the most destructive foliar diseases in cowpea (Chandrasheker et al., 1989). The pathogen invades the leaf tissues and generates urediniospores on the surfaces, which can be rapidly disseminated by the wind to cause premature senescence of leaf and severe yield loss of pods and seeds later on. To date, most of asparagus bean varieties in the Chinese market are susceptible to rust. Therefore, there is an urgent need to develop elite rust resistant varieties to curb this disease.

Previous studies have reported various modes of inheritance of rust resistance genes in cowpea. In the African type cowpea (ssp.unguiculata), rust resistance was controlled by dominance genes with additive effects (Rangaiah, 1997), recessive genes (Uma and Salimath, 2004), or polygenes located at different loci (Uma et al., 2016). Earlier studies in asparagus bean revealed that the rust resistance was controlled by a single dominant gene. (Zhang et al., 1997; Li et al., 2007). While a few SCAR and SSR markers were known to be linked to rust resistance (Li et al., 2007; Uma et al., 2016). No genetic or physical gene map information of these genes has been reported so far.

The recent technological advancement facilitated the availability of genomic resources of cowpea provides great opportunities for disentangling genetic architecture of rust resistance (Muñoz-Amatriain et al., 2017). A cowpea iSelect Consortium Array (Illumina, Inc) that targets over 50k SNPs has been shown to be a very powerful tool for mapping complex traits (Xu et al., 2017). The present study, focuses on to map the rust resistance gene with SNP chip in a RIL population.

MATERIALS AND METHODS
Plant materials: “ZN016”, “Zhijiang 282” and an 119-line F₆ recombinant inbred line (RIL) population derived from these two accessions were used in this study. ZN016 is a highly resistant asparagus bean landrace to Uromyces vignae. Zhijiang 282 is a highly susceptible to above pathogen with excellent pod yield and quality traits.

Rust evaluation in tunnel greenhouse: Two independent artificial inoculation tests were conducted on 119 RILs in 2011 winter at the tunnel greenhouse of Haining (HN) experimental station (30ºN, 120ºE) of Zhejiang Academy of Agricultural Sciences. Six seeds each line were sown in a plastic pot (20-cm diam.) and three uniform plants per pot.

*Corresponding author’s e-mail: peixu@mail.zaas.ac.cn
were retained later for phenotyping. For inoculation, the whole plants were carefully sprayed with a urediniospores suspension (5 × 10^5 conidia per milliliter) when the third trifoliate leaves of the plants had fully expanded. The urediniospores were collected from naturally infected asparagus bean plants grown in Hangzhou (30ºN, 120ºE), Zhejiang Province. After inoculation, the plots were covered with plastic sheets to maintain humidity for 24 hours. Data on disease assessments were made after 21st day of inoculation. Lesion types of the first to fifth trifoliate leaves were recorded on a 0-5 scale and the disease indexes (DI) were calculated according to Li et al. (2007).

**DNA extraction and genotype identification**: Genomic DNA of the 119 RILs, the parents “ZN016” and “Zhijiang 282”, were extracted from young leaves of one month old seedlings using a DNA extraction kit (Qiagen, Hilden, Germany). The genotypes of the 119 RILs were retrieved from Xu et al. (2017), where 7,988 high quality SNP data were acquired by using the Cowpea iSelect Consortium Array which represents a publicly accessible resource for screening 51 128 SNPs. The Cowpea iSelect Consortium Array is available from Illumina (Illumina Inc., San Diego, CA, USA; http://www.illumina.com/areas-of-interest/agrigenomics/consortia.html).

**Genetic mapping and QTL analysis**: A linkage map for the RIL population earlier published (Xu et al.2017) contained 7,964 SNPs that were mapped to 697 bins in 11 linkage groups. To reduce redundancy in mapping, only one SNP marker each bin was retained to construct a new map. MapQTL V5 was used for QTL detection. The QTLs were first detected by interval mapping (IM), and then the markers closest to the QTL peak were fixed as cofactors in the multiple-QTL model (MQM) to confirm the QTLs and to scan for new QTLs. A LOD significance threshold of 3.0 was adopted as a commonly used standard.

**Comparing physical positions of the QTL and a previous mapped SCAR marker**: The sequences of the QTLs-flanking SNPs as detected in this study and a SCAR marker previously reported to link with rust resistance (Li et al., 2007) were used to blast cowpea IT97K-499-35 reference genome on Phytozome 12 (https://phytozome.jgi.doe.gov/pz/portal.html#info?alias=Org_Vunguiculata_er), in order to determine and compare their physical positions.

**RESULTS AND DISCUSSION**

**Inheritance of rust resistance in ZN016**: In the tunnel greenhouse tests, “ZN016” exhibited no rust symptoms, while “Zhijiang 282” displayed susceptible phenotype releasing more sporulation. Their F_1 progeny showed a resistant phenotype similar to “ZN016”. The DI of the 119 RILs varied broadly, from 0 to 0.88 in the 2011HN1 experiment and 0 to 0.96 in the 2011HN2 experiment, respectively (Table 1). The correlation coefficient between the two trials was 0.85 (P< 0.05). As shown in Fig 1, the DI of these RILs exhibited a two-peaked distribution in the two trials, while it was skewed toward the lower DI, indicating the existence of a major QTL for rust resistance. These findings are in agreement with the findings of Li et al. (2007) who proposed a single dominant gene mode for rust resistance in ZN016.

**Mapping of the rust resistance gene**: Under the LOD significance threshold of 3, a major QTL was detected to be associated with DI variation in both the 2011HN1 and 2011HN2 experiments. This QTL, designated as Ruv1, was located in a 12.483 cM interval on LG9 flanked by the SNP markers 2_01772 and 2_03292. The peak marker 2_04336 accounted for 34.8% of the phenotype variation (Table 2, Fig 2). Two minor QTLs designated as Ruv2 and Ruv3 associated with DI variation in both trials, were mapped to a 7.01 cM interval between the SNP markers 2_00934 and

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**Table 1: Rust disease index in the parental lines and the RIL population.**

<table>
<thead>
<tr>
<th>Trials</th>
<th>Parent</th>
<th>RIL</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>ZN016</td>
<td>ZJ282</td>
</tr>
<tr>
<td>2011HN1</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>2011HN2</td>
<td>0</td>
<td>0.65</td>
</tr>
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</table>

**Fig 1**: Frequency distribution of rust disease index in the RIL population in 2011HN1 (a) and 2011HN2 (b) experiments.
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2_12503 on LG7, and a 6.19 cM interval between the SNP markers 2_37041 and 2_07847 on LG8, respectively. 

Ruv2 had a maximum LOD score of 3.71 at the peak in the 2011HN1 trial, contributing 12.5% of the phenotypic variation. The LOD score of 3.28 was observed for Ruv3 in 2011HN1 trail, and it explained 11.9% of the phenotypic variation.

According to the cowpea genome assembly V1.1, Ruv1 was located in a genomic region spanning 1,266,531 to 12,644,493 bp on chromosome 11. Ruv2 was located in an interval from 25,637,691 bp to 26,166,990 bp on chromosome 2 and Ruv3 was in the interval from 11,513,685 bp to 28,652,848 bp on chromosome 9. In a previous study, we have identified a SCAR marker ABRS\textsubscript{AAG/CTG98} linked to rust resistance in “ZN016” by using a bulked segregant analysis (BSA) in an F\textsubscript{2} population (Li et al., 2007).

ABRS\textsubscript{AAG/CTG98} was found to locate at the position of 1,346,163 bp on chromosome 11, which falls well within the physical interval of Ruv1 as delimited by the flanking marker 2\_03292 (at 1,266,531 bp) and the QTL peak marker 2\_04336 (at 2,330,997 bp). Hence the present SNP-based QTL mapping validates the previous BSA analysis that was based on the earlier generation AFLP technology. Classic BSA has been demonstrated to be an efficient approach to map resistance genes based on the linkage of DNA markers to phenotypes for many qualitatively inherited traits (Michelmore and Kesseli 1991). However, for rust resistance, the classification of the resistant and susceptible phenotypes can be subjective, which may severely confound the BSA mapping result. To avoid this problem, QTL mapping approach are often used to identify the rust resistance genes/QTLs by using the original DIs data (Vuông et al., 2016; Leal-Bertioli et al., 2015). Benefiting from this strategy, two more minor QTLs controlling rust resistance in “ZN016”, which have likely been missed in the previous BSA mapping study in Li et al. (2007), were detected in the current study.

In conclusion, due to the recent availability of the cowpea Consortium SNP Array, a major QTL and two minor QTLs associated with rust resistance were detected in the Chinese vegetable cowpea landrace ZN016. The tightly linked molecular markers to each QTL are valuable for marker-assisted selection of rust resistance plants, breaking of linkage drag, pyramiding of multiple resistance and/or agronomically favorable genes, and will finally accelerate the development of new elite cultivars with rust resistance.

**ACKNOWLEDGEMENT**

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**Table-2: Rust resistance QTL detected in 2011HN1 and 2011HN2.**

| Trials     | QTL | LGs | Flanking markers | Genetic distance (cM) | Marker | Position (cM) | LOD  | R\(^2\)(%)
<table>
<thead>
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</thead>
<tbody>
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<td>2011HN1</td>
<td>Ruv1</td>
<td>LG9</td>
<td>2_01772 - 2_03292</td>
<td>49.80-62.28</td>
<td>2_04336</td>
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<td>49.80-62.28</td>
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<td>LG7</td>
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<td>30.83</td>
<td>3.19</td>
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<tr>
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<td>LG8</td>
<td>2_37041-2_07847</td>
<td>37.05-43.24</td>
<td>2_00497</td>
<td>38.79</td>
<td>3.28</td>
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