Biotechnology Reports 15 (2017) 132–137

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Full Length Article

SSR markers associated to early leaf spot disease resistance through selective genotyping and single marker analysis in groundnut (*Arachis hypogaea* L.)

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ARTICLE INFO

Keywords:
Groundnut
Early leaf spot
Simple sequence repeat
Single marker analysis
Selective genotyping

ABSTRACT

Groundnut (*Arachis hypogaea* L.) is an important oilseed and food crop of the world. Breeding for disease resistance is one of major objectives in groundnut breeding. Early leaf spot (ELS) is one of the major destructive diseases worldwide and in West Africa, particularly in Burkina Faso causing significant yield losses. Conventional breeding approaches have been employed to develop improved varieties resistant to ELS. Molecular dissection of resistance traits using QTL analysis can improve the efficiency of resistance breeding. In the present study, an ELS susceptible genotype QH243C and an ELS resistant genotype NAMA were crossed and the F_2 population genotypic and F3 progenies phenotypic data were used for marker-trait association analysis. Parents were surveyed with 179 simple sequence repeat (SSR) markers out of which 103 SSR markers were found to be polymorphic between the parents. These polymorphic markers were utilized to genotype the F_2 population followed by marker-trait analysis through single marker analysis (SMA) and selective genotyping of the population using 23 resistant and 23 susceptible genotypes. The SMA revealed 13 markers while the selective genotyping method identified 8 markers associated with ELS resistance. Four markers (GM1911, GM1883, GM1000 and Seq13E09) were found common between the two trait mapping methods. These four markers could be employed in genomics-assisted breeding for selection of ELS resistant genotypes in groundnut breeding.

1. Introduction

Groundnut or peanut (*Arachis hypogaea* L.), originated in South America, is one of the most important oilseeds and food crops cultivated in the semi-arid tropics. The cultivated groundnut is tetraploid ($2n = 4x = 40$). It is member of genus *Arachis* and family Leguminosae [1]. The agro-morphological diversity within the crop, particularly the differences in the branching pattern and presence of reproductive node on the main stem, allowed to distinguish the two cultivated subspecies i.e. *A. hypogaea* subsp. *hypogaea* and *A. hypogaea* subsp. *fastigiata*. The subspecies are further divided into botanical varieties. The subspecies *hypogaea* is divided into *hypogaea* (virginia) and *hirsuta,* while the subspecies *fastigiata* into *fastigiata* (valencia), *vulgaris* (spanish), *peruviana* and *aequatoriana* [1].

In 2014, groundnut was grown in 115 countries covering a total area of about 26.54 million (M) hectares (ha) with a global production of about 43.91 M tons and an average yield of about 1655 kg/ha [2]. The Asian continent ranks first with over 58.3% of world production, followed by the African continent (31.6%), American continent (10.0%) and Oceania (0.1%). The major producing countries are China (16.55 M tons), India (6.56 M tons), Nigeria (3.41 M tons), USA (2.35 M tons) and Sudan with 1.77 M tons [2]. In Africa, groundnut production has grown significantly from year 1990 to 2000. This growth is mainly due to increased production in West African countries such as Nigeria, Senegal, Ghana, Burkina Faso and Mali [3]. For example Nigeria, the third largest producer in the world, accounted for about a fourth of groundnut production in Africa in 2014 [2].

Groundnut is a good source of fat, protein and minerals and hence it plays important role in human nutrition. Its seed contains 48–55% oil and 26–28% protein, and is a rich source of dietary fiber, minerals and vitamins [4]. The haulms and groundnut cake are important sources of animal feed. In addition, groundnut has ability to fix atmospheric nitrogen to the soil to help in the maintenance of soil fertility. The hardiness, plasticity, the multiplicity of uses of groundnut makes it one

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http://dx.doi.org/10.1016/j.btre.2017.07.005
Received 16 May 2017; Received in revised form 27 July 2017; Accepted 28 July 2017
Available online 10 August 2017

of the most useful legume crops.

Despite its importance, the productivity of groundnut is severely constrained by several biotic and abiotic factors. The yield of groundnut in Africa is very low, around 1 ton/ha, compared to global average yield of about 2 ton/ha. Among the major constraints, biotic factors particularly foliar diseases constitute a serious yield limiting challenge in groundnut production. Early leaf spot (ELS) caused by *Cercospora arachidicola* Hori and late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Bert and Curtis) Deigton are the most destructive foliar fungal diseases [5]. Groundnut yield losses pertaining to these two diseases are estimated to reach up to 50–70% along with adverse effects on the quality of the produce [6].

In order to reduce the impact of these diseases, control methods include use of chemical and resistant varieties among others. The usage of fungicides allows good control, however majority of smallholder farmers cannot use them since they lack the financial resources and technical expertise required to use them [7]. Moreover, the use of fungicides is not a cost-effective approach for smallholder farmers. In addition, use of fungicides has negative effects on the environment as well as on human health. Genetic approach involving breeding for innate foliar disease resistance are considered sustainable and cost effective to reduce the impact of leaf spots. Studies were conducted to identify or develop resistant or tolerant varieties to these diseases through conventional breeding. The complex nature of inheritance with recessive genes conferring resistance has hindered the progress of disease resistance breeding [8].

The breeding efficiency for disease resistance can be enhanced by employing new biotechnological tools such as use of DNA markers for mapping and tagging of the markers with desirable traits [8–10]. Several studies have demonstrated that molecular technology assisted breeding has significant advantages than conventional breeding particularly for traits which are difficult to manage through phenotypic selection [11,12]. Among the molecular markers, microsatellites or simple sequence repeats (SSRs) have received extensive attentions owning to their advantages of high reproducibility, co-dominant inheritance and high information content [13]. Constructing a molecular linkage map is now routine to trace the valuable alleles in a segregating population. Mapping population plays a crucial role in linkage map construction. Genetically diverse parents are selected for developing a mapping population to generate complete linkage map with large number of molecular markers.

Selective genotyping offers an alternative resourceful approach for deciphering trait linked markers, in which DNA markers are assayed only on the most genetically informative progeny. Hence those with extremely high and/or low phenotypic values for a trait of interest are only subjected to the marker-trait analysis. This allocation of genotyping resources only to selected progeny can reduce genotyping costs with little loss of information, and/or for validation and fine-mapping of QTL that have been detected. This concept was introduced by Lebowitz et al. [14], who used the term 'trait-based analysis' to refer to approaches to QTL mapping in which marker allele frequencies are compared between groups of progeny selected based on trait values. Lander and Botstein [15] introduced the more general term 'selective genotyping' for QTL mapping based on selected groups of progeny, and suggested that QTL analysis in this case could also be based on the usual marker-based approaches that compare phenotypic values among marker genotype classes.

Sun et al. [16] indicated that QTL mapping based on selective genotyping is more powerful than simple interval mapping method but less powerful than composite interval mapping method. Lebowitz et al. [14] and Gallais et al. [17] have discussed the theory and experimental design for analysis of marker allele frequencies in classes of progeny defined on the basis of quantitative trait values. Both authors concluded that trait-based analysis of selectively genotyped progeny can be a useful alternative to marker-based analysis of all individuals in a population, when only one quantitative trait is of interest. Xu et al. [18] have also concluded from simulation analyses that selective genotyping can be used to replace the entire population genotyping approach.

The present study was conducted to identify SSR markers associated to ELS disease resistance through selective genotyping.

2. Materials and methods

2.1. Mapping population

The F_2 mapping population comprising of 82 $F_{2:3}$ lines developed from the cross QH243C × NAMA was used for this study. QH243C belongs to Spanish bunch and is a high yielding cultivar in Burkina Faso; however it is susceptible to ELS. The genotype NAMA belongs to Virginia bunch and is highly resistant to ELS. The mapping population was developed at ICRISAT Mali. The F_2 and F_3 progenies were used for genotyping and phenotyping, respectively. The field experiment for phenotyping was carried out at ICRISAT Mali research station while the genotyping was done at ICRISAT Patancheru, India.

2.2. Phenotyping for early leaf spot disease

A set of 82 F_2 individual plant and 46 F_3 mapping population (23 resistant and 23 susceptible) along with the parental genotypes was phenotyped for ELS disease resistance. Phenotyping of mapping population was done during 2013 rainy season for F_2 population and 2014 rainy season for F_3 mapping population at ICRISAT Mali station under natural infestation. This station has been known to be a hotspot for ELS. The 23 resistant and 23 susceptible genotypes were obtained from F_2 individual plant phenotyping. Seed of each F_3 progeny was planted in a 4 m row spaced at 50 cm, and intra row spacing was 15 cm. Randomized complete block design with 3 replications was used to raise the F_3 population. The seeds were treated with the fungicide APRON STAR 42W before sowing. Disease scoring for ELS was done at 40 days (ELS_I), 60 days (ELS_II) and 80 days (ELS_III) after sowing, by using a modified 9-point scale [19]. Disease score of 1 was given if there was 0% infection; 2 for 1–5%; 3 for 6–10%; 4 for 11–20%, 5 for 21–30%; 6 for 31–40%; 7 for 41–60%, 8 for 61–80% and 9 for 81–100% infection were recorded. Plants with a disease score of 1–3, 4–6 and 7–9 were designated as being resistant, moderately resistant and susceptible, respectively [20].

2.3. DNA extraction and genotyping with SSR markers

Firstly, young leaf tissues of the F2 plants were sampled and kept in a freezer at −80 °C. Then, for genotyping, only DNA of extreme progenies (i.e., 23 resistant and 23 susceptible) along with two parental lines were subsequently used. DNA was extracted using modified cetyltrimethyl ammonium bromide (CTAB) extraction method [21]. DNA quality and quantity were checked on 0.8% agarose gels and DNA concentration was normalized to get 5 ng/µl for further genotyping work.

Initially the parents QH243C and NAMA were screened for polymorphism by using 179 available SSR markers [21–26]. One hundred three (103) markers were found to be polymorphic between the parents QH243C and NAMA. Based on the phenotyping data, the 46 F_2 lines were selected for genotyping with the103 polymorphic SSR markers.

Polymerase chain reactions (PCR) were performed as described by Varshney et al.[27] with some modifications. The final reaction volume was 7 µl. The recipes for PCR reaction mixture for all the labeled and unlabelled primers were common except the volume of sterile distilled water. The PCR reaction was prepared in 384-well plates containing 2 µl template DNA (5 ng), 0.7 µl of 10× Taq buffer containing $MgCl_2$ (50 mM), 0.7 µl of dNTP (2 mM), 0.7 µl of primers (5pm/µl) (forward and reverse), 0.04 µl of Taq polymerase (Genei 5U/µl), 0.25 µl of dye (2pm/µl) and 2.41 µl, 2.66 µl of sterile double distilled water for unlabelled and labeled primers, respectively.