C. R. Biologies xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Comptes Rendus Biologies

www.sciencedirect.com

Molecular biology and genetics/Biologie et génétique moléculaires

Association mapping for yield and yield-contributing traits in barley under drought conditions with genome-based SSR markers

Salah Fatouh Abou-Elwafa

Agronomy Department, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt

ARTICLE INFO

Article history:
Received 20 December 2015
Accepted after revision 14 March 2016
Available online xxx

Keywords:
Association mapping
Drought
Barley
Hordeum
SSR markers

ABSTRACT

Drought negatively affects plant development, growth, yield, and ultimately production of crop species. Association analysis of yield and yield-contributing traits was conducted for a barley germplasm collection consisting 107 wild (*Hordeum spontaneum* L.) genotypes, originating from 12 countries using 76 SSR markers. Phenotypic evaluations were performed for days to heading, plant height, number of tillers/plant, spike length, thousand kernel weight, single plant yield under well-watered and drought-stress conditions. Highly significant differences between well-watered and drought-stress conditions were observed in all measured traits. Association analysis revealed a total of 83 significant marker–trait associations for all six measured traits. The results revealed that several chromosomal regions significantly influence more than one trait, suggesting a possible existence of pleiotropic or indirect effects. The phenotypic variation explained by individual marker–trait associations ranged from 5.08 to 27.84%. The results demonstrated that wild barley is a valuable source for improving yield and yield-contributing traits for drought tolerance. Our data provide a tool kit for the potential application of marker-assisted selection for drought tolerance in barley.

1. Introduction

Barley (*Hordeum vulgare* subsp. *vulgare*), the most drought tolerant of the small grain cereals and a major crop in the Mediterranean region, is considered a model species for physiological and genetic studies, because of its diploid nature, with a relatively small number of large chromosomes and has been widely used as a genetic model [1]. Since selection processes in many breeding programs limit the level of diversity, a wide and representative collection of germplasm is required in order to supply genetic diversity [2]. Cross compatibility and shared genome between wild and cultivated barleys introduces wild barley as a good source for valuable alleles to barley breeders for crop improvement and to enrich the barley germplasm pool available [3]. Recent advances in genetic and genomic techniques and technologies have led to explosive advances in new genetic and genomic approaches for the dissection of various quantitative traits and the determination of their chromosomal locations, which facilitated and advanced the identification of a number of marker–trait associations in various crop species, including barley. Genetic diversity in wild barley and its promising contribution as a source of favorable alleles for a number of agronomic traits such as plant height, grain yield and drought tolerance have been numerously reported [4–11].

Drought tolerance is defined as the ability of a plant to survive, grow, and produce a harvestable yield with limited water supply or under periodic conditions of water deficit [12]. Increased frequency of droughts

Email address: elwafa75@aun.edu.eg.

http://dx.doi.org/10.1016/j.crvi.2016.03.001
1631-0691/

negatively affect plant development, growth, yield, and ultimately production of crop species. *In the light of climate changes*, other factors that affect the sustainability of the world's resources and its consequences on food security, development of drought tolerant cultivars is therefore essential for maintaining yields under climate change conditions and for the extension of agriculture to sub-optimal cropping areas [13]. Given the complexity of the genetic control of drought tolerance (polygenic inheritance, low-heritability, and high G × E interactions), marker-assisted breeding has not contributed significantly to crop improvement for drought tolerance. However, progress has been made in the last two decades in the understanding of the genetic basis of drought tolerance in crop plants, which is a prerequisite for the application of marker-assisted breeding in the development of cultivars with improved tolerance. Barley seems to be relatively well adapted to water deficit; therefore, it is regarded as being a model to study and understand the genetic basis and mechanisms of drought tolerance [14–17].

Most quantitative trait loci (QTL) associated with drought tolerance in barley have been identified through yield and yield-contributing traits under drought-stress conditions [18–21]. Although the development of molecular markers and genome sequencing approaches should accelerate positional cloning of genes responsible for drought tolerance, the genomic regions associated with those QTLs are still relatively large and are usually inappropriate for screening in a breeding program. To the best of our knowledge, up to now there is no report available regarding the application of marker-assisted selection for drought tolerance in barley as well as in any other crop species. The limited success of the molecular breeding approaches until now suggests a careful rethinking about new plant genetic and genomic approaches and platforms that may allow us to overcome this limitation and improve our understanding and breeding for drought tolerance [22]. Therefore, considering the drawbacks of classical QTLs analysis, association mapping provides the opportunity to identify QTL with high mapping resolution as well as a lesser research effort. In barley, association mapping has been successfully employed in identifying molecular markers significantly associated with numerous phenotypes in a number of plant species, including salt tolerance, yield and yield stability under drought conditions, spot blotch, drought stress (DS) related traits in wild barley [23–28].

In the present study, we aimed to implement association mapping analysis to identify significant association between molecular markers and six yield and yield-contributing traits under drought-stress conditions.

2. Material and methods

2.1. Plant materials, planting conditions, and stress treatments

Ninety-four barley accessions (*Hordeum vulgare* ssp. *spontaneum*) collected from 12 different countries were provided by National Small Grains Germplasm Research Facility, USDA, ARS, Idaho, USA [29]. Drought stress experiments were conducted in 2014/2015 at the Asyut University Experimental Farm at latitude of 27° N. Experiments were conducted essentially as described by Abou-Elwafa [29]. In brief, nine seeds were sown in three rows in round plastic pots with a diameter of 25 cm and 40 cm depth filled with 12–13 kg of clay soil. Plants were fertilized three times with 250 ml of NPK liquid fertilizer containing 9% N, 3% P_2O_5, and 6% K_2O in each pot. Sowing dates were staggered so that accessions would experience stress at the flowering stage of development. A well-watered (WW) and severe DS experiments were carried out. The WW experiments were irrigated with about 500 ml of water per pot each day by drip irrigation. The DS experiments were also irrigated as WW experiments until 20 days before anthesis, when water was drastically decreased to 125 ml twice a week.

The following traits were recorded: i) days to heading (DH), date when 50% of plants have begun heading, ii) plant height (PH), height of main stem at maturity, iii) number of tillers/plant (NoT), iv) spike length (SL) in cm excluding the awns, v) thousand kernel weight (TKW) in g, and vi) single plant yield (SPY) in g. Drought tolerance indices (DTIs) were calculated for all studied traits by dividing the trait value under DS by the trait value under the control.

2.2. Genotyping and marker analysis

SSR primers were selected from published linkage map of barley as revealed by Marcel et al. [30]. SSRs were screened by using eight diverse accessions and finally, a total of 76 markers were selected based on clear polymorphic banding patterns (Supplementary Table 2). The SSR markers were identified based on their uniform distribution in the genome, quality of their PCR product and polymorphism level from the public sequences of Karakousis et al. [31], Ramsay et al. [32], and Rostoks et al. [33]. The number of alleles that resulted from each one of the 76 SSR markers was counted, and the frequency of each allele was computed across the whole set of accessions. Markers with an allele frequency less than 5% in the population (rare alleles) were treated as missing data and excluded from further analyses.

2.3. Association and statistical analyses

DNA marker-quantitative trait (SSR-trait) associations were identified using the general linear model (GLM) in TASSEL (http://www2.maizegenetics.net/). The model used to detect SSR-trait associations considers the effect of the genetic marker (M), the environment (E), and the interaction (M × E). The mean squares of M × E were used as an error term for the estimation of the F-statistic for each marker main effect. The mean squares of the residuals were used to calculate the F-statistic for the M × E effect. An SSR-trait association was considered real when the marker main effect was significant at $P \leq 0.01$ [34]. The presence of an SSR-trait association depending on the environment was identified when the M × E was significant at $P \leq 0.01$. Association analysis was performed using drought tolerance indices of six traits. Analysis of variance (ANOVA) of all studied traits was performed using

Please cite this article in press as: S.F. Abou-Elwafa, Association mapping for yield and yield-contributing traits in barley under drought conditions with genome-based SSR markers, C. R. Biologies (2016), http://dx.doi.org/10.1016/j.crvi.2016.03.001