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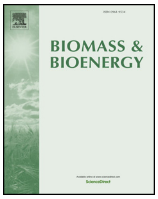

Research paper

Dynamic quantitative trait loci (QTL) for plant height predict biomass yield in hybrid rye (*Secale cereale* L.)

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ABSTRACT

The biomethane production from agricultural biomass will be a key component of the future energy mix. Rye (*Secale cereale* L.) is ideally suited for this purpose due to its high adaptability to a wide range of soils, weather and cropping conditions. Its high biomass yield is achieved with the lowest input of fertilizer and pesticides of all small-grain cereals. For the selection of cultivars with high kernel and biomass potential in the same breeding program, a prediction of biomass yield by indirect traits would increase the selection progress. To analyze quantitative-trait loci (QTL) for plant height in three developmental stages (BBCH 32, BBCH 51–55, BBCH 73) and biomass yield, 258 experimental rye hybrids were grown at three and four locations in Germany in 2011 and 2012, respectively. A genetic linkage map was constructed with 911 molecular markers. The QTL analysis identified a total of 18 QTL for plant height at the three assessment dates and of three QTL for dry matter yield at milk ripening. A major QTL on chromosome 2R explained 46, 12, and 25% of the total genetic variance for plant height at BBCH 51–55, BBCH 73, and for biomass yield, respectively. Furthermore, two large-effect QTL for plant height in two growth stages and dry matter yield were identified on chromosomes 3R and 5R. For eleven of the identified QTL in rye the respective QTL/genes on syntenic rice chromosomes were detected that could serve as candidate genes in follow-up experiments. A set of sequence-tagged site (STS) markers flanking the three QTL alleles for dry matter yield (*QDmy-2R, QDmy-3R,* and *QDmy-5R*) enables to the achievement of an indirect selection gain for biomass yield. This marker-assisted strategy allows for the screening of large hybrid rye populations already at early developmental stages without the resource-demanding harvest for biomass yield.

1. Introduction

The European Union aims for a reduction of greenhouse gas emissions by 40% by 2030 [1]. Even more ambitiously, the German Federal Government adopted a Climate Action Plan for a greenhouse-gas-neutral country by 2050 [2]. Switching energy supplies to renewables is the key to reach these targets and the contribution of plant biomass is of particular relevance. The anaerobic digestion of organic substrates for biogas production is a robust and highly flexible system where a wide range of diverse crops can be used [3]. Plants for energy production were grown in Germany on 24,000 km^2 in 2016, thus covering 20% of the total arable land [4]. Of this area, 15,000 km^2 (62.5%) were devoted to plants for biogas (biomethane) production, with maize still contributing 10,000 km^2 (41.7%) [4]. It is, therefore, politically and agronomically highly desirable to diversify the crop rotation by cultivating alternative cereals [5]. To achieve this goal, the maximum input of maize silage as a substrate in biogas plants was limited to 44% of the dry mass fraction till 2021 [6].

Winter rye (*Secale cereale* L.) is a cereal crop, which is mainly grown in Central and Eastern Europe. This close relative of wheat is ideally suited for biomass production due to its high adaptability to a wide range of environmental and cropping conditions. Its high biomass yield is achieved with the lowest input of fertilizer and pesticides of all small-grain cereals. In comparison to wheat and barley, rye proved to be the most effective species with a potential to reduce nitrate leaching by up to 93% at the field scale [7]. Thus, the cultivation of cereal rye contributes to reducing nitrogen losses and greenhouse gas emissions. In view of climate change, both factors are serious challenges [8,9] for sustainable agriculture, which aims to ensure food security for a growing human population given the need to manage the world's

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Abbreviations

BBCH	Code for describing developmental stages of crops. Abbreviation for Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie (= Federal Biological Agency [now Julius-Kuehn Institut], Federal Variety Protection Office, chemical industry)
cM	centi Morgan
COS	Conserved ortholog set (markers)
CV	Cross validation
DArT	Diversity array technology (markers)
DMY	Dry matter yield
DS	Data set
ES	Estimation set in CV
GY	Grain yield
LOD	logarithm of odd
MAS	Marker assisted selection
PH 1	Plant height measured at harvest stage BBCH 32
PH 2	Plant height measured at harvest stage BBCH51-55
PH 3	Plant height measured at harvest stage BBCH73
QTL	Quantitative trait locus/loci; SSR simple sequence repeat
STS	sequence tagged site
TS	Test set in CV

rapidly increasing demand for energy and water. As a consequence of its unique agronomic performance, winter rye is gaining increasing attention as a feedstock for lignocellulosic biomass to produce biofuels within corn–soybean rotations in the United States corn belt [10,11].

In Germany, about 20% of the rye harvest is already used for bioenergy production, predominantly for anaerobic digestion [12]. About 60% is applied to feed livestock and only the last 20% is used for human nutrition and other purposes. Therefore, the cultivation of this traditional cereal for energy use is not directly competing against bread making for the limited resources of land and water. Hybrid varieties are cultivated on the major part of the acreage in Germany. Currently, six hybrid and two population cultivars, which are harvested before reaching full maturity, are available options for farmers supplying feedstocks to biogas plants [13]. Hybrid breeding is particularly successful in rye due to two genetically divergent, heterotic pools (Petkus and Carsten) and the effective use of a cytoplasmic male sterility (CMS) system, both allowing for the exploitation of a maximal hybrid performance [14].

Quantitative trait loci (QTL) can help to accelerate and alleviate the breeding steps for developing new hybrid rye cultivars. Until recently, however, genomics-assisted breeding in the outbreeding and highly heterozygous rye was largely unexplored due to missing genomic tools. This has changed with the advance of microarray-based genotyping methods. QTL for important traits of testcross progeny within the Petkus gene pool were recently described in an initial genome-wide analysis of hybrid rye [15]. One to nine QTL were detected for the agronomic traits grain yield and thousand-kernel weight, as well as for the quality traits test weight, falling number, protein, total and soluble pentosane, and starch contents [15]. The falling number, also referred to as the Hagberg Number, is an internationally standardized method for determining pre-harvest sprouting [16]. Pentosanes, and more specifically the arabinoxylans, reduce the nutrient digestibility for livestock [17] but are highly important for baking quality [18]. For all traits, a high genotype × environment interaction variance was observed [15]. Recently, we reported on 22 QTL with significant effects on grain yield, heading date, tiller number, and thousand-grain weight across seven environments in testcross progeny between the Petkus and the Carsten gene pool [19]. Previous QTL mapping studies were based on the line *per se* performance for individual agronomic traits in rye, for example, dwarfing genes [20,21] or α-amylase activity [22–24]. An in-depth analysis of plant height and biomass yield in a triticale (× *Triticosecale* Wittmack) population of 647 doubled-haploid lines detected a major QTL explaining 42% of the genetic variance in plant height and 36% in the biomass yield on rye chromosome 5R [25–30]. This major QTL represented the dominant rye dwarfing gene *Ddw1* [20,31,32] that reduces the plant height and biomass yield considerably.

To achieve high biomass yielding hybrid rye cultivars it is possible to use plant height (PH) as an indirect trait for selection as judged from a phenotypic correlation study [33]. This is especially interesting if the grain and biomass yield are to be simultaneously improved in the same breeding program, because an indirect selection would avoid conducting additional experiments for biomass evaluation. Indeed, this additional effort is currently necessary, as efficient and non-destructive phenotyping methods are yet not available in rye to assess the biomass yield at the milk-ripening stage (BBCH73 according to [34]). In rye, a large array of QTL each with small effects was reported to govern plant height [15,35]. In contrast to wheat and triticale, dwarfing genes have yet not been used in practical rye breeding in Germany [15]. Therefore, modern elite rye is currently the tallest small-grain cereal with plant heights of 1.3–1.6 m, depending on the environment. This is especially a challenge when using genetic resources for introgressing biomass traits into elite breeding populations [36]. Consequently, rye still has a lower harvest index than wheat, that is, it produces more straw relative to kernel mass [37]. This renders rye particularly attractive as a biogas substrate, where the whole dry matter yield is the main breeding goal [5]. The exploitation of high biomass yielding rye hybrid cultivars holds great potential [15,36], but knowledge about the genetic basis of biomass yield in rye is still lacking. We report the first genome-wide QTL study of an elite interpool hybrid population (Petkus x Carsten) for plant height and biomass yield. The objectives of our study were to (1) identify the QTL of complex inherited agronomic traits focused on biomass yield, (2) identify the dynamics of plant height QTL through assessment in three growth stages, (3) detect potential co-located QTL for the analysed traits in order to test the possibility of breeding for biomass and grain yield in the same selection program, and (4) identify candidate genes for both traits based on a comparative genomics approach between rye and rice.

2. Materials and methods

2.1. Plant material

A biparental cross between two highly inbred parents from the Carsten (pollinator) gene pool (HYB201 and HYB202) was established by HYBRO Saatzucht GmbH & Co.KG and forwarded by selfing to $F_{2:3}$ lines as previously described [19,33]. In total, 258 $F_{2:3}$ lines were crossed with a cytoplasmatically male-sterile single cross tester of the Petkus (seed parent) gene pool resulting in three-way interpool hybrids. They were tested along with both testcrossed parents (each parent was repeated four times) and six checks were done for completing the field design. The plant material is described in Ref. [33] and is proprietary of HYBRO Saatzucht GmbH & Co.KG (Kleptow Nr. 53, 17291 Schenkenberg, Germany).

2.2. Field experiments and traits

Field experiments were conducted in 2011 and 2012 in Hohenheim, Baden Württemberg (48°72‘N, 9°20‘W), Groβ Lüsewitz, Mecklenburg-Western Pomerania (54°07‘N, 12°33‘W), Wulfsode, Lower Saxony (53°06‘N, 10°24‘W) and in Bornhof, Mecklenburg-Western Pomerania (53°49’N, 12°89′W). In total, data from seven environments (location x year combinations) were available, because the experiments in Bornhof