

Potential for Marker-Assisted Simultaneous Improvement of Grain and Biomass Yield in Triticale

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Abstract Triticale is a promising crop for agricultural biomass production but breeding has until now mainly focused on grain yield. Here, we evaluated the potential of marker-assisted simultaneous improvement of grain yield and biomass yield. To this end, we employed a large triticale doubled haploid population with 647 individuals derived from four families that were phenotyped for grain yield and biomass yield, as well as thousand-kernel weight, tiller density, and plant height in multi-environment field trials. Employing an association mapping approach, we identified quantitative trait loci (QTL) for all the five traits. The phenotypic correlation between grain yield and biomass yield was low, and we detected only one overlapping QTL suggesting different genetic architectures underlying both traits. Our results indicate that a marker-based selection for either grain yield or biomass yield does not adversely affect the other traits. Furthermore, an improvement of the multiplicative yield traits can to some extent also be achieved by selection for QTL identified for the component traits. Taken together, our results suggest that marker-

assisted breeding can assist the establishment of dual-purpose triticale cultivars with high grain and biomass yield.

Keywords Triticale · Grain yield · Biomass yield · QTL · Association mapping · Marker-assisted breeding

Introduction

Agriculturally produced crop biomass holds a huge unexplored potential for biomass production for industrial purposes (e.g., bioenergy, biofuels, biomaterials) and thus for a sustainable bio-economy. In recent years, energy crops have grown in acreage resulting in a competition of arable land for food, feed, or biomass production, which is often controversially discussed. To date, crops are usually grown for one purpose only but seldom for dual-purpose, i.e., harvest of grain and remaining biomass.

Triticale (\times *Triticosecale* Wittmack) can produce a higher biomass yield for a comparable grain yield as compared to other small-grain cereals such as wheat, barley, or rye [1]. Some studies therefore indicated that triticale may be suited for a joint use of grain and biomass yield [2–4]. While the biomass yield of triticale is lower compared to that of maize, it appears ideally suited to diversify crop rotation in regions where maize is the predominant crop grown for biomass production [5]. Nevertheless, at present, grain yield is the major breeding goal in triticale breeding programs, and limited effort has been targeted towards improvement of biomass yield. Most of the available triticale cultivars therefore have a high grain yield but perform poorly with regard to biomass yield. Conversely, the few available forage cultivars bred for high biomass yield tend to have low grain yield. Two approaches therefore appear suited to exploit the potential of triticale as a source for crop biomass production: The development of

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triticale cultivars with high grain yield and high biomass yield as dual-purpose cultivars or the breeding of two types of triticale cultivars, grain yield types and biomass yield types. However, breeding of both, dual-purpose and biomass yield types, has until now been hampered by the destructive analysis of biomass yield and the consequent need for separate breeding programs which requires major investment. Furthermore, whether competitive dual-purpose cultivars can be bred also depends on the correlation and the extent of shared genetic architecture between grain and biomass yield, as both need to be improved concurrently.

Biomass yield is evaluated in field trials by destructive analysis whereby the plots are harvested with a field chopper. The disadvantage of this approach is that no other traits, including grain yield, can be assessed on these plots and biomass is consequently not routinely assessed in breeding programs. This routine assessment of biomass yield potential can nowadays be facilitated by precision phenotyping platforms. Busemeyer et al. [6, 7] have recently reported the development of a multi-sensor platform and its use for the non-invasive prediction of biomass yield in triticale. This prediction by the “BreedVision” platform yielded high prediction accuracies illustrating the utility of precision phenotyping platforms for plant breeding.

Molecular markers linked to quantitative trait loci (QTL) can be used to assist classical breeding to enhance selection gain per time unit. This marker-assisted selection requires the identification of QTL for the trait of interest and the subsequent development of diagnostic markers. Association mapping is often done in diversity panels but the methodology can also be applied to QTL mapping in multiple segregating families [8, 9]. While multiple-line cross QTL mapping is an identity-by-descent approach based on linkage mapping methodology, association mapping is an identity-by-state approach that has recently been shown to perform well in a model comparison study [10]. The genetic architecture underlying grain yield and biomass yield in triticale has recently been investigated by multiple-line cross QTL mapping [11–13]. These analyses revealed that both traits must be regarded as complex traits controlled by few large- or medium-effect QTL and many small-effect QTL.

The aim of this study was to investigate the potential of a marker-assisted simultaneous improvement of grain yield and biomass yield in triticale. The study was based on a large triticale mapping population with 647 doubled haploid lines derived from four families that were evaluated for grain yield, biomass yield, and component traits in multi-environment trials. In particular, the objectives of this study were to (1) investigate phenotypic correlations between grain yield and biomass yield as well as thousand-kernel weight, tiller density, and plant height; (2) identify QTL for these traits by association mapping; (3) assess the co-localization of the QTL identified for the different traits; and (4) evaluate the potential to

improve grain yield or biomass yield by marker-assisted selection based on the identified QTL for these traits or the component traits.

Materials and Methods

Plant Material, Field Trials, and Phenotypic Data

The plant material and the field trials underlying this study have been described previously [6, 12, 14–17]. In brief, a mapping population consisting of 647 doubled haploid (DH) triticale lines was used [18, 19]. The population consisted of four families designated as DH06 ($n = 131$), DH07 ($n = 120$), EAW74 ($n = 200$), and EAW78 ($n = 196$) as described by Alheit et al. [20]. The DH lines were grown in partially replicated designs [21] including common checks with 960 plots per location, at two locations in 2 years, i.e., four environments. Total above-ground biomass yield (Mg ha^{-1}) was obtained by non-invasive prediction based on the “BreedVision” precision phenotyping platform at BBCH stage 81 (very early dough development) [7, 22]. At full maturity, data were recorded for grain yield (GY, Mg ha^{-1}), thousand-kernel weight (TKW, g), tiller density (TD, tillers per m^2), and plant height (PH, cm). The data on biomass yield, grain yield, thousand-kernel weight, and plant height were described by Alheit et al. [11] and by Liu et al. [13] and analyzed employing linkage mapping methodology.

Phenotypic data were analyzed by ordinary lattice analysis of variance [23]. Variance components were determined by the restricted maximum likelihood (REML) method assuming a full random model. Heritability (h^2) on an entry-mean basis was estimated from the variance components as $h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{G \times E}^2}{\text{env}} + \frac{\sigma_e^2}{\text{env} \times \text{rep}}}$, where σ_G^2 refers to the genotypic variance, $\sigma_{G \times E}^2$ to the genotype-by-environment interaction variance, σ_e^2 to the error variance, env to the number of environments, and rep to the number of replications per environment, which in this study with a partially replicated design equaled 1.45 [24]. Best linear unbiased estimates (BLUES) were estimated across environments assuming fixed effects for the genotype. All statistical analyses were performed using ASReml-R 3.0 [25].

Association Mapping in Multiple Families

The DH lines were genotyped with DArT markers, and QTL mapping was done based on the integrated consensus linkage map described by Alheit et al. [20]. For QTL mapping, an additive genetic model was chosen, and association mapping was done with a biometric model that performed well in a recent comparison of models for association mapping in multiple families [26] and that

incorporates a family effect, cofactors, and a SNP effect across families:

$$Y = 1\mu + X_f M_f + X_q b_q + \sum_{c \neq q} X_c b_c + \varepsilon.$$

In this model, Y is a $N \times 1$ column vector of the BLUEs of N DH lines, coming from F families ($F = 4$); 1 is a $N \times 1$ column vector containing constant 1; μ is the intercept; X_f is a $N \times F$ matrix whose elements are 1 or 0 according to whether or not an individual belonged to family f and M_f is a $F \times 1$ vector of family effects; X_q (X_c) is a $N \times 1$ column vector containing the marker information of each DH at marker q (cofactor c); b_q (b_c) is the expected allele substitution effect of marker q (cofactor c); and ε is the vector of the residuals of the model.

We applied a two-step procedure for QTL detection. In the first step, stepwise multiple linear regression was used to select a set of cofactors based on the Schwarz Bayesian criterion (SBC) with a model including a family effect and cofactors [27]. Cofactor selection was performed using the GLMSELECT procedure of SAS® [28]. In the second step, we calculated a P value for the association of each marker with the phenotypic value for the F test with a full model (with marker effect) against a reduced model (without marker effect). The Bonferroni-Holm procedure [29] was used to detect markers with significant ($P < 0.05$) main effects. QTL were declared as overlapping between the traits if they fell within an arbitrarily defined 10-cM interval surrounding each QTL.

The proportion of the within-family genotypic variance explained by the detected QTL was estimated as described previously [30]. To obtain asymptotically unbiased estimates of QTL parameters, and for the QTL frequency distributions, we employed fivefold cross-validation [30, 31].

Results

In the triticale mapping population with 647 doubled haploid lines derived from four families, significant genotypic and genotype-by-environment interaction variances were observed for grain yield, biomass yield, thousand-kernel weight, tiller density, and plant height (Table S1). The ratio between genotypic and genotype-by-environment interaction variances ranged between 0.3 for tiller density and 9.6 for plant height and was higher for biomass yield (5.3) than for grain yield (1.5). The heritability was only moderate for tiller density with 0.47 but high for the other four traits ranging between 0.78 and 0.95. Grain yield was significantly positively correlated with biomass yield ($r = 0.11$, $P < 0.01$), thousand-kernel weight ($r = 0.36$, $P < 0.01$), and tiller density ($r = 0.09$, $P < 0.05$) but not with plant height ($r = -0.02$) (Fig. S1). Biomass yield was significantly correlated with grain yield,

tiller density ($r = 0.28$, $P < 0.01$), and plant height ($r = 0.86$, $P < 0.01$). The correlation between grain yield and biomass yield was significant but rather low with 0.11 ($P < 0.01$) and varied between families, ranging from 0.04 in DH06 to 0.42 in EAW78 (Fig. S1).

Joint association mapping in the four families identified QTL for all the five traits (Table 1, Fig. S2). The highest number of QTL (35) was detected for plant height whereas only five QTL were found for tiller density. The proportion of genotypic variance jointly explained by the detected QTL was 15.1% for grain yield and 59.5% for biomass yield, and was maximum for plant height with 79.7%. The proportion of genotypic variance explained by each single QTL ranged from 1.6 to 4.7% for grain yield, from 1.0 to 34.4% for biomass yield, from 0.5 to 9.6% for thousand-kernel weight, from 4.8 to 10.2% for tiller density, and from 0.2 to 42.0% for plant height (Table S2–S6). The Manhattan plots indicated that some chromosomal regions were identified as QTL for several traits (Fig. S2). We arbitrarily defined QTL as overlapping between two traits if they fell within a 10-cM interval surrounding the QTL. Using this criterion, grain yield and biomass yield showed one overlapping QTL as illustrated by the Venn diagram (Fig. 1). This QTL affecting both traits was located on chromosome 5R. We found, however, several QTL overlapping between grain yield or biomass yield and the component traits. Grain yield shared three QTL with thousand-kernel weight, one with tiller density, and two with plant height, while biomass yield had in common six QTL with thousand-kernel weight, one with tiller density, and eight with plant height.

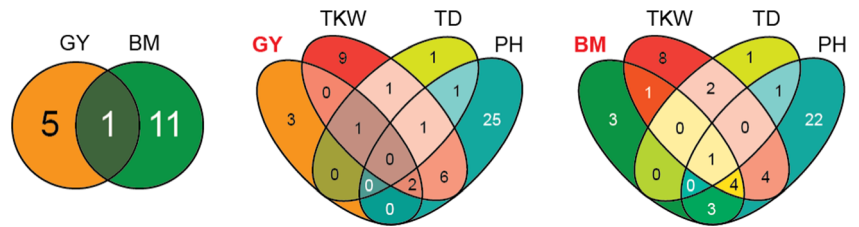
We employed a fivefold cross-validation approach to obtain asymptotically unbiased estimates of the genotypic variance explained by the detected QTL (p_G). This revealed a strong relative bias in p_G for grain yield and for tiller density (Table 1). The cross-validated predictive power for the proportion of genotypic variance explained by the identified QTL

Table 1 Results of QTL mapping and fivefold cross-validation for grain yield (GY), biomass yield (BM), thousand-kernel weight (TKW), tiller density (TD), and plant height (PH)

	GY	BM	TKW	TD	PH
QTL _{DS}	6	12	20	5	35
p_{G-DS} (%)	15.1	59.5	65.6	35.8	79.7
QTL _{ES}	4.8	10.1	18.6	5.2	19.5
p_{G-ES} (%)	15.4	55.9	64.8	36.1	74.2
p_{G-TS} (%)	2.6	39.0	49.0	12.8	59.1
Relative bias (%)	78.4	30.3	24.1	60.6	20.3

Number of detected QTL (QTL_{DS}), proportion of genotypic variance explained by the detected QTL across all families in the data set (p_{G-DS}), number of QTL (QTL_{ES}) and proportion of genotypic variance averaged over estimation sets (p_{G-ES}) and averaged over test sets (p_{G-TS}), and relative bias in the estimation of p_G

Fig. 1 Venn diagrams for trait-specific and overlapping QTL detected for grain yield (GY) and biomass yield (BM), as well as between grain yield or biomass yield and thousand-kernel weight (TKW), tiller density (TD), and plant height (PH)



ranged between 2.6% for grain yield and 59.1% for plant height. The QTL frequency distributions derived from the cross-validation revealed that some QTL detected with the full data set were supported by their identification in a high number of runs whereas others were only identified in few runs (Fig. S2).

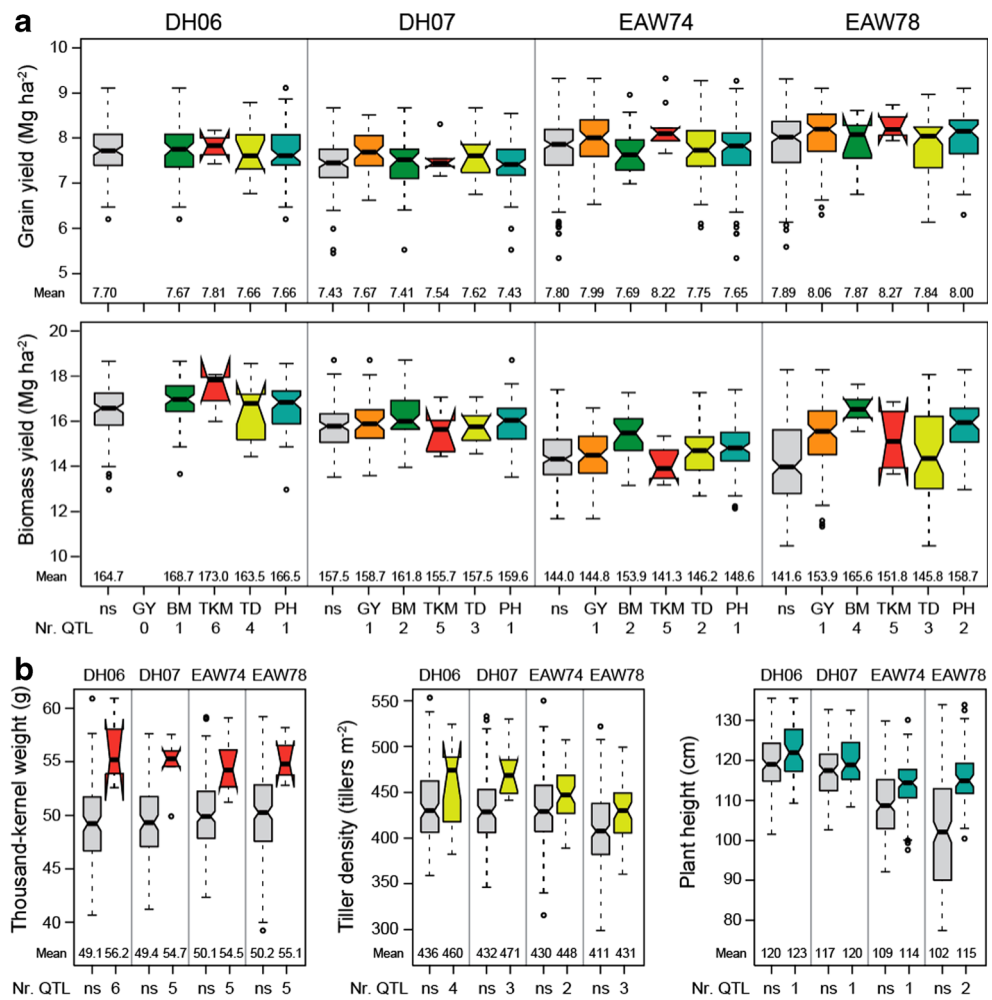
We next assessed the effect of marker-assisted selection on grain yield and biomass yield based on QTL explaining more than 3% of the genotypic variance (Fig. 2a). For grain yield, marker-assisted selection based on the grain yield, and in some families on the thousand-kernel weight QTL, increased the means of the families, whereas selection for the biomass yield, tiller density, or plant height QTL had no effect. Likewise, selection on the biomass yield QTL increased the

biomass level of the selected fraction, especially in family EAW78 in which also selection on the plant height QTL had a strong effect on biomass yield. The effect of selection on QTL of the other component traits depended on the family and especially in family EAW78 also increased biomass yield. Selection on the thousand-kernel weight, tiller density, or plant height QTL increased these traits in each family (Fig. 2b).

Discussion

In the last decade, the exploitation of agricultural biomass for the production of bioenergy has increased strongly in Central

Fig. 2 a Effect of marker-assisted selection on grain yield and biomass yield. The boxplots show the phenotypic values in each family after selection for the favorable alleles of all detected grain yield (GY), biomass yield (BM), thousand-kernel weight (TKW), tiller density (TD), or plant height (PH) QTL explaining more than 3% of the genotypic variance and segregating in the family. **b** Effect of selection on these QTL on the respective traits. *ns* no selection



Europe [32]. In addition to bioenergy, crop biomass also offers the potential for industrial purposes to establish a sustainable bio-economy. Triticale is a promising crop for biomass production owing to its high biomass yield and available genetic variation [5]. In this study, we investigated the potential for a simultaneous improvement of grain yield and biomass yield by marker-assisted breeding.

Breeding for Grain and Biomass Yield

Triticale breeding has until now mostly focused on grain yield. The majority of the available triticale cultivars therefore possess a shorter stature owing to the utilization of height-reducing genes, allowing higher doses of nitrogen fertilizer to be applied. These cultivars thus enable higher grain yields and often also possess a better grain yield stability as compared to the taller forage cultivars. Dual-purpose cultivars for a joint grain and biomass harvest are interesting for two reasons. On the one hand, harvesting the straw is of commercial interest in several regions for use in animal production or generally as renewable source for bioenergy, i.e., second-generation biofuels. On the other hand, cultivars that have a high biomass yield at early to late dough stage increase the flexibility of the farmer as the decision for either biomass harvest or grain harvest can be made in spring depending on the better return on investment. The alternatives are pure biomass type cultivars. It must be noted, however, that these also require a certain grain yield to minimize the costs for seed production.

The investment required for separate grain yield and biomass yield programs has thus far been the largest obstacle preventing selection gain in triticale biomass yield. In this study, we evaluated biomass yield through non-invasive prediction by the “BreedVision” precision phenotyping platform [6]. This enables the assessment of both early biomass yield and grain yield on the same plots and consequently obliterates the need for two separate breeding programs. An important criterion for the breeding of economically competitive dual-purpose cultivars is the correlation between grain yield and biomass yield, as both traits must be optimized concurrently. A positive correlation would thereby enable an indirect improvement of one trait by selection on the other, whereas a negative correlation would be problematic as improvements of one trait would come at the expense of the other. We observed a significant but rather low positive correlation between grain and biomass yield in all the families except one (Fig. S1). This suggests that both traits can be improved in parallel and improvements of one trait do not negatively impact selection for the other.

Gowda et al. [5] have recently reported that grain yield, early plant height, heading time, spikes per square meter, and thousand-kernel weight are key variables that allowed the prediction of early biomass yield. This shows that biomass

yield, just as grain yield, is a multiplicative trait with different component traits contributing to it. The heritability estimates are comparable to those reported for triticale or its two parents, wheat and rye, for grain yield (e.g., 0.78 in wheat by [33] or 0.70 in rye by [34]), biomass yield (e.g., 0.51 in triticale by [5] and 0.49 in rye by [35]), thousand-kernel weight (e.g., 0.85 in triticale by [5], 0.92 in wheat by [36], and 0.85 in rye by [34]), and plant height (e.g., 0.90 in triticale by [5], 0.85 in wheat by [37], and 0.92 in rye by [34]). The lowest heritability was observed for tiller density, a trait that is known to be difficult to score reliably and as illustrated here, also shows a strong genotype-by-environment interaction. Nevertheless, as the heritabilities of the traits examined here were generally high, this data set is well suited to study the potential of marker-assisted breeding in triticale.

Detection of Trait-Specific and Overlapping QTL

High-density genotyping which is available nowadays for triticale [20, 38] enables genomic approaches and subsequently marker-assisted breeding to assist classical phenotypic selection [9]. QTL mapping in multiple families promises to enhance QTL detection power and can be done by association mapping or by multiple-line cross QTL mapping. Both have been shown to perform well and to yield in part complementary results regarding identified QTL [10]. The mapping population underlying this study has recently been used to identify QTL for grain yield, thousand-kernel weight, biomass yield, and plant height by multiple-line cross QTL mapping [11, 13]. Here, we used an association mapping approach for multiple families which identified QTL for all the five traits (Table 1). The cross-validated proportion of genotypic variance explained by the detected QTL was comparable to the previous results but based in part on different QTL. This further supports the conclusion that association mapping and multiple-line cross QTL mapping are both suited for QTL detection in multiple families and possess different but complementary characteristics, resulting in different QTL being detected.

While the cross-validated proportion of genotypic variance was low for grain yield, it amounted to considerable 39.0% for biomass yield (Table 1). This may indicate that grain yield is an even more complex trait than biomass yield, controlled almost exclusively by small-effect QTL that escape detection in QTL mapping. The cross-validated predictive power was low for tiller density but moderately high for thousand-kernel weight and plant height. Thus, the component traits with their presumably less complex genetic architecture and oftentimes higher heritability may offer an avenue for a marker-assisted improvement of the complex yield traits.

We observed one overlapping QTL between grain yield and biomass yield which is consistent with the low phenotypic correlation between both traits (Fig. 1). This QTL was located on chromosome 5R and has recently been identified as *Ddw1*,

a major height-reducing gene from rye [11, 39]. Despite the low proportion of genotypic variance explained by the detected grain yield QTL, this low overlap in detected QTL indicates a different genetic architecture underlying grain yield and biomass yield. This further supports the conclusion that a simultaneous improvement of both traits should be feasible, also if assisted by marker-based approaches. By contrast, we found that some QTL regions associated with either grain or biomass yield were also identified as QTL for the component traits. This suggests that the observed significant phenotypic correlations are in part based on a shared genetic control.

Prospects for Marker-Assisted Improvement of Grain Yield and Biomass Yield

We investigated the potential of marker-assisted selection to improve grain yield or biomass yield based on the detected QTL explaining more than 3% of the genotypic variance (Fig. 2). Selection for the favorable alleles resulted in increased family means for both traits. Notably, selection for biomass yield QTL did not adversely affect the grain yield performance of the families and vice versa. This is in agreement with the low phenotypic correlation between both traits and the finding that their genetic architecture likely shares only few QTL. Consequently, a marker-assisted improvement of either of these two traits by selection based on molecular markers appears possible without negative effects on the other trait.

A recent study in maize indicated that a marker-assisted improvement of the multiplicative trait grain yield can be achieved by selection on QTL for the component traits kernel number and 100-kernel weight [40]. In our study, selection for thousand-kernel weight QTL resulted in some families in a similar increase of grain yield as compared to selection for the grain yield QTL (Fig. 2a). This comparable effect of the thousand-kernel weight QTL on grain yield is likely due to the higher number of QTL and the much higher proportion of genotypic variance explained by these QTL as compared to the grain yield QTL. This in combination with the high phenotypic correlation of thousand-kernel weight with grain yield, and the finding that half of the grain yield QTL were shared between the two traits illustrates that the complex trait grain yield can be improved by marker-assisted selection for the component trait thousand-kernel weight (Figs. 1 and 2). For biomass yield, a similar picture was observed, as selection for component trait QTL could improve biomass yield in some families. Notably however, the effect of selection on component trait QTL to increase grain or biomass yield was family-specific, and this approach therefore cannot be expected to always yield the desired effect. Furthermore, many of the identified QTL were family-specific and thus cannot be employed at random for marker-assisted selection in any family of a breeding program. Taken together, our results

nevertheless illustrate the feasibility of a separate as well as a simultaneous improvement of grain yield and biomass yield by marker-assisted breeding.

Conclusions

Crop biomass holds a huge yet unexplored potential for a sustainable bio-economy. The advent of precision phenotyping platforms nowadays enables the non-invasive assessment of biomass yield in small-grain cereals and thus a routine assessment of this trait. In this study, we show that breeding for increased biomass can be further assisted by genomic approaches such as marker-assisted selection to enhance selection gain per time unit. However, owing to the genetic complexity of grain and biomass yield, the predictive power for the proportion of explained genotypic variance was limited, and consequently, genomic selection might be an attractive alternative that warrants further research. Our results further revealed that grain yield and biomass yield were only weakly positive correlated and appear to be mainly controlled by different genetic architectures. This suggests that besides high yielding forage types, dual-purpose cultivars with high grain yield and high biomass yield can be established, either through concurrent phenotypic selection on both traits, or assisted by genomic approaches.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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