The da1 mutation in wheat increases grain size under ambient and elevated CO2 but not grain yield due to trade-off between grain size and grain number

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Abstract

Grain size is potentially yield-determining in wheat, controlled by the ubiquitin pathway and negatively regulated by ubiquitin receptor DA1. We analysed whether increased thousand grain weight in wheat da1 mutant is translated into higher grain yield and whether additional carbon provided by elevated (e)CO2 can be better used by the da1, displaying higher grain sink strength and size. Yield-related, biomass, grain quality traits and grain dimensions were analysed by two-factorial analysis, regarding genotype and eCO2. da1 increased grain size but reduced spikes and grains per plant, grains per spike and spikelets per spike, independent of eCO2 treatment, leaving total grain yield unchanged. eCO2 increased yield and grain number additively and independently of da1, but did not overcome the trade-off between grain size and number observed for da1. eCO2 but not da1 impaired grain quality, strongly decreasing concentrations of several macro- and micro-elements. In conclusion, intrinsic stimulation of grain sink strength and grain size, achieved by da1, is not benefitting total yield unless trade-offs between grain size and numbers can be overcome. The results reveal interactions of yield components in da1-wheat under ambient and eCO2, thereby uncovering limitations enhancing wheat yield potential.

Introduction

Wheat (*Triticum* spp.) accounts for 30% of the global grain production and of 45% of the cereal nutrition, thus representing a major food crop species (FAOSTAT, 2019). Despite significant breeding progress, yield levels of wheat remain below the required amounts to cope with the growing human population and with climate change consequences (Ray, Mueller, West, & Foley, 2013). Grain yield is the result of different components that interact in a complex and multiplicative manner (Schulthess et al., 2017; Sukumaran, Dreisigacker, Lopes, Chavez, & Reynolds, 2015) covering two main traits, related to grain number and grain dimensions. Various agronomic traits have contributed to grain yield improvement in the past such as plant height, harvest index, total biomass, number of productive tillers, grain number per spike, spike length, grain number per spike and per area and thousand grain weight (TGW). From these, harvest index and grain number per spike and per area are most important (Foulkes et al., 2011; Philipp et al., 2018). Multiple interactions and compensatory mechanisms exist among the different yield components, depending on genotype x environment interactions (Slafer, Savin, & Sadras, 2014).

Thousand grain weight is one component of yield and thus a potential target for wheat yield improvement (Brinton & Uauy, 2019; Foulkes et al., 2011; Mohler et al., 2016; Tshikunde, Mashilo, Shimelis, & Odindo, 2019). Genes involved in the ubiquitin pathway are central regulators of grain size for a number of plants (reviewed by Li and Li, 2014). Ubiquitin covalently binds to target proteins and triggers their degradation in the 26S proteasome complex (Vierstra, 2003). Notably, the ubiquitin proteasome pathway promotes

irreversible proteolysis of a set of regulatory proteins absolutely required for cell-cycle phase transitions (Genschik et al., 2014).

DA1 encodes an ubiquitin receptor containing two ubiquitin-interacting motifs (UIMs) and one zinc-binding LIM domain. DA1 binds polyubiquitinated proteins mediating their degradation by the 26S proteasome (Verma et al., 2004). Arabidopsis *da1-1* was isolated from a genetic screen by showing increased seed and organ size and by producing larger and heavier seeds resulting from enlarged sporophytic integuments (Li *et al.,* 2008). Thus, the size of the maternal outer layers can determine final grain size by setting a physical limit on the available space for the growing filial seed organ (Adamski*et al.,* 2009; Hasan *et al.,* 2011).

In spring wheat, the TaDA1 homeologs reveal high sequence similarities to AtDA1, especially in the UIMs and LIM domains, implying similar protein structures and functions and conserved interaction in the ubiquitin-proteasome pathway in plants (Liu et al., 2020). While TaDA1-B and TaDA1-D were predominantly expressed in the vegetative organs leaves and roots, TaDA1-A was principally expressed in young spikes at prior to anthesis. DA1-overexpressing plants contained fewer cells in the outer pericarp. On the other hand, DA1-RNAi plants had more outer pericarp cells, produced a wider pericarp cell layer and increased TGW by around 10%. This confirmed that TaDA1 functions to restrict early maternal cell proliferation (Liu et al., 2020). However, whether this increased TGW can be translated into a higher wheat grain yield remained unclear. Moreover, increases in grain weight have been frequently reported to have little impact on wheat grain yield often due to the trade-off between grain weight and grain number (Brinton et al., 2017; Philipp et al., 2018; Song, Huang, Shi, Zhu, & Lin, 2007; W. Wang et al., 2018). However, in some cases grain yield improvement in wheat has been significantly associated with increased TGW (Brinton & Uauy, 2019; Tshikunde et al., 2019).

Nevertheless, the positive effect of *da1* on seed size, as shown in wheat and Arabidopsis plants, may provide approaches to improve seed yield. In addition, higher grain size in wheat can positively affect grain composition, flour extraction and/or quality (Nuttall et al., 2017; Wiersma et al., 2001).

Higher grain size and therefore improved sink strength can be relevant to the response to eCO_2. This is especially important, given the fact that due to anthropogenic activities, the atmospheric CO_2 concentration is predicted to increase to 550 ppm by 2050 with profound consequences for crop growth (Lemonnier and Ainsworth, 2018; Uddling et al., 2018). While eCO_2 frequently improves carbon assimilation and increases plant biomass in many species (Taub et al., 2008), sink limitation often occurs, leading to photosynthetic feed-back inhibition (White et al., 2016). Grain yield in wheat is predominantly sink-limited during most of the grain filling period when grains grow under saturated source supply (Borras, Slafer, & Otegui, 2004; Sofield, Evans, Cook, & Wardlaw, 1977). Thus, a more efficient photosynthesis under eCO_2 conditions could be possible at less-limiting sink capacity and increasing the grain sink strength could be promising to use additional CO_2 (Wang et al., 2013).

It is hypothesized that grain yield in the da*1* mutant could potentially benefit from additional source stimulation by eCO_2 when compared to ambient conditions. Wheat genotypes with increased grain size, as achieved in the *da1* mutant, exhibit higher sink capacity/strength at the level of the individual grains. eCO_2 stimulates source activity, which in many plants stimulates photosynthesis and induces faster growth and biomass accumulation (Amthor, 2001; Jablonski, Wang, & Curtis, 2002).

In this study, the *da1* wheat mutant was characterised, which provides a suitable model to better understand intrinsic yield determinants in wheat. First, we asked whether the increased TGW in *da1* can be translated into higher wheat grain yield and what is the relationship of grain size with other yield-related factors. Second, we want to find out whether the additional carbon provided by eCO_2 can be better used by the *da1* mutant displaying higher sink strength at the level off individual grains. Using such an approach, simultaneously combines increases of both source (eCO_2) and sink strength (*da1*). To this end, yield-related and biomass traits, grain dimensions and grain quality traits were collected and analysed in a two-factorial analysis, regarding genotype and eCO_2 treatment.

Material and methods

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Generation of the *da1* mutant

The *da1* mutant derived from an ethyl methanesulfonate-mutant population established in spring wheat (*Triticum aestivum,*variety Trappe) by KeyGene (www.keygene.com). The mutant population has been screened for mutants in the *DA1* gene. Positive lines have been back-crossed (BC3) with Trappe to reduce background mutations. The mutations in the different A, B and D genomes have been combined by crossing.

The *da1* wheat mutant, under analysis in this study, possesses mutations in alleles of the wheat genomes A, B and D and in all three cases the mutation affects the DA1-domain either by blocking its transcription or by amino acid exchange (Fig. S1).

Plant growth

To simulate field-related conditions, plants were grown under semi-controlled conditions in 4 small greenhouses (6.1 x 3.4 m) in soil beds with regular irrigation and without supplemental light or temperature regulation for the duration of the experiment (Saalbach et al., 2014). The gables of the greenhouses consist of meshes to ensure optimal ventilation. Outside temperature was recorded throughout the experiment (Fig. S2). Grains of *da1* and Trappe were sown on April 3rd, 2018 and harvested at full maturity at July 18th, 2018 on the IPK campus, Saxony-Anhalt, Germany. A randomised block design was used with six blocks (= replications) per greenhouse in two-fold repetition (2 greenhouses for both ambient and eCO$_2$). Experimental plots (0.5 x 1m) consisted of four rows (= 160 seeds, which resulted in a density of 360 grains per m^2) Soil N content was determined and adjusted with fertilisation to 180 kg N ha^{-1} before the experiment with no further fertilisation during the experiment. eCO$_2$ treatment was started at the one-leaf stage in 2 greenhouses and continued until physiological maturity, with daily exposition from 5.00 a.m. to 10.00 p.m. During that time, CO$_2$ was continuously supplied from gas cylinders via a pipe system surrounding the interior of the respective greenhouses. Levels within the greenhouses were recorded and adjusted to 600 ppm using 2 CO$_2$ sensors per greenhouse. The other 2 greenhouses served as an ambient control.

Experimental design and data analysis

A two-factorial experiment was performed. The factors were: (i) spring wheat (*Triticum aestivum* L. cv. Trappe) versus the *da1* mutant and (ii) ambient (approx. 380 ppm) versus eCO$_2$(600 ppm). In order to avoid border effects at the front and the back of the plots, all samplings and measurements were performed only for the interior of the 2 inner rows (30 plants of each inner row = 60 plants per plot), resulting in a single measurement value per plot (except for spikelets per spike). Mature spikes from 6 main tillers per plot were harvested from both *da1* and Trappe under ambient and eCO$_2$ (6 spikes x 6 blocks x 2 greenhouses ambient x 2 greenhouses eCO$_2$ x 2 genotypes (*da1* , Trappe) = in total 288 spikes) and were harvested and investigated for the total number of spikelets per spike. Harvest index was calculated from each plot separately. The total dry weights of the 60 harvested plants of the inner rows per plot were determined and divided by the respective total grain weights

The experiment allowed to study the effect of each factor on response variables related to plant dimensions, yield-related traits, biomass traits, grain composition and spike-related traits. Interactions between the two factors were calculated for all combinations but were not significant. The data were analysed by two-way ANOVA using OriginPro 8.1 software (www.originlab.com/) and the statistical software program R (www.r-project.org/).

To verify the results, the experiment with *da1* and Trappe was repeated in 2019 under the same management conditions with eight plots of each genotype. However, the eCO$_2$ treatment was omitted in the 2019 experiments.

Analysis of grain morphology, sucrose, starch, carbon, nitrogen and micro-elements

Grain dimensions, thousand grain weight (TGW), grain width, length and area were determined on mature dry grains using the digital seed analyser MARVIN (www.marvitech.de). The traits harvest index, grain yield per spike, grain yield per spikelet and grain number per spikelet were calculated.

From each plot a sample of mature grains (app. 50 g) were ground by ball-mill to a fine flour, which was used for subsequent analysis of grain components, macro- and microelements. Starch and sucrose contents were determined using a coupled enzyme assay as described (Weigelt et al., 2009). Total carbon and nitrogen in dried wheat flour were determined with the Vario EL Elemental analyser (www.elementar.de).

Milled flour from multiple mature grains (see above) were weighed into PTFE digestion tubes and digested in HNO_3 under pressure using a microwave digester (UltraCLAVE IV; MLS). Macro- and microelements were measured by inductively coupled plasma optical emission spectrometry (ICP-OES, iCAP 6500, Thermo Fisher Scientific, Germany) combined with the CETAC ASXPRESS PLUS rapid sample introduction system, and a CETAC autosampler (CETAC Technologies, Omaha, NE, USA). Element standards were prepared from certified reference materials from CPI international (Eroglu et al., 2017).

Results

Sequences alignment and phylogenetic analysis

DA1 from the genome B was selected to perform a blastp search of orthologs on EnsemblPlants (https://plants.ensembl.org/index.html). Those sequences with a percentage of identity higher than 60% were selected for the following analyses i.e. *Zea mays* Zm00001d035844_T012, *Hordeum vulgare* HORVU2Hr1G002700.14, *Sorghum bicolor*SORBI_3010G064600:KXG19479, *Triticum dicoccoides*TRIDC2AG001610.3, *Brachypodium distachyon*BRADI_4g42580v3:KQJ92266, *Glycine max* GLYMA_17G247700:KRH05774,*Triticum turgidum* TRITD2Av1G003900.3, *Oryza sativa Japonica group* Os06t0182500-02, Arabidopsis thaliana AT1G19270.1, Aegilops tauschii AET0Gv20035900.10, *Triticum urartu*TRIUR3_12237:TRIUR3_12237-T1, *Medicago truncatula*MTR_5g018900:AES94835, *Manihot esculenta*MANES_10G022200:OAY38533, *Cucumis sativus* Csa_2G286500:KGN62017 and *Beta vulgaris* BVRB_6g138060:KMT08523). Amino acid alignment was performed with CLUSTALW followed by the construction of an unrooted phylogenetic tree using MEGA-X v10.1.5 with the ML (Maximum Likelihood) and NNI (Nearest-Neighbour-Interchange) JTT model on a 2000 bootstrap method (Kumar, Stecher, Li, Knyaz, & Tamura, 2018).

The ClustalO alignment indicated that the sequences TraesCSU02G00780.1, TraesCS2B02G007700.1 and TraesCS2D02G0169.1 correspond to DA1 genes of the genome A, B and D respectively and share 99% identity (Fig. 1). The DA1 gene in wheat encodes 504 amino acids and harbours a DA1-typical domain, ubiquitin interacting motif domain (UIM) and LIM-type zinc finger domain (Znf_LIM), (Fig. S3). Wheat DA1 has high identity to the homeologs of barley (98%), rice (88%), maize (86%), millet (86%), and Arabidopsis (60%), which suggests similar functions across plant species.

Plant performance and grain dimensions

After sowing the germination rate was determined as 99% to 100% with no apparent genotype and treatment effects. Days to anthesis (app. at 61 d after sowing) and length of flowering (app. 3.5 d) were found to be not dependent on either genotype or treatment.

The analysis of grain dimensions of mature grains revealed that thousand grain weight (TGW) for *da1* was increased by 8% (*P = 2E-5*). eCO_2 further enlarged TGW for *da1* and Trappe, each by another approx. 8% (*P = 3E-6,* Fig. 2). The increased TGW by both genotype and treatment was reflected by parallel and highly significant increases of grain length, width and area (Fig. 2). Thus, the*da1* mutant exhibited increased TGW compared to Trappe, and eCO_2 further increased TGW in a similar manner in both genotypes.

Yield-related traits

Grain yield calculated as tons ha^{-1} was not different between *da1* and Trappe but was significantly increased by 28% and 22% upon eCO_2 treatment for *da1* and Trappe, respectively (Fig. 3). Spike number per plant was unchanged by eCO_2 treatment but the genotype effect revealed 6% more spikes per plant for Trappe compared to *da1* .

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While grain number per plant was significantly enhanced under eCO_2 by 19% and 13% for Trappe and *da1* respectively, there was a negative effect of the genotype resulting in 10% and 15% lower grain number per plant for *da1* under ambient and eCO_2 conditions compared to Trappe, respectively. Similarly, the trait grain number per spike increased upon eCO_2 in both Trappe and *da1* by 9% and 8%, respectively, whereas the genotype effect decreased grains per spike in *da1* by app. 6% under ambient and eCO_2 conditions. Taken together, the *da1* mutant displayed reduced spikes per plant, grains per plant and grains per spike, independent of the treatment, which could outcompete the higher TGW (Fig. 2), resulting in no gain of grain yield per area of *da1* compared to Trappe. The results also indicated a higher response of Trappe to eCO_2 conditions compared to Trappe, for the traits spikes per plant, grain number per plant and grains per spike.

Biomass traits

eCO_2 in many plants stimulates source activity, photosynthesis and induces faster growth and biomass accumulation (Jablonski et al., 2002). On the other hand, DA1 is supposed to be a regulator of cell proliferation in maternal seed organs (Liu et al., 2020). Therefore, possible effects of eCO_2 treatment and genotype on biomass traits were analysed (Fig. 4). Tiller number at the beginning of the stem elongation stage was not different between the genotypes but increased similarly by 7% and 9% upon eCO_2 for Trappe and *da1* , respectively. Whereas plant height at anthesis was not different between genotypes at either treatments (data not shown), eCO_2 increased plant height at 10 days after anthesis by 6% and 7% for Trappe and *da1* , respectively, compared to ambient CO_2. Plant biomass was independent of the genotype but increased upon eCO_2 by 22% and 17% for Trappe and *da1* , respectively. Harvest index (HI) did not changed by genotype but was slightly higher upon eCO_2 treatment by 4% for both *da1* and Trappe.

Taken together, the results revealed no differences in the biomass traits analysed between *da1* and Trappe. As expected, eCO_2 treatment leads to an increase in these traits, with no apparent differences in the response between *da1* and Trappe.

Spike-related traits

The results described above indicated specific changes at the level of grain number and grain size. We therefore analysed spike related traits. The genotype and treatment effects are shown in Fig. 5. Grain yield per spike did not differ between *da1* and Trappe for both ambient and eCO_2 conditions, lacking a genotype effect. However, levels increased significantly upon eCO_2 treatment by 18 and 16% for *da1* and Trappe, respectively, showing a clear treatment effect. Spikelets per spike did not show significant treatment effects upon eCO_2, but with a trend to lower levels due to eCO_2 (not significant at $P < 0.05$). However, the genotype effect led to approx. 3% less spikelets per spike in *da1* compared to Trappe.

Grain yield per spikelet was not changed between *da1* and Trappe for both ambient and eCO_2 conditions, lacking a genotype effect. Grain number per spikelet behaved similar, lacking a genotype effect. However, both, grain yield per spikelet and grain number per spikelet increased upon eCO_2 by 15% and 8%, respectively.

Taken together, the results indicated reduced numbers of spikelets per spike in *da1,* which is probably balanced by a higher TGW (Fig. 2) resulting in no gain of grain yield per area of *da1* compared to Trappe.

Grain composition

While eCO_2 can increase grain yield of wheat there is often a shift in grain components and functional properties (Fangmeier, Grüters, Högy, Vermehren, & Jäger, 1997; P. Högy et al., 2009). Likewise, differences in grain size can change the relationship between seed organs affecting grain composition and quality (Nuttall et al., 2017). Therefore, possible effects of treatment and genotype on the composition of mature grains were analysed.

Grain starch content was increased by approx. 2% in *da1* grains compared to Trappe under ambient conditions and by 3% in response to eCO_2. Sucrose content in mature grains was 9% lower for *da1* compared to

Trappe under ambient conditions and 6% under eCO_2. The results showed that both *da1* effects and eCO_2 only slightly affected starch and sucrose levels. Both factors resulted in a small increase of the starch to sucrose ratio. Grain total carbon content of mature grains was unchanged between *da1* and Trappe but was slightly lower upon eCO_2 treatment, however with only 0.5%. Grain total nitrogen content was not different between *da1* and Trappe but decreased significantly upon eCO_2 treatment by as much as 15% in both *da1* and Trappe (Fig. 6).

The concentrations of major macro- and microelements were measured by ICP-OES (Table 1). The analysis of flour from mature grains revealed that *da1* did not differ considerably from Trappe with respect to important grain nutrient and micro-element concentrations including Fe, Zn, S, Mn, Mg, K, Ca, Na and N. Significant genotype-dependent differences were found only for Na with 8% lower values in *da1* . In contrast, eCO_2 treatment strongly decreased the concentrations of several of the essential elements, including Fe, Zn, S, Mn and N by 7, 12, 12, 10 and 15%, respectively, whereas levels of Mn, K, Ca and Na were unchanged.

Validation on ambient conditions

In order to confirm the results from the 2018 experiment, the field trial was repeated in the following year 2019 with *da1* and Trappe, while the eCO_2 treatment was omitted. Fig. 7 shows the data as a comparison between *da1* and Trappe and between the two years. According to the first-year's results, *da1* again exhibited significantly increased TGW, grain length, width and area. Similar to the 2018 experiment, grain yield and spike number per plant were not different between *da1* and Trappe. Thus, the trial in 2019 confirmed the differences between *da1* and Trappe. Grain morphology traits such as TGW, grain length, area and width behaved similarly as in the 2018 trial, thus confirming the increased grain size phenotype of *da1* mutants. Likewise, yield parameter results were consistent between both experiments, grain yield per spike was not different between both lines. The *da1* mutant exhibited increased grain size but decreased grain number per plant and spike.

Discussion

Grain size in wheat is a potential target to improve yield potential. The ubiquitin pathway affects organ growth and seed size, which is negatively regulated by the ubiquitin receptor DA1 restricting early maternal cell proliferation in Arabidopsis and crop plants such as canola, maize and wheat (N. Li & Li, 2014; Liu et al., 2020; J. L. Wang et al., 2017; Xie, Li, Ran, Wang, & Zhang, 2018). The *da1* wheat mutant increases grain size and provides a suitable model to better understand intrinsic yield determinants in wheat. The aim was to study whether the increased TGW in *da1* can be translated into higher grain yield and what is the relationship between the different yield-related traits. In order to alleviate possible source limitation, it was also tested whether the additional carbon provided by high CO_2, expected in the future, can be better used by the *da1* mutant displaying higher sink strength at the level off individual grains. The results show that increased grain size in *da1* wheat is interacting with other yield-related components at both eCO_2 and ambient conditions, leaving the final grain yield unchanged.

The *da1* mutation and elevated CO_2 independently increase grain size

The *da1* wheat mutant exhibited 8% increased TGW compared to Trappe (Fig. 2). This increase is related to both grain length and width and indicated higher sink strength of individual grains. Accordingly, the Arabidopsis *da1-1* mutant produces larger and heavier seeds resulting from enlarged sporophytic integuments (Y. Li, Zheng, Corke, Smith, & Bevan, 2008). In wheat, grain size is associated with carpel size (Calderini, Abeledo, Savin, & Slafer, 1999) and with variations in the ovary wall size, which is related to cell number rather than cell size (Reale, Cerri, Ayano, & Benincasa, 2017). Furthermore, grain length and pericarp cell length are associated (Muñoz & Calderini, 2015; Pielot et al., 2015). TaDA1 has been described as a negative regulator of grain size. DA1-RNAi plants resembles the wheat *da1* mutant and produced more outer pericarp cells at 15 DAP, formed a wider pericarp cell layer and displayed increased grain size by around 10% (Liu et al., 2020). It was also shown that TaDA1-A is predominantly expressed in young spikes at pre-anthesis (Liu et al., 2020). Cell division in the cereal pericarp is terminated as early as two days after fertilization (Radchuk, Weier, Radchuk, Weschke, & Weber, 2011). Thus, it is hypothesised that the impact

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of *da1* on grain size most probably comes from a prolonged cell division phase in the early maternal grain tissue, probably before anthesis. Hence, the increased maternal grain layers may overcome physical barriers to endosperm expansion, thereby increasing the available space for endosperm growth, which finally leads to increased grain size (Hasan, Herrera, Lizana, & Calderini, 2011).

Our results reveal that eCO_2 further increased TGW in a similar manner for both genotypes by app. 8%, independently from the *da1* -effects (Fig. 2). In plants, eCO_2 has been shown to enhance cell division, to shorten the duration of the cell cycle, and to promote cell production as well as expansion (Gamage et al., 2018). Thus, eCO_2 could possibly further stimulate the cell proliferation in the developing grains. This is probably due to the eCO_2-mediated carbon "fertilisation effect", which mediates an assimilate and/or sugar supply effect stimulating cell proliferation within the developing grain (Lastdrager, Hanson, & Smeekens, 2014; H. Weichert et al., 2017).

Improvement of grain size in *da1* is compensated by lower grain number

The finding that biomass traits such as tiller number, plant height at 10 days after anthesis, harvest index and spike number per plant were not changed in *da1* indicates that the mutation does not change vegetative parameters but preferentially affects grain development around anthesis. While grain size was increased in *da1* compared to Trappe (+ 8%, Fig. 2), some of the other important yield components were decreased (Fig. 3) such as spikes per plant (- 6%), grains per plant (- 12%) and grains per spike (- 6%). However, total grain yield was not altered (Fig. 3). These results reveal that the improvement of grain size is counter-balanced in a way that final grain yield is not altered. Thereby, the larger grain size was compensated by several other traits related to grain number per spike such as less spikes per plant, lower grain number per spike, lower number of spikelets per spike and, eventually less grains per spikelets, even the latter was not significant here (Fig. 5). Altogether, this results in a lower number of grains per plant and per area in *da1* compared to Trappe. The comparison between *da1* and Trappe confirms the well-known trade-off between grain size and grain number, which finally maintains grain yield stability (Sadras, 2007).

The *da1* mutation and elevated CO_2 additively increase grain size but not grain yield

Both *da1* and eCO_2 independently stimulate sink activity, which leads to a larger grain size. However, it can be hypothesised that *da1* acts only on early grain development by sustaining cell proliferation without directly affecting source strength or assimilate availability. This may cause competition for assimilates between individual grains within the spike, which opens up in a compensation of the larger grain size by less grains per plant and per spike. In contrast, eCO_2 operates at the whole plant level including grain development by a metabolite/assimilate effect, which becomes evident by a stimulation of different biomass traits (Fig. 4) such as higher grain number per plant (+ 7%), grain yield (+ 14%, Fig. 3) and grain number per spikelet (+ 8%, Fig. 6). Additional supply of eCO_2 produced a significant increase of grain size in Trappe and *da1* each by 8% (Fig. 2), indicating that TGW in both genotypes can benefit in a similar manner from eCO_2. Thus, the CO_2 treatment and the *da1* mutation increased grain size additively leading to an increase in TGW of 17% in the *da1* x eCO_2 combination compared to Trappe at ambient CO_2. However, the reduction of grain number is still maintained in the *da1* x eCO_2 combination compared to Trappe x eCO_2. In consequence, total grain yields of *da1* or Trappe at eCO_2 remained unchanged. In summary, even though eCO_2 increases yield and grain number in general, it cannot overcome the negative effect of the *da1* genotype, which goes back to a trade-off between grain size and grain number. Thus, total yield in the genetic background of a *da1* mutation cannot profit from source stimulation by higher CO_2, expected in the future due to climate change, as final yield gain remained unchanged between *da1* and Trappe in eCO_2.

Grain quality is not altered by *da1* but impaired by elevated CO_2

Grain starch and sucrose contents were only slightly altered by both *da1* mutation and eCO_2, leading to a small increase of the starch to sucrose ratio in *da1* (Fig. 6). Moreover, the concentration of essential macro- and microelements in *da1* grains were not changed with respect to Trappe (Table 1). In contrast, eCO_2 treatment strongly decreased several of the macro- and micro-elements similarly in *da1* and Trappe, such as Fe, Zn, S and Mn by 7, 12, 12 and 10%, respectively. However, other elements remain constant such as

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Mg, K, Ca and Na. C3 plants such as wheat generally respond to eCO_2 with increased photosynthesis, reduced stomatal conductance and a significant reduction in the amount of essential elements in grains (Amthor, 2001; Petra Högy & Fangmeier, 2008; Pleijel & Högy, 2015). It has been suggested that reduced transpiration-driven mass flow of nutrients under eCO_2 contributes to decreases in seed concentrations of several elements (Houshmandfar et al., 2018). Especially the grain N concentration decreased upon eCO_2 by 15% (Table 1). CO_2 enrichment causes lower N and protein levels in non-leguminous C3 species and alters acquisition, remobilisation, redistribution, and accumulation of N (Taub & Wang, 2008). eCO_2 physiologically induces N deficiency, reducing both nitrate uptake from soil and nitrate reduction while ammonium uptake is favoured (Bloom, Burger, Kimball, & Pinter Jr, 2014).

Enhancing grain size via *da1* is not a suitable way to increase yield potential in wheat

The negative relationship between grain size and grain number is an intrinsic property of many crop and non-crop plants (Acreche & Slafer, 2006; Quintero, Molero, Reynolds, & Calderini, 2018). From an evolutionary point, the adjustment between these two traits is important and guarantees yield stability (Sadras, 2007). This trade-off is probably not easy to overcome by conventional breeding and results from the complex interaction of source limitation such as shortage of assimilate supply to grains, sink limitation such as inability of each grain to unload and/or accumulate assimilates, and translocation limitation such as inefficient delivery of assimilates from leaves to grains (Seki et al., 2015).

The breeding process in the past essentially increased grain number per area by enhancing grains per spike and spikelets, however without much gain in TGW (Philipp et al., 2018). An increase of grain number per spike strongly depends on assimilates allocated to the spike (Ghiglione et al., 2008). Competition for assimilates occurs within the spike and is controlled by assimilate loading and unloading within the vascular system and the short-distance transport within the spike, rachis and spikelets. Possible limitations in transport capacities and competition for assimilates between spikelets and/or florets could impact on biomass distribution among individual tissues within the spike (Reynolds et al., 2009). A potential issue could be to target resistance to assimilate movement imposed by the vascular system of the spike. Resistance to assimilate movement within the spike, and particularly that within the spikelets, may be an important component of spike 'sink activity' and a possible limitation to yield (Bremner & Rawson, 1978). Disparity in number and dimensions of vascular bundles in different spike segments could be critical affecting ultimate size and grain number along the rachis (Asli & Houshmandfar, 2011). In rice, simultaneous increases in sink size and translocation capacity through the vascular bundles increased the number of vascular bundles and contributed to increased grain yield (Fujita et al., 2013; Terao, Nagata, Morino, & Hirose, 2010).

Eventually, genetic yield gain during breeding has not been accompanied by similar increases in the vasculature size of the wheat spike. Accordingly, no clear association was found between the genetic improvement and magnitude of vascular systems in peduncles of the wheat spike (Lopez-Garrido, Molina-Quiros, De la Puerta-Lopez, Vidal-Bernabe, & Garcia-Del-Moral, 2001). Hence, the spike architecture in terms of the relative distribution of grain yield and number along the spike is surprisingly stable and has not been improved by breeding in the past (Philipp et al., 2018).

The results in this study give valuable insights into the interactions among yield components related to grain size and grain number and the possible limitations of yield potential in wheat. Apparently, the intrinsic stimulation of sink strength and/or growth by increasing cell proliferation, as achieved in *da1* grains, is not expedient towards benefiting total yield, unless the trade-off between grain size and grain number can be overcome. While eCO_2 increased yield and grain number additively and independently of *da1*, it did not prevail the trade-off between grain size and number in *da1*. The attempt to increase grain sink strength by ectopic expression of a sucrose transporter in the wheat endosperm increased individual grain weight but also decreased grain number per spike, thereby confirming the predominant trade-off between grain size and number (Saalbach et al., 2014; N. Weichert et al., 2010). This firm relationship supports the conclusion that the improvement of grain yield is best achieved through an integrated approach targeting several yield-component traits in parallel (Wurschum, Leiser, Langer, Tucker, & Longin, 2018).

Supplementary data

Fig. S1, Overview on the location of the mutations in the *da1* -wheat.

Fig. S2, Mean day temperature as recorded by the IPK Gatersleben whether station 2018. **Fig. S3,** Schematic representation of the DA1 wheat sequence and its domains.

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

References

Acreche, M. M., & Slafer, G. A. (2006). Grain weight response to increases in number of grains in wheat in a Mediterranean area.*Field Crops Research, 98* (1), 52-59. doi:https://doi.org/10.1016/j.fcr.2005.12.005

Amthor, J. S. (2001). Effects of atmospheric CO2 concentration on wheat yield: review of results from experiments using various approaches to control CO2 concentration. *Field Crops Research, 73* (1), 1-34. doi:http://dx.doi.org/10.1016/S0378-4290(01)00179-4

Asli, D. E., & Houshmandfar, A. (2011). An anatomical study of vascular system of spikelet supplying translocates to differentially growing grains of wheat. *Advances in Environmental Biology* , 1597-1602.

Bloom, A. J., Burger, M., Kimball, B. A., & Pinter Jr, P. J. (2014). Nitrate assimilation is inhibited by elevated CO2 in field-grown wheat.*Nature Climate Change, 4* (6), 477-480.

Borras, L., Slafer, G. A., & Otegui, M. E. (2004). Seed dry weight response to source-sink manipulations in wheat, maize and soybean: a quantitative reappraisal. *Field Crops Research, 86* (2-3), 131-146. doi:10.1016/j.fcr.2003.08.002

Bremner, P., & Rawson, H. (1978). The Weights of Individual Grains of the Wheat Ear in Relation to Their Growth Potential, the Supply of Assimilate and Interaction Between Grains. *Functional Plant Biology, 5* (1), 61-72. doi:https://doi.org/10.1071/PP9780061

Brinton, J., Simmonds, J., Minter, F., Leverington-Waite, M., Snape, J., & Uauy, C. (2017). Increased pericarp cell length underlies a major quantitative trait locus for grain weight in hexaploid wheat. *New Phytologist, 215* (3), 1026-1038. doi:10.1111/nph.14624

Brinton, J., & Uauy, C. (2019). A reductionist approach to dissecting grain weight and yield in wheat. *J Integr Plant Biol, 61* (3), 337-358. doi:10.1111/jipb.12741

Calderini, D., Abeledo, L., Savin, R., & Slafer, G. A. (1999). Effect of temperature and carpel size during pre-anthesis on potential grain weight in wheat. *The Journal of Agricultural Science, 132* (4), 453-459.

Eroglu, S., Giehl, R. F. H., Meier, B., Takahashi, M., Terada, Y., Ignatyev, K., . . . von Wiren, N. (2017). Metal Tolerance Protein 8 Mediates Manganese Homeostasis and Iron Reallocation during Seed Development and Germination. *Plant Physiology, 174* (3), 1633-1647. doi:10.1104/pp.16.01646

Fangmeier, A., Gruters, U., Hogy, P., Vermehren, B., & Jager, H. J. (1997). Effects of elevated CO2, nitrogen supply and tropospheric ozone on spring wheat-II. Nutrients (N, P, K, S, Ca, Mg, Fe, Mn, Zn).*Environ Pollut, 96* (1), 43-59. doi:10.1016/s0269-7491(97)00013-4

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Foulkes, M. J., Slafer, G. A., Davies, W. J., Berry, P. M., Sylvester-Bradley, R., Martre, P., . . . Reynolds, M. P. (2011). Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. *J Exp Bot, 62* (2), 469-486.

Fujita, D., Trijatmiko, K. R., Tagle, A. G., Sapasap, M. V., Koide, Y., Sasaki, K., . . . Kobayashi, N. (2013). NAL1 allele from a rice landrace greatly increases yield in modern indica cultivars.*Proceedings of the National Academy of Sciences, 110* (51), 20431-20436. doi:10.1073/pnas.1310790110

Gamage, D., Thompson, M., Sutherland, M., Hirotsu, N., Makino, A., & Seneweera, S. (2018). New insights into the cellular mechanisms of plant growth at elevated atmospheric carbon dioxide concentrations.*Plant, Cell & Environment, 41* (6), 1233-1246. doi:10.1111/pce.13206

Ghiglione, H. O., Gonzalez, F. G., Serrago, R., Maldonado, S. B., Chilcott, C., Cura, J. A., . . . Casal, J. J. (2008). Autophagy regulated by day length determines the number of fertile florets in wheat. *The Plant Journal, 55* (6), 1010-1024. doi:10.1111/j.1365-313X.2008.03570.x

Hasan, A. K., Herrera, J., Lizana, C., & Calderini, D. F. (2011). Carpel weight, grain length and stabilized grain water content are physiological drivers of grain weight determination of wheat.*Field Crops Research, 123* (3), 241-247. doi:http://dx.doi.org/10.1016/j.fcr.2011.05.019

Hogy, P., & Fangmeier, A. (2008). Effects of elevated atmospheric CO2 on grain quality of wheat. *Journal of Cereal Science, 48* (3), 580-591. doi:http://dx.doi.org/10.1016/j.jcs.2008.01.006

Hogy, P., Wieser, H., Kohler, P., Schwadorf, K., Breuer, J., Franzaring, J., . . . Fangmeier, A. (2009). Effects of elevated CO2 on grain yield and quality of wheat: results from a 3-year free-air CO2 enrichment experiment. *Plant Biol (Stuttg), 11 Suppl 1* , 60-69. doi:10.1111/j.1438-8677.2009.00230.x

Houshmandfar, A., Fitzgerald, G. J., O'Leary, G., Tausz-Posch, S., Fletcher, A., & Tausz, M. (2018). The relationship between transpiration and nutrient uptake in wheat changes under elevated atmospheric CO2. *Physiologia Plantarum, 163* (4), 516-529. doi:10.1111/ppl.12676

Jablonski, L. M., Wang, X., & Curtis, P. S. (2002). Plant reproduction under elevated CO2 conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytologist, 156* (1), 9-26. doi:10.1046/j.1469-8137.2002.00494.x

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms.*Mol Biol Evol, 35* (6), 1547-1549. doi:10.1093/molbev/msy096

Lastdrager, J., Hanson, J., & Smeekens, S. (2014). Sugar signals and the control of plant growth and development. *J Exp Bot, 65* (3), 799-807. doi:10.1093/jxb/ert474

Li, N., & Li, Y. (2014). Ubiquitin-mediated control of seed size in plants. *Frontiers in Plant Science, 5* , 332. doi:10.3389/fpls.2014.00332

Li, Y., Zheng, L., Corke, F., Smith, C., & Bevan, M. W. (2008). Control of final seed and organ size by the DA1 gene family in Arabidopsis thaliana. *Genes Dev, 22* (10), 1331-1336. doi:10.1101/gad.463608

Liu, H., Li, H., Hao, C., Wang, K., Wang, Y., Qin, L., . . . Zhang, X. (2020). TaDA1, a conserved negative regulator of kernel size, has an additive effect with TaGW2 in common wheat (Triticum aestivum L.).*Plant Biotechnol J, 18* (5), 1330-1342. doi:10.1111/pbi.13298

Lopez-Garrido, E., Molina-Quiros, S., De la Puerta-Lopez, P. G., Vidal-Bernabe, M., & Garcia-Del-Moral, L.-F. (2001). Quantification of vascular tissues in the peduncle of durum wheat cultivars improved during the twentieth century. *International Journal of Developmental Biology, 45* (S1), S47-S48.

Mohler, V., Albrecht, T., Castell, A., Diethelm, M., Schweizer, G., & Hartl, L. (2016). Considering causal genes in the genetic dissection of kernel traits in common wheat. *Journal of applied genetics, 57* (4), 467-476.

Munoz, M., & Calderini, D. F. (2015). Volume, water content, epidermal cell area, and XTH5 expression in growing grains of wheat across ploidy levels. *Field Crops Research, 173* , 30-40. doi:https://doi.org/10.1016/j.fcr.2014.12.010

Nuttall, J., O'Leary, G., Panozzo, J., Walker, C., Barlow, K., & Fitzgerald, G. (2017). Models of grain quality in wheat—A review. *Field Crops Research, 202* , 136-145.

Philipp, N., Weichert, H., Bohra, U., Weschke, W., Schulthess, A. W., & Weber, H. (2018). Grain number and grain yield distribution along the spike remain stable despite breeding for high yield in winter wheat. *PLoS One, 13* (10), e0205452. doi:10.1371/journal.pone.0205452

Pielot, R., Kohl, S., Manz, B., Rutten, T., Weier, D., Tarkowska, D., . . . Weschke, W. (2015). Hormone-mediated growth dynamics of the barley pericarp as revealed by magnetic resonance imaging and transcript profiling. *J Exp Bot, 66* (21), 6927-6943. doi:10.1093/jxb/erv397

Pleijel, H., & Hogy, P. (2015). CO_2 dose–response functions for wheat grain, protein and mineral yield based on FACE and open-top chamber experiments. *Environmental Pollution, 198* , 70-77. doi:http://dx.doi.org/10.1016/j.envpol.2014.12.030

Quintero, A., Molero, G., Reynolds, M. P., & Calderini, D. F. (2018). Trade-off between grain weight and grain number in wheat depends on GxE interaction: A case study of an elite CIMMYT panel (CIM-COG). *European Journal of Agronomy, 92* , 17-29. doi:https://doi.org/10.1016/j.eja.2017.09.007

Radchuk, V., Weier, D., Radchuk, R., Weschke, W., & Weber, H. (2011). Development of maternal seed tissue in barley is mediated by regulated cell expansion and cell disintegration and coordinated with endosperm growth. *J Exp Bot, 62* (3), 1217-1227. doi:10.1093/jxb/erq348

Ray, D. K., Mueller, N. D., West, P. C., & Foley, J. A. (2013). Yield Trends Are Insufficient to Double Global Crop Production by 2050. *PLoS One, 8* (6), e66428. doi:10.1371/journal.pone.0066428

Reale, a., Cerri, a., Ayano, a., & Benincasa, a. (2017). Ovary size in wheat (Triticum aestivum L.) is related to cell number. *Crop Science, 57* (2), 914-925.

Reynolds, M., Foulkes, M. J., Slafer, G. A., Berry, P., Parry, M. A., Snape, J. W., & Angus, W. J. (2009). Raising yield potential in wheat. *J Exp Bot, 60* (7), 1899-1918. doi:10.1093/jxb/erp016

Saalbach, I., Mora-Ramirez, I., Weichert, N., Andersch, F., Guild, G., Wieser, H., . . . Weber, H. (2014). Increased grain yield and micronutrient concentration in transgenic winter wheat by ectopic expression of a barley sucrose transporter. *Journal of Cereal Science, 60* (1), 75-81. doi:10.1016/j.jcs.2014.01.017

Sadras, V. O. (2007). Evolutionary aspects of the trade-off between seed size and number in crops. *Field Crops Research, 100* (2-3), 125-138.

Schulthess, A. W., Reif, J. C., Ling, J., Plieske, J., Kollers, S., Ebmeyer, E., . . . Jiang, Y. (2017). The roles of pleiotropy and close linkage as revealed by association mapping of yield and correlated traits of wheat (Triticum aestivum L.). *J Exp Bot, 68* (15), 4089-4101. doi:10.1093/jxb/erx214

Seki, M., Feugier, F. G., Song, X. J., Ashikari, M., Nakamura, H., Ishiyama, K., . . . Satake, A. (2015). A mathematical model of phloem sucrose transport as a new tool for designing rice panicle structure for high grain yield. *Plant Cell Physiol, 56* (4), 605-619. doi:10.1093/pcp/pcu191

Slafer, G. A., Savin, R., & Sadras, V. O. (2014). Coarse and fine regulation of wheat yield components in response to genotype and environment. *Field Crops Research, 157* , 71-83.

Sofield, I., Evans, L., Cook, M., & Wardlaw, I. (1977). Factors Influencing the Rate and Duration of Grain Filling in Wheat. *Functional Plant Biology, 4* (5), 785-797. doi:https://doi.org/10.1071/PP9770785

Song, X. J., Huang, W., Shi, M., Zhu, M. Z., & Lin, H. X. (2007). A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nat Genet, 39* (5), 623-630. doi:10.1038/ng2014

Sukumaran, S., Dreisigacker, S., Lopes, M., Chavez, P., & Reynolds, M. P. (2015). Genome-wide association study for grain yield and related traits in an elite spring wheat population grown in temperate irrigated environments. *Theor Appl Genet, 128* (2), 353-363. doi:10.1007/s00122-014-2435-3

Taub, D. R., & Wang, X. (2008). Why are Nitrogen Concentrations in Plant Tissues Lower under Elevated CO2? A Critical Examination of the Hypotheses. *J Integr Plant Biol, 50* (11), 1365-1374. doi:10.1111/j.1744-7909.2008.00754.x

Terao, T., Nagata, K., Morino, K., & Hirose, T. (2010). A gene controlling the number of primary rachis branches also controls the vascular bundle formation and hence is responsible to increase the harvest index and grain yield in rice. *Theor Appl Genet, 120* (5), 875-893. doi:10.1007/s00122-009-1218-8

Tshikunde, N. M., Mashilo, J., Shimelis, H., & Odindo, A. (2019). Agronomic and Physiological Traits, and Associated Quantitative Trait Loci (QTL) Affecting Yield Response in Wheat (Triticum aestivum L.): A Review. *Frontiers in Plant Science, 10* (1428). doi:10.3389/fpls.2019.01428

Wang, J. L., Tang, M. Q., Chen, S., Zheng, X. F., Mo, H. X., Li, S. J., . . . Tan, X. L. (2017). Down-regulation of BnDA1, whose gene locus is associated with the seeds weight, improves the seeds weight and organ size in Brassica napus. *Plant Biotechnol J, 15* (8), 1024-1033. doi:10.1111/pbi.12696

Wang, W., Simmonds, J., Pan, Q., Davidson, D., He, F., Battal, A., . . . Akhunov, E. (2018). Gene editing and mutagenesis reveal inter-cultivar differences and additivity in the contribution of TaGW2 homoeologues to grain size and weight in wheat. *Theor Appl Genet, 131* (11), 2463-2475. doi:10.1007/s00122-018-3166-7

Weichert, H., Hogy, P., Mora-Ramirez, I., Fuchs, J., Eggert, K., Koehler, P., . . . Weber, H. (2017). Grain yield and quality responses of wheat expressing a barley sucrose transporter to combined climate change factors. *J Exp Bot* . doi:10.1093/jxb/erx366

Weichert, N., Saalbach, I., Weichert, H., Kohl, S., Erban, A., Kopka, J., . . . Weber, H. (2010). Increasing sucrose uptake capacity of wheat grains stimulates storage protein synthesis. *Plant Physiol, 152* (2), 698-710. doi:10.1104/pp.109.150854

Weigelt, K., Kuster, H., Rutten, T., Fait, A., Fernie, A. R., Miersch, O., . . . Weber, H. (2009). ADP-glucose pyrophosphorylase-deficient pea embryos reveal specific transcriptional and metabolic changes of carbon-nitrogen metabolism and stress responses. *Plant Physiol, 149* (1), 395-411. doi:10.1104/pp.108.129940

Wurschum, T., Leiser, W. L., Langer, S. M., Tucker, M. R., & Longin, C. F. H. (2018). Phenotypic and genetic analysis of spike and kernel characteristics in wheat reveals long-term genetic trends of grain yield components. *Theoretical and Applied Genetics, 131* (10), 2071-2084. doi:10.1007/s00122-018-3133-3

Xie, G., Li, Z., Ran, Q., Wang, H., & Zhang, J. (2018). Over-expression of mutated ZmDA1 or Zm-DAR1 gene improves maize kernel yield by enhancing starch synthesis. *Plant Biotechnol J, 16* (1), 234-244. doi:10.1111/pbi.12763

Figure Legends

Fig. 1, Phylogenetic tree of selected DA1 proteins.

Wheat DA1 sequences are shaded. *Zea mays* Zm00001d035844_T012,*Hordeum vulgare* HORVU2Hr1G002700.14, *Sorgum bicolor*SORBI_3010G064600:KXG19479, *Triticum dicoccoides*TRIDC2AG001610.3, *Brachypodium distachyon*BRADI_4g42580v3:KQJ92266, *Glycine max* GLYMA_17G247700:KRH05774,*Triticum turgidum* TRITD2Av1G003900.3, *Oryza sativa*Japonica group Os06t0182500-02, *Arabidopsis thaliana* AT1G19270.1,*Aegilops tauschii* AET0Gv20035900.10, *Triticum urartu*TRIUR3_12237:TRIUR3_12237-T1, *Medicago truncatula*MTR_5g018900:AES94835, *Manihot esculenta*MANES_10G022200:OAY38533, *Cucumis sativus* Csa_2G286500:KGN62017 and *Beta vulgaris* BVRB_6g138060:KMT08523.

Fig, 2, Grain Dimensions

Box plots show the distribution of 4 grain dimension-related traits, measured for *da1* wheat and wildtype Trappe. P-values indicate statistical significance between values. Two factors, genotype and treatment influencing grain dimension traits.

Fig. 3, Yield-related traits.

Box plots show the distribution of 4 yield-related traits, measured for *da1* wheat and wildtype Trappe. P-values indicate statistical significance between values. Two factors, genotype and treatment influencing yield-related traits.

Fig. 4, Biomass traits.

Box plots show the distribution of 4 biomass-related traits, measured for *da1* wheat and wildtype Trappe. P-values indicate statistical significance between values. Two factors, genotype and treatment influencing biomass-related traits.

Fig. 5, Spike-related traits.

Box plot show the distribution of 4 spike-related traits, measured for *da1* wheat and wildtype Trappe. P-values indicate statistical significance between values. Two factors, genotype and treatment influencing grain composition-related traits.

Fig. 6, Grain composition.

Box plots show the distribution of 4 grain composition-related traits, measured for *da1* wheat and wildtype Trappe. P-values indicate statistical significance between values. Two factors, genotype and treatment influencing grain composition-related traits.

Fig. 7, Repetition experiment.

Box plots show the distribution of grain dimension-, yield-, biomass-, and grain composition-related traits, measured for da1 wheat and wildtype Trappe. P-values indicate statistical significance between values. Percentages gives the change of values for da1 compared to Trappe. Table insert shows a comparison between experiments from 2018 and 2019 in percentage, (Trappe = 100 %), green and red colour designate significant higher and lower values of da1 compared to Trappe. n = 8; bold, significant differences; *, P<0.05; **P<0.01; ***, P<0.001.

Table 1, Grain macro- and micro elements.

Factors genotype and eCO_2 treatment influencing the concentration of nutrient elements in mature grains of *da1* and Trappe.

$\mu\gamma \ \gamma\text{-}1$	ambient CO_2 Trappe MW	ambient CO_2 Trappe SD	ambient CO_2 da1 MW	ambient CO_2 da1 SD	ambient CO_2 P	eCO_2 Trappe MW	eCO_2 Trappe SD	eCO_2 da MW
Fe	43.57	3.33	43.51	4.45	*0.970*	38.81	3.61	39
Zn	41.63	2.98	42.04	5.23	*0.820*	35.51	3.32	36
S	1849.34	84.90	1843.99	108.94	*0.089*	1613.93	125.97	16
Mn	43.40	5.19	42.98	4.20	*0.830*	37.18	3.70	37
Mg	1461.40	115.42	1481.99	139.07	*0.670*	1334.60	106.99	14
K	6546.57	397.98	6472.35	564.25	*0.710*	6566.76	400.75	65
Ca	618.97	90.75	623.27	80.29	*0.900*	540.57	55.65	56
Na	12.78	1.33	11.64	1.32	*0.050*	12.92	1.40	12
N	2373.72	105.18	2328.41	58.44	*0.210*	2008.45	124.99	19

n = 24, P-values in bold, significant differences according to two-way ANOVA, P < 0.05.

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