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Gas exchange-yield relationships of malting barley genotypes treated with fungicides and biostimulants

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ABSTRACT

Optimization between photosynthetic carbon assimilation and stomatal water loss is the key in order to breed crops for future climate. We analyzed stomatal conductance (gs), CO_2 assimilation rate (Anet), fungal diseases, grain yield and yield components of seven European malting barley genotypes treated with fungicides alone or together with biostimulants in the field over three consecutive seasons. Stomatal conductance, net assimilation rate and grain yield were affected by genotype, treatment and year. We then examined which of these traits are most strongly correlated with yield. Grain yields ranged between 6000-8300 kg ha^{-1} in 2015 and 2017, but only 3500–6000 kg ha^{-1} in 2016 due to hotter and drier weather. The benefits of treatments with fungicides or fungicides together with biostimulants were greater in 2016, when the treatments increased yields by 20–21% on average, compared to 0–11% in 2015 and 2017. In 2016, gs and Anet were correlated with grain yield, indicating that in hotter and drier than average season maintaining higher transpiration and photosynthesis resulted in higher yield. In 2015 and 2017, average values of instantaneous gs and Anet were not significantly correlated with grain yield. Pooling all years, longer pre-heading period, higher grain numbers per ha and per ear, larger 1000-kernel weight and higher water use efficiency were associated with higher grain yield, whereas a negative correlation was detected between grain yield and the number of ears per area. Thus, developing fewer productive tillers per area, but more and heavier grains per ear led to higher grain yield. Major barley fungal pathogens had a negative effect on grain yield via shortening of grain filling period.

1. Introduction

The increase in global grain yield during 1961–2000 was achieved by extending or increasing photosynthesis per land area through irrigation and fertilization and by increasing harvest index through plant breeding with no genetic improvement in the CO_2 assimilation rate (Richards, 2000). A further rise in crop production will be needed to meet the food demand of the growing human population (9.8 billion predicted by 2050; http://esa.un.org/wpp/). Globally, barley is the fourth most cultivated crop by land usage and production (Ahmed et al., 2013), whereas in EU, barley is ranked second between wheat and grain maize (EUROSTAT, 2011), and is grown mainly for beer production and animal feed. During the 1990s, barley and wheat yields stagnated in almost all central and northern European countries. This stagnation was associated with declines in crop price and changes in agricultural management within the EU towards environmental friendly production using less fertilizer (Peltonen-Sainio et al., 2009; Finger, 2010; Peltonen-Sainio et al., 2015; Wiesmeier et al., 2015). On the other hand, suboptimal environmental conditions, lack of nutrients, diseases and physiological disorders can also limit yield by causing stress and reducing the size and duration of photosynthetic area (Araus, 2002). Net blotch (*Pyrenophora teres*) and spot blotch (*Cochliobolus sativus*) are the most prevalent fungal diseases of barley leaves in Estonia (Sooväli and Koppel, 2010), whereas *Fusarium* species colonize ears and grains of spring barley and reduce the yield and grain quality (Wegulo et al., 2015). Fungicides reduce fungal infections, maintain the green leaf area and increase grain yield when the risk of disease is severe, but can occasionally induce stress in plants in drought conditions (Dias, 2012). Biostimulants help plants to circumvent or tolerate stress; these biostimulants include different compounds or microorganisms that are

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applied to plants or soils to improve crop nutrition, vigor, yield, quality and tolerance to abiotic stress (http://www.biostimulants.eu/; Calvo et al., 2014). Thus, the combined use of biostimulants and pesticides may help to overcome potential pesticide-induced stress (Le Mire et al., 2016).

Carbon assimilation in photosynthesis and water loss via transpiration are key physiological processes that affect biomass production; grain yield is an integrated measure of assimilates produced over photosynthesizing organs (leaves, stems, ears) and time. Stomata are small pores surrounded by guard cells on the leaf surfaces that control plant water loss and leaf cooling through transpiration and photosynthesis through CO_2 influx. The stomata of cereals show large potential conductance and rapid responses to environmental changes (Hetherington and Woodward, 2003; Büchsenschütz et al., 2005; Franks and Farquhar, 2007; Kollist et al., 2014; Merilo et al., 2014). Stomatal conductance (gs) is defined as the transpiration rate divided by leaf-to-air vapor pressure deficit, which is the driving force for transpiration. Average stomatal conductance is significantly correlated with average yield in wheat, indicating that gs could serve as an indirect selection criterion for yield (Fischer et al., 1998). One reason for the strong correlation between gs and yield might be leaf cooling in warmer climate providing heat resistance and favoring higher yields (Lu et al., 1994; Hetherington and Woodward, 2003). It has been shown that gs of important crops (wheat, cotton, soybean) has increased over the last 80 years with positive correlations detected between gs, year of release and yield (Roche, 2015).

A better understanding of the physiological basis of crop yield and identification of the traits associated with grain yield serve to improve the efficiency of crop breeding (Araus et al., 2008). Despite their importance, net CO_2 assimilation rate (Anet) and gs are relatively rarely measured together with yield in multi-year experiments. We present the results of field experiments with seven European malting barley genotypes treated with fungicides alone or together with biostimulants over three consecutive seasons. We aimed to examine 1) the variation induced by treatment, genotype and year in leaf gas exchange and crop phenological traits together with grain yield and yield components; 2) the correlations between different traits. We hypothesized to find a positive effect of treatments on yield and gas exchange traits and a positive correlation between gas exchange traits and grain yield. As fungicides alone or together with biostimulants were used to increase the variation in studied traits, we also present data about leaf fungal and *Fusarium* spp. infections.

2. Material and methods

2.1. Experimental design and agronomic management

The experiment was carried out in a spring barley (*Hordeum vulgare*) field at the Estonian Crop Research Institute, in Jõgeva, Estonia (58°45'N, 26°24'E) in 2015–2017. Seven malting barley genotypes from Germany (Breustar, Conchita, Grace), Denmark (Iron) and UK (Propino, Quench, NFC Tipple) were sown (500 germinating seeds per 1 m^2) in randomized plots of 5 m^2 with row spacing of 12.5 cm (Supplemental Fig. 1). All studied genotypes were released post-2000. Before sowing, the seeds were treated with fludioxonil and cyproconazole (trademark Maxim Star 025FS), 1.5 L Mg^{-1}. A complex fertilizer (N 22; P_2O_5 7; K_2O 12) was applied before sowing in all years at a rate of 409 kg ha^{-1} (N_{90} P_{13} K_{41}). Recommended weed control was applied uniformly across the field. Supplemental Table 1 gives further details of agricultural practices.

The experimental setup consisted of control and treated (fungicides applied alone or together with biostimulants) plots with three replicates per treatment. Using fungicides is a common practice for growing malting barley within EU; in the fungicide (F) treatment, boscalid (233 g L^{-1}) and epoxiconazole (67 g L^{-1}) compounds (trademark Bell) were applied once, 1.5 L ha^{-1}, at cereal flag leaf growth stages 37–39

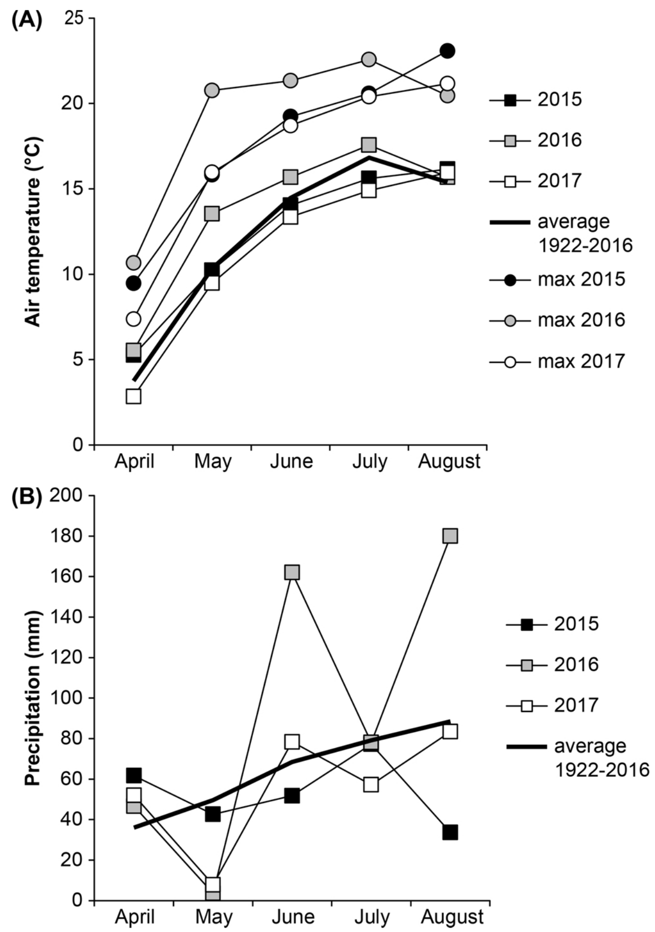

Fig. 1. Air temperature (A) and precipitation (B) for 2015–2017 and long-term averages (April-August) in Jõgeva. Average monthly diurnal and maximum temperatures are presented for 2015–2017.

(BBCH-scale) according to Meier (2001). Of the genotypes studied, Grace, Iron, Conchita and Breustar are moderately resistant to net and spot blotch, whereas Quench, Propino and NFC Tipple are moderately sensitive to these fungal pathogens (ETKI, 2018; Scandagra, 2018). Another treatment, FB, included application of biostimulants twice and fungicide once: biostimulant Ruter AA (free amino acids 7%, total N 5.5%, organic matter 15%, P_2O_5 5%, K_2O 3.5%, CaO 8.7%, Fe-EDDHA 0.036%, Mn-EDTA 0.05%, Zn-EDTA 0.07%, Mo 0.1%) was applied (3 L ha^{-1}) at tillering stage (BBCH 23–25). Then, a simultaneous application of biostimulant Delfan Plus (free amino acids 24%, total N 9%, organic matter 37%, organic C 23%; 1 L ha^{-1}) and fungicide Bell (1.5 L ha^{-1}) applied at BBCH 37–39 followed. The fungicides and biostimulants were applied at full doses registered for use in Estonia. No treatment was done in control plots.

2.2. Growing conditions

The soil of the experimental field was *Calcaric (Eutric) Cambisol* (FAO classification), with sandy loam texture, organic C content was 1.5–1.9%, available P 148–173 mg kg^{-1}, K 139–206 mg kg^{-1}, Ca 1406–1422 mg kg^{-1} and with pH_{KCl} 5.7–6.0. The climate data were obtained from the Meteos Compact weather station (Pessl Instruments GmbH, Austria) located in Jõgeva next to the trial site. Air temperature and precipitation were close to the long-term averages (1922–2016) in 2015, only August was drier than average (Fig. 1). Nevertheless, the top soil profile (0–20 cm) remained drier than optimal in the first half of June, 2015 (Fig. 2A). The 2016 and 2017 seasons were characterised by dry periods in May (4 mm and 9 mm of precipitation, respectively),