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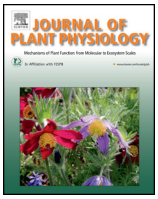

Rapid seedling establishment and a narrow root stele promotes waterlogging tolerance in spring wheat

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ABSTRACT

Improving the waterlogging tolerance of wheat varieties could alleviate yield constraints caused by excessive rain and poor soil drainage. In this study, we investigated root and shoot growth as well as anatomical traits of six spring wheat genotypes with contrasting waterlogging tolerance properties. Our aim was to identify root traits that differentiate tolerant from sensitive genotypes. Two experiments were conducted using rhizoboxes and photography for data acquisition. In experiment one, root growth of the genotypes was studied during seedling establishment and a subsequent waterlogging treatment, starting at the 3-leaf stage and maintained for seven days. In the second experiment, root and shoot growth of previously waterlogged plants was compared between the genotypes during seven days of recovery. At harvest of experiment two, root segments were sampled to investigate genotype differences of root cross sectional area, root cortex area, stele area and percentage of aerenchyma. The results show that tolerant, in contrast to sensitive genotypes, developed seminal roots faster in the seedling establishment phase and more nodal roots during the waterlogging treatment. NK93602 and Bjarne were the best performing genotypes. Bjarne in particular had a narrower relative stele size of nodal (13.4%) and seminal roots (11.7%) compared to other genotypes (e.g. 16.3% in nodal roots and 13.9% in seminal roots of sensitive Quarna). The results from this study suggests that early vigor is an important trait for waterlogging tolerance in the field. Anatomical root traits, such as a narrow stele and aerenchyma may contribute to improving waterlogging tolerance furthermore.

1. Introduction

Improving the waterlogging tolerance of dryland crops is becoming increasingly important as climate change is projected to increase the precipitation and the frequency of floods and heavy rainfalls in parts of the world (Parry et al., 2007; Barua et al., 2014; Bailey-Serres et al., 2012). Wheat (*Triticum aestivum* L.), one of the world's staple crops, is poorly adapted to waterlogging and substantial yield losses may be a consequence. The severity of the stress and subsequent yield loss depends on factors such as the duration of the event (Marti et al., 2015), the developmental stage at the onset (De San Celedonio et al., 2014), soil and climate conditions (Mcdonald et al., 2006; Watson et al., 1976) as well as the genetic background. Genotypes of wheat are known to tolerate waterlogging stress differently (Mcdonald et al., 2006; Sayre et al., 1994), and the grain yield loss of waterlogged, relative to drained controls may vary from 18 to 81% (Setter et al., 1999). Improving the waterlogging tolerance of wheat has been a longstanding objective. Yet, the advances have been limited, likely due to the complexity of the trait and the dependency on environmental conditions.

Oxygen deficiency in the rhizosphere is the dominant cause of waterlogging stress. It arises as water fills the soil pore space, causing either complete absence (anoxia), or partial absence of oxygen (hypoxia) (Ricard et al., 1994). Until the soil drains, re-supply from the atmosphere will be limited and other gases such as ethylene may accumulate as a result of impeded gas diffusion (Sasidharan and Voesenek, 2015). Plant cells exposed to anaerobic conditions convert to anaerobic respiration and the ethanolic fermentation pathway (Ricard et al., 1994). The conversion curtail ATP production and a subsequent energy shortage can cause cell death or limit energy demanding processes such as nutrient uptake (Colmer and Greenway, 2011), photosynthesis and growth (Malik et al., 2001).

Maintaining oxygenated conditions of the root apices is crucial for stress prevention in the roots. Rice and other plant species native to wetlands may constitutively form intercellular gas spaces in the roots

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Abbreviations: C, control; WL, waterlogging; 2dfp-11dfp, 2–11 days from planting; 1dWL-7dWL, 1–7 days of waterlogging; 1dyr-7dyr, 1–7 days of recovery; RCS, root cortical senescence

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known as aerenchyma (Mcdonald et al., 2002). Induced by ethylene and reactive oxygen species (ROS), aerenchyma, sometimes reported as root porosity, is also commonly found in waterlogged roots of wheat (Xu et al., 2013; Yamauchi et al., 2014b) and barley (Broughton et al., 2015; Zhang et al., 2016). Aerenchyma have been associated with stress alleviation (Huang et al., 1994b; Thomson et al., 1992) and maintenance of yield (Setter et al., 1999). However, it is also evident that the mere presence of aerenchyma does not enable cereal crops to persist waterlogging stress. For instance, Zhang et al. (2016) investigated the relationship between a waterlogging tolerance score and a visual aerenchyma score of nodal roots. A significant correlation between scores of aerenchyma and waterlogging tolerance was found among 177 double haploid barley lines, but the correlation coefficient (r) was limited to 0.2 after 7 days of anaerobic stress treatment. In a QTL-mapping study, Broughton et al. (2015) found root porosity of barley nodal roots to be positively correlated with several plant growth parameters including fresh, dry, relative and absolute weight of roots and shoots. Root porosity was significantly correlated with several of these traits ($r = 0.25$ at the most, for root fresh weight). The authors identified a QTL which was determined to be syntenic with the submergence tolerance gene *Sub1* in rice, and a QTL (*Qaer1.02-3*) associated with aerenchyma in maize. The QTL in question accounted for 39% of the phenotypic variation in root porosity. Similarly, a QTL identified by Zhang et al. (2016) accounted for 44% of the genotypic variation. These results indicate the relevance of aerenchyma for waterlogging tolerance, and that additional traits are likely also involved.

Aerenchyma have been found in both seminal and nodal roots of wheat. However, the extent of it and the ability to act as a diffusion pathway appears to be age and length limited. Thomson et al. (1990) found that neither seminal nor nodal roots that were longer than 200 mm increased in porosity if they had emerged in aerobic nutrient solution and then transferred to anaerobic conditions. Barrett-Lennard et al. (1988) and Trought and Drew (1980) found that nodal roots did not exceed a certain length when emerging under anaerobic conditions. According to their own assumptions, as well as to theory cited by them (Armstrong, 1980), the elongation ceased when the oxygen supply to the root tip was limited by radial oxygen loss (ROL) and internal consumption. Similarly, Huang et al. (1997) found contrasting abilities of two wheat genotypes to increase the root porosity in nodal roots that already existed when a hypoxic treatment was imposed. While seminal roots may cease growth or even senesce under anaerobic conditions (Malik et al., 2002; Thomson et al., 1990), an increased number of nodal roots per tiller, or per unit shoot fresh weight may be found (Malik et al., 2001; Watkin et al., 1998; Thomson et al., 1992). Nodal root emergence is likely triggered by ethylene (Voesenek and Sasidharan, 2013) and is considered as a beneficial trait as they may partly compensate for the loss of functionality of seminal roots (Thomson et al., 1992).

The longitudinal O_2 diffusion and the functionality of aerenchyma could further be improved if coupled with a barrier to oxygen loss (ROL) (Colmer, 2003). Suberization of the hypodermis to prevent ROL is common among wetland species but comparably absent in common wheat. *Hordeum marinum*, a wild relative to wheat and native to wetlands, have a strong ROL barrier (Mcdonald et al., 2001). Through wide hybridization, the trait has been successfully transferred to *H. marinum*-wheat amphiploids (Malik et al., 2011) but further work would be necessary in order to improve the fertility of the offspring (Islam et al., 2007). The prospects of introducing ROL barrier traits from *H. marinum* to wheat was further dismissed by Konnerup et al. (2017), who found that a barrier to ROL was not expressed in disomic addition lines produced from *H. marinum*-wheat amphiploids, nor did they develop more aerenchyma than their wheat parents. Moreover, it is unclear whether improving the barrier to ROL entail tradeoffs such as reduced water and nutrient uptake, or impediments for O_2 entry in the roots once the conditions are oxygenated (Colmer and Greenway, 2011).

Oxygen that is not lost through ROL diffuses in a source-sink manner within roots, where the oxygen concentration may decrease both longitudinally and radially (Armstrong, 1980). The stele tissue is considerably more dense than the surrounding cortex, and stelar anoxia can arise at oxygen concentrations otherwise sufficient for aerobic respiration in cortical cells (Gibbs et al., 1998). The respiration rate of stele tissue may be several times higher than the cortex (Aguilar et al., 2003) and modelling have suggested that a narrower stele may prevent stelar anoxia (Armstrong and Beckett, 1987). Thicker roots and a large cortex area could also be beneficial traits as it would provide more space where aerenchyma can develop (Yamauchi et al., 2014a; Visser et al., 2000).

The majority of previous physiological studies related to anaerobic stress have focused on root and shoot growth under or immediately after the treatment (Striker, 2012). A period of subsequent recovery is less often considered although the ability to recover may differ among genotypes of both wheat (Huang et al., 1994b) and barley (Pang et al., 2004). Contrasting abilities to recover has also been found in field experiments. For instance, Sundgren et al. (2018) found that foliar chlorosis recorded during a waterlogging treatment, did not necessarily correspond with the ability to recover aboveground biomass growth post treatment.

We hypothesize that anatomical root traits of waterlogging sensitive and tolerant wheat genotypes differ and that these traits influence their ability to tolerate waterlogging. We tested this hypothesis by conducting a controlled environment study, investigating the root properties of six spring wheat genotypes with contrasting waterlogging tolerance. Our objective has been to identify root growth and anatomical traits or characteristics that may contribute to the presumed waterlogging tolerance of these genotypes. Two separate greenhouse experiments were conducted to undertake this objective. The first experiment included a seedling establishment phase and a seven days long waterlogging treatment. In the second experiment, the genotypes were compared in their ability to recover from the previous waterlogging treatment.

2. Materials and methods

2.1. Plant material

Six genotypes were used in the experiments, three considered to be tolerant (Bjarne, Zebra and NK93602) and three sensitive (Naxos, Quarna and T9040) to waterlogging. The assumed tolerance properties of the genotypes have been determined in a previous hillplot field screening trial conducted in Norway (Sundgren et al., 2018). Briefly, the tolerance of the genotypes was determined based on measurements of plant height (height under waterlogged conditions relative to drained conditions), the relative number of spikes, the delay in heading date and visual scores of foliar chlorosis as well as a score reflecting their condition at maturity, thus indicative of their ability to recover and to produce yield. Bjarne, NK93602 and T9040 are genotypes with Norwegian origin, while Zebra originates from Sweden, Naxos from Germany and Quarna from Switzerland. Evenly sized seeds were pre-germinated for approximately 65 h in a dark growth cabinet (20 °C) on petri dishes with moist filter paper before transplanting them to the rhizoboxes.

2.2. Growth conditions, preparation of rhizoboxes and experimental setup

The described experiments were conducted in a greenhouse facility at Institute of Bio- and Geosciences (IBG-2 (Plant Sciences); Forschungszentrum Jülich GmbH, Jülich, Germany), at 16 h day length and day/night temperatures of ~20/18 °C. Plants were grown under natural light conditions and supplied with artificial lighting (SON-T AGRO 400, Philips, Amsterdam, The Netherlands) to maintain light intensity $> 300\,\mu\text{mol photons m}^{-2}\,\text{s}^{-1}$.

The rhizoboxes used in the experiments were constructed by molded