Plant growth regulators ameliorate or exacerbate abiotic, biotic and combined stress interaction effects on *Zea mays* kernel weight with inbred-specific patterns

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ABSTRACT

Plant growth regulators have documented roles in plant responses to single stresses. In combined-stress environments, plants exhibit novel genetic architecture for growth traits and physiological responses can display synergistic effects. Synergistic responses have been used to optimize agronomic production and recommendations for adaptation to weather and field conditions can be used to improve selective breeding for higher yielding crops. We hypothesized that alterations in hormone balance would alter phenotypic responses to abiotic and crowding stress in a genotype-specific manner. We investigated the role of plant growth regulators in modulating combined-stress growth responses in *Zea mays*. Maize inbreds were exposed to all single, double and triple combinations of the following stressors in a field environment: drought, nitrogen deprivation, and increased plant density. Growth regulator chemical treatments were applied in a factorial design using a range of inbred genotypes. We found significant differences between the seed weights of plants given different chemical treatments and when single-stress environments were compared to combined stress environments. Different inbred genotypes exhibited contrasting patterns of response to combined stress and plant growth regulator treatment. Plant growth regulators altered combined-stress response pathways in maize inbreds; predictions of growth regulator effects would be improved by better characterization of stress environments and genotype-environment interactions.

1. Introduction

Of all of the world's grains, maize production is the largest by weight, and the United States is the top exporter of this grain (Capehart, n.d.; USDA, 2016). Geneticists aim to select for traits that will result in better protection against pests, more resistance to harsh environmental conditions, and for grain that is a more nutritious food source (Carena et al., 2010). With the continued growth of the world's population the agriculture industry faces a higher demand for grains and smaller land resources to meet this demand. Therefore, the overarching goal is to produce crops of a higher quality at higher quantity. Knowledge about plant physiological response to stresses is key to meeting this demand, as crops are frequently exposed to stress (Lobell and Gourdji, 2012).

Exposure of plants to stress at certain points during development can have detrimental impacts on growth and crop yield (Carena et al., 2010). Significant decreases in corn grain yield and plant biomass can result from limitations in nitrogen availability, which is especially important in low-input smallholder settings, regions with limited access to

fertilizer, irrigation and agricultural chemicals (Weber et al., 2012). Loss of plant biomass can also be seen in response to varying plant density, even in some modern maize hybrids (Tokatlidis et al., 2011). Source-sink balance is a key determinant of the final harvest weight of maize kernels, typically with an interaction seen between genotypes and varying environmental limits across years (Boomsma et al., 2009; Borrás et al., 2004; Sala et al., 2007). Kernel weight is less affected by late abiotic stress than kernel number, and the kernel weight environmental response varies across genotypes (Slafer and Otegui, 2000; Borrás et al., 2009). This makes kernel weight a useful trait for both basic research and applied agronomic experimentation (Kesavan et al., 2013; Zhang et al., 2016). However, kernel weight is a complex trait composed of several underlying processes (Prado et al., 2014) and thus parametric approaches that assume test normality may not be the most powerful for kernel weight data.

Increased attention has been given recently to plant responses to combined stresses (Ramakrishna and Kumari, 2017; Zandalinas et al., 2017). Information about response to combined-stresses is especially

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relevant to agricultural production, because crops growing in the field encounter multiple stresses simultaneously rather than being exposed to one stress in an otherwise controlled environment. Plant physiological responses to combined-stress are not additive; when plants are exposed to two simultaneous stresses, portions of the two individual single-stress response physiological and signaling pathways are expressed, but not all (Mittler, 2006; Suzuki et al., 2014; Zandalinas et al., 2017). A signaling network has been proposed by Makumburage et al. (Makumburage et al., 2013) in which loci within individual ultraviolet radiation and drought stress response physiological pathways repress loci in different stress response pathways. This proposed pathway was derived from the observed novel genetic architecture in response to combined stress, relative to the architecture of genetic response to a single stress. Makumburage et al. (2013) observed that the interaction between two stress-response pathways in maize allowed improved growth under combined-stresses compared to what would be expected − a synergistic response. This combined-stress-genotype interaction is not unique to drought and ultraviolet radiation; drought and heat and the combination of drought and heat interact distinctly with maize genetic variation, leading to different selection recommendations for combined stress environments (Cairns et al., 2013). Combining abiotic and biotic stressors, specifically plant density, also results in non-additive responses (Rossini et al., 2011). A synergistic response to drought and low nitrogen maize can be present and, in maize, has been exploited for production via agronomic advice to reduce nitrogen fertilizer application under drought conditions (Bennett et al., 1989; Sadras and Richards, 2014; Weber et al., 2012), though this synergistic response is also genetically variable and thus would not apply to all production settings. We now have many examples of the genotype and combined stress interaction specificity and current research recommendations focus on ways to exploit these interactions (Souza et al., 2017; Wani et al., 2016).

Plant hormones have been long known to be mediators between the external environment and the internal activities of plants (Wilkinson et al., 2012). Hormones are involved in cross-talk between other pathways within the plant (Mittler et al., 2011), and often play an integrator role between multiple pathways (Gómez-Cadenas et al., 2014; Jaillais and Chory, 2010). Different plant hormones contribute positively and negatively to modulation of plant responses to single and combined stresses (Suzuki, 2016); for example, gibberellic acid interacts with ethylene and abcisic acid to sensitize or ameliorate plant responses to different abiotic stresses, as summarized in (Wani et al., 2016). Due to their role as pathway integrators, we have focused on hormones as candidates for the non-additive interactions seen between stress responses when multiple stresses were applied together.

Plant growth regulator is a term given to a large group of chemicals used to alter intrinsic levels of plant hormones. Many of these chemicals are sold commercially for treating disease or altering growth, and target the biosynthesis or degradation of plant hormones. Externally applied gibberellins influence plant growth and development and play a role in modulation of abiotic stress (Colebrook et al., 2014). Many of the widely used plant growth regulators are triazoles (Rademacher, 2015). Some triazole compounds were originally used as fungicides (by limiting gibberellic acid synthesis in fungi), and were later recognized for their effects on plant growth (Rademacher et al., 1992; Rademacher, 2015). Paclobutrazol is a triazole commonly used to limit stem elongation in crops; the compound inhibits synthesis of gibberellic acid by preventing formation of the precursor molecule kaurenoic acid (Hedden and Graebe, 1985). Paclobutrazol has recent been shown to increase drought tolerance in tomato, with concomitant modification in metabolic and gene expression profiles (Pal et al., 2016). Uniconazole has been shown to increase drought tolerance in *Arabidopsis thaliana* (Saito et al., 2006). Treatment of maize with propiconazole also results in dwarf phenotypes, via inhibition of brassinosteroid synthesis (Hartwig et al., 2012). Many of the effects of these plant growth regulators are poorly documented and much of the information is available only in

summary form from agricultural companies and extension agencies.

Non-linear combinatoric stress responses can be used to group maize genotypes into high and low input optimal types (Ruffo et al., 2015). The inputs investigated by Ruffo et al. (2015) included plant growth regulators and nitrogen fertilizer; these inputs interacted in a synergistic pattern with different maize hybrid genotypes. A similar pattern of input-genotype interaction has also been seen in analyses of nitrogen fertilizer and plant hormone inputs (specifically, externally applied indole-acetic acid and kinetin), which affect different maize genotypes differently (Otie et al., 2016).

In this study we investigated the potential role of hormones in maize responses to combined stresses, via chemical treatment of plants grown in single-stress and combined-stress environments. A diverse set of maize inbreds were tested for chemical-combined stress interactions, as variation in genetically controlled responses is key to selective breeding. We hypothesized that an alteration in hormone balance would alter phenotypic response differently in single and combined stress environments.

2. Materials and methods

2.1. Field design and implementation of stress conditions

Plants were grown in an experimental plot at the Central Crops Research Station in Clayton, North Carolina, Latitude 35.66979°, Longitude −78.4926° from April 12 to August 30, 2013. The field was arranged in a strip plot design, in which the plants were exposed to up to three of the following stresses: nitrogen deprivation, drought, and high-density stress. The field was divided into eight sections as shown in Fig. 1, and each of the sections received a combination of between zero and three of the stresses previously mentioned, so that all possible stress combinations were included. Drought stress was imposed by lack of irrigation to stressed sections; rainfall in this field site is adequate for early season maize growth and supplemental irrigation is only available for late-season (anthesis) stages. Water was supplied to irrigated portions as needed during silking and grain fill, using an overhead watering system. A nitrogen-stressed environment was created by skipping nitrogen application in nitrogen-stress field blocks. Other nutrient-containing fertilizers were applied equally across all sections of the field, in accordance with standard maize growth practice at this site and soil test results. Density stress was implemented during planting, with seeds spaced four inches apart in stressed sections, rather than the standard agronomic spacing of eight inches apart in regular-density control sections. The nitrogen and water stress conditions were selected based on agronomic practice for this field site and prior data on these maize inbreds (Makumburage and Stapleton, 2011); this field experimental design results in increasing stress over the growth season, with water and nitrogen limiting during the developmentally sensitive windows around anthesis and seed fill. All plants were self-pollinated using standard maize nursery procedures, with equal pollination effort across the entire experiment. This field design results in n = 5 overall, with the check B73 inbred having additional replication.

2.2. Genotypes

For this study, we selected a range of genotypes that were from temperate, tropical and mapping populations. The B73 and Mo17 inbreds are widely studied; B73 in particular was a key to improved, higher-yielding germplasm in the single-cross hybrid era of maize breeding (Carena et al., 2010). Improved tropical genotype CML103 was selected by CIMMYT breeders and is included in key diversity panels such as the NAM (McMullen et al., 2009). Ex-plant variety protection inbreds are also part of diversity and mapping panels; we used the LH132 ex-PVP line for our experiment (Bari and Carena, 2015; Nelson et al., 2008; Romay et al., 2013). We also chose a few genotypes from a widely used IBM mapping population, which was derived from