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Heterosis for water status in maize seedlings

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ABSTRACT

Heterosis is one of the greatest practical achievements of plant breeding and has been extensively used in crop improvement in maize. However, the physiological basis of heterosis still remains poorly understood despite its manifestation at early stages of the plant life cycle. Therefore, a better understanding of the physiological mechanisms associated with heterosis may enable further exploitation of this phenomenon. Five maize hybrids and their parental lines were grown in a greenhouse under two water regimes (well watered and water stressed). Plant growth, different root traits, plant water use and water use efficiency and gas-exchange and chlorophyll fluorescence, nitrogen content and stable oxygen, carbon and nitrogen composition and stomatal density of leaves were measured. Plant height, shoot biomass and leaf area were higher in hybrids than in their respective parents in both control and stress conditions. Moreover, hybrids showed a better water use and water use efficiency for biomass than inbred lines. Significant heterosis was also found for photosynthetic rate, stomatal conductance, transpiration rate and the C_i/C_a ratio under water stress conditions, whereas for control conditions the differences were not significant. Likewise, root weight density and root length density were higher in hybrids than parents, especially underwater stress. No heterosis occurred for stable isotope composition under either water regime. The results do not support a constitutively better water status as a cause of heterosis under well watered conditions.

1. Introduction

Heterosis or hybrid vigour is the foundation of modern maize breeding programs. It is defined as the advantage of a hybrid's performance over the parents in terms of viability, growth and productivity (Tollenaar et al., 2004) and in the case of maize breeding, heterosis has been widely used for decades because it increases yield potential and improves adaptation to stress. Heterosis is most evident for adult traits like final plant biomass or yield but it is also apparent in the embryo (Meyer et al., 2007) and during early seedling development (Arkin and Monk, 1979; Hoecker et al., 2006). Despite the importance of heterosis in maize production, the genetic mechanisms, and even more importantly the physiological mechanisms of this phenomenon are still to be elucidated (Blum, 2013).

Several explanations have been developed to describe the underlying genetic mechanisms that lead to heterosis, including dominance, over dominance and epistasis hypotheses (Birchleret al., 2003, 2006; Hochholdinger and Hoecker, 2007). All these hypotheses suggest that the contribution of many genes is responsible for the more vigorous phenotypes of hybrids over inbred lines. At the physiological level, numerous physiological hypotheses have been proposed to explain the metabolic superiority of the hybrid over the inbred parents and the subsequent effects on growth, plant size, yield and other development characteristics (Tollenaar et al., 2004; Tollenaar and Lee, 2006). According to some authors, heterosis for growth is mediated by embryo size (Ashby, 1932, 1936) or by the endogenous concentration of gibberellins (Rood et al., 1988). Others explain heterosis as a dynamic attribute affected by both the environment and the stage of development (Tollenaar et al., 2004; Tollenaar and Lee, 2006). Tollenaar et al. (2004) reported that heterosis for grain yield in maize can be attributed to: (i) heterosis for dry matter accumulation (DMA) before silking, which results mainly from greater light interception due to increased leaf size; (ii) heterosis for DMA during the grain filling period, which results from greater light interception due to a greater maximum leaf area index and increased stay green, and (iii), heterosis for harvest index. More recently, Araus et al. (2010) have proposed that heterosis in subtropical maize was mediated by constitutive differences in water status, with hybrids exhibiting a better water status

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than the parental lines regardless of the water growing conditions. Water stress may negatively affect growth and productivity not just through a decrease in the photosynthetic rates per unit leaf area but also reducing cell expansion, leaf growth and thus total plant photosynthesis, biomass and yield (Ashraf and Mehmood, 1990; Aslam et al., 2013).

Understanding the physiological mechanisms of heterosis might open up opportunities for increasing not just yield potential but also stress adaptation. In this sense, drought is the major abiotic factor that limits agricultural crop production (Golbashy et al., 2010). The present study was designed to assess in subtropical maize seedlings the heterosis manifested by the hybrids over mid-parents for different morpho-physiological traits under well watered and water stressed conditions. To that end shoot growth and root architecture related to water uptake (Elazab et al., 2012), together with photosynthetic and transpiratory gas-exchange, stomatal density, nitrogen concentration and accumulated water used were studied. In addition, the signature of stable carbon, oxygen and nitrogen isotopes, which are considered indicators of water, transpirative status and nitrogen status in C_3 plant such as wheat (Cabrera-Bosquet et al., 2011; Yousfi et al., 2012) and in C_4 plants such as maize (Araus et al., 2010; Cabrera-Bosquet et al., 2009a,b) were analysed. The final objective was to evaluate if differences in water status are behind the heterosis of maize seedlings under both water regimes. This study complements previous work undertaken on similar subtropical inbreds and hybrids growing in the field where inbreds produced about 30–40% and 15–30% of hybrid yield under well watered and water stressed conditions, respectively (Araus et al., 2010; Cairns et al., 2012).

2. Material and methods

2.1. Plant material and growing conditions

The experiment was conducted in subtropical maize (*Zea mays* L.), provided by the Global Maize Program at the International Maize and Wheat Improvement Center (CIMMYT) (Table 1). Five female lines, one male line (tester) common to the five females and their respective F1 hybrids were used.

The experiment was started on 30 September 2013 in a greenhouse at the experimental fields of the University of Barcelona, Spain, using a randomized complete block design with three replications for treatment (control and water stress) and genotype and ended on 8 November 2013, when inbreds and the corresponding hybrids reached on average the 6 and 8 leaf stages, respectively. Two seeds of each genotype were sown in pots of 9 dm^3 volume and 24 cm height. The soil was composed of a mix of 50% perlite, 25% vermiculite and 25% peat. Three weeks after plantlet emergence seedlings were thinned to one per pot. After sowing, control plants were irrigated to keep pots to 100% water capacity for a period of five weeks. In the stress treatment pots were irrigated up to 60% of the water capacity for three weeks and then watering was decreased for one week to reach 40% of water capacity, ending with 30% water capacity for one week. Water was added every 2–3 days if needed. The process involved weighing each pot and then applying the required amount of water.

The absolute minimum and maximum air temperatures during the growing period were 12 °C and 31.9 °C, respectively and the maximum photosynthetic photon flux density (PPFD) was 1450 $\mu mol\, m^{-2}\, s^{-1}$.

2.2. Gas-exchange measurements

Leaf gas-exchange and chlorophyll fluorescence were measured using a portable open gas-exchange system (Li-6400; Li-Cor, Inc., Lincoln, NE, USA) equipped with a light source (Li-6200-02B LED; Li-Cor). Net CO_2 assimilation rates (A), stomatal conductance (g_s), transpiration rate (E) and the ratio of intercellular to ambient CO_2 concentration (C_i/C_a) were measured in recently fully expanded leaves before harvest. Environmental conditions in the leaf chamber consisted of a PPFD of 1500 $\mu mol\, m^{-2}\, s^{-1}$, an air temperature of 25 °C and an ambient CO_2 concentration of 400 $\mu mol\, mol^{-1}$.

2.3. Stomatal and epidermal cell densities

Leaf surface replicas were used to determine leaf stomatal density, which was expressed as the number of stomata per unit leaf area (Radoglou and Jarvis, 1990). The density of epidermal cells was also calculated in the same way. The leaves selected were those in which gas exchange had been measured. The adaxial and abaxial epidermises of the leaves were carefully smeared with nail varnish from the mid-area between the central vein and the leaf edge and allowed to dry for at least 10 min. Then the dried layer of nail varnish was peeled away using adhesive tape stuck onto a glass slide. The number of stomata (s) and epidermal cells (e) were counted under the microscope, and the stomatal density (SD, pores mm^{-2}) and the epidermal cell density (ECD, cells mm^{-2}) were calculated as follows:

$$SD = \frac{s}{0.152}$$

$$ECD = \frac{e}{0.152}$$

where 0.152 mm^2 is the surface area of the microscopic field (which has a radius of 0.22 mm). For each leaf side four microscopic fields were measured. Additionally, the stomatal index (defined as SD ECD^{-1}) was calculated, for each leaf side.

2.4. Plant growth and shoot biomass

At the end of the experiment, plant height was determined by direct measurement from the soil surface to the tip of the last leaf, using a ruler. Then leaves were harvested and scanned (Canon PIXMA/MP140 scanner, Fukushima, Japan), with image obtained at 300 dots per inch, and total aerial plant biomass was calculated using ImageJ, a public domain Java image processing and analysis program (National Institutes of Health, Bethesda, Maryland, USA). Fresh organs were then dried at 60 °C for 48 h, weighed and ground finely for stable isotope analysis.

2.5. Root traits

Plants were uprooted and roots were carefully separated from the soil, washed with tap water followed by rinsing with deionised water and gently dried with paper tissue. The four types of roots (primary, seminal, crown and brace roots) were processed separately. The fresh weight was then calculated as the sum of the fresh weight of the four classes of roots, and the total volume was calculated by the sum of the volume of the four types of roots. To determine the total root length (the cumulative length of an entire plant root system), roots were scanned using a flatbed scanner at a resolution of 300 dots per inch (Canon PIXMA/MP140 scanner, Fukushima, Japan). To scan, root samples were placed in a rectangular glass dish (200 mm × 1500 mm) with a 4–5 mm deep layer of water to untangle the roots, while minimizing root overlap. When necessary, root agglomerates were separated into subsamples until they could be placed into the rectangular glass dish. The images were analysed by the root image analysis software EZ-Rhizo (Glasgow University, UK) as shown elsewhere (Armengaud et al., 2009). After determining the morphological characteristics of the root, the