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Functional mechanisms of drought tolerance in maize through phenotyping and genotyping under well watered and water stressed conditions

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ABSTRACT

Developing tolerant genotypes is crucial for stabilizing maize productivity under drought stress conditions as it is one of the most important abiotic stresses affecting crop yields. Twenty seven genotypes of maize (*Zea mays* L.) were evaluated for drought tolerance for three seasons under well watered and water stressed conditions to identify interactions amongst various tolerance traits and grain yield as well as their association with SSR markers. The study revealed considerable genetic diversity and significant variations for genotypes, environment and genotype × environment interactions for all the traits. The ranking of genotypes based on drought susceptibility index for morpho-physiological traits was similar to that based on grain yield and principal component analysis. Analysis of trait – trait and trait – yield associations indicated significant positive correlations amongst the water relations traits of relative water content (RWC), leaf water potential and osmotic potential as well as of RWC with grain yield under water stressed condition. Molecular analysis using 40 SSRs revealed 32 as polymorphic and 62 unique alleles were detected across 27 genotypes. Cluster analysis resulted in categorization of the genotypes into five distinct groups which was similar to that using principal component analysis. Based on overall performance across seasons tolerant and susceptible genotypes were identified for eventual utilization in breeding programs as well as for QTL identification. The marker-trait association analysis revealed significant associations between few SSR markers with water relations as well as yield contributing traits under water stressed conditions. These associations highlight the importance of functional mechanisms of intrinsic tolerance and cumulative traits for drought tolerance in maize.

1. Introduction

Maize is an important cereal and fodder crop cultivated across the world (White and Johnson, 2003). Drought is one of the most important abiotic stresses limiting crop yields including maize (Prasanna, 2012; Mir et al., 2012). The adverse effects of various abiotic stresses including drought and high temperature are likely to be further accentuated by the impending climate change. Although a complex trait controlled by genotype × environment (G × E) interactions, improvement of grain yield in maize under harsh environments is an urgent priority to meet the increasing demands for food of ever increasing population. A major approach to identify drought tolerant genotypes is to assess tolerance on the basis of yield stability or drought susceptibility index (Sinha et al., 1986) as grain yield under water-deficit stress is the ultimate culmination of various physiological and metabolic functions in plant. It is well known that duration, intensity of drought stress as well as stage of crop growth determine the extent of yield losses. In maize, anthesis-silking interval (ASI) is known to be an indirect selection criterion for grain yield (Magorokosho et al., 2003) and it is indeed the most critical stage adversely affected by drought (Bolaoos and Edmeades, 1993). It is also well known that in maize the final ker-

Abbreviations: DSI, drought susceptibility index; LWP, leaf water potential; PCA, principal component analysis; PIC, polymorphism information content; QTL, quantitative trait loci; RWC, relative water content; SAHN, sequential, agglomerative, hierarchical and nested clustering methods; SSR, simple sequence repeat; UPGMA, unweighted pair group method using arithmetic means.

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Table 1a
Soil physical and chemical characteristics in the experimental plot.

Texture	Sandy loam
Sand (%)	59.3
Silt (%)	14.9
Clay (%)	16.8
pH	7.9
Electrical conductivity (m mhos cm^{-1})	0.20
Organic carbon (%)	0.35
Total nitrogen (%)	192.4
Available phosphorus (kg ha^{-1})	28.7

Table 1b
Soil moisture content in the experimental plot in different seasons.

Season	Soil moisture (%)	
	Well-watered	Water stressed
2012 rainy season	12.0	7.3
2012–13 post rainy season	14.1	7.3
2013 rainy season	12.0	5.1

nel number at harvest is tightly controlled by the available leaf area at anthesis. (Khanna Chopra and Maheswari, 1998). Although grain yield is the most commonly used criterion for drought tolerance in several crop improvement programmes, dissecting this complex trait into several contributing ones for use in screening is of paramount importance. Plethora of information exists on the mechanisms of drought tolerance as well as on G × E interactions (Gong et al., 2015; Maheswari et al., 2012; Yue et al., 2005).

Several morpho-physiological traits have been identified which contribute to water deficit stress tolerance in crops. Also drought related markers and genes which regulate metabolic and physiological responses under stress have been identified (Setter et al., 2011; Kakumanu et al., 2012; Pandey et al., 2013; Thirunavukkarasu et al., 2014; Shanker et al., 2014). Water-deficit stress often leads to reduction in plant height and increase in anthesis silking interval and an eventual yield reduction by adversely affecting realization of both source and sink potentials. Further intrinsic traits mainly related to water relations such as relative water content, leaf water potential, osmotic potential as well as stomatal conductance and transpiration are also negatively impacted by water-deficit stress. Thus, selection for grain yield as well as the intrinsic tolerance traits needs to be addressed for genetic enhancement of drought tolerance. Characterization and identification of useful traits is essential for efficient utilization of available diverse germplasm. Identification of molecular markers such as SSRs and SNPs associated with yield and tolerance traits is another important approach to accelerate the genetic enhancement of drought tolerance in maize (Nguyen et al., 2012; Thirunavukkarasu et al., 2014; Beyene et al., 2015).

In this context, the present study was aimed at deciphering the functional mechanisms underlying trait-trait, trait-yield and marker-trait associations in response to water-deficit in a set of genotypes assembled from diverse sources. Further the association of a few SSR markers with traits conferring drought tolerance was examined to identify crucial metabolic functions to cope with water-deficit stress.

2. Materials and methods

2.1. Location

Field experiments were conducted in the crossing block area, Central Research Institute for Dryland Agriculture, Hyderabad, Telangana, India (17° 22′ N and 78° 28′ E) during the rainy (wet) season of 2012, 2013 and post rainy (dry) season of 2012–2013 which represented different weather conditions to analyse the responses of maize germplasm to water-deficit stress. Soil physical, chemical properties are presented in Table 1a. Soil moisture content of the surface soil (0–15 cm) was measured gravimetrically for both well watered and water stressed plots and the average soil moisture level at stress point is presented in Table 1b.

2.2. Experimetal design and agronomic details

The genotypes were sown in a randomized complete block design (RCBD) with three replications at a single row plot of 10 plants with row spacing of 60 cm and plant to plant spacing of 25 cm. In each season, the genotypes were grown under two different water regimes (management) i.e., well watered, in which plants were irrigated at regular intervals so as to maintain the plants in non-stressed condition and water stressed treatment in which plants received irrigation till they reached early vegetative stage i.e. 45 days after sowing (DAS) and subsequently no water was applied till maturity. The recommended dose of fertilizers 60 kg N ha^{-1} and 60 kg P ha^{-1} as diammonium phosphate, 30 kg K ha^{-1} as muriate of potash was applied as basal dose; second dose of 30 kg N ha^{-1} at vegetative stage (30 DAS) and third dose of 30 kg N ha^{-1} as urea and 30 kg K ha^{-1} as muriate of potash was side dressed at flowering stage (55 DAS). The crop was maintained pest and disease free with plant protection measures. The weekly average minimum and maximum temperature ranged from 16 °C to 33.1 °C and the relative humidity varied from 64–89% during 2012 and 2013 wet seasons, While the weekly minimum and maximum temperature recorded during the dry season was 12 °C and 36 °C respectively with the relative humidity ranging between 54–75%. Total rainfall received during the crop growth period of 2012 and 2013 wet seasons was 700 mm and 660 mm respectively while only one rain event of 10 mm was recorded during the dry season (Fig. 1).

2.3. Genetic materials

Twenty seven maize genotypes of diverse origin and differing in their yield stability were used for the study. Out of these, ten genotypes (HKI-161, HKI-164-7-4, HKI-164-D4, HKI-7660, HKI-L-287, LM-6, RJR-198, RJR-208, RJR-363, RJR-385) were obtained from Directorate of Maize Research (DMR), New Delhi, five (NSJ-211, NSJ-245, NSJ-366, PSRJ-13122, SNJ2011-26) from National Bureau of Plant Genetic Resources (NBPGR), Regional Station, Hyderabad and twelve (Z101-15, Z32-12, Z32-62, Z32-87, Z40-183, Z40-19, Z49-65, Z59-17, Z60-72, Z60-87, Z93-194, Z96-5) from CIMMYT Regional Centre, Hyderabad.

2.4. Phenotyping

Data was recorded on three representative plants of each genotype for various morphological traits *viz*., plant height (PH), anthesis silking interval (ASI), total biomass (TB), grain yield/plant (GY) and 100 seed weight (SW). A dry spell of 10 days coincided with the anthesis silking stage. Plant height was measured from the soil surface to the tip of the central axis. Anthesis silking interval was recorded as the difference between the number of days for tassel emergence and visible silks. Data was also recorded on three plants for each genotype on various physiological traits *viz*., relative water content (RWC), leaf water potential (LWP), osmotic potential (OP), transpiration rate (TR), stomatal conductance (SC), leaf temperature (LT), SPAD chlorophyll meter reading (SCMR) and canopy