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Screening of Provitamin A Maize Inbred Lines for Drought Tolerance using β-carotene Content, Morpho-physiological and Biochemical Traits

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Introduction

Drought is one of the major abiotic constrain to maize's (*Zea mays* L.) productivity, the most common staple crop in sub-Sharan Africa. On the other hand, lack of vitamin A in maize has been deemed a contributory factor to high prevalence of vitamin A deficiency (VAD) in this region, especially among rural communities who cannot afford diversified diets. Provitamin A maize is a yellow/orange endosperm maize, which was recently identified as a complementary solution to vitamin A deficiency (VAD) among maize consuming communities (Bouis et al., 2011). It contents three provitamin A carotenoids which are β-carotene, α-carotene and β-cryptoxanthin (Fierce et al., 2008). Among the three carotenoids, β-carotene has higher provitamin A activity, which is twice than that of α-carotene and β-cryptoxanthin, therefore is considered the most efficient and important carotenoid (Harrison, 2015). One way to promote the adoption of provitamin A maize is to enhance its tolerance to drought stress.

Drought stress affects maize at almost all growth stages, but flowering and grain filling stages are the most susceptible, with yield losses of over 90% reported when drought coincide with these growth stages (Lu et al., 2011). Genetic improvement of maize for drought tolerance through breeding is a sustainable solution to reduce the impacts of drought. However, breeding for drought tolerance is a complex task because the trait is controlled by many genes and is highly affected by genotype by environment interaction (GEI). Plants respond to drought stress through morphological, physiological (morpho-physiological) and biochemical changes. The level and rate of response to drought varies genetically, a character manipulated by plant breeders when developing drought tolerant cultivars. Therefore, there is need to assess the level of the available genetic variation as part of the pre-breeding activities. This further helps plant breeder to employ a suitable breeding strategy.

Screening for drought tolerance and provitamin A content using integrated approaches is an important step in developing drought tolerant provitamin A maize. The main objective of this study was to screen and select candidate drought tolerant provitamin A inbred lines based on their grain yield, β-carotene content, morpho-physiological and biochemical performances under drought stress and optimum conditions.

Material and Methods

A total of fifty maize inbred lines, which included forty-three provitamin A experimental lines, three provitamin A checks and four non-provitamin A drought tolerant checks, were screened. The research study was carried out in 2016/17 season, across three environments (Env), which are two greenhouse trials and one field trial in the KwaZulu-Natal province of South Africa. A 5 x 10 alpha lattice design was used in all the three environments with two replications and two water regimes (water stress; WS and optimum conditions; WW). The WS treatment was implemented following the CIMMYT protocol (Bänzinger et al., 2000). Eleven morpho-physiological traits were measured across all the three environments namely grain yield (GY), days to antheisis (DA), anthesis silking interval (ASI), ears per plant (EPP), leaf rolling (LR), leaf senescence (SEN), chlorophyll content (CC), stomatal conductance (G_s), β-carotene content (BCC) and proline content (PC). Majority of the traits were measured following the CIMMYT protocol except PC and BCC, which were measured following methods described by Bates et al. (1973) and Menkir et al. (2008), respectively.

Combined analysis of variance of β-carotene, all morpho-physiological and biochemical traits evaluated in this study were analysed following the lattice procedure of SAS 9.4 after homogeneity test of variances. Correlation analysis, principal component analysis (PCA) and PCA biplots were computed using Genstat 18.

Results and Discussion

The observation that 39.1% of the experimental provitamin A inbred lines performed better than the best provitamin A check, supports the hypothesis of this study that there are shortages of drought tolerant provitamin A maize germplasm. Combined ANOVA showed significant differences among the means of all the morpho-physiological and biochemical traits used in this study. This indicated the presence of a rich source of variation available to plant breeders to develop drought cultivars. Drought induced GY reduction can be largely associated with combined effect of changes in EPP, ASI, PH, G_s, CC, SEN and LR as illustrated by the principle component biplot (Figure 1). The observed high correlation between GY and EPP ($r = 0.78$), and ASI ($r = 0.61$) could be attributed to the fact that at reproductive stage, drought stress induced delayed silking, kernel abortion and poor grain filling which then resulted in reduced number of ears with fully developed kernels and wider ASI. Thus, selecting for many EPP and short ASI can boost yield under both conditions contrary to the findings by Monneveux et al. (2008), who did not find any significant association between GY and ASI. Our study confirms the findings by Cairns et al. (2012) that ASI is still an important trait to be used for selection in maize drought stress breeding.

The observed positive correlation between GY and PH under both stress and optimum conditions infers that selection for taller plants could be an indirect selection for high yielding. However, this disagrees with findings by Bolaños and Edmeades (1996) who reported an increase in drought tolerance with decrease in PH. Our findings can, therefore be explained from the standpoint that PH is one of the "sinks", a product of dry matter accumulation and is a key indicator of growth rate in maize (Lee & Tollenaar, 2007). Thus, plant channels photosynthetic assimilates to PH effecting growth, a process which is experienced before flowering especially by determinate maize cultivars.

Stomatal conductance (G_s) also largely contributed to the total observed variation, especially under optimum conditions as illustrated by the principal component biplot (Figure 1b) and the observed high correlation with GY ($r = 0.86$). The observed huge decrease in G_s due to drought stress supports the suggestion by Grzesiak et al. (2006), that G_s is the major physiological trait that discriminate among drought tolerant and susceptible maize and wheat genotypes. Thus, drought tolerant genotypes are more efficient in conserving tissue water status via decreased G_s, which in turn reduces transpiration rate and water loss in contrast to the susceptible genotypes.

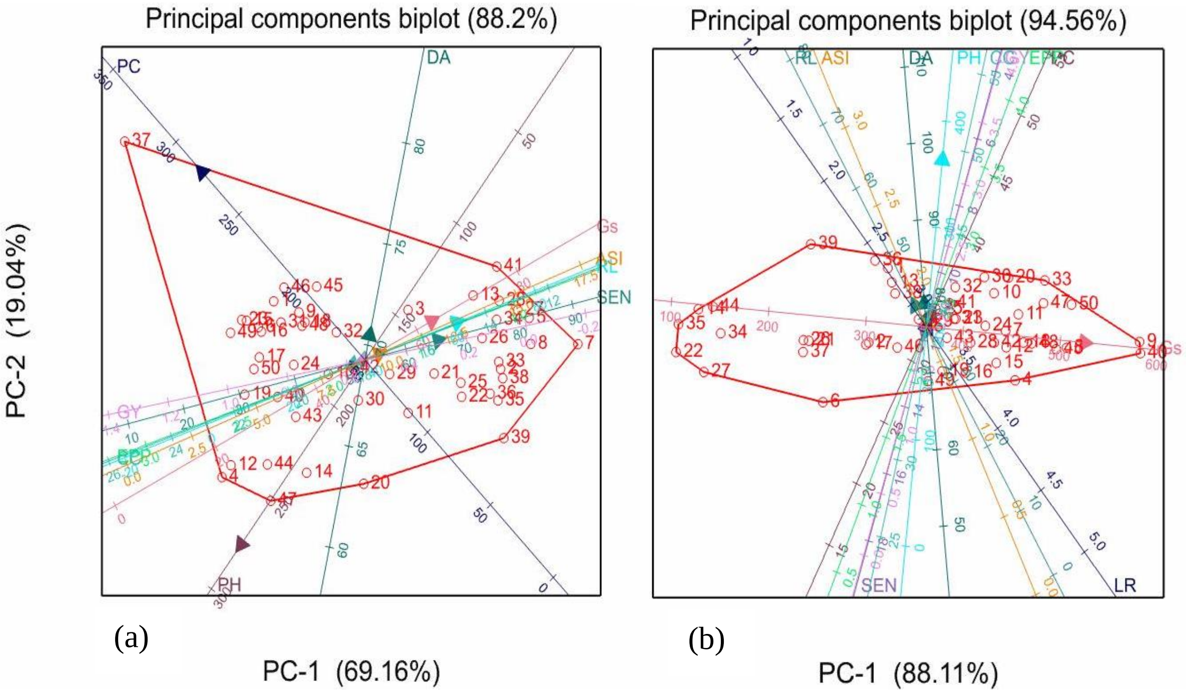

Figure 1: Principal component biplots showing genotypic clustering under (a) WS and (b) WW conditions. ASI - anthesis silking interval; CC - chlorophyll content; DA - days to anthesis; EPP - Ears per plant; GY - grain yield; Gs - stomatal conductance; PH - Plant height; LR - Leaf rolling; PC - proline content

The observed significant increase (79%) in free PC after exposure to drought and the strong positive correlation between GY and PC (r = 0. 58) under stress suggest that maize genotypes that exhibited high free PC can be selected as drought tolerant. Our results, therefore support the claim by a number of plant physiologist who reported that under drought conditions proline is released to effect plant cell osmotic adjustments which helps to conserve cell turgor which is essential for plant continued growth and productivity under water limiting conditions (Hong-Bo et al., 2006; Changhai et al., 2010; Marcińska et al., 2013).

Conclusions and Outlook

This study proved that the morpho-physiological and biochemical traits applied in the screening of provitamin A inbred lines were effective in discriminating among genotypes for drought tolerance. The highly ranked genotypes in the SI ranking are drought tolerant provitamin A maize inbred lines from which parents for the downstream hybridization programme were selected. Additionally, the highly ranked genotypes can be used as drought tolerance donors in drought tolerance breeding programmes in which backcross approaches can be utilised. The study also revealed that apart from the traditionally applied traits in drought tolerance studies (GY, EPP, and ASI), G_s is a key trait in differentiating genotype's response to drought tolerance. It is, therefore recommended to carry further studies to investigate the genetics of G_s especially the inheritance level and related genetic parameters. Furthermore, the application of free PC analysis in maize screening needs further investigation across more than one field sites and exploring fast and easy way of detecting free PC in plant samples.

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