doi:10.20944/preprints202004.0083.v1

Review

Strengthening the Practical Application of Experiments Assessing Maize Drought Tolerance

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Abstract

Drought is among the most important abiotic stressors influencing food-crop production worldwide. Currently, drought-tolerant maize materials are rarely used for actual breeding because corn production primarily focuses on heterosis to generate desired varieties. In this article, we reviewed current work on assessing maize drought tolerance. We suggested that the development of enhanced screening techniques must clearly consider the connection between theory and application. We strongly recommend that agricultural scientists focus on translating the results of laboratory experiments into practical methods for improving crop productivity.

Keywords: drought-tolerance characteristics; maize breeding; maize drought tolerance; molecular markers; phenotype; screening of drought-tolerant plants

Urgent need for practical application of drought-tolerant varieties

Drought is among the most important abiotic stressors influencing food-crop production worldwide. The most efficient way to address the problem of drought-related decreases in productivity is focusing on improving crop adaptation to a given environment, rather than attempting to provide “optimal” growth environments (Boyer, 1982). Discovering germplasm resources with improved productive capacity under drought is a more effective long-term solution than temporary measures such as irrigation systems to alleviate soil moisture deficiency (Salekdeh et al., 2009). Therefore, breeders and agronomists are deeply interested in cultivating drought-tolerant varieties and uncovering drought-tolerant characters (Avramova et al., 2016). To this end, many materials with excellent drought-resistance have been found, including hybrid, inbred, and transgenic lines.

Drought-tolerant plants are typically found through a comparison of variance in relevant indices. Despite the existence of numerous highly targeted proposals for guiding experiment design, current drought evaluation systems for maize remain ambiguous (Gilbert and Medina, 2016). Currently, drought-tolerant maize materials are rarely used for actual breeding because corn production primarily focuses on heterosis to generate desired varieties. Likewise, the development of drought-tolerant germplasm in research also employs heterosis. Thus, existing processes for identifying drought-tolerant plants must be optimized to strengthen the practical applications of scientific research.

A PubMed search for drought-related plant studies revealed that the screening and identification of drought-tolerant varieties or traits is a research hotspot. However, despite these numerous scientific advancements, reports rarely mention the application of their findings to successful breeding of drought-tolerant crops. The primary reason for this translation gap is a lack of efficient methods to realize most research findings in a mass-production setting. There is an urgent need for rapid, cost-effective screening of drought-tolerant varieties so that they can be applied to grain production.

Characterizing the mechanism underlying drought resistance in most crops is particularly challenging, because the drought-resistance traits are controlled by multiple loci. Occasionally, selecting for specific stress-tolerant alleles may yield unexpected results. Moreover, the frequency of these alleles may gradually decrease during the breeding process (Blum, 1988). A large evaluation system has been constructed to mine experimental data for the purposes of clarifying whether a germplasm resource has drought tolerance characteristics. Previously, we proposed that drought-tolerance experiments should be conducted under field/production conditions (Wu et al., 2017). In this article, we focus on how field experimental results can help direct crop production, specifically in maize.

Piecemeal maize research under laboratory and field conditions

PubMed literature search yielded 44 articles closely related to maize drought research. They could be classified into three major categories. The first group of studies focused on comparing physiological and biochemical indices of drought resistance across multiple varieties to identify those with high tolerance to water deficit. The second set of studies focused on screening for molecular markers (e.g., chromosomal fragments or genetic loci) associated drought resistance. The third category classified maize varieties according to phenotypic characteristics associated with high drought resistance and tested the reliability of these indicators under normal and drought conditions. Most of the experiments aimed to identify drought resistance of maize were conducted indoors, while others were conducted integrating with field trials (Table 1). In addition, we found several articles that did not confirm with existing best practices and guidelines for such research (Salekdeh et al., 2009), suggesting the need to emphasize optimization before beginning an experiment. Notably, however, QTL research and genome-wide association studies mainly focused on field cultivation of materials. These field-based outcomes tend to be more reliable for practical application than laboratory drought tests. Nonetheless, the fact that most studies are laboratory-oriented is likely because building a controlled, outdoor experimental site that meets the criteria for investigating complex traits is extremely expensive. Therefore, researchers attempt to lower costs while still obtaining usable data through smaller-scale, indoor appraisals of drought resistance.

The laboratory studies on maize drought-resistance evaluated macro-level physiological or biochemical traits of maize lines grew under drought conditions. However, focusing on genetic markers (e.g., drought-resistant genes) may be more practical, as breeding based on marker-assisted selection (MAS) has high screening efficiency and has resulted in useful varieties (Beyene et al., 2016). Unfortunately, MAS-derived varieties have not found their way into actual production. Furthermore, although numerous drought-resistant molecular markers have been developed and marked in the maize genome, they have typically been employed in QTL and GWAS research. The nature of such studies focuses on fragmented and small genetic contributions to a given phenotype; thus, their practical contribution to breeding is limited (Buckler et al., 2009; Messmer et al., 2009). More effort should instead be aimed at identifying drought-resistant varieties that can reliably transmit the trait of interest to multiple generations.

Emphasizing “omics” technologies should prove beneficial in this regard, as this field uses a wide range of markers to identify multiple causes of phenotypic differences and distinguish specific genotypes. Drought-response mechanisms are particularly in need of an omics perspective, given high inter-variety phenotypic differences under water deficient conditions. Strengthening the links between different research schema through an integrative omics-focused lens should provide new perspectives on the screening of drought-tolerant varieties (Figure 1). Improved screening will bring new materials for heterosis-based breeding, currently the main frame work used in maize production. In the next section, we discuss how screening methods can be enhanced through an integrative approach.

Establishing an efficient and comprehensive evaluation system

First, to improve the credibility of results, drought evaluations should be performed on carefully selected targets, those with suitable reference materials for comparison. For a genome as complex as that of maize, test materials should exhibit common characteristics and differ only in their growth performance underwater deficiency for a more controlled experimental comparison. Moreover, researchers should avoid using only a single, simplistic index for drought resistance. For instance, some studies have employed changes in relative water content of various plant materials as a proxy for drought tolerance (Ku et al., 2016). However, plants react differently across developmental different stages and depending on drought intensity or duration; therefore, a single indicator cannot fully capture the extent of a given variety's drought response, leading to potentially inaccurate tolerance assessments (Gilbert and Medina, 2016; Chen et al., 2015). Recently, comparisons of inbred lines with hybrids (e.g., Xiang et al., 2017) are also suboptimal for practical breeding purposes given the vast difference in drought regulation mechanisms (Cairns et al., 2012).

Second, trait heritability must be explicitly verified in a candidate material. Drought tolerance among existing commercial varieties should be compared to provide specific suggestions regarding which materials are best suited for breeding. Next, research should focus on crossbreeding commercial species that all exhibit high drought tolerance so that these drought-resistant genes are transferred to the next generation of hybrids. Currently, very few experiments employing inbred lines for breeding have incorporated hybridization to verify whether traits of interest occur in the next generation. This is a necessary step because heterosisis likely to obscure beneficial parental genotypes (Wei et al., 2011).

Thirdly, screening programs should explore drought resistance mechanisms in candidate materials that have been identified via the first two improvements described. Fortunately, the continuous advancement of high-throughput technologies is elevating our understanding of the molecular mechanisms underlying drought stress responses. Both proteomics (Hu et al., 2015) and transcriptomics (Aravind et al., 2017) are effective in describing changes to the molecular regulation network in maize under drought conditions. The specific protein- or gene-expression patterns uncovered are valuable evidence for drought resistance.

Indeed, based on these results from high-throughput omics studies, appropriate molecular markers can be developed to provide efficient and accurate guidance for breeding. Although capable of accurate genotype screening, existing genetic markers such as SNPs and SSRs are limited because they may regulate multiple phenotypes simultaneously, thus contributing less to drought resistance individually (Buckler et al., 2009; Messmer et al., 2009). To meet rapid-screening requirements, improved markers should be tightly linked to the trait of interest, while also accounting for spatiotemporal specificity in protein or gene expression (Shanker et al., 2014).

Concluding remarks and outlook

Stress tolerance assessment is vital to effective breeding selection for crop improvement. Currently, numerous drought-tolerant varieties have been bred and evaluated, but screening methods with increased efficiency and accuracy are necessary to use the materials in practical breeding programs. The development of enhanced screening techniques must clearly consider the connection between theory and application. In conclusion, we hope that the proposals described here are instructive to researchers of crop drought tolerance. We strongly recommend that agricultural scientists focus on translating the results of laboratory experiments into practical methods for improving crop productivity.

Acknowledgements

Our work was supported by the National Natural Science Foundation of China (U1904107).

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Table 1 A summary of representative researches on drought-tolerance in maize

Purpose	**Treatment methods**	**Treatment period**	**Maize materials**	**Selection criteria**	**Ref.**
Identifying drought-tolerance varieties	Greenhouse: withholding water for 17 days and recovering for five days	8-leaf stage	Nine inbred lines and one hybrid line	Drought resistance, recovery and adaptability	Chen et al., 2016
	Greenhouse and field: withholding soil water content of the soil at 15%–16% for 16 days	10-leaf stage	Drought-tolerance transgenic lines (TsVP and betA) and their wild-type; hybrid line of TsVP and betA	Several agronomic traits, e.g., anthesis-silking interval, yield	Wei et al., 2011
Exploring new drought-tolerance indicators	Greenhouse: withholding water until measure; Field: using rainout shelters and withholding water	Greenhouse: 5-day old seedlings; Field: 35-40-day old seedlings	Recombinant inbred lines	Large root cortical cells, small file number of cortical cells, and few but long lateral roots were related high yields. Other priority traits included rooting depth, acquisition of deep soil water, plant water status, leaf photosynthesis, and growth rate etc.	Chimungu et al., 2014a; Chimungu et al.,2014b; Zhan et al., 2015
	Greenhouse: without watering until the 4th leaf was fully expanded	4-leaf stage	Inbred lines and hybrids, differing in drought tolerance	The agronomic traits related to drought resistance were analyzed, e.g., leaf elongation rate, leaf width	Avramova et al., 2016
	Field: withholding irrigation 2-4 weeks	One month after sowing	Hybrids	Hybrids with lower kernel $\Delta^{18}O$ under well-watered and intermediate water stress had higher yields	Cabrera-Bosquet et al., 2009
Screening drought-related molecular markers	Field: withholding irrigation before anthesis	Flowering stage	Inbred lines	Molecular markers related to agronomic traits, e.g., plant height, leaf senescence, chlorophyll content, root capacitance, yield	Messmer et al., 2009; Almeida et al., 2014; Thirunavukkarasu et al., 2014; Xu et al., 2014; Li et al., 2016;
			Inbred lines and their offspring from crossing with a common inbred line	Molecular markers related to metabolic and physiological traits (e.g., abscisic acid, proline, total sugar, dry mass, leaf weight) and field traits (e.g., grain yield, anthesis-silking interval)	Xue et al., 2013; Zhang et al., 2016

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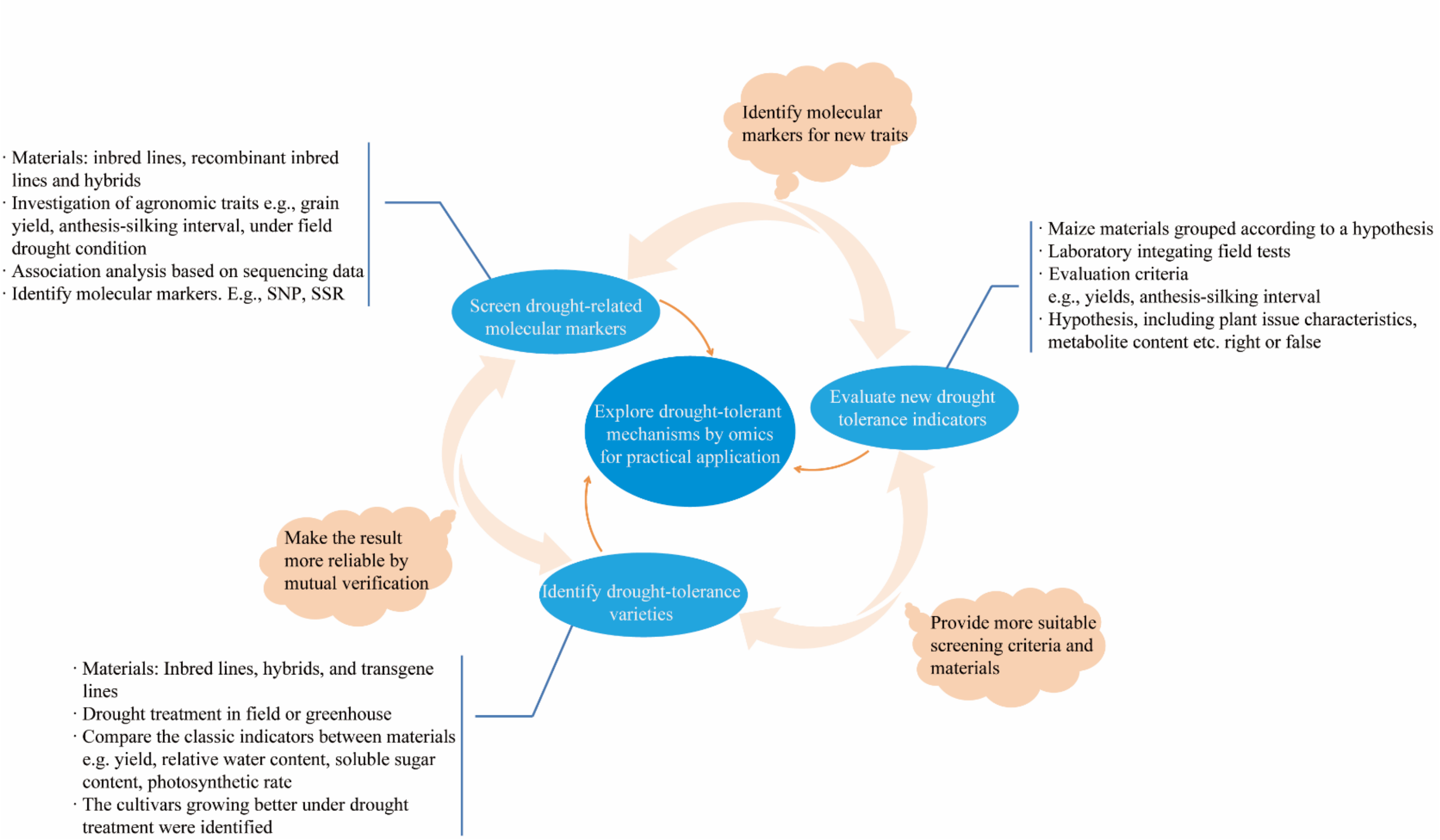

Figure 1 A conceptual flowchart for strengthening the practical application of experiments assessing maize drought tolerance.