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Inter-disciplinary Collaborations Demanded for the Exploration of Applicable Indicators in Abiotic Stress Study-the Case of Tomato

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Abstract

The pace of human population growth will take us beyond 9 billion mark by 2050 while arable land is shrinking, thus food security will be a great challenge in the future. In crop production, one major factor limiting yield is abiotic stress such as drought and salinity, which can reduce the yield by more than 50%. Genetic improvement of crops to various abiotic stresses will be an inevitable road to achieve global food security. Crop tolerances to abiotic stresses are controlled by Quantitative trait loci (QTLs). The various stress indicators like morphological, plant growth, physiological and biological, and molecular play a vital role in assessing the tolerance of genotype to abiotic stresses and has been used in tomato to identify tolerant genotypes. However, intensive collaborations among breeders, botanist, physiologist, molecular biologist, statistician, bioinformatic specialist and other experts are critical for crop abiotic stress research. Breeders are likely to have higher priority in telling which germplasm performs better under stress circumstance and what kind of mechanism might be underlying. In the present scenario, the inter-disciplinary collaborations is demaded for development and execution of fruitful crop improvement programmes to combat the abiotic stresses.

1. Introduction

Global population is expected to exceed 9 billion by 2050 while arable land is shrinking, thus food security will be a great challenge in the future. In crop production, one major factor limiting yield is abiotic stress such as drought and salinity, which can reduce the yield by more than 50%. Drought is one of the most critical challenges worldwide. Disastrous drought frequently happened all over the world. For example, the United States experienced the biggest drought in 2012 since 1956. Salinized land accounts about 6% of the world's total land and 20% of irrigated land, thus adversely affects crop production worldwide (Hasanuzzaman et al., 2013). Secondary salinization is even increasing this number; for instance, millions of horticulture land in China were threatened. Temperature range largely determines the distribution of plants, and cold or chilling stress impairs the growth and survival of every crops. Genetic improvement of crops to various abiotic stresses will be an inevitable road to achieve global food security.

Crop tolerances to abiotic stresses are controlled by Quantitative trait loci (QTLs). Genetically dissection of the complicated mechanisms of crops under abiotic stresses is sure to accelerate the breeding of better varieties. QTL and/ or association mapping are general approaches to develop markers for breeding program or explore genes responsible for stress tolerance. This will unavoidably depends on the precisely genotyping as well as phenotyping of plant materials. Currently, with the booming of technologies of next generation of sequencing, the genomes of a large number of crops have been decoded, and de novo sequencing of large amount of germplasms are accomplished or undergoing. Thus, genotyping will not be a bottle neck for crop abiotic stress study. On contrast, phenotyping would be bothering most researchers in the community of abiotic-stress research. Here, we take tomato as an example to briefly review the indicators used for phenotyping regarding abiotic stresses of drought, salt and low temperature.

Tomato (*Solanum lycopersicum*) is one of the most important crop species grown worldwide and a biological research model. Cultivated tomato are generally sensitive to drought, salinity and low temperature, while tomato has 16 related wild species, which are adapted to different growth conditions such as sea water, extreme dry area or high mountain.

2. Stress Indicators

2.1. Morphological indicators

Morphological indicator under stress condition is economic and easy to track. Tomato might be a promising model in this regard. As compared to cultivated tomato, wild tomato species span a wide range of environmental conditions, from arid deserts to wet humid rainforests (Grandillo et al., 2011).

Stomatal density and stomatal pore size may affect the water loss under drought stress, so they may be used as indicators. For instance, the stomatal density of drought-tolerant tomato *S. pennellii* is only about 70% of that of cultivated tomato *S. lycopersicum*. The wild species *S. pennellii* also exhibits a 54% thicker leaf than cultivated tomato.

2.2. Plant growth indicators

Since every abiotic stress will impair plant growth, indicators directly related to growth are assumed to be able to reflect plant tolerance to stress. Germination rate and germination potential are wildly used indicators for evaluation of stress tolerance, and this might be the least controversial indicator in stress-tolerance assessment. They are successfully utilized in QTLs identification regarding salt, drought and cold tolerance. For example, more than one dozen of QTLs contributing to rapid germination under salt stress, were identified in genetic populations derived from cultivated tomato and its related wild species (*S. pennellii* and *S. pimpinellifolium*), in which salt-stress level of 150-175 mM NaCl were applied in the evaluation. Also, seed germination is used as the indicator in QTLs exploitation of tomato drought tolerance, in which polyethyleneglycol was used to mock drought stress (Foolad et al., 2003), and in cold-stress related QTL mapping (Foolad et al., 1998). However, limitations in this approach include-

a. It may not be applicable for certain populations when it is difficult to get enough seeds.
b. Tolerance at germination stage may be poorly correlated with tolerance at vegetative or reproductive stage.
c. Considerable differences in germination speed are expected for distinct germplasms from the same species. In addition, high quality seeds have to be used in the experiment.

During seeding stage, possible indicators could be used are plant height, root length, relative growth rate, and accordingly the biomass (determined by fresh weigh or dry weight). Survival rate is a good indicator once there is significant difference in growth performance among the materials. Repeated drought and rehydration treatment on five-leaf-stage seedlings was used to identify drought-tolerant tomato introgression lines (Gong et al., 2010). In cold stress, survival rate would be significantly higher for a tolerant germplasm as compared to a sensitive one (Liu et al., 2012). Accordingly, stress-related index (e.g. salt tolerance index, or chilling injury index) based on the visual performance of plants in a line is employed to have a roughly quantitative assessment of germplasms (Li et al., 2011). This type of indicator is useful for primary QTL mapping work but may have difficulty for QTL fine mapping. In accordance with this, biomass is generally considered as good candidates of quantitative indicators in stress-tolerance assessment.

Yield as one of the most important goal of crop breeding, is no reason to be excluded in abiotic stress study. Breto et al. (1994) identified six markers associated with QTLs affecting fruit yield under salt stress.

2.3. Physiological and biochemical indicators

Upon abiotic stresses, series of physiological and biochemical changes in metabolic pathways occur in plants to combat the adverse effects of stress, including osmotic adjustment, protection of photosynthesis, ion homeostasis and redox homeostasis, etc. Thus, physiological and biochemical parameters involved in those process were employed in evaluation of stress damage or stress tolerance. Various physical or biochemical indicators were used to evaluate stress tolerance of tomato landrace and transgenic plants, including photosynthesis, pollen viability, relative water content, ions, ion leakage, chlorophyll content, proline, malondialdehyde (MDA) and H_2O_2 level and detoxifying enzymes (POD, CAT, and SOD etc).

Using shoot wilting as indicator, QTLs associated with shoot turgor maintenance under chilling stress are found in *S. hirsutum* and a major QTL is even fine mapped to a 2.7 cM interval on chromosome 9 (John et al., 2005). Photosynthesis parameters (chlorophyll fluorescence parameters) are also indicators for growth, and thus for stress-tolerance evaluation. Pollen quality or pollen viability in stress condition would also be potential candidate indicators to tell if a germplasm performs better than another.

Since ion homeostasis is critical for plants growing in saline environment, measurement of ions (especially Na^+, and their counterparts, K^+) content and their distribution is generally considered as important indicators for salt stress study. Saranga et al., (1993) analyzed concentrations of Na, K, Cl, Ca, and Mg in leaves and stems of cultivated and wild species of tomato irrigated with saline water, and found that the dry matter of the plant above-ground parts is positively correlated with the $K^+ : Na^+$ ratio in stems and negatively correlated with the Cl^- concentrations in leaves and stems. Thus these indicators have been utilized in QTL analysis. Further, genes encoding high-affinity K^+ transporters (HKT) are found highly likely to be responsible for a major QTL on chromosome 7 of a wild species of tomato (Asins et al., 2013).

Relative electrolyte leakage and lipid peroxidation (estimated by MDA content) were generally used to evaluate the cell membrane damage. In tomato cold stress, parameters including electrolyte leakage, lipid peroxidation, proline, soluble sugars, lipid peroxidation, chlorophyll fluorescence parameters and reactive oxygen species (O_2^-, H_2O_2 etc) are all somewhat related to cold response, and relative electrolyte leakage, MDA and maximum quantum efficiency of Photosystem II (Fv/Fm) might be better indictors in cold tolerance evaluation (Liu et al., 2012).

ABA is considered as a stress hormone and ABA accumulation would improve plant's tolerance, however, this might depends where the ABA accumulates. In wheat, drought-tolerant wheat accumulates much less ABA in reproductive tissues as compared to sensitive genotypes (Ji et al., 2011). So, ABA may act like a double-edged sword and its homeostasis is critical for plant to combat abiotic stresses. Other stress-related hormones include cytokinine, brassinolide and polyamines could be potential stress indicators, but hormones are not easy to measure, especially for large-sale experiment.

2.4. Molecular indicators

Gene expression or the level of specific protein in abiotic stress should be good candidates in exploration of stress-related indicators. Unlike disease resistance study, pathogenesis-related proteins are intensively employed as indicators (Nambeesan et al., 2012), there have been few reports of gene indicators under stress condition in plants. Available evidence suggests that tomato dehydrin can be used as a transcriptional marker of cold stress (Weiss et al., 2009). Noticeably, microarray analysis has been conducted on tomato for exploitation of responsive genes under salt, drought and cold stress (Liu et al., 2012). Proteome analysis was also carried out on tomato under salt stress. With the advancement of "omics" research, it is hopeful to find more molecular indicators under different stresses.

3. Challenges and Prospects

Enhancing the crop productivity from abiotic stress-affected lands will be one of the greatest challenges for researchers in this era of global climate change. Simple screening technologies are highly demanded for crop abiotic-stress research.

Statistical tools like correlation analysis are logically considered as good tools to explore stress-related indicators. Ideally, a collection of tolerant germplasms will be considered in a correlation analysis to find a suitable indicator for the evaluation of the stress tolerance. However, this kind of analysis has prerequisites, e.g. 1) there are at least a few of stress-tolerant germplasms could be included, 2) the mechanisms underlying of the tolerance should be similar (if the indicator is closely associated to the corresponding mechanism). Unfortunately, tolerance often appeared in rare germplasms, and the mechanisms maybe different. So it is not easy to meet these prerequisites. On the other side, false correlation will be easily established upon using indicators under different level of stress treatment (e.g. different concentration of salt stress). Principal component analysis may be workable in rating stress-tolerant related traits (Farshadfar and Elyasi, 2012).

What kind of indicator should be preferentially considered in a study will largely depend on our hypothesis. For example, if the hypothesis is "drought tolerance due to robust root system", then indicators related to root system (e.g. fresh and dry root mass) would be applicable. Under this hypothesis, using small-pot in actual experimental process wouldn't be appropriate as it will restrict the performance of the root system.

4. Conclusion

At times, when it is increasingly becoming difficult to identify simple and apt indicators of abiotic stress, intensive collaborations among breeders, botanist, physiologist, molecular biologist, statistician, bioinformatic specialist and other experts are critical for crop abiotic stress research. Breeders are likely to have higher priority in telling which germplasm performs better under stress circumstance and what kind of mechanism might be underlying. This information would be very important for forming a better hypothesis. Nature has all her secrets, the only thing we need to do is really collaborate nationally and internationally to reveal them, with our grand respect.

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