

Drought resistance – is it really a complex trait?

Abraham Blum

www.plantstress.com, PO Box 16246, Tel Aviv, Israel. Email: ablum@plantstress.com

Abstract. Drought resistance is being increasingly labelled as being a ‘complex trait’, especially with the recent expansion of research into its genomics. There is a danger that this label may turn into an axiom that is liable to damage education on the subject as well as research and the delivery of solutions to the farmer. This opinionated review examines whether there is grounds for such an axiom. Drought resistance is labelled as a ‘complex trait’ mainly when viewed by molecular biologists from the gene discovery platform. This platform is capable of expressing hundreds and thousands of drought-responsive genes, which are up- or down-regulated under dehydration stress according to growth stage, plant organ or even time of day. Sorting out the ‘grain out of the chaff’ in order to identify the function of the candidate genes towards drought resistance is difficult and, thus, the idea that drought resistance is complex is raised. However, when drought resistance is viewed from the physiological and agronomic whole-plant and crop platform, it appears much simpler; its control, whether constitutive or adaptive, is rather obvious with respect to manipulation in breeding and crop management. The most important and common drought resistance traits function to maintain plant hydration under drought stress due to effective use of water (EUW). The state of our knowledge and the achievements in breeding for drought resistance do not support labelling drought resistance as a complex trait. The genomics road towards drought resistance is complex but we already know that the destination is much simpler.

Additional keywords: abiotic stress, drought stress, drought tolerance, gene discovery, gene expression.

Introduction

Interest in drought resistance, in its basic or applied aspects, has been growing recently. Concerns about climate change and water scarcity in agriculture are important reasons for expanding this research area. The increasing global demand for food while water resources for agriculture are diminishing dictates that dryland productivity must rise. Ample funding has been a major impetus for attracting new scientists and laboratories into this domain. Many molecular genetics laboratories were drawn into this research area by both scientific interest and generous research funding.

Subsequently, it has become common to find research proposals, research reports and literature reviews that state ‘drought resistance is a (very) complex trait’. The high exposure of this statement creates the impression that this is an axiom. Labelling drought resistance as a ‘complex trait’ in a research report might be a candid subjective impression of the researcher or a real scientific fact in the specific case. However, accepting this label at face value may turn it into an axiom and, subsequently, cause detrimental implications towards education on the subject, the direction of future research and its cost and the delivery of solutions to the farmer.

The determination or measurement of the level of complexity is complex. Bennet (1990) discussed the possibility of measuring complexity independently of intuition. His discussion implies that it might be possible in computational physics but not in biological processes involving function. Although we have no objective measure of complexity in functional plant biology, it

should not be left to plain intuitive notions, which can vary with personality, education and experience. Therefore, this opinionated review attempts to capture some of the facts that can help resolve, even intuitively, whether drought resistance is a complex trait.

View of drought resistance from the genomics platform

Drought resistance is considered to be complex mainly when it is viewed from the genomics platform. Most recorded conclusions and complaints about drought resistance being complex arise from studies of gene mining and gene expression, as well as from some molecular mapping attempts of drought resistance.

It is not uncommon for hundreds if not thousands of genes found to be up- or down-regulated under dehydration stress in the laboratory. These genes may be associated with a large number of metabolites and proteins or seem to have no identified apparent function – to date. Gene expression may also vary with different plant parts or growth stage, which adds to the impression of complexity. A typical example leading to an impression that drought resistance is complex can be seen in the dissection of gene expression under drought stress in rice (*Oryza sativa* L. ssp. *indica*) as performed by Zhou *et al.* (2007). The number of genes that were up-regulated in the shoot, flag leaf and panicle was 1020, 301 and 448, respectively. Further, 547, 614 and 1145 genes were down-regulated in the shoot, flag leaf and panicle, respectively. However, only a small proportion of these genes were common to all plant parts. In another striking study (Wilkins *et al.* 2010), transcription factor abundance in *Arabidopsis*

thaliana (L.) Heynh. subjected to well-watered and drought-stress conditions was found to vary extensively with the time of day. Even DNA methylation and demethylation was found on certain rice genomic sites in response to drought stress and rehydration and these seemed to differ between a resistant and a susceptible genotype (Wang *et al.* 2011). Furthermore, this genome-wide DNA methylation in response to drought stress was specific with respect to plant growth stage and plant organ. Taking all of the above together – drought resistance appears to be complex.

Passioura (2006) raised the importance of scale when physiology and metabolism are related to whole-plant performance. Gene expression under drought stress can be apparent on a cellular or subcellular scale and on a time scale of minutes or days, but these expressions may not necessarily materialise towards any effect on a whole-plant scale. Molecular geneticists tend to regard it almost as axiomatic that any modification seen at the cellular or subcellular level must impact the whole plant. This axiom has no proof.

It must, therefore, be realised that the view from the genomics platform is not of drought resistance but rather of molecular signalling and networking in responses to dehydration mostly on a subcellular scale and often for a short time period in an organ and a time specific manner. It can be correctly assumed, unless proven otherwise, that among the hundreds and thousands of dehydration-responsive genes, only a very small proportion actually have any real significance towards drought response and drought resistance in terms of whole-plant growth and its productivity. Many genes might be involved in plant modifications that do not concern plant production or even survival under drought stress. What the biologist might consider as certain adaptive responses to drought can be, for example, a process such as programmed cell death and the associated metabolism, which prepare the plant for a system shut-down rather than to sustained growth or even sustained life. If one may assume an anthropomorphic interpretation, the plant is designed by evolution to be pessimistic about drought stress, resolving to system shutdown and reductionist processes in order to prepare for a further increase in water shortage. This is what signalling is about. It is expressed, for example, in root-sourced ABA signals, which cause stomatal closure, inhibit assimilation and reduce shoot growth even before leaf water status is reduced (e.g. Davies *et al.* 2005). Furthermore, ABA also induces abscission of flowers and fruit buds in order to decrease the physiological load of fruit or grain on the stressed plant and the demand for photosynthate (Blum 2011). In contrast, plant production in an agricultural eco-system relies on a more 'optimistic' design as compared with a native plant facing natural selection. The farmer who is supported by the agricultural scientist has effective means for coping by crop management and plant design. Therefore, not all cellular responses or even cellular adaptations to dehydration translate into drought resistance in the agronomic sense.

The gene discovery and gene expression approach to understanding drought resistance is the complex issue in terms of making sense of these huge numbers of responsive genes expressed all over the plant at various growth stages and times. The complexity is brought about the need to sort the 'grain out of the chaff' in order to capture the few candidate genes that might

control the relatively significant, direct and most effective components of whole-plant drought resistance. Bioinformatics helps in sorting the grain and in linking some of the genes to known metabolites. But even if the function of certain genes are linked to certain metabolites this does not substantiate yet a link between the given gene and drought resistance. Thus, the complexity is not in drought resistance *per se* but in the process of gene discovery. Whereas genomics relies on discovering genes after which they must be assessed for functionality in the whole plant, it must also be realised that this road is complex. This does not necessarily imply that the destination is complex.

In certain cases where the gene discovery process has identified a potential candidate and proceeded to test it rigorously, the results led to a simple drought resistance mechanism, which is reconciled with established facts acquired by years of plant physiology and breeding research. An example is seen in the review by Peleg and Blumwald (2011). Isopentenyltransferase (IPT) catalyses the first reaction in the biosynthesis cytokinins. Cytokinins delay drought stress-induced senescence (Vaadia 1976). Transgenic tobacco (*Nicotiana tabacum* L.) plants expressing isopentenyltransferase gene driven by a stress- and maturation-induced promoter were created. This gene suppresses drought-induced leaf senescence resulting in what is recognised as the non-senescence or stay-green trait in plant breeding (Rosenow *et al.* 1983; Thomas and Smart 1993). This transformation resulted in dehydration avoidance as shown by high leaf water status and retained photosynthetic activity and growth under prolonged drought stress after flowering. The road to proving the function of this gene in tobacco plants under drought stress was long. Non-senescence has been known for many years as an effective drought resistance mechanism in plant breeding. Years of continuous sorghum breeding for this trait produced many sorghum hybrids adapted to dryland conditions, which carry the trait (Rosenow and Dahlberg 2000). Therefore, drought resistance in the specific example of non-senescence is not a complex destination when approached by both gene discovery work and plant breeding experience.

It is regretful that molecular gene discovery work is still plagued by occasional inadequate experimental procedures, which can produce confusing results. These problems are mostly derived from a lack in understanding of basic plant-water relations and their interaction with the environment. Flawed experimental practice and poor drought resistance phenotyping can result in incomprehensible results, which can lead to the conclusion that the subject matter is complex. Following are a few typical examples most of which are discussed elsewhere (Blum 2011; www.plantstress.com).

Gene expression studies are performed by comparing different varieties or mutants under drought stress. Stress is often applied for a given number of days rather than to the same level of plant water status in all genotypes. Thus, a dehydration-avoidant genotype will most likely express less (or different) stress-responsive genes than a non-avoidant genotype on the given date. The requirement for the same level of plant water status when all genotypes are sampled for DNA is rarely observed. To the same extent, different plant parts might differ in their water status on the given day. Thus, the difference in gene expression

observed among different plant parts is not necessarily a function of the specific organ, but can be a function of a difference in organ water status. Pinheiro and Chaves (2011) performed a meta-analysis of some 450 reports dealing with photosynthesis under drought stress. They found that experimental results were mostly non-comparable and that it was difficult to relate molecular events to photosynthesis across all reported data. It is suggested here that part of the observed complexity in that analysis could be simply explained by the most likely fact that different experiments were performed under different levels and rates of leaf water deficit. Standardising experiments for evaporative conditions and soil moisture as suggested by Collins *et al.* (2008) is not enough. Gene expression and metabolic function experiments should be standardised for plant and tissue water status in order to be compared or repeated.

Slow normal progress of dehydration as it happens in the field induces minimal expression of stress-responsive genes while more rapid stress in pots with restricted roots will result in greater gene expression (Barker *et al.* 2005). The difference is in the effect of the rate of stress *per se* on gene expression. Thus, the phenomenon of hundreds and sometimes thousands of expressed genes can be partly accounted for by the abnormal rapid stress imposition typical of laboratory studies (see <http://www.plantstress.com/Devil/devils.htm>).

Although pots are essential and often legitimate vessels for plant research, they can be fallible in studies of drought response. The most common fault is the effect of plant size on water use in a pot. Large plants use water faster than smaller plants. Thus, when irrigation is stopped to induce drought stress a large plant will wilt before a small one. This can override the effect of a studied gene and even bias the main results. It certainly may cause greater gene expression in the larger than in the smaller plant in line with their different water status.

When stomatal closure is affected by ABA in pot experiments, plants closing stomata will conserve moisture and delay wilting. A plant that is ABA insensitive or lacking in ABA production will continue transpiration and quickly use up all available water in the pot and wilt. Thus, on a given date it is possible to show that an ABA-accumulating transgenic plant is turgid while the wild type is wilted. In the field the results may be totally different, if not the inverse. Sustained transpiration is in most cases the desirable phenotype towards plant production under drought stress (Blum 2009). Depending on the drought scenario and traits other than ABA such as deep roots or osmotic adjustment, the drought-resistant phenotype will sustain transpiration and produce greater yield. This is in contradiction with several pot experiments where ABA was considered to improve drought resistance because it reduced transpiration (e.g. Iuchi *et al.* 2001). Furthermore, ABA is widely recognised (as reviewed by Blum 2011) to restrict growth and cause severe reproductive failure.

Molecular mapping of drought resistance in order to enable marker assisted selection for the trait has been expanding explosively in recent years, for a good reason. However, as indicated by experts in this discipline (Collins *et al.* 2008; Varshney *et al.* 2011) the molecular approach has a great potential but actual results and delivery towards water limited environments are meager. Reasons are being discussed in these

and other reviews, where the ‘complexity’ of drought resistance is given as one.

When drought resistance is being phenotyped in terms of yield under stress it should not come as a surprise that it is almost always found to be associated with too many small effect QTLs and epistatic inheritance, leading to the conclusion that drought resistance is complex. This is also well expressed in the high genotype \times environment interaction for yield, which seems to be the rule. Although yield under stress is important, the fact that it is biologically not a true heritable trait *per se* should lead one to address those traits that underlie yield under stress.

Even simple traits such as plant height can be viewed as a complex genetic entity when processed by molecular mapping. Cereal breeders have long been highly successful in manipulating the well known few major height (e.g. sorghum (*Sorghum bicolor* (L.) Moench)) or dwarfing (e.g. wheat (*Triticum aestivum* L.)) genes in cereal breeding. Molecular mapping of cereal plant height (Yu *et al.* 2002; Zhang *et al.* 2008) describe a much more complex inheritance of this trait as we knew it, including epistasis. This cannot be not reconciled with the *de facto* ease of plant height manipulation in breeding programs. Therefore, in the eye of the breeder a simple trait such as plant height can become a complex issue when marker-assisted selection (MAS) is considered.

When drought resistance traits other than yield are considered for mapping there is often a considerable lack of understanding about the crucial traits for the specific crop in the specific drought profile. When finally the appropriate traits are being mapped, poor phenotyping can very often cause failure (Xu and Crouch 2008; Varshney *et al.* 2011). Poor phenotyping of drought resistance traits can lead to confusing and disappointing results with the inevitable conclusion (if not the excuse) that drought resistance is complex.

With the recent advent of sensor technologies and cheap robotics we see the emerging new facilities and services for ‘high throughput plant phenotyping’ as outlined by Furbank (2009). Fast phenotyping is indeed the ‘Holy grail’ for plant breeders, but when drought resistance is considered the quality of phenotyping is still an overriding consideration with respect to recognising and targeting the correct trait and phenotype under the exact relevant stress conditions. This is where we encounter most failures.

View of drought resistance from the whole-plant platform

Experienced agronomists, plant breeders and crop physiologists who have been gaining long and extensive experience with crop plants under drought stress do not unanimously agree that drought resistance should be categorically labelled as a complex trait. This is partly due to our success in field research towards coping with drought problems by genetic and crop management solutions. When the problem of coping with drought stress is approached in the field there are more than a few cases showing that simple solutions and simple plant traits can support a crop under drought stress. For example, numerous reports give evidence to the crucial role of deep roots in ascribing drought resistance to upland rice. The impact of roots where deep soil moisture is available is as simple as a long rope in a deep well. So in this case, as an example, drought resistance in upland rice depends on a simple trait and it is

not complex. What can be complex sometimes is the inheritance of deep roots or the developmental interaction of the root with the drying soil and its physical strength. But for the upland rice breeder the solution for drought resistance in most cases is not complex.

Another example is the anthesis to silking interval (ASI) in maize (*Zea mays* L.), which increases under drought stress due to the delay in silking. Drought resistance at flowering is often linked to a sustained short interval under drought stress. This is due to the avoidance of delayed and/or slow silk growth under drought, which might be linked to water status, hormonal status or carbon status under stress. The allele for short ASI was found to be that as for leaf growth (Welcker *et al.* 2006), indicating simpler rather than a complex model for ASI regulation. ASI is being used very effectively in field selection for drought resistance in maize.

Yet another example towards the relative simplicity of drought resistance is osmotic adjustment. This stress-responsive and adaptive dehydration avoidance trait is important in many crop plants because it has been repeatedly linked to yield under drought stress (Blum 2011). It is also an important component of drought resistance of a released Australian wheat cultivar (Munns and Richards 2007). Osmotic adjustment in wheat is controlled by one major and a few minor genes (Morgan 1991) with high heritability (Moinuddin *et al.* 2005). The successful use of breeding for drought resistance based on osmotic adjustment cannot be reconciled with statements that drought resistance is universally complex.

Effective drought resistance in crop plants is largely achieved by dehydration avoidance. (Blum 2011). Dehydration tolerance that involves sustained function or the survival of plants under extreme dehydration is less important in crop production under limited water supply. Dehydration tolerance is important in seed embryos, resurrection plants and certain desert vegetation. It is also important for freezing tolerance and winter survival.

Dehydration avoidance traits conserve plant water status and turgor through the effective use of water (EUW) (Blum 2009). Turgor maintenance is not driven by hundreds and thousands of genes. Turgor maintenance is controlled by accumulated cell-compatible solutes leading to osmotic adjustment or by cell wall bio-physical traits, which can be regulated under stress. Osmotic adjustment can be driven by the uptake of ions from soil such as potassium or by metabolically derived cell compatible solutes. These are not very complex functions.

Discovered genes are tested for function using transgenic technology. Most reports of such studies display photographs of the transgenic plant versus the wild type under drought stress. Careful inspection of dozens of such published photographs and/or the verbatim description of the modified phenotype indicates that these transgenic plants maintained turgor while the wild type was wilted under drought stress. Thus, many if not most discovered genes reported to enhance drought resistance in transgenic plants target one main and primary trait, namely dehydration avoidance via turgor maintenance. This cannot be reconciled with the claim that drought resistance is universally complex.

The gene discovery platform understandably ignores constitutive plant traits as determinants of whole-plant drought resistance. Constitutive traits can be affected by drought stress but they do not require dehydration-responsive genes for their

expression. Relatively simple heritable constitutive plant morphological and developmental traits can have a decisive effect on crop performance and productivity under drought stress. Most constitutive traits that impact drought resistance, again, operate mainly through dehydration avoidance and EUW. Examples are root depth, plant leaf area as determined by leaf size or tillering, early flowering, leaf surface properties and even certain morphological features of the reproductive system, which influence fertility under stress (Blum 2011). When drought resistance is probed by functional genomics through the study of stress-responsive genes, such simple albeit effective constitutive traits are ignored.

When viewed from the whole-plant and crop platform, certain components of drought resistance are considered as very effective while they may not be fully understood. Such traits can still be used in breeding while awaiting the full resolution of their *modus operandi*. For example, stem reserve utilisation for grain filling under drought stress is a unique and effective component of dehydration tolerance in most grain crops. However, we do not fully understand reserve mobilization signalling under stress and non-stress conditions. We do not understand how the interaction between source and sink affect mobilisation and what metabolic or hormonal signals are involved. We know that stem reserve mobilisation to the grain should be maximised in a grain producing plant in order to enhance its post-flowering drought resistance (Yang and Zhang 2006). Better understanding in this respect should help breeding for the trait. Gene expression narrowly targeting reserve mobilization signalling is an example of a potentially important genomic research in support of breeding for the trait.

In conclusion, the genomics road towards drought resistance is complex but we already know that the destination is much simpler.

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