# Bio Technology

# Signal Transduction One of the Current Molecular Approaches in the Management of Drought Stress in the Sub-Saharan Region

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**Abstract:** Drought affects maize production in Africa leading to up to 70% crop loss and in certain cases total crop loss. Several breeding interventions have been attempted with little success. Thus alternative complementation approaches have been necessitated. The current review looks at genetic engineering as a suitable complement to molecular breeding with emphasis on signal transduction. Complex signaling processes have been exploited and several genes encoding signaling factors that function in the drought response have been identified. The successfully understanding and utility of these upstream transcription factors which demonstrate various signal in adverse condition will greatly enable us effectively exploit these genes. The aim of this review is to understand the molecular mechanism and the function of these genes and to see on how best we can exploit them in current and future studies on development of drought tolerant maize crop.

**Key words:** Maize, drought, signal transduction, genetic engineering

## INTRODUCTION

In Sub-Saharan region maize yield and production is often inadequate due to abiotic stress such as drought, aluminum toxicity, or scarcity of nutrients; biotic stress being mainly pests and diseases (Ajanga and Hillocks, 2000; Kanampiu et al., 2002; Schechert et al., 1999). Of the a biotic stresses, drought is a major stress affecting productivity of maize in Africa leading to up to 70% crop loss and in certain cases total crop loss (FAOSTAT, 2002). Erratic rainfall pattern and drying up of rivers due to extensive deforestation of water catchments areas have led to long dry spells making irrigation an impractical alternative. Depletion of the ozone layer leading to global warming, the climate change has further compounded maize problems greatly affecting the rainfall pattern and the seasonal temperatures in the process aggravating maize production. On an economical perspective maize production has declined as a result of land sub-division due to population pressure and high farm input costs, making maize production expensive and unprofitable especially to smallholder farmers who use it both as a food and a cash crop (World Bank, 1995). In Kenya for example, Maize yield and production is 1.7 t ha$^{-1}$ and 2.7 million tons annually which is not adequate to meet its demand of the 34 million growing population, making it a net importer of 427,000 tons of maize per annum (CIMMYT, 1999; Kanampiu et al., 2002).

Maize is grown in a wide range of agroecological zone Table 1 (Corbett 1998), but due to the adverse effects of temperature change/rainfall pattern; tropics farming pattern in Africa which wholly relies on the climate has led to recurrent drought leading to low production of maize. Therefore, to increase production of maize, there is an urgent need to conduct research into methodologies that can cub drought stress. The methodologies currently in use include marker assisted selection, conventional breeding and genetic engineering. Conventional breeding has had limited success in overcoming these constraint hence the need to use molecular breeding and genetic transformation as an alternative approach to overcome the constraint.

## PHYSIOLOGICAL/MOLECULAR CHANGES DURING DROUGHT STRESS IN PLANT

Water is a fundamentally important component of the metabolism of all living organisms, facilitating many vital biological reactions by being a solvent, a transport medium and evaporative coolant (Bohnert and Jenson, 1996). In plants and other photo-autotrophs, water plays the additional role of providing the energy necessary to drive photosynthesis. Water molecules are split, in a process termed autolysis, to yield the electrons that are used to drive the energy yielding photosystem II reaction centre (Salisbury and Ross, 1992).

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Table 1: Maize agro-ecological production zones and associated climatic conditions in the Tropics (Corbett, 1998)

| Agro-ecological zone | Elevation (m) | Rainfall (mm) Mar.-Aug. | Temperatures (DC) Mar.-Aug. | | |
| --- | --- | --- | --- | --- | --- |
| | | | Mean (°C) | Average minimum (°C) | Average maximum (°C) |
| Lowland tropics dry | >700 | 300-550 | 25.4 | 20 | 30 |
| Moist | <400 | >550 | 25.8 | 20 | 31 |
| Dry mid-altitude | 700-1400 | 300-550 | 22.0 | 14 | 33 |
| Dry transitional | 1100-1700 | <550 | 19.7 | 11 | 27 |
| Moist transitional | 1200-2000 | >500 | 19.7 | 11 | 29 |
| Highland tropics dry | 1600-2300 | <550 | 16.6 | 8 | 26 |
| Moist | 1600-2700 | >550 | 16.7 | 7 | 27 |
| Cool | 2000-2900 | <1000 | 13.8 | 5 | 22 |
| Moist mid-altitude | 1200-2000 | >500 | 22.1 | 13 | 30 |
| Extreme water stress | 400-1100 | <400 | 23.8 | 16 | 32 |

One of the major consequences of drought stress is the loss of protoplasmic water leading to the concentration of ions such as $Cl^-$ and $NO_3^-$. At high concentrations these ions effectively inhibit metabolic functions (Hartung *et al.*, 1998). Additionally, the concentration of protoplasmic constituents and the loss of water from the cell lead to the formation of what is termed a glassy state. In this state whatever liquid is left in the cell has a very high viscosity, increasing the chances of molecular interactions that can cause protein denaturation and membrane fusion (Hartung *et al.*, 1998; Hoekstra *et al.*, 2001). To counter these effects so as to maintain cell turgor and metabolic functions, plants generate reactive oxygen species, including hydrogen peroxide ($H_2O_2$), the superoxide anion and hydroxyl radicals (Inze and Montagu, 1995). Accumulation of $H_2O_2$ can, in turn, induce the expression of detoxification and stress protection genes such as Heat Shock Proteins (HSPs), Glutathione-S-Transferases (GSTs), peroxidases, superoxide and Pathogenesis-related proteins, thus protecting plants from stress damages (Kovtun *et al.*, 2000). HSPs have been reported to serve as molecular chaperones that participate in ATP-dependent protein unfolding or assembly/disassembly reactions and prevent protein denaturation during stress (Pelham, 1986). Correlations between expression of HSPs and thermo-tolerance have been found in maize, tomato and creeping bentgrass (Park *et al.*, 1996; Preczewski *et al.*, 2000; Ristic *et al.*, 1998). GSTs are enzymes that detoxify endobiotic and xenobiotic compounds by covalent linkage of glutathione to hydrophobic substrates. In general drought stress which leads to a signal transduction resulting in accumulation of proteins not only protect the plant from drought stress but also other environmental stresses like chilling and heat (Li *et al.*, 2003; Ristic *et al.*, 1998; Roxas *et al.*, 1997; Sabehat *et al.*, 1998) as shown in the general example on Fig. 1. To achieve this series of coordinated molecular reactions are involved and studies in genomic, proteomics and trascriptomics all in a concerted effort can provide a solution to drought stress.
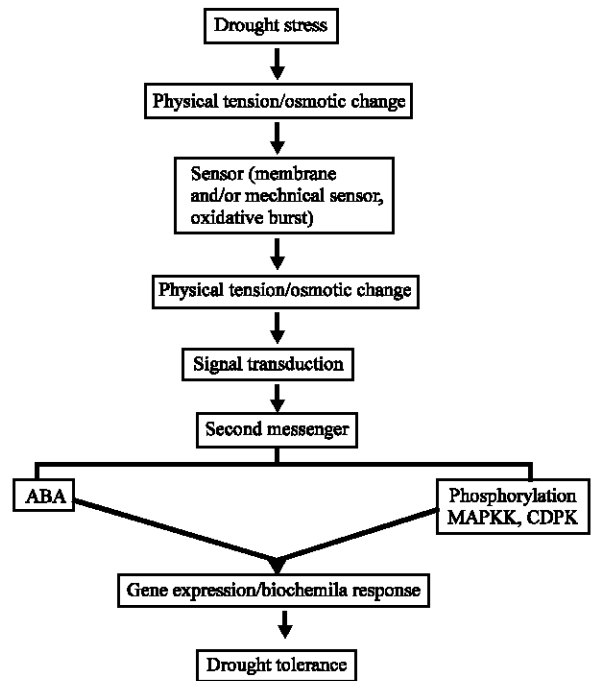


Fig. 1: Drought stress and signal transduction leading to drought stress tolerance in plant

## MOLECULAR APPROACH TO DROUGHT STRESS TOLERANCE

Identification of key gene(s), whose manipulation will ultimately affect crop performance in response to drought stress, is highly complex and difficult to decipher because of the polygenic nature of drought response. In addition, the plant's response to each stress is unique and thus the response to multiple stresses will also be different. Indeed, global expression profiling of a plant's response to stress conditions has shown that, although overlap may occur for different abiotic stresses, such as cold, salt, dehydration, heat, high light and mechanical stress, a set of genes unique to each stress response is also seen (Ingram and Bartels, 1996; Shinozaki *et al.*, 2003;

Thomashow, 1999). It is apparent that in understanding drought responsive network will require a considerable amount of time and resources, but a systematic and concerted effort will ensure that only the most suitable genes are identified for crop improvement. The task can be shortened by integrating the information already available and by avoiding the repetition of effort or branching away from the main focus.

The basic strategy of genetic engineering for drought tolerance is to introduce functional genes that are directly involved in these events. With the advancement of DNA microarray technology, which allows high-throughput analysis of differential messenger RNA expression, several hundred stress-induced genes have been identified as candidate genes for genetic engineering. Among the genes identified, several were classified as regulatory genes, such as protein kinases and transcription factors, in addition to functional genes.

Although, the conventional approaches in both plant breeding and physiology are of great importance (Flowers, 2004; Vinocur and Altman, 2005). The genetic engineering of key regulatory genes that govern a subset of stress-related genes appears to be one of the most promising strategies for enabling scientists to minimize the deleterious effects associated with drought in a more certain approach.

## IMPORTANT PROTEINS AND METABOLITES DURING DROUGHT STRESS

Several successful attempts to engineer drought tolerance have transferred functional genes that encode enzymes associated with the synthesis of osmotically active compounds, transporters, chaperones and Reactive Oxygen Species (ROS) scavengers, as shown in (Chaves and Oliveira, 2004). In drought-tolerant transgenic plants, many genes involved in the synthesis of compatible solutes-organic compounds such as amino acids (e.g., proline), quaternary and other amines (e.g., glycinebetaine and polyamines) and a variety of sugars and sugar alcohols (e.g., mannitol, trehalose and galactinol) that accumulate during osmotic adjustment-have been used to date (Chen and Murata, 2002).

Stress-responsive proteins such as the Late-Embryogenesis-Abundant (LEA) class of proteins, have also been thought to function in the detoxification and alleviation of cell damage during dehydration (Bartels and Sunkar, 2005). Over expression of some LEA genes has been reported to result in enhanced tolerance to dehydration, although the precise mechanism is still unknown (Bartels and Sunkar, 2005; Vinocur and Altman, 2005). Recent biochemical analysis demonstrated that

LEA proteins can prevent protein aggregation induced by desiccation as well as freezing (Goyal *et al.*, 2005). Taken together with recent computational studies (Wise and Tunnacliffe, 2004), LEA proteins are proposed to function as chaperone-like protective molecules and act against cellular damage (Vinocur and Altman, 2005).

Apart from proteins other important metabolites produced in response to drought stress is the plant hormone ABA (Bartels and Sunkar, 2005; Machuka *et al.*, 1999). Recently, in addition to a key ABA biosynthesis gene (NCED3), a cytochrome P450 CYP707A family has been identified as ABA 80-hydroxylases, which play a central role in regulating ABA levels during seed imbibitions and dehydration stress conditions (Kushiro *et al.*, 2004; Luchi *et al.*, 2001; Saito *et al.*, 2004). The result may enable us to control ABA levels under conditions of drought stress and ultimately contribute to advancement in the engineering of drought tolerance. Indeed, an insertional mutant of CYP707A3, which was expressed most abundantly among four CYP707A members under stress conditions, exhibited elevated drought tolerance with a related reduction of transpiration rate (Umezawa *et al.*, 2006).

Thus, unraveling the mechanisms underlying the action of metabolites and proteins involved in drought tolerance could facilitate the creation of stress-tolerant plants. In addition, the recent progress of metabolome analysis should also contribute to the discovery of novel stress resistance systems that can be utilized for stress engineering (Oksman-Caldentey and Saito, 2005).

## TRANSCRIPTION FACTORS FOR REGULON BIOTECHNOLOGY

Transcriptome analyses using microarray technology, together with conventional approaches, have revealed that dozens of Transcription Factors (TFs) are involved in the plant response to drought stress (Bartels and Sunkar, 2005; Vinocur and Altman, 2005). Most of these TFs fall into several large TF families, such as AP2/Ethylene response factors, dehydration-response element binding protein, basic leucine-zipper protein, zinc-finger protein and waxy production. The expression of TFs regulates the expression of downstream target genes that are involved in the drought stress response and tolerance. Recent progress in TF study has led to the engineering of drought tolerance using both transcriptional activators and repressors.

Transcriptional activators upregulate stress-responsive genes have been utilized to produce drought-tolerant transgenic plants (Fig. 2). In addition to the enhancement of drought tolerance, overexpression of
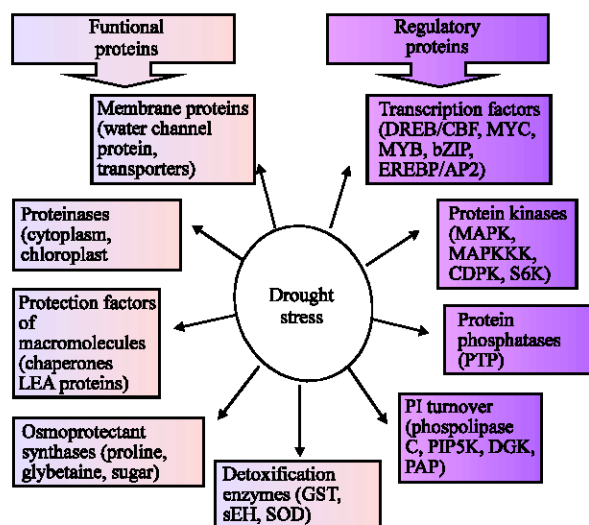
Fig. 2: Drought stress-inducible genes and their possible functions in stress tolerance and response

the DREB1/CBF3 (dehydration-responsive element binding protein/CRTbinding factor) TF in Arabidopsis controlled many stress inducible target genes (Maruyama *et al.*, 2004; Seki *et al.*, 2001) and increased tolerance to freezing and high salt exposure (Kasuga *et al.*, 1999). In a more recent example, transgenic plants expressing a drought-responsive AP2-type TF, SHN1-3 or WXP1, induced several wax-related genes and resulted in enhanced cuticular wax accumulation and increased drought tolerance (Aharoni *et al.*, 2004; Zhang *et al.*, 2005). Thus, it has been shown that the overexpression of some drought-responsive transcription factors leads to the expression of downstream genes and the enhancement of drought tolerance (Zhang *et al.*, 2005).

Over expression of DREB2A carrying a small internal deletion of 30 amino acids induces the expression of downstream genes under untreated conditions and enhances drought tolerance (Sakuma *et al.* personal communication). Interestingly, the deleted region contains a transcriptional inhibitory region with a PEST sequence, which is generally known to play a role in the degradation of the protein, although the actual inhibitory mechanism of DREB2A is still unknown (Sakuma *et al.* personal communication).

In addition to the deletion of inhibitory regions, the use of point mutations is an important strategy to produce active forms of transcriptional activators for engineering drought tolerance. A point mutation mimicking the phosphorylation of a rice bZIP transcription factor, TRAB1, from serine to aspartic acid at a phosphorylation site, significantly increased the level of transcriptional activation in the absence of the inducer ABA in a rice protoplast transient assay (Kagaya *et al.*, 2002). Recently, transgenic plants expressing a phosphorylated active form of AREB1 with multisite mutations also resulted in the induction of many ABA-responsive genes without exogenous ABA application.

Constitutive expression of a Cys2/His2-type zinc-finger transcriptional repressor, STZ, which was upregulated by dehydration, high salt, cold stress and ABA treatment, increased tolerance to drought stress (Sakamoto *et al.*, 2004). Transgenic Arabidopsis plants expressing an STZ ortholog (CAZFP1), which normally functions as a transcriptional repressor in yeast, also showed tolerance to drought stress and exhibited resistance against bacterial infection (Kim *et al.*, 2004). Overall, in addition to the conventional overexpression of TFs in regulon biotechnology, the modification of TFs might one day allow us to create TF-modified plants that confer novel traits.

These facts support the supposition that stress-dependent activation or deactivation of signal components might function as a molecular switch for the biotechnological manipulation of the stress response in plants.

## MOLECULAR APPROACHES FOR DROUGHT MANAGEMENT USING SIGNAL TRANSDUCTION IN PLANT TRANSFORMATION LABORATORY, KENYATTA UNIVERSITY, KENYA

Upstream TFs of various signal transduction systems function in abiotic stress responses, involving protein phosphorylation and/or dephosphorylation, phospholipids metabolism, calcium sensing, protein

Table 2: Engineering drought tolerance using signaling factors

| Classification | Gene name | Transgenic | Origin | Expression | Experiments | Parameters | Years |
|---|---|---|---|---|---|---|---|
| Protein kinases | | | | | | | |
| CDPK | OsCDPK7 | Rice | Rice | CaMV35SP | water withholding | Shoot growth, Fv/Fm, wilting, gene expression | 2000 |
| GSK3/Shaggy | AtGSK1 | Arabidopsis | Arabidopsis | CaMV35SP | Water withholding | Survivability | 2001 |
| MAPKKK | NPK1 | Maize | Tobacco | CaMV35SP | Limiting water supply | Leaf number, kernel yield | 2004 |
| SnRK2 | SRK2C | Arabidopsis | Arabidopsis | CaMV35SP | Water withholding | Survivability, gene expression | 2004 |
| Others | | | | | | | |
| Calcium sensor | CBL1 | Arabidopsis | Arabidopsis | Agrobacterium MAS | Water withholding | Survivability, gene expression | 2003 |
| 14-3-3 Protein | GF14l | Cotton | Cotton | CaMV35SP | Limiting water supply | Senescence, Chl content, photosynthesis | 2004 |
| CC-NBS-LRR | ADR1 | Arabidopsis | Arabidopsis | CaMV35SP | Water withholding | Survivability, gene expression | 2004 |
| Farnesyl-transferase | ERA1 | Arabidopsis, canola | Arabidopsis | CaMV35SP/ RD29AP (antisense) | Water withholding, field test | Survivability, water loss, seed yield, oil content | 2005 |

degradation and are being studied (Bartels and Sunkar, 2005; Boudsocq and Lauriere, 2005; Muoma, 2009; Vinocur and Altman, 2005). Although, these complex signaling processes are not yet fully understood, several genes encoding signaling factors that function in the drought response have been identified (Shinozaki *et al.*, 2003; Zhang *et al.*, 2005). Several drought inducible genes have been identified and some of these factors are currently available for engineering for drought tolerance (Fig. 2).

Of these factors, the manipulation of signaling factors is of merit in that they can control a broad range of downstream events, which results in superior tolerance for multiple aspects. For example, in PTL, Kenyatta University Kenya, a tobacco mitogen-activated protein kinase kinase kinase (MAPKKK), NPK1, which was truncated for constitutive activation, has been found to activate oxidative signal cascade and led to cold, heat, salinity and drought tolerance in transgenic plants (Kovtun *et al.*, 2000; Muoma, 2009; Shou *et al.*, 2004).

Although, recent progress in this field has identified several signaling factors related to the drought stress response in plants, these findings also highlight the complexity of the signal transduction network with cross-talk, feedback and physical interactions delivering appropriate signals to suitable targets at the correct time. As with the yeast 'scaffold' hypothesis, signaling proteins often have multiple signal outputs, an occurrence that explains the different functions of the MAPKKK, Ste11p (Park *et al.*, 1996; Posas and Saito, 1997). For example, AtMPK6 functions in at least two different MAPK cascades, MKK2-MPK6 and MKK4/MKK5-MPK6 and each combination can transmit or transduce different signals from cold or salt stress and bacterial pathogens, respectively (Nakagami *et al.*, 2005). Further functional or biochemical analyses will be required to gain a precise understanding of the functions of various signaling factors in plants which can then be used to promote the engineering of drought tolerance using signaling factors. Currently a range of signal factors have been identified with positive results in model plants and a number of important food crops Table 2.

## STATUS OF MOLECULAR APPROACHES AT PTL, KU, KENYA

Molecular biologists have identified several factors related to the plant response to drought stress and many of these factors have already been shown to be effective for engineering drought tolerance in model plants. Such demonstrated success under experimental conditions has encouraged the use of this strategy to engineer drought tolerance capability in crop species.

With increase of in-depth understanding of functional genomics approaches, the identification of candidate genes related to drought response has increased, with very explicit strategies availed Fig. 3. for genetic engineering. Plant Transformation Laboratory (PTL) Kenyatta University is exploiting the availability of these genes and techniques to improve the drought tolerance, nutritional value and adaptability of major food crops in Africa. This is being done by optimizing and improving the available plant transformation methodologies so that they can be adapted and applied to plant species used as food crops in the tropical environs (Ombori *et al.*, 2008). Currently, we are only able to produce transgenic plants with a limited selection of maize inbred lines and just started working on selected tubers. Emphasis on use of inbred lines for studies in PTL has been encouraged as they tend to provide consisted results unlike the segregated hybrids and cultivars (Muoma *et al.*, 2008; Ombori *et al.*, 2008). The second advantage of using inbred is that they are the source of the hybrid seeds availed to farmers hence improving their quality will have a direct impact on the end product sent to farmers.
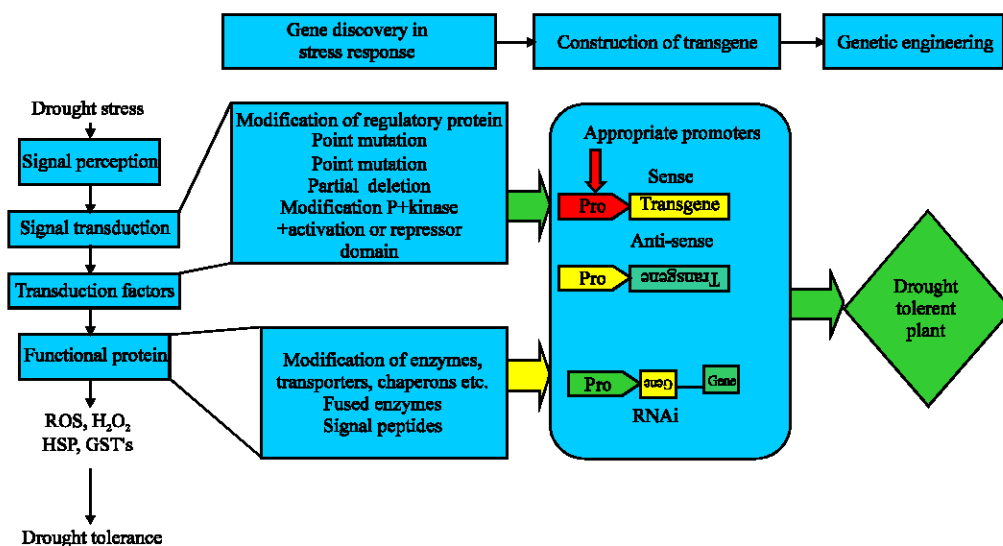
Fig. 3: Strategies for drought tolerance gene identification, isolation and cloning

Constitutive promoters such as the CaMV35S promoter have tended to be unreliable as they are not always functional or can have negative effects on transgenic plant growth or development (Kasuga *et al.*, 1999; Yamaguchi-Shinozaki and Shinozaki, 2005). The lab is thus working with collaborators on availing and using ubiquitous promoters in plants so as to check this effects which can be detrimental to the study of transgenic events and more so transgenic food crops.

A major factor which comes into play limiting the utility of transformed products as food is the selection system. An effective selection system, including suitable promoters, is required for the transformed food crops to be utilized as food. The use of non-antibiotic marker selection system has been emphasized in the development of tropical maize transformation studies as the end products are mainly used as food. The use of phosho-mannose selection system has been successfully achieved with the PTL lab in conjunction with collaborators in Syngenta U.S and Cape town University South Africa having optimized the protocol for transformation. Other selection systems like D-amino acid oxidase (DAAO) which depends on the D-amino acid used, -or+selection has been successfully used in Arabidopsis is being exploited in tropical maize. In all, the discoveries in Arabidopsis and tobacco have provided a stepping stone and availed gene for use in introgression of drought tolerance gene and the -omics of the products. To broaden our working horizon, we are studying the post-transcriptional regulation involving protein modification, protein degradation and RNA metabolism, which enable us understand the gene activation/silencing in tropical plants. Such studies will allow us to create novel approaches which will enable us fine-tune drought tolerance according to the time and circumstances found in the tropical region.

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