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Review Article
Comparative Analysis of Gene Expression in Selected Drought Resistant Vegetables

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Abstract

Drought is an environmental stress that is known worldwide to be a major concern in the production of quality food crops. Several characteristics of drought tolerance have been confirmed in *Amaranthus hypochondriacus*, *Amaranthus tricolor*, *Amaranthus hybridus*, *Vigna unguiculata* and have been utilized in the breeding of drought-tolerant genotypes of several vegetables. Four techniques were described in this study for gene expression in *Amaranthus*. In sunflower, RNA sequencing by rtPCR is the major method used in gene expression. However, in *Arabidopsis*, microarray technology which includes cDNA or oligonucleotide microarrays is the technique analysed in the study. Several other vegetables have shown different ways of adapting to drought and the biochemical and physiological effects have been observed in some other neglected vegetables. Not much is available in the literature on a comparative review of expressed genes for drought tolerance in literature. This review aims to bridge such a gap by comparing gene expression analyses and providing a single front of information for a user.

Key words: Vegetables, *Amaranthus*, *Arabidopsis*, sunflower, gene expression, primers, oligonucleotide microarrays, hydrology

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INTRODUCTION

Several definitions according to different fields have been adopted when defining drought. These are meteorology, the economy of water resources and hydrology. Meteorologically, drought can be defined based on duration and dryness period, it is a period that is averagely more than a particular number of days with rainfall or water less than a specified amount[1]. Hydrologic drought is majorly concerned with the effects of dry spells on subsurface or surface hydrology. The severity and frequency of hydrologic drought are completely based on its influence on river basin[1,2] while agricultural drought is experienced when moisture in the soil is lost to the level that yields from crops and pasture are significantly affected. Agricultural drought links various features of meteorological drought to effects on agriculture and it focuses on rainfall shortages, evapotranspiration, departures from normal etc[1].

Drought is one of the most important environmental stress in crop production. Pressure on the demand for food crops and agricultural products continues to outweigh production from year to year, leading to food insecurity in many parts of the world[3]. To improve yield and resilience in crops and agricultural plants, there is a relentless effort the study the effect of drought and stress on crops[4]. Physiological response and molecular adaptability of plants to water stress have been exposed via fundamental research[5], but there is a gap between maximum production and stress. The ability to close this gap is of utmost importance to guarantee food in the nearest future[5].

Drought tolerance: Is plants' ability to continue biomass output under arid or drought circumstances[6,7]. Several plants possess the natural ability to adapt to dry environments, through mechanisms such as desiccation tolerance, detoxifying and xylem embolism repair[7]. Stress as a result of both temperature and drought can exist together or singly during any stage during the growth and development of a plant thereby causing grain weight reduction and loss in yield[8]. Exposing a plant to a particular type of stress always gives rise to an increase in tolerance of such plant to an other type of stress that shows similar effects even at the cell level. For example, extremely low (freezing) temperatures, in-availability of water and high salt level can cause low osmotic potential and as a result, can give rise to a response to osmotic stress[9]. Therefore, promoters that possess transcription factors for sequence, actively associated with cold, drought and response to salt are good indicators of converging points at the molecular level[10]. At the molecular level, these include gene overlaps which then suggest complex coordination of response to the combination of stress in the plant even at the molecular level[11-14].

The tolerance of plants to extreme drought conditions can be said to be an example of a quantitative trait. Single genes, like genes responsible for flowering time, the height of the plant and ear type can play important roles in adaptation to drought-prone environment[15]. There is a steady increase in molecular biology research, particularly on drought-resistant mechanisms, which are targeted at improving tolerance to drought in certain plants[16] worked on the expression of up to five genes that induce drought in the shoot of sunflower using PCR and rtqPCR[17] clone a DREB gene which is a drought-related transcription factor and as HaDREB2 that is found in sunflower by using gene interaction analyses technique. This was carried out to improve the ability of sunflowers to resist drought regimes, Sauca and Lazar[18] added these genes that are drought resistant into inbred lines of sunflowers using the embryo rescue technique.

Certain plants including vegetables have over time shown the ability to withstand a period of drought, this regime of drought, however, allows plants to exhibit certain characteristics that are peculiar to drought-resistant crops. In underdeveloped and developing countries majorly in Africa, climate change is a major issue and it is a great hindrance to the cultivation and growth of quality food crops, it is very important to study genes that can withstand drought and their application where necessary to the improvement of crops.

This study was designed to specifically analyse the mode of expression of the drought-resistant gene in *Amaranthus*, sunflower and *Arabidopsis* and comprehensive comparative analysis of all these methods.

Genes conferring drought tolerance: Profiling of gene expression has helped to identify hundreds of genes induced when plants experience stress[11,19-21]. Several genes that are drought tolerant have been detected in model species and have been successfully engineered into other crop species, these genes have been expressed in cereal crops and confirmed via field trials. Traits including yield and drought performances were however successfully improved and do not in any way affect plant growth negatively. $HaHB_4$ which is a transcription factor for sunflower and is a stress-inducible promoter of a homologous gene was expressed in soybean, transgenic plants possess characteristics such as delayed senescence, increase yield both in the absence or presence of drought as a result of stress[22], this same gene has been engineered into bread wheat with a similar result as observed

in soybean[23,24]. In maize, CspA, CspB expressed plants have reduced leaf area, improved photosynthetic rate and high chlorophyll content, the best lines have however been commercialised for example by Monsanto (now Bayer) in 2010 as described by Castiglioni *et al*.[25] and Nemali *et al*.[26].

So many different genes were detected and have been engineered to improve the quality of crops, these genes are NF-YB1, NF-YB2[27] and TPS/TPP, TsVP[28] and OsNACs, OsERF71, HVA1, DRO1 in maize and rice, respectively[29]. Severally research especially in maize and rice shows that drought adaptation by plants and resulting cellular dehydration induces molecular response actively in plants. The response observed improve significantly the ability to be tolerant to negative constraints using transcriptional control[5,30].

The transcriptome is literarily the collection of RNA in a cell or tissue, it is the best way to show a good representative of the cellular state of a plant. Also, transcriptome analysis provides ease of genome-wide profiling, this has made it an important part of a lot of genomic studies which include studies on biological processes and diseases.

As previous studies showed that large transcriptome analyses have revealed how complex molecular response to drought in the plant is[31-33]. Information from the genetic makeup of model plants is now being moved to other crops by exploiting genome synteny, by taking advantage of molecular pathways that are conserved which also include the genes controlling tolerance (stress). Studies showed that if this approach is followed, drought response components can be identified and searched in plants[34-37]. Earlier developed transgenic plants can regulate upward stress response or replicate physiological processes that have been shown previously to have a direct relationship with drought tolerance, this was evident in certain physiological studies.

Transcription factors and their components that coordinate the expression of regulons (downstream) are the optimal targets for traits like stress tolerances. These are transgenic crops that possess genes that encode DREBs/CBFs transcription factors these are tomato[38], rice[39,40] and wheat[41]. Transgenic plants showed increased stress tolerance in addition to over induction of stress genes that are downstream related and probably greater contents of proline and sugars that are soluble. A report showed that overexpression of SNAC1 in rice enhanced drought tolerance and increased potential in the field. Leaves that overexpresses SNAC1 lost water slowly by showing an increase in closure of stomata and ABA sensitivity[42].

Expression of the gene in selected vegetables: This study was designed to analyse several drought-resistant genes expressed by selected drought resistant vegetables also there are agronomic and physiological effects majorly on yield. Also, methods used in the extraction of these genes and the economic viability in this part of the world to agriculturists and its general influence on the sustainability of food security within and outside the shores of Africa.

Gene expression in *Amaranthus*: Because vegetable plants of traditional origin can only withstand a short time of scarcity of water and it's critical to understand how dryness affects their growth, metabolism, development and production. This is due to the variation in various traditional crops grown for a range of purposes, therefore, water requirements must be assessed for each unique crop[43].

Severe drought results in a reduction of CO_2 which is a result of a huge decrease in stomatal conductance, this is because of a huge proportion of light energy that remains inactive photochemically and it is an anti-oxidative reaction[43]. This often results in electrons used in photosynthesis being redirected and the formation of super-toxic oxide becomes the end product of this important process in plant growth response. In *Amaranthus*, there is a correlation between the period of formation of free oxygen radicals and the period of the commencement of the activities of AP, GR and SOD enzymes[43].

Amaranths modulate leaf area which subsequently helps in reducing water loss, this adjustment enables a higher effective control over the usage of water, this may also help in the process of desiccation avoidance under extreme conditions of drought. After 15 hrs of re-watering of these cultivated *Amaranthus*, a huge recovery of leaf area, RWC and LWP were observed.

Several techniques have been identified for the study of genes and have been confirmed to have many advantages over transgenic techniques for target gene(s) analysis, these techniques include: Clustered Regularly Interspace Short Palindromic Repeat (CRISPR)-Cas9 system, Virus-Induced Gene Silencing (VIGS), targeting induced local lesions in genomes (TILLING) and expressed sequence tags (ESTs)[44-47]

Virus-Induced Gene Silencing (VIGS): Figure 1 shows the path of VIGS in plants, VIGS technique is a very highly rated potent tool for the investigation and mining of genes implicated in intolerance (drought). It is used as a Post-Transcriptional Gene Silencing (PTGS) technique in the study of endogenous genes.

A 200-400 bp of the target gene is used in the VIGS technique and then cloned to a vector which is later used to infect plants and silence that gene[44-49], a report by Senthil-Kumar and Mysore[44] also showed that one of the most significant advantages of VIGS technology is the absence of

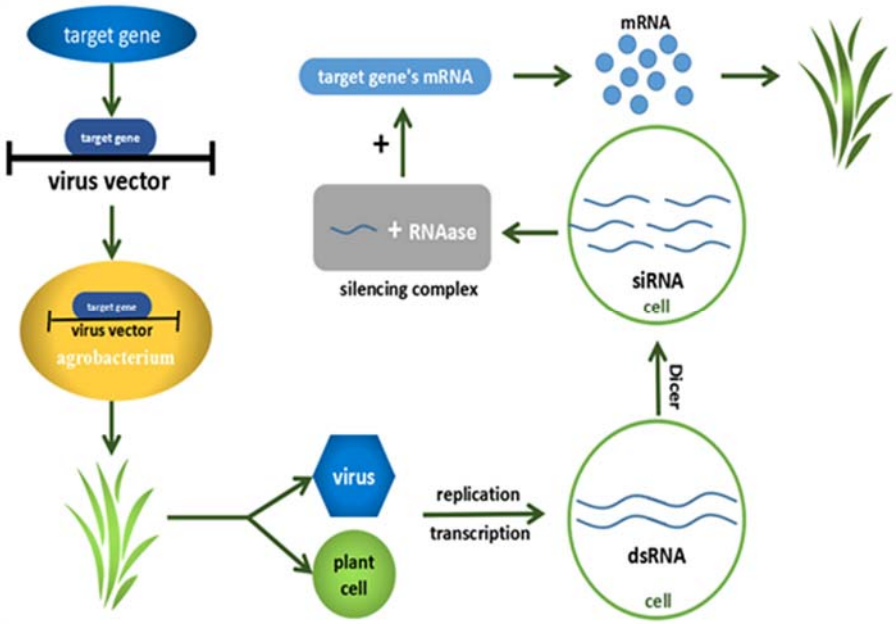

Fig. 1: Virus-induced gene silencing in plants[48]

the need for stable plant transformants. A report by scientists[50,51] also established that several different genes can be investigated at the same time and a single target can be silenced using this approach. The target gene and VIGS vectors can be introduced into the plants by prick inoculation, needless syringe inoculation and biolistic inoculation among other ways[44,52].

Plants have a variety of molecular mechanisms to deal with abiotic stress and other stress-related genes are activated in response to stress[53,54]. An important enzyme for growth and development in the plant, Mitogen-Activated Protein Kinases (MAPKs), which are also very important in signal transduction under severe conditions[55-59].

Mitogen-Activated Protein Kinases (MAPKs) are protein that converts extracellular stimuli into a range of cellular responses, they are part of the oldest signal transduction pathways and are generally used in evolution[60]. Eukaryotic cells have diverse and multiple pathways for MAPK, these pathways regulate gene expression, motility, mitosis, metabolism etc.

The important part played by different MAPKs under drought stress has been studied through VIGS technology these roles include regulating drought tolerance, response to extreme temperature stress, response to salt stress and responses to biotic stresses in horticultural plants[61]. The VIGS of GhWRKY27a increased cotton tolerance to drought stress. In addition, another transcriptional factor family, NAC, plays a key function in drought[62,63].

Aside from these, a degradation process that is a major protein is called autophagy is activated in plants when responding to stimuli that are environmentally influenced, this has been implicated in drought stress because of the presence of autophagy-related genes (ATG). Multiple abiotic stressors trigger the ATG8 gene that is observed in wheat, as well as ATG6 and its orthologs, which are also observed in rice, barley and wheat. The functionality of a VIGS system based on the Barley Stripe Mosaic Virus (BSMV) was tested under drought stress. The findings suggested that ATG genes are involved in a variety of drought-related survival processes[64-66].

Despite this, weeds and wild species of major cultivars have been shown to have many drought-tolerant genes. For example, the ApDRI15 gene observed in *Alternanthera philoxeroides* is now known as a drought-tolerant gene by VIGS[67-70].

Expressed Sequence Tags (ESTs): ESTs can be created from cDNA libraries and are a sequence-based tool for identifying and studying genes. This approach can deliver cost-effective results for functional analyses of certain genes[71]. The first step in the identification of responsive genes is to create cDNA libraries from stressed plants and then ESTs are found by sequencing the clones[71,72]. ESTs have high-quality transcripts that can be used to investigate genes as functional markers under stressful situations.

Drought-responsive genes have been found and examined by ESTs in a variety of crops over the last two

Table 1: Gene expression analysis techniques

Gene expression methods	Technique used	Economic importance	Ease of use	Plant examples
Virus-Induced Gene Silencing (VIGS)	It is a post-transcriptional gene silencing technique	Expensive	Simple	Cotton and tomato
Expressed Sequence Tags (ESTs)	It is a sequence-based tool for identifying study genes from cDNA libraries	Cost-effective	Simple	Wheat, millet, sweet potato, etc
TILLING	It is a non-transgenic method for studying allelic differences in a target gene from a mutant population using PCR	Inexpensive	Simple	Amaranths
CRISPR	It is based on plant anti-viral defence mechanism and it is a nuclease protein-based technology (Cas9)	Cost-effective	Very simple	Amaranths

decades, including common beans[73], barley[71,74], chickpea, sorghum[75,76], rice[77-79], *Camelina sativa*[80], wheat[81,82], Kodo millet[83], pearl millet[84,85], sweet potato[86], rapeseed[87], Peanut[88] and *Ammopiptanthus mongolicus*[89]. To determine the most promising ESTs, BLASTX or qRT-PCR analysis might be used.

TILLING: Genomes of huge varieties of crops are now available thanks to advances in high-throughput techniques, opening up a slew of new possibilities for traditional mutation-based reverse genetic procedures[90]. TILLING is a non-transgenic method for studying allelic differences in a target gene in a mutant population, as well as the effect of the mutant gene on plant phenotypes[91-93]. It is a simple and inexpensive method for finding Single Nucleotide Polymorphisms (SNPs) in a target sequence, PCR can detect these point mutations in the target genes.

Furthermore, independent of ploidy levels, this approach can be used on any plant species with a genome sequence. Chemical mutagens that create random mutations are employed in TILLING to induce mutations in plant genomes. Ethyl methanesulfonate (EMS) is employed as a mutagen in most of the experiments to generate the TILLING population[45]. However, a modified approach known as EcoTILLING was created to analyze the polymorphism that evolved as a result of environmental circumstances. It appears that studying genes related to abiotic stressors is a more promising technique, under these circumstances, a modified approach known as EcoTILLING has been created[94,95].

It appears that studying genes related to abiotic stressors is a more promising technique.

Clustered regularly interspace short palindromic repeat technology: Clustered Regularly Interspace Short Palindromic Repeat also known as CRISPR is associated with nuclease protein (Cas 9) technology and is majorly focused on the antiviral mechanism that is a defence based in plants and has provided researchers with several new opportunities. In comparison to older approaches for the same objective, it is a very easy, less cytotoxic and highly efficient targeted genome editing tool[96,97].

CRISPR/CAS9 makes use of CAS9 endonuclease that was derived from Streptococcus pyogenes, in addition to a guide RNA which helps to direct CAS9 to the target sequence, both of which work together to form double-stranded DNA breaks, which are later repaired using either the Non-Homologous End Joining (NHEJ) method or the Homology-Directed Repair (HDR) pathway[98,99].

Table 1 above shows common gene expression analysis techniques.

Gene expression in sunflower: In a study by Liang *et al.*[100] where RNA sequencing is used to identify genes that are expressed differentially in the shoots of sunflowers under drought stress. They used 12 sunflower samples, three of both leaves and roots treated with polyethylene glycol (PEG) and three leaves and roots of control plant treatments, which were mixed to create a transcriptome library to serve as a reference DGE sequence library.

The study showed that 296 and another 876 genes were expressed differentially in drought-stressed treated plants in the shoots and control, respectively. The study also involved a comparison between shoots under drought stress. While experiencing drought, 805 and 198 (differential expressed genes) DEGs were confirmed to be specific for the leaf part of the plant and the root respectively, out of which 71 genes were expressed differentially in both roots and leaves.

This study also showed that roots and leaves showed genes in lower numbers which are downwardly regulated in comparison to the upwardly regulated genes. However, lots of stress-induced genes got regulated within tissues which means that these genes are likely to play roles as part of mechanisms that cope with drought stress. Another 71 genes revealed similar expression in leaves against the roots showing that there is a clear overlap at the transcriptional level which is in response to drought stress[100].

Polyethylene glycol (PEG): Polyethylene glycol (PEG) is a non-plasmolyzing osmoticum for stimulating embryo maturation. Effects of PEG impersonate water stress that is natural particularly on seeds during the final stages of maturation. A work by Ćali-Dragosavać *et al.*[101] on white spruce embryos

after application of PEG, observed an increase of 3-fold as observed in its maturation frequency, these embryos closely look like related zygotes in low moisture levels and their capacity to tolerate desiccation.

Analysis of gene expression in *Arabidopsis* using microarrays: Profiling and analyzing cDNAs using microarray technology is another way of gene expression in plants exposed to drought, salinity or cold[11,12]. Two major kinds of microarray technology that can be employed are:

- cDNA microarray
- Oligonucleotide microarray using Affymetrix GeneChip

cDNA microarray: This method is frequently used to identify differential gene expression patterns which are used to evaluate changes in expression levels of mRNA across similar cells exposed to different stimuli, or between distinct cellular phenotypes or developmental stages[102] possesses characteristics that make it the most commonly used approach for mRNA expression profiling.

To construct a microarray containing thousands of components, segments of DNA representing gene collection to be tested are amplified by PCR and physically spotted at high density on glass microscope slides using very simple x-y-z stage robotic devices. It is simple to create microarrays containing the whole set of genes from a microbial genome or tens of thousands of eukaryotic cDNA clones.

The microarrays are queried using at least two fluorescently labelled probes produced from mRNA from the cellular phenotypes of interest in a co-hybridization assay[103]. Based on the ratio with which each probe hybridizes to each array element, the kinetics of hybridization allows relative expression levels to be measured.

Hybridization is measured by a confocal laser scanner, which enables the simultaneous assessment of the expression levels of the genes represented in the array. The invention and execution of several scientific techniques and strategies for fluorescence intensity normalization are required for proper expression analysis using microarrays.

The three stages of the expression analysis procedure include array manufacturing, probe preparation and hybridization, data collection, standardization and analysis. Hybridization of RNA produced from cell lines has been used to evaluate and optimize this method, resulting in repeatable and consistent results. Various additional protocols can be used as alternatives[104].

PCR-amplified cDNA clones or genes are arrayed at high density on derivatized glass microscope slides to create microarrays. In most eukaryotes, expressed sequence tag (EST) data are the most comprehensive source of information for gene identification. ESTs are single pass, incomplete sequences of cDNA clones that are widely employed in humans and other organisms for gene discovery and mapping. The EST method has been the widely utilized, generated EST sequences account for more than 71% of all GenBank entries and 40% of individual nucleotides in the database[105].

cDNA clones are often chosen to represent as many different transcripts as possible. Numerous analyses of these data have been performed to identify distinct human transcripts within the EST data, the two most often utilized are UniGene Boguski M.S. and UniGene Boguski M.S.

The Human Gene Index was created in 1995 by the National Center for Biotechnology Information (NCBI) and The Institute for Genomic Research (TIGR) at the National Center for Biotechnology[106]. UniGene and HGI are based on EST clustering, tentative human consensus (THC) are produced by the TIGR protocol by assembling the ESTs within the clusters. Research where[107] used the TIGR HGI to select cDNA clones for array building as part of a program to compile a 30,000-gene clone set. THCs were chosen for inclusion in the clone set with a bias for those that included known genes or had mapped locations, THCs with known genes or mapped locations were chosen for inclusion in the clone set.

The 299 drought inducible genes, 54 cold-inducible genes, 245 ABA inducible genes and 213 high salinity inducible genes were identified by a 7000 full-length cDNA microarray in *Arabidopsis* according to a study carried out[12,13]. The study further revealed that only 10% of the drought-inducible genes were also induced by cold stress.

A way to validate microarray reproducibility results according to research by Wei *et al.*[108] is by quantitative real-time reverse transcription, where six rice variety differential expression levels of 10 randomly selected DEGs were assessed.

Consistently, qRT-PCR results were observed to have coincided with what was obtained from microarrays. Two genes which are *LOC_Os08g43390* and *LOC_Os12g08760* showed clearly expression patterns with expression in one of the varieties. However, results obtained using the qRT-PCR technique showed good agreement with the results obtained using the microarray technique.

A lot of genes that induce stress were identified with the Affymetrix GeneChip array which contains oligonucleotides and represents about 8000 *Arabidopsis* genes that are independent[11]. Genes that induce stress that were gotten from the cDNA microarray analysis did not overlap with the ones

identified through GeneChip analysis. This inconsistency in products is majorly due to gaps known to be between gene-sets arrayed in the two systems when compared with stress treatment and growth conditions[109].

Drought-associated miRNAs: The miRNAs as identified by Barrera-Figueroa *et al.*[110] are drought associated and also carried out a test on differential expression of miRNAs in drought stress and control samples in each genotype. In the study, these criteria were used in the identification of drought-related miRNAs:

- Adjusted p-value was observed to be <0.01 in at least one out of the two that were compared
- Normalized counts (TPTM) value was seen to be at least 100 in one out of the four libraries
- Log2 ratio of normalized counts between control and drought libraries were taken to be >1 or <-1 in one of the two genotypes

In an analysis that involves differential expression, unique mature miRNAs were considered because they are the major active form of miRNA. The study also revealed 44 drought-related mature miRNAs which belong to 28 families in total, the flow of statistically significant change was similar in both genotypes for all 44 drought-related miRNAs, showing that miRNA gene expression in *IT93K503-1* and *CB46* had a very close total response to drought stress. However, 30 of 44 miRNAs were up-regulated in the condition of drought, also 14 were downregulated in at least one. Of the 44 miRNAs that are drought associated, 22 miRNAs expressions in 17 different families in drought conditions were altered at least two-fold in stack comparison to what was obtained in the control for both genotypes.

It is also reported by Barrera-Figueroa *et al.*[110] that gene expression regulated using sequence-specific interaction between miRNAs and target mRNAs shows accuracy and mechanisms that are inheritable for plants which make them respond to environmental stimuli.

Carrying out deep sequencing from two different genotypes of the cowpea of small RNA libraries identified 157 miRNAs. However, when the comparison is carried out between the level of expression of miRNAs in control and drought treated samples, 30 miRNAs that were up-regulated in the drought conditions and 14 down-regulated were identified. miRNAs that are drought associated include families of miRNA that have been confirmed to have an association with drought in other plants, meaning they are deeply involved in drought response pathways that are conserved.

Most conserved miRNAs have their targets conserved, for example, the study revealed that in response to drought in cowpea miR156 was upregulated. MiR156 responds to abiotic stresses and also can target SPB transcription factors in wheat, rice, *Arabidopsis* and maize[111-115]. This particular miRNA is very much involved in the developmental regulation at the vegetative phase change[114], which indicates that development reprogramming is crucial in plants to adapt to stress caused by drought. miRNA and miR169 were down-regulated in these cowpea genotypes. It is also important to note that *Arabidopsis* and miR169 were downregulated and nuclear factor Y and transcription factor NFYA5 which was its target were both induced by drought stress[116].

DISCUSSION

The study highlighted several methods used for gene expression in several kinds of research involving drought resistance in the vegetable. This study particularly revealed methods of expression such as microarrays, (VIGS), (CRISPR)-Cas9 system, (TILLING) and (ESTs) whose application varies from gene identification, the study of endogenous genes and molecular study of variations in mutant population, also the table in the study conforms with various studies as regards gene expression[44,49]. In *Amaranthus*, where several techniques have been identified for gene study, each of the identified techniques has its peculiarity. For example, the VIGS technology has a superior advantage over other methods since it can be used to investigate different genes at a time and the fact that this technique does not necessarily need a stable plant transformant[44]. Also, it has a comparative advantage in terms of cost-effectiveness, a factor that is very important in a low-income area such as Africa. Expressed sequence tags (ESTs) technique which is another technique described in this review study has a huge advantage over other methods of gene expression because it is relatively cheap to carry out and maintain, this is in correlation with the study of Blair *et al.*[71].

The ability to work with genes in a mutant population is a unique feature of TILLING, this technique can also be used for a huge number of crops when compared relatively to other methods of gene expression this is supported by Kurowska *et al.*[90], this technique is also cheap and very simple.

In sunflower, RNA sequencing with rtPCR using gene-specific primers as described in this study as a method of identifying differentially expressed genes. This method also shows the specificity of drought in the plant parts or tissue, the major advantage of this technique is the fact that it reveals both up and down-regulated genes.

A major way of gene expression analysis expression in *Arabidopsis* that was described in this study is the microarray technology which can either be by cDNA or Oligonucleotide microarray. Aquaporins perform a major role in solute and water transport and thus maintain water homeostasis while responding to stress which is also found in *Arabidopsis*. Aquaporin families in plants are complex and are made up of a great number of genes[117].

The major advantage of the microarray technology is the fact that it can be used to identify the gene of different origins viz a viz drought, high salinity and cold-inducible genes according to Kreps *et al*.[11] and Seki *et al*.[12]. It also shows the cross-relationship between these genes.

Stress makes plants develop peculiar molecular mechanisms to survive different stress factors, with the different morphological and genetic features found in certain plants[118]. However, climate change in this part of the world is a menace that can be solved using a molecular technique that is affordable and at the same time reliable. Also, to meet the great demand for improved crops, it is necessary to adopt innovative technologies that can ensure the production of important crops[119].

CONCLUSION

The ease of use, techniques and economic importance of the various methods that can be used in analyzing gene expression were critically explained in the study, this is of utmost importance in developing countries particularly Africa where the scourge of drought is due to climate change and access to current techniques in agriculture is extremely limited due to low level of income and lack of investment in the science of production of the crop.

SIGNIFICANCE STATEMENT

This study was carried out majorly to critically analyse the common gene expression techniques and their relevance to third world countries in the world, especially Nigeria. Many crops cannot withstand drought and yet are major food crops that needed to be produced throughout the year, this study however gave major and popular molecular techniques used in gene expression and proper comparative work was also carried out to look at the effectiveness, availability and more importantly ease of access of these techniques to researchers in Nigeria and how it can be transmitted and translated to the production of more drought-resistant crops which will, in turn, reduce the problem of food insecurity in third world countries around the world.

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