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Research Application Summary

Identification of drought related and drought responsive genes in cassava

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Abstract

Conventional cassava breeding has yielded a lot of progress for many traits including pest and disease resistance and high yields but little progress has been made with respect to drought tolerance. It is believed that molecular breeding offers a possibility of efficiency and precision in introgression of drought tolerant genes. Several studies show that several genes respond to drought stress and some of them have been used to improve stress tolerance of plants through gene transformation. Unfortunately such studies have not been carried out in cassava. This research is aimed at identifying genes responsive to drought stress in cassava using Real-Time PCR. Identification of drought tolerance genes will help in understanding the mechanisms of drought tolerance in cassava and may be useful in developmental of drought tolerant crops through genetic transformation. The research has only just started by first observing response of 53 cassava genotypes under water stress conditions.

Key words: Cassava, drought tolerant genes, water stress

Résumé

La reproduction traditionnelle du manioc a réalisé beaucoup de progrès pour beaucoup de traits comprenant la résistance au parasite et à la maladie et les rendements élevés mais peu de progrès a été réalisé concernant la tolérance à la sécheresse. On pense que la reproduction moléculaire offre une possibilité d'efficacité et de précision dans l'incorporation des gènes tolérant la sécheresse. Plusieurs études prouvent que plusieurs gènes répondent à l'effort de sécheresse et certains d'entre eux ont été utilisés pour améliorer la tolérance au stress des plantes par la transformation de gène. Malheureusement de telles études n'ont pas été effectuées pour le manioc. Cette recherche a visé d'identifier les gènes sensibles à l'effort de sécheresse dans le manioc en utilisant PCR en temps réel. L'identification des gènes de tolérance de sécheresse aidera

dans la compréhension des mécanismes de tolérance à la sécheresse pour le manioc et peut être utile dans développemental des cultures tolérant la sécheresse par la transformation génétique. La recherche a juste seulement commencé en observant premièrement la réponse de 53 génotypes de manioc dans des conditions de stress de l'eau.

Mots clés: Manioc, gènes tolérants de sécheresse, stress de l'eau

Background

Drought is one of the main environmental constraints to agricultural productivity worldwide, and most climate-change studies indicate an expansion of arid zones. Therefore, a large part of the world will become arid as a result of global warming. This means that water stress or drought will be one of the major crop production constraints. Cassava is staple crop that has the potential to mitigate the climate change effects due to its inherent ability to grow in semi-arid and drought-prone areas. Though cassava has been recognized as a hardy crop with ability to withstand moderate moisture stress, research to improve its water efficiency has been limited. More effort on drought research has been placed on commercial seed crops such as maize and rice where seed sales can profit the private seed companies. Because cassava is vegetatively propagated, it is regarded as a less profitable crop and has been left to public institutions, which in most cases are under funded, especially in Africa.

Although conventional cassava breeding has yielded a lot of progress for many traits including pest and disease resistance and high yields (Kawano, 2005), little progress has been made with respect to development of drought tolerant varieties. Breeding for drought tolerance in cassava has been an extremely difficult task due to high genotype by environment (GxE) interaction (Ceballos *et al.*, 2004), the heterozygous nature of the crop, its long growth cycle and poor knowledge of the crop's diversity (Fregene *et al.*, 2001). Thus, the development of a new variety can take between eight and twelve years. Molecular breeding through marker assisted selection (MAS) offers a possibility of efficiency and precision in introgression of desired traits because it would reduce the generation time.

One of the most productive molecular approaches to establishing the basic responses of plants to drought is by studying candidate genes and comparing the expression of genes thought to be

important for drought tolerance. Many efforts have been made to clarify the mechanisms of drought tolerance in plants through molecular and genomics approaches. Studies by Bray (1993); Liu and Baird (2004); and Talamè *et al.*(2007), revealed that there are several genes that respond to drought stress at the transcriptional level. Some of these genes have been reported to play important roles in protecting plants from drought stress through stress perception, signal transduction, transcriptional regulatory networks in cellular responses, or tolerance to dehydration (Nakashima and Yamaguchi-Shinozaki, 2006; Umezawa *et al.*, 2006). It has also been reported that some of the putative drought-tolerance genes have been used to improve stress tolerance of plants through gene transformation (Pellegrineschi *et al.*, 2004; Umezawa *et al.*, 2006). Although some progress has been made with other traits, the molecular basis of plant tolerance to drought stress remains to be elucidated (Bruce *et al.*, 2002; Umezawa *et al.*, 2006). This research targets to unveil the molecular patterns of drought tolerance in cassava.

Literature Summary

Several hundred genes that respond to drought stress at the transcriptional level have been identified through gene expression studies in model crop Arabidopsis by microarray technology and other means (Shinozaki and Yamaguchi-Shinozaki, 2007). Gou *et al.*(2009), performed gene expression experiments using drought-tolerant and drought-sensitive barley genotypes to compare differences in transcription levels between drought-tolerant and drought-sensitive genotypes under drought-stress conditions. Indeed Guo *et al.* (2009) were able to separate drought-tolerance-related genes from drought-responsive genes. Similar studies have been successfully undertaken in other species including Arabidopsis and maize (Bruce *et al.*, 2002). Unfortunately such studies have not been carried out in cassava and cassava is not sufficiently close phylogenetically to any of these species to take advantage of these studies through comparative genomics. This research is aimed at understanding of molecular mechanisms of drought tolerance in cassava. The specific objectives of the study are to; i) identify candidate genes responsive to drought stress and ii) evaluate expression of candidate genes in cassava in response to water stress.

Research Approach

Gene identification and primer design. Seventy genes related to tolerance to moisture stress in different crop and non crop plants were identified from literature. Out of these genes, thirty genes that have been demonstrated to contribute to

drought tolerance (Table 1) were selected for expression analysis. Gene sequences of these genes were obtained using the Basic Local Alignment Search Tool (BLAST) program through database querying/searching, or database mining using plant databases of the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/). The obtained gene sequences were then BLASTed into the cassava genome data base (http://www.phytozome.net) to obtain gene homologs in cassava. The cassava homolog sequences were used to design primers for the respective genes. When the gene belonged to a gene family or when a gene had different cassava homologs, all the family members/cassava homologs were aligned together using the EBI clustalw2 tool (http://www.ebi.ac.uk/Tools/clustalw2/index.html) and primers specific to the gene of interest manually designed (Fig.1). Two primer pairs were designed for each gene.

RNA extraction. Root and leaf samples will be collected from water stressed and well-watered plants on the 10th day of 25% moisture content. Samples will be collected between 2:00-2:30 pm from the third leaf fully expanded from the growing tip sampled in each plant and at least three fibrous root tips randomly collected from each plant. Samples from each replication for each treatment will be treated separately as biological replicates (three biological replicates for qPCR). Samples will be immediately stored in a -80^{o}C freezer for RNA extraction. The trizol method will be used to extract RNA. The concentration of RNA from each sample will be determined by UV spectrophotometry at A260, while the quality of total RNA will be analysed by 1% ethidium-bromide agarose-gel electrophoresis.

Real time PCR. Two step RT-PCR will be used. The extracted mRNA will be converted to cDNA libraries by the reverse transcriptase enzyme (superscript II/III) (RT phase). Stock cDNA will be stored at -80^{o}C for different gene quantifications (30 candidate genes, Table 1). Three technical replicates (qPCRs) will be performed for each gene pair in each biological replicate of each genotype and stress treatment. PCR will be performed with the 7900HT Fast Real-Time PCR system of ABI-PRISM® (USA) using the SYBR Green detection method. All the qPCR reactions will be normalised with internal reference genes. Care will be taken to ensure that the reference genes chosen do not change their expression under the experimental conditions or between different tissues. The

Table 1. Genes selected for gene expression studies in cassava.

Gene name	Accession	References
Arabidopsis thaliana putative senescence-related protein	At2g17840/BT001230	Matsui *et al.,* 2008
Arabidopsis thaliana germin-like protein	At1g72610/AY081576	Kreps *et al.,* 2002
Arabidopsis thaliana GBF3 (G-BOX BINDING FACTOR 3);	NM_180118	Matsui *et al.,* 2008
Arabidopsis thaliana late embryogenesis abundant group 1	AT5G06760	Hundertmark and Hincha, 2008
Arabidopsis thaliana GER3 (GERMIN 3	NM_122070	Kreps *et al.,* 2002
Arabidopsis thaliana RAB18 (RESPONSIVE TO ABA 18)	NM_126038	Hundertmark and Hincha, 2008
Arabidopsis thaliana chitinase, putative	AT2G43570	Shou *et al.,* 2004
Arabidopsis thaliana dehydrin family protein	AT1G54410	Hundertmark and Hincha, 2008
Nicotiana tabacum mRNA for protein kinase,	D26601	Shou *et al.,* 2004
Zea mays transcription factor subunit NF-YB2	NM_001112582	Nelson *et al.,*2007
Zea mays phospholipase C (PLC), mRNA	NM_001111784	Wang *et al.,* 2008
Datura stramonium mRNA for arginine decarboxylase 1	AJ251898	Capell *et al.,* 2004
Arabidopsis thaliana ABF3	NM_179159	Ito *et al.,* 2006
Arabidopsis thaliana DREB1A	NM_118680	Oh *et al.,* 2005; Liu *et al.,* 1998
Arabidopsis thaliana MYC2	At1g32640	Matsui *et al.,* 2008
Arabidopsis thaliana ATMYB102	AT4G21440	Matsui *et al.,* 2008
Pisum sativum manganese superoxide dismutase	U30841	Wang *et al.,*2005
Oryza sativa DREB1	AY064403	Ito *et al.,* 2006
Hordeum vulgare subsp. vulgare cultivar Strider HvCBF4	AY785851	Oh et al; 2007
Oryza sativa Japonica Group CBL-interacting protein kinase 12	EU703798	Xiang *et al.,* 2007
Oryza sativa Japonica Group zinc finger protein ZFP252	AY219847	Xu *et al.,* 2008
Arabidopsis thaliana RD28	NM_129274	Yamaguchi-Shinozaki *et al.,* 1992
Hordeum vulgare NADP-dependent malic enzyme	EU977179	Hundertmark and Hincha, 2008
Oryza sativa stress-induced transcription factor NAC1 (snac1)	DQ394702	Hu *et al.,* 2006
Arabidopsis thaliana protein farnesyl transferase	U46574	Wang *et al.,* 2005
Tobacco mRNA for manganese superoxide dismutase	X14482	McKersie *et al.,*1996
Arabidopsis thaliana AVP1;	NM_101437	Gaxiola *et al.,* 2001; Park *et al.,* 2005
Arabidopsis thaliana putative receptor protein kinase, ERECTA	At2g26330/AY035110	Garg *et al.,*2002
Arabidopsis thaliana 14-3-3 protein GF14 lambda (GRF6)	AF145298	Yan *et al.,*2004
Maize NADP-dependent malic enzyme (Me1)	J05130	Laporte *et al.,*2002

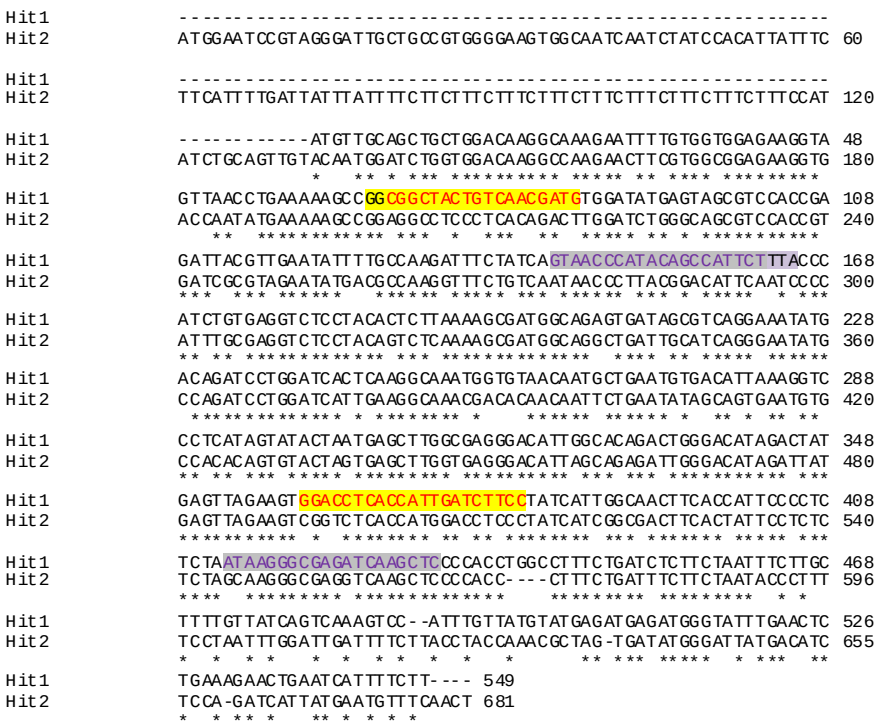

Figure 1. Manual designing of the primer pairs for Arabidopsis thaliana LEA14 which is drought related gene.

expression level of the genes will be estimated at a certain point of the run (ct value) as the amount detected above the threshold is directly related to the initial amount of target gene product in the sample. The linear correlation between PCR product and SYBR green fluorescence intensity in the exponential phase of the PCR reactions will be used to calculate the amount of template present at the beginning of the reaction from the formula;

$$N_n = N_0 X (E)^n$$

(where N_n=number of molecules/copy numbers at n^{th} cycle/ct value, N_0= number of molecules/copy numbers at cycle 0 i.e. initial copy numbers; E= PCR amplification efficiency and n=number of cycles).

Thus the initial copy numbers will be calculated as; $N_0 = N_n / (E)^n$

Research Application

Identification of drought tolerance genes will help in understanding the mechanisms of drought tolerance in different cassava genotypes. The candidate genes are also useful in developmental of drought tolerant crops through genetic transformation, i.e., will enable effective use of genetic and

genomic approaches to breed cassava with improved water use efficiency.

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