

## Expression analysis of Aldehyde Dehydrogenase (GDI\_E\_37) gene in groundnut under different abiotic stress

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**Abstract:** Abiotic stresses such as drought, salt and temperature stresses are limiting factors for crop productivity in arid and semi-arid regions and influences many aspects of plant growth and development. Aldehyde dehydrogenases (ALDHs) are a family of enzymes that are involved in plant metabolism and contribute to aldehyde homeostasis in plants when exposed to abiotic stress and involved in detoxification processes to eliminate toxic aldehydes. To gain a better understanding of the abiotic stress responses at molecular level, we carried out a genomic analysis of stress-responsive genes/transcripts in drought-tolerant cultivar K-134. As a first step toward characterization of stress-responsive genes, previously we constructed, analyzed and classified subtracted cDNA library from drought tolerant groundnut cultivar (K-134). In this present study we analyzed expression profiles of aldehyde dehydrogenase (GDI\_E\_37) under different abiotic stress levels. Our result showed that aldehyde dehydrogenase (GDI\_E\_37) was expressed differentially under different abiotic stress conditions. However it was expressed maximum at severe stress conditions over the controls. Results and discussions were discussed in this paper.

**Key words:** Abiotic stress, aldehydes, stress tolerance, Soil moisture level (SML), Groundnut

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### I. Introduction:

Agriculture is a major user of water resources in many regions of the world. Generally plants are frequently encounter unfavorable conditions such as drought, salinity, high temperature, freezing, nutrition deficiency and chilling, which adversely affect their growth, development, and productivity. Among the abiotic stresses drought is one of the most important stress constraints to global agricultural production. More than 10% of arable land is affected by drought and salinity [1]. With increasing aridity and growing population, water will become an even scarce commodity in the near future. Development of superior high yielding crops led to green revolution in the past has tremendously boosted agricultural productivity worldwide. However, world population will touch the eight billion more in about 25 years from now. As per the United Nations prediction by 2020 we will need 40 per cent more grains than what we produced today [2]. Therefore understanding the plant tolerance to drought is a fundamental research to meet food demands of increasing population and to cope with drastic climatic conditions.

Abiotic stresses such as drought, salt, heat, cold and temperature stresses are major limiting factors which cause severe changes in the morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity [3, 4]. Among all abiotic stresses drought is a major factor that limits crop production, it can be simply defined as a period of below normal precipitation that limits plant productivity in a natural or agricultural system [5]. Plants experience drought stresses either when the water supplies to roots become difficult or when the transpiration rate becomes very high. These conditions often coincide under arid and semi-arid regions. Crops which are generally cultivated in these regions dependent on current rainfall rate and suffer from intermittent drought stress during vegetative or reproductive growth period. Plants are varying tremendously in their ability to withstand abiotic stresses, both between species and within populations of a single species [1] and have developed different adaptive mechanisms to meet the adverse environmental conditions. Plants often exhibit avoidance and tolerance characters to resist drought stress. One of the important avoidance characters of crop plants exhibit is deep root system that penetrate the water table [6], besides, leaf reflectance characters to decrease heat load, including fleshy leaves, thick cuticles or pubescence, biosynthesis of epicuticular wax, sunken stomata, and other stomatal variations to regulate the

transpiration through stomata [7]. In addition plants are well adapted and have developed several biochemical and molecular mechanism to deal with various environmental cues. Signaling cascades and cellular pathways leading to the production of proteins, molecular chaperons, ROS scavenging systems, accumulation of compatible solutes in response to stress [3]. One of the major molecular responses plants exhibit to water deficit is altered gene expression. Significant progress has been made in studying the plants systems to understanding the role of various genes, transcription factors, cellular macromolecules and the components of the signal transduction cascades during stress [8].

Several studies have been reported that a number of genes to be induced by various abiotic stresses such as drought, high-salinity, and low temperature, and their products are thought to be function in stress tolerance and response [9, 10]. The molecular and transgenic studies have revealed [11, 12, 13] that the identification of novel genes, determination of their expression patterns in response to the drought stress, and an improved understanding of their functions in stress adaptation will provide us the basis of effective engineering strategies to improve stress tolerance in crop plants [14]. Therefore, the potential stress tolerance can be achieved by the isolation of novel stress responsive genes from stress tolerant crop species and cloned into susceptible species to improve the stress tolerance level of susceptible species.

Aldehyde dehydrogenase (ALDH) enzymes are belong to a family of NAD (P)<sup>+</sup> dependent enzymes, that have substrate specificity and catalyse the oxidation of various aldehydes to the corresponding carboxylic acids, thus reducing the peroxidation of lipids. ALDH genes are considered to be 'aldehyde scavengers' to eliminate surplus aldehydes [15, 16]. Aldehyde molecules are common intermediates in most cellular pathways such as carbohydrate, amino acid, protein, lipid or steroid metabolism [17, 16]. Lipid peroxidation resulting in the loss of membrane integrity or modification of proteins subsequently causing cellular and developmental arrest [18, 19, 20]. On the other hand, recent findings suggest that some aldehydes may also have a signaling function [21]. The accumulation of aldehydes leads to the production of reactive oxygen species (ROS) such as singlet oxygen, hydroxyl radical, superoxide and H<sub>2</sub>O<sub>2</sub> [22, 23]. They are involved in stress adaptation to abiotic and biotic environments and regulate aldehyde homeostasis under stress conditions. Various plant ALDH genes have been reported to be activated by environmental stress such as dehydration, salinity or excessive light [24, 15, 25]. Stress related members of ALDH genes have been investigated in *Arabidopsis* [26]. Several studies reported aldehydes dehydrogenases (ALDH3F1, ALDH3I1 or ALDH7B4 genes) were involved in stress tolerant mechanism in plants by a reduction of H<sub>2</sub>O<sub>2</sub> and malondialdehyde (MDA) [27]. The involvement of ALDH genes in stress tolerance was corroborated by the analysis of *Arabidopsis* ALDH T-DNA knock-out (KO) mutants was reported in stress tolerant *Arabidopsis* [28]. In the present study we analyzed expression profiles of aldehyde dehydrogenase (GDI\_E\_37) gene under various abiotic stresses such as water, salt and heat stress.

## **II. Material And Methods:**

### **2.1. Experimental Design and Plant material:**

Seeds of groundnut (*Arachis hypogaea* L.) cultivars namely (cultivar K-134 and (cultivar JL-24) were procured from Andhra Pradesh Agricultural Experimental Station Kadiri, Anantapur district. Seeds were surface sterilized with 0.1% (w/v) sodium hypo chlorite solution for 5 minutes, thoroughly rinsed with distilled water, germinated in plastic pots containing 2 kg of soil: sand (2:1) mixture and allowed to grow for nineteen days. The pots were maintained in the departmental botanic garden under natural photoperiod (10-12 hours; temperature 28 ± 4<sup>o</sup>C). Nineteen-day-old groundnut plants were subjected to a progressive water stress by withholding water. Plant soil water status (per cent soil moisture level) was measured at regular intervals by gravimetric method to obtain required soil moisture levels (100, 50 and 25% SMLs).

### **2.2. Total RNA Isolation and Subtractive hybridization:**

Total RNA was isolated from the control (100 % SML) and stressed (25% SML) leaf samples according to the protocol described by Datta et al., (1989). Then Subtractive hybridization was performed between biotinylated control single stranded cDNA and stressed mRNA [29]. Then the cDNA library was constructed by subtractive hybridization between control cDNA and stressed mRNA. First strand cDNA synthesis was performed with subtracted mRNA. Subtractive mRNA was used as template and primed with Oligo dT designed with a "GAGA" sequence to protect the *Xho*I restriction enzyme. First strand cDNA was synthesised using lambda UNI-ZAP XR cDNA synthesis kit (Stratagene, USA) components and incubated at 42<sup>o</sup>C for 1 hour in the presence of template, primer, dNTPs, reaction buffer and enzyme (strata script) reverse transcriptase as per the instruction manual.

Primer for first strand cDNA synthesis

5'-GAGAGAGAGAGAGAGAGAGAACTAGTCTCGAGTTTTTTTTTTTTTTTTTTT-3' GA"  
XhoI poly (dT)

The second stand of the cDNA is made using the random priming of the first strand cDNA by RNA primers produced by limited digestion of the mRNA template by *E. coli* RNase H followed by transcription with *E. coli* DNA polymerase I at 16°C for 2.5 hours. The subtracted pool was made into ds-cDNA and cloned and packaged into Uni-ZAP XR vector according to the packaging instructions (Stratagene, USA).

### 2.3. cDNA sequencing, data base and homology analysis using bioinformatics approach:

The 3' end of cDNA sequence was determined using DYEnamic ET Dye terminator kit (Amersham Pharmacia, Uppsala, Sweden) and ABI 3700 automated sequencer (Perkin Elmer, USA). Sequences are processed using the NCBI developed vector screening programme (<http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen.html>) to remove vector and cloning oligo sequences and various contaminants, and to trim to a high quality region. Based on the qualified sequences, the predicted amino acid sequences were used to search for similar peptide sequences in public databases NCBI (National Center for Biotechnology Information, Bethesda, USA) using the BLASTX algorithm <http://www.ncbi.nlm.nih.gov> [30] and default parameters of the program were used in all cases. The similarity scores between the cDNA clones and known sequences were represented by the BLASTX probability E-values. The cDNAs were classified according to the E-values generated in the BLAST searches. Largest open reading frame was determined using ORF finder program ([www.ncbi.nlm.nih.gov/gorf.html](http://www.ncbi.nlm.nih.gov/gorf.html)) and used for multiple homology analysis. Nearly six proteins for each insert that were showing homology were aligned with each other using multiple alignment program *clustalW* ([www.ebi.ac.uk/clustalw](http://www.ebi.ac.uk/clustalw)). Homology searching tools reveals that the EST clone aldehyde dehydrogenase (GDI\_E\_37) was shown homology with stress related genes in other plant species. So we selected aldehyde dehydrogenase (GDI\_E\_37) gene for their expression analysis under different abiotic stress conditions.

### 2.4. Expression analysis of aldehyde dehydrogenase (GDI\_E\_37) under various abiotic stresses through northern blot:

19-day-old pot grown groundnut plants were subjected to different abiotic stresses such as drought (until soil moisture levels reached to 50 and 25%), Salt stress was imposed to the seedlings by supplementing 50 and 100 mM of NaCl solution to the plants for three days and Heat stress was imposed by keeping the plants at 45°C and 55°C for 8 hours. RNA was isolated from all stress treated leaf samples by [31]. The samples were flash frozen in liquid nitrogen and stored at -80°C for further use. For expression analysis total RNA (20 µg) was separated by electrophoresis in denaturing formaldehyde 1% (w/v) agarose gels [31] and then transferred to Hybond-N+ nylon membrane (Amersham Pharmacia Biotech, Uppsala, Sweden). Plasmid containing GDI\_E\_37 (aldehyde dehydrogenase) insert was selected from our cDNA library. Inserts were released from the plasmid DNA and Probe (GDI\_E\_37) was amplified with T3 and T7 primers in the presence of p32dCTP radio label. Hybridization was carried out at 55°C for 12-16 hours. After washing, membrane was exposed to X-ray film.

## III. Results:

The major objective of the present investigation is molecular cloning, characterization and expression of stress responsive genes from a stress tolerant crop species groundnut (*Arachis hypogaea* L. cultivar K-134) under water stress. Hence, the expression patterns of subtracted cDNA clones were analyzed by northern blot analysis. In this particular objective we took and studied expression analysis of aldehyde dehydrogenase (GDI\_E\_37) gene under different abiotic stress conditions.

### 3.1. Cloning and sequencing of drought stress-induced aldehyde dehydrogenase (GDI\_E\_37) gene from groundnut.

Since we aimed to identify differentially expressed drought-induced genes in drought-tolerant groundnut cultivar (K-134), cDNA subtraction protocol was followed. A subtracted cDNA library was constructed with poly (A) RNA isolated from drought stressed (25% SML) (since the known stress-responsive genes Gdi-15 and Elip were shown maximum expression at 25% SML) and unstressed (100% SML) leaf samples by subtractive hybridization as described earlier. The length of the subtracted cDNA products visibly ranged from 400 to 2,000 bp (not shown). From the subtracted cDNA library, we selected 200 clones randomly and sequenced from their 5' ends. After removing of vector backbone, 120 high-quality EST sequences were generated. The EST sequences generated from this subtracted cDNA library were analyzed against the current GenBank database using the BLASTX algorithm. Drought induced genes, named as GDI, representing groundnut drought stress induced were deposited in the public domain through NCBI dbEST division database (Genbank). Sequence homology search via NCBI database revealed that the clone (GDI\_E\_37) had shown

significant homology with aldehyde dehydrogenase gene in *Brassica rapa L.* so the EST (GDI\_E\_37) identified as putative aldehyde dehydrogenase with this accession number (EC391300) figure.1.

**Figure 1. Nucleotide sequence, deduced amino acid sequence of putative aldehyde dehydrogenase (GDI\_E\_37) and its homology analysis**

**Nucleotide sequence of GDI\_E\_37**

GCTGGAGCTCCCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCCCGGGCTGCAGGAATTCGGCA  
 CGAGGGAATTTGCTCCAAAAGTATAAATGCTTTGAAAGAAGAGTTGAAGCAATCTTTGGCGAA  
 GATCGATGGAATCAAAAAGACATGTCTCGTATTGTGTCCCCGACCCAGTTTTTCGCGGCTGGTGAAGC  
 TCTTGGATGAAGATAAAGTATCTGACAAAATTGTTTTGGAGGTCAGAGGGATGAGATGAAACTA  
 AAGATTGCACCAACTATCATATTGGATGTTCCAGATGATGCAATGGTGTGCAAGAAGAGATATTT  
 GGGCCAATAATGCCAATCATCACTGTAGAAAACATAGAAGATAGCTTTGGCATAATCAAATCTAA  
 GCCAAAACCTCTTGCTGCTTATCTCTTTACAAAACATGAGCAGTTAAAGAAGGCATATGTGAAAA  
 TATATCTTCTGGAGGGATGCTCATCAATGACACTGTCATACATGTTGCGACTCGTGGTTTTGCCTTT  
 GGAGGAGTTGAAGAAAGTGAATGGGATGTTACCACGGGAAGTTCTCTTTTGATAGTTTCAGCCAT  
 AAGAAGTCTGTCCTCTATAGAAGTTTTGATGCAGATTCATCCTTAAGGTTCCCTCCATATACACCCG  
 AGAAGGAAAAATTGTTGAAGGCCATTTTCAGTGGCAACATTATTCGCATAATTCTTACTTTGCTTG  
 GATGGTCTTAGATTAGAATAAACGCATATACAAGGAACATCC

**Translated product (+1 frame: 136 to 735)**

MESKDMSRIVSPTQFSRLVKLLDEDKVS DKIVFGGQRDEMCLKIAPTIILDV PDDAMVMQEEIFGPIPIIT  
 VENIEDSFGIIKSKPKPLAAYLFTNNEQLKAYVENISSGMLINDTVIHVATRGLPFGGVEESGMGCYH  
 GKFSFDSFSHKKSVLYRSFDADSSLRFPYPYTPPEKEKLLKAIFSGNIIRIILTLLGWS

**Multiple sequence alignment**

gi|7270374 ITTKDFASKLIDALKTELETFFGQNALESKDLSRIVNSFHFKRLESMLKENG VANKIVHG 60  
 gi|83701643 -----LKSKDVS RIVNSFHFKRLESMMKENG VANKIVHG 34  
 gi|77553820 ITTKSFAPKLLALEKVLKVFYGRDPLRSSDLSRIVNSNHFNRLLKLMDDENVS DKIVFG  
 60  
 GDI\_E\_37 FGTREFAPKLINALKEELKQFFGEDRW NQKTCLVLCPRPSFRGWSSWMKIKYLT KLFLEV  
 60  
 ... : \* . . . : : :

gi|7270374 GRITEDKLIKISPTILLDVPEASSMMQEEIFGPLLPIITVQKIEDGFQVIRSKPKPLAAYL 120  
 gi|83701643 GQTMEDKLIKISPTILVDVPEESSMMQEEIFGPLLPIITVSKIEDGFQVIRSKPKPLAAYL 94  
 gi|77553820 GQRDEHQLKIAPTIFMDVPLDSGIMKEEIFGPLLPIITVDKIHESFALINSMTKALAAAYL 120  
 GDI\_E\_37  
 RGMNRNLHQLSYWMFQMMQWCKKRYLQGCQSSLKTKIALASNLSQNLLLLISLQTMSSRR 120  
 : : : : . : ..\*.\*\* : : : : :

gi|7270374 FTNNKELEKQFVQDVSAGGITINDTVLHVTVKDL PFGGVGESGIGAYHGKFSYETF SHKK  
 180  
 gi|83701643  
 FTDNKVLQNR FVENVSAGGMGINETVLHVTLKDL PFGGVGESGIGAYHGKFSYETF SHKK 154  
 gi|77553820  
 FTKDSKLQEQYEA AISAGMLVNDTAVHLTNQYLPFGGVGESGMGAYHGRFSFEAF SHKK 180  
 GDI\_E\_37 HMWKIYLLEG-CSSM TLSYMLRLVVC LLEELKKVEWDVTTGSSLLIVSAIRSLSSI---- 175  
 . . \* : : : : : : : : : : \* : . \* : :

gi|7270374 GVLYRSFSGDADLRYPPYTPKKMVLKALLSSNIFAA ILAFFGFS 225  
 gi|83701643 GVLYRSFDGSDLRYPYTPPEKRVL KALLSSDIFGAILAFFGFS 199  
 gi|77553820 AVLVRRFAGEAAARYPPYSPAKLKILRGV LKGNLGAMIKAILGF- 224  
 GDI\_E\_37 EVLMQIHPG-----SLHIHPRRKNCR PFSVATLFAFLCLDG-- 212  
 \*\* : \* : : : : : : : \*

gi|7270374 : putative aldehyde dehydrogenase [*Arabidopsis thaliana*]

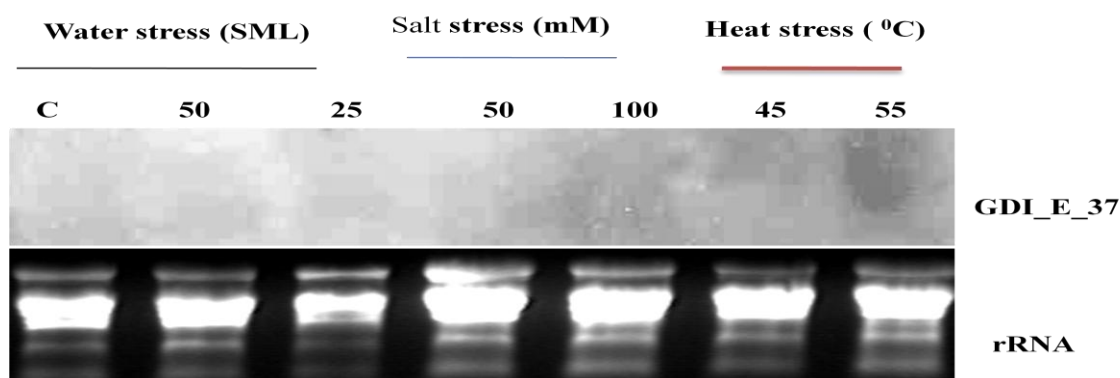
gi|83701643 : putative aldehyde dehydrogenase [*Brassica rapa*]  
 gi|77553820 : aldehyde dehydrogenase, putative [*Oryza sativa*]

Determination of nucleotide sequence, deduction amino acid sequence and homology analysis of GDI\_E\_37

### 3.2. Expression analysis of Aldehyde dehydrogenase (ALDH) under different abiotic stress conditions through Northern blot analysis.

The important molecular response of plants to stress is expression of stress-responsive genes. In this way in the present study we analyzed expression analysis of Aldehyde dehydrogenase (GDI\_E\_37) under different abiotic stress conditions by Northern blot analysis. Total RNA obtained from leaves of control (100% SML) and various abiotic stress treatments such as water stress (at 50 and 25% SML), NaCl stress (50 and 100 mM), and heat stress (45°C and 55°C for 12 h) and transferred on to nylon membranes and hybridized with labeled selected clone (GDI\_E\_37) such as EC391300 (Aldehyde dehydrogenase). Northern blot analysis revealed that Aldehyde dehydrogenase gene was upregulated with increasing specific stress conditions and share common gene expression due to water, NaCl and temperature stresses studied (Fig. 2).

**Figure 2.** Northern expression analysis pattern of groundnut drought induced aldehyde dehydrogenase (GDI\_E\_37) under different abiotic conditions. Total RNA isolated from leaves of 19-day-old groundnut plants under control (C) and different abiotic stress treatments water stress (Control, 50 and 25 (SML); Salt stress 50 and 100 mM; Heat stress (45°C and 55°C for 12 h) was resolved on gel and transferred onto nylon membrane. Blots were hybridized with <sup>32</sup>P labeled cDNA probe. rRNA used as loading control as shown in the panels below each blot.



## IV. Discussion:

Aldehyde dehydrogenases (ALDHs) play a major role in the detoxification processes of aldehydes generated in plants when exposed to abiotic stress. Aldehyde molecules are common intermediates in most cellular pathways such as carbohydrate, amino acid, protein, lipid or steroid metabolism [17, 16]. However, when produced in excessive amounts, they can have detrimental effects on cellular metabolism because of their chemical reactivity [32]. Aldehydes can cause genotoxic effects (i.e. chromosomal aberrations and DNA adducts) [33, 34, 35], lipid peroxidation resulting in the loss of membrane integrity or modification of proteins subsequently causing cellular and developmental arrest [32, 36, 18, 37]. On the other hand, recent findings suggest that some aldehydes may also have a signaling function [38]. Therefore, maintaining the in vivo concentration of reactive aldehydes at well-balanced, non-toxic levels is crucial for organisms.

Several workers reported that the roles of ALDHs in various organisms [39, 40, 41]. The first plant ALDH3 genes were isolated from the desiccation-tolerant resurrection plant *Craterostigma plantagineum* (Cp-ALDH) and *A. thaliana* (ALDH3I1 and ALDH3H1) [41]. After then several plant ALDH genes have been reported to be activated by environmental stress such as dehydration, salinity or excessive light by [24]. Accumulation of aldehydes in plants in response to abiotic stress leads to the production of reactive oxygen species (ROS) such as singlet oxygen, hydroxyl radical, superoxide and H<sub>2</sub>O<sub>2</sub> [23]. Under excessive generation of ROS, the capacity of the electron transport chain exceeds the consumption of reduction equivalents delivered to the stroma side of the chloroplastic thylakoid membranes leading to oxidative stress/damage [42]. The molecular relationship of all ALDH genes was examined recently in *Arabidopsis thaliana* [27]. The over expression of ALDH3I1 in transgenic *Arabidopsis* against abiotic stress was reported [19]. Above all works support that Aldehyde dehydrogenase (ALDHs) genes were upregulated with abiotic stress. Similarly our study clearly indicates that the Aldehyde dehydrogenase (GDI\_E\_37) was expressed with increasing stress than control samples. More over it was expressed maximum at 25% soil moisture level and higher salt (100mM) and

heat stress levels (55°C). These results will lead to better understanding of the biological functions of the aldehyde dehydrogenase in plants under abiotic stresses.

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