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Gene Expression of Dehydrin (DHN1) Gene From Genotypes of Maize And Enhances Drought Tolerance Stress

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Abstract: *Maize is very important crop which is mainly used for food and human consumptions as well as wide adaptation to biotic and abiotic stress such as drought , heat , diseases and insects tolerant .It's can be used to stabile yield under growing conditions change when exposed it to extreme droughtstress. This is very big problem for maize genotypes, especially in tropics and subtropics areas. This study has been designed and carry out in greenhouse of university Texas A&M, USA to investigate the effects of three period of irrigation on DHN1gene expression and increasing of the drought tolerance in maize genotypes under this study. The chlorophyll content trait shown higher more than others genotypes in the Pioneer31B13 (52.23 ,46.02 and 41.11) respectively for three periods of irrigation. Whilethe highest number of stomatal conductance shown in the Dekalb6867 in the second third period (63. mmol.m-2. s-1). The increasing of number of stomatal conductance with the increasing of the period of irrigation and followed that increasing of the leaf temperature with increasing of the same of periods. The genotypes of Pioneer31B13 observed stabile across the three periods of more than others of genotypes and gave values (90.81,73.17 and 63.12 gm per individual plant) for three periods of irrigationrespectively.Also, the Pioneer31B13 has gave (2040 fold of expression) to drought stress more than others under the same periods of irrigation because of the nature of the genetic modified for the climate changes in USA. we concluded that the Pioneer31B13 would be eligible genotype to derived theinbreeds to be more efficient for producing cultivars with converge between high yield and stability under prone environmental factors like drought, heat and sufficient of nutrient element.*

Keywords: *gene expression,drought, Maize*

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I. Introduction

Corn (*Zea mays L*) is one of the most important cereal crops which is mainly used food,livestock feed, forage and biofuel (Kumar et al, 2005).Maize is well adapted to different abiotic stresses that which including heat and drought, heat,moisture,wind and other environmental conditions. Also biotic stresses such as diseases, insects and viruses (Setimela et al, 2005). The change in environmental climate lead to significant loss of yield. It's mainly the plant production is adversely affected by various biotic and abiotic stress (Kaini , 2013) . In many countries of the world, most of maize genotypes are grown in dry condition. The amount of water from rainy not enough to growing conditions for maize to both seasons spring and autumn. Over period of years it is observed that the regions of maize growing experience serve drought in the spring as well as intermittent drought in autumn, and grain yield reduction from 10 to 40 depending on servity and time of drought through development stage of plant cycle life (Loffler et al,2005).Water deficit stress is major lethal factor at all stages of plant and curbed of growing and plant productivity (Coballero et al, 2005). Drought stress causes physiological changes which include change in evapotranspiration via closing of the stomata that which lead to reduce of carbon assimilation and eventually, loss of total dry matter (Demir et al, 20006).Drought stress is one of abiotic stress, which that effects on the all of stage of plant by decrease of chlorophyll content and carotenes in plant tissues (Kuroda et al., 1990). Plant breeders divided the genotypes of wheat into groups depend on the chlorophyll content and Carotenoids (resistant and sensitive) and they are reported that the tolerant genotypes have ability to maintain chlorophyll pigments under stress (Zaeifizade and Goliov ,2009). The use of gene expression concept and molecular work will provide new information about tolerant and intolerant crop andaccelerate to improve genotypesbecause gene expression technique rapidly made the researchers to determinate the level and timing of activation of these genes during development of plant stage and response to various environmental factors through the life cycle crops(Zhang et al ,1999).The main function of dehydrin genes to protect cells and tissues from damages caused by drought stress, which is induced dehydration and play major roleto prevent the protiens from aggeragation and maintain cell membranes from damages (Erikson and Harryson ,2011). The aim of this study to isolated DHN gene of genotypes of maize and elucidate the drought tolerance in these genotypes.

II. Material And Methods

Four genotypes of maize were sown on 1 April 2016 under three periods of irrigation (4 , 8, 10 days) with thee pots for each genotype . the experiment was conducted in greenhouse university of Texas A&M, USA. This experiment was carry out according to RCBD as factorial experiment, the first factor was maize genotypes and the second factor was the periods of irrigation. pots were placed in the green house under condition 35 c°. when the seedlings reached 8 leaves, the period of irrigation were applied. The amount of water of each plot were (77, 38.5 ,30.8Liter from water irrigation) to the three period of irrigation respectively . Samples were taken at flowering stages 17 ,22, and 29 June , then grinned with Liquid nitrogen (-280 c°) by using a morter and pestle in order to get 100 mg from each genotypes. The amount of 100 mg were placed into 2 ml micro centrifuge tube in order to extract the RNA using RNasemini kit(Qiagen/USA) . Then use 2 µl from RNA in a reaction to reach the volume 20 µl . The solution of this reaction including (10 µl RT buffer ,1.5 µl enzyme and 4 µl from RNA and 4.5 µl RNase free water) to complete the solution to 20 µl. The solution placed in the centrifuge to eliminate the air bubbles. the solution incubated in the thermocycler at 37 c° for one hour. Then the reaction was stopped via the temperature to 90 c° for 5 minutes .The samples were storied at -80 c°to the use of RT -PCR . The primer was designed by Blast software as following DHN1 :forward primer1: CTGGGTTTGAGCACGGCAT reverse CTTCCTCCTTCCGCCCATAC forward primer 2 :GCAGCATGGAGTACGGTCAG Reverse primer: TTCCACCCATGCCAGACT. The standerd primer was 18s rRNA forward : TGTGCCGCTAGAGGTGAAATT revers: TGGCAAATGCTTTCGCTT.And then the reaction of Real time PCR was performed by using 10 µl of SYBR green Kit and 1 µl from each primer and 0.3 from reverence dye and 8 µl from the RNase free water according(Agilent technologies kit/USA . The thermos cycle was applied 1 minute at 95 ° (1 cycle) , 5 seconds at 95 °c and 60 ° c at 15 cycles . eventually the data was used to account the fold of gene expression (wenjiang et al,2006).

III. Results

Photosynthesis and cell division are reduced by water deficit stress, therefor. according the data in Table (1). It has shown that chlorophyll content is more affected by decreasing of period irrigation from 4 to 10 days and its varied among all genotypes under this study. the genotype pioneer 31B13 shown the highest significant differences in chlorophyll index between first and third period of irrigation (52.23, 46.02 and 41.0 SPAD respectively). while the genotype TS8811 gave the lowest significant in chlorophyll content under three periods of irrigation (37.0, 35 and 30.01 respectively). The effect of drought on stomatal conductance muddles through restriction of the CO2 moleculesfrom diffusion into chloroplasts and lead to determinate the number of stomatal opening, Therefore. It has shown that number of stomatal conductance significantly increased from 8 t0 10 days in all genotypes under study. However, the lowest of number of stomatal conductance was observed in leave of genotype pioneer 31B13 (10 mmol m2.s-1) . while the highest value (46 mmol m2.s-1) in genotype Asgrow RX913 . Generally, the genotype Asgrow RX913 showed the higher stomatal conductance across all period of irrigation (Table 1) . Normally. the number of stomatal conductance reduce the process of respiration and lead to increase of heat leave (Table 1). It has shown the heat leave was increased by period of irrigation, and lead to prove that heat leave correlated with stomatal conductance positively. The genotype of maize Asgrow RX913 in the two period of irrigation appeared the highest value of photosynthetic active radiation(PAR) about 57.21 µmol.m-2.s-1 .while the genotype Pioneer BIB13 gave the value 30.97 µmol.m-2.s-1 under duration three of irrigation Table(1).

Table 1. The Effect Of Duration Of Irrigation On Chlorophyll, Stomata Conductance And Leaf Temperature In Maizegenotypes.

Duration of irrigation	**Genotypes**	**Chlorophyll index**	**Stomatal conductance (mmol $m^{-2}.s^{-1}$)**	**Leaf temperature**	**Photosynthetic active radiation(PAR) µmol.$m^{-2}.s^{-1}$**
4	Pioneer31B13	52.23	10.0	31.20	43.34
	Dekalb6867	43.44	14.0	25.0	32.65
	Asgrow RX913	47.60	21.0	35.33	35.8
	TS 8811	37.50	18.0	27.23	40.10
8	Pioneer BIB13	46.02	30.0	37.0	34.4
	Dekalb6867	38.34	25.0	30.1	36.20
	Asgrow RX913	34.80	23.0	37.0	57.21
	TS 8811	35.10	38.0	38.2	32.7
10	Pioneer BIB13	41.11	39.0	43.2	30.97
	Dekalb6867	34.00	63.0	39.8	35.20
	Asgrow RX913	27.30	46.0	46.30	41.87
	TS 8811	30.20	51.0	44.7	35.39
L.S.D 5%		6.44	13.10	4.32	4.97

Table 2.The effect of period irrigation on grain weight and individual plant yield in maize genotypes.

Period of irrigation per days	Genotypes	Seed weight (mg)	Yield gm /plant
4	Pioneer31B13 Dekalb6867 Asgrow RX913 TS 8811	282.30 274.01 288.70 264.32	90.81 75.62 80.64 81.64
8	Pioneer31B13 Dekalb6867 Asgrow RX913 TS 8811	271.37 262.00 276.30 242.00	73.17 66.02 71.20 68.16
10	Pioneer31B13 Dekalb6867 Asgrow RX913 TS 8811	263.0 242.00 256.21 234.23	63.12 52.75 59.39 62.98
L.S.D 5%		10.75	6.74

Table 2. showed grain weight and individual plant yield. It is obvious that the most component of yield decreased as the period of irrigation increases from 4 to 10 days in all genotypes. However, the decreasing was significant where the genotype pioneer 31B13 gave 282.30, 271.37 and 263.0 mg of the grain weight in three periods of irrigation respectively. While the genotype TS8811 gave the lowest means across the three periods of irrigation 264.32, 242 and 234.23 gm for grain weight. it was pellucid that the genotype Pioneer31B13 was superior genotype compared with others under all periods of irrigation. The data presented in Table 2 . shown that the yield of plant decreased with increasing of periods of irrigation. the significant difference between the genotypes was observed under all periods of irrigation where the genotype Pioneer31B13 gave the highest value (90.8 gm per plant under 4 days of period of irrigation in comparison with the genotype of Dekalb6867 under 10 days which gave the lowest value (52.75 gm per plant). This result depends on the role of chlorophyll content in maize which correlated with increasing of yield per plant. The observation that done on the genotype of Pioneer31B13 was much better than others in all period of irrigation under study. Therefore, the high yield of the genotype Pioneer31B1 might reflect the high chlorophyll content and number of stomatal conductance in leave of plant. In this study, water deficit stress -induced the DHN genes, that which is isolated and tested in term of gene expression in well-watered and stressed maize. the expression of this gene increased in stress plants. Therefore, the DHN gene is notified to express about drought tolerance in plant of maize in flowering stage of plant cycle life. The data presented in Table 3. Shown that the transcripts of DHN gene is higher in Pioneer31B13 under two periods of irrigation and that will lead to overcome from the problems of drought.It's obvious the data shown in Table 3 that DHN genes were expressed in 2048 and 523.84-fold in two periods of irrigation in genotype Pioneer31B13. while the genotype Asgrow RX913 gave 129.84-fold under third period of irrigation comparison with all genotypes across two periods of irrigation.

Table 3. The effect of period irrigation (8 and 10 days) on DHN gene expression.

Period of irrigation per days	Genotypes	Gene expression (fold) for primer 1	Gene expression (fold) for primer 2	S. E
8	Pioneer31B13 Dekalb6867 Asgrow RX913 TS 8811	2048 .00 1.5454 2.093 4.134	512.00 4.594 17.508 8.57	254.03
10	Pioneer31B13 Dekalb6867 Asgrow RX913 TS 8811	523.84 2.298 129.84 3.595	125.365 13.288 70.034 221.320	61.63

IV. Discussion

In this study. four genotypes of maize were evaluated under three periods of irrigation. Plant breeders are seeking to invest of establishment green leaf area with high chlorophyll content to maintain the photosynthesis across the stage of plant (seedling and maturity), well as reducing power of production of plant under water deficit stress (Farooq et al.,2009). The characteristics of leave of plant have been relied on the photosynthesis apparatus and yield (Khayatnezhad et al.,2011), and because of the green leaves considered to be one of the component that is determining the filling grain in cereal crops (Ahmadi ,1985). The water deficit stress induce the senescence of leaves in plants, and lead to express of senescence, as well as breakdown of chlorophyll content with the decline of photosynthesis metabolite (Kuroda et al ,1990 ; Zobeyed et al,2005).Late of senescence in plant crops will be enhance fixed carbon and nitrogen remobilizing because of the senescence will impacts on all organs of plant across whole plant life cycle. Its play role in remobilizing and transferring the metabolites into vegetative tissue (cakir ,2004 ;Greziask et al,2006).Water stress affects on several of traits of plant including gas exchange , stomata conductance , CO_2assimaltiom ,xylem embolison ,

cell dehydration and growth of plant (Medina et al ., 1999).The water stress caused inhibition on the photosynthesis process and reduce of CO_2 as well as less of activity photosynthesis via inahibted of enzymes such sa carboxylase and oxygenase(Medina et al ., 2000).The results of this study have confirmed the association between the senescence delay and increasing of number stomatal conductance with grain weight and individual plant yield and this consistent with the finding of (Leuning et al. 1995; Bruce et al. 2002). The drought stress caused change temperature of leaf and in the wide range of the biochemical processes including :photosynthesis ,chlorophyll contents ,electron transports ,CO_2 reduction in mesophyll tissues (Begonia and Begonia ,2002) ,and lead to reduce the carbon fixation ,anatomy of leaves and photorespiration with the high temperature rate, ultimately the plant growth and development relied on the chloroplast because it formed the core of photosynthesis apparatus , in which the light and dark reaction occurred in this organelle and the increased of heat temperature out of optimum limit will caused the disruption of photosynthetic metabolite (Crafts-Brandner andSalvucci , 2002) .The increasing photosynthetic active radiation(PAR) is mainly relied on the chlorophyll pigment content, canopy structure light use , photosynthetic pigment content , LAI and leaf angle .Also all these traits will involve in receive of the light and contributed to formatting total dry matter (Gallo et al,1985.,Ciganda et al , 2009). The increasing of PAR in the genotype Asgrow RX913 more than the others genotypes under three period of irrigation , but the low of yield it because of the efficiency of the transformation from source to sink , ultimately the yield of this genotype low(Teal et al ,2006).

The stay green in maize is correlated with induced of *ipt* gene which is controlled on cytokinin production by delay leaf senescence (Rivero et al al ,2009). which lead eventually to high yield in plants. The results are confirmed that the evaluated of Pioneer31B13 was signicantly better than other genotypes, especially under water deficient stress. The results indicated the response of the Pioneer31B13 genotype in the field conditions and expression of drought tolerance genes where this genotype have 2048 fold expressions to express about the ability to tolerate under drought stress, this result agree with others when they found 366 folds in some of genotypes subjected to drought stress (Dong et al , 1998). A lot of work have been done from breeders to produce genotypes from maize with high chlorophyll content and number of stomata conductance well as investigate the correlated between these traits with yield. Breeders have been used chlorophyll content and number of stomata conductance in selection more than others traits in fields under drought conditions and the found its more difficulty to depend on the yield and its components to select drought tolerant genotype.

A major challengeto conventional plant breeding method for drought tolerance is to identification the effective selection criterion to the tolerant drought cultivar. Several methods of plant breeding and selection criteria relied on the drought index (DTI) includes: stomatal conductance, heat temperature leaf , chlorophyll content index(CCI) and photosynthetic active radiation(PAR) . This approach allows us to use the morphological traits as a rapid screening for the genotypes to identify which genotype is suitable to the extremely drought conditions. Recent studies in gene expression techniques have contribute greatly to best to understanding the genetic nature of varieties and their biochemical pathways under drought stress. Also the changes of the biotic stress, which allow to induce the genes, protiens , enzymes plant of crop and enhance of tolerant to drought stress (Anjum et al,2003). The induce of DHN genes will enhance to produce the reactive oxygen species (singlet O_2), super -oxide(OH) and hydrogen peroxide(H_2O_2) , lead to elevent the damage on the membrane lipid , chloropyll proteins and decrease of the photosynthesis functions in plants (Asada , 2006). The studies are focused to determinate the partitioning of the assimilate products from sink to source, that which lead to increasing of tolerant in crops under water deficit stress, and application of the plant genomics with gene expression will help to improve the the inbeds , cultivars and synthetic varities with economic yield under drought stress.

References

[1]. Ahmedi N.1985. Plant physiology (photosynthesis and nutrition. First Publication. pp14-16.

[2]. Anjum F.,M.yaseen , E.Rasul , A.Wahid , Anjum. 2003b .Water stress in barely (Hordeum Vulgare L.).II.Eeffect on chemical composition and chlorophyll contents .pak.J.Agric .Sci.,40:45-52.

[3]. Asada K.2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions . plant physiol 141:391-396.

[4]. Begonia, G. B and M.T.Begonia. 2007. Plant photosynthesis production as controlled by leaf growth, phenology, and behavior. photosynthetica 45:321-333.

[5]. Bruce, W. B., Edmeades, G. O., and Barker, T. C. (2002). Molecular and physiological approaches to maize improvement for drought tolerance. Journal of Experimental Botany, 53(366), 13–25.

[6]. Caballero, J.I., C.V. Verduzco, J. Galan and E.S.D. Jimenez, 2005. Proline accumulation as a symptom of drought stress in maize: A tissue differentiation requirement. *J. Exp. Bot.,* 39: 889–897.

[7]. Cakir, R. (2004). Effect of water stress at different development stages on vegetative and reproductive growth of corn. Field Crops Research, 89(1), 1–16

[8]. Ciganda V., A.A.Gitelson and J.Schepers .2009. Non -destructive determination of maize leaf and canopy chlorophyll content. J Plant physiol 168;157-167.

[9]. Crafts-Brander, S.J.,M.E.Salvucci . 2002. Senesitivity of photosynthesis in a C_4 Plant maize to heat -plant physiol.129:1773-1780.

[10]. Demir, A.O., A.T. Goksoy, H. Buyukcangaz, Z.M. Turan and E.S. Koksal, 2006. Deficit irrigation of sunflower (*Helianthus annuus* L.) in a sub-humid climate. *Irrig. Sci.,* 24: 279–289

[11]. Dong L-Y, Masuda T, Kawamura T, Hata S and Izui K (1998) Cloning, expression, and characterization of a root-form phosphoenolpyruvate carboxylase from Zea mays: comparison with the C4-form enzyme. Plant Cell Physiol 39: 865–873.
[12]. Erriscson ,S.K and Hayrryson P.D.2011 .Molecular Biology, structure and function ,In :Luttege U,Beck E.Bartels D.B.(eds) plant dessication tolerance .springer –verlag ;pp :289-305.
[13]. Farooq M., S.M.A.Basra , A. Wahild , Z.A.Cheema ,M.A.Cheema ,A.Khaild .2008.Physiological role exogenously applied glycinebetaine in improving drought tolerance of fine grain aromatic rice (oryza sativa). J.Agron.Crop .Sci., 19: 325-333.
[14]. Gallo K.P, C.S.T. Daughtry and M.E. Bauer. 1985. Spectreal estimation of absorbed photosynthetically active radiation in corn canopies. Remote Sens Environ 17: 221-232.
[15]. Gamon J.A, J.A.Barrey .2012.Facultative and constitutive effects on the photochemical Reflectance Index(PRI) in sun shade conifer needles .Plant sci.60:85-95.
[16]. Grzesiak M.T,S.Grzesiak , A. Skoczowski.2006.Change of leaf water potential and gas exchange during and after drought in triticale and maize genotypes differing tolerance .Photosynthetica 44:561-568
[17]. Kamar R., J.Kamul , R.B.Dubey , N.Singode ,G.K.Chikka , A.Manivannan and M.K.Denath. 2015. Assessment of drought tolerance in maize (zea mays L) based on differnet indices .Journal of Breeding and Genetics 47 (3) :291-298.
[18]. Khayatnezhad M., R.Gholamin ,S.H.Jamaatie-e-Somarin ,R.Zabihie-Mahmoodabad .2011.The leaf chlorophyll content and stress resistance relationship considering in corn cultivars (Zea mays) . Adv.Environ .Biol.,5(1):118-122.
[19]. Kiani M. 2013 .Screening drought criteria in maize .Asian Agric .Rual Dev.3(5) :290-295
[20]. Kranz, W. L., Irmak, S., Van Donk, S. J., Yonts, C. D., and Martin, D. L. (2008). Irrigation Management for Corn. Neb Guide, University of Nebraska, Lincoln.
[21]. Kuroda M, Qzawa T, Imagawa H (1990). Changes in chloroplast peroxidase activities in relation to chlorophyll loss in barley leaf segments. Physiologia plantarum, 80: 555-560.
[22]. Kuroda M., T.Qzawa , H.Imagawa . 1990. Change in chloroplast perioxidase activaties in relation to chlorophyll loss in barely leaf segments .physiologia plantarum ,80:555-560.
[23]. Leuning R , F.M .Kelliher , D.C.G. de pury and E.D.Schulze .Leaf nitrogen , photosynthesis , conductance and transpiration scaling from leaves canopies .plant cell ,Environmental 1183-1200.
[24]. Loffler C.M ., J.Wai , T.Fast J.Gogerty , S.Langton , M.Bergman ,B.Merrill and M.Cooper . 2005. Classification of maize environment using crop simulation and Geographic information systems . Crop Sci. 1708- 1716.
[25]. Media C.L., E.C.Machodo ., M.M.A Gomes .1999. Stomata conductance, transpiration and photosynthesis rates in Valencia orange tree submits to water stress Braz.J.plant physiol: 11:29-34.
[26]. Medina C.L., E.C.Machado ., J.M.Pinto 1998.photosynthesis of Valencia Orange tree grafted on four rootstocks and submitted to water deficit stress. Bragantia 57:1-14.
[27]. Rivero RM, Shulaev V, E. Blumwald .2009. Cytokinin-dependent photorespiration and the protection of photosynthesis during water deficit. Plant Physiol. (150):1530-1540.
[28]. Setimela P., Z.Chitalu ., J.Jonazi ., A.Mambo , D.Hodson , M.Banziger .2005. Environmental Classification of maize -testing sites in the SADC regions collaborative maize breeding stratagies in the subcontinent . Ephytica 145:123-132.
[29]. Teal R.K., B.Tubana, K.Girma , K.W.Freeman ,D.B.Arnal ,O.Walsh, W.R.Rauna. 2006. In season predication of corn grain yield potential using normalized difference vegetation index. Agron .J. 98: 1488-1494.
[30]. Vina A., A.A.Gitelson .2005.New development in the remote estimation fraction of absorbed photosynthetically active radiation in crops .Geophys Res Lett 2005:32 :L17403.
[31]. Winjaing F., Jianbo H. , T.Spencer .,R.Carrol , G.Wu.1999. Statistical models in assessing fold change in Real time RT-PCR experiments .Computing Biology and chemistry 30:21-26.
[32]. Zaeifizade M, Goliov R (2009). The Effect of the Interaction between Genotypes and Drought Stress on the Superoxide Dismutase and Chlorophyll Content in Durum Wheat Landraces. Turk. J. Boil., 33: 1-7
[33]. Zhang ,J.,N.Y.Kleueva and H.T.Nguyen .1999.plant adaptation and crop improvement for arid and semiarid environments .In .I.R.Trayor ,H.Dregne and K.Mathis (eds).proceeding of the fifth international conference on Desert Development:The Endless fronter ,12-17 Aguest 1996 .Lubbock ,TX.(USA);Texas Tech University .Vol .II.Pp.604-620.
[34]. Zobayed S., T. Afreen , T. Kozai .2005. Temperature stress can alter the photosynthetic efficiency, and secondary metabolite concentration in St. Johanwort. Plant Physiol:Bioch., 43:977-984.

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