Genetics and Plant Physiology – 2014, Volume 4 (3–4), pp. 182–190
Special Issue (Part 2) – Conference "Plant Physiology and Genetics – Achievements and Challenges"
24 - 26 September 2014 – Sofia, Bulgaria

# EVALUATION OF TOLERANCE TO OSMOTIC STRESS OF EMMER GENOTYPES (*TRITICUM DICOCCON* SCHRANK) USING INDIRECT PHYSIOLOGICAL METHOD

***Chipilski R.*[*], *B. Kyosev, G. Desheva***

*Institute of Plant Genetic Resources "Konstantin Malkov", Druzhba str. 2,*
*4122 Sadovo, Bulgaria*

Received: *16 September 2014* Accepted: *17 March 2015*

**Summary:** Thirty eight emmer genotypes (*Triticum dicoccon* Schrank**)** were evaluated with regard to their tolerance to osmotic stress. Evaluation was made by applying the indirect physiological method, recognizing the growth depression in seedlings cultivated in solution with increased osmotic pressure (atm). The osmotic stress induced by adding 0.5 M and 1 M solution of sucrose after germination, inhibited seedling growth in all genotypes studied. Positive and significant at 0.05 and 0.01 level correlations between the two osmotic concentrations in root (r=0.386) and shoot (r=0.757) were observed. On the basis of the obtained common average values, a negative regression dependence between the growth of root/shoot and the solution concentration with increasing osmotic pressure was established. The most tolerant genotypes to osmotic stress were characterized by a low depression of root/shoot growth. The accessions BGR32748, BGR17310, BGR31904, BGR 22611 and BGR32746 demonstrated the best ability of osmotic regulation.

**Citation:** Chipilski R., B. Kyosev, G. Desheva, 2014. Evaluation of tolerance to osmotic stress of emmer genotypes (*Triticum dicoccon* Schrank) using indirect physiological method. *Genetics and Plant Physiology*, Conference "Plant Physiology and Genetics – Achievements and Challenges", 24-26 September 2014, Sofia, Bulgaria, Special Issue (Part 2), 4(3–4): 182–190.

**Keywords:** Depression coefficient; drought tolerance; osmotic stress; emmer.

**Abbreviations:** Atm – atmosphere; M – molarity; OA – osmotic adjustment.

## INTRODUCTION

Drought is the major factor limiting crop productivity world-wide and cultivating plants with increased resistance to this stress appears critical to keep yields at a sufficient level (Peleg et al., 2005). The screening of such cultivars, based on their productivity, is a long and tedious task that would undoubtedly be accelerated if traits that could be reliably related to water stress were identified. Drought resistance is the result of various morphological, physiological and biochemical characteristics. Its genetic improvement in crop plants

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[*]Corresponding author: radotch@abv.bg

requires the identification of appropriate drought resistance mechanisms and particularly the development of suitable methodologies for their measurement in large breeding populations (Bajii et al., 2000). Osmotic adjustment (OA) is considered to be an important component of drought tolerance mechanisms in plants (Zhang et al., 1999). According to Blum et al. (1996) OA is usually defined as a decrease in cell sap osmotic potential resulting from a net increase in intracellular solutes rather than from a loss of cell water. Plants under different environmental stresses accumulate low molecular weight organic solutes generically termed as compatible solutes, which include amino acids and sugars. In addition to these organic substances, some inorganic solutes are also a significant fraction of the osmotically active solutes present in plant cells (Zhang et al., 1999). Two indirect methods for osmoregulation measurement: a method for measuring the coleoptile length in seedlings exposed to osmotic stress and a method for measuring the osmotic regulation in the pollen grains (Morgan, 1988; Morgan, 1999) have potential to substitute the complicated physiological methods. The method for coleoptile growth measurement under water deficit developed by Morgan (1988) is based on the fact, that genotypes with better potential for osmoregulation are able to maintain better turgor and associated physiological processes, such as a more intensive cells increase in response to water deficit. The above maintained indirect methods have been recently used in drought resistance studies (Moud and Yamagishi, 2005; Eivazi et al., 2007; Moud and Maghsoudi, 2008). Genotypic differences in terms of osmoregulation ability have been reported in various crops. Significant variations in this trait were observed in wheat (Morgan 1983; Blum et al., 1996), sunflower (Jamaux et al., 1997), shorgum (Ackerson et al., 1980), millet (Henson, 1982), rice (Lilley and Ludlow,1996; Babu et al., 1998), barley (Blum 1989, Ganusheva et al., 2011) and wild species from Gramineacea (Bozhanova et al., 2005; Uhr et al., 2007).

The objective of this study was to evaluate emmer accessions (*Triticum dicoccon* Schrank) in terms of their tolerance to osmotic stress, by using the indirect physiological method.

## MATERIALS AND METHODS

The study was conducted in the Physiological laboratory at the Konstantin Malkov Institute of Plant Genetic Resources in Sadovo, Bulgaria. Thirty eight accessions of emmer (*Triticum dicoccon* Schrank) were investigated. The accessions are maintained in the *ex-situ* field collection in IPGR-Sadovo. Katya variety was used as a standard of drought tolerance. It was defined as a standard variety according to international studies in drying conditions under CIMMYT, Turkey and ICARDA-Syria.

The reactions of roots and shoots to two levels of osmotic stress were estimated by applying the method of Bozhanova (1997). Seeds from all genotypes included in the research were sterilized and put for germination on wet filter paper in Petri dishes with 20 ml distillated water in a thermostat for 72 h at 25°C, in the dark. After germination the seedlings were divided in three variants:

1. Control-seedlings were kept in distilled water;
2. Moderate osmotic stress-seedlings were transferred to 0.5 M solution of sucrose, which provoked osmotic stress with pressure of 12.23 atm;
3. Strong osmotic stress-seedlings were transferred to 1 M solution of sucrose, which provoked osmotic stress with pressure of 24.45 atm.

The seedlings from all variants were put on wet filter paper turned to rolls, which were put in a thermostat for 48 h at 25°C. The lengths of roots and shoots were measured in cm. The biometrical measurements were carried out on 20 seedlings per accession.

The osmotic pressure of the sucrose solution was calculated according to Todd Helmenstine (http://chemistry.about.com/od/workedchemistryproblems/a/Osmotic-Pressure-Example.htm).

Osmotic pressure, atm = iMRT, where:

i – van't Hoff factor of the solute;

M – molar concentration in mol/L;

R – universal gas constant = 0.08206 L atm/mol K;

T – absolute temperature in K.

The coefficient of depression was calculated according to Blum et al. (1980):

Coefficient of depression,

% = [(A-B)/A] x 100, where:

A – average length of roots /shoots in the control variant, cm;

B – average length of roots/shoots in the osmotic stress variant, cm.

The data were processed by the method of correlation and regression analysis (Lidanski, 1988). Statistical analyses were performed using the statistical program SPSS 13.0 and Statistica-6.

## RESULTS AND DISCUSSION

The osmotic stress induced by adding solution of sucrose applied after germination at concentrations of 0.5 M and 1 M inhibited the growth of seedlings from all genotypes studied.

The average coefficient of shoot growth depression was 26.8 % in the trial with moderate osmotic stress and 37.9 % in the trial with strong osmotic stress, while for roots these values were 26.4 % and 38.2 %, respectively (Table 1). According to Marcheva et.al. (2013) and Chipilski et al. (2014), water deficit influenced to a greater extent the roots of young seedling of *Triticum durum* Defs. and *Triticum aestivum* L., while other authors (Bozhanova and Dechev, 2010; Bozhanova and Hadzhiivanova, 2010; Ganusheva et al., 2011) established an opposite trend for *Triticum, Aegilops and Hordeum* species.

When applying moderate osmotic stress to young wheat plants the coefficient of shoot growth depression ranged from 1.44 % for BGR26767 to 48.08 % for BGR30015, while the value for the depression coefficient in the standard variety Katya was 47.99 %. Depression of root growth ranged from 4.84 % for BGR30039 to 47.93 % for BGR30015, and all accessions except for BGR19034, BGR26765, BGR19038 and BGR30039 showed lower values for the coefficient of root growth depression in comparison with the standard variety Katya.

In response to strong osmotic stress, the coefficient of shoot growth depression ranged from 15.16 % for BGR22611 to 52.61 % for BGR19038 whereas for root growth depression the coefficient values ranged from 24.40 % for BGR30016 to

**Table 1-1.** Response to osmotic stress of 38 emmer genotypes.

| Accession | Root length [cm] | | | Shoot length [cm] | | | Depression coefficient [%] | | | | Average depression coefficient [%] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0M sol. sucrose | 0.5M sol. sucrose | 1.0M sol. sucrose | 0M sol. sucrose | 0.5M sol. sucrose | 1.0M sol. sucrose | 0.5 M sol. sucrose | | 1.0 M sol. sucrose | | 0.5M sol. sucrose Seedling | 1.0M sol. sucrose Seedling | Average of Seedling |
| | | | | | | | Root | Shoot | Root | Shoot | | | |
| Katya-st | 10.62 | 6.72 | 5.96 | 7.75 | 4.03 | 3.78 | 36.72 | 47.99 | 43.93 | 51.20 | 42.35 | 47.57 | 44.96 |
| BGR19047 | 8.11 | 5.86 | 4.31 | 7.36 | 6.57 | 4.56 | 27.76 | 10.73 | 46.83 | 38.04 | 19.25 | 42.44 | 30.84 |
| BGR30039 | 6.32 | 6.01 | 2.53 | 6.99 | 5.94 | 3.51 | 4.84 | 15.05 | 59.90 | 49.74 | 9.94 | 54.82 | 32.38 |
| BGR30018 | 4.39 | 3.70 | 2.46 | 5.75 | 4.44 | 4.20 | 15.83 | 22.81 | 44.00 | 27.01 | 19.32 | 35.51 | 27.41 |
| BGR30017 | 9.24 | 6.16 | 5.22 | 8.84 | 6.84 | 4.94 | 33.32 | 22.61 | 43.47 | 44.10 | 27.97 | 43.79 | 35.88 |
| BGR26766 | 7.97 | 5.11 | 4.87 | 7.75 | 5.68 | 5.32 | 35.90 | 26.70 | 38.98 | 31.46 | 31.30 | 35.22 | 33.26 |
| BGR26765 | 8.69 | 5.21 | 4.22 | 7.85 | 5.71 | 4.88 | 40.07 | 27.22 | 51.46 | 37.80 | 33.65 | 44.63 | 39.14 |
| BGR26767 | 7.12 | 5.94 | 4.41 | 5.92 | 5.84 | 4.08 | 16.59 | 1.44 | 37.98 | 31.00 | 11.39 | 36.16 | 23.77 |
| BGR26764 | 7.24 | 5.76 | 4.50 | 8.02 | 5.92 | 4.42 | 20.48 | 26.15 | 37.84 | 44.89 | 23.32 | 41.37 | 32.34 |
| BGR10998 | 7.57 | 6.38 | 5.58 | 7.84 | 6.42 | 4.64 | 15.74 | 18.13 | 26.25 | 40.81 | 16.93 | 33.53 | 25.23 |
| BGR10995 | 8.68 | 7.05 | 6.34 | 7.12 | 5.71 | 4.78 | 18.76 | 19.77 | 26.92 | 32.77 | 19.26 | 29.85 | 24.56 |
| BGR32748 | 8.26 | 6.77 | 6.05 | 7.59 | 6.68 | 5.53 | 18.04 | 12.09 | 26.75 | 27.15 | 15.07 | 26.95 | 21.01 |
| BGR31904 | 6.26 | 5.15 | 4.29 | 5.84 | 5.26 | 4.13 | 17.67 | 9.98 | 31.38 | 29.39 | 13.83 | 30.39 | 22.11 |
| BGR32746 | 6.42 | 5.66 | 3.98 | 5.68 | 4.92 | 4.04 | 11.86 | 13.47 | 37.99 | 28.87 | 12.67 | 33.43 | 23.05 |
| BGR17310 | 7.48 | 6.63 | 5.52 | 5.69 | 4.66 | 3.92 | 11.42 | 18.21 | 26.25 | 31.09 | 14.82 | 28.67 | 21.74 |
| BGR30016 | 7.36 | 5.92 | 5.56 | 7.17 | 4.52 | 4.48 | 19.55 | 36.90 | 24.40 | 37.56 | 28.22 | 30.98 | 29.60 |
| BGR17309 | 6.44 | 4.50 | 4.36 | 6.24 | 5.21 | 4.71 | 30.10 | 16.63 | 32.30 | 24.50 | 23.36 | 28.40 | 25.88 |
| BGR32747 | 7.46 | 5.15 | 5.03 | 7.96 | 4.47 | 4.33 | 30.90 | 43.93 | 32.60 | 45.64 | 37.42 | 39.12 | 38.27 |
| BGR17305 | 8.80 | 6.51 | 6.03 | 7.81 | 5.43 | 4.75 | 25.96 | 30.52 | 31.44 | 39.21 | 28.24 | 35.33 | 31.78 |

**Table 1-2.** Response to osmotic stress of 38 emmer genotypes (continued).

| Accession | Root length [cm] | | | Shoot length [cm] | | | Depression coefficient [%] | | | | Average depression coefficient [%] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0M sol. sucrose | 0.5M sol. sucrose | 1.0M sol. sucrose | 0M sol. sucrose | 0.5M sol. sucrose | 1.0M sol. sucrose | 0.5 M sol. sucrose | | 1.0 M sol. sucrose | | 0.5M sol. sucrose Seedling | 1.0M sol. sucrose Seedling | Average of Seedling |
| | | | | | | | Root | Shoot | Root | Shoot | | | |
| BGR19038 | 7.30 | 4.05 | 3.56 | 8.75 | 5.08 | 4.14 | 44.54 | 41.94 | 51.29 | 52.61 | 43.24 | 51.95 | 47.60 |
| BGR30019 | 5.86 | 4.16 | 3.64 | 5.45 | 4.57 | 3.68 | 28.97 | 16.28 | 37.97 | 32.47 | 22.63 | 35.22 | 28.92 |
| BGR30015 | 12.20 | 6.35 | 5.86 | 9.21 | 4.78 | 4.60 | 47.93 | 48.08 | 51.92 | 50.12 | 48.01 | 51.02 | 49.51 |
| BGR17311 | 7.52 | 6.90 | 5.24 | 6.29 | 4.71 | 4.42 | 8.30 | 25.21 | 30.31 | 29.76 | 16.75 | 30.03 | 23.39 |
| BGR19048 | 9.85 | 7.37 | 6.98 | 8.23 | 5.01 | 4.34 | 25.24 | 39.17 | 29.13 | 47.31 | 32.21 | 38.22 | 35.21 |
| BGR17308 | 8.01 | 5.71 | 5.40 | 7.77 | 4.63 | 4.37 | 28.69 | 40.38 | 32.62 | 43.81 | 34.54 | 38.22 | 36.38 |
| BGR17306 | 10.00 | 6.70 | 6.08 | 8.86 | 5.12 | 4.96 | 32.99 | 42.24 | 39.18 | 44.04 | 37.61 | 41.61 | 39.61 |
| BGR19046 | 9.02 | 6.26 | 6.17 | 7.38 | 4.97 | 4.54 | 30.60 | 32.70 | 31.59 | 38.42 | 31.65 | 35.01 | 33.33 |
| BGR19045 | 9.36 | 6.95 | 5.94 | 6.47 | 5.23 | 4.76 | 25.79 | 19.11 | 36.59 | 26.39 | 22.45 | 31.49 | 26.97 |
| BGR19044 | 6.81 | 5.79 | 3.80 | 6.58 | 4.72 | 4.16 | 14.96 | 28.34 | 44.20 | 36.80 | 21.65 | 40.50 | 31.08 |
| BGR19043 | 10.68 | 6.90 | 5.55 | 7.94 | 5.74 | 4.97 | 35.41 | 27.65 | 47.99 | 37.38 | 31.53 | 42.68 | 37.11 |
| BGR19042 | 9.32 | 6.59 | 5.64 | 9.01 | 5.49 | 4.83 | 29.30 | 39.03 | 39.51 | 46.43 | 34.17 | 42.97 | 38.57 |
| BGR19041 | 6.75 | 4.72 | 4.15 | 5.30 | 4.37 | 3.51 | 29.96 | 17.51 | 38.46 | 33.84 | 23.73 | 36.15 | 29.94 |
| BGR19040 | 9.04 | 6.69 | 5.90 | 8.35 | 5.66 | 5.06 | 26.04 | 32.31 | 34.69 | 39.46 | 29.17 | 37.08 | 33.12 |
| BGR19039 | 8.39 | 5.84 | 4.54 | 6.91 | 4.65 | 4.18 | 30.42 | 32.64 | 45.90 | 39.49 | 31.53 | 42.69 | 37.11 |
| BGR19037 | 8.97 | 6.00 | 4.74 | 8.56 | 5.22 | 4.38 | 33.10 | 39.02 | 47.19 | 48.86 | 36.06 | 48.02 | 42.04 |
| BGR19036 | 9.12 | 6.34 | 6.02 | 8.95 | 5.06 | 5.05 | 30.47 | 43.49 | 34.03 | 43.56 | 36.98 | 38.79 | 37.89 |
| BGR22611 | 9.71 | 6.75 | 5.87 | 5.49 | 5.14 | 4.66 | 30.51 | 6.42 | 39.54 | 15.16 | 18.47 | 27.35 | 22.91 |
| BGR19034 | 9.49 | 5.77 | 5.32 | 7.45 | 4.91 | 4.07 | 39.18 | 34.06 | 43.95 | 45.34 | 36.62 | 44.64 | 40.63 |
| BGR19035 | 7.67 | 5.66 | 5.06 | 6.19 | 5.28 | 4.31 | 26.26 | 14.66 | 34.06 | 30.38 | 20.46 | 32.22 | 26.34 |
| Average | 8.19 | 5.94 | 5.04 | 7.30 | 5.25 | 4.46 | 26.42 | 26.80 | 38.23 | 37.88 | 26.61 | 38.05 | 32.33 |

59.90 % for BGR30039. The coefficient values for shoots and roots in the standard variety Katya were 51.20 % and 43.93 %, respectively.

The average values for the depression coefficients in the genotypes as an expression of the proneness to osmotic regulation at the whole plant level are presented in Table 1 and Fig. 1.

According to the obtained data of the average coefficients of seedling growth depression only BGR 19038 and BGR 30015 showed values higher than the standard variety Katya. These genotypes were most sensitive to osmotic stress as compared to all other samples. Likewise, in the standard var. Katya the estimated value for the coefficient of seedling growth depression was by 12.6% higher than the common average coefficient of depression.

The accessions BGR 32748, BGR 17310, BGR 31904, BGR 22611, BGR 32746 and BGR 17311 showed lower average coefficients of depression in comparison with the common average depression value. These accessions demonstrated the best ability of osmotic regulation.

Table 2 shows the correlation coefficients between some traits (length of root, length of shoot, depression of root length and depression of shoot length) calculated for all molar concentrations. A positive correlation between length of root and length of shoot was found, more pronounced and statistically significant at 0 M and 1 M solution of sucrose, r=0.628 and r=0.532, respectively. The induced osmotic stress caused genotypic differences by reducing the intensity of growth of the seedlings. This was confirmed by the results on depression of root and shoot growth, where positive and significant at 0.05 and 0.01 level

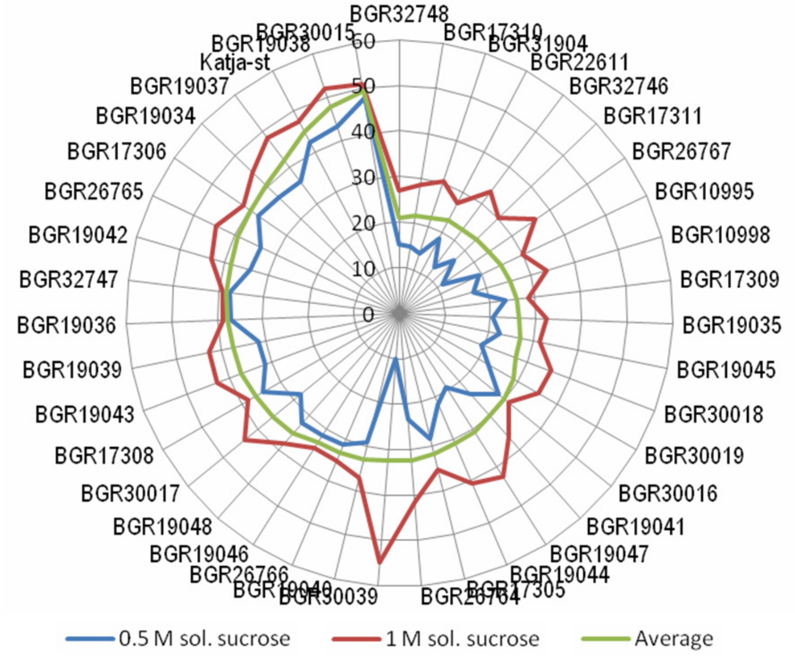

**Figure 1.** Ability for osmotic regulation in 38 emmer genotypes expressed through the average values of depression coefficients at two levels of osmotic stress.

**Table 2.** Correlation between traits at two levels of osmotic stress.

| Traits | Shoot length 0 M | Shoot length 0.5 M | Shoot length 1.0 M | Depression of root at 1 M | Depression of shoot at 1 M |
|---|---|---|---|---|---|
| Root length 0 M | 0.628** | - | - | - | - |
| Root length 0.5 M | - | 0.246n.s. | - | - | - |
| Root length 1.0 M | - | - | 0.532** | - | - |
| Depression of root at 0.5 M | - | - | - | 0.386* | - |
| Depression of shoot at 0.5 M | - | - | - | - | 0.757** |

n.s. – No significant correlation; *– Correlation is significant at the 0.05 level; **– Correlation is significant at the 0.01 level.

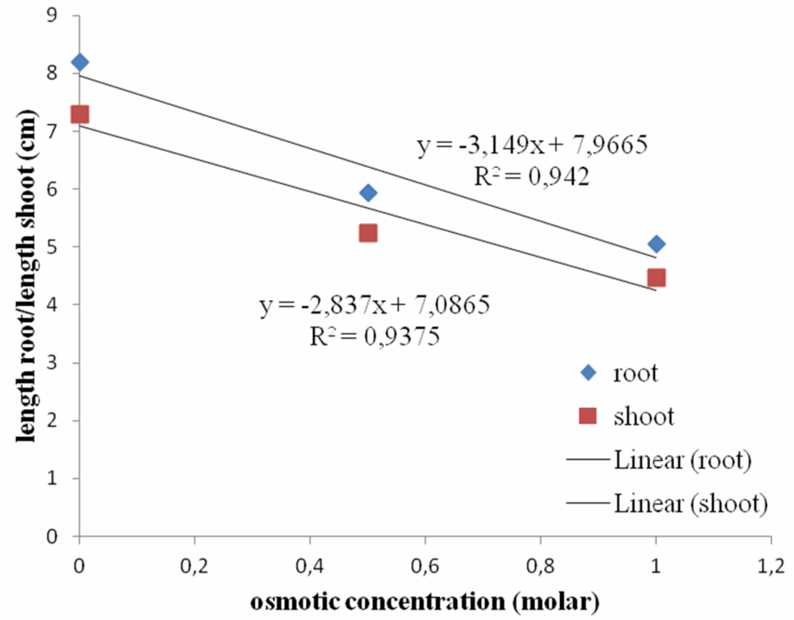

**Figure 2.** Linear relationship between the intensity of seedling growth and the osmotic concentration of the solution.

correlations between the two osmotic concentrations for root (r=0.386) and shoot (r=0.757) were observed.

The linear relationship between the intensity of seedling growth and the osmotic concentration of the solutions is shown in Fig. 2. A negative regression dependence between the growth of root/shoot and the solution concentration with increasing the osmotic pressure was established. The equations confirmed the strong limitation role of the osmotic pressure in seedling growth.

## CONCLUSION

Osmotic stress induced by adding solution of sucrose at concentrations of 0.5 M and 1 M after germination inhibited the growth of seedlings in all genotypes studied. A positive correlation between length of root and length of shoot, more pronounced and statistically significant at 0 M and 1 M solution of sucrose was established. The induced osmotic stress caused genotypic differences by reducing the intensity of seedling growth. A negative regression dependence between the growth of root/shoot and the solution concentration with increasing osmotic pressure was established. The equations confirmed the strong limitation role of the osmotic pressure in seedling growth. The accessions BGR32748, BGR17310, BGR31904, BGR 22611 and BGR32746 demonstrated the best ability for osmotic regulation. Further screening is needed by studying more physiological and agronomical characteristics connected to growth and productivity of plants in response to drought.

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