# Wheat Grains Germination and Seedling Growth Performance under Drought Condition

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**Abstract:** Drought is one of the most critical abiotic factors especially in warm dry areas yielding limited crop. Ten wheat genotypes tested for drought tolerance at germination stage. Polyethylene Glycol-8000 used to induce -1.7 and -3.5 bars osmotic potential as compared to control treatment with three replications in factorial experiment with Completely Randomized Design (CRD). The studied parameters showed a decreasing in style of response to increment of PEG concentration. The lowest Final Germination Percentage (FGP) mean recorded was 73.23 % under -3.5 bars as compared to control. Means of Daily Germination time (MDG), Germination Index (GI) and Coefficient of Velocity Germination (CVG) have been decreased from 4.04, 3.23 and 81.64 at control treatment to 2.46, 1.77 and 69.21 at severe drought level. But the highest CVG; 83.34 recorded by Azady under 160 g/ L PEG. In addition under -3.5 bar treatment lowest means of shoot and root length recorded; 3.34 and 1.92 cm as compared to control treatment. While, lowest shoot weight and whole seedling weight; 0.07 and 0.079 g recorded by Abu-ghreb, but lowest root dry weight 0.008 g was recorded by Adena. While, a significant increase observed in proline content in all genotypes at 160 g PEG-8000/ L medium. Ezz, Sham-6, Azady, Rabeaa and Riagary seedlings' accumulated more proline as compared to Tammuz-2, Adena, Abu-ghreb, Abehade and Ebba-99. According to the studied parameters a dendrogram constructed. The genotypes classified into two groups. Resistant; include Ezz, Sham-6, Azady, Rabeaa and Rizgary. Sensitive; includes Tammuz-2, Adena, Abu-ghreb, Abehade and Ebba-99.

Keywords: PEG, GI, MDG, CVG, Shoot dry weight, Proline.

## Introduction

Agricultural lands about 25% are now affected by environmental stress around the world. Thus Agricultural productivity is subject to crop failure and average yields lose more than 50% (Fathi & Tari, 2016). Drought regarded as one of the most destructive environmental stresses. Its influence depends on the extent, intensity and the development phase (Lamaoui *et al*., 2018). Seed germination is a great trouble for cereal production, because it's vigor to limit the green area per land area in arid and semi- arid regions. Water absorption is the first stage of germination and the absorption rate depends on the chemical component of the seed.

Proteins, mucilage and pectin are more hydrophilic colloid and absorb more water than starch (Fathi & Tari, 2016).

Water availability performs a huge role in activation and reaction of variety of enzymes as well as it has a role in metabolites solubilize and transport and also act as a reagent in hydrolytic breakdown of stored proteins lipids, and carbohydrates in germinated seeds (Biaecka & Kepczynski, 2010). As instance, amylase enzymes hydrolyzing the endosperm starch into simple sugars, which provide the energy for roots and shoots development. Thus the activity of such enzymes is reduced in the absence of water and causes a negative effect on carbohydrate metabolism (Zeid & Shedeed 2006). Ultimately affects delay germination and many aspects of plant growth; limits the root and shoot growth and reduces the reserved dry matter (Shekari et al., 2000).

Selecting drought tolerance genotypes are crucial in dry land areas. As the inducement of drought stress in the field cannot be controlled and not easily carried out. In addition there is no a precise technique for testing huge numbers of genotypes under uncontrollable field condition (Shaheen & Hood-Nowotny, 2005). Seedling development under laboratory conditions has been accepted as a suitable growth stage to proof wheat adaptability under osmotic stress condition. As well as using Poly Ethylene Glycol (PEG) accepted as a suitable method to efficiently test large sets of germplasm with good rigor. For quite a while now, PEG recognized as non-penetrating, sluggish, non-ionic and high molecular weight osmoticum. It can lower the water potential of nutrient solutions without passing or being phytotoxic (Manoj & Uday, 2007). Khakwani et al. (2011) demonstrated that germination is a useful criterion in detecting water stress tolerance. They tested six varieties of wheat; those were tolerant to drought during in vitro germination tests were similarly tolerant in field conditions. Manoj & Uday (2007) used PEG to induce drought stress by reducing water potential results in decreasing germination percentage and uttering seedling growth.

The objectives of the current study are to compare the ability of ten bread wheat genotypes to tolerate drought stress using PEG-8000 as an osmoticum at germination stage. This will provide a theoretical base to improve drought resistance abilities of susceptible genotypes by inserting responsible genes in dryland farming in arid and semiarid regions.

## Materials and methods

An experiment was conducted to study the effects of drought, using PEG-8000 (Poly Ethylene glycol-8000) as an osmotic substrate, on germination indices and seedling growth parameters as well as osmotic adjustment prediction, through determining endogenous proline in ten local bread wheat genotypes; Ezz, sham-6, Azady, Tammuz-2, Rabeaa, Adena, Abu-ghreb, Rizgary, Abehade and Ebaa-99 cultivating in Kurdistan region. The grains of the ten genotypes were obtained from Agriculture research Centers of Erbil, Dohuk and Sulaimania. Grains subjected to two drought stress level of PEG-8000; 80 and 160 g/l to induce -1.7 and -3.5 bars osmotic potential according to IST and compared to control treatment. PEG-8000 prepared by dissolving the required amount of PEG in distilled water at $30°C$. Grains surface sterilized using sodium hypochlorite solution (10%) for 30 seconds. After the treatment, the grains washed two times with distilled water. 10 grains from each genotype germinated on two layers of filter paper in 9cm Petri dishes with respective treatment from PEG-8000. The Petri dishes wrapped well to deny evaporation and moisture loss under laboratory condition ($24±2 °C$) for eight days. Germination of seeds deemed when their radicle elongated of about greater than 2 mm (ISTA, 1999). The experiment organized as factorial test, using a completely randomized design (CRD) with three replications. Least significant difference test (LSD) applied at one percentage level of probability to compare among means as explained by Steel & Torrie (1980). Coefficients of similarity among genotypes calculated according to Sneath & Sokal (1973) based on characteristic features mean values of germination indices

under drought stress. IBM SPSS Statistics version 25 used to analyze the data Dice similarity coefficients calculation; similarity grade converted to distance and used for dendrogram generation with Ward's method (Takezaki & Nei, 1996).

**Studied parameters:**

**Germination indices**

After seed soaking, germinated grains checked each 24 hours within the experiment period to decide the germination parameters. Number of germinated seeds obtained after 8 days as; Final Germination Percentage (FGP) according to (ISTA, 1999), where FGP= $N_g$ / $N_t$ x 100, $N_g$=Total number of germinated seeds, $N_t$=Total number of seeds evaluated. Mean Germination Time, MGT = $\Sigma D$ n/ $\Sigma$ n, Where n is the number of seeds, which were germinated on day D, and n is the days number required for germination, calculated according to Sadeghi *et al.* (2011). Germination Index (GI) calculated by following formula: GI=no. of germinated seed/Days of first count+.....+? No. of germinated seed/ Days of final count (AOSA, 1990). Coefficient of Velocity of Germination (CVG) calculated according to the mathematical manipulation; CVG= $\Sigma Ni$/ $\Sigma NiTi$ x 100, Where Ti is the number of days after sowing, Ni is the number of seeds germinated on *i*th day, and *S* is the total number of seeds used according to Scott *et al.* (1984).

**Growth parameters**

At the end of the 14th day after grains soaking, five seedlings were randomly selected (from each replicate) and traits including shoot length and dry weight, root length and dry weight and total dry weight have been measured. The dry weight (DW) obtained after drying the seedlings for 48 h at 72°C (Bağci *et al.*, 2003).

**Determination of proline content**

Proline (mg/g fresh weight) amounts were determined according to following method of Bates *et al.* (1973). 0.1 g of fresh sample of leaves added in 5 ml of 3% sulfosalicylic acid in a test tube, ground and then allowed to settle. Then 2 ml from supernatant was mixed with 2 ml of glacial acetic acid and 2 ml ninhydrin reagent and was boiled for 1 h in water bath at 100°C. After 1 h, the reaction was stopped in ice and finally 4 ml of toluene was added, vortexed and the absorbance capacity read at 520 nm on the UV Spectrophotometer.

## Results and Discussion

The survival ability of the ten wheat genotypes determined during germination stage as a drought tolerance screening tool using PEG. Data pertain the effect of PEG induced stress on final germination percentage (FGP), Mean of daily germination (MDG), germination index (GI) and coefficient of velocity germination (CVG) is given in Table (1). For all genotypes, the final germination percentage was highest at control treatment and started to decrease as the drought level increased. Azady recorded the highest FGP; 92.29 % compared to Abehade; that recorded the lowest FGP; 78.74 %. The genotypes responded to the osmotic stress treatments owing to their genetic variation that could be utilized to develop new drought adaptable genotype to dry land areas (Chen *et al.*, 2016). The lowest mean of FGP recorded was 73.23% under severe drought stress 160 g/ L PEG effect compared to control. With due attention to combination effect of genotypes x drought levels; 100% FGP recorded by Ezz, Azady, Rabeaa and Rizgary at control treatment. While the lowest FGP; 61.35 % was recorded by Ebba-99 at severe drought stress level. This is consistent with the finding of Liu *et al.* (2015), which showed significant reduction in germination percentage due to PEG treatments occurs as a result of decreasing potential gradient between seeds and their environment. That led to impede the imbibition process and decreased germination percent and seedling viability

For other germination parameters no significant variation observed under the genotype treatment. But under drought an inverse relationship was observed between drought and daily germination time (MDG),

**Table (1): Effect of genotypes, drought levels and their combination on germination indices of eight ten wheat genotypes.**

| Parameters | PEG g/ L | Ezz | Sham-6 | Azady | Tammuz-2 | Rabeaa | Adena | Abu-ghreb | Rizgary | Abehade | Ebaa-99 | Mean | LSD (0.01) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Final germination percentage (FGP) | 0 | 100 | 95.60 | 100 | 97.33 | 100 | 90.34 | 90.12 | 100 | 90.34 | 90.24 | 95.40 | |
| | 80 | 95.33 | 85.23 | 96.44 | 90.43 | 95.12 | 82.45 | 80.34 | 93.56 | 80.33 | 84.66 | 88.39 | 16.21 |
| | 160 | 78.44 | 77.44 | 80.44 | 65.33 | 80.55 | 72.34 | 70.43 | 80.45 | 65.55 | 61.35 | 73.23 | |
| Mean | | 91.26 | 86.09 | 92.29 | 84.36 | 91.89 | 81.71 | 80.30 | 91.34 | 78.74 | 78.75 | LSD (0.01) = 13.54 | |
| LSD (0.01)= 10.23 | | | | | | | | | | | | | |
| Mean daily germination (MDG) | 0 | 4.45 | 4.02 | 4.78 | 3.89 | 4.23 | 3.76 | 3.65 | 4.11 | 3.76 | 3.78 | 4.04 | |
| | 80 | 4.34 | 3.89 | 4.11 | 3.01 | 3.21 | 2.89 | 2.95 | 3.65 | 2.65 | 2.14 | 3.28 | 1.53 |
| | 160 | 4.01 | 3.34 | 3.54 | 2.07 | 2.17 | 1.78 | 1.89 | 3.21 | 1.34 | 1.23 | 2.46 | |
| Mean | | 4.27 | 3.75 | 4.14 | 2.99 | 3.20 | 2.81 | 2.83 | 3.66 | 2.58 | 2.38 | LSD (0.01)= 1.11 | |
| LSD (0.01)= N.S | | | | | | | | | | | | | |
| Germination Index (GI) | 0 | 3.89 | 3.65 | 3.97 | 3.34 | 3.76 | 3.17 | 2.92 | 3.7 | 3.35 | 2.95 | 3.23 | |
| | 80 | 3.43 | 3.31 | 3.56 | 2.78 | 3.22 | 2.71 | 2.48 | 3.01 | 2.41 | 2.81 | 2.97 | 1.24 |
| | 160 | 2.58 | 2.45 | 2.67 | 1.04 | 2.46 | 1.02 | 1.16 | 2.26 | 1.04 | 1.06 | 1.77 | |
| Mean | | 3.30 | 3.14 | 3.40 | 2.39 | 3.15 | 2.30 | 2.19 | 2.99 | 2.27 | 2.27 | LSD (0.01)= 1.12 | |
| LSD (0.01)= N.S | | | | | | | | | | | | | |
| Coefficient velocity germination ( CVG) | 0 | 83.34 | 81.78 | 83.23 | 82.34 | 82.43 | 80.23 | 81.21 | 82/45 | 80.11 | 80.12 | 81.64 | |
| | 80 | 79.11 | 76.12 | 81.11 | 76.23 | 78.45 | 75.34 | 73.56 | 76.45 | 73.21 | 70.23 | 75.98 | 10.11 |
| | 160 | 72.13 | 70.11 | 76.34 | 68.54 | 74.23 | 65.56 | 68.32 | 70.34 | 64.22 | 62.34 | 69.21 | |
| Mean | | 78.19 | 76.00 | 80.23 | 75.70 | 78.37 | 73.71 | 74.36 | 73.40 | 72.51 | 70.90 | LSD (0.01)= 8.34 | |
| LSD (0.01)= N.S | | | | | | | | | | | | | |

germination index (GI) and coefficient of velocity germination (CVG). Their mean values have been decreased from 4.04, 3.23 and 81.64 at control treatment to 2.46, 1.77 and 69.21 at 160 g/L from PEG, respectively due to the drought stress effect. Also the combination of the two treatments had significant effect. The highest MDG and GI; 4.78 and 3.97 were recorded by Azady under control treatment. But the highest CVG; 83.34 recorded by Ezz under 160 g/ L PEG.

Previous studies by Alaei *et al.* (2010), Metwali *et al*. (2011) and Almaghrabi (2012) reported that wheat cultivars respond variously to water deficit and scored different germination indices values under water various water deficit conditions. Thus germination indices regarded as valuable tool for selecting drought resistant ability of genotypes (Jajarmi, 2009; Dodd & Donovan, 1999).

It is apparent from table (2) that increased concentration of PEG during the seedling growth inhibits the growth parameters and survival of wheat seedlings. A significant decrease observed under drought conditions. Under severe drought stress (160 PEG- 8000 g /L) treatment lowest mean of shoot and root length recorded; 3.34 and 1.92 cm as compared to control treatment.

As well as the combination effect of genotypes and drought stress levels had a significant effect on both shoot and root length.

**Table (2): Effect of genotypes, drought levels and their combination on growth parameters of ten wheat genotype.**

| Parameters | PEG g/ L | Ezz | Sham-6 | Azady | Tammuz-2 | Rabeaa | Adena | Abu-ghreb | Rizgary | Abehade | Ebaa-99 | Mean | LSD (0.01) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shoot length (cm) | 0 | 7.56 | 8.61 | 7.83 | 6.66 | 7.61 | 7.43 | 7.38 | 75.0 | 71.0 | 6.16 | 7.38 | 1.59 |
|  | 80 | 7.32 | 6.01 | 6.13 | 4.45 | 6.85 | 6.18 | 5.01 | 6.21 | 4.13 | 4.12 | 5.64 |  |
|  | 160 | 4.54 | 4.21 | 4.45 | 2.34 | 4.19 | 2.21 | 3.15 | 4.28 | 2.12 | 1.87 | 3.34 |  |
| Mean |  | 6.47 | 6.28 | 6.14 | 4.48 | 6.22 | 5.27 | 5.18 | 6.00 | 4.45 | 4.05 | LSD (0.01) = 2.05 |  |
| LSD (0.01)= N.S |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Root length (cm) | 0 | 3.5 | 3.63 | 3.28 | 3.58 | 3.33 | 3.46 | 2.95 | 3.44 | 2.96 | 2.99 | 3.31 | 1.11 |
|  | 80 | 3.21 | 3.48 | 3.21 | 3.26 | 3.12 | 3.33 | 2.87 | 3.36 | 2.59 | 2.29 | 3.07 |  |
|  | 160 | 2.59 | 2.76 | 3.01 | 1.06 | 2.83 | 1.08 | 1.07 | 2.75 | 1.02 | 1.01 | 1.92 |  |
| Mean |  | 3.10 | 3.29 | 3.17 | 2.63 | 3.09 | 2.62 | 2.30 | 3.18 | 2.19 | 2.10 | LSD (0.01) = 1.17 |  |
| LSD (0.01)= N.S |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Shoot dry weight (g) | 0 | 0.31 | 0.32 | 0.31 | 0.27 | 0.31 | 0..29 | 0.29 | 0.31 | 0.3 | 0.28 | 0.30 | 2.01 |
|  | 80 | 0.24 | 0.28 | 0.26 | 0.21 | 0.28 | 0.27 | 0.27 | 0.28 | 0.26 | 0.25 | 0.26 |  |
|  | 160 | 0.22 | 0.21 | 0.21 | 0.08 | 0.18 | 0.1 | 0.07 | 0.23 | 0.11 | 0.11 | 0.15 |  |
| Mean |  | 0.26 | 0.27 | 0.26 | 0.19 | 0.26 | 0.19 | 0.21 | 0.27 | 0.22 | 0.21 | LSD (0.01) = 0.35 |  |
| LSD (0.01)= 0.28 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Root dry weight (g) | 0 | 0.122 | 0.143 | 0.131 | 0.12 | 0.129 | 0.101 | 0.129 | 0.131 | 0.129 | 0.119 | 0.13 | 0.32 |
|  | 80 | 0.119 | 0.128 | 0.126 | 0.085 | 0.108 | 0.097 | 0.095 | 0.128 | 0.118 | 0.099 | 0.11 |  |
|  | 160 | 0.085 | 0.095 | 0.081 | 0.007 | 0.056 | 0.008 | 0.009 | 0.093 | 0.009 | 0.009 | 0.05 |  |
| Mean |  | 0.110 | 0.120 | 0.110 | 0.070 | 0.100 | 0.070 | 0.080 | 0.120 | 0.090 | 0.080 | LSD (0.01) = 0.06 |  |
| LSD (0.01)= N.S |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Seedling dry weight (g) | 0 | 0.432 | 0.463 | 0.441 | 0.390 | 0.439 | 0.391 | 0.419 | 0.441 | 0.429 | 0.399 | 0.424 | 0.131 |
|  | 80 | 0.359 | 0.408 | 0.386 | 0.295 | 0.388 | 0.367 | 0.365 | 0.408 | 0.378 | 0.349 | 0.370 |  |
|  | 160 | 0.305 | 0.305 | 0.291 | 0.087 | 0.236 | 0.108 | 0.079 | 0.323 | 0.119 | 0.118 | 0.197 |  |
| Mean |  | 0.365 | 0.392 | 0.373 | 0.257 | 0.354 | 0.289 | 0.288 | 0.391 | 0.309 | 0.289 | LSD (0.01) = 0.19 |  |
| LSD (0.01)= N.S |  |  |  |  |  |  |  |  |  |  |  |  |  |

The lowest shoot and root length; 1.87 and 1.01 cm recorded by Ebba-99. In contrast Sham-6 recorded the highest shoot and root length; 8.61 and 3.63 cm under control treatment. Shoot and root length reduction under water deficit condition belong to an inhibition of cell division and elongation led to a kind of tuberization and lignification of the root system. Thus the plant enters a slow-down state, whereas looking forward to the conditions to become favorable (Taiz & Zeiger, 2006). The decreasing trend in shoot and root length was also reported by Ahmad *et al.* (2013) and Chachar *et al.* (2014) under drought condition.

Growth and seedling biomass depend on elongation and division ability of cells as well as their differentiation. That involves genetic, physiological, ecological and morphological events and their complex interactions, which they are affected by water deficit (Taiz & Zeiger, 2006). There was no significant difference in the shoot and root dry weights under the genotype effect. While, drought stress condition caused a significant decrease in both trends. Lowest mean values for shoot and root dry weights; 0.15 and 0.05 g recorded due to 160 PEG- 8000 g /L growth condition as compared to control. In addition the interact factor of genotype and drought

stress levels have also caused a great significant difference. The highest shoot, root and seedling dry weight; 0.32, 0.143 and 0.463g were recorded by Sham-6 under control treatment. The lowest shoot weight and whole seedling weight; 0.07 and 0.079 g recorded by Abu-ghreb, but lowest root dry weight 0.008 g was recorded by Adena. Vegetative division is one of the most drought susceptible physiological processes due to turgor pressure decrease. That can be inhibited by interruption of water flow from the surrounding area to pro-xylem under severe water deficiency (Nonami, 1998). Shoot and root dry weight decreasing trend consistent with the other researchers (Ahmad *et al.,* 2013); who found a significant reduction of shoot and root dry matter under water stress.

The osmolyte content increase is one of the adaptation reactions during water stress that protecting the enzyme system in drought tolerant genotypes (Besma & Mounir 2010). Significant increase in proline content was observed in all genotypes in the presence of PEG at the concentrations of 160 g PEG-8000/ L. Ezz, Sham-6, Azady, Rabeaa and Riagary seedlings' accumulated more proline under drought conditions as compared to Tammuz-2, Adena, Abu-ghreb, Abehade and Ebba-99. (Fig. 1). It regarded as an adaptation mechanism to take control the unfavorable condition and provide energy for growth and sustain (Sankar *et al.*, 2007). The finding is consistent with findings of past studies by Bowne *et al.* (2012) and Mwadzingeni *et al.* (2016). They reported proline accumulation in wheat genotypes exposed to water stress.
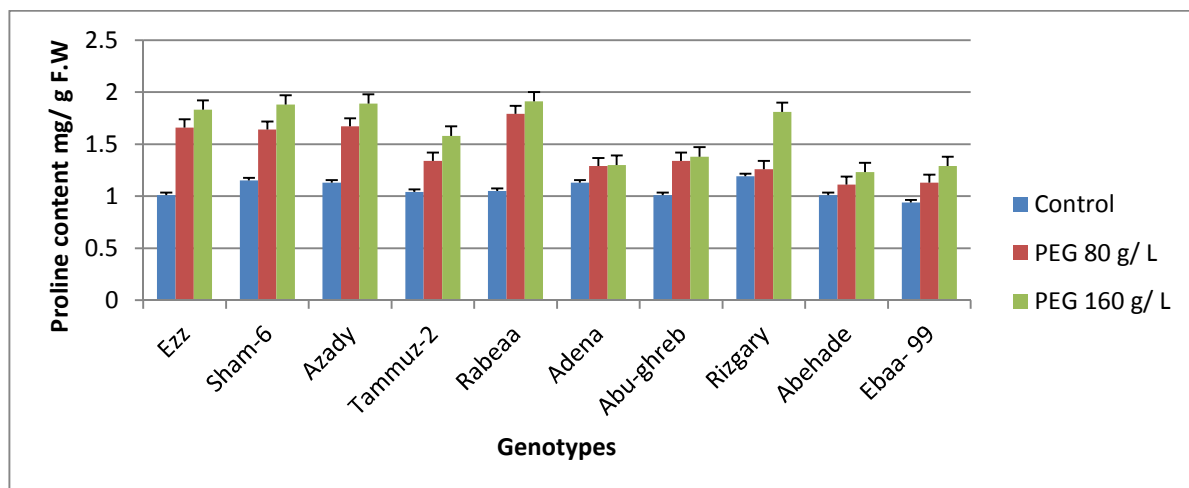


**Fig. (1): Effect of genotypes, drought levels and their combination on proline accumulation in fresh seedlings.**

A dendrogram generated based on the studied characters at germination stage under extreme water deficit condition. The ten genotypes mainly grouped into two main clusters (Fig. 2). The first cluster gathered five tolerant genotypes; Ezz, Sham-6, Rizgary, Azady and Rabeaa. The most similar genotypes in this group are ezz, Sham-6 and Rizgary with lowest resealed combine distance value. Cluster 2; includes five

susceptible genotypes; Adena, Abu-ghreb, Tammuz-2, Abehade and Eba-99. The most similar genotypes are Adena and Abu-ghreb that have lowest distance. The results are similar to Kumar *et al.* (2011); whom assorted sixty wheat genotypes to three groups, tolerant, moderately tolerant and sensitive based on morpho physiological traits. As well as Qadir *et al.* (2016) used dendrogram to classify 15 bread wheat genotypes based on in
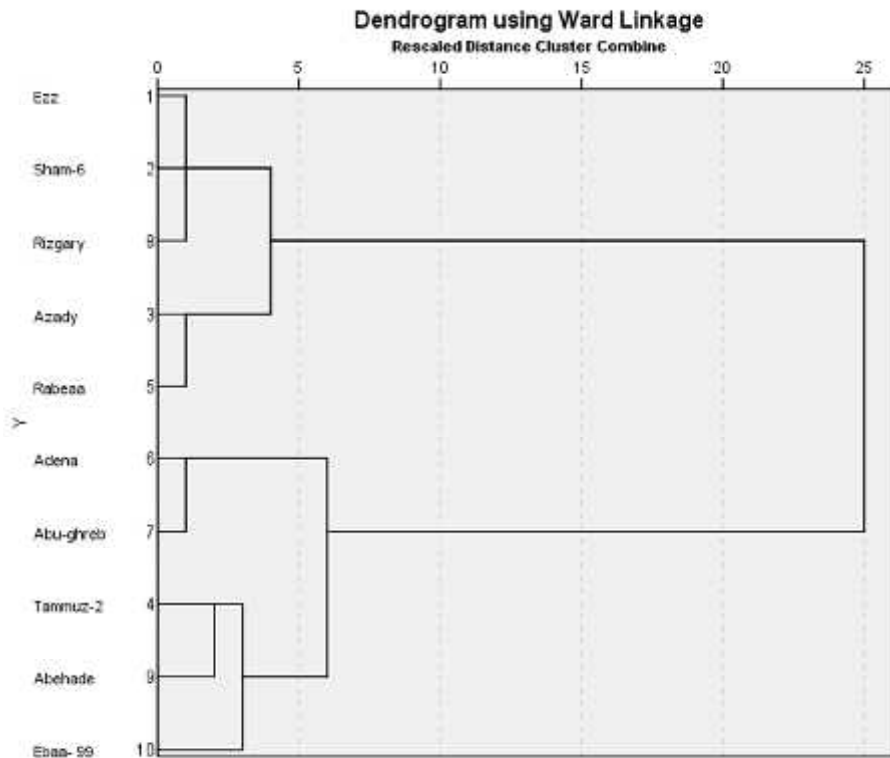
**Fig (2). Dendrogram cluster analyses for ten wheat genotypes revealed by UPGMA based on germination characters under drought condition.**

vitro culture characters under drought stress condition.

## Conclusion

Germination stage in laboratory experiment would appear to be suitable for screening large population to predict drought tolerance prior to field study for yield testing. Germination indices, seedling growth traits and proline accumulation can be used as a selectable tool to discrimination between tolerant and susceptible genotypes under drought stress. According to the traits measured in this study, we found that the ten genotypes can be classified into two groups depends on their ability to tolerate the osmotic stress as follow: first (adaptive group); include Ezz, Sham-6, Azady, Rabeaa and Rizgary. The second (sensitive group) include; Tammuz-2, Adena, Abu-Ghreb, Abehade and Ebba-99.

## Acknowledgements

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