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Screening of Tomato Inbred-Lines for Drought Tolerance at Germination and Seedling Stage

Kibreab Mehreteab Ghebremariam[1], Yan Liang[1], Cui Li[1], Yunzhou Li[1] & Lei Qin[1]

[1] Northwest A&F University, College of Horticulture, Yangling 712100, Shaanxi, PR China

Correspondence: Yan Liang, Northwest A&F University, College of Horticulture, Yangling 712100, Shaanxi, PR China. Tel: 86-29-87082179. E-mail: liangyan@nwsuaf.edu.cn

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Abstract

This investigation was carried out to screen 45 tomatoes inbred-lines' response to drought at germination and seedling stage. These inbred-lines which were collected from different countries were screened with osmotic concentration of 12.5% polyethylene glycol 6000 while their respective control treatments were treated using distilled water for twelve days at germination stage. Relative germination energy, relative germination rate, relative germination index, relative vitality index and the relative PEG injury rate were computed to identify the most tolerant inbred-lines on germination stage. Inbred-lines TTI1103B-2, A0911, Roma, L12, and TTD210B were found to be the most tolerant. While inbred-lines L9, TTI1109B-1-2-1, A4, B6 and A7 were the most sensitive to drought. Moreover, analysis of variance revealed significant differences among the inbred-lines studied. The experiment was repeated for confirmation and the result was similar. An additional study was conducted on 30 days old seedlings to observe their water stress response at their seedling stage. A total of 30 inbred-lines, 10 inbred-lines each from tolerant, moderate and sensitive; were selected based on the results found at germination stage. After proper watering for 30 days, watering was stopped for the following 10 days. After that, the dead and alive was counted. The seedlings were then re-watered for the following one week and those recovered from dead were counted again. Finally, the dead percentage and recovery percentage was computed as an indicator of water stress tolerance. And the result was very similar to the experiment done on germination stage.

Keywords: water stress, germination indexes, relative PEG injury rate, Polyethylene glycol 6000

1. Introduction

Abiotic stress, particularly drought stress is a common problem occurring all over the world, seriously limiting global crop production (Pan, Y. Wang, G. Wang, Cao, & J. Wang, 2002; Hassan, Shaaban, Hashem, & Seleem, 2004). As one of the main causes of yield loss in the world, drought stress accounts for a decrease in the average yield of more than 50% of many crops (X. Wang, Vinocur, & Altman, 2003; Bayoumi, Manal, & Metwali, 2008). Therefore, there is no doubt how crucial role water plays in the production of vegetables. Pessarakli (1999) reported that plants can be affected by drought at any time of their life, but the critical stages are at germination and seedling growth. Most tomato cultivars are drought sensitive at all stages of plant development, with seed germination and early seedling growth being the most sensitive stages (Foolad, Zhang, & Subbiah, 2003; Nuruddin, Madramootoo, & Dodds, 2003). Not only drought sensitivity is critical in seed germination and seedling growth, but their characters in relation to drought response are extremely important factors in determining yield (Rauf, 2007).

Selection of drought tolerance at the early seedling stage is usually accomplished by inducing chemicals like polyethylene glycol 6000 (Almaghrabi, 2012). Polyethylene glycol molecules are inert, non-ionic, and virtually impermeable to cell membranes and can induce water stress uniformly without causing direct physiological damage (Lu & Neumann, 1998; Kulkarni & Deshpande, 2007). Dodd and Donovan (1999) explained that PEG hinders absorption of water by seeds, but penetrable ions reduce potential inside a cell and results in water absorption then starts to germinate.

In this investigation, forty five (45) tomato inbred-lines were screened using polyethylene glycol 6000 to study their response to drought resistance at germination stage and 30 out of these 45 inbred-lines were included in the seedling stage drought tolerance investigation. Their growth parameters, germination indexes, dead percentage,

and recovery percentage were studied to identify the most tolerant inbred-lines in this early stage of plant growth.

2. Materials and Methods

2.1 Materials and Methods in the Germination Stage Experiment

This section of the experiment was designed to observe the effect of water stress on seedling growth parameters and germination indexes of tomato at germination stage. First, forty five tomatoes inbred-lines with good yield, red in color as well as big and medium fruit size were chosen based on their previous characteristics found by the tomato germplasm resource bank, college of horticulture, Northwest A and F University, China. Those inbred-lines have 7 different countries of origin as presented in Table 1.

Table 1. Names and source of countries/ provinces of the sample inbred-lines

No.	Inbred-line's name	Source country	No.	Inbred-line's name	Source country
1	TTI1213A	Nanjing*	24	TTI1101B-1	Taiwan*
2	TTI1114A-1-2-2	Taiwan*	25	A4	China*
3	TTI1103B-2	Taiwan*	26	L9	Taiwan*
4	TTI1217A	Shaanxi*	27	A7	Shaanxi*
5	TTI1216A	Shaanxi*	28	ZB1	Israel
6	HV-083	Taiwan*	29	ZB2	Israel
7	A0910	Yinchang*	30	B5	Shaanxi*
8	A6	Shaanxi*	31	B2	Shaanxi*
9	Roma	USA	32	B6	Shaanxi*
10	TTI1210B	Shaanxi*	33	M82	USA
11	A0911	Yinchang*	34	TTD203B	USA
12	A0909	Yinchang*	35	ZB5	USA
13	A5	Shaanxi*	36	TTI1109B-1-2-1	Taiwan*
14	TTI1214-A	Nanjing*	37	L12	Taiwan*
15	TTI1212B	Taiwan*	38	TTI1117A-1	Taiwan*
16	F0818	Germany	39	A0907	USA
17	TTI1229A	Shaanxi*	40	TTD210B	Egypt
18	TTI1108B-1-1-1-1	Taiwan*	41	L142	USA
19	A0916	USA	42	A2	Shaanxi*
20	A0919	Xinjiang*	43	ZB3	USA
21	A0917	Xinjiang*	44	TTD211B	Egypt
22	F0820	France	45	TTI1105B-1	Taiwan*
23	TTI1211B	Taiwan*			

Note: asterisk (*) indicates the source country is China.

L12 and L9 are wild type tomato. L12 was taken as a control for a drought tolerant while, M82 was taken as control for sensitive to water stress. 300 uniform size, full and without damage seeds of each the material were soaked in an initial temperature of 55°C warm water for twelve hours, and then divided into 6 replications of 50 seeds each, and placed on a moistened plastic petri-dishes of 9 cm diameter. Three replications were treated with osmotic concentration of 12.5% PEG 6000 and the other 3 replications by distilled water as a control. All the treatments were kept in an incubator with a temperature of 25°C and relative humidity 90%. Distilled water and PEG were added regularly when required. Germinated seeds were first counted after 36 hours and then counted

every day at the same time for 12 days. A length of 10 seedlings from every replication was measured after 12 days. Germination rate, germination energy, germination index, vitality index and their relative indexes as well as relative PEG-injury rate were calculated as follows (Li, 2008):

$$Germination\ rate\ (GR) = a/b*100;\ Germination\ energy\ (GE) = c/b*100 \quad (1)$$

$$Germination\ index\ (GI) = \sum Gt/\ day\ t;\ Relative\ PEG\ injury\ rate = (e-d)/e \quad (2)$$

Where,

a= total number of germinated seeds in PEG concentration or distilled water in 12 days.

b= total number of seeds evaluated in one replication.

c= total number of germinated seeds in PEG concentration or distilled water in six days.

Gt = germinated seeds in day t.

t = 1, 2, 3……, 12.

SL = seedling length.

d= germination percentage in PEG concentration.

e= germination percentage of control.

Vitality index was calculated as follows by referring to Lihua, Wen, and Fen (2005):

$$Vitality\ index\ (VI) = GI*SL \quad (3)$$

And the relative germination indexes were calculated by the following formulas:

$$Relative\ germination\ rate\ (RGR) = GR\ in\ PEG/GR\ in\ water*100 \quad (4)$$

$$Relative\ germination\ energy\ (RGE) = GE\ in\ PEG/GE\ in\ control\ *100 \quad (5)$$

$$Relative\ germination\ index\ (RGI) = GI\ in\ PEG/GI\ in\ control*100 \quad (6)$$

$$Relative\ vitality\ index\ (RVI) = VI\ in\ PEG/VI\ in\ control\ *100 \quad (7)$$

SPSS software and Origin software were used in the analysis. Data was subjected to analysis of variance (ANOVA) and significance of differences among treatments was tested using the least significant difference (LSD) at 5%.

Table 2. Sample of the inbred-lines included in the seedling stage water stress experiment

Samples for drought tolerant inbred-lines	Samples for drought moderate inbred-lines	Samples for drought sensitive inbred-lines
TTI1103B-2	A0917	L9
A0911	TTI1105B-1	TTI1109B-1-2-1
Roma	ZB5	A4
L12	TTI1210B	B6
TTD210B	B2	A7
TTI1214-A	TTI1217A	A2
TTI1101B-1	TTI1117A-1	TTI1213A
A0919	A0909	B5
TTD211B	L142	TTI1114A-1-2-2
ZB3	HV-083	M82

2.2 Materials and Methods in the Seedling Stage

To carry out the drought tolerance examination in the seedling stage, 10 inbred-lines from each the drought tolerant, drought moderate and drought sensitive were selected based on their drought tolerance performance found on germination stage. 30 seeds of each the inbred-lines were grown in seed trays at room temperature and 16 hour day time photoperiod in three replications. A particular attention was given to the tray position, flatness

and uniformity since the water needs to be dispersed equally. Equal amount of soil was spread levelly on equal size of trays. After planting the germinated seeds in the trays, they were properly watered for 30 days. At this stage, watering was stopped for the following 10 days and, the dead and alive was counted by the end of the 10th day. After re-watered for the following one week, these recovered seedlings were counted. Finally, the dead percentage and recovery percentage was calculated from the data recorded. A list of the inbred-lines used in the seedling stage drought tolerance experiment is given in Table 2.

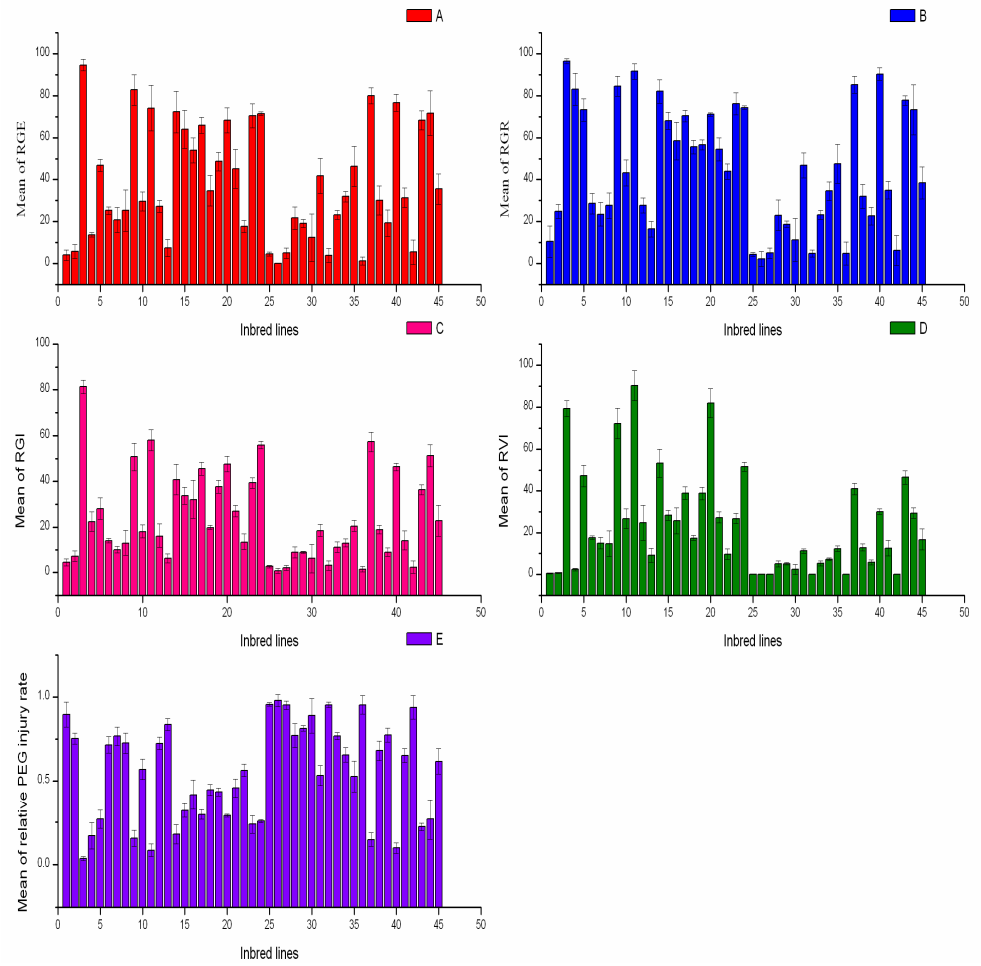

Figure 1A-E. Inbred-lines versus their percentage relative germination energy, mean of relative germination rate, mean of relative germination index, mean of relative vitality index and their mean of relative PEG injury rate

3. Result

3.1 Germination Stage Water Stress Tolerance

In this experiment, RGE, RGR, RGI, RVI and relative PEG injury rate, which are important parameters used to identify the drought tolerance of the inbred-lines on their germination stage were assessed in order to evaluate and rank our sample inbred-lines according to their drought tolerance. The first four parameters were used to rank the inbred-lines according to their tolerance to drought and the last parameter was used to check the accuracy of the arrangement found based on former four parameters. The result of the above stated five parameters for the 45 inbred-lines is summarized graphically in Figures 1A-E.

Moreover, ANOVA analysis was used to evaluate the accuracy of the data obtained from the experiment. Based on this parameter, the sample inbred-lines were significantly different from each other at 99% confidence level.

3.1.1 Relative Germination Energy (RGE)

As it is explicitly shown in Figure 1-A, the highest RGE was observed in the inbred-lines TTI1103B-2, Roma, L12, TTD210B and A0911 with their respective values of 94.5, 82.7, 79.9, 76.5 and 74.1 percent. Considering the relative germination energy parameter alone, the inbred-line TTI1103B-2 is the most tolerant to water stress. The wild type tomato L12, which was control for drought tolerant, stood third following to Roma. The lowest values were obtained in the inbred-lines L9, TTI1109B-1-2-1, B6, TTI1213A, and A4 with their values of 0, 1.1, 3.8, 4 and 4.6 percent respectively. The wild type tomato L9 registered the lowest value and it didn't germinate at all up to 6 days under PEG treatment. The control for drought sensitive, M82, has relative germination energy of 23.2%. This shows that there were other inbred-lines in the sample which are more drought sensitive than it.

3.1.2 Relative Germination Rate (RGR)

TTI1103B-2, A0911, TTD210B, L12 and Roma have the highest RGR with 96.3, 91.5, 90.3, 85.3, 84.4 percent respectively. The control for drought tolerant inbred-line L12 was among the inbred-lines that have the highest values. While the lowest values were found in the inbred-lines L9, A4, TTI1109B-1-2-1, B6 and A7 with their RGR 2.1, 4.4, 4.7, 4.8, and 4.9 percent respectively. The M82, control for drought sensitive has got a RGR of 23.2%, the same value as its RGE.

3.1.3 Relative Germination Index (RGI)

The highest RGI was recorded in the inbred-lines TTI1103B-2, A0911, L12, TTI1101B-1 and TTD211B with their values of 81.4, 58, 57.3, 55.9 and 51.3 respectively. The inbred-lines L9, TTI1109B-1-2-1, A7, A2 and A4 have the lowest values of RGI 0.64, 1.42, 2.06, 2.34 and 2.64 respectively.

3.1.4 Relative Vitality Index (RVI)

The highest relative vitality index was found in inbred-lines A0911, A0919, TTI1103B-2, Roma and TTI1214A with 90.1, 81.8, 79.2, 72.2 and 53.2 values respectively, while the lowest values occurred in TTI1109B-1-2-1, B6, A4, A7 and L9 inbred-lines. Each of these inbred-lines has equal value of RVI which equals zero. Particularly, the inbred line TTI1103B-2 got an outstanding performance with the highest values in the parameters RGE, RGR and RGI and third highest in terms of RVI.

3.1.5 Relative PEG-Injury Rate

As it is portrayed in Figure 1-E, these drought tolerant inbred-lines have the lowest relative PEG injury rate and these susceptible to water stress have higher relative PEG injury rate. Correspondingly, TTI1103B-2, A0911, TTD210B, L12 and Roma have the lowest relative PEG injury rate with their values 0.036, 0.085, 0.097, 0.147 and 0.156 respectively. And the highest PEG injury rate recorded in L9, A4, TTI1109B-1-2-1, B6 and A7 with their respective values of 0.979, 0.956, 0.953, 0.952 and 0.951.

3.2 Seedling Stage

Since drought can affect the growth of tomato at seedling stage severely, it is very important to study the effect of water stress at seedling stage. As it is shown in Tables 5 and 6, samples of 10 inbred-lines from drought tolerant, moderate and drought sensitive was taken and 30 seedlings of each of the inbred-lines were studied. Generally, the dead percentage was more in these sensitive to water stress and was relatively less in these drought tolerant inbred-lines. The lowest dead percentage was registered in the inbred-lines TTI1103B-2, L12, Roma, A0911, and TTD210B with their value of 10, 13.33, 16.67, 20, and 20 respectively. The highest dead percentage was recorded in the inbred-lines L9, TTI1109B-1-2-1, A4, B6 and A2 with their respective value of 76.67, 73.33, 73.33, 70 and 66.67. The highest recovery percentage was recorded in L12, TTI1103B-2, A0911, TTD210B and Roma inbred-lines with their value of 75, 66.67, 66.67, 66.67 and 60 respectively. And the lowest recovery percentage was found in the inbred-lines L9, TTI1109B-1-2-1, A4, A7 and TTI1213A with their respected value of 17.39, 22.73, 22.73, 26.32 and 27.78. In general, those inbred-lines with lower death rates have higher recovery rates.

4. Discussion

Drought is one of the most important environmental stresses limiting crop productivity. Nowadays due to global warming and climate change unpredictable drought is happening in different parts of the world. And previous studies proved water deficit to affect seed germination and seedling growth negatively (Van den berg & Zeng, 2006). PEG being the most commonly used laboratory experimental material to determine the susceptibility of a seed for drought at its germination stage, applying the optimum amount is crucial to determine the closest potential effect of drought to the seed and for this reason, 12.5% PEG 6000 were used (Cui, Jitao, Yan, & Lei, 2011). RGE, RGR, RGI and RVI which are important parameters used to identify the drought tolerance of the

inbred-lines on their germination stage were assessed in order to evaluate and rank our sample inbred-lines according to their drought tolerance.

The 45 inbred-lines were ranked based on their aggregated score of RGE, RGR, RGI and RVI parameters. The rank of each inbred line is shown in Table 4. TTI1103B-2, A0911, TTD210B, L12 and Roma were the top five inbred-lines in the rank which is an indication of their tolerance to water stress. Among them, TTI1103B-2 is identified to be an outstanding material for it was founded as the top drought tolerant out of our sample inbred lines and Lei & Yan (2011) have explored it as cold tolerance tomato. Moreover, Roma which is found to be drought tolerant in our investigation is also famous by its Fusarium wilt tolerant characteristics. L12, which was selected as a control for drought tolerant has also performed well and ranked fourth out of the 45 inbred-lines. On the other hand, the inbred-lines L9, TTI1109B-1-2-1, A4, B6 and A7 are the most sensitive to water stress. The control for drought sensitive, M82 ranked 32 out of the 45 inbred-lines. This reveals that there were other 13 inbred-lines more sensitive to water stress than M82. The overall ranking of the inbred-lines were consistent as the individual's scores on the four different parameters fall in narrow ranges. However, the insight given in the discussion here is only in comparison within the 45 sample inbred lines included in our investigation. Generally, as we can observe in Figure 1 A-D, the inbred-lines which are tolerant to water stress have the highest values and those which are sensitive to water stress have the lowest values of RGE, RGR, RGI and RVI.

Table 3. ANOVA analysis result

		Sum of squares	df	Mean square	F	Sig.
RGE	Between groups	96718.081	44	2198.138	63.196	.000
	Within groups	3130.460	90	34.783		
	Total	99848.541	134			
RGR	Between groups	111069.422	44	2524.305	88.621	.000
	Within groups	2563.593	90	28.484		
	Total	113633.015	134			
RGI	Between groups	49785.794	44	1131.495	68.012	.000
	Within groups	1497.308	90	16.637		
	Total	51283.103	134			
RVI	Between groups	82778.528	44	1881.330	63.774	.000
	Within groups	2655.006	90	29.500		
	Total	85433.534	134			
Relative.PEG.injury.rate	Between groups	11.135	44	.253	87.894	.000
	Within groups	.259	90	.003		
	Total	11.394	134			

Note: RGE = Relative germination energy; RGR = relative germination rate; RGI = relative germination index; RVI = relative vitality index; PEG = polyethylene glycol.

The relative PEG injury rate was a good parameter which illustrates the effect of PEG or water stress in the germination capacity of the inbred-lines. Even though, the inbred-lines were planted under the same conditions and the amount of PEG applied was equal, much difference was observed in terms of germination response. This result was similar to those found on wheat research by Majid and Roza (2011) where the two genotypes of durum wheat which were planted under the same condition had distinct responses to salinity and drought stress. The difference observed in our investigation could be because of the variation in water stress tolerance of the inbred-lines. As it is illustrated in Figure 1-E, these drought tolerant inbred-lines have smaller relative PEG injury rate and these susceptible to water stress have higher relative PEG injury rate. This means the induction of PEG related water stress in drought tolerant inbred-lines were lower compared to the inbred-lines susceptible to drought. Accordingly, TTI1103B-2, A0911, TTD210B, L12 and Roma have the lowest relative PEG injury rate and the inbred-lines L9, A4, TTI1109B-1-2-1, B6 and A7 recorded the highest relative PEG injury rate.

Moreover, the soundness and correctness of the experimental result were evaluated using ANOVA, LSD and Duncan's analysis using SPSS software. Analysis of variance revealed significant differences among inbred-lines for RGE, RGR, RGI, RVI and relative PEG injury rate. More specifically, the F value obtained is significant at least at the alpha level less than 0.001, as indicated by the significance level of .000 reported in the last column of Table 3, which falls well below the required 0.05 alpha level. Thus, we can conclude that the differences found between our groups are significant and there is less than 1 in a 1000 chance that the differences we found are the result of sampling error.

Table 4. Inbred-lines ranked in terms of their water stress response at germination stage

Inbred-line	Rank	Inbred-line	Rank	Inbred-line	rank	Inbred-line	rank
TTI1213A	39	A0911	2	A0917	17	B2	22
TTI1114A-1-2-2	37	A0909	25	F0820	30	B6	42
TTI1103B-2	1	A5	36	TTI1211B	12	M82	32
TTI1217A	23	TTI1214-A	6	TTI1101B-1	6	TTD203B	28
TTI1216A	13	TTI1212B	15	A4	43	ZB5	19
HV-083	27	F0818	16	L9	45	TTI1109B-1-2-1	44
A0910	31	TTI1229A	11	A7	41	L12	4
A6	29	TTI1108B-1-1-1-1	18	ZB1	34	TTI1117A-1	24
Roma	3	A0916	14	ZB2	35	A0907	33
TTI1210B	21	A0919	8	B5	38	TTD210B	5
L142	26	A2	40	ZB3	10	TTD211B	9
TTI1105B-1		19					

Note: rank was computed based on the averaged score in the RGE, RGR, RGI and RVI.

Table 5. Dead and recovered percentage at seedling stage for the drought tolerant and moderate inbred-lines

Name	Dead %	Recovery %	Rank at germination stage out of 45	Name	Dead %	Recovery %	Rank at germination stage out of 45
TTI1103B-2	10	66.67	1	A0917	36.67	54.55	17
A0911	20	66.67	2	TTI1105B-1	30	55.55	19
Roma	16.67	60	3	ZB5	33.33	40	19
L12	13.33	75	4	TTI1210B	36.67	36.36	21
TTD210B	20	66.67	5	B2	40	41.67	22
TTI1214-A	23.33	42.85	6	TTI1217A	40	33.33	23
TTI1101B-1	26.67	50	6	TTI1117A-1	46.67	35.71	24
A0919	23.33	42.85	8	A0909	43.33	30.77	25
TTD211B	23.33	57.14	9	L142	43.33	30.77	26
ZB3	30	55.55	10	HV-083	46.67	28.57	27

In terms of the drought tolerance of the inbred lines at seedling stage, their performance was comparable to their germination stage records. As revealed in Tables 5 and 6, the inbred-lines which are drought tolerant at germination stage have lower dead percentage and higher recovery percentage at their seedling stage and these which are sensitive to water stress at germination stage have higher dead percentage and lower recovery percentage at their seedling stage. Thus, TTI1103B-2, L12, Roma, A0911, and TTD210B which are the most

drought tolerant inbred-lines on germination stage have a low dead percentage and high recovery percentage during the seedling stage. While the inbred-lines sensitive to water stress at the germination stage such as the L9, TTI1109B-1-2-1, A4, B6, A2, A7 and TTI1213A recorded higher dead percentage and lower recovery percentage at seedling stage. This indicates that inbred-lines which are tolerant to water stress at germination stage are also tolerant to water stress at seedling stage and those inbred-lines which are sensitive to water stress at germination stage are sensitive to water stress at their seedling stage as well. In general, the dead percentage was increasing as the rank to drought tolerance of the inbred-lines on their germination stage was increasing whereas; the recovery percentage was decreasing as the rank to drought stress at germination stage was increasing. Thus, most of the inbred-lines studied have similar tolerability and susceptibility to water stress during germination and seedling stages.

Table 6. Dead and recovery percentage for the drought sensitive inbred-lines

Name	Dead percentage	Recovered percentage	Rank at germination stage out of 45
L9	76.67	17.39	45
TTI1109B-1-2-1	73.33	22.73	44
A4	73.33	22.73	43
B6	70	28.57	42
A7	63.33	26.32	41
A2	66.67	30	40
TTI1213A	60	27.78	39
B5	53.33	31.25	38
TTI1114A-1-2-2	56.67	35.29	37
M82	50	33.33	32

Moreover, in the 30 days old seedling we visually observed that the leaves of the tolerant inbreed lines was less and with the smaller leaf area when it is compared with those of the sensitive inbred-lines. This may help the drought tolerant seedlings to have a less rate of evapo-transpiration and the drought susceptible inbred lines tend to have a high amount of evapo-transpiration though this observation needs further investigation.

The most drought tolerant inbred-lines found from this investigation will be used for further breeding program.

5. Conclusion

From this study, it can be concluded that the inbred-lines TTI1103B-2, A0911, TTD210B, L12 and Roma are the most drought tolerant while L9, TTI1109B-1-2-1, A4, B6 and A7 are the most sensitive to water stress. Generally, the tomato inbred-lines studied responds to water stress were similar at their germination stage and their early seedling growth stage.

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