



Effect of Polyethylene glycol, Sorbitol and Absolute Ethanol on Achene Dormancy to Enhance Achene Germination in Sunflower

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ABSTRACT

Sunflower is economically important oilseed crop in Pakistan. Ten sunflower genotypes were evaluated with three treatments in laboratory of department of Plant Breeding and Genetic, University of Agriculture Faisalabad, during 2018. Genotypes were sown in Complete Randomized Design as well as factorial structured treatment with two replications. Two achenes for each genotype per replication per treatment were sown in the trays filled with organic soil. One control and three different treatments of polyethylene glycol, sorbitol and ethanol with two concentrations of 0.5% and 1.0 were used to break achene dormancy. After fourteen days germination data was recorded on various traits (leaves area, root length, shoot length, shoot fresh and dry weight and root fresh and dry weight). All treatments were highly significant for all traits except shoot length. T1 (Polyethylene glycol) showed maximum effect on root length and shoot dry weight and provide maximum values for these traits. T2 (Sorbitol) showed significant effect on leaf area, shoot length, shoot fresh weight, root fresh weight, shoot dry weight and provide maximum values for these traits. While T3 (Ethanol) showed significant effect only on root dry weight.

Keywords: Sunflower, Achene dormancy, Polyethylene glycol, Sorbitol, Ethano

1. Introduction

Oilseed crops are essential crops for human beings after cereals and sugars in the daily life. Sunflower is one of the most important oil crop which is cultivated throughout the world. It belongs to Asteraceae family. Fats are present in sunflower oils that are very important for our diet. Sunflower (*Helianthus annuus*) produces lower amount of saturated fatty acid palmitic (4.76-9.60%) and stearic acid (0.23-7%) as well as a high source of unsaturated fatty acid as well as oleic acid (9.88-18.15%), linoleic acid (21.08-80.13%) and linolenic acid (0.11-19.96%) with basic protein content of 27% (Nasreen *et al.*, 2015). Sunflower oil used for cooking purposes in kitchen. It contains high amount of vitamin A, D, E and K. Sunflower oil has very important value when used as food because its oil has oxidative stability and respectable nutritional superiority (Razzaq *et al.*, 2017).

Dormancy of seeds is one of the major issues that reduce its germination. The phenomenon of seed dormancy is common in the seed plants (gymnosperms and angiosperms). It is a survival mechanism developed throughout growth that allows seeds to suspend germination until favorable conditions occur (Gandy *et al.*, 2015). Embryo becomes inactive to germinate because of seed upper coat due to lack of water contents. There is limited diffusion of oxygen to embryo by lipid bilayer which covers the embryo and in this way this covering is not clear and there is distance to radical and pericarp so distribution of oxygen and water is not occur fully due shortage of humidity, so seed become dormant. Due to anatomy of seed upper covering, there are differences in seed dormancy stages (Weiss *et al.*, 2013).

Seed dormancy differs from seeds to seeds in different plant species. Two types of seed dormancy

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studied in sunflower seeds. Primary dormancy developed due to accumulation of abscisic acid at the time of ripening of seeds because ABA consider as inhibitor of seed germination (Maiti *et al.*, 2006). Thickening of seed coat and its impermeability to water inside the embryo is also cause of seed dormancy. Secondary dormancy occurred due to fluctuations in environment such as photoperiod, water and low temperature (Chilling) and high temperature also affects the seed germination (Vujakovic *et al.*, 2012). Different techniques have already been used to break the seed dormancy in cultivated sunflower species.

Seeds of sunflower can be treated with polyethylene glycol, sorbitol and ethanol to break the seed dormancy. These play important role in breaking seed dormancy in sunflower. Polyethylene glycol, sorbitol, and ethanol are mostly use to break the seed dormancy and improve germination. Polyethylene glycol has been used in priming of achenes of sunflower to improve its productivity. Priming means to elevation achene germination performance (Hamidi *et al.*, 2013).

The purpose of break the achene dormancy is that to increase its productivity (Razzaq *et al.*, 2017). Ethanol can also be used in this lab experiment to break the achene dormancy of sunflower (*Helianthus annuus* L.). Ethanol has been used for fractionation of sunflower to obtain lecithin that is food product. Third treatment was sorbitol to break achene dormancy. Sorbitol was also introduced to wheat plant for callus induction (Hassan *et al.*, 2009).

2. Materials and Methods

2.1 Collection of germplasm

Ten accessions of sunflower were collected from Oilseed Research Lab of Department of Plant Breeding and Genetics, University of Agriculture Faisalabad. These accessions were used in this experiment. These used accessions are mention in the Table 1.

Table 1: Name of accessions of sunflower used in experiment

Sr. No#	Accessions	Sr. No#	Accessions
1	William-82	6	00201/5
2	NARC-15	7	00103/3
3	NARC-2	8	00308/6
4	00205/3	9	00107/1
5	00105/5	10	00202/4

2.2. Preparation of solutions to break seed dormancy

Three chemicals were used to conduct experiment. These chemicals were polyethylene glycol, sorbitol and ethanol. These chemicals were taken from the Sunflower Research Lab of Plant Breeding and Genetics, University of Agriculture, Faisalabad.

2.2.1. Preparation of Polyethylene glycol (PEG-6000) solutions

Two levels of polyethylene glycol solutions were prepared by dissolving 5 g and 10 g of PEG-6000 in 100 ml tap water in two beakers of 500ml. Two seeds from each accession were put into 10 petridishes. About 10 ml from each level of solution was poured in the seed containing petridishes.

2.2.2. Preparation of Sorbitol solutions

Solutions of sorbitol with two levels were prepared by dissolving 5 g and 10 g of sorbitol in 100 ml tap water in two beakers. About 10 ml from each level of solution was poured in 10 petridishes that was already poured with seeds taken from each accession.

2.2.3. Preparation of Ethanol solutions

Two levels of Ethanol solutions were also prepared by dissolving 5 ml and 10 ml of ethanol in 100 ml tap water in beakers. Two seeds were put into 10 petridishes and these petridishes were poured by the taken 10 ml solution from each prepared solution of ethanol.

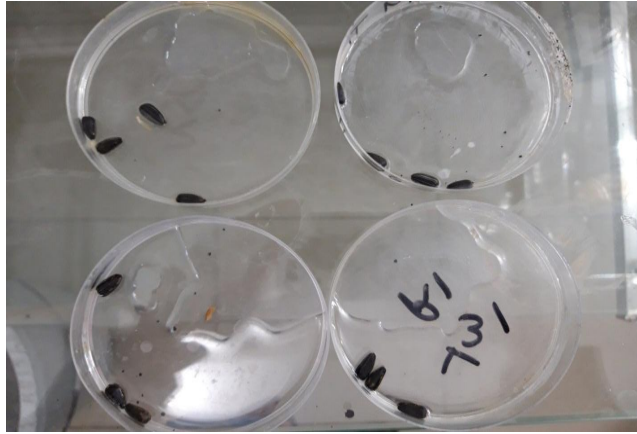


Fig. 1: Seed soaking in prepared solution in Petri dishes

2.3. Sowing of treated seeds in trays

Experiment was completed by using Complete Randomized Design (CRD) with factorial structured treatments. Two plastic cavity trays were used to conduct this experiment. Each tray had 105 units, holes, or cavities. Each row in each tray had 15 cavities and column had 7 cavities. Each unit also had a hole in bottom for water drainage. Units of each tray were filled with compost soil i.e. organic. Two seeds for each accession per replication were sown in each unit of a tray. Daily watering was done and germinated seeds were counted.



Fig. 2: A view of sunflower seedling after 09 days of sowing in trays

2.4. Recording of Data

Data of different parameters (leaf area, shoot length, root length, fresh shoot weight, fresh root weight, dry shoot weight and dry root weight) were recorded at seedling stage after 14 days of sowing.

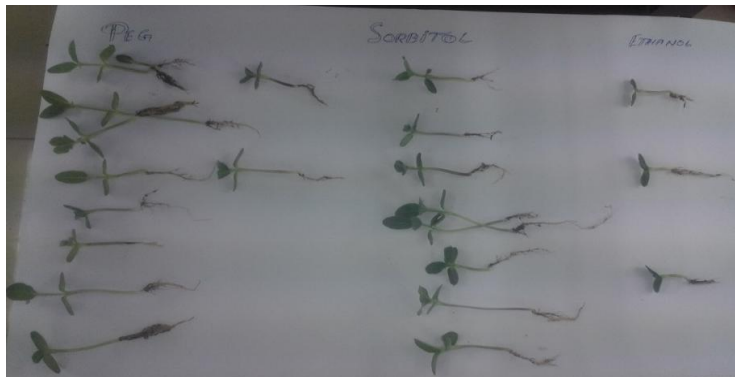


Fig. 3: A view of fractionated seedling from trays after 14 days of sowing

2.5. Statistical Analysis

Recorded data of sunflower seedlings were subjected to the analysis of variance (Steel *et al.*, 1997). Tukey's test was used to compare mean values of various accessions.

3. Results

3.1. Leaf Area

Analysis of variance of leaf area for treatments was significant but for Genotypes and their interactions with treatment was non-significant as shown in Table 3(a) (Beyaz *et al.*, 2018). Leaf area of all genotypes was compared by applying Tukey's test. Mean comparison for leaf area among sunflower genotypes showed significant differences as shown in Table 3(b). Graphical representation of leaf area showed that range of leaf area was from 0.59 to 1.85. Genotype 205/3 had maximum leaf area under treatment T2 (1.0) followed by the 308/6 under treatment T2 (0.5). So T2 (Sorbitol) showed a significant effect on leaf area.

Table 3(a): Analysis of variance for leaf area

SOV	DF	SS	MS	F
Genotype	9	0.27424	0.03047	0.85
Treatment	3	0.29324	0.09775	2.72*
G×T	27	0.62766	0.02325	0.65
Error	100	3.59309	0.03593	
Total	139			

SOV = Source of variance,
 MS = Mean sum of square

DF = Degree of freedom,
 F_{cal} = F-calculated.

SS = Sum of square,
 * = Significance difference at 0.05 probability

** = Highly Significance difference at 0.01 probability

Table 3(b): Mean comparison for leaf area among sunflower genotypes

Genotype	Mean	Homogeneous group
205/3	1.0513	A
308/6	1.0409	A
William-82	1.0300	A
NARC-16	1.0156	A
202/4	1.0019	A
105/5	0.9900	A
201/5	0.9719	A
103/3	0.9313	A
107/1	0.9231	A
NARC-2	0.9231	A

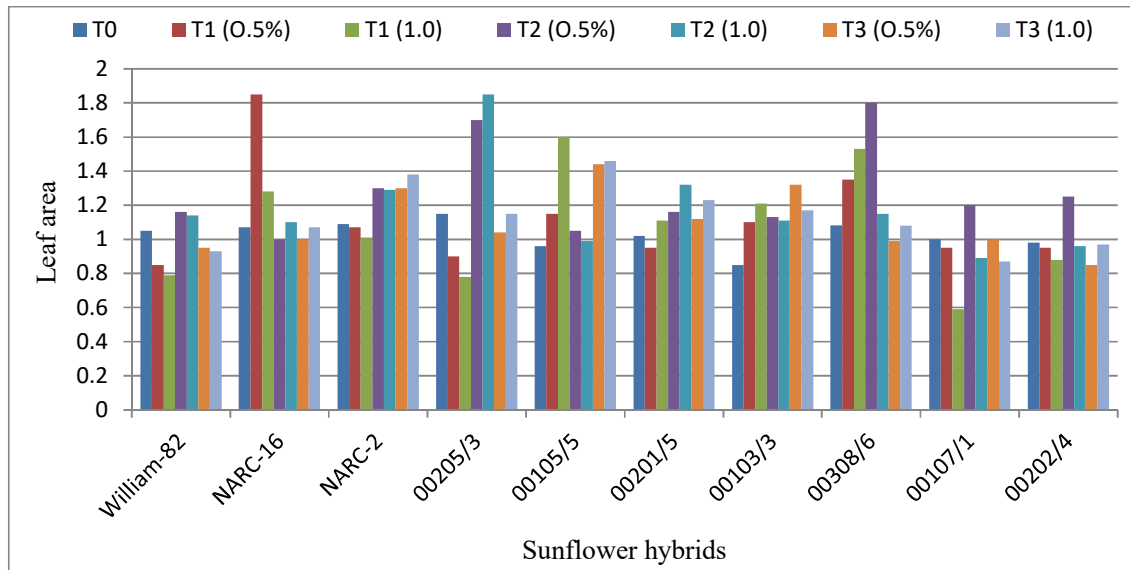


Fig. 4: Graphical representation of means for leaf area among sunflower genotypes under different treatments

3.2. Shoot length

ANOVA showed significant results for length of vegetative portions at seedling stage in accessions of sunflowers as mentioned in Table 4(a). Genotypes, treatments and their interactions, all of these factors were non-significance. Mean comparison for shoot length among sunflower genotypes showed significant differences as shown in Table 4(b). The graphical representation of shoot length showed that range of shoot length of all sunflower genotypes was from 0.87 to 7.9. Accession 107/1 showed maximum shoot length under treatment T2 (0.5).

Table 4(a): Analysis of variance for shoot length

SOV	DF	SS	MS	F
Genotype	9	11321	1257.91	0.67
Treatment	3	5014	1671.32	0.89
G×T	27	40773	1510.13	0.80
Error	100	188679	1886.79	
Total	139			

Table 4(b): Mean Comparison for Shoot Length among Sunflower Genotypes

Genotype	Mean	Homogeneous group
205/3	4.656	A
NARC-2	4.164	A
201/5	3.915	A
William-82	3.625	A
202/4	3.559	A
103/3	3.515	A
107/1	3.493	A
308/6	3.361	A
105/5	2.389	A
NARC-16	2.266	A

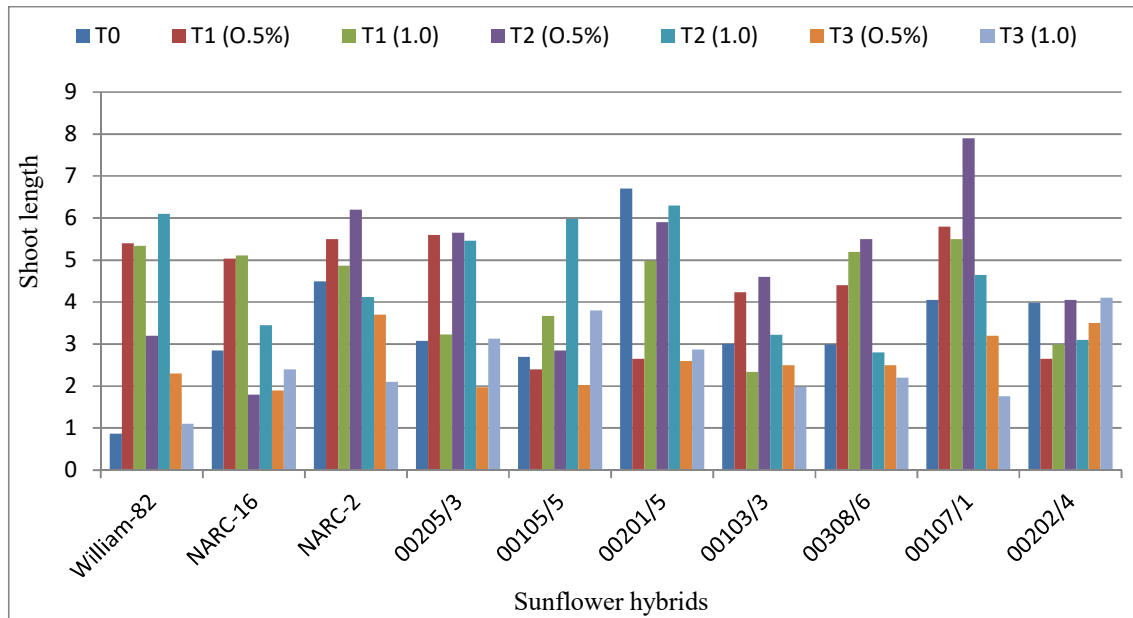


Fig. 5: Graphical representation of means for shoot length among sunflower genotypes under different treatments

3.3 Root length

Analysis of variations for root length at seedling stage showed highly significant results for treatments but genotypes and interactions were non-significant as shown in Table 5(a) (Mahmood and Basra 2009). Mean comparison of different genotypes is described in table 5(b), showed significant differences. The graphical representation of root length showed that range of root length of all sunflower genotypes was from 1.09 to 5.4. Accession NARC-2 showed maximum root length followed by accession 205/3 under treatment T1 (0.5). It showed that T1 (Polyethylene glycol) had significant effect on root length.

Table 5(a): Analysis of variance for root length

SOV	DF	SS	MS	F
Genotype	9	18.168	2.0187	1.05
Treatment	3	49.377	16.4591	8.52**
G×T	27	54.863	2.0320	1.05
Error	100	193.129	1.9313	
Total	139			

Table 5(b): Mean comparison for root length among sunflower genotypes

Genotype	Mean	Homogeneous group
103/3	2.8019	A
NARC-2	2.7069	A
201/5	2.6581	A
205/3	2.5931	A
William-82	2.5856	A
107/1	2.4869	A
202/4	2.3050	A
308/6	2.0837	A
105/5	1.9794	A
NARC-16	1.5406	A

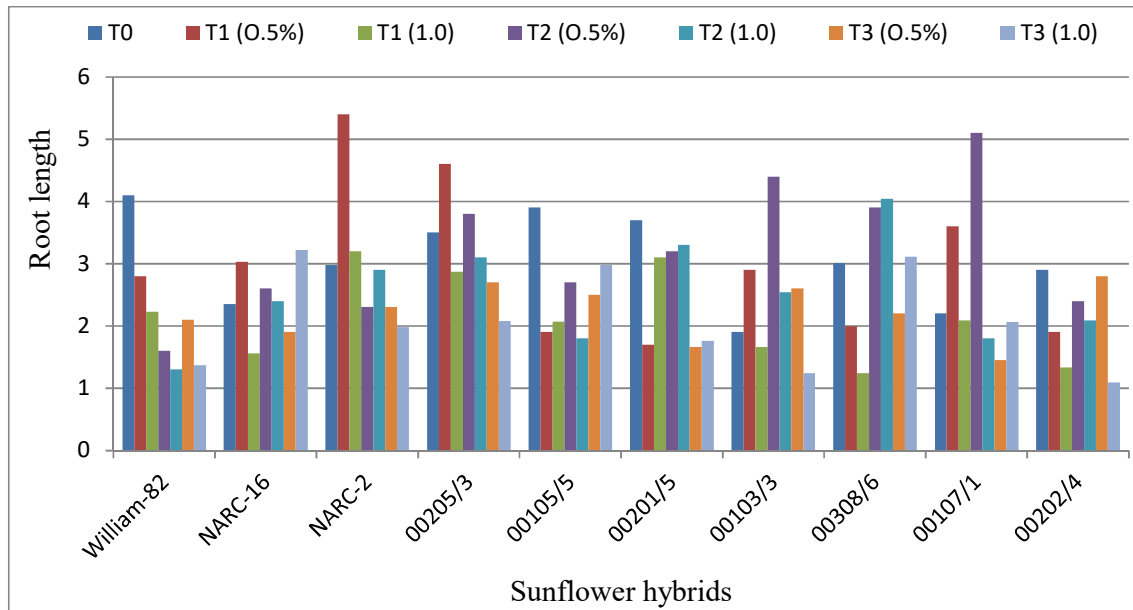


Fig. 6: Graphical Representation of Means for Root Length among Sunflower Genotypes under Different Treatments

3.4. Shoot fresh weight

Analysis of variations for shoot fresh weight showed highly significant results for treatments but genotypes and interactions were non-significant as shown in Table 6(a) (Kandil *et al.*, 2017). Mean comparison of different genotypes is described in table 6(b), showed significant differences. The graphical representation of shoot fresh weight showed that range of all sunflower genotypes was from 0.54 to 2.6. Accession 107/1 showed maximum shoot fresh weight followed by accession 308/6 under treatment T2 (0.5). It showed that T2 (Sorbitol) had significant effect on shoot fresh weight.

Table 6(a): Analysis of variance for shoot fresh weight

SOV	DF	SS	MS	F
Genotype	9	1.6246	0.18051	0.63
Treatment	3	4.1073	1.36910	4.79**
G×T	27	3.6809	0.13633	0.48
Error	100	28.5791	0.28579	
Total	139			

Table 6(b): Mean comparison for shoot fresh weight among sunflower genotypes

Genotype	Mean	Homogeneous group
103/3	1.4444	A
William-82	1.4269	A
308/6	1.3294	A
107/1	1.3219	A
202/4	1.2937	A
201/5	1.2150	A
NARC-2	1.1975	A
205/3	1.1781	A
NARC-16	1.1281	A
105/5	1.1006	A

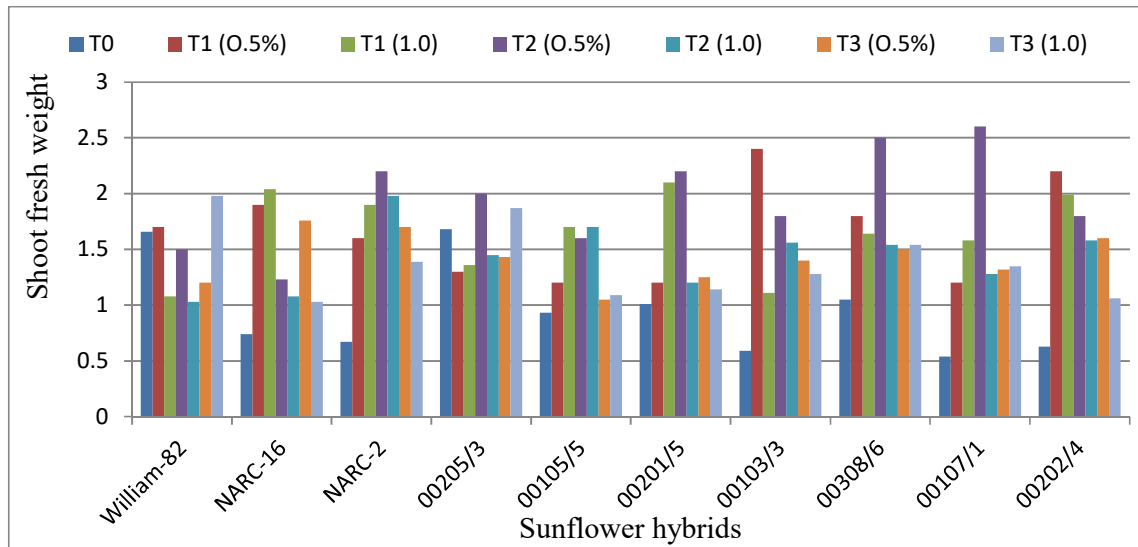


Fig. 7: Graphical representation of means for shoot fresh weight among sunflower genotypes under different treatments

3.5 Root Fresh weight

Analysis of variance for fresh root weight among sunflower accessions is mentioned in Table 7(a). Treatments were highly significant and genotypes were significant for root fresh weight trait while interactions were non-significant among different sunflower genotypes (Kandil *et al.*, 2016). Mean comparison of different genotypes is described in table 7(b), showed significant differences. The graphical representation of root fresh weight showed that range of all sunflower genotypes was from 0.11 to 0.98. Accession 107/1 showed maximum value for root fresh weight under normal conditions. But under treatments 205/3 showed maximum value under T2 (1.0).

Table 7(a): Analysis of variance for root fresh weight

SOV	DF	SS	MS	F
Genotype	9	2.6556	0.29507	2.28*
Treatment	3	1.9282	0.64272	4.96**
G×T	27	4.3555	0.16132	1.25
Error	100	12.9464	0.12946	
Total	139			

Table 7(b): Mean comparison root fresh weight among all accessions

Genotype	Mean	Homogeneous group
NARC-16	0.8088	A
105/5	0.7369	A
308/6	0.6576	AB
202/4	0.5731	AB
205/3	0.5693	AB
107/1	0.5693	AB
103/3	0.5297	AB
William-82	0.5154	AB
201/5	0.4708	AB
NARC-2	0.2536	B

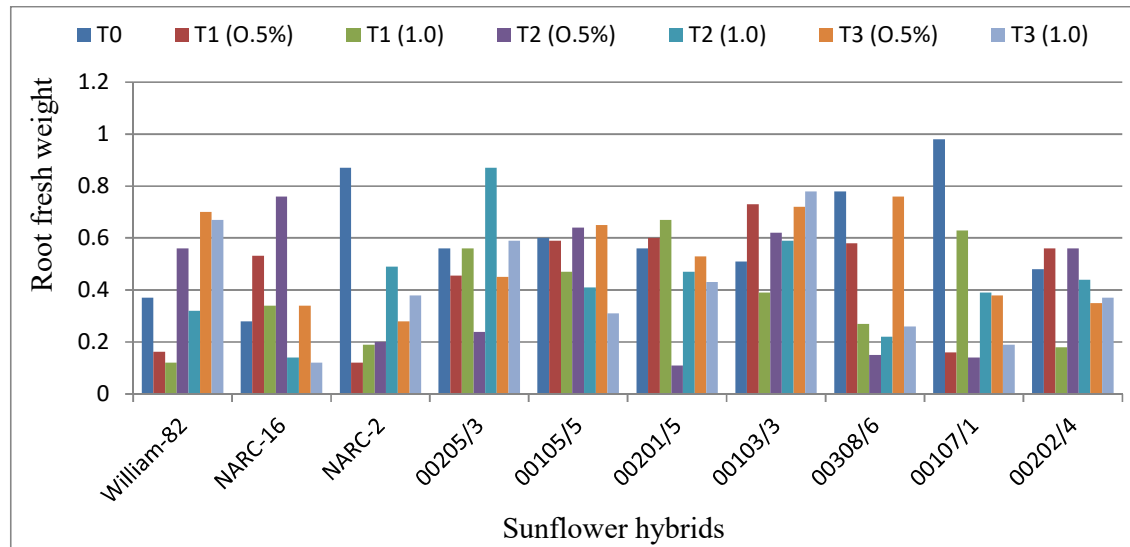


Fig. 8: Graphical representation of means for root fresh weight among sunflower genotypes under different treatments

3.6 Shoot Dry weight

Analysis of variations for shoot dry weight showed highly significant results for treatments but genotypes and interactions were non-significant as shown in Table 8(a) (Pirmani *et al.*, 2013). Mean comparison of different genotypes is described in table 8(b), showed significant differences. The graphical representation of shoot dry weight showed that range of all sunflower genotypes was from 0.004 to 0.09. Accession 103/3 showed maximum shoot dry weight under treatment T2 (1.0) and T1 (0.5). It showed that T1 (Polyethylene glycol) and T2 (Sorbitol) had significant effect on shoot dry weight.

Table 8(a): Analysis of variance for shoot dry weight

SOV	DF	SS	MS	F
Genotype	9	2.9053	0.32282	1.60
Treatment	3	2.7164	0.90547	4.49**
G×T	27	5.7435	0.21272	1.06
Error	100	20.1629	0.20163	
Total	139			

Table 8(b): Mean comparison for shoot dry weight

Genotype	Mean	Homogeneous group
NARC-16	0.7641	A
105/5	0.5781	AB
308/6	0.5751	AB
202/4	0.4611	AB
205/3	0.4569	AB
107/1	0.4537	AB
103/3	0.4016	AB
William-82	0.3989	AB
201/5	0.3953	AB
NARC-2	0.1501	B

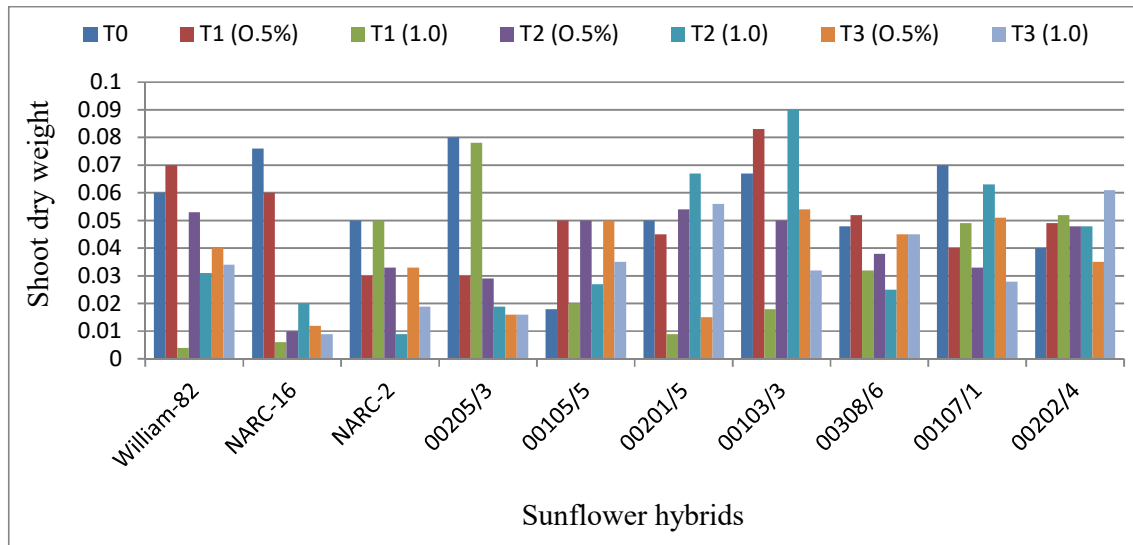


Fig. 9: Graphical representation of means for shoot dry weight among sunflower genotypes under different treatments

3.7 Root Dry weight

Analysis of variance for dry root weight among sunflower accessions is mentioned in Table 9(a). Treatments were highly significant while genotypes and interactions were non-significant among different sunflower genotypes (Pirmani *et al.*, 2013). Mean comparison of different genotypes is described in table 9(b), showed significant differences. The graphical representation of root dry weight showed that range of all sunflower genotypes was from 0.0012 to 0.023. Accession 308/6 showed maximum value for root dry weight followed by 107/1 under treatment T3 (0.5).

Table 9(a): Analysis of variance for root dry weight

SOV	DF	SS	MS	F
Genotype	9	2.9492	0.32769	1.52
Treatment	3	2.8487	0.94957	4.42**
G×T	27	6.0096	0.22258	1.04
Error	100	21.5028	0.21503	
Total	139			

Table 9(b): Mean comparison for root dry weight among sunflower accessions

Genotype	Mean	Homogeneous group
NARC-16	0.7519	A
308/6	0.5646	AB
105/5	0.5639	AB
107/1	0.4443	AB
202/4	0.4420	AB
205/3	0.4408	AB
William-82	0.3875	AB
103/3	0.3795	AB
201/5	0.3786	AB
NARC-2	0.1354	B

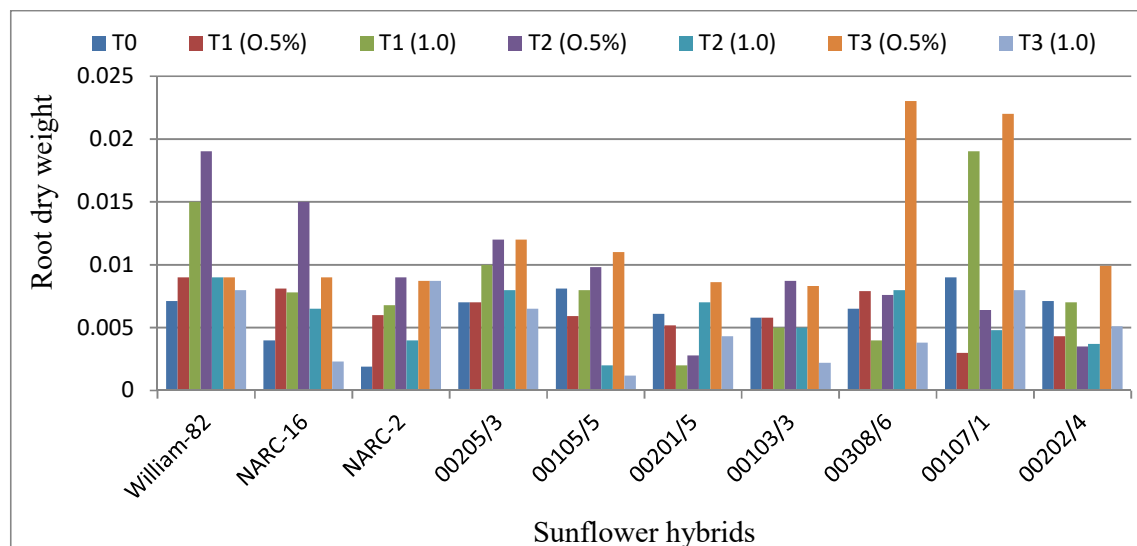


Fig. 10: Graphical representation of means for root dry weight among sunflower genotypes under different treatments

4. Conclusion

Results of this experiment showed that T1 (Polyethylene glycol) had maximum effect on root length and shoot dry weight and provide maximum values for these traits. T2 (Sorbitol) showed significant effect on leaf area, shoot length, shoot fresh weight, root fresh weight, shoot dry weight and provide maximum values for these traits. While T3 (Ethanol) showed significant effect only on root dry weight. So from this experiment it can be concluded that sorbitol provides maximum results for number of traits and it can be used in future programs to break seed dormancy in various crops.

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