



Research Article

Deciphering drought tolerance potential of sweet corn genotypes through polyethylene glycol induced drought stress

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Abstract

Drought is a restraining factor for the major dependent factors like yield. Pertaining to this issue, analyzing the drought potential of the sweet corn is a major concern. The present investigation was carried out to reveal the drought tolerant potential of sweet corn genotypes by *invitro* screening under four levels of osmotic stress (0, -2, -4 and -6 bar) using PEG. The material used for the study included six lines and five testers and their corresponding thirty hybrids synthesized in a line x tester mating design. The seedlings exhibited a significant variation for all the traits and indices analyzed. Under high stress, SVI of the selected entries were found to be higher because of increase in germination percentage and seedling length which would ultimately lead to better field emergence. Performance of the hybrids WNC12039-1×45503, WNC12039-1×45678 and SC11-2×45679 excelled over the commercial check *i.e.* Misthi when subjected to lower water potential. Based on the preliminary study, the identified inbreds WNC 12069-2, SC 11-2 and the hybrids WNC12039-1×45503, WNC12039-1×45678 and SC11-2×45679 could be further evaluated in the field.

Keywords

Drought, sweet corn, PEG 6000 and Seedling Vigour index.

INTRODUCTION

Sweet corn (*Zea mays* var. *saccharata*) is a natural mutant of maize with high sugar content. In recent times, there is a steep rise in the demand of globally for sweet corn. It is mostly consumed at an immature stage at 20-24 days after pollination. Apart from its numerous conventional usages as fresh and canned kernels, other novel products like sweet corn milk and soups are currently gaining their prominence in many countries. Hence breeding programs, for improving the sweet corn towards the changing climatic scenarios will enable the farmers and the industries to rely on them.

Among the innumerable abiotic factors restraining the crop production, drought is one of the most distressing environmental stresses (Yue *et al.*, 2006). In the year 2017, approximately 19 per cent of the losses in agricultural production globally declined due to drought and this constituted to more than 17 billion dollars in the United States (FAOSTAT 2017). Current progressive approaches put forth by IPCC, 2018 also reports that the

yield of maize in many regions has been affected negatively owing to the recent climate changes. This persuades the need for drought tolerance in mainstream crops.

Drought undermines the plant growth from seedling to maturity, and the studies have unveiled that the harmful impact of drought results in crops due to the damages perceived during crucial stages of development namely, germination, seedling development and flowering stages (Tsago *et al.*, 2014). Hence, higher sensitivity of the crop to water stress at early growth stages can be detrimental to the crop growth. Biometric elements and indices at an early growth stage could be employed in designing the selection criteria to figure out the drought tolerant genotypes (Queiroz *et al.*, 2019).

As screening of a large number of lines under field conditions is laborious, an artificially simulated drought condition in lab could be used to identify outstanding

genotypes from a source population. PEG efficiently decreases the water potential which further decreases the water absorption by plant roots. In laboratory screening, PEG offers a significant advantage over other low molecular weight osmolytes (e.g.,mannitol) where the later reflects a certain negative implications in plant growth and development (Hohl *et al.*, 1991). Therefore, in this study, *invitro* screening was carried out using PEG to induce varying osmotic pressure in the seedling stages in a set of inbreds and hybrids to identify the potential inbreds and hybrids for further drought screening in field conditions.

MATERIALS AND METHODS

A. Genetic Materials

The experiment comprised of six lines, five testers and their thirty hybrids obtained from a line x tester mating design in the Department of Millets, Tamil Nadu Agricultural University. The seeds of these genotypes along with a commercial check, Misthi were subjected to variable concentrations of PEG 6000.

B. Experimental Methods and Design

Sampled seeds of uniform sizes were surface sterilized with 1 per cent sodium hypochlorite for five minutes followed by rinsing with distilled water twice. Roll paper towel method of germination was used to grow the seedlings for the study. Ten seeds per replication were arrayed equidistantly in a proper way to avoid contact between the seeds on moistened paper towel. A moistened germination paper was placed above it to cover the seeds. The paper towels were rolled with a polythene sheet below and were placed in beakers comprising varying PEG concentrations. Distilled water was used as control (0 bar). Drought stress was imposed at -2, -4 and -6 bar by dissolving 119.5g, 178.4g and 223.6g of PEG 6000 respectively in 1000ml of distilled water. The corresponding concentrations for employing the respective stress were estimated using the formula of Michel and Kaufmann (1973) at 25°C.

$$\text{os} = -(1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) C^2 T$$

Where,

os = Osmotic potential (bar)

C = Concentration (g L^{-1}) PEG 6000 in distilled water)

T = Temperature ($^{\circ}\text{C}$)

The experiment was carried out with 30 hybrids, commercial check, Misthi and eleven inbreds and drought tolerance potential was elucidated by subjecting the genotypes to four levels of osmotic potential (0, -2, -4, and -6 bar) elicited by PEG 6000 with two factors (genotypes and PEG treatment) in completely randomized design with two replications.

C. Data Collection and Analysis

The seeds were counted as germinated when the length of radicle and plumule were around 2mm. At the end of the seventh day, seedling observations like the number of normal and abnormal seedlings, shoot length, root length, seedling length were recorded. The above observations were used to calculate various tolerance indices as follows:

- ✓ Germination Percentage GP(%) (Scott *et al.*, 1984)

$$\text{GP(%)} = \frac{\text{Number of germinated seeds}}{\text{Number of viable experimental seeds}} \times 100$$

- ✓ Seedling Vigor Index =
Germination Percentage \times Seedling Length

$$\text{✓ Root to shoot ratio (R/S)} = \frac{\text{Root length}}{\text{Shoot length}}$$

- ✓ Stress tolerance index (Raj *et al.*, 2019 and Partheeban *et al.*, 2017)

$$\text{Stress tolerance index} = \frac{\text{Trait of stressed plant}}{\text{Trait of control plant}} \times 100$$

- a. PHSI (%)

$$= \frac{\text{Plant height of stressed plant}}{\text{Plant height of control plant}} \times 100$$

Where, PHSI is Plant height stress tolerance index

- b. RLSI (%) =

$$= \frac{\text{Root length of stressed plant}}{\text{Root length of control plant}} \times 100$$

Where, RLSI is Root length stress tolerance index.

The data obtained were subjected to analysis of variance using AGRES 7.1 and SPSS 16.0 statistical softwares.

RESULTS AND DISCUSSION

Sweet corn is an emerging economic crop and is expected to be affected by the adverse climatic conditions. Pertaining to this all the inbreds and hybrids, **Table 2** showed significant variation among the genotypes and treatments for all the traits including the seedling parameters and indices except root to shoot ratio which was non-significant among treatments. This states that, all inbreds and hybrids significantly responded to all the treatments in terms of shoot length, seedling length, plant height stress tolerance index, seedling vigor index. It was also observed that all the sweet corn inbreds and hybrids, exhibited progressive decline for their seedling growth with increasing concentration of PEG and this shows the effect of the osmotic stress in the seedlings(**Table 3**).

Table 1. Lines and testers used in the study

Lines		Testers	
L₁	SC 11-07	T₁	45503
L₂	SC 11-2	T₂	45530
L₃	SC 1421-5-2-1	T₃	45678
L₄	WNC 12069-2	T₄	45679
L₅	WNC 12039-1	T₅	45683
L₆	USC 1-2-3-1		

Table 2. ANOVA for different seedling parameters in sweet corn entries under drought stress

INBREDS									
Source	Df	GP (%)	SL (cm)	RL (cm)	SEL (cm)	PHSI (%)	RLSI (%)	R/S	SVI
Genotype	10	469.55**	7.48**	125.48**	157.24**	1116.91**	2053.2**	1.92**	918458.67**
Treatment	3	3131.44**	36.79**	33.01**	143.53**	3655.15**	2883.90**	0.04	2967822.59**
G × T	30	248.94	1.34**	5.22	9.21*	213.66**	472.6013	0.10*	144823.64*
Error	44	151.14	0.54	3.10	4.57	88.13	335.1293	0.05	69373.39
HYBRIDS									
Genotype	30	901.29**	24.13**	56.83**	112.45**	812.04**	465.40**	0.55**	1920132.15**
Treatment	3	4557.39**	234.75**	437.70**	1365.00**	8007.10**	6437.84**	0.10	26299772.92**
G × T	90	164.34**	11.34*	25.25**	43.09**	307.44**	325.60*	0.23**	512284.66**
Error	124	90.73	7.23	15.18	25.48	171.87	221.38	0.13	318940.20

*: Significant at 5 % level; ** Significant at 1% level

GP- Germination Per centage, SL - Shoot Length, RL - Root Length, SEL - Seedling Length, PHSI – Plant Height Stress Index ,RLSI – Root Length Stress Index , R/S – Root to Shoot Ratio, SVI – Seed Vigour Index

a. Germination Percentage (GP %)

Most of the inbreds and hybrids showed reduction in their germination with an increase in stress levels which was also substantiated by the results of Djemel *et al.* (2018) and Partheeban *et al.* (2017). Among the inbreds, under controlled condition, L₅ (95%) and T₃ (75%) recorded maximum and minimum germination per cent. Under a mild stress (-2 bar), inbred L₄ (85%) performed well and while L₂ (40%) was found to be lower in its germinability. In moderate stress (-4 bar), tester T₄ (85%) followed by line L₄ recorded maximum values while the line L₁ (45%) recorded minimum value. Under high stress (-6 bar), L₄ (85%) had established a higher germination percentage. This confronts that, the line L₄ had an inherent potential to overcome the osmotic stress and exhibited a higher germination percentage.

Among the hybrids under controlled condition, L₁×T₃, L₁×T₄, L₁×T₅, L₄×T₃, L₄×T₅, L₅×T₁, L₅×T₂, L₅×T₃, L₆×T₁, L₆×T₂, L₆×T₃, L₆×T₄, L₆×T₅ recorded 100 per cent germination and the hybrid L₃×T₅(75%) reported a poorest germination percentage. Under a mild stress (-2 bar), L₅×T₁, L₅×T₂, L₆×T₁, L₆×T₃, L₆×T₄ were on par with control, while L₃×T₄, L₃×T₂ had an average of 65 percentage germination. Considering the highest osmotic level *i.e.* -4 bar, L₅×T₁, L₅×T₂ were observed with a maximum germination, L₃×T₂ having a poor germination. Seedlings that perceive the stress and germinate at a higher osmotic level are known to inhabit a drought potential in them and owing to this, at higher stress, hybrid L₅×T₁ (95%) performed well. Comparing the commercial check

performance, other than L₃×T₅ all the hybrids recorded a higher germination percentage than Misthi. So, it can be inferred that L₅×T₁ were good performers under all stress levels which implies that this hybrid could perform well under unfavorable water deficit conditions as they had a higher germinability under stress conditions.

b. Root Length (RL), Shoot Length (SL), R/S ratio:

Higher the root length, greater the penetration ability of the plant to acquire more water from deeper layers (Oh *et al.*, 2011). Therefore, root length is one of the component traits for drought tolerance (Govindaraj *et al.*, 2010). In addition to the reduction in root length the effect of drought is also evident mostly on shoot, which bears the economic part of the plant.

In the present study, it was revealed that the root, shoot and seedling length were significantly reduced with increasing stress level (**Table 3**) which was substantiated by the results of Petcu *et al.* (2018) and Khayatnezhad *et al.* (2010). Under controlled condition (0 bar), the mean shoot length varied from 11.17cm (L₆) to 7.13cm (L₃) and RL varied from 20.11cm (L₂) to 5.61cm (L₁). Among the hybrids, shoot length was highest in L₁×T₅ (29.2cm). RL was highest in L₂×T₁ (43.83cm). When the genotypes were subjected to -2 bar, SL was found to be the highest in L₂ (10.08 cm) and lowest in T₁ (5.34cm). RL were highest in L₄ (15.43cm) and lowest in T₅ (2.74cm). Among the hybrids, L₁×T₃ recorded highest SL and RL (14.83 and 26.32cm respectively). At moderate stress (-4 bar), SL and RL were highest in L₄ (10.01cm and 14.27cm

Table 3. Mean Comparison of Main Effects of Drought Stress Levels in Inbreds and Hybrids

INBREDS								
Drought stress	GP (%)	SL (cm)	RL (cm)	SEL (cm)	PHSI (%)	RLSI (%)	R/S	SVI
0 bar (control)	83.182	9.045	10.498	19.599	100.000	100.000	1.168	1622.236
- 2 bar	66.364	6.812	8.646	15.458	78.800	81.919	1.262	1030.873
- 4 bar	60.455	6.569	8.231	14.800	75.442	78.057	1.244	919.073
- 6 bar	55.909	6.163	7.671	13.782	70.900	74.072	1.239	792.141
HYBRIDS								
0 bar (control)	93.55	14.62	23.92	38.46	100.00	100.00	1.72	3600.47
- 2 bar	83.39	11.22	19.45	30.67	80.86	84.84	1.76	2577.06
- 4 bar	82.26	10.77	19.22	29.94	77.48	81.75	1.81	2502.04
- 6 bar	72.58	10.38	17.78	27.68	75.01	76.18	1.81	2061.02

respectively). While comparing all the hybrids, highest SL and RL were reported in $L_2 \times T_1$ (13.46cm) and $L_5 \times T_1$ (26.55cm) respectively. At higher stress (-6 bar), maximum SL and RL were recorded in L_4 (8.24cm and 13.76 cm respectively). Among the hybrids, highest SL and RL were recorded in $L_6 \times T_3$ (19.21cm) and $L_4 \times T_4$ (23.25cm) respectively. Though the shoot length was maximum for L_2 and $L_1 \times T_5$ under controlled condition, it was reduced drastically when subjected to stress, which in turn affects their photosynthetic efficiency.

The higher dosages of PEG (-6 bar) negatively affected root, shoot and ultimately seedling length for most of the genotypes. But the inbreds L_4 , L_5 , T_2 recorded higher root length when compared to control with RLSI greater than 100. The genotype L_4 at higher stress performed well with higher RL, SEL which states that it can tolerate stress, which is specified by less reduction in shoot length and higher root length, so they can penetrate deep into

the soil to draw more water from the deeper layers which can also be supported by the findings of Guoxiong *et al.* (2002). L_1 was noted to be underperforming with limited SL, RL, SEL and RLSI. With respect to hybrids, $L_6 \times T_3$ was with higher shoot length but the root length was highly affected and consequently, R/S ratio was low at higher stress level. In terms of stress tolerance index (PHSI and RLSI), at higher drought stress level (-6 bar) L_4 performed well with 98.07 per cent PHSI. With regard to RLSI, inbreds T_2 and L_4 recorded the values of 112.3 and 102.08 per cent respectively. Among the hybrids, it was found that $L_2 \times T_2$ had higher RLSI (105.25%) and $L_6 \times T_3$ and $L_4 \times T_5$ had higher PHSI 160 and 96.5 per cent respectively. PHSI of $L_1 \times T_5$ was very less when compared to other hybrids. This indicated that at higher stress shoot length was highly reduced in addition to root length and so it is less adaptable to drought environment.

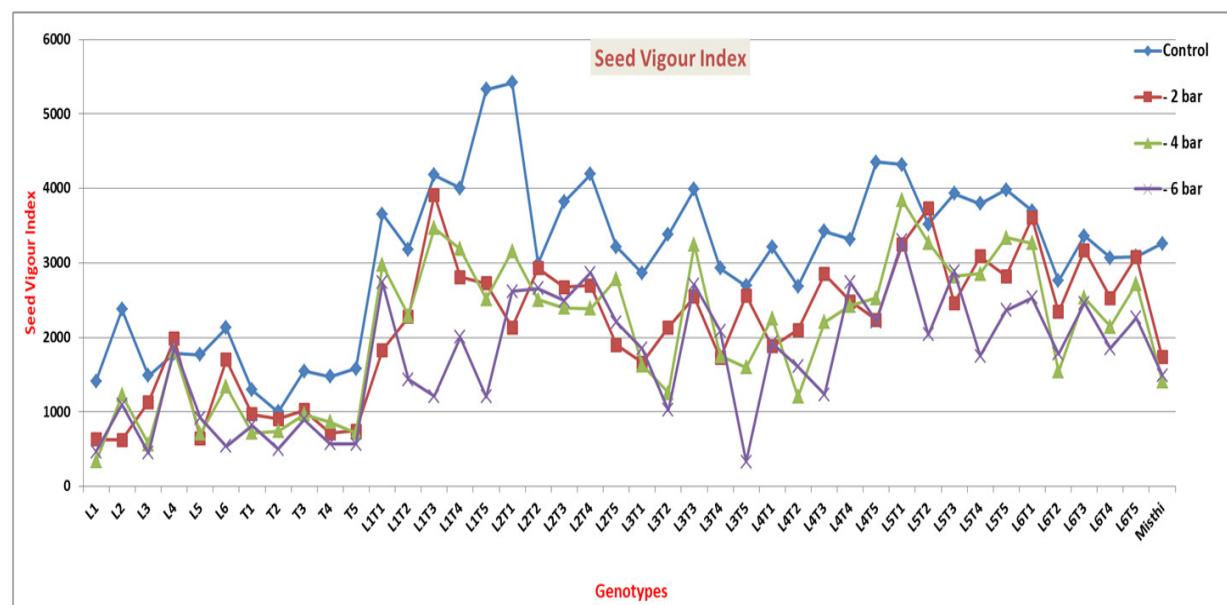


Fig. 1. Relative performance of parents and hybrids for seed vigour index under various PEG 6000 induced osmotic stress

In the inbreds, R/S ratio was observed to be 1.168, 1.262, 1.244 and 1.239 under 0, -2, -4 and -0.6 bars water potential respectively (**Table 3**). In hybrids by reducing the water potential, R/S ratio was found to increase from 1.729 (0 bar) to 1.81 (-6 bar). In control, high root/shoot ratio was recorded in several test entries but under drought stress they failed to exhibit the same trend. L_1 , L_3 , L_4 , L_5 , T_1 and hybrids $L_2 \times T_4$, $L_3 \times T_1$, $L_3 \times T_4$, $L_3 \times T_5$, $L_4 \times T_2$, $L_5 \times T_2$, $L_5 \times T_3$, $L_5 \times T_4$, $L_5 \times T_5$, $L_6 \times T_1$ on imposing mild stress R/S

ratio is found to be increased. This is due to the increase in root length over shoot length. Among the hybrids, $L_1 \times T_3$, $L_1 \times T_5$, $L_5 \times T_1$, $L_6 \times T_5$ showed increased R/S ratio up to -4 bar and decreased at higher stress level (-6 bar). In L_5 , T_2 , T_3 , $L_3 \times T_3$, $L_2 \times T_5$, $L_3 \times T_1$, $L_4 \times T_1$ and Misthi showed an increasing trend with lowering water potential. This increasing trend of R/S ratio corroborates with the findings of Queiroz et al. (2019) in maize and sorghum.

Table 4. Mean comparison of main effects of sweet corn genotypes under different levels of moisture stress for various plant traits.

INBREDS	GP (%)	SL (cm)	RL (cm)	SEL (cm)	PHSI (%)	RLSI (%)	R/S	SVI
L_1	62.50	7.14	3.41	10.55	67.16	60.72	0.46	712.13
L_2	60.00	7.72	14.50	22.22	75.35	72.45	1.86	1373.63
L_3	63.75	5.44	8.13	13.57	77.64	78.51	1.49	915.28
L_4	82.50	8.64	14.24	22.66	107.78	106.08	1.66	1859.50
L_5	63.75	8.31	7.00	15.31	82.04	84.43	0.84	1014.08
L_6	63.75	7.69	13.64	21.33	80.09	88.24	1.84	1445.53
T_1	73.75	6.01	6.89	12.90	89.77	101.16	1.18	955.48
T_2	58.75	6.22	7.25	13.32	86.64	100.59	1.22	796.94
T_3	60.00	7.33	11.00	18.33	88.65	93.37	1.53	1111.53
T_4	76.25	6.93	4.36	11.66	67.11	59.81	0.63	909.43
T_5	66.25	7.20	5.96	13.16	71.91	73.28	0.82	908.40
HYBRIDS								
$L_1 \times T_1$	85.00	12.38	20.11	32.49	79.59	80.42	1.62	2798.08
$L_1 \times T_2$	73.75	11.67	18.22	29.27	67.02	73.21	1.62	2295.98
$L_1 \times T_3$	83.75	13.59	22.85	36.44	74.23	89.12	1.72	3193.38
$L_1 \times T_4$	86.25	12.98	21.65	34.63	83.84	88.08	1.68	3002.48
$L_1 \times T_5$	78.75	15.23	20.07	35.30	61.17	83.22	1.62	2944.15
$L_2 \times T_1$	90.00	12.37	24.59	36.95	86.33	67.72	1.93	3333.93
$L_2 \times T_2$	88.75	12.28	18.69	30.96	92.57	95.88	1.55	2772.70
$L_2 \times T_3$	87.50	12.17	20.30	32.47	91.22	75.69	1.67	2847.98
$L_2 \times T_4$	82.50	15.64	20.91	36.55	68.86	89.89	1.50	3036.10
$L_2 \times T_5$	81.25	9.98	20.93	30.91	87.04	86.26	2.11	2526.98
$L_3 \times T_1$	71.25	8.93	18.71	27.64	70.00	81.43	2.17	1997.78
$L_3 \times T_2$	62.50	10.80	19.34	29.73	91.65	82.50	1.79	1948.00
$L_3 \times T_3$	87.50	12.96	22.59	35.55	71.80	94.28	1.84	3121.38
$L_3 \times T_4$	73.75	8.77	19.53	28.30	79.93	85.04	2.26	2123.95
$L_3 \times T_5$	57.50	11.19	18.86	27.88	77.50	87.41	1.68	1797.53
$L_4 \times T_1$	80.00	9.42	19.18	28.59	76.38	91.51	2.12	2319.88
$L_4 \times T_2$	70.00	8.71	17.39	26.10	90.44	92.04	1.99	1901.03
$L_4 \times T_3$	81.25	9.39	19.45	28.84	77.79	87.97	2.12	2428.30
$L_4 \times T_4$	85.00	10.18	22.06	32.24	88.99	87.06	2.17	2742.61
$L_4 \times T_5$	86.25	13.13	19.64	32.27	85.29	69.74	1.63	2834.75
$L_5 \times T_1$	98.75	12.29	24.93	37.23	82.81	88.40	2.05	3678.35
$L_5 \times T_2$	95.00	11.85	20.98	32.83	93.85	93.19	1.79	3140.80
$L_5 \times T_3$	87.50	12.69	21.60	34.29	85.74	89.78	1.72	3024.53
$L_5 \times T_4$	86.25	12.64	20.66	32.60	78.87	86.36	1.65	2873.28
$L_5 \times T_5$	86.25	12.37	23.62	35.98	81.96	88.60	1.94	3127.90
$L_6 \times T_1$	91.25	12.67	23.12	35.79	93.67	98.72	1.84	3275.25
$L_6 \times T_2$	92.50	10.76	12.14	22.56	92.28	77.88	1.12	2106.80
$L_6 \times T_3$	95.00	13.37	16.81	30.18	111.43	77.63	1.38	2882.43
$L_6 \times T_4$	93.75	10.02	15.40	25.42	88.76	81.09	1.53	2399.74
$L_6 \times T_5$	95.00	11.90	17.39	29.29	1.67	97.27	1.48	2788.15
Misthi (c)	57.5	11.92	21.09	33.00	80.75	89.12	1.80	1975.45

a.Seedling Vigor Index (SVI):

According to the International Seed Testing Association (ISTA) seedling vigor is the sum total of all the properties of seed which determines its performance and activity in wide environment. Analysis of Seedling vigour Index which included shoot length, root length and germination per cent recorded in the experiment indicated that in inbreds SVI ranged from 2376 (L_2) to 1001(T_2) (Fig. 1). In -2 bar, SVI varied from 1983.05 (L_4) to 627 (L_2) and in -4 bar SVI varied from 1821 (L_4) to 631 (L_1). At higher stress level (-6 bar), highest SVI was recorded for L_4 (1870). Among the hybrids, highest SVI was reported in $L_2 \times T_1$ (5416.6) and lowest SVI in $L_4 \times T_5$ (4356) in control (0 bar). At mild stress (-2 bar), maximum SVI was expressed in $L_1 \times T_3$ (3913.7) and minimum in $L_3 \times T_1$ (1659). At moderate stress, $L_5 \times T_1$ had maximum SVI of 3844 and $L_4 \times T_2$ had minimum value of 1207.9. $L_5 \times T_1$ reported higher SVI (3300.4) and $L_3 \times T_5$ reported lowest SVI (332) under high stress (-6 bar). Most of the hybrids, excluding $L_1 \times T_2$, $L_1 \times T_3$, $L_1 \times T_5$, $L_3 \times T_2$, $L_3 \times T_5$, $L_4 \times T_3$ recorded higher SVI than standard hybrid Misthi at higher stress (-6 bar).

The genotypes with higher seedling vigour index under stress could be inferred to perform well in the field since, seedling vigor index is the single concept behind several characters which determine the quality and emergence potential of seedlings. Though $L_1 \times T_5$ recorded higher SVI under control, they could not perform well under high stress level because of drastic reduction in germination and shoot length. Among the inbreds, L_4 followed by L_2

was found to be tolerant under higher stress. Among the hybrids, $L_5 \times T_1$, $L_5 \times T_3$ followed by $L_2 \times T_4$ were also found to be tolerant under drought stress. The identified genotypes have proven to be with highly vigorous seeds so that, they could establish quickly, reduce evaporation from soil to certain extent and sustain under drought condition. Also, results from table 4 shows that the selected line L_4 and the hybrid $L_5 \times T_1$ recorded higher mean performances for most of the traits selected over various levels of drought stress. These could be further screened in the field for validation of their drought tolerant potential.

On grasping the genetics mechanism of seed germination which is imperative to upgrade the crop genetics to develop drought tolerant crop which can only ascertain "more crop per drop" possible to real life few genotypes have been identified. Considering the seedling vigour index, the lines WNC12069-2 and SC11-2 possessed an inherent potential to survive under higher osmotic conditions and the hybrids WNC12039-1×45503, WNC12039-1×45678 and SC11-2 ×45679 had better seedling parameters than the other hybrids including the check. Hence, these genotypes could be further evaluated in the field for their expressivity under *invivo* condition.

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