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Gradient *in vitro* Testing of Tomato (*Solanum lycoersicon*) Genotypes by Inducing Water Deficit: A New Approach to Screen Germplasm for Drought Tolerance

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Abstract: A new concept was tested for screening germplasm under *in vitro* condition using Polyethylene Glycol (PEG-6000) under 2 induced stress conditions by developing gradient with two replications in factorial CRD in MS medium. Important seedling growth parameters were recorded. Drought resistant mutant derivatives performed significantly superior for root characters over cultivated genotypes. Decrease in seedling growth was worth notice with increasing concentration of PEG indicating precise nature of *in vitro* screening. Under normal environment also, mutant exhibited better seedling growth than cultivated genotypes in respect to root traits with higher magnitude of initial stress root growth was severely retarded. Initially mild and later on increasing stress exhibited more root growth and reduced vegetative growth indicating assimilate translocation more towards root growth than shoot growth. In general mutant derivatives performed better than cultivated genotypes under all levels of water stress. This new screening method validated mutants to be drought tolerant at all levels of water stress. These *in vitro* studies were complemented by screening all genotypes under field condition and similar performance was observed for drought tolerance. This new Gradient *in vitro* (GIV) screening method may prove to be potential to study response of genotypes under stress.

Key words: Drought, *Solanum lycopersicon*, Gradient *in vitro*, Polyethylene Glycol (PEG), dry matter, stability

INTRODUCTION

The identification and selection of genotypes with improved drought tolerance will play an important role in developing Tomato genotypes with better yield and persistence during drought stress periods. Root penetration and leaf osmotic regulation under low water potential are most important drought avoidance traits. New Gradient *in vitro* method was developed to determine the stability of deep root production under two artificial water stress situations for validating its practical implementation. Using proposed method mutants were screened with aim of genetic enhancement in relation to drought tolerance.

The most widely used solute is the polymer, Polyethylene Glycol (PEG). PEG compounds have been used with monocots, dicots, gymnosperms, fungi and yeasts. PEG is described as a non-ionic water-soluble polymer, which is not expected to penetrate intact plant tissues rapidly and is widely used to induce water stress in higher plants (Nepomuceno *et al*., 1998). The decrease in seedling growth as a result of the decrease in osmotic potentials with increasing PEG concentration has been reported in Tomato (Manoj and Uday, 2007; Pillay and Beyl, 1990; Srinivasa and Bhatt, 1991), Rice (Pirdashti *et al*., 2003), Wheat (Dhanda *et al*., 2004), Barley (Kocheva and Georgiev, 2003), Ryegrass (Stacy *et al*., 2004), Black Gram (Geetha *et al*., 1997), Potato (Gopal and Iwama, 2007) and Cowpea (Abaye *et al*., 2004). Manoj (2005) reported 10 mg average root dry weight in drought tolerant mutant as compared to 6.5 mg average root dry weight in susceptible cultivated genotypes. Polyethylene Glycol (6000 MW) concentration of 20, 40 and 60 g L^{-1} was used in MS medium along with normal MS medium as control for seedling growth study.

Present research advocates new PEG-6000 based screening method for stress tolerance evaluation. Increasing concentration from top (0, 20, 40 and 60 g L^{-1}) represents initially available moisture and increasing stress levels with root growth. Decreasing concentration from top (60, 40, 20 and 0 g L^{-1}) of PEG-6000 represents conditions for initial sever stress and gradual moisture availability as root grows. Stability of 18 tomato genotypes under two different stress levels was studied and further confirmed by field screening. Present objective is to validate new Gradient *in vitro* screening method for seedling growth in tomato genotypes under water stress conditions.

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MATERIALS AND METHODS

Seedling Gradient *in vitro* culture: Present research was carried out between June 2005 to October 2006 at Vidya Pratishthan's school of Biotechnology, Baramati, India. For *in vitro* screening of tomato genotypes, MS basal media with various concentrations of PEG at the rate of 0, 20, 40 and 60 g L^{-1} were prepared individually and autoclaved at 121°C and 15 lb/sq inch for 15 min. Four layers of increasing and decreasing (PEG + Basal MS medium) were prepared with concentration of (0, 20, 40 and 60 gL^{-1} of PEG-6000) with layer of 1.5 cm each. After solidification of one layer, next layer was poured (Fig. 1). The seeds were surface sterilized with 70% ethanol for 1 min and then with mercuric chloride (0.1%) for 10 min and thoroughly washed with sterile distilled water for three times. The seeds were presoaked with sterile water for one day and the next day inoculated onto autoclaved media at the rate of 5 seeds per bottle. The genotypes under study includes naturally occurring mutant, its derivatives, hybrids developed using this mutant as one of the parent and some cultivated genotypes along with their hybrids (Table 1). All these inoculated culture bottles were maintained under optimum culture conditions at 16 photoperiod (70 µmol m^2 sec^{-1}) and 28°C temperature. Seedling growth study was recorded 30 days after inoculation. Root and shoot length (cm) as well as their respective fresh and dry weight (mg) were recorded for *in vitro* grown seedlings.

Field screening: Tomato genotypes under study were grown for evaluating field performance. Observations of 10 plants were taken and average were used for analysis. All the 18 genotypes were transplanted in RBD design with two replications having the spacing 60×60 cm. Normal cultivation practices were followed and plants were grown up to maturity. Morphological and anatomical observations were taken 90 days after transplanting. The results of field screening are presented in Table 1.

Statistical analysis: The mean, regression coefficient (bi) and mean square deviation from linear regression line (S^2d) were calculated using stability parameters proposed by Eberhart and Russel (1966) in their stability model. Genotypes were categorized according to regression coefficient (bi) i.e., (bi<1-susceptible, bi = 1-moderate, bi>1-tolerant). Greater the regression coefficient value tolerant the genotype.

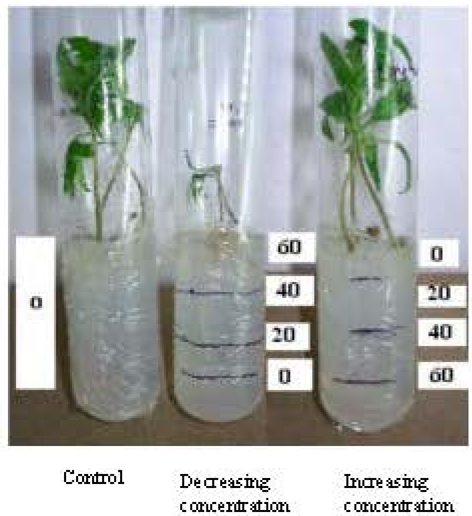

Fig. 1: Gradient PEG media (Hybrid-3)

Table 1: Comparative genotype analysis under field condition in relation to drought tolerance

Entry	Source/pedigree	Palisade mesophyll thickness (µm)	Shoot length (cm)	Root length (cm)	Root shoot ratio	Xylem vessels/ root cross section
MTG 1-1	Mutant derivative (M_1)	248.0*	52.0	67.0*	1.29	38.0
MTG 1-2	Mutant derivative (M_1)	236.0	58.0	39.0	0.67	31.0
MTG 1-3	Mutant derivative (M_1)	213.0	56.0	43.0	0.76	33.0
MTG 1-5	Mutant derivative (M_1)	95.0	32.0	17.0	0.53	13.0
TG 42	Pure line	162.0	60.0	32.0	0.53	16.0
TG 2-3	Pure line	137.0	130.0	30.0	0.22	21.0
Hy-1	TG-42 X TG 2-3	182.0	125.0	49.0	0.39	39.0
Hy-2	TG-42 X Mutant 1	168.0	72.0	47.0	0.65	28.0
Hy-3	Mutant 1 X TG-42	239.0*	75.0	71.0*	0.88	36.0*
MTG 1-4	Mutant derivative (M_1)	241.0*	49.0	62.0*	1.31	37.0*
Hy-4	TG-80 X TG-64	147.0	68.0	37.0	0.54	18.0
Hy-5	TG-5 X TG-13	151.0	132.0	40.0	0.30	20.0
TG-5	Pure line	126.0	132.0	32.0	0.24	23.0
TG-13	Pure line	163.0	62.0	27.0	0.43	19.0
TG-80	Pure line	167.0	60.0	38.0	0.46	16.0
TG-64	Pure line	126.0	65.0	35.0	0.53	18.0
Wild-2	Wild cherry tomato	168.0	95.0	45.0	0.47	21.0
Wild-4	Wild cherry tomato	176.0	105.0	49.0	0.46	25.0
SE		14.7	6.8	1.6	2.30	
CD (5%)		44.4	17.1	4.7		5.2
Population mean		188.0	73.3	45.4	0.66	28.0

*: Significant at 5% level

RESULTS AND DISCUSSION

Growth in basal MS medium (control): Mutant genotypes MTG 1-4, MTG 1-1 exhibited rapid root penetration as compared to other pure lines and hybrids. Restricted shoot growth of mutant genotypes as compared to more vegetative growth in pure lines and hybrids is worth mention (Table 2). Hybrid-3 (MTG 1 -4 X TG- 42) had longest roots (13 cm) as compared with range in other genotypes (4-8 cm). Significantly superior difference in root fresh weight and dry weight was also observed between mutant and cultivated genotypes. Root: Shoot length ratio ranged between 1.022-1.310 in mutants whereas it was lower in cultivated genotypes ranging 0.483-0.775. Higher magnitude of fresh biomass and dry matter production was also in similar trend. Mutants developed faster secondary roots as well as true leaves. Present results are in accordance with Pillay and Beyl (1990).

Increasing concentration gradient: Mutant (MTG-1-4) and its derivatives (MTG 1-4, MTG 1-3, MTG-1-2) roots crossed third layer (i.e., 40 g L^{-1} PEG concentration layer) indicating its ability to continue root growth (4.8-8.1 cm) with increasing water stress. Cultivated genotypes (TG-64, TG-23, TG-5) root growth under this condition was found to be poor ranging in between 2.5-4 cm only. Present results are in line with El Sayeed *et al*. (2002), Rao and Bhatt (1991) and Pillay and Beyl (1990). Root dry matter accumulation in mutants MTG 1-4, Hy-3 (MTG-1 X TG-42) and MTG 1-1 was found to be superior (9.23-10.71%) as compared to susceptible cultivated genotypes under study (7.98-8.86%). When compared with control overall reduction in root length (33%), number of secondary roots (42%) in mutant genotypes was observed as compared to 49.4 and 59% in cultivated genotypes, respectively (Table 3). Superior shoot growth was observed in genotype MTG 1-3 as compared to genotype TG-64 and TG-13 (Fig. 2). Overall increase in root dry matter was observed in all genotypes as compared to normal situation. These results are in accordance with El Sayeed *et al*. (2002).

Decreasing concentration gradient: This condition could discriminate all genotypes clearly in categories of tolerant, moderate and susceptible. It was interesting to note

TG-64 S   TG-13 S   MTG-13 R

Fig. 2: Effect of Increasing concentration in gradient media on seedling growth

Table 2: Performance of genotypes under normal MS medium (Control)

Entry	Root length (cm)	Shoot length (cm)	Root: Shoot ratio	Root Fresh wt. (mg)	Root Dry wt. (mg)	Root Dry matter (%)	Shoot Fresh wt. (mg)	Shoot Dry wt. (mg)	Shoot Dry matter (%)	No. of secondary roots	No. of true leaves
MTG 1-1	9.50	9.30	1.022	72	5.5	7.63	779	65.50	8.90	9.0	6.0
MTG 1-2	7.50	8.10	0.926	73	5.1	6.98	813	71.20	8.36	8.0	6.0
MTG 1-3	7.10	8.30	0.855	79	5.9	7.46	648	52.20	8.02	7.0	4.0
MTG 1-5	3.00	6.10	0.492	31	2.1	6.77	333	25.10	7.50	2.0	3.0
TG 42	9.00	12.30	0.732	72	5.4	7.60	473	38.20	8.03	6.0	5.0
TG 2-3	7.20	13.30	0.541	73	5.3	7.26	513	40.10	7.82	5.0	7.0
Hy-1	6.10	14.50	0.421	67	4.9	7.31	539	41.10	7.63	7.0	4.0
Hy-2	6.40	13.10	0.489	44	2.9	6.59	467	35.30	7.58	4.0	2.0
Hy-3	11.20	9.60	1.167*	102	8.5	8.33*	895	76.25	8.52	10.0*	8.0
MTG 1-4	9.30	7.10	1.310*	85	6.9	8.11*	867	72.90	8.41	10.0*	8.0
Hy-4	7.50	14.10	0.532	77	5.6	7.27	437	34.60	7.93	3.0	6.0
Hy-5	7.00	14.50	0.483	70	5.1	7.28	469	36.86	7.86	3.0	4.0
TG-5	7.10	13.30	0.534	51	3.5	6.86	339	25.40	7.51	3.0	5.0
TG-13	8.20	13.30	0.617	75	5.9	6.93	431	34.56	8.02	8.0	6.0
TG-80	8.00	11.70	0.684	79	6.2	7.97	635	50.22	7.91	4.0	5.0
TG-64	4.10	9.70	0.423	70	4.9	7.00	338	24.70	7.31	2.0	4.0
Wild-2	9.30	12.00	0.775	72	5.7	7.11	722	57.60	7.98	7.0	9.0
Wild-4	7.20	10.10	0.713	50	3.2	6.00	352	24.70	7.02	5.0	6.0
SE	0.21	0.28				0.11					
CD (5%)	0.62	0.83				0.32					

*: Significant at 5% level

Table 3: Performance of genotypes under increasing concentration of PEG in MS medium (i.e., 0, 20,40 and 60 g L^{-1})

Entry	Root length (cm)	Shoot length (cm)	Root shoot ratio	Root Fresh wt. (mg)	Root Dry wt. (mg)	Root Dry matter (%)	Shoot Fresh wt. (mg)	Shoot Dry wt. (mg)	Shoot Dry matter (%)	No. of secondary roots	No. of true leaves
MTG 1-1	5.10	5.30	0.943	52	4.8	9.23	336	79.0	8.63	4	4
MTG 1-2	5.10	5.90	0.864	54	4.9	9.02	361	32.0	8.86	3	4
MTG 1-3	4.80	6.10	0.787	48	4.1	8.54	318	28.0	8.81	3	3
MTG 1-5	2.10	3.40	0.618	22	1.9	8.60	151	13.0	8.62	1	1
TG 42	6.50	8.00	0.813	63	6.0	9.52	229	19.0	8.29	9	3
TG 2-3	3.50	8.10	0.432	38	5.1	13.10	229	22.0	9.61	5	5
Hy-1	4.10	8.30	0.494	44	3.9	8.86	262	22.0	8.32	3	2
Hy-2	1.10	7.00	0.143	5	0.5	10.01	41	3.5	8.53	2	---
Hy-3	8.10	8.50	0.953*	75	8.3	11.10*	365	35.1	9.06	7	5
MTG 1-4	6.90	5.20	1.327*	64	6.7	10.46*	351	32.7	9.34	6	5
Hy-4	3.20	7.10	0.429	56	6.0	10.71	211	22.0	10.42	5	5
Hy-5	4.30	5.40	0.796	47	5.1	8.52	235	22.0	9.36	2	2
TG-5	3.50	5.20	0.700	34	2.9	9.52	156	15.0	9.61	5	3
TG-13	4.50	9.10	0.500	42	4.1	9.51	213	17.0	7.98	4	4
TG-80	3.90	6.30	0.619	48	4.0	8.43	292	25.3	8.66	3	3
TG-64	2.50	5.30	0.472	32	2.7	8.43	147	14.1	9.59	2	2
Wild-2	4.10	7.50	0.533	53	7.0	13.20	335	33.0	9.85	11	6
Wild-4	4.10	5.50	0.745	22	1.8	8.18	166	13.0	7.83	5	3
SE	0.16	0.34				0.15					
CD (5%)	0.47	1.03				0.47					

*: Significant at 5% level

Table 4: Performance of genotypes under decreasing concentration of PEG in MS medium (i.e., 60, 40, 20 and 0 g L^{-1})

Entry	Root length (cm)	Shoot length (cm)	Root shoot ratio	Root Fresh wt. (mg)	Root Dry wt. (mg)	Root Dry matter (%)	Shoot Fresh wt. (mg)	Shoot Dry wt. (mg)	Shoot Dry matter (%)	No. of secondary roots	No. of true leaves
MTG 1-1	4.90*	4.60	1.065	73	6.50	8.90	52	4.11	7.88	3	1
MTG 1-2	4.70	4.60	1.022	72	6.40	8.88	54	4.05	7.41	3	1
MTG 1-3	4.20	4.40	0.955	67	6.00	8.95	50	3.81	7.62	2	1
MTG 1-5	1.10	1.10	0.909	21	1.60	7.61	23	1.20	5.21	----	----
TG 42	3.50	2.00	1.750	70	7.50	10.42	12	0.99	8.25	----	----
TG 2-3	3.10	3.10	1.033	43	3.90	9.06	45	3.99	8.66	----	----
Hy-1	3.30	2.90	1.138	42	3.70	8.80	43	3.20	7.44	----	----
Hy-2	3.20	2.80	1.143	31	2.80	9.03	38	2.91	7.63	----	----
Hy-3	5.20*	5.10	1.020*	85	7.70	9.05	72	6.31	8.75	5	2
MTG 1-4	5.10*	4.70	1.064*	78	7.30	9.35	68	5.92	8.67	4	2
Hy-4	2.30	3.30	0.697	44	3.80	8.63	42	3.11	7.38	----	----
Hy-5	3.00	3.10	0.968	53	4.30	8.11	46	3.02	6.52	----	----
TG-5	3.50	1.10	3.500	8	0.70	8.75	29	2.31	7.93	----	----
TG-13	3.80	3.90	0.974	68	6.70	10.42	12	1.05	8.45	----	----
TG-80	4.10	4.10	1.000	71	6.60	9.29	32	2.03	6.56	4	1
TG-64	2.00	2.30	0.870	22	2.00	9.09	23	1.91	8.26	----	----
Wild-2	2.10	3.20	0.700	4	0.49	12.25	6	0.52	8.73	----	----
Wild-4	0.30	3.10	0.100	2	0.20	10.00	8	0.88	10.03	----	----
SE	0.13	0.21				0.20					
CD(5%)	0.38	0.64				0.60					

*: Significant at 5% level

that, high level of initial stress situation leads to dramatic reduction in root growth. Under this stress situation also, mutant genotypes MTG 1-4, Hy-3, MTG 1-1 exhibited superior root length (4.9-5.2 cm), shoot length (4.6-5.1 cm) and superior dry matter accumulation [root (8.9-9.35%) and shoot (7.3-8.6%)] as compared to susceptible genotypes TG-64, T-5 and TG-2-3 (Table 4). It was also observed that growth of secondary roots and true leaves is adversely affected if initially high level of water stress is imposed during seedling growth and establishment. Present results are as per reports of Abaye *et al.* (2004) and Manoj and Uday (2007).

This experiment was an attempt to mimic two soil conditions *in vitro* i.e., increasing and decreasing moisture gradient. Increasing gradient represented situation where initially moisture is available for seedling growth but as root penetrates moisture availability goes on reducing. Decreasing gradient situation provided water stress for seedling growth at higher level initially and moisture availability gradually increased if seedling could establish early. Practically first situation rarely exists in field condition.

Under normal condition mutant genotypes exhibited characteristic feature of drought tolerant genotype by rapid root growth, controlled vegetative growth, more dry matter accumulation, more number of secondary roots and faster leaf growth. Overall root and shoot growth under increasing concentration gradient was hampered remarkably as compared to control. Root growth was more affected than shoot growth. Increase in dry matter (1-2%) as compared to control was observed in about all genotypes indicating assimilation of photosynthate from leaves to roots under water stress situation. Findings of de Quiroz *et al*. (1997), El Sayeed *et al*. (2002), Abaye *et al*. (2004) and Manoj and Uday (2007) supports these results. Increasing concentration gradient screening where moisture is available for initial growth but as roots grow, the moisture availability goes on decreasing. This is an ideal condition to study ability of roots to continue growth under increasing water stress situation.

Decreasing concentration reflected situation when drought condition prevails at the time of initial seedling growth. Increased root length and biomass as compared to shoot were observed under this condition. Present results are in accordance with Srinivasa and Bhatt (1993). Mutant genotype MTG 1-4 and its derivatives (MTG 1-1, MTG 1-2, MTG 1-3) were able to maintain root: shoot ratio greater than one whereas other susceptible genotypes had significantly lower root: shoot ratio indicating their inability to continue root growth under water stressed situation. Root dry matter content was found to be increased and shoot dry matter found to be decreased due to water stress situation. The main adverse effect was on secondary root development. Overall reduction in number of secondary roots under increasing concentration gradient was 13.6% whereas under decreasing concentration gradient was 76.4%. These results puts some more light on adverse effect of sever initial water stress on seedling establishment. Overall root development and expansion of true leaf development was significantly reduced under decreasing water stress gradient situation. Present results are in accordance with El Sayeed *et al*. (2002) and Abaye *et al*. (2004). Relatively higher root length and root biomass production by mutant and its derivatives indicated their remarkable ability to continue root growth under sever water stress situation.

Early seedling establishment is an important attribute for raising successful vegetable crop under water scarcity area. Under normal situation mutant and its derivatives were noted with highest root: shoot ratio (1.1-1.3) as well as root dry matter production (7.3-8.3%) their early vigor for seedling growth. This is one of the most important feature for selecting water stress tolerant genotypes.

Stability of genotypes: Eberhart and Russel (1966) statistical analysis model was used to calculate stability of root penetration ability (root growth) under three varying environments. A genotype with high mean value of regression coefficient more than one (bi>1) and deviation not significantly differing from zero ($S^2di = 0$) would be stable for drought resistance under various environments. The data pertaining to mean values of root length obtained in all three environments, average mean values, stability parameters viz. regression coefficient (bi) and mean square deviation from linear regression coefficient ($S^2di = 0$) are depicted in Table 5. Figure 3

Table 5: Performance of genotypes over gradient *in vitro* environments and stability parameters for root length

	Environment (E)					
	Root length (cm)			Stability parameters		
Genotypes	E_1 (Control)	E_2 (Increasing PEG concentration)	E_3 (Decreasing PEG concentration)	Mean	bi	S^2di
MTG 1-1	9.50*	5.00	4.90*	6.47*	1.15	0.53
MTG 1-2	7.60	5.10	4.70	5.80	0.70	0.03
MTG 1-3	7.10	4.80	4.30	5.40	0.67	0.00
MTG 1-5	3.00	2.10	1.00	2.03	0.42	0.24
TG 42	9.00	6.50	3.50	6.33	1.15	1.84
TG 2-3	7.00	3.50	3.10	4.53	0.95	0.13
Hy-1	6.00	4.10	3.30	4.47	0.62	0.01
Hy-2	6.00	1.00	3.20	3.40	0.87	4.87
Hy-3	13.00*	8.00*	5.20*	8.73*	1.74	0.63
MTG 1-4	11.00*	6.90*	5.00*	7.63*	1.36	0.16
Hy-4	7.00	3.00	2.30	4.10	1.13	0.07
Hy-5	7.00	4.30	3.00	4.77	0.90	0.08
TG-5	7.00	3.50	3.50	4.67	0.88	0.37
TG-13	8.00	4.50	3.80	5.43	1.00	0.03
TG-80	8.00	3.90	4.00	5.30	1.01	0.60
TG-64	4.00	2.50	2.00	2.83	0.46	-0.01
Wild-2	9.00	4.00	2.10	5.03	1.59	0.05
Wild-4	7.00	4.00	0.30	3.77	1.40	2.86
Pop. Mean	7.57	4.26	3.29	5.04	1.00	0.23
SE	0.21	0.16	0.10	0.59		
CD (5%)	0.62	0.47	0.29	1.76		

*: Significant at 5% level

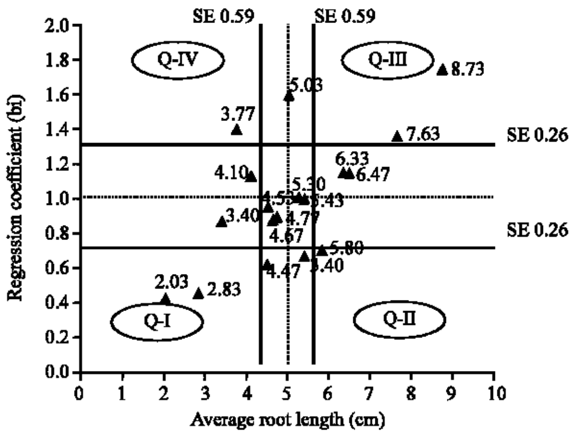

Fig. 3: Mean performance and stability of genotypes for root length

shows categorization of genotypes according to their average performance under three varying situations. Genotypes in QIII are most stable for their root growth under all situations. It is noted that higher the average root length and regression coefficient, higher the stability. Lower average root length and regression coefficient indicates susceptibility to water stress.

Overall average performance for root length was found to be best in Hy-3 (8.73 cm) in which mutant genotype MTG 1-4 was used as female parent. Resistant genotype mean root length ranged between 6.47-8.73 cm whereas susceptible genotypes ranged between 2.83-5.43 cm only. This difference under varying environments confirms ability of mutant genotypes (MTG 1-4, MTG 1-1, MTG 1-4) and Hybrid-3 ability for water stress tolerance. Most of mutant genotypes exhibited more than unity indicating tolerance and almost all cultivated genotypes had regression coefficient significantly less than unity indicating susceptibility. Furthermore, *in vitro* screening results were confirmed by field screening under stress situation to all genotypes and similar results were obtained.

The present investigation was an attempt to suggest an improvement over earlier screening methods using PEG-6000 for water stress tolerance. This Gradient *in vitro* method is having advantage of studying genotypic performance under varying environments. Furthermore, screening results can be subjected to stability analysis to compare genotypes for their potential of water stress tolerance levels.

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