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Full Length Research Paper

In Vitro screening of tomato genotypes for drought resistance using polyethylene glycol

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Drought is a major abiotic factor that limits plant growth and productivity. Tomato is an important vegetable crop and area under production is limited by irrigation water scarcity. Effort was made to screen tomato germplasm under *in vitro* condition using polyethylene glycol (PEG) at four concentrations (0, 20, 40 and 60 g/l) with two replications in factorial CRD. Important seedling characters like root length and weight; shoot length and weight were recorded. Drought resistant mutant derivatives and hybrid produced using mutant derivative as female parent performed significantly superior for root characters. Decrease in seedling growth was worth notice with increasing concentration of PEG indicating precise nature of the *in vitro* screening. Mutant hybrid and its derivatives were observed with outstanding ability to continue root growth under *in vitro* stress conditions indicating there ability to fight with sever water stress situation. These results were further confirmed for early indication traits in raised bed seedlings and fully-grown mature plants under field conditions. At all three experimental conditions, mutant derivatives and hybrids performed better than cultivated genotypes under all levels of water stress. Based on results, Hy-3 and MTG 1-4 were found to be drought resistant due to there remarkable performance at all levels of water stress. This *in vitro* screening method is potential and cost effective method to screen large set of germplasm within very less time period and accurately.

Key words: Drought, tomato, polyethylene glycol, xylem, anatomy.

INTRODUCTION

Rain fed vegetable production is need of the day to cope up with increasing demand for vegetable crops considering increasing world population. Water is a major constraint in tomato production under rain fed condition in case of dry spell during production. Water stress creates elevated osmotic pressure in the root zone and reduces availability of water and nutrients to plant limiting growth. Tomato (*Solanum lycopersicon*) is one of the most important vegetable crops grown in India. With an annual production of 5.4 million Mt, the country is the sixth largest tomato producer in the world. To fulfill increasing demand for this nutritionally important vegetable, rain fed tomato cultivation is advocated.

The existence of genotypic variability reported for diffe-rences to drought resistance in solanaceous vegetable (Srinivasa Rao and Bhatt, 1991), tomato (Pillay et al., 1990), Black gram (Geetha et al., 1997) and many researchers worldwide. In the present investigation, 16 tomato genotypes were screened for drought resistance using *in vitro* technique and efforts were made to study effect of water stressed situation on seedling growth. Polyethylene glycol (6000 MW) was used for *in vitro* screening of tomato germplasm to study response of these lines at different water stress levels. Seedling growth on raised beds and crop growth under normal field conditions up to maturity was also compared to see overall performance of genotypes under study.

MATERIALS AND METHODS

For *in vitro* screening of tomato genotypes, Murashige and Skoog (1962) MS basal media with various concentrations of PEG; 0, 20, 40 and 60 g/l were prepared, poured in bottles and autoclaved at 121^{0}C and 15 lb/sq. inch for 15 min. The seeds were surface sterili-

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zed 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 for one day and the next day inoculated onto autoclaved media at 5 seeds per bottle. All these inoculated culture bottles were maintained under optimum culture conditions at 16 photoperiod (70 µ mol M^2 s^{-1}) and 28^0C temperature. Seedling growth study was recorded 30 days after inoculation. Root and shoot length (cm) as well as their respective weight (mg) were recorded for *in vitro* grown seedlings. Factorial CRD analysis was carried out using MAU stat software package.

Seedlings were grown on raised beds to screen field performance. Proper care of seedlings was taken to avoid disease pest incidence. Observations were taken after 30 days of sowing. All the genotypes were transplanted in RBD design with two replications having the spacing 60 x 60 cm. Normal cultivation practices were followed and plants were grown up to maturity. Morphological and anatomical observations were taken 90 days after transplanting.

RESULTS AND DISCUSSION

In *in vitro* experiment, root length and weight were characters of special interest. Significant differences were observed under different PEG concentrations of 0, 20, 40 and 60 g/l. Seedlings were at stage of first true leaf initiation stage. Results of root characters are depicted in Table 1 and shoot characters in Table 2

Root length

Early and rapid elongation of roots is important indication of drought resistance. Ability of continued elongation of root under situation of water stress was remarkable character of mutant genotypes. At 20 g/l concentration of PEG, mutant hybrid exhibited 8.5 cm long roots whereas genotype TG –5 had only 2.2 cm long roots. Mutant and its cross with determinate line (i.e. Hy -3) showed lowest reduction in length whereas other susceptible lines reduced length in root. Performance of genotype TG-80 was worth to note amongst cultivated germplasm. It was interesting to note that this cultivated genotype maintains stay greenness throughout the growth in field conditions also.

Root weight

Highest root weight was recorded by drought resistant Hy–3 (102, 44, 30, 22 mg) whereas lowest weight was noted in susceptible cultivar TG–5 (20, 18, 14, 10 mg) when tested at 0, 20, 40 and 60 g/l, respectively (Figure 3). Resistant genotypes managed to maintain root growth at this highest concentration also. Mutant derivatives and hybrid indicated extra ability of roots to extract water in water stressed situation by maintaining growth at artificial drought condition. Our results are in confirmation with Tyagi et al. (1995)

Primary root initiation

Drought resistant genotypes produced early and more primary roots. Invariably all drought resistant lines had 8 – 10 primary roots as compared to 3 - 4 in drought susceptible genotypes. These extra primary roots give an increased ability to these plants for early establishment of seedlings in field condition in drought situation and impart increased vigor. At 20 g/l PEG concentration there was reduction in number of primary roots in all genotypes. Reduction was more in drought susceptible cultivars as compared to drought resistant cultivars. In drought resistant cultivars, only 1 - 2 primary roots were reduced whereas in susceptible genotypes average 3 - 4 primary roots reduced. At highest concentration of PEG 60 g/l, only mutant and its hybrid could produce primary roots whereas none of genotype could produce primary roots.

Shoot Length

Root length is more affected to drought condition than shoot length. Balanced growth was observed in drought resistant mutant derivatives and hybrid as demonstrated through restricted vegetative growth and lower magnitude of shoot length. Drought resistant mutant (MTG 1-4) was noted with 7 cm shoots whereas genotype TG – 13 recorded 10 cm shoot length. Drastic reduction in shoot growth was observed with increasing PEG concentration, which was considerably lower in mutant derivatives and hybrid, hence resistant (Figure 1).

Cultivars with indeterminate growth habit showed more reduction in length as compared to determinate type. It indicates that determinate tomato can be well suited to drought areas than indeterminate growth habit. Turner (1979) has recommended indeterminate growth habit for drought tolerance but our results advocate determinate growth habit better for drought resistance.

Figure 1. *In vitro* grown seedlings of tomato genotypes during drought resistance using polyethylene glycol.

Shoot weight

Highest weight was 213 mg in mutant plant MTG 1-4 whereas lowest was observed in line TG-5 (37 mg).

Table 1. Effect of water stress on root characters of tomato genotypes during drought resistance using polyethylene glycol.

Genotypes	Root length (cm)					Weight length (mg)				
	Control	20 g/l PEG	40 g/l PEG	60 g/l PEG	Mean	Control	20 g/l PEG	40 g/l PEG	60 g/l PEG	Mean
MTG 1-1	9.5	5.8	4.3	2.1	5.43	106	48	36	26	54*
MTG 1-2	6.7	4.8	3.9	2	4.35	82	38	18	6	36
MTG 1-3	7.1	3	2.7	1.8	3.65	68	44	34	30	44
MTG 1-5	3.2	2.5	2	1.5	2.30	26	20	16	14	19
TG 42	9.3	3	2	1	3.83	56	24	8	6	23.5
TG 2-3	8	3	1	1	3.25	64	10	6	6	21.5
Hy-1	6.3	3	1	1	2.83	26	18	14	6	16
Hy-2	6.1	2.5	2	1.5	3.03	38	26	20	14	24.5
Hy-3	10.25*	8*	5	3.1	6.59*	102	44	30	22	49.5*
MTG 1-4	9.1	6.5	4.5	2.5	5.65	96	34	24	18	43*
Hy-4	7.2	3.5	1.5	1.5	3.43	74	22	14	14	31
Hy-5	7.1	3.5	1.6	1	3.30	34	24	22	16	24
TG-5	7.4	2	1.9	1.7	3.25	20	18	14	10	15.5
TG-13	5.3	4	1.5	1.45	3.06	54	26	12	12	26
TG-80	4.5	4	1.7	1.5	2.93	32	30	2	2	16.5
TG-64	4.2	3	2	1	2.55	24	22	10	8	16
	G	G X L							G	G X L
SE ±	1.43	2.87							4.8	9.7
CD (5%)	3.96	7.94							13.2	27.01

Figure 2. Field grown seedlings of tomato genotypes during drought resistance using polyethylene glycol.

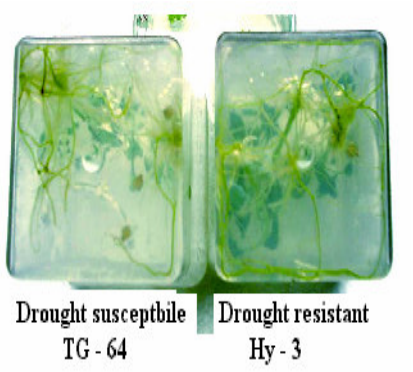

Figure 3. Comparative root growth at maturity of tomato genotypes during drought resistance using polyethylene glycol.

Resistant lines weight ranged between 150-200 mg while susceptible lines weight was between 40-100 mg. In MS medium with highest concentration of PEG (60 g/l), except drought resistant cultivars near about all genotype seedlings ceased growth. MTG 1-4 and Hy–3 indicated growth indicating highest ability to establish seedlings under this concentration also. Our results are in accordance with Rao and Bhatt (1991).

Seedling and field anatomical studies

The data depicted in Table 3 clearly indicate performance of genotype from seedling growth pattern in field condition. Statistically significant differences in root length, number of feeder roots and fresh weight of roots can be observed. Similarly stress tolerant genotype exhibits controlled shoot growth and more stem girth as compared to susceptible genotypes (Figure 2). We calculated a simple parameter here, which can be useful as preliminary screening of large number of genotypes for drought resistan-

Table 2. Effect of water stress on shoot characters of tomato genotypes during drought resistance using polyethylene glycol.

Genotypes	Shoot length (cm)					Shoot weight (mg)				
	Control	20 g/l PEG	40 g/l PEG	60 g/l PEG	Mean	Control	20 g/l PEG	40 g/l PEG	60 g/l PEG	Mean
MTG 1-1	8.2	6.2	4.1	1.2	4.93	192	113	79	17	100.3
MTG 1-2	6.3	4.5	3.8	3.3	4.48	158	101	81	7	86.8
MTG 1-3	6.2	5.5	4.5	4	5.05	149	95	70	52	91.5
MTG 1-5	4.5	3.8	3.5	3	3.70	85	63	41	29	54.5
TG 42	9.2	6.5	2.2	1.8	4.93	203	55	16	7	70.3
TG 2-3	6.1	5.3	3.2	2.5	4.28	167	41	29	11	62.0
Hy-1	10.1	6.2	4.2	3.5	6.00	190	97	72	43	100.5
Hy-2	9.3	8.1	5.3	3.9	6.65	164	61	39	22	71.5
Hy-3	7.5	6.3	5.4	4.3	5.88	198	156	102	48	126.0
MTG 1-4	7.6	6.5	5.3	3.9	5.83	213	179	113	65	142.5
Hy-4	8.8	7.4	4.5	4	6.18	159	59	32	32	70.5
Hy-5	8.5	5.3	3.5	2.8	5.03	58	26	20	12	29.0
TG-5	6.2	4.5	3.5	1.8	4.00	37	3	2	1	10.8
TG-13	10.1	9.6	5.5	2.7	6.98	128	81	41	34	71.0
TG-80	7.6	6.5	3.8	2.5	5.10	59	52	13	6	32.5
TG-64	5.5	4.2	2	1.8	3.38	40	33	17	10	25.0
		G X E							G	G X E
SE ±	2.36	4.7							22.9	29.87
CD (5%)	6.5	13.08							63.43	82.76

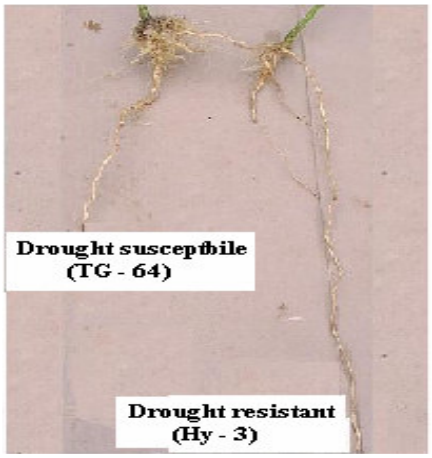

Figure 4. Comparative root growth of tomato genotypes under drought resistance field screening.

ce: Root vigor index (RVI) = Length of root (cm) X fresh weight of root (g) X Number of feeder roots/5. Seedlings were scored on basis of RVI. These results were compared with field screening results and *in vitro* results as well as other stress tolerance parameters like anatomical screening (Table 4). The important leaf parameters identified were leaf thickness, palisade mesophyll height and leaf strength index (LSI). Stomatal parameters like number of stomata on lower side of leaf, distance between stomata also plays crucial role in increasing water use efficiency of plants (Kulkarni and Deshpande, 2006a). Kulkarni et al. (2007) reported anatomical variability in wine purpose grape genotypes for water use efficiency. The various anatomical and morphological traits were correlated with chlorophyll content as well as yield.

Stem acts as main reservoir of stored starch during stress situation of plant for survival as well as optimum yield levels. Stem parameters like stem girth, secondary phloem width plays important role in dry matter partitioning ability of plants for sustaining water stress situation (Kulkarni and Deshpande, 2006b).

The strength and ability of moisture absorption is dependent like total root length, number of xylem poles in roots, number of feeder roots etc. These parameters are genetically governed and can be introgressed (Kulkarni and Deshpande, 2006c). Figure 5 shows root length variability studied at three different growth stages i.e. *in vitro* seedlings, field-grown seedlings and root length at maturity i.e. 90 days after transplanting. It is clear from graph that mutant genotypes (drought resistant) exhibits superior performance in all three situations if compared with cultivated genotypes (drought susceptible). The stable performance under normal field condition, water stressed field situation as well as higher magnitude of average *in vitro* root growth was observed under various levels of stress. Early indications at seedling conditions,

Table 3. Comparitive performance of seedling of tomato genotypes under field condition.

Genotype	Root length (cm)	Shoot length (cm)	Root Weight (g)	Shoot Weight (g)	Number of feeder roots	Secondary roots	Root vigor Index (RVI)
MTG 1-1	12.3	7.3	0.193	1.397	28	3	66.469
MTG 1-2	11	6.3	0.182	1.262	27	3	54.054
MTG 1-3	9.7	6	0.179	1.301	24	2	41.671
MTG 1-5	3.9	4.2	0.082	0.503	2	----	0.640
TG 42	6.5	7.5	0.115	0.904	11	1	8.223
TG 2-3	7.1	6.3	0.129	1.092	12	1	10.991
Hy-1	9	6.8	0.168	1.273	11	2	16.632
Hy-2	12	6	0.181	1.404	12	2	26.064
Hy-3	12.5	7.5	0.201	1.393	29	3	52.461
MTG 1-4	13	7.3	0.215	1.403	36	3	100.620
Hy-4	7.8	6.3	0.193	1.013	19	1	28.603
Hy-5	8	6.8	0.137	1.107	21	1	23.016
TG-5	6.3	6.5	0.129	1.023	18	1	14.629
TG-13	7.1	6.5	0.148	1.139	24	1	25.219
TG-80	7.9	6.8	0.153	1.208	21	1	25.383
TG-64	6	5.9	0.126	1.157	13	1	9.828
S.E	0.26	0.353	5.45	26.58	2.19		
C.D at 5%	0.79	1.05	16.32	79.57	6.56		
Population mean	4.62	7.72	5	3.28	16.94		

Table 4. Genotypic correlation and anatomical screening of tomato genotypes during drought resistance under field condition.

Plant part	Trait	Genotypic Correlation	Resistant range	Susceptible range	Reference
Root	Length (cm)	0.795	40 - 71	35 – 49	Kulkarni (2005)
	Number of xylem poles	0.630	31 - 39	18 - 23	
	Tertiary roots	0.791	39 - 49	19 -27	
	Fresh weight of roots (g)	0.687	39 - 49	19 -24	
Stem	Secondary phloem width (µm)	0.706	403-693	252 –378	(Kulkarni and Deshpande, 2006b)
Petiole	Distance from lower epidermis to phloem (µm)	0.467	907 - 1134	693 – 858	(Kulkarni and Deshpande, 2006a)
	Phloem width (µm)	0.590	693 - 957	567 – 643	
	Xylem number	0.576	14 - 18	12 – 15	
Leaf	Number of stomata on lower side of leaf	- 0.510	102 - 132	192 – 255	(Kulkarni and Deshpande, 2006a)
	Distance between stomata on lower side of leaf (µm)	0.487	189 - 223	100 – 162	
	Leaf thickness (µm)	0.682	463 - 550	432 – 487	
	Palisade mesophyll height (µm)	0.743	182 - 239	126 – 163	
Seedling	Root length (cm)	0.641	9-13	6 – 8	Kulkarni (2005)
	Root weight (m)	0.741	126 – 137	182 215	
	Number of feeder roots	0.746	27 - 36	13 – 21	

stable root growth under water stressed situation (Figure 4) and screening of genotypes under *in vitro* conditions simultaneously can give clear discriminative categorization of drought susceptible and drought resistant genotypes.

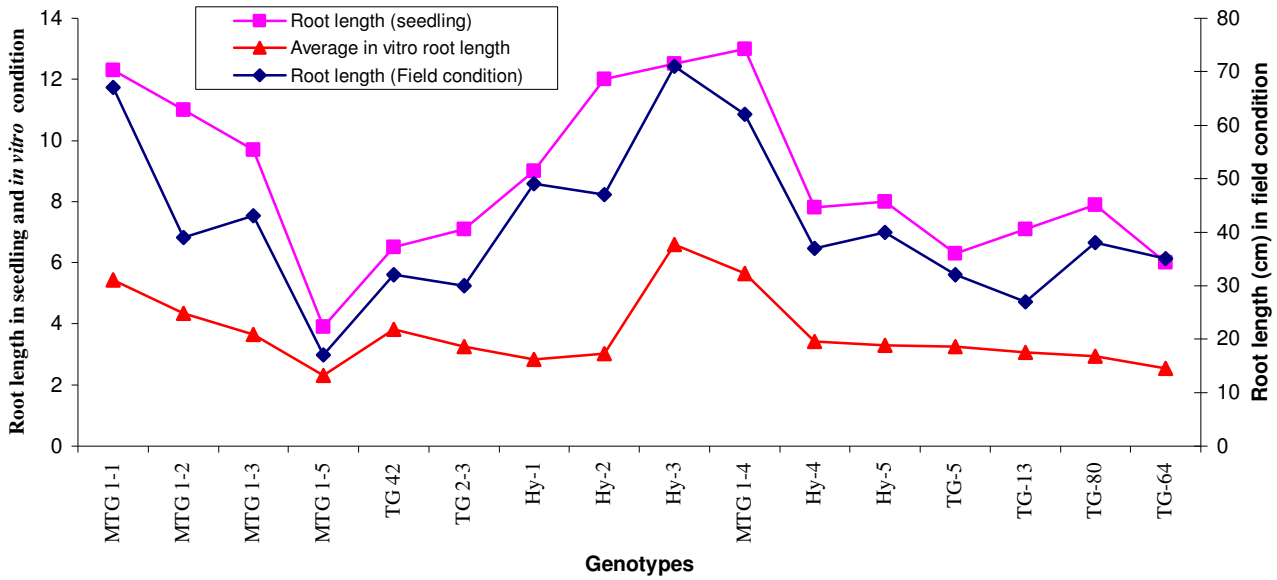

Figure 5. Comparative root growth studies of drought resistance of tomato genotypes using polyethylene glycol.

Conclusion

Drought resistance screening under field condition involves lot of environmental factors that affects phenotypic expression of genotype. These effects cannot be completely avoided in dorught resistance screening experiments. This *in vitro* screening method proves to be an ideal method to screen large set of germplasm with less efforts and accuraetly. *In vitro* growth pattern differences are due to genotypes and least environmental influence is advantage.The correct and clear expression of genotype can be evalueated by this method using different concentrations of polyethylene glycol (PEG). These experiments can be additionally supported by field seedling and crop evaluation methods to validate drought resistant genotypes.

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