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Comparative Studies in Stem Anatomy and Morphology in Relation to Drought Resistance in Tomato (*Lycopersicon esculentum*)

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Abstract: Anatomical and morphological aspects are integral part of drought resistance studies. The yield of a crop under water stressed situation is combined effect of anatomical, physiological and bio-chemical processes, which are controlled by genetic make up of plant and prevailing environment. Stem plays a major role as storage organ of food material for plants, which is useful in abiotic stress situation like drought. Substantial variation in stem girth (2.0 to 4.3 cm), number of xylem vessels per stem cross section (33 to 110), secondary phloem width (252-882 μm) was observed. Comparative dry matter production in leaf, stem and roots was studied in water stressed conditions. Present investigation aimed at highlighting stem anatomical and morphological aspects, which plays major role in imparting drought resistance.

Key words: Stem, tomato, xylem, secondary phloem, drought, anatomy

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops grown in India. With an annual production of 5.4 million Mt, the country is sixth largest tomato producer in the world. Many morphological, physiological and biochemical differences in plant varieties have been reported to be concerned with drought resistance. However reports related to the anatomical features imparting drought resistance are scanty. Anatomical features are stable features over seasons and years. These parameters are genetically governed and can be introgressed.

Blum (1966) reported genetic improvement of stress reserve and utilization as a potent mechanism of drought resistance. The ability to transport and partition a high proportion of assimilates into economically important organs like fruits will have a major impact on crop productivity and is one of the major dehydration tolerance process in plants (Hsiao, 1973). Remobilization of starch reserves stored in stems contribute significantly to grain yields of legumes. (Constables and Hearn, 1978). Because translocation is more tolerant than photosynthesis and respiration to moisture deficit (Boyer, 1976), the ability to store and mobilize large quantities of carbohydrates for osmotic adjustment or fruit growth under terminal drought should improve the ability of a cultivar to perform better under drought conditions (Blum *et al*., 1983). Mobilization could also play a crucial role in determining the sink strength by preventing mega-gamet sterility, thus protecting reproductive development, if stress occurs at the flowering stage (Boyer, 1992). Remobilization of stored reserves can influence the performance of a genotype in both intermittent and terminal moisture deficit environment. In intermittent moisture deficit situation, stored carbohydrates determine the ability of a genotype to recover from stress.

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Stem and root characters play a decisive role in maintaining a desirable water balance between the plant and soil thereby contributing towards drought resistance. In tomato, root dry weight increased and shoot dry weight decreased when stressed but after recovery there was a mobilization of assimilates from root to shoot (IIHR Annual Report, 1990). The differential growth of root and shoot in tomato was related to osmotic adjustments (Shashidhar *et al*., 1991).

Evidence from Wheat (*Triticum annum*) shows that certain genotypes use reserves extensively for grain filling even under non-stress conditions. In this sense, stem reserves are constitutive as a backup source for grain filling under stress (Blum, 2000). Various methods like selection under stress (Mahalaxmi *et al*., 1994), Chemical desiccation (Nicolas and Turner, 1993; Blum *et al*., 1994; Haley and Quick, 1993) were used for screening germplasm to correlate stem reserve in relation to drought resistance. But our method is novel and unique, as we have used anatomical markers to screen tomato genotypes in relation to drought resistance.

Materials and Methods

Plant Material

One colchiploid mutant having drought resistant attributes was identified during germplasm screening. M_2 progeny was grown and 5 plants resembling original mutant were selected for further study. For present investigation sixteen genotypes including 5 mutant derivatives, 3 hybrids developed using mutant as one of parent (Male as well as female parent), 6 cultivated genotypes, 2 hybrids developed by crossing cultivated genotypes were subjected for analysis using anatomical and morphological parameters. Study aimed to reconfirm molecular and *in vitro* polymorphism for drought resistance amongst genotypes. The genotypes included in this investigation, along with key morphological and anatomical characteristics are listed in Table 1.

Methodology

Temporary rain out shelter was prepared using Polyethylene sheet in Kharif-2004 season. ICRISAT guidelines were used for experiment. Seedlings of germplasm were raised on beds covered with polyethylene tunnel. Seedlings were transplanted after 25 days into experimental fields. Two Replications for control plot and two replications for stress plot were transplanted. After 60 days of

Table 1: Key morphological and anatomical features of germplasm in relation to drought resistance

Genotype	Source/pedigree	Stomata per sq mm (lower side of leaf)	Palisade mesophyll hiegth (µm)	Root length (cm)	Root: Shoot ratio (fresh weight g)	No. of root hairs/5 (cm)
MTG 1-1	Mutant derivative (M_2)	102	248	67	0.08	49
MTG 1-2	Mutant derivative (M_2)	116	236	39	0.06	43
MTG 1-3	Mutant derivative (M_2)	132	213	43	0.05	39
MTG 1-5	Mutant derivative (M_2)	206	95	17	0.07	11
TG 2-3	Pure line	216	137	32	0.04	31
Hy- 1	Mutant M_1 × TG -5	109	182	49	0.05	47
Hy-2	TG -42 × Mutant M_1	140	168	47	0.07	33
Hy-3	Mutant M_1 × TG -42	122	239	71	0.05	39
TG-42	Pure line	216	162	32	0.04	31
MTG 1-4	Mutant derivative	110	241	62	0.04	44
Hy-4	TG-80 × TG-64	176	147	37	0.04	27
Hy-5	TG -5 × TG -13	192	151	40	0.04	24
TG-5	Pure line	235	126	32	0.04	19
TG-13	Pure line	198	163	27	0.04	23
TG-80	Pure line	205	167	28	0.04	32
TG-64	Pure line	222	126	35	0.04	29
	SE	10.95	14.74	2.36		1.77
	CD at 5%	32.79	44.47	5.02		5.34

transplanting water stress was imposed on plots by withholding irrigation for 20 days. Two plots were kept as control with regular irrigation schedule. The ambient temperature ranged between 32-35°C and relative humidity 70-75% in the rainout shelter.

For recording observations, five plants from each plot in each replication were randomly selected and uprooted. These plants were used for recording observation on stem fresh and stem dry weight.

Stem was dried in oven at 105°C for 8 h, till the samples showed constant dry weight. Data is expressed as mean dry wt. g/plant. Anatomical observations were taken by preparing TS of stem sections and observations were taken using ocular micrometer. The standard procedure mentioned by Jenson (1962) was used for taking anatomical observations.

Results and Discussion

Width of stem epidermis was predominantly more in drought resistant genotypes as compared to drought susceptible ones. Cells of epidermis were of bigger size in drought resistant genotypes. In drought resistant genotypes, it ranged between 20-22 µm whereas in susceptible between 8-14 µm. Hy-3 was with highest epidermis thickness of 23.9 µm. TG-64 was observed with thinner layer of epidermis with 11.3 µm thickness.

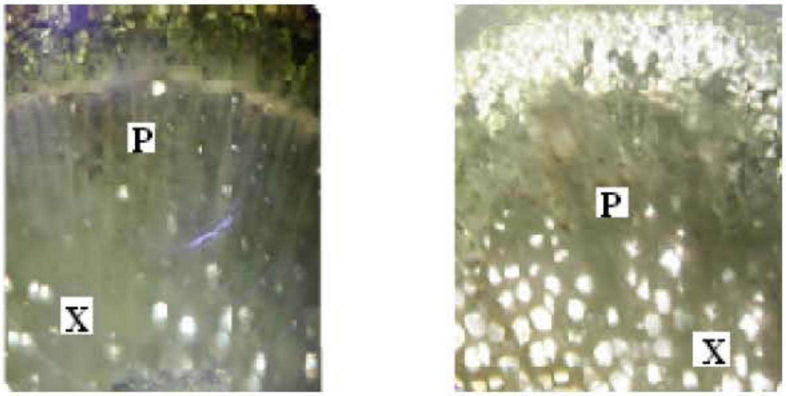
Width of cortex was more in drought resistant genotypes as compared to susceptible ones. It ranged between 300-376 µm in resistant genotypes whereas it was between 225-252 µm in susceptible genotypes. Hy - 3 was noted with 376 µm cortex width whereas TG-5 with 226.8 µm cortex width.

Secondary phloem was with more width in stem of drought resistant mutant derivatives indicating their increased ability of conducting more food material. It was between 693-882 µm.in drought resistant genotypes whereas it ranged between 252-403 µm. in susceptible genotypes. Susceptible genotypes TG - 64 (252 µm) and TG-42 (252 µm) were noted with lower magnitude for width of secondary phloem. Mutant derivative MTG 1-4 (693 µm) and Hy-3 (882 µm) observed with higher width of secondary phloem (Table 2).

Susceptible genotypes had smaller and less number of xylem vessels whereas resistant genotypes have xylem vessels with bigger size and are more in number (Fig. 1). Mutant derivative MTG 1-4 xylem diameter is 166.3 µm and Hy-3 exhibited 170.1 µm diameter. Diameter of bigger xylem vessels

Table 2: Stem anatomical features in relation to drought resistance (in µm)

Entry	Epidermis	Cortex	Secondary phloem	Vascular cambium width at xylem poles	Pith	Average diameter of xylem vessels		Total No. of xylem/ section
						Big	Small	
MTG 1-1	22.6	378	630	1008	3906	165	-	78
MTG 1-2	21.4	315	693	882	4410	151.2	-	68
MTG 1-3	20.1	352.8	630	945	4158	149.9	-	65
MTG 1-5	7.5	252	252	630	2520	-	73.08	33
TG 42	11.3	252	252	1008	4662	144.9	76.86	52
TG 2-3	8.8	189	378	758	4284	-	64.2	110
Hy-1	21.4	252	252	756	4032	154.9	-	72
Hy-2	18.9	189	504	1008	5166	-	91.9	78
Hy-3	23.9	376	882	1260	4410	170.1	-	68
MTG 1-4	21.4	352.8	693	1134	3654	166.3	-	73
Hy-4	12.6	352.8	403.2	882	4788	136	65.5	65
Hy-5	7.5	252	378	756	2896	-	78.1	121
TG-5	11.3	226.8	315	630	2583	-	73.08	107
TG-13	8.8	315	312.8	1008	3528	-	91.9	92
TG-80	10	252	315	756	4347	117.1	61.7	78
TG-64	11.3	252	252	756	4914	136	76.8	69
S.E	3.4	7.3	2.73					2.34
CD at 5%	10.1	14.6	8.19					7.02



Drought resistant mutant MTG 1-4 **Drought susceptible cultivated type TG-64**

(X . Xylem, P. Phloem)

Fig. 1: Stem xylem vessel variability in relation to drought resistance in tomato

in resistant genotypes ranged between 136-165 μm. In susceptible xylem are of 64-91 μm diameter i.e., smaller in size as compared to mutants. Xylem vessel diameter is easy criteria for selection of genotypes for drought tolerance. Assimilate retranslocation is universal trait with intermediate heritability (Richards *et al.*, 1999).

Increased width of epidermis is desirable as it decreases rate of transpiration. In drought resistant genotypes width of epidermis was found to be more. Xerophytes have features of such increased epidermal cell size. Along with this, xerophytes are reported to be having 2-3 layers of epidermis for increased drought resistance. This multiple layer epidermis was not observed in tomato genotypes. This is desirable and significantly important character.

Width of cortex was more in drought resistant genotypes than susceptible ones. Width of secondary phloem and number, diameter of xylem vessels was more in drought resistant genotypes than susceptible genotypes. This is in relation to increased capacity and use of food and water from these conducting tissues, respectively. Predominantly drought resistant genotypes were recorded with bigger size xylem vessels and susceptible ones with smaller size xylem vessels. Deshpande and Kulkarni (2005) reported increased xylem diameter and number of xylem poles in tomato roots associated with drought resistance.

Close association of anatomical parameters with dry matter production was observed which plays major role for imparting drought resistance. It revealed that almost all the anatomical characters expressed their positive correlation with total dry matter produced by plant. Epidermis width ($r = 0.607$), number of xylem vessels ($r = 0.703$) and secondary phloem width ($r = 0.706$) are important stem anatomical features worth to mention significantly affecting dry matter production and ultimately drought resistance in tomato.

Dry matter partitioning in tomato was observed to be 50:40:10 in leaf, stem and roots respectively in susceptible genotypes. Hy-3 (Resistant × Susceptible) was found to be with modified dry matter partition with increased dry matter in stem and roots (40:50:10). There was extra 10% dry matter in stem of drought resistant genotypes as compared to susceptible ones. Stem dry matter was highest in MTG 1-4 (70.28 g) and it was lowest in genotype TG-64 (15.94 g).

Stem is major site for storage of food material from photosynthesis. Thick stem is considered to be advantageous in relation to drought resistance. It is indication of extra capacity to store food material, which is useful during moisture stress situation. MTG 1-4 and Hy-3 were noted with extra

Thick srem: resistant Mutant MTG 1-4

Thin stem: susceptible TG-64

Fig. 2: Stem girth variations in tomato genotypes (90 days after transplanting)

Table 3: Stem morphological and dry matter content variability

	Stressed plot			
	Stem			
Genotype	Fresh wt. (g)	Dry wt. (g)	Total dry matter (g)	Stem girth (cm)
MTG 1-1	83	14.359	36.60	3.6
MTG 1-2	95	15.295	33.50	3.7
MTG 1-3	91	14.282	31.70	3.2
MTG 1-5	33	4.323	8.40	1.4
TG 42	81	9.153	23.00	4.3
TG 2-3	89	11.303	25.80	4.2
Hy-1	98	14.798	31.40	4
Hy-2	129	21.027	36.50	4.2
Hy-3	135.6	26.848	53.00	4.3
MTG 1-4	142.8	28.702	51.00	5
Hy-4	97	19.497	33.90	3.9
Hy-5	113	20.114	39.10	3.1
TG-5	117	21.177	36.70	3
TG-13	81	18.063	29.80	2.9
TG-80	113	28.589	46.10	4.1
TG-64	82	14.186	25.50	2
SE	4.46	2.99	2.20	0.22
CD at 5%	9.51	6.74	4.68	0.46

stem (5.6 and 5.1 cm) girth indicating robustness of plant. Among susceptible genotypes diameter was lowest in TG -64 and measuring 2.9 cm only, indicating weaknesses of stem (Fig. 2).

Slight smaller stem girth was observed in water stressed plot. This may be due to increased rate of transpiration. Stem provides dry matter to roots during water stress so they loose fresh wt as well as dry matter. In water stress situation, drought resistant mutant was observed with 5.4 cm girth followed by 5.0 cm girth in Resistant × Susceptible hybrid (Hy -3).

Translocation of food from stem to economic part is important drought resistance process in plants (Boyer, 1976). Traits that contribute to drought resistance include long and thick stem internodes, with extra storage tissue perhaps in the form of solid stems. In studies where crosses where made between lines contrasting in the solid stem trait, the solid-stem progeny contained more soluble carbohydrate per unit of stem length (Ford *et al*., 1979). We have got similar results in relation to anatomical features. A significant positive relationship exists between rate of stem dry matter loss after anthesis and grain production capacity under drought conditions across a range of genetic material (Rawson *et al.*, 1977). In case of sorghum, harvesting index was highest in the drought tolerant M-35-1 genotype compared to others under several moisture stress conditions. In several crops, genotypic variations were reported for the ability to store and mobilize carbohydrates for seed feeling during terminal moisture stress (Subba Rao *et al.*, 1995).

Dry matter partitioning was around 40:50:10 in leaves, stem and roots of tomato plant respectively. More dry matter in leaves, stem and roots of drought resistant mutant and its hybrid (Hy-3) as compared to other susceptible genotypes was observed. Another hybrid (Hy-2) could not perform superiorly for amount of dry matter production may be due to their lower efficiency at biochemical levels and poor combining ability of male parent (Table 3). TG-42 is a determinate genotype used as female parent in hybridization and was used as male parent to test the performance of hybrid combination.

In water stressed plot; dry matter partitioning was modified to cope up with increased water stress imposed upon plants. There was decreased stem fresh and dry weight and increased fresh and dry weight of roots. The results obtained are in accordance with Srinivasa Rao and Bhatt (1993). They reported decreased shoot dry matter and increased dry in matter in roots. Drought resistant mutant genotype and its hybrid indicated slightly lower dry matter. This may be due to more amount of xylem vessels in these shoots and lower pith area. In general, cultivars with more pith area in shoots could store more assimilate and were observed with more increase in shoot dry matter.

In wheat genotypes, sources have been identified with high chlorophyll at heading (Hede *et al.*, 1999), high leaf conductance (Villhelmsen *et al.*, 1999), high pubescence (Trethowan *et al.*, 1998), peduncle volume, stay green and heat tolerance. Searches are currently under way for long awns, high osmotic adjustment and biomass under drought and high temperature stress. Anatomical features identified by us plays important role regarding dry matter portioning, drought avoidance, stay greenness and lodging.

Conclusions

It was indicative from present investigation that thicker stem and more number of conducting tissues (xylem and phloem) plays vital role in drought resistance. Dry matter portioning also plays key role to cope up yield during water stress situation. Substantial anatomical variability was observed in drought resistant mutant genotypes as compared with cultivated genotypes. These unique anatomical features were correlated with dry matter production. It was observed that width of secondary phloem in stem plays key role in imparting drought resistance. Anatomical markers are simple and cost effective characterization methodology for screening germplasm against drought resistance.

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