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Anatomical Breeding for Altered Leaf Parameters in Tomato Genotypes Imparting Drought Resistance Using Leaf Strength Index

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Abstract: Present investigation was carried out with aim to understand role of leaf anatomy in drought resistance. Seventeen tomato genotypes were screened for petiole, leaf anatomy and stomatal features to understand the secret of drought resistance. Total petiole thickness, compact parenchyma and more width of phloem vessels, more number of xylem vessels were important characters of resistant genotypes. Leaves of resistant type had longer palisade mesophyll cells with compact arrangement, thin spongy mesophyll layer leading to higher tissue ratio (Palisade mesophyll: Spongy mesophyll). Thickness of leaves was invariably more in drought resistant genotypes (500-550 μm) as compared to susceptible ones (400-450 μm). Remarkably reduced number of stomata, bigger size and more distance between them was highlighting character of drought resistant genotypes as compared to abundant, smaller size and closely spaced stomata in susceptible genotypes. We introduce here a new parameter " Leaf Strength Index" which is simple to study and cost effective. This can be potential method for screening large number of genotypes for categorizing them in relation to their ability of drought resistance. These findings will be a new light towards breeding drought resistant genotypes giving emphasis on anatomical features.

Key words: LSI, tomato genotype, drought resistance, anatomical features

INTRODUCTION

Building genetic resistance against abiotic stresses like drought in tomato genotypes had been the dream in tomato breeding. Use of resistant varieties leads to judicious use of available water resources, which in turn increase area under cultivation and helps to cope up with increasing demand of popular vegetable crop like tomato.

Anatomical parameters are rather little explored in relation to drought resistance. These parameters are genetically governed and can be introgressed. Some workers have studied the inheritance and genetics of various morphological characters responsible for drought resistance.

The tomato plant has compound leaves. Leaflets are connected through leaf rachis. Leaf is connected to stem by petiole. Just inside the epidermis of petiole lie several layers of collenchyma tissues followed by layer of parenchyma tissues. The vascular tissue is arranged is semi cylinder in center of petiole. The bundles are bicollateral (Hayward, 1938) with phloem on the inner and outer regions of the vascular semi cylinder. Most of leaves are protected by a thin outer cuticle. Just inside lies the epidermis on both sides of leaves. Inside epidermis lies oblong, upright palisade mesophyll cells and loosely arranged spongy mesophyll cells. Midrib anatomy is similar to petiole. Tomato leaf exhibits reticulate venation. Lateral veins branch off of the midrib. No cell is more than six cells away from a vascular bundle. The leaf epidermis contains numerous stomata. Stomata openings are controlled by two guard cells. Surrounding guard cells are subsidiary cells (www.ucdavis.edu). Parenchymatous tissues are thin walled and polyhedral shape and concerned with vegetative activities of plant. Plant posses two kind of intercellular spaces systems continuous and discontinuous. More intercellular spaces lead to more losses of water through stomata. The amount of parenchyma indicates strength and honeycomb structure is desirable. Only 1% water is used by plant for growth and development and 99% lost through transpiration through stomata.

Various stomatal characters such as sunken type and less number per unit area are associated with drought resistance (Davis, 1977). Guard cell size ranged between 78-92 μm in winter wheat genotypes and was important selection criteria for cold hardiness (Limin and Fowler, 1994). Water evaporates through intercellular spaces of parenchyma. From there water

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vapor escapes into atmosphere through stomata (90%) or cuticle (10%). Cuticle restricts transpiration and is chemically stable (Dicher, 1963). The density of stomata has been established as 100 to 300 per square mm for leaves of many species. (Stalfelt, 1956). Parenchymas are principally storage tissues polyhedral in shape mainly related to storage, excretion like vegetative activities of plant. Apparently the potentiality to divide may be retained by parenchyma cells for many years (Barker, 1953). Many different food substances are synthesized and stored by parenchyma cells. These substances are stored in vacuolar sap or they may be discrete solid or fluid bodies in cytoplasm Amides, protein and sugar are dissolved in cell sap (Netolitzky, 1953)

Water is abundant in all active vacuolated parenchyma cells so that the parenchyma plays a major role as water reservoir. In a study of species of bamboo, the variation in moisture content of the different parts of culms were found to be clearly associated with the proportion of parenchyma cells in the tissue system (Liese and Grover, 1961). Parenchyma may be rather specialized as a water storage tissue. Many succulent plants such as the Cacteceae, Aloe, Agaves contain in their photosynthetic organs chlorophyll free parenchyma cells full of water. Collenchyma is thick walled kind of parenchyma. It is immediately between the epidermises. Sometimes entire epidermal cells are collenchymatous. Collenchyma walls may contain over 60% water based on fresh weight; over 200% based on dry weight (Cohn, 1892). We have reported root morphological and anatomical parameters imparting drought resistance in tomato (Deshpande and Kulkarni, 2005). Resistant genotype root length ranged between 60-70 cm whereas susceptible group had only 30-40 cm long roots. Number of xylem per cross section of root were 36.5 in resistant genotypes against 26.8 in susceptible groups.

Anatomical parameters in midrib cross-section having compact and long parenchyma in trispecies derivatives of cotton (Genus *Gossypium*) were reported (Ansingkar *et al*., 2004). Stability of anatomical parameters over seasons along with their genetics was discussed (Rajendran, 2004). In cotton, small and thick leaves with thick layers of palisade tissue are associated with drought resistance (Bhatt and Andal, 1979; Singh *et al*., 1990; Singh and Narayanan, 1993). Additive as well as nonadditive effects for anatomical traits like number of stomata, number of epidermal cells, guard cell length and stomatal width were studied (Lakshminarayana *et al*., 1979). Thick leaves, high level of electrolyte, high chlorophyll content, few stomata and vigorous root growth are related to heat tolerance in Chinese Cabbage (Kuo *et al*., 1988).

MATERIALS AND METHODS

Plant material: One naturally occurring mutant having drought resistant attributes was identified during germplasm screening. M_2 progeny was grown and 4 plants resembling original mutants were selected for further study. For present investigation sixteen genotypes including 3 mutant derivatives, 3 hybrids, 3 cultivated genotypes were subjected for genetic diversity analysis using anatomical features. Study aimed to reconfirm morphological and RAPD polymorphism (Data not given) for drought resistance amongst genotypes at anatomical level.

Anatomy and micrometry: Leaf anatomy of all the genotypes was studied in detail using light microscopy by adopting standard methods of investigation (Jenson, 1962).

Leaf sampling: The fully opened young leaf of 10th internode to 12th internode was sampled to study the leaf anatomy.

Fixation: Leaf samples from similar stage of crop growth (Same internodes) were fixed in the standard fixative FAA (90 mL of 90 per cent alcohol + 5 mL formalin (40% formaldehyde) + 5 mL glacial acetic acid). The leaf samples were allowed to remain in fixative for 48 h. Afterwards the samples were transformed to 70% alcohol to store them for further studies. Proper care was taken to maintain uniformity in leaf midrib size and portion.

Affixing the sections: One percent gelatin (SD fine chem.) with little quantity of potassium dichromate was used as an adhesive. A few drops of gelatin were poured on the surface of a pre cleaned micro slides with the help of filter. The paraffin ribbons were cut into convenient lengths with the help of blade and placed on the adhesive surface. Gelatin could enable the sections to adhere to the slide, even at high temperature. The slides were then warmed over the warming plate maintained at nearly 45°C for a standard period of 2 h to facilitate flattening stretching of ribbon. The excess adhesive was poured out and slides were later dried for 72 h under room temperature in dust free environment.

Micrometry: Magnification field of microscope with the help of calibrated ocular meter standardized with the help of stage micrometer. Observations on anatomical parameters of leaf petiole and stomata were recorded using ocular micrometer (Table 1).

Table 1: Anatomical features of petiole cross-section

Entry	Cuticle (µm)	Collenchyma layer (µm)	Epidermis (µm)	Lower epidermis to phloem (µm)	Phloem thickness (µm)	Phloem to upper epidermis (µm)	Arrangement of Parenchyma (µm)	Diameter of parenchyma (µm)	Xylem vessels
MTG 1-1	3.2	189	22.6	1134	957.6	2167.2	Compact	151.2	18
MTG 1-2	2.8	151.2	21.4	630	756	2016	Compact	163.8	17
MTG 1-3	2.2	126	20.1	819	756	1704	Compact	151.2	14
MTG 1-5	1.5	126	7.5	478.8	509	882	Loose	90.72	8
TG 42	2.3	138.6	11.3	756	655.2	1648	Loose	167.5	14
TG 2-3	1.9	151.2	8.8	567	705.6	1323	Loose	148.6	10
Hy-1	3.9	189	21.4	756	819	1701	Very compact	163.8	18
Hy-2	2.6	37.8	18.9	781.2	693	1764	Compact	167.5	14
Hy-3	3.8	214.2	23.9	907.2	781.2	2520	Very compact	153.7	16
MTG 1-4	4	252	21.4	986.5	858	2711.5	Very compact	148.6	17
Hy-4	2.9	163.8	12.6	858	631.2	1611.5	Loose	161.2	12.5
Hy-5	3.4	138.6	7.5	835.3	643.8	1801.8	Semi compact	112.4	12
TG-5	3.3	151.2	11.3	630	655.2	1423.8	Loose	152.4	12
TG-13	3.5	163.8	8.8	869.4	756	2142	Compact	165	13
TG-80	3.1	176.4	10	945	819	1953	Loose	151.2	12.7
TG-64	3.5	163.8	11.3	693	567	1562.4	Loose	151.2	13.1
SE			3.4	47.25	52.23	42.21			1.06
CDat 5%			10.1	141.75	156.37	126.504			3.19
Population Mean	2.83	158.29	17.26	788.52	738.35	1822.56		151.62	14.41

Number of stomata (per microscopic field): The micrometric observations were recorded at 100 × (10x × 10 x) magnification field of microscope in area of one square millimeter.

RESULTS

Petiole cross-section: Thick cuticular layer is desirable trait for increased water use efficiency. Highest cuticular thickness was observed in mutant MTG 1-4 (3.3 µm) whereas lowest observed in cultivated type TG-13 (1.9 µm). Drought resistant genotype cuticle thickness ranged between 2.8 to 4 µm range. Susceptible genotypes have thin cuticle ranging between 2.2 to 3.1 µm thickness. Thick cuticle is chemically stable so always desirable (Dicher, 1963). Collenchyma layer was predominantly present in drought resistant cultivars. Comparatively thinner layer of collenchyma was noted in susceptible genotype TG-42 (138.6 µm). The genotype MTG 1-4 highly resistant to drought was with most thick Collenchyma layer (252 µm width).

These tissues play a key role in transpiration rate and water storage ability of plants. These cells have special ability of absorbing and retaining water (Barker, 1953; Liese and Grover, 1961). Drought resistant genotypes were with very compact and uniform shaped and parenchyma cells with no intercellular spaces. Absence of inter cellular spacer reduces water loss through transpiration. Drought susceptible genotypes have loose parenchyma, slightly bigger in size (i.e., around 12-15 µm diameter) with abundant intercellular spaces. These types of germplasm are characterized by increased water loss through transpiration under water stress situation (Fig. 1).

Phloem width was comparatively more in drought resistant cultivars ranging between 700-1000 µm with highest in mutant derivative MTG 1-1 i.e., 957.6 µm. Comparatively less width was observed i.e., 500-700 µm in cultivated genotypes. Cultivar TG-80 possessed more phloem width 819 µm indicating good ability to flow cell sap but cultivar is fairly tolerant. There was not much different in phloem cells per 1260 µm between drought susceptible and resistant cultivars so this character may be not of much importance. More number of xylem vessels in petiole is desirable as indicates increased ability of conducting water to leaves. Number of xylem in petiole cross-section conducting tissue was more (15-18) in drought resistant genotypes. In susceptible cultivars, it ranged between 12-14. Mutant derivative MTG 1-4 (17) and Hy-3 (16) were noted with higher number of xylem vessels, whereas susceptible genotypes TG-64 (13.1) and TG-5 (12) observed with relatively lower number of xylem vessels in petiole.

Leaf cross section: Thick leaved cultivars ranging between 501- 550.3 µm thickness with highest thickness 528 µm in Hy- 3. Genotypes with comparatively thin leaves ranged between 432-487 µm thickness was susceptible to water stress condition (Table 2). Genotypes have considerable variability regarding length of palisade mesophyll cells. Highest length was in MTG 1-4 i.e., 241 µm. Heterosis for increased palisade mesophyll was observed in Hy.-3 with palisade mesophyll length 239.5 µm (Fig. 1).

Presence of lower palisade mesophyll layer is an important character regarding drought resistance. In *Gossypium arboreium*, this layer of lower palisade

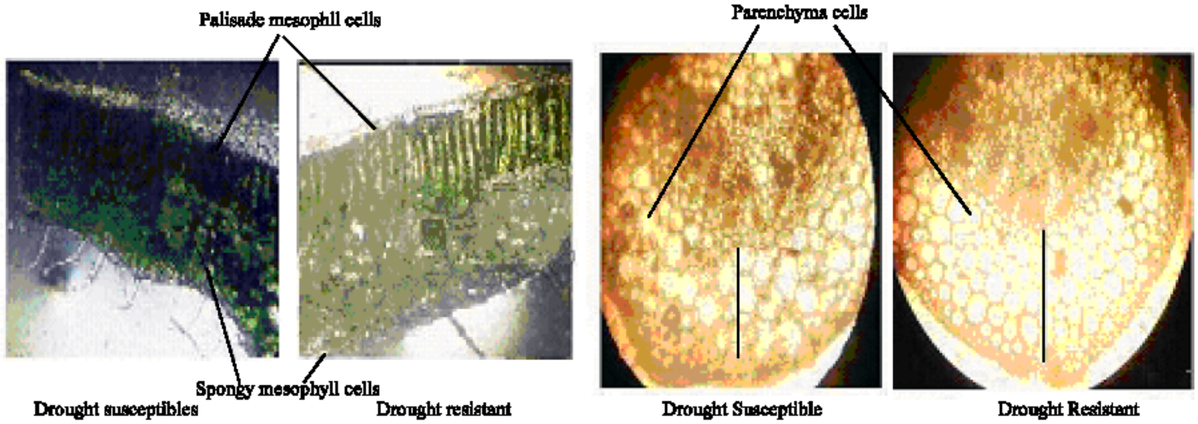

Fig. 1: Comparative leaf anatomy variation in leaf and petiole cross section

Table 2: Leaf cross-section anatomical features

Entry	Epidermis thickness (µm)	Palisade mesophyll height (µm) Upper	Lower	Spongy mesophyll height (µm)	Total thickness of leaf lamina (µm)	Tissue ratio	Per 100 unit palisade mesophyll cells	Average intercellular space between palisade (µm)
MTG 1-1	22.6	248.3	Absent	302	550.3	0.82	42	15.2
MTG 1-2	21.4	236.5	Absent	312.5	549	0.76	39	16.8
MTG 1-3	20.1	213.1	Absent	298.3	511.4	0.71	41	16.5
MTG 1-5	7.5	95.2	Absent	112.5	207.7	0.85	17	58.7
TG 42	11.3	162.9	Absent	251.6	414.5	0.65	36	21.3
TG 2-3	8.8	137.4	Absent	311.2	448.6	0.44	31	25.6
Hy-1	21.4	182.6	Absent	303.9	486.5	0.60	49	11.3
Hy-2	18.9	168.3	Absent	202.6	370.9	0.83	39	17.8
Hy-3	23.9	239.5	Absent	288.5	528	0.83	46	13.2
MTG 1-4	21.4	241	Absent	260.5	501.5	0.93	48	11.7
Hy-4	12.6	147.2	Absent	316.5	463.7	0.47	31	25.4
Hy-5	7.5	151.3	Absent	281.4	432.7	0.54	34	12.9
TG-5	11.3	126.5	Absent	336.5	463	0.38	29	29.5
TG-13	8.8	163.5	Absent	240.1	403.6	0.68	32	15.6
TG-80	10	167.9	Absent	276.2	444.1	0.61	37	19.8
TG-64	11.3	126.8	Absent	360.7	487.5	0.35	30	29
SE	3.4	14.742			23.31			
CD at 5%	10.1	44.478			67.158			
Pop. Mean	17.26	188.36		269.10	457.46	0.72	37.7	21.23

Table 3: Anatomical features of Stomata and Guard cells

Entry	Lower side of leaf: No. of Stomata	Lower side of leaf: Distance between Stomata (µm)	Upper side of leaf: No. of Stomata	Upper side of leaf: Distance Between Stomata (µm)	Guard Cell size: Length (µm)	Guard Cell size: Width (µm)
MTG 1-1	102	201.6	60.3	478.8	75.6	63.2
MTG 1-2	116	239.4	59	453.6	63	63.5
MTG 1-3	132	189	51	403.2	75	63.9
MTG 1-5	206	100.8	53	529.2	37.8	37.8
TG 42	216	113.9	65	352.8	100.8	38.8
TG 2-3	260	75.6	68	415.8	113.4	37.2
Hy-1	109	191.52	39	567	100.8	50.4
Hy-2	140	167.58	48	415.8	100.8	37.8
Hy-3	122	192.78	59	403.2	88.2	63.5
MTG 1-4	110.2	223.02	54.6	399.42	88.5	62.9
Hy-4	176.6	156.24	70.9	301.14	101.6	50.4
Hy-5	192.2	162.24	77.6	287.28	103.2	37.2
TG-5	235	88.2	72	478.8	100.9	37.6
TG-13	198	126	59	365.4	113.4	25.2
TG-80	205	100.8	63	403.2	100.5	37.9
TG-84	222	100.8	68	415.8	102.1	37.4
SE	10.95	16.632	3.48	53.802		
CD at 5%	32.79	39.69	10.42	161.154		

Table 4: Leaf Strength Index variability amongst tomato genotypes

Entry	Average leaf area (sq cm)	Total thickness of leaf lamina (mm)	No. of stomata per sq mm (Lower side of leaf)	Leaf strength Index (LSI)	Category of genotype	Root length (cm)
MTG 1-1	27.47	0.55	102	0.148	Resistant	67
MTG 1-2	26.66	0.54	116	0.124	Moderately resistant	39
MTG 1-3	33.5	0.51	132	0.129	Moderately resistant	43
MTG 1-5	9.8	0.20	206	0.009	Susceptible	17
TG 42	27.39	0.41	216	0.051	Susceptible	32
TG 2-3	26.26	0.44	260	0.044	Susceptible	30
Hy-1	39.01	0.48	109	0.171	Resistant	49
Hy-2	30.4	0.37	140	0.080	Susceptible	47
Hy-3	46.41	0.52	122	0.197	Resistant	66
MTG 1-4	40.81	0.50	110.2	0.185	Resistant	64
Hy-4	24.18	0.46	176.6	0.062	Susceptible	37
Hy-5	31.59	0.43	192.2	0.070	Susceptible	40
TG-5	23.76	0.46	235	0.046	Susceptible	32
TG-13	26.91	0.40	198	0.054	Susceptible	27
TG-80	28.81	0.44	205	0.061	Susceptible	28
TG-64	17.16	0.48	222	0.037	Susceptible	35
S.E		0.02	10.95			
C.D. at 5%		0.06	32.79			
Population Mean		0.45	102			43.3

Categories: 0.040- 0.100 (Susceptible), 0.101- 0.160 (Moderately resistant), Above 0.160 (Resistant)

mesophyll is predominantly present which impart drought resistance to this deshi cotton genotype (Singh *et al*., 1996). In present investigation no tomato cultivar was found with presence of this lower palisade mesophyll layer. So identification of land races or wild type with this character and introgression in cultivated tomato type will be important aspect in tomato breeding to develop drought resistant cultivars. Considerable variation was also observed for characters like epidermis thickness, spongy mesophyll cell thickness etc.

Number of palisade mesophyll cells (per 1260 μm), length and observed compactness of palisade mesophyll cells, which are directly related to drought resistance ability of genotypes. Palisade mesophyll cells were between 40-50 in drought resistant cultivars whereas it was lower i.e., 29-36 in susceptible cultivars. In susceptible cultivars, palisade mesophyll cells have considerable intercellular spaces as compared to compact palisade mesophyll cells in resistant types.

Tomato leaves, like the tomato petiole, have bicollateral bundles. The xylem is in the center of the vein with the phloem distributed on both the adaxial and abaxial sides of the bundle (Hayward, 1938).

Stomatal variation: Stomata ranged between 100-300 per sq mm in studies of Stailfelt, 1956. Xerophytes may have as low as 10-15 stomata per sq mm. Lower number of stomata increases water use efficiency of plants by reducing rate of stomatal transpiration in situation of water stress. Drought resistant plant has as less as up to 50% reduced number of stomata per square millimeter in comparison to susceptible cultivars with abundant stomata. Resistant cultivars with high water use efficiency were with 100-132 stomata per square millimeter with lowest in mutant derivative (MTG 1-4) with only 102 stomata per microscopic field (Table 3). Drought susceptible genotype has abundant stomata ranged between 170-260. Distance between stomata ranged between 170-200 μm in resistant cultivars whereas it was lower i.e., 100-156 μm in susceptible cultivars. Similar results were obtained upper side of leaf for number of stomata and distance between stomata. Number of stomata is around only 30% in upper side of leaf as compared to lower side of leaf. P. Venkateswara Rao, (Unpublished ICRISAT Data) reported that there are far number of stomata on the lower surface of the leaf than on the upper surface in *Cajanas cajan.* This adaptation in plants is due to more exposure to sunlight or temperature from upper side of leaf and increased water loss from upper side. Mostly upper side stomata were found to be sunken and more protected than lower side stomata.

Leaf strength Index (LSI): We propose here a new index i.e., Leaf Strength Index (LSI) which can be calculated as

Leaf Strength Index = Leaf Area (sq cm) X Leaf thickness (mm)/Number of stomata per sq mm.

Larger leaf area leads to increased total photosynthesis per plant. Earlier it was proposed that smaller sized leaves are associated with drought resistance but this will lead to reduced final economic yields due to reduced area for photosynthesis. As palisade tissues are associated with photosynthesis and rate of transpiration, longer palisade tissues along with compact architecture facilitates more rate of photosynthesis and reduced rate of water loss through intercellular spaces. Cultivated genotypes with loose palisade tissues and smaller size are characterized by lower rate of photosynthesis and more water loss through extra intercellular spaces. Reduced number of stomata helps in maintaining high water potential in the tissues by reducing transpiration rate. Number of stomata per sq.mm and distance between stomata shows strong positive association.

Leaf Strength Index variability amongst different tomato genotypes under study are depicted in Table 4. This new index divides genotypes into three distinct categories. Information about root length of respective genotypes is also provided as supportive information.

DISCUSSION

Drought resistant plants were found to be with thick leaves as compared to thinner leaves in susceptible lines. Thickness of leaves is due to thicker lower and upper epidermis, more length of palisade mesophyll cells and spongy mesophyll cells as compared to thin leaves in susceptible. Compactness of palisade mesophyll cells along with less intercellular spaces imparts resistance to drought. Xerophytes have increase in thickness of cell wall and greater density of both vascular systems along with increased development of palisade tissues at expense of spongy tissue (Wylie, 1949). Leaves with loose palisade mesophyll cells, along with more intercellular spaces will evaporate more water through stomatal transpiration and get affected by water stress condition. Compact, long palisade mesophyll cells are desirable presence of lowers palisade mesophyll is very ideal character for drought resistance that is seen in some xerophytes but in present study not a single genotype was found to be having that character.

The density of stomata has been established as 100-300 per sq mm for leaves of many species (Stailfelt, 1956). Shrinivasa Rao and Bhatt (1991) counted 146 to 190 stomata at top, 109 to 143 in middle and 90 to 119 in the bottom position of leaves of tomato. In present study, as number of stomata reduced per microscopic field the resistance for drought increased. Drought resistant genotypes were found to be having nearly 50% less number of stomata per microscopic field. As number of stomata reduced, distance of stomata was found to be more as compared to be drought susceptible cultivars in which stomata were closely spaced

Shrinivasa Rao and Bhatt (1991) concluded that tomato genotypes having a higher threshold water potential for stomatal closure would be more suitable for rainfed condition. Rate of transpiration was high at top of plant which gradually decreased stomata closes as leaf water potential decreases.

Table 5: Genotypic correlations of anatomical features in tomato with total dry matter production under water stressed situation

Plant Part	Parameter	Correlation coefficient with dry matter production
Leaf	No. of stomata (Lower side)	-0.518
	Distance between stomata (Lower side)	0.487
	Leaf thickness	0.682
	Palisade mesophyll height	0.743
Petiole	Distance from lower epidermis to phloem	0.467
	Phloem width	0.590
	No. of xylem vessels	0.576

Association and contribution of different anatomical, morphological parameters to drought resistance: The data depicted in Table 5 clearly indicated close association of anatomical parameters imparting the drought resistance. It revealed that almost all the anatomical characters expressed their positive correlation with total dry matter produced by plant (Data not given). Amongst leaf parameters, distance between stomata ($r = 0.487$), leaf thickness ($r = 0.682$) and palisade mesophyll height ($r = 0.743$) showed their strong positive association. However, number of stomata per leaf from lower side expressed significant negative correlation with dry matter production. It clearly indicated role of density of stomata with plant vigor, yield and dry matter production and stay greenness. To breed genotype resistant to drought, selection pressure with higher value for above anatomical parameters is needed. Petiole is main part of leaf from which food and water conduction takes place. All petiole parameters studied revealed positive correlation with dry matter production i.e., distance from lower epidermis to phloem ($r = 0.467$), Phloem width ($r = 0.590$) and xylem number ($r = 0.576$). The results obtained in present investigation invigorate the drought resistance evident in the mutant and its derivatives. Negative relationship between number of stomata and tomato dry matter production was noticed in present study. This fact sufficiently explains for cause of drought resistance in mutant and its derivatives. The present genotypic correlation investigation with total dry matter production has clearly indicated the important role of anatomical parameters for imparting drought resistance. Ultimately, it is mandatory to give due weightage to these parameters while planning a project to develop drought resistant genotype against abiotic stresses like drought.

Present investigation was an effort to validate role of anatomical parameters imparting drought resistance in tomato mutant genotypes. Substantial importance of characters like thick leaves, longer and compact palisade mesophyll cells, lower number of stomata and compact parenchyma cells in relation to drought resistance is clearly indicated. Henceforth, emphasis is to given for anatomical parameters in drought resistance breeding. Our newly proposed parameter of Leaf Strength Index (LSI) is cost effective way for characterizing large set of germplasm in relation to drought resistance. Plant anatomy is potential field in life sciences underutilized for enhancement of germplasm. Solutions to many unsolved problems of abiotic stresses like drought, flood, cold, high temperature can be solved successfully studying anatomical parameters. Prospectus is rich in biotic stresses like sucking pest, Viruses and disease resistance also.

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