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Mining Anatomical Traits: A Novel Modelling Approach for Increased Water Use Efficiency under Drought Conditions in Plants

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Abstract: Crop yields are reduced by 70–80% due to a water stress situation specifically during the reproductive stage and are not able to fulfil the needs of food requirement in developed and developing countries of the world. Earlier work was mainly focused on the use of morphological or physiological and molecular aspects for improved stress tolerance. Efforts are being made to overcome this problem with the help of today's sophisticated and advanced technology through genomics, proteomics and metabolomics. The presented model summarizes our work in the last five years to mine anatomical parameters as a novel approach to further improving introgression or exploitation of stress adaptive traits. We have focused on some key anatomical traits playing a substantial role in water stress tolerance. This new conceptual model encompasses increased palisade mesophyll height, higher leaf strength index (LSI), higher number of conducting tissues with increased diameter in leaf, stem and root and controlled transpiration rate due to a lower number of stomata per unit leaf area along with the increased guard cell size. Different plants viz. *Lycopersicon esculentum, Capsicum annuum,* and *Calotropis gigantea* were screened by developing polyploids to validate this model approach. Genotypes of *Vitis vinifera* and *Solanum melongena* were also screened. Wild relatives like *Lycopersicon esculentum* var. *cerasiforme* and *Solanum khasianum* were evaluated for comparison. These observations were further correlated with various stress adaptation traits like yield under stress, *in vitro* screening, chlorophyll content, transpiration heating and cooling, molecular markers etc. A new scoring method is proposed which will be helpful to screen a large set of germplasms on a preliminary basis to discriminate genotypes for drought tolerance. There is an urgent need to study the genetics of these stress adaptive traits using high throughput molecular markers to make them more useful for a higher magnitude of genetic gain.

Keywords: drought; palisade mesophyll; xylem; water use efficiency; polyploids

Drought tolerance is a complex trait involving cascades of stress-induced genes. Reports based on morphological, physiological or molecular aspects give an insight into the complexity of drought tolerance mechanism in plants. The reason for slow progress in genotype development may be due to complex physiological response to drought and expression of trait affected mainly by genotype × environment (G × E) interaction. Most abiotic stress related traits are quantitative, thus making them less suitable for genetic engineering than simply inherited monogenic traits.

Parameters for physiological and morphological aspects like transpiration efficiency (Condon *et al.* 1990), dissociation tolerance mechanism (Zhang *et al.* 1999), grain filling under stress (Blum 2000), assimilate translocation (Richards *et al.* 1999), stem reserve (Kulkarni & Deshpande 2006b), stay greenness (Borrel *et al.* 2000), seedling thermo tolerance (Yadav *et al.* 1999), root traits (Shashidhar *et al.* 1991), root morphology (Kamoshita *et al.* 2002) and root length Kulkarni and Deshpande, (2007b) are well discussed.

Genomic approach has also been extensively used for drought tolerance studies including molecular markers, QTL's and candidate gene approach for different traits. Genes induced by abiotic stress response were reviewed by Abdin *et al.* (2002). Molecular markers for important traits in relation to drought tolerance like stay greenness are well discussed (McIntyre *et al.* 1997; Borell *et al.* 2000). One of the major QTL reports includes root depth (Kamoshita *et al.* 2002) and stay greenness trait.

Along with these morphological, physiological and molecular aspect studies, the important traits of plants, i.e. anatomical traits, are mostly neglected during drought tolerance studies. Data are focused rather on physiological traits. Based on results of our experimentation during the last five years, we strongly recommend the incorporation of anatomical traits in drought tolerance studies (Kulkarni 2005; Kulkarni & Deshpande 2006a, b, c; Kulkarni & Deshpande 2007a, b; Kulkarni *et al.* 2007). Reports related to genetic variability of anatomical traits imparting drought tolerance are scanty. Anatomical features are stable over years, genetically governed and can be introgressed (Rajendran 2004). Polyploids as well as wild relatives were studied as whole plant anatomical approach for screening. The correlation of these anatomical features with stress adaptive traits helped us to validate our model. It seems practically impossible to breed drought tolerant genotypes without considering these traits in drought tolerance genetic enhancement research programmes.

Whole plant anatomical basis for drought tolerance

Studies were performed with different genotypes of chilli, tomato, *Calotropis*, *Solanum* species and grape. Polyploids of tomato, chilli and *Calotropis* were generated by treatment with chemical mutagen (colchicine); different anatomical and biochemical parameters were studied from selected mutants; data are published (Kulkarni 2005; Kulkarni & Deshpande 2006a, b, c, 2007a, b; Kulkarni *et al.* 2007). Based on these studies and supporting evidences (discussing all supporting literature is out of scope of this paper), the present model is proposed by dividing it into four different components on the basis of functions of different plant parts although this model is based on experiments conducted for five different plants. Validity for other plants needs to be confirmed experimentally for supporting the utility of this model. The drought tolerance mechanism can be divided into the following four sub-headings:
Traits related to photosynthesis
Evapotranspiration
Food reservoir mechanism
Moisture uptake mechanism

Traits related to photosynthesis

Palisade mesophyll (PM) cells are a site for 90% of active photosynthesis of plant, increased height of palisade mesophyll positively increases the rate

Table 1. Cellular variation in the petiole cross-sections of five plants under observation

Plant	Collenchyma layer (μm)		Phloem width (μm)		Xylem bundles/section		Arrangement of parenchyma	
	resistant	susceptible	resistant	susceptible	resistant	susceptible	resistant	susceptible
Tomato[I]	189–252	138–176	819–917	567–756	14–18	12–13	very compact	loose
Chilli	189–250	126–163	781–844	516–567	22–28	15–17	very compact	loose
Eggplant	151–214	126–163	378–567#	630–705	17–24	14–18	very compact	loose
Grape[II]	242–432	152–216	326–356	280–305	35–47	27–32	very compact	loose
Calotropis	185–226	145–168	358–376	278–296	72–82	50–62	very compact	loose

– resistant genotypes being wild (*Solanum khasianum*) the petiole is thinner in size
[I]Kulkarni and Deshpande (2006a); [II]Kulkarni *et al.* (2007)

Table 2. Comparative analysis of leaf anatomy affecting the rate of photosynthesis

Plant	Palisade mesophyll height (µm)		Spongy mesophyll height (µm)		Tissue ratio (PM:SM)		Palisade mesophyll cells/1000 µm	
	resistant	susceptible	resistant	susceptible	resistant	susceptible	resistant	susceptible
Tomato[I]	213–219	126–162	260–316	240–281	0.71–0.93	0.35–0.68	39–48	29–37
Chilli	252–315	189–252	176–201	138–163	1.43–1.56	1.36–1.54	35–40	25–28
Eggplant	214–254	151–189	138–239	302–352	1.05–1.55	0.50–0.53	32–39	20–26
Grape[II]	107–113	70–90	70–90	80–112	1.25–1.56	0.80–0.87	82–100	67–78
Calotropis	250–287	175–252	325–382	320–357	0.59–0.76	0.54–0.65	121–132	81–90

[I]Kulkarni and Deshpande (2006a); [II]Kulkarni *et al.* (2007)

of photosynthesis. Thicker layer of collenchyma, larger phloem width, higher number of xylem poles per cross-section and compact parenchyma are features worth mentioning (Table 1). Polyploids studied in tomato depicted stay greenness, initial vigorous growth and thicker leaves in a mutant (248 µm), which were mainly due to an increased height of palisade mesophyll cells as compared to a susceptible genotype (132 µm) (Figure 1A). The petiole cross-sectional anatomy was observed to play a significant role in water stress tolerance. This has a major positive contribution to the rate of photosynthesis as indicated by a significant positive correlation with total chlorophyll content (Borse *et al.* 1998; Kulkarni *et al.* 2007) in grape (Table 2).

Intercellular spaces between palisade mesophyll cells increase the volume of the leaf which comes in contact with air leading to a higher loss of water from palisade mesophyll cells, increasing transpirational losses and ultimately resulting in reduced growth and rate of photosynthesis. As a result, cellular growth is also hampered, which mainly depends on the maintenance of turgor pressure.

In addition to the increased height of palisade mesophyll cells, compactness also affects the rate of photosynthesis. Polyploids of tomato, chilli and *Calotropis* exhibited compact arrangement of palisade mesophyll cells as compared to their control. Heterosis for an increased palisade mesophyll height was also observed in tomato and was directly related with Leaf Strength Index (LSI) (Kulkarni & Deshpande 2006a). Stress susceptible control genotypes comparatively showed less developed palisade mesophyll cells which were loosely arranged.

Comparison of grape cultivars (all cultivated types) also revealed similar results. The genotypes with greater palisade mesophyll height were observed to be stay-green, with higher chlorophyll content and highest magnitude of juice yield per ha as compared to genotypes with shorter palisade mesophyll cells which are loosely arranged (Kulkarni *et al.* 2007). Leaf thickness was 163.9 µm in highly drought resistant genotypes and it was 118.8 µm in susceptible genotypes in mulberry (Sushellamma & Datta 1995). This difference in leaf thickness imparted the moisture retention capacity of 66.7% in resistant and 50.2% in susceptible genotypes.

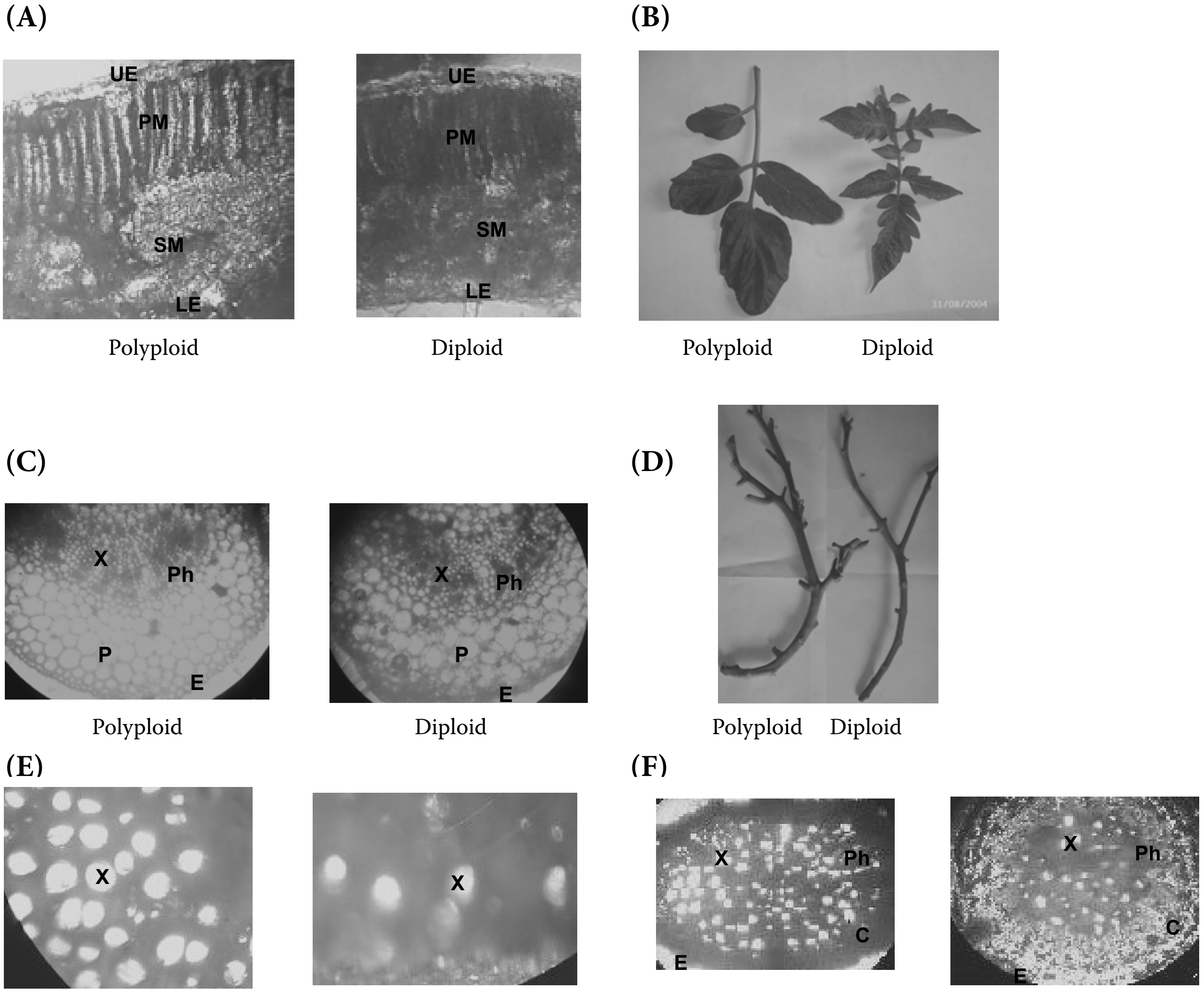
The ratio of palisade mesophyll to spongy mesophyll (PM:SM) plays a major role in indicating the photosynthetic ability of leaves. The higher the ratio, the higher the rate of photosynthesis, which is clearly observed in tomato, chilli, grape and *Calotropis* (Kulkarni & Deshpande 2006a; Kulkarni *et al.* 2007). The ratio of PM:SM was 2.18 in highly drought resistant mulberry genotypes as compared to as low as 0.86 in susceptible genotypes (Sushellamma & Datta 1995).

The leaf cell arrangement in cultivated eggplant (*Solanum melongena*) was compared with a wild relative (*Solanum khasianum*). It was observed that cultivated genotypes were characterized by a higher amount of spongy mesophyll cells and lower height of palisade mesophyll cells. The drought resistant wild relative (*Solanum khasianum*) was characterized by the higher tissue ratio (1–1.5), which was only 0.50–0.53 in cultivated eggplant genotypes. Comparatively, the tissue ratios in chilli and tomato were not so significantly higher but drought tolerant polyploids exhibited a higher value of the tissue ratio as compared to cultivated genotypes.

The presence of the lower palisade mesophyll layer is an important character which imparts drought tolerance and is observed in diploid genotype of the cotton *Gossypium arboreum* (Singh *et al.* 1996). We are of an opinion that light cannot penetrate enough to the lower side of the leaf, so the lower palisade mesophyll cells are majorly involved in reducing evapotranspirational losses by increasing the palisade mesophyll cell density in total leaf volume. The photosynthesis carried out by these cells provides an additional advantage.

These results lead to a conclusion that the compactness of palisade mesophyll cells and reduced air spaces by the smaller area of spongy mesophyll cells can be an ideal trait for the highest photosynthetic ability of leaves. If palisade mesophyll cells are well developed, the transpiration rate lowers due to the compactness of the tissue. On the other hand, if loose spongy mesophylls are well developed (like in the case of drought susceptible genotypes), the rate of transpiration is greater, which ultimately leads to the reduced rate of photosynthesis. Compact parenchyma with honeycomb structure was also observed to play an important role in controlling a moisture loss (Figure 1C).

Our results are supported by studies in coconut (Kumar *et al.* 2000), wild tomato *Lycopersicon pennellii* (Kebede *et al.* 1995), Chinese cabbage



Polyploid Diploid Polyploid Diploid

Polyploid Diploid Polyploid Diploid

Polyploid Diploid Polyploid Diploid

Figure 1. Key morpho-anatomical features in relation to drought tolerance in polyploid and diploid tomato: (A) leaf ultrastructre, (B) leaf size, (C) compact parenchyma in petiole, (D) stem size difference, (E) xylem density variation in stem, (F) root xylem density (UE – upper epidermis, LE – lower epidermis, PM – palisade mesophyll, SM – spongy mesophyll, Ph – phloem, X – xylem, P – parenchyma, E – epidermis, C – cortex)

(Kuo *et al.* 1988), mulberry (Sushellamma & Datta 1995) and peanut (Rao & Wright 1994) which agree with the statement that the increased palisade mesophyll height increases the water use efficiency of plants under a water stress situation.

Evapotranspiration

In grape, genotypes with higher juice yield per ha and stay greenness had on average 380–402 stomata per mm^2 as compared to a higher frequency in lower yielding genotypes (Kulkarni *et al.* 2007). This variation proves the importance of stomatal frequency and distribution at an increased rate of photosynthesis ultimately resulting in increased yields. Stomatal frequency was observed to be significantly reduced in polyploids of tomato, chilli and *Calotropis* by 50–60% as compared to cultivated genotypes. As evident from Table 3, an increase in the size of guard cells, increased pore size and increased distance between stomata were observed in polyploids while in cultivated genotypes the presence of abundant, closely spaced, smaller size stomata is responsible for higher evapotranspirational losses.

In an eggplant wild relative (*Solanum khasianum*) the stomatal number was 45–50% less as compared to cultivated genotypes on the lower as well as the upper side of the leaf greatly reducing evapotranspirational losses. An additional advantage was the greater distance between stomata and their sunken nature providing a benefit to wild relatives. This genotype is a potential donor for drought tolerance traits in eggplant (Table 3).

Polyploidy induced an increase in the guard cell size of stomata and they were observed to be 36–40 μm long and 18–28 μm wide as compared to 24–28 μm length and 12–18 μm width in the mother plant in *Calotropis*. This increase in size is related to a reduced number of stomata per unit area. In studies of 92 genotypes of mulberry, Sushellamma and Datta (1995) reported the mean stomatal size of 48.8 μm in highly drought resistant genotypes as compared to 19.2 μm in susceptible genotypes.

The rate of evapotranspiration reduced by the lower magnitude of stomata distribution can be induced by polyploidy or introgressed from wild relatives. A lower number of stomata reduces evapotranspirational losses and increases the water use efficiency, i.e. the rate of photosynthesis. Guard cells with increased stomata size are also the area of interest as the turgor pressure maintenance and increased cell size are correlated with proper gas exchange and stomatal opening and closing. The guard cell size regulates the exchange of gases (e.g. CO_2, O_2, H_2O).

Similar results were observed in wild tomato *Lycopersicon pennellii* (Kebede *et al.* 1995), Chinese cabbage (Kuo *et al.* 1988), wheat (Limin & Fowler 1994) and *Quercus velutina* (Ashton & Berlyn 1994).

Food reservoir mechanism

An increase in root dry weight due to stress remobilization of assimilates from stem to root has been reported (Shashidhar *et al.* 1991). Evidence from wheat shows that some genotypes use reserve

Table 3. Variation in stomatal anatomy affecting the rate of evapotranspiration

Plant	Number of stomata per mm^2				Distance between stomata (μm)			
	upper side of leaf		lower side of leaf		upper side of leaf		lower side of leaf	
	resistant	susceptible	resistant	susceptible	resistant	susceptible	resistant	susceptible
Tomato[I]	50–60	59–77	102–132	192–235	403–478	365–415	187–223	86–123
Chilli	57–69	84–113	130–186	199–310	201–264	252–322	142–185	58–88
Eggplant	42–56	101–108	90–117	180–216	154–192	115–147	88–97	68–86
Grape[II]	142–156	182–198	380–402	502–745	105–122	117–136	88–101	52–71
Calotropis	80–92	143–164	128–145	162–217	60–83	101–147	48–61	72–86

[I] Kulkarni and Deshpande (2006a); [II] Kulkarni *et al.* (2007)

photosynthates extensively for grain filling even under non-stress conditions. In this sense, the stem reserve is a constitutive backup source for grain filling under stress (Blum 2000).

In tomato, a polyploid mutant was observed with higher cortex thickness (352–376 µm) and larger secondary phloem area (630–882 µm) as compared to susceptible genotypes (252–315 µm and 252–378 µm, respectively). The higher cortex thickness facilitates large storage of photosynthates whereas the larger secondary phloem area is an indication of the conduction of photosynthates in higher amounts (Kulkarni & Deshpande 2006b). Xylem vessels of increased diameter and their higher number were also observed in the stem of the polyploid mutant as compared to cultivated genotypes (Table 4). The contribution of these traits clearly indicates efficient translocation, conductivity and storage of photosynthates in polyploids as compared to cultivated plants imparting adaptability to drought.

The average stem girth of drought-resistant polyploids in tomato (3.9–5.0 cm), chilli (5.2–6.0 cm), Calotropis (8.3–9.0 cm) as compared to their control, i.e. 2–2.9 cm, 2.6–3.3 cm and 5.6–6.5 cm respectively, indicates the importance of the trait in the stress tolerance mechanism. Close association of anatomical parameters with dry matter production was observed in tomato. The number of xylem vessels (r = 0.703) and secondary phloem width (r = 0.706) are key stem anatomical features which show significant positive correlations with dry matter production and ultimately with drought tolerance in tomato (Kulkarni & Deshpande 2006b). Similar results were observed in chilli and *Calotropis* (unpublished data).

Drought tolerant polyploids altered dry matter partitioning (40:50:10) as compared to susceptible cultivated genotypes (50:40:10) for leaf, stem and root, respectively, in tomato (Table 4). This 8–10% extra dry matter storage ability in the stem of polyploids plays a crucial role in the water stress tolerance mechanism (Figure 1D). The number of xylem vessels in the stem also plays an important role in the stress tolerance mechanism being higher in tolerant genotype (110) as compared to susceptible genotype (62) as depicted in Figure 1E. Blum (2000) discussed genotypic variation for the ability to store and mobilize carbohydrates for seed filling during terminal moisture stress. Differences in the combining ability of genotypes for this trait contribution were also observed. Earlier efforts emphasized traits like high chlorophyll at heading (Hede *et al.* 1999), high leaf conductance, high pubescence (Treathown *et al.* 1998) and peduncle volume for drought tolerance in wheat.

Moisture uptake mechanism

Efficient palisade mesophyll cells facilitate optimum water utilization for maximum photosynthetic activity. Under highly efficient photosynthesis, the regulated transpiration mechanism is facilitated by a lower number of stomata per unit area. This is a possible mode of maintaining the proper 'negative' tension for higher moisture absorption through wider xylem poles under water stress situations while maintaining the optimum rate of photosynthesis.

Wild genotypes in tomato, chilli and eggplant roots showed wider xylem poles of increased den-

Table 4. Root:shoot ratio differences imparting stay greenness in plants

Plant	Root length (cm)		Shoot length (cm)		Root:shoot length ratio	
	resistant	susceptible	resistant	susceptible	resistant	susceptible
Tomato[III]	40–71	27–38	52–68	60–132	0.7–1.0	0.2–0.5
Chilli	36–44	20–31	60–82	50–78	0.5–0.6	0.3–0.4
Eggplant	57–74	20–27	35–45	50–71	1.6–2.7	0.3–0.4
Grape*	–	–	–	–	–	–
Calotropis	40–55	28–32	68–89	85–108	0.6–1.2	0.3–0.4

*As same rootstock is used data do not vary significantly

[III] Kulkarni and Deshpande (2006b)

Table 5. Root cross-sectional anatomy responsible for variable ability of water uptake

Plant	Xylem poles/cross section		Xylem diameter (μm)		Number of feeder roots/5 cm	
	resistant	susceptible	resistant	susceptible	resistant	susceptible
Tomato[III]	33–45	16–20	180–200	100–125	39–49	23–32
Chilli	82–95	47–75	176–192	140–155	23–36	13–19
Eggplant	41–48	31–38	170–186	130–146	–	–
Grape[II]	38–45	27–33	124–132	81–92	–	–
Calotropis	51–63	31–39	120–135	88–96	26–35	19–23

[II]Kulkarni *et al.* (2007); [III]Kulkarni and Deshpande (2006b)

sity. The roots of polyploids in tomato (33–39), chilli (82–89) and *Solanum khasianum* (eggplant wild relative; 41–48) also exhibited a higher number of xylem poles per cross-section as compared to their controls (Table 5). Figure 1F clearly shows more and wider xylem poles in polyploid tomato (38) as compared with diploid mother plant (21). In grapes, stay green and high-yielding genotypes had a higher number of xylem poles as compared to stress susceptible and lower-yielding genotypes (Kulkarni *et al.* 2007). Similar results of increased xylem poles were obtained in polyploids of Calotropis.

Root anatomy in cotton is extensively studied and found to have a wide range of diversity (Reinhardt & Rost 1995). A high number of xylem poles was initially thought to be involved in improved capabilities for water transport associated with increased xylem elements (Ooserhuis & Wullschleger 1987). But the study by McMicheael *et al.* (1999) revealed it to be more directly associated with a greater potential for improved lateral root growth. This fact was also described in studies with upland rice (Courtois *et al.* 1996).

Root anatomical parameters like number of xylem vessels and diameter of xylem vessels were positively correlated with dry matter production under stress whereas traits like distance between xylem poles were negatively correlated in tomato (Kulkarni 2005).This gives a better insight into the role of xylem vessels in relation to drought tolerance. A direct correlation was observed between the number of xylem poles in the taproot of cotton seedling and the number of lateral roots produced growth (McMicheael *et al.* 1987). Yamboo *et al.* (1992) reported the metaxylem radius ranging between 20 and 32 μm in rice. Root stocks of grafted grape genotypes showed 3–4 times wider xylem poles as compared to normal ones, which increased the water conduction ability 10–15 times compared to normal genotypes and increased the rate of photosynthesis (Dr. Robwalker, CSIRO Industry, Australia – personal communication).

Morpho-anatomical correlations for drought tolerance

Besides anatomical features some morphological aspects of roots affect drought tolerance. All drought tolerant genotypes invariably exhibit larger roots along with higher density of secondary or tertiary roots. The basis for a difference between cultivated and normal genotypes was in the root growth habit. Resistant genotypes were observed to show deep and vertical growth habit whereas cultivated genotypes showed shallow growth and a lower number of roots. Courtois *et al.* (1996) studied variability in the root depth of upland rice, which is used as a potential donor for longer root traits. Lafine *et al.* (2001) reported highly significant genotypic variance for both maximum root depth and deep root weight comparing traditional Australian varieties and temperate Japonicas. Yadav *et al.* (1997) reported 0.77 broad-sense heritability for root length. The root depth and deep root weight are quantitatively controlled in a doubled haploid population from *Indica* × *Japonica* segregants.

Resistant genotypes exhibited longer and deeper roots whereas the root growth of cultivated genotypes was shallow. Prospects would be better if both types of root growth habits could be incorporated into a single genotype which will have an advantage of moisture absorption during frequent rain situations whereas the part of the deeper root

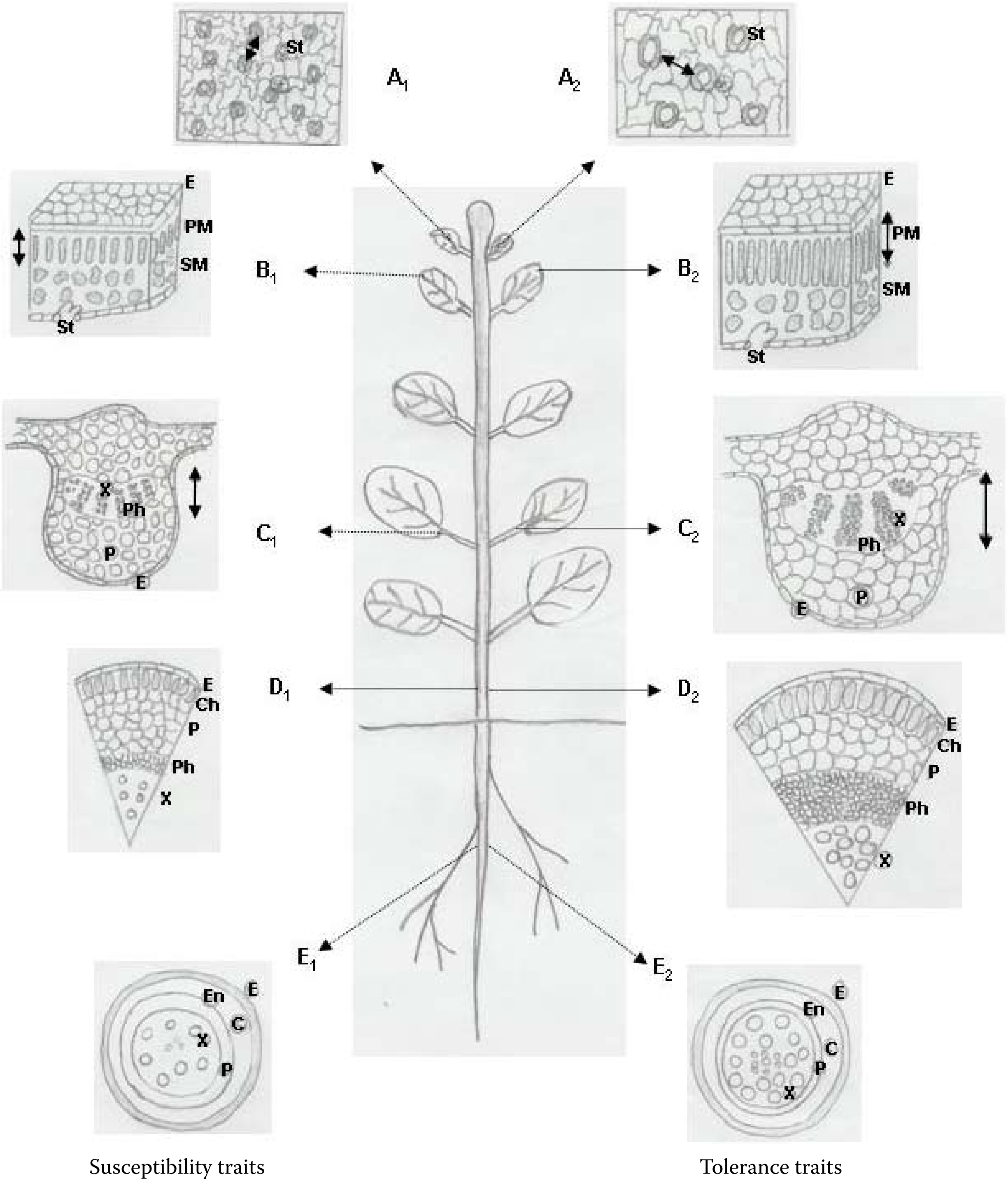


Figure 2. Model based on anatomical features for drought tolerance in plants. A_1 and A_2: variation in size of stomata; B_1 and B_2: variation in palisade mesophyll cells size and leaf thickness; C_1 and C_2: petiole cross section showing difference in parenchyma compactness; D_1 and D_2: variation in secondary phloem width of stem; E_1 and E_2: xylem vessel comparison (St – stomata; E – epidermis; PM – palisade mesophyll; SM – spongy mesophyll; Ph – phloem; X-xylem; P – parenchyma; Ch – collenchyma; En – endodermis; C – cortex)

system is utilized for drought situations. This may prove to be an ideotype root system for plants under both drought and normal conditions.

The number of feeder roots actively engaged in moisture absorption was also observed to be higher in drought resistant polyploids as compared to their controls (Table 4). High root:shoot ratio is an important drought adaptive trait. Genotypic correlations for root morphological parameters like root length (r = 0.795), feeder roots/5 cm (r = 0.583) and tertiary roots (r = 0.791) were significantly positively correlated with dry matter production under stress conditions in tomato (Kulkarni & Deshpande 2007). Sushellamma and Datta (1995) reported correlation coefficients of anatomical traits with yield under stress in mulberry. Stomatal size (0.278) and total leaf thickness (0.268) were correlated positively whereas stomatal frequency was correlated negatively (–0.167) with yield under stress.

These anatomical traits were also correlated with chlorophyll content in grape (Kulkarni *et al.* 2007), *in vitro* seedling growth studies in tomato (Kulkarni & Deshpande 2006c) and chilli, yield under stress in tomato (fruit yield), grape (juice yield), RAPD markers in tomato (Kulkarni & Deshpande 2006a) to validate the functional importance of these anatomical traits. The proposed model based on the above morpho-anatomical studies is represented in Figure 2.

Proposed ideotype for increased water use efficiency (WUE) under water stress in plants

Donald (1968) proposed the use of crop ideotype breeding as a way to increase crop yields. The ideotype breeding method offered the framework for applying concepts of light interception and plant competition in development of high-yielding cultivars. It emphasized selection for specific characteristics that affect photosynthesis in a plant and may contribute to higher yields, such as leaf size and position, plant height and larger tillering capacity. Reynolds *et al.* (1999) emphasized leaf anatomical traits like thickness along with physiological aspects, i.e. reduced evapotranspiration rate and reduced photoinhibition risk associated while evaluating a conceptual model for drought tolerance in wheat. Our proposed model considers aspects earlier reported with deeper insight into anatomical aspects directly related to drought tolerance.

Highlights of proposed model

- Longer palisade mesophyll cells
- Reduced air spaces in leaves
- Larger size and lower number of stomata
- Petiole with a higher number of conducting tissues
- Compact parenchyma cells
- Higher proportion of secondary phloem and conducting tissues in stem
- Deeper roots with wider and dense xylem poles
- Higher number of feeder roots and root hairs

Utilization of model in crop yield improvement

- Drought tolerance
- Sucking pest tolerance
- Improved nutrient use efficiency
- Disease tolerance
- Salinity tolerance
- Cold tolerance

Applications

- Identification of sources with a high expression of drought adaptive traits
- Pre-screening of haploid/diploid/polyploid genotypes for development of new drought tolerant genotypes with a higher level of water use efficiency
- Studies of genotypic correlations of various anatomical traits with yield under stress
- Identification of QTL's for drought tolerance

CONCLUSIONS

The present report confirms anatomical features playing a key role for increased water use efficiency and drought tolerance in plants. Investigation of five (tomato, chilli, eggplant, grape and Calotropis) different plant groups representing diverse growth habits having characteristic cellular arrangement outyielded highlighting principles for the drought tolerance mechanism. Interestingly, similar principles in polyploid and wild relatives of cultivated species governed drought tolerance. Efforts concentrated on understanding the function of each cell and studies of cell specific differential gene expression will put more light on this complex trait in plants.

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References

Abdin M.Z., Rehman R.V., Israr M., Srivastava P.S., Bansal K.C. (2002): Abiotic stress related genes and their role in conferring tolerance in plants. Indian Journal of Biotechnology, **1**: 225–244.

Ashton P.M.S., Berlyn G.P. (1994): A comparison of leaf physiology and anatomy of *Quercus* (Section *Erythrobalanus –Fagaceae*) species in different light environments. American Journal of Botany, **81**: 589–597.

Blum A. (2000): Drought tolerance is it a complex trait? In: Proc. Int. Workshop on Field Screening for Drought Tolerance in Rice. ICRISAT, 17–22.

Borell A.K., Bidinger F.R., Sunita K. (2000): Does maintaining green leaf area in sorghum improve yield under drought. 1. Leaf growth and senescence. Crop Science, **40**: 1026–1037.

Borse T.H., Maheshwari V.L., Baviskar M.P. (1998): Inhibition of photosystem II (PSII) by Metribuzin. Indian Journal of Experimental Biology, **36**: 800–804.

Condon A., Faruhar G.D., Richards P.A. (1990): Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat leaf gas exchange and whole plant studies. Australian Journal of Plant Physiology, **17**: 9–22.

Courtois B., Chainep W., Moolsris, Sinha P.K., Trebuil G., Yadav R. (1996): Drought tolerance and germplasm improvement: on going research in upland rice research consortium: In: Piggin C., Courtois B., Smith V. (eds): Upland Rice Research in Partnership IRRI Discussion Paper Series. 16. International Rice Research Institute, Los Baros , 154–175.

Donald C.M. (1968): The breeding of crop ideotypes. Euphytica, **17**: 385–403.

Hede A., Skovmand B., Reynolds M., Crossa J., Vilhelmsen A., Stoelen O. (1999): Evaluating genetic diversity for heat tolerance traits in Mexican wheat landraces. Genetic Resources and Crop Evolution, **46**: 37–45.

Kamoshita A., Wade C.J., Ali M.L., Zhang J., Sarkarung S., Nguyen H. (2002): Mapping QTL's for root morphology of a rice population adapted to rainfed conditions. Theoretical and Applied Genetics, **104**: 880–893.

Kebede H., Martin B., Nienhuis J., King G. (1995): Leaf anatomy of two tomato species with contrasting gas exchange properties. Crop Science, **34**: 108–113.

Kulkarni M. (2005): Polymorphism studies in tomato for germplasm enhancement in relation to water use efficiency. [PhD Thesis.] Swami Ramanand Teerth Marathwada University, Nanded.

Kulkarni M., Deshpande U. (2006a): Anatomical breeding for altered leaf parameters in tomato genotypes imparting drought tolerance using Leaf Strength Index. Asian Journal of Plant Sciences, **5**: 414–420.

Kulkarni M., Deshpande U. (2006b): Comparative studies in stem anatomy and morphology in relation to drought tolerance in tomato (*Lycopersicon esculentum*). American Journal of Plant Physiology, **1**: 82–88.

Kulkarni M., Deshpande U. (2006c): RAPD based fingerprinting of tomato genotypes for identification of mutant and wild cherry specific markers. Journal of Plant Sciences, **1**: 192–200.

Kulkarni M., Deshpande U. (2007a): *In vitro* screening of tomato genotypes for drought tolerance using polyethylene glycol. African Journal of Biotechnology, **6**: 691–696.

Kulkarni M., Deshpande U. (2007b): Root anatomical and morphological basis for drought tolerance in Tomato (*Solanum lycopersicon*). Bioinfolet, **3**: 292–296.

Kulkarni M., Borse T., Chaphalkar S. (2007): Anatomical variability in grape (*Vitis venifera*) genotypes in relation to water use efficiency (WUE). American Journal of Plant Physiology, **2**: 36–43.

Kumar Naresh S., Rajagopal V., Anitha K. (2000): Leaf let anatomical adaptations in coconut cultivars for drought tolerance. In: Muraleedharan N., Rajkumar R. (eds): Recent Advances in Plantation Crops Research. Allied Publishers Ltd., New Delhi, 225–229.

Kuo C.G., Shen B.J., Chen H.M., Chen H.C., Opena R.T. (1988): Association between heat tolerance, water consumption and morphological characters in Chinese cabbage. Euphytica, **39**: 65–78.

Lafine H.R., Champoux M.C., McLaren G., O'Toole J.C. (2001): Rice root morphological traits are related to isozyme groups and adaptation. Field Crops Research, **71**: 57–70.

Limin A.E., Fowler D.B. (1994): Relationship between guard cell length and cold hardiness in wheat. Canadian Journal of Plant Science, **74**: 59–62.

McIntryre C.C., Tao Y.Z., Jordan D.R., Henzell K.G.(1997): Application of molecular markers to sorghum breeding in Australia. International Sorghum and Millets Newsletter, **38**: 11–15.

McMichael B.L., Wosterhuis D.M., Zak J.C., Beyrounty C.A. (1999): Growth and development of root systems. In: Stewart J.M., Oosterhuis D.M., Heithol J. (eds): Cotton Physiology. Book II. National Cotton Council, Memphis.

Ooserhuis D.M., Wullschleger S.D. (1987): Water flow through cotton roots in relation to xylem anatomy. Journal of Experimental Botany, **38**: 1866–1874.

Rajendran T.P. (2004): Challenges for management of sap sucking pests in cotton. In: Int. Symposium of Strategies for Sustainable Cotton Production. A Global Vision. Crop Protection. U.A.S., Dharwad, 37–41.

Rao Nageshwara R.C., Wright G.C. (1994): Stability of the relationship between specific leaf area and carbon isotope discrimination across environment in peanut (*Arachis hypogea* L.). Crop Science, **34**: 48–103.

Reinhardt D.H., Rost T.L. (1995): Primary and lateral root development of dark and light grown cotton under salinity stress. Botanica Acta, **108**: 457–465.

Reynolds M., Skovmand B., Trethowan R., Pfeiffer W. (1999): Evaluating conceptual model for drought tolerance. In: Ribaut J.M., Poland D. (eds): Molecular Approaches for Genetic Enhancement of Cereals for Stable Production in Water-Limited Environments. CIMMYT Headquarters, Mexico. 49–53.

Richards R.A., Rebetzke G.J., Appels R., Condon A.G. (1999): Physiological traits to improve the yield of rainfed wheat: can molecular genetics help? In: Workshop on Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-Limited Environments. June 21–25, 1999, CIMMYT Headquarters, Mexico.

Shashidhar H.E., Mervat E.S. Hebhar B.K. Prasad T.G., Udayakumar M. (1991): Root osmotic adjustment promotes higher water status in shoots. Evidence for a link between drought tolerance and avoidance mechanism in two crop species. In: National Seminar of Young Scientists on Environmental Physiology. Garhwal, Srinagar.

Singh J., Bhardwaj S.N., Singh M. (1996): Leaf size and specific leaf weight in relation to its water potential and relative water content in upland cotton. Indian Journal of Agricultural Sciences, **60**: 215–216.

Sushellamma B.V., Datta R.K. (1995): Breeding for drought tolerance in Mulberry. In: Sharma B. (ed.): Genetic Research and Education in Current Trends and the Next Fifty Years. Indian Society of Genetics and Plant Breeding, New Delhi, 641–650.

Trethowan R.M., Reynolds M.P., Skovmand B., van Ginkel M. (1998): The effect of glume pubescence on floret temperature in wheat. In: Agronomy Abstracts 1998. October 18–22, 1998, ASA, Baltimore.

Yadav R., Courtois B., Huang N., McLaren G. (1997): Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. Theoretical and Applied Genetics, **94**: 619–632.

Yadav R.S., Hash C.T., Bidinger F.R., Howarth C.J. (1999): QTL analysis and marker assisted breeding of traits associated with drought tolerance in pearl millet. In: Ito O., O'Toole J., Hardy B. (eds): Genetic Improvement of Rice for Water Limited Environments, IRRI, Manila.

Yamboo E.B., Ingram K.T., Real T.G. (1992): Root xylem influence on the water relations and drought tolerance of rice. Journal of Experimental Botany, **43**: 925–932.

Zang T., Kluwa N.Y., Nguyen H.T. (1999): Plant adaptation and crop improvement for arid and semiarid environments. In: Traylor I.R., Dregne H., Mathis K. (eds): Proc. 5th Int. Conf. Desert Development: The Endless Frontier, August 12–17, 1996, Texas Tech. University, Lubbock, 604–620.

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