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Identification of drought tolerant turmeric (*Curcuma longa* L.) genotypes with sustainable yield

C V Nazmin Banu & K S Krishnamurthy*
ICAR–Indian Institute of Spices Research, Kozhikode, 673 012, Kerala
**Email: kskrishnamurthy@gmail.com*

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Abstract

Turmeric (*Curcuma longa* L.) (Zingiberaceae), is native to south west India. Water stress is the most common adverse environmental condition which severely affects crop productivity. Here we have analysed morphology, stomatal density, relative water content, electrolyte leakage, epicuticular wax content and rhizome yield of 50 elite turmeric genotypes for identification of genotypes with differential response (tolerance and susceptibility) to water stress. Results showed that water stress during rhizome development stage (90-120 days after planting) significantly decreased the leaf relative water content and increased membrane permeability. Epicuticular wax content varied significantly among the genotypes. Genotypes with lower leaf area, higher relative water content, lesser electrolyte leakage, higher wax content and fewer stomata than other genotypes were shortlisted as tolerant. From among these shortlisted ones, four tolerant genotypes (IISR Pragati, SL 5, Suguna, and Suvarna) with higher yield, along with two susceptible genotypes (IISR Alleppey Supreme and IISR Kedaram), were further evaluated in field conditions. The results indicated that, in terms of yield and physiological parameters, the tolerant genotypes significantly outperformed the susceptible ones, showcasing superior drought tolerance traits. These genotypes with contrasting characters can be used for further studies to elucidate the mechanism of drought tolerance.

Keywords: Electrolyte leakage, epicuticular wax, relative water content, stomatal density, stress response

Introduction

A wide range of biotic and abiotic stresses that crop plants experience substantially impair their ability to grow and develop which may lead to a decline in productivity, posing serious threat to agriculture (Sharma & Lavanya, 2002). One-third of world's population resides in regions with water shortage. Climatic models have predicted that severity and duration of drought stress is expected to increase due to elevated CO_2 in atmosphere and on-going global climate change scenarios (IPCC, 2007). Thus, water shortage is one of the major limitations to productivity worldwide (Lambers *et al*., *2008).* Drought stress impacts the equilibrium of water within the plant, disturbs cellular-level metabolic reactions, and diminishes both ATP synthesis and respiration (Upadhyaya *et al*., 2021).

Turmeric (*Curcuma longa* L.) belonging to the family *Zingiberaceae* is a triploid, vegetatively propagated medicinal spice crop, widely used as food preservative, natural dye, as well as a drug (Krup *et al*., 2013).Turmeric has also been traditionally used in ayurvedic medicine and against various malignant diseases, diabetes, allergies, arthritis and Alzheimer's disease. Medicinal properties associated with turmeric are due to curcumin, one of the important secondary metabolites (Aggarwal *et al*., 2007). Recent studies indicate that turmeric has a greater potential for use in the production of pharmaceuticals and cosmetics. Hence, it is important to find good practices to increase the growth parameters of such a valuable spice crop (Mohamed *et al*., 2014). Turmeric requires assured availability of irrigation water especially during the dry season (Tripathi *et al*., 2018).

Abiotic stress factors such as drought was found to adversely affect growth and productivity of the plants (Mostajeran *et al*. , 2014). Water shortage has a considerable impact on agricultural systems, and thus the capacity of plants to resist this stress is of great economic importance (Sankar *et al*., 2007; Reddy *et al*., 2004) . In this regard, the development of cultivars which are tolerant to water deficit has become a priority. When crops are subjected to drought stress, numerous changes occur at the physiological, metabolic, and molecular levels in comparison with crops grown under non stressed conditions. To cope with water deficit, plants have evolved physiological and biochemical adaptations and they respond to desiccation at the biochemical, physiological, cellular, and molecular levels (Shinozaki &Yamaguchi, 2007). Across plant species, drought imposes various physiological and biochemical limitations and adverse effects (Pirasteh-Anosheh *et al*., 2016). The response to environmental stresses varies among genotypes within a species (Sakata & Higashitani, 2008)

Drought tolerance is a complex trait, which involves interaction of morphological, physiological, and biochemical processes. However, a high yield potential under drought conditions is an obvious target for improvement strategies (Wang *et al*., 2013). Identification of genotypes with differential response (tolerance and susceptibility) under drought stress will facilitate

identification and characterisation of important physiological, biochemical and molecular mechanisms involved in drought response (Chaudhary *et al.* 2020). The current study was carried out to identify drought tolerant turmeric genotypes with sustainable yield suitable for drought prone areas.

Materials and methods

Plant material and experimental methods

Present study comprised of fifty turmeric genotypes which included popular released varieties, a few promising genotypes for yield and quality and short duration genotypes maintained in the germplasm repository of ICAR-IISR Experimental Farm, Peruvannamuzhi, Kerala, India. Planting was taken up in the month of June 2019, in a completely randomized design with three replicates in grow bags at ICAR-Indian Institute of Spices Research (ICAR-IISR), Kozhikode, Kerala. Recommended package of practices of ICAR-IISR were followed for crop cultivation.

Seed rhizomes (25g) of 50 indigenous turmeric germplasm accessions were planted in polythene grow bags (15 cm × 30 cm) containing potting mixture (2:1:1 of soil: sand: farmyard manure), which had the permanent wilting point at 7.0 to 7.5% moisture content, maintained in a greenhouse at a temperature of 30 ± 3°C (day) and relative humidity of 80 ± 5%. Plants were irrigated to field capacity, with a soil moisture content (SMC) of 21%, which was analysed according to the standard methods as described in AOAC (2005), till 90 days after planting (DAP). Plants were subjected to water stress treatment from 90 to 120 DAP, by suspension of irrigation for 30 days. SMC was reduced to 11 % after 30 days of moisture stress (at 120 DAP). Again, the plants were irrigated to field capacity till harvest. The genotypes were evaluated using morphological and physiological parameters to assess their tolerance attributes to water stress and also the yield under the water stress condition.

In the second round, the field experiment was conducted during 2022-23 at ICAR-Indian Institute of Spices Research, Kozhikode, Kerala. The experimental materials consisted of four shortlisted drought tolerant genotypes (IISR Pragati, SL 5, Suguna and Suvarna) and two susceptible genotypes (IISR Alleppey Supreme and IISR Kedaram) based on the results of the earlier experiment.

Morphological characterization

Morphological characters such as plant height (cm), number of tillers, number of leaves, leaf length (cm) and breadth (cm) were recorded at 120 DAP after one month of stress treatment (90-120 DAP). Leaf area per plant (cm^2 $plant^{-1}$) was determined as per Panja *et al*. (2005) as follows,

$$\text{Leaf area} = 5.71 + 0.72\ (\text{length} * \text{breadth})$$

Where, 5.71 and 0.72 are the intercept and regression coefficient of leaf area estimated using least-square linear regression analysis. Length and breadth are the maximum length and breadth of the leaf (cm).

Analysis of physiological characters

The youngest fully matured leaf (fourth leaf from shoot apex) of plants labelled for observation purpose was used for physiological assays.

Relative water content (RWC)

RWC was determined at 10 days intervals at four different soil moisture levels *viz.* 21 % (control), 17.10% (10 days after treatment (DAT)- early stress), 13.5% (20 DAT- moderate stress) and 11% (30 DAT- severe stress), following the method of Barrs & Weatherley (1962). For this, approximately 150 mg fresh weight of leaf discs ($2cm^2$) from control and stress were floated in double distilled water in a Petri dish for three hours to gain full turgidity and their turgid weight was determined. The turgid samples were dried in hot air oven at 80^0C to a constant weight and dry weight of the leaf discs was recorded and RWC was calculated using the formula given below.

RWC (%) = [(FM - DM)/(TM - DM)] * 100,

Where, FM, DM, and TM are the fresh, dry and turgid masses, respectively, of the tissue.

Electrolyte leakage (EL)

Electrolyte leakage was determined at different soil moisture levels as that of RWC as per Blum & Ebercon, (1981). Leaf discs of 1cm diameter were collected from control and 10, 20 and 30 days stressed plants, washed in distilled water, blotted on filter paper and then incubated in 5 ml of distilled water for three hours. The electrical conductivity (EC) of the solution was measured using EC-TDS analyser (initial electrical conductivity). The leaf discs were then boiled for fifteen minutes, cooled to room temperature and the EC of the solution was measured again (final EC). The cell electrolyte leakage was computed using the formula

EL (%) = (Initial EC/ Final EC) x 100,

Where, EC = Electrical conductivity.

Stomatal density (SD)

After 30 days of stress treatment, at 120 DAP, stomatal density was determined on the abaxial and adaxial surfaces of the leaf using the method of Mahareli *et al*. (2002). Mature leaf samples were thoroughly cleaned to remove the dust adhering to the surface, then applied nail polish, the replica fluid, in a thin and uniform film (by spreading a drop or two on the leaf surface) and allowed it to dry completely. The replica was then carefully peeled off with the aid of cello tape, and it was positioned on the slide such that the imprinted surface was on the upper side. All the stomata that were visible in 0.9604 view field of microscope at 10X magnification were counted. The stomatal density was expressed as number of stomata mm^{-2}.

Leaf epicuticular wax (wax)

Leaf epicuticular wax estimation was performed at 120 DAP as per Ebercon *et al*. (1977). A wax reagent was prepared by combining 20 grams of potassium dichromate with 40 ml of deionized water and 1 litre of concentrated H_2SO_4. The wax reagent was boiled on a hot water bath until a clear solution was obtained. Carnauba

wax was used for standard graph preparation.

Leaf sample (2 cm^2 area) was immersed in 15 ml of distilled chloroform for 20 seconds (to extract only epicuticular wax as longer periods of immersion may extract the inner lipids). The chloroform extract was boiled on water bath to dryness. Wax reagent (5 ml) was added to it and boiled for thirty minutes in hot water bath (100°C). It was cooled to room temperature and 12 ml of deionized water was added. The mixture was filtered using Whatman No 1 filter paper and the filtrate was collected. The intensity of the colour was determined using Spectrophotometer at 590 nm.

Rhizome yield

Crop (planted in June) that underwent moisture stress treatment for one month (90 to 120 DAP) was harvested eight months after planting and the fresh rhizome yield was recorded.

Scoring

Based on ideal genotype concept for drought tolerance (reduced leaf area, lesser stomata, higher wax content, higher RWC and lower electrolyte leakage), cut off values for tolerant, moderate and susceptible categories were assigned for each parameter in such a way that tolerance category was assigned the highest score (30) followed by moderate (20) and susceptible was assigned the least (10). Based on the importance of the parameter in drought tolerance, weightage was assigned to each parameter (RWC 0.3, electrolyte leakage 0.2, wax content 0.10, stomatal density (abaxial) 0.15, stomatal density (adaxial) 0.10 and leaf area 0.15) and the sum total of all the weightages was equal to unity (one). Then, the product of the score and weightage for each parameter and its sum total for each genotype was determined which is the weighted score for each genotype. For rhizome yield, genotypes with higher yield (>200 g/plant) was assigned the highest score (30) followed by moderate score (20) for plants with 100-200 g yield per plant and the lowest score (10) for plants with <100 g yield per plant. For yield alone, separate weightage (0.5) was assigned. Genotypes were ranked based on weighted score of both the morpho-physiological parameters as well as yield.

Field evaluation

Of the nine shortlisted tolerant lines (Table 3), four genotypes (IISR Pragati, SL 5, Suguna and Suvarna) with the highest yield, along with two susceptible ones (IISR Alleppey Supreme and IISR Kedaram), were evaluated for physiological parameters and rhizome yield under field conditions during 2022–2023 at ICAR-Indian Institute of Spices Research, Kozhikode, Kerala. The experiment was laid out in a randomized block design (RBD) with four replications in August 2022. Each genotype was planted on the raised beds of size 1.5× 1×0.30 m (l×b×h) with plant to plant spacing of 30 cm and row spacing of 25 cm, accommodating 20 plants bed^{-1}. Recommended package of practices for the crop cultivation were followed. Irrigation was withheld from November (2022) to February (2023) in stress treatment for a period of four months and observations were recorded on physiological parameters (RWC and EL) at

three successive growth stages (120, 150 and 180 DAP) and the rhizome yield in both control as well as stress treatments. Soil moisture content of 18.5 to 19.0 % (near field capacity) was maintained in control and soil moisture was 12.5 % (in November 2022) which reduced to 11.0 % (near wilting point) in February 2023. There were six rainy days (132 mm) in November and four (97mm) in December which alleviated the stress to some extent during November-December but the stress effect was more during January-February.

Drought susceptibility index was calculated to express the decrease in yield of a cultivar under drought condition with respect to the mean reduction in the yield of all the cultivars under consideration as per the equation given by Fisher & Maurer (1978).

$$DSI = [1 - (Yd/Yp)]/D$$

Where, Yd = yield in the non-irrigated treatment, Yp = yield in the irrigated control.

D = Drought intensity = 1-[(mean Yd of all genotypes in the non-irrigated treatment)/ (mean Yp of all genotypes in the irrigated control)].

Statistical analysis

The results presented are the means of three replicates in the first experiment and the means of four replicates in the field experiment. One way ANOVA was conducted in the first experiment and Factorial ANOVA was conducted in the field experiment using R software. This was followed by Duncan Multiple Range Test (DMRT) at 95% confidence level, for mean separation.

Results and discussion

Plant responses to drought are greatly affected by the morphological and physiological characters. The primary method for describing and classifying any crop species is morphological characterisation (Smith & Smith, 1989). In the present experiment in turmeric, the data on morphology such as leaf area and plant height showed significant variation ($P < 0.05$) among different genotypes.

Morphological characters

Genotypes varied significantly ($p < 0.05$) for all the morphological characters studied. Of the morphological characters analysed, plant height ranged from 79 cm (Acc 8) to 167.67 cm (Megha Turmeric) with a mean of 125.19 cm. Number of tillers ranged from 1(ACC 66) to 7 (BSR 1) with a mean of 2.91. Number of leaves ranged from 5.33 (Kanthi) to 15.33 (Salem Erigoor) with a mean of 9.97. The leaf area per plant ranged from 1849 cm^2 (PH 2) to 11402 cm^2 (Salem Erigoor).The mean leaf area was 4722.6cm^2 (Table 1).

Drought stress is expected to be intensified due to global climate change, which will lower the yield potential of crops (Chadalavada *et al*., 2021). Leaves are the main organs of transpiration and assimilation in plants. Genotypes with lower leaf area were assumed to be tolerant as they lose less water during transpiration due to lower transpirational area. The variation in leaf area directly affects plant photosynthesis which consequently reduces the yield (Walter *et al*., 2009). In order to adapt to drought, plants often reduce leaf area, which results in fewer stomata which

will reduce transpiration (Tezara *et al.*, 1999). These aspects were considered in the present study also to identify drought tolerant types with higher rhizome yield.

Table 1. Morpho-physiological parameters of turmeric genotypes 120 days after planting.

Genotype	Plant height (cm)	Number of tillers	Number of leaves	Leaf area (cm^2 $plant^{-1}$)	Stomatal density (mm^{-2})		Wax (μg cm^{-2})
					Abaxial	Adaxial	
Acc 66	117.3^{qrs}	1.00^{k}	14.3^{bc}	4145.5^{jklmn}	36.3^{ab}	8.6^{a}	6.24^{q}
Acc 8	79.00^{z}	2.00^{j}	8.3^{mn}	2545.5^{qrst}	$35.6b^{cd}$	7.6^{bcd}	10.55^{lmnop}
Acc 849	150.0^{cdefg}	1.00^{k}	8.6^{lm}	4416.3^{jkl}	32.6^{klm}	7^{defg}	11.18^{klmno}
Amballur	123.2^{nop}	2.33^{ij}	13^{de}	8212.1^{c}	26.3^{w}	6.3^{ghi}	$12.03^{fghijklm}$
BSR II	154.3^{cd}	4.33^{cd}	15^{ab}	9289.6^{b}	31^{pqr}	5.6^{ijk}	17.20^{a}
BSR I	149.3^{defg}	7.00^{a}	14.3^{bc}	4694.1^{ij}	28.6^{uv}	5^{klm}	$12.80^{defghijk}$
BSR II White	115.3^{rs}	4.67^{c}	14.4^{abc}	7073.2^{def}	30.3^{rs}	5.6^{ijk}	14.22^{bcde}
CO- 1	147.6^{efghi}	3.67^{ef}	6.3^{p}	2170.2^{stu}	35.3^{cde}	5^{klm}	14.20^{bcde}
CO- 2	117.8^{pqrs}	4.33^{cd}	5.6^{pq}	1904.2^{u}	31.6^{nop}	4.3^{m}	13.85^{cdefg}
Duggirala Red	150.4^{cdefg}	3.00^{gh}	7.6^{no}	4316.2^{jklm}	31.3^{opq}	6^{hij}	13.60^{defghi}
IISR Alleppey Supreme	123.6^{no}	4.67^{c}	10^{ijk}	7401.1^{d}	35.3^{cde}	7.6^{bcd}	10.41^{lmnop}
IISR Kedaram	118.8^{opqr}	4.00^{de}	14^{c}	8253.1^{c}	34.6^{efg}	8^{abc}	8.99^{p}
IISR Prathibha	161.3^{b}	4.00^{de}	12.6^{ef}	5851.5^{h}	31.6^{nop}	5.3^{jkl}	$13.41^{defghij}$
IISR Prabha	155.6^{bc}	4.00^{de}	10.4^{i}	4543.9^{ijk}	27^{w}	5^{klm}	$11.92^{ghijklm}$
IISR Pragati	101.6^{vwx}	2.00^{j}	10^{ijk}	2998.3^{pqr}	33.3^{ijk}	6^{hij}	$11.97^{ghijklm}$
Sudarsana	130.6^{lm}	2.33^{ij}	8.3^{mn}	2885.9^{qr}	35^{def}	7.3^{cdef}	9.29^{op}
Suguna	95.47^{yz}	2.00^{j}	9.3^{kl}	3941.3^{klmn}	33^{jkl}	6.6^{fgh}	11.37^{jklmn}
Suvarna	123.2^{nop}	1.33^{k}	10.6^{hi}	6334^{gh}	33.3^{ijk}	5.3^{jkl}	$13.08^{defghijk}$
Kanthi	127.9^{mn}	1.00^{k}	5.3^{q}	3083.2^{pq}	34^{ghi}	7.3^{cdef}	11.40^{jklmn}
Megha Turmeric	148.1^{efgh}	2.33^{ij}	10.5^{hi}	7182.2^{def}	32.3^{lmn}	7.3^{cdef}	10.60^{lmnop}
NDH 1	128.0^{mn}	2.00^{j}	7.4^{no}	3552.5^{nop}	31.6^{nop}	6.3^{ghi}	10.62^{lmnop}
NDH 98	150.3^{cdefg}	2.33^{ij}	7.6^{no}	2227.5^{stu}	35.3^{cde}	6.6^{fgh}	10.35^{lmnop}
NTC 188	167.6^{a}	2.00^{j}	11.4^{gh}	4677.0^{ij}	31.3^{opq}	5^{klm}	13.54^{defghi}
NTC 189	118.1^{opqrs}	2.00^{j}	8.6^{lm}	2019.4^{tu}	35^{def}	8.3^{ab}	10.06^{mnop}

Panth Peetab	112.3st	3.00^{gh}	8.8^{lm}	2409.7^{rstu}	33.3^{ijk}	7.3^{cdef}	12.83^{defghijk}
PH 1	96.0^{xyz}	3.67^{ef}	7.3^{o}	2035.5^{tu}	36.3^{ab}	8.6^{a}	11.68^{ijklm}
PH 2	97.6^{wxy}	4.00^{de}	8.6^{lm}	1849.0^{u}	34^{ghi}	7^{defg}	9.51^{nop}
Rajendra Sonali	97.6^{wxy}	5.33^{b}	5.6^{pq}	2042.1^{tu}	31^{pqr}	6.3^{ghi}	12.24^{efghijkl}
Rajendra Sonia	96.8^{wxyz}	2.33^{ij}	10.3^{ij}	3111.7^{opq}	31.6^{nop}	5.6^{ijk}	14.03^{bcdef}
Ranga	105.5^{uv}	2.00^{j}	8.6^{lm}	2735.8^{qrs}	36^{abc}	7.6^{bcd}	10.16^{mnop}
Rasmi	105.3^{uv}	3.33^{fg}	8.6^{lm}	2614.8^{qrst}	36.3^{ab}	6^{hij}	10.34^{lmnop}
Roma	136.6^{jk}	3.33^{fg}	12.8^{de}	6718.1^{efg}	28.6^{uv}	4.6^{lm}	10.15^{mnop}
Salem Erigoor	134.6^{kl}	5.33^{b}	15.3^{a}	11401.9^{a}	28^{v}	5.6^{ijk}	13.12^{defghijk}
SC 61	123.3^{nop}	1.33^{k}	7.3^{o}	3881.7^{lmn}	33.6^{hij}	5.3^{jkl}	11.77^{hijklm}
SL 1	150.6^{cdef}	4.00^{de}	12.6^{ef}	8377.5^{c}	29.3^{tu}	5^{klm}	16.03^{ab}
SL 10	151.3^{cdef}	3.67^{ef}	11.4^{gh}	6645.1^{fg}	32.6^{klm}	4.6^{lm}	13.01^{defghijk}
SL 2	141.9^{ij}	2.00^{j}	8.6^{lm}	6214.5^{gh}	33.4^{ij}	7.6^{bcd}	13.64^{defghi}
SL 3	153.3^{cde}	4.00^{de}	11.8^{fg}	7030.3^{def}	30st	5^{klm}	14.36^{bcd}
SL 4	144.8^{ghi}	3.00^{gh}	13.6^{cd}	7327.3^{de}	32^{mno}	6^{hij}	15.96^{ab}
SL-P389/ 1	84.6^{a}	4.00^{de}	8.6l^{m}	3903l^{mn}	32.6^{klm}	8^{abc}	16.65^{a}
SL 6	150.6^{cdef}	4.00^{de}	7.4^{no}	4465.3^{ijkl}	30st	5^{klm}	13.71^{cdefghi}
SL 5	109.3^{tu}	3.00^{gh}	7.6^{no}	4365.7^{jkl}	30.6^{qrs}	5.6^{ijk}	13.81^{cdefgh}
SL 7	146.3^{fghi}	2.00^{j}	10.7^{hi}	5051.0^{i}	36.3^{ab}	5.3^{jkl}	15.7^{1abc}
SL 8	102.3^{vw}	3.67^{ef}	10^{ijk}	4498.3^{ijkl}	31^{pqr}	6.3^{ghi}	13.61^{defghi}
SL 11	143.3 ^{hij}	2.33	9^{lm}	3975.9^{klmn}	34.6^{efg}	8^{abc}	9.15^{op}
Sobha	131.3^{klm}	2.67^{hi}	9.4^{jkl}	3717^{mno}	34^{ghi}	5.4^{jk}	10.17^{mnop}
Sugantham	122.3^{nopq}	1.33^{k}	8.3^{mn}	4044.3^{klmn}	36.6^{a}	7.4^{cde}	8.78^{p}
Suranjana	120.3^{opqr}	2.00^{j}	9^{lm}	2241.6^{stu}	34^{ghi}	8^{abc}	11.24^{klmno}
Suroma	123.6^{no}	3.00^{gh}	9.3^{kl}	4202^{jklm}	34.3^{fgh}	6.8^{efg}	9.19^{op}
Varna	91.0^{z}	2.00^{j}	12.6^{ef}	7553.8^{d}	35^{def}	7^{defg}	10.60^{lmnop}
General Mean	125.2	2.91	9.9	4722.6	32.7	6.3	12.11
CV (%)	2.88	13.76	5.90	8.21	1.43	7.1	1.56
CD (P=0.05)	5.83	0.65	0.95	628.43	0.76	0.73	0.31

The mean value (n=3) followed by different letters within a column are significantly different (P< 0.05).

Physiological parameters

Relative water content (RWC)

Leaf relative water content (RWC) is one of the most reliable indicators for defining drought tolerance of crop plants. Drought stress causes plant to lose water through transpiration and thus reduces its relative water content (Lugojan & Ciulca 2011).

In control, RWC was highest in BSR II White (95.9%), followed by IISR Prathibha (93.49%), and lowest in Sobha (80.17%) and Rasmi (80.26%), with a mean value of 86.74%. At early stress, it ranged from 87.25% (Amballur) to 65.28% (Acc 66) which was on par with 65.34% (Rasmi) with a mean of 76.36%, and at moderate stress, RWC varied from 76.49% (SL 5) followed by 76.18% (IISR Prabha) to 60.26% (Acc 66) with a mean of 67.48%. RWC further decreased under severe stress, ranging from 67.7% (IISR Prabha) to 53.32% (Acc 66), with a mean value of 61.58% (Table 2). Genotypes with higher RWC during water stress are supposed to have higher water stress tolerance than others.

In this study, an increase in stress intensity resulted in a significant reduction ($P < 0.05$) in relative water content compared to control. Drought tolerant genotypes maintained higher relative water content as compared to sensitive genotypes due to more osmoregulation (Keyvan, 2010). Similar results were reported by Bian & Jiang (2009) in *Poa pratensis* and Wang & Huang (2003) in Kentucky bluegrass. Maintenance of leaf turgor plays an important role in stomatal regulation and photosynthetic activities under water-deficit conditions (Lipiec *et al*., 2013). It indicates a positive correlation between RWC and photosynthetic rate (Moayedi *et al*., 2010) thus the yield.

Electrolyte leakage (EL)

A significant increase in membrane permeability of leaf tissue with increased drought stress was observed. Elevated levels of drought intensity are accompanied by an increase in electrolyte leakage due to increased cell permeability (Blum & Ebercon, 1981). In the untreated plants, EL was lowest in NTC 188 (9.82%) which was on par with Amballur (10%) and highest in Suranjana (15%) followed by SL 11(14.4%) (Mean=12.12%). At early stress, it ranged from 13(NTC 188) to 19.1% (IISR, Alleppey Supreme) with a mean of 15.8%. Under moderate stress there was an increment in EL from 14% (Amballur) to 22% (Acc 66) (Mean 17.82%). At severe stress, it was highest in Roma (15.7%) and lowest in Acc 66 (24.3%) with a mean value of 19.36% (Table 2). Genotypes with lower EL during water stress are supposed to have higher water stress tolerance than others.

Increase of EL% with development of drought stress has been reported in rice and Kentucky bluegrass (Guo *et al*., 2006; Liu *et al*., 2008). In our study the drought tolerant genotypes showed less EL % increase compared to susceptible ones. This finding was inconsistent with that in rice (Larkunthod *et al.,* 2018) and in wheat (Selim *et al.,* 2019). Under water deficit, the cell membrane is subjected to changes such as penetrability which decreased photosynthetic rate and sustainability (Blokhina *et al.,* 2003).

Stomatal density

Stomatal density was the highest in PH 1 (8.67 mm^{-2}) and the lowest in CO 2(4.33 mm^{-2}) with an average of 6.38 mm^{-2}on adaxial side. It ranged from 26.3 mm^{-2} (Amballur) to 36.67 mm^{-2} (Sugantham) with an average of 32.76 mm^{-2} on abaxial side (Table 1). Stomatal density was more on abaxial side (fivefold) than on the adaxial side. Genotypes with fewer stomata were assumed to be tolerant as they reduce the rate of transpiration, thus maintaining higher leaf water status. Adaptation to drought in plants can be found in the form of reduction of stomatal density and size (Ouyang *et al*., 2017). Reduced number of stomata helps to prevent the rapid rate of water loss in plants (Chaves *et al.,* 2009).

In this study there was more number of stomata on abaxial side than that on adaxial side. Windarsih *et al.* (2022) observed a higher number of stomata on abaxial surface of *Zingiberaceae* leaves. Genotypes with sustainable yield under drought are likely to have less number of stomata compared to susceptible ones. Similar findings were reported in Arabidopsis (Hepworth *et al.,* 2015) and in rice (Caine *et al.,* 2019).

Genotypes with least number of stomata have a reduction in yield compared to high yielders such as Suguna and IISR Pragati which had a moderate number of stomata. Because the extreme reduction in number of stomata favouring drought resistance may lead to reduction in photosynthesis, hence the productivity (Cal *et al.,* 2019; Yang *et al.,* 2010). Drought response based on stomatal morphology may vary with different genotypes (Liu *et al.,* 2006).

Epicuticular wax content

Epicuticular wax content was the highest for BSR II (17.2ug cm^{-2}) and the lowest for Acc 66 (6.24ug cm^{-2}) (Table 1) with an average of 12.11ug cm^{-2}. In general, higher epicuticular wax content provides greater tolerance to drought. The outermost covering with epicuticular wax on leaves reduces surface transpiration and improves crop water use efficiency (Ni *et al.,* 2012) and thus yield under water stress conditions. The relationship between higher epicuticular wax content and drought resistance is reported in maize (Meeks *et al.,* 2012), wheat (Bi *et al.,* 2017) and rice (Islam *et al.,* 2009). In our study the genotypes categorised as susceptible ones (IISR Kedaram, Acc 66 and IISR Alleppey supreme) had a lower epicuticular wax content.

Yield

In general, rhizome yield was very less due to water stress during the critical growth period (90 to 120 DAP) which coincides with the rhizome development period. Rhizome yield was the highest in Suguna (295 g $plant^{-1}$) followed by IISR Pragati (290 g $plant^{-1}$) and was lowest in Acc 66 (62 g $plant^{-1}$) with a mean yield of 178.1g $plant^{-1}$ (Table 2). Drought severely affects plant growth and development with substantial reductions in crop productivity (Farooq *et al.,* 2009). Plants exposed to drought experience rapid stomatal closure to reduce transpiration rate. This response has consequently decreased the carbon dioxide intake, which may lead to a decline in productivity (Cal *et al.,* 2019).

Table 2. Changes in relative water content, electrolyte leakage and rhizome yield of fifty turmeric genotypes subjected to water stress during rhizome development stage**.**

Genotype	RWC (%)				EL (%)				Yield (g plant^{-1})
	Control	10 DAT	20 DAT	30 DAT	Control	10 DAT	20 DAT	30 DAT	
ACC 66	80.79^{d}	65.28^{p}	60.26^{l}	53.32^{h}	12.4^{l}	18.4^{c}	22^{a}	24.3^{a}	62^{y}
ACC 8	83.99^{x}	76.77^{u}	60.58^{k}	58.53^{y}	14^{d}	17.4^{g}	21.4^{c}	22.1^{g}	85^{x}
ACC 849	86.67^{p}	77.73^{s}	64.5^{yz}	63.75^{jk}	14.35^{b}	18^{d}	19.5^{h}	20.2^{mn}	185^{lmn}
Amballur	92.95^{c}	87.25^{a}	75.43^{c}	65.25^{f}	10^{y}	13.4^{z}	14^{b}	16.8^{b}	95^{x}
BSR 2	90.87^{g}	80.86^{m}	73.43^{h}	65.57^{e}	11.93^{no}	15.3^{q}	15.9^{u}	16.3^{c}	185^{lmn}
BSR I	92.95^{c}	82.55^{i}	74.16^{f}	65.01^{g}	10.9^{stu}	13.7^{wx}	15.2^{x}	18.2^{w}	198^{jk}
BSR 2 White	95.9^{a}	82.38^{j}	72.6^{j}	63.7^{jk}	10.8^{uv}	14.2^{u}	16.6^{s}	17.2^{a}	120^{uvw}
CO 1	91.48^{f}	74.95^{z}	68.076^{p}	63.75^{jk}	13.21^{f}	17.2^{h}	19.4^{h}	20.5^{k}	179.7^{mno}
CO 2	87.16^{n}	83.08^{g}	73.18^{i}	62.86^{n}	10.7^{v}	13.6^{xy}	14.7^{z}	17.5^{yz}	175no
Duggirala Red	83.3^{y}	70.13^{l}	68.08^{op}	58.93^{x}	10.84^{tuv}	14^{v}	17.1^{qr}	18.4^{uv}	180^{mno}
IISR Alleppey Supreme	86.67^{p}	70.28^{k}	62.53^{g}	57.97^{a}	12.6^{k}	19.1^{a}	21.2^{d}	22.5^{e}	140st
IISR Kedaram	83.38^{y}	68.17^{n}	61.54^{i}	56.33^{e}	11.8^{op}	16.8^{j}	20^{f}	20.9^{j}	125^{uv}
IISR Prathibha	93.49^{b}	81.09^{l}	74.39^{e}	66.67^{b}	12.9^{hi}	16^{o}	17^{r}	18.3^{vw}	243^{cd}
IISR Prabha	90.42^{i}	81^{l}	76.18^{b}	67.7^{a}	12.64^{k}	16^{o}	16.2^{t}	18.4^{uv}	235^{def}
IISR Pragati	86.31^{q}	73.52^{f}	68.37^{n}	66.4^{c}	13^{gh}	16.3^{lm}	18.7^{j}	19.7^{p}	290^{ab}
Sudharsana	83.3^{y}	70.12^{l}	62.97^{f}	57.77^{b}	11.8^{op}	14^{v}	17.8^{m}	21.1^{i}	195^{jkl}
Suguna	80.79^{d}	73.87^{e}	67.61^{q}	63.8^{j}	11.7^{p}	14.4^{t}	17.3^{op}	19^{s}	295^{a}
Suvarna	86.63^{p}	76.81^{u}	65.3^{w}	63.99^{i}	12.7^{jk}	16.2^{mn}	17.5^{n}	18.8^{t}	268.7^{b}
Kanthi	85.24^{u}	75.13^{y}	64.69^{x}	59.55^{v}	10.9^{stu}	14.4^{t}	17.4^{no}	18.3^{vw}	220^{gh}
Megha Turmeric	86.69^{p}	73.83^{e}	64.32^{ab}	59.4^{w}	12.4^{l}	16.4^{l}	18.6^{j}	19.9^{o}	223.3^{fg}
NDH 1	85.85st	73.36^{g}	64.38^{za}	63.4^{m}	12.84^{ij}	16.2^{mn}	18.3^{k}	19.6^{p}	250^{c}
NDH 98	90.88^{g}	73.15^{h}	63.33^{e}	60.14^{u}	13.19^{f}	16.3^{lm}	19.8^{g}	20.2^{mn}	185^{lmn}
NTC 188	84.37^{w}	74.23^{ab}	65.3^{w}	57.59^{c}	9.82^{z}	13^{b}	16.2^{t}	17.4^{z}	203.3^{ij}
NTC 189	80.94^{c}	72.84^{i}	63.73^{d}	61.39^{r}	13.2^{f}	16.9^{ij}	17.4^{no}	20.3^{lm}	147^{rs}
Panth Peetab	89.54^{j}	75.12^{y}	65.47^{v}	63.99^{i}	10.7^{v}	14^{v}	17.1^{qr}	19^{s}	180^{mno}
PH 1	80.63^{e}	70.52^{j}	62.07^{h}	57^{d}	12.2^{m}	16.2^{mn}	17.3^{op}	19.4^{q}	130^{tu}
PH 2	82.13^{a}	74.3^{a}	64.56^{xy}	58.97^{x}	13.44^{e}	16.6^{k}	17^{r}	20.9^{j}	188.3^{klm}
Rajendra Sonali	85.24^{u}	73.98^{d}	63.64^{d}	59.5^{vw}	13.2^{f}	16.3^{lm}	17.5^{n}	19.1^{rs}	230^{efg}
Rajendra Sonia	92.76^{d}	78.93^{p}	70.3^{m}	66.33^{c}	13.1^{fg}	17.6^{f}	19.8^{g}	20.1^{n}	240^{cde}
Ranga	82.91^{z}	75.93^{w}	68.21^{o}	60.71^{t}	11.4^{q}	15.6^{p}	17^{r}	19.6^{p}	224.7^{fg}

Rasmi	80.26^{f}	65.34^{p}	63.93^{c}	60.65^{t}	11.7^{p}	14.7^{s}	17.5^{n}	20.4^{kl}	200^{ijk}
Roma	91.5^{f}	85.03^{b}	66.25^{u}	63.67^{k}	10.2^{wx}	13.2^{a}	14.5^{a}	15.7^{e}	109^{w}
Salem Erigoor	90.35^{i}	74.17^{bc}	70.93^{k}	63.56^{l}	13^{gh}	16.6^{k}	19.7^{g}	20.8^{j}	225^{fg}
SC 61	84.94^{v}	78.09^{r}	63.276^{e}	55.4^{g}	12^{n}	16.3^{lm}	17.2^{pq}	15.9^{d}	92.3^{x}
SL 1	90.74^{h}	83.25^{f}	70.27^{m}	65.6^{e}	11.7^{p}	14.7^{s}	15.5^{w}	17.6^{xy}	160^{pq}
SL 10	91.62^{e}	83.8^{d}	74.75^{d}	66.15^{d}	11.4^{q}	15.2^{qr}	16.2^{t}	17.7^{x}	250^{c}
SL 2	85.95^{s}	78.36^{q}	72.486^{j}	64.07^{i}	10.1^{xy}	13.2^{a}	15.7^{v}	16.8^{b}	118^{vw}
SL 3	87.03^{o}	82.75^{h}	73.71^{g}	62.86^{n}	12^{n}	15.1^{r}	18.3^{k}	19.2^{r}	150.3^{qrs}
SL 4	85.77^{t}	80.1^{o}	67.11^{s}	62.66^{o}	11.2^{r}	14.7^{s}	15.3^{x}	16^{d}	91^{x}
SL-P 389_1	87.79^{m}	77.58^{t}	63.29^{e}	59.4^{w}	10.95st	13.8^{w}	17.4^{no}	18.5^{u}	200.3^{ij}
SL 6	88.74^{k}	83.36^{e}	74.46^{e}	61.75^{q}	11^{s}	14.6^{s}	18.1^{l}	19.25^{qr}	155^{qr}
SL 5	92.8^{d}	84.09^{c}	76.49^{a}	65.29^{f}	11.2^{r}	14.3^{tu}	15^{y}	17.1^{a}	262^{bc}
SL 7	88.35^{l}	80.51^{n}	70.63^{l}	64.29^{h}	13.11^{fg}	17^{i}	18^{l}	19.7^{p}	130^{tu}
SL 8	83.28^{y}	74.11^{c}	66.47^{t}	61.24^{s}	10.3^{w}	13.5^{yz}	15.3^{x}	16^{d}	125^{uv}
SL 11	85.95^{s}	81.25^{k}	74.63^{d}	62.467^{p}	14.4^{b}	17.8^{e}	20.7^{e}	21.9^{h}	130^{tu}
Sobha	80.17^{f}	75.48^{x}	63.2^{e}	57.03^{d}	14.29^{bc}	18^{d}	20^{f}	22.9^{c}	210^{hi}
Sugantham	87^{o}	76.61^{v}	61^{j}	57.92^{a}	12^{n}	16.1^{no}	19^{i}	20.2^{mn}	170^{op}
Suranjana	86.17^{r}	69.4^{m}	67.4^{r}	61.37^{r}	15^{a}	18.8^{b}	20^{f}	23.5^{b}	162^{pq}
Suroma	82.91^{z}	70.07^{l}	60.66^{k}	56.14^{f}	14.2^{c}	18.7^{b}	21.6^{b}	22.7^{d}	140st
Varna	81.2^{b}	67.78^{o}	64.24^{b}	58.23^{z}	13.4^{e}	18^{d}	21.2^{d}	22.3^{f}	177^{mno}
General Mean	86.74	76.37	67.49	61.58	12.16	15.76	17.8	19.36	178.1
CV (%)	0.08	0.08	0.12	0.10	0.75	0.60	0.57	0.59	4.1
CD(P=0.05)	0.11	0.10	0.13	0.10	0.15	0.15	0.17	0.18	11.7

RWC= Relative water content, EL= Electrolyte leakage; The mean value (n=3) followed by different letters within a column are significantly different (P< 0.05). 10 days after treatment (DAT) = early stress, 20 DAT= moderate stress, 30 DAT= severe stress.

Scoring

Genotypes were scored based on all the morpho physiological parameters studied as well as yield and their weighted scores were determined. Weighted score ranged from 16.5 to 39.5 and nine genotypes with higher weighted score (≥33.5) were identified as tolerant ones with sustainable yield. They included SL 10, SL 5, IISR Prabha, IISR Prathibha, IISR Pragati, NDH 1, Suguna, Suvarna and Rajendra Sonia. Genotypes with weighted score of ≤ 25.5 were classified as water stress susceptible genotypes. IISR Alleppey Supreme, IISR Kedaram and Acc 66 belonged to this category (Table 3). They showed higher yield reduction with respect to the highest yielder (Suguna) compared to other genotypes.

Table 3. Turmeric genotypes identified as tolerant and susceptible to drought

SL. No.	Genotype	weighted *score (total)	Yield g plant^{-1}	Drought response
1	IISR Prabha	39.5	235	Tolerant
2	IISR Pragati	39.5	290	Tolerant
3	IISR Prathibha	39.0	243	Tolerant
4	SL5	37.5	263	Tolerant
5	Rajendra Sonia	36.5	240	Tolerant
6	SL 10	36.5	250	Tolerant
7	Suguna	36.0	295	Tolerant
8	Suvarna	33.5	268	Tolerant
9	NDH 1	33.5	250	Tolerant
10	IISR Kedaram	25.5	125	Susceptible
11	IISR Alleppey Supreme	22.0	140	Susceptible
12	Acc 66	16.5	62	Susceptible

The genotypes identified as tolerant ones with sustainable yield had shown better morpho - physiological performance under drought condition compared to other genotypes. Reduced (low to moderate) leaf area, less number of stomata (low to moderate), higher wax content, higher RWC % and lower EL % may lead to higher resistance to drought stress which resulted in increased yield in tolerant ones under drought condition. Identification of contrasting genotypes with differential response to drought condition facilitate characterization of agronomically important genes and biochemical mechanisms involved in stress response (Nutan *et al*., 2017; Xu & Bassel 2020).

Field analysis

From the nine shortlisted tolerant lines (Table 3), four genotypes (IISR Pragati, SL 5, Suguna and Suvarna) with highest yield along with two susceptible ones (IISR Alleppey Supreme and IISR Kedaram) were evaluated for yield and physiological characters in field condition. The genotypes showed significant variation with respect to yield and physiological parameters.

RWC and EL varied significantly among the genotypes (Figure 1a, b). RWC (%) which decreased with stress treatment was lowest at 180 DAP where tolerant genotypes maintained higher RWC than the susceptible genotypes (highest RWC of 69.5% in Suguna which was on par with

IISR Pragati (68.7%). EL (%), which increased with stress treatment and was highest at 180 DAP, and tolerant genotypes showed lesser increase. EL was the least in SL 5 (23.8%) followed by IISR Pragati (24.2%) and Suguna (25.2%). Higher RWC and lower to moderate EL are desirable characters for sustainable yield under drought condition as these characters enable crops to withstand water deficiency and maintain turgor.

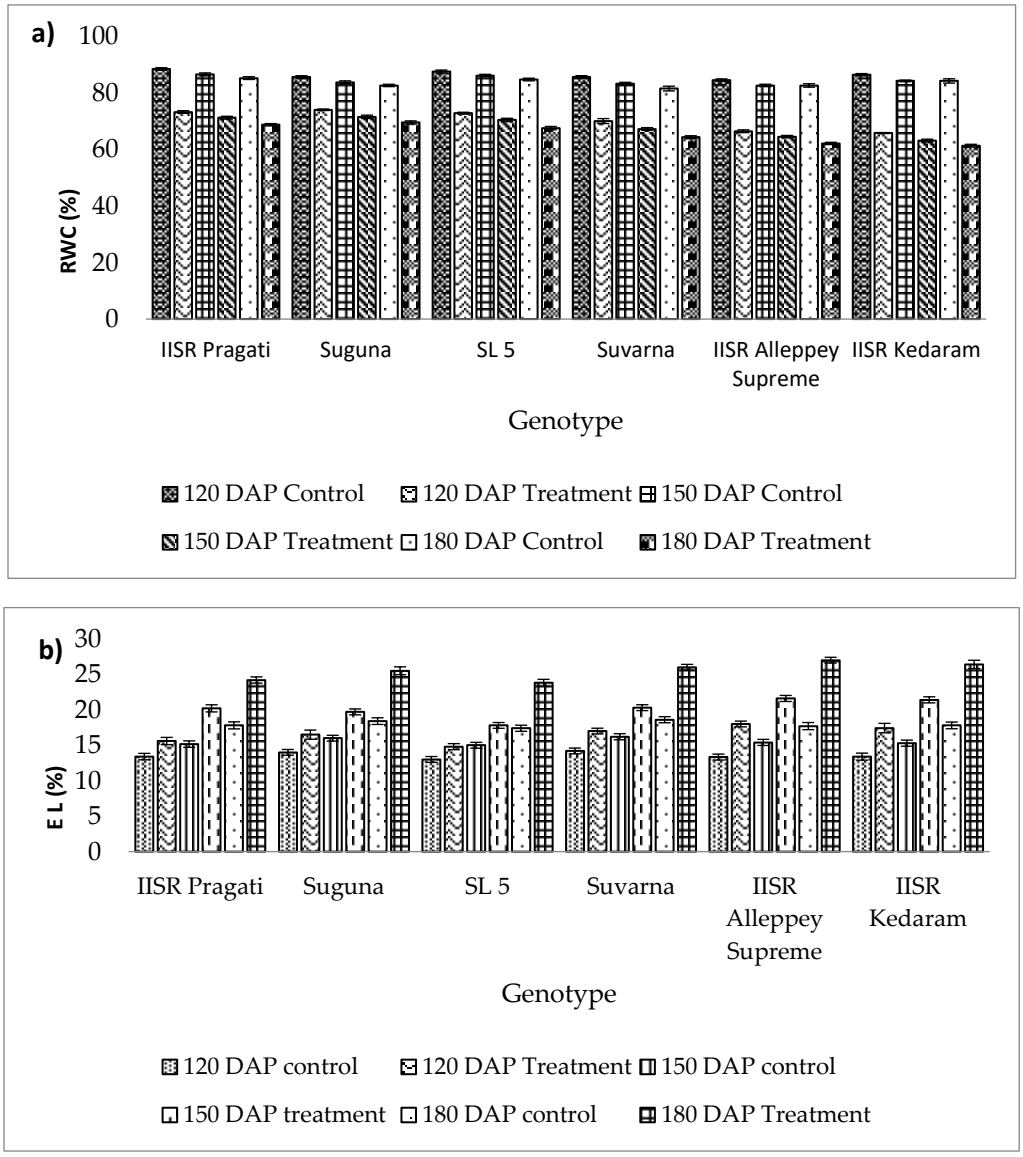

Fig. 1. Effect of drought stress on relative water content (RWC %) and electrolyte leakage (EL %) of leaf in turmeric. DAP (Days after treatment); leaf relative water content (a); electrolyte leakage (b); Values are mean ± SE of four replicates. Genotypes differed significantly ($P<0.01$), for RWC % and EL % at 120, 150 and 180 DAP. Significant difference ($P <0.001$) was also observed between control and stress treatments.

Rhizome yield showed significant variation among the genotypes (Table 4). Maximum rhizome yield was recorded in IISR Pragati (215g plant^{-1}) followed by Suguna (190g plant^{-1}) under drought condition. The variation in yield may be due to genetic variation among the genotypes.

SI ranged from 0.02 to -0.56. Genotypes with DSI <0 was considered as tolerant and those with DSI >0 as susceptible. DSI of the genotypes identified as tolerant ranged between -0.46 (Suvarna) to -0.56 (IISR Pragati) and the order of drought tolerance was IISR Pragati> SL 5 >Suguna>Suvarna (Table 4). Genotypes identified as susceptible showed DSI of 0.01 and 0.02. Considering our results from this study on morpho-physiological characters and yield, four tolerant genotypes (IISR Pragati, Suguna, SL 5 and Suvarna) with sustainable yield and two susceptible genotypes (IISR Alleppey Supreme and IISR Kedaram) were identified for further investigation on the mechanism of drought tolerance in turmeric.

Table 4. Changes in rhizome yield of six turmeric genotypes subjected to water stress in field condition

Genotype	Yield (g plant^{-1})		DSI
	Control	Treatment	
IISR Pragati	242^{a}	215^{bc}	-0.56
Suguna	223^{b}	190de	-0.49
SL 5	208^{bc}	182^{ef}	-0.53
Suvarna	172^{f}	143^{g}	-0.46
IISR Alleppey Supreme	206^{cd}	117^{i}	0.010
IISR Kedaram	216^{bc}	121^{h}	0.020
Mean	211	161	
CV (%)	4.0		
CD (P= 0.05)	10.6		

DSI= Drought susceptibility index; The mean value (n=4) followed by different letters within a column are significantly different (P< 0.05)

Conclusion

Fifty elite turmeric genotypes were screened for identification of physiologically superior genotypes with better yield performance under water stress. Results revealed that water stress during rhizome development stage significantly decreased the leaf relative water content and increased membrane permeability. Epicuticular wax content varied significantly among the genotypes. Genotypes with lower leaf area per plant, higher relative water content, lesser electrolyte leakage, higher wax content and fewer stomata than other genotypes were shortlisted as tolerant. From among these shortlisted ones, four tolerant genotypes with higher yield, and two susceptible genotypes were further evaluated under

field conditions. The results showed that tolerant genotypes significantly outperformed the susceptible ones in terms of drought tolerance traits as well as yield. These genotypes with contrasting characters can be used for further studies to elucidate the mechanism of drought tolerance in turmeric.

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