



Hydraulic dynamics and photosynthetic performance facilitate rapid screening of field grown mulberry (*Morus* spp.) genotypes for drought tolerance

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ARTICLE INFO

Keywords:

Biomass
Chlorophyll *a* fluorescence
Drought stress
Hydraulic conductance
Mulberry
Photosynthesis

ABSTRACT

Impact of drought stress on terrestrial plants is predicted to increase in future due to rapid increase in air temperature and changes in precipitation patterns. Mulberry (*Morus* spp.) is an economically important perennial crop in sericulture industry. The present study was aimed at using hydraulic dynamics and photosynthetic performance for screening drought tolerance among mulberry genotypes by using non-invasive, rapid and efficient methods. Plant hydraulic variables were measured with commercially available sap flow meter, stem and leaf psychrometers in one year old mulberry genotypes. Plant leaf gas exchange parameters and chlorophyll *a* fluorescence were measured in control and stressed plants to assess their efficiency. Based on hydraulics and photosynthetic parameters, mulberry genotypes were characterized into 3 functional groups including high, average and poor performing groups (HPG, APG and LPG respectively). HPG genotypes maintained significantly better leaf water status (ψ_{md}), stem hydraulic conductance (K_s) and sap flow rate (F) which resulted in enhanced photosynthetic efficiency compared to APG, LPG under prolonged drought regimes as well as after recovery. Hydraulic parameters were positively correlated with photosynthetic performance of each group under drought. Our data suggest that plant water transport efficiency plays a major role in regulating photosynthetic performance which in turn determines the biomass yields under drought. Hydraulic dynamics based screening will be highly useful for rapid selection of efficient mulberry genotypes.

1. Introduction

Drought is a predominant environmental stress factor which is known to affect the physiological responses of terrestrial plants, by inhibiting carbon assimilation and which in turn affects the plant growth as well as yield of terrestrial plants (Nolf et al., 2015). Rapid increases in fossil fuel consumption, deforestation and industrialization contribute towards significant variations in global average air temperature and precipitation patterns, which are predicted to influence the frequency and severity of drought events in many regions of the world, particularly in mediterranean and subtropical climates (Martorell et al., 2014). Thus, it is highly crucial to screen drought tolerant varieties and understand the precise mechanism of drought tolerance (DT) which could be utilized for crop improvement programs. DT can be explained on the basis of plant's ability to survive under water deficit conditions and maintain optimum biomass production along with leaf gas exchange physiology (Fang and Xiong, 2015; Chen

et al., 2016). Further, DT is also determined by the ability of plants to persist and recover efficiently from drought, which usually varies among species as well as between genotypes belonging to the same species (Martorell et al., 2014). From the past few decades, research has been focused towards identifying fast-growing and drought tolerant tree species, which are economically important and can be easily cultivated under various agro climatic conditions. These selected genotypes act as potential source of genes for carrying out breeding programs for drought tolerance in agricultural and forestry crops (Sapeta et al., 2013; Christersson, 2010).

Terrestrial trees are known to survive even under severe drought stress, by regulating various morphological, biochemical and physiological processes and which in turn determine carbon assimilation rates and growth patterns (Chaves et al., 2003; Zhang et al., 2013). Hydraulic dynamics determine water transport through stem or leaves and play a key role in determining plant adaptive responses under drought stress (Addington et al., 2006; Poorter et al., 2009). Hydraulic conductivity,

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<https://doi.org/10.1016/j.envexpbot.2018.10.038>

Received 6 August 2018; Received in revised form 31 October 2018; Accepted 31 October 2018

Available online 02 November 2018

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as such was found to be regulated via xylem integrity and hydraulic architecture (xylem vessel density, vessel size) (Costa et al., 2004). Under water deficit conditions, stomatal conductance was reported to be strongly associated with stem xylem functions in tree species (Zhang et al., 2013). Primary response to drought includes stomatal closure, which prevents transpirational water loss and at the same time reduces the photosynthetic carbon uptake, leading to reduced photosynthetic rates (Hubbard et al., 2001; Cochard et al., 2002). Simultaneously, drought also decreases leaf water potential leading to enhanced sap flow tension in xylem vessels which results in cavitation and inhibits water transport capacity (Hirasawa et al., 1989; Quick et al., 1992; Pons and Welschen, 2003). Hence, drought induced inhibition of plant water transport efficiency reduces carbon assimilation and results in slower growth rate in various plant species (Brodribb and Field, 2000). Therefore, monitoring hydraulic dynamics along with photosynthetic performance under drought stress are the crucial tools for screening of drought tolerant genotypes.

DT traits are crucial to understand the ability of plants to recover efficiently upon rehydration (Sapeta et al., 2013). Recovery from drought stress is a complex process and includes readjustment of photosynthetic machinery, metabolic pathways and repair of the drought induced damage to retain optimum plant growth (Chen et al., 2016). Monitoring plant responses to drought/recovery is essential to screen and identify better performing species/genotypes under drought. To perform such non-invasive, rapid and efficient screening, changes in hydraulic dynamics and photosynthetic performance of plants under drought stress, followed by recovery could be highly useful and hence would contribute significantly towards crop improvement programs under severe water deficit regimes.

Along with photosynthetic gas exchange parameters chlorophyll *a* fluorescence is also known for screening photochemically efficient plant species/genotypes under adverse environmental conditions (Sekhar et al., 2015). At leaf level, dissipation of excess light energy as heat is termed as non-photochemical quenching (NPQ) which serves as one of the principle mechanism in plants to avoid drought induced damage (Santos et al., 2009; Ruban and Horton, 1995). Thus, analyzing the photosystem-II efficiency under drought provides a rapid indication of variations in DT ability of plants which is used as an efficient screening tool. However, correlation of photosynthetic gas exchange and chl *a* fluorescence with hydraulic parameters under drought conditions is not well characterized. Moreover, measurement of hydraulic characteristics provides crucial information on plant's ability to transport water to photosynthetic and growing tissues, which can postulate sensitivity to abiotic stress factors and affects distribution of species (Brodribb 2009). For the past few decades, destructive methods were used to measure the stem hydraulics (K' from accurate flow rate at applied pressure gradient) in the laboratory as well as in the field by using high pressure flow meter (HPFM), ultralow flow meter (ULFM) and hydraulics flow meter (Melcher et al., 2012). For rapid screening, the non-destructive method to measure hydraulic conductivity (K_h) is an essential prerequisite, which can be measured by Hydraulic conductivity meter (HCM; ICT International, Australia).

Mulberry (*Morus* spp.) is a fast growing and semi deciduous tree species, cultivated to feed the silkworm culture in sericulture industry. However, some of the Asian and European countries also cultivate mulberry as a forage crop for animal husbandry, as mulberry leaves are known to be highly nutritious and devoid of toxic elements (Guha et al., 2010b). Recently it was reported that mulberry can be grown as a potential bio-energy crop, which is suitable for future climatic conditions owing to fast growth and higher photosynthetic rates (Sekhar et al., 2014, 2015 and 2017). However, majority of mulberry cultivating areas in south India are facing frequent and severe abiotic stress factors, especially drought, which adversely affects the plant growth and leaf yield. In order to overcome drought induced yield limitations, it is important to screen and cultivate DT genotypes. Thus, in the present study, we aim to identify genotypic variations within the species based

on their hydraulic dynamics and physiological characteristics. We also aim to correlate hydraulic parameters with photosynthetic performance in different mulberry genotypes under drought conditions and recovery.

2. Materials and methods

2.1. Plant material and stress treatments

Mulberry stem cuttings were collected from varied agro-climatic conditions of India and grown in experimental fields of University of Hyderabad for one year. Based on our preliminary drought screening experiments, all the 12 mulberry genotypes taken for the present study were divided in to three different groups; Selection13 (S13) and Victory1(V1), Selection-1 (S1) and Selection-34 (S34) were considered as high performance group (HPG); Mysore Local (ML), Kollegal Local (KL), Selection-1635 (S1635) and Mildew Resistant-2 (MR2) were included under average performance group (APG) and Kanva-2 (K2), Selection-36 (S36), Dehradun (DD) and Papua New Guinea (PNG) were categorized under poor performance group (PPG) (Chaitanya et al., 2003; Guha et al., 2010b). For each group two separate plots were prepared for selected mulberry genotypes. Four plants ($n = 4$) per each genotype in both the plots were arranged in a completely randomized block design (CRBD). The variations in local climate (air temperature ($^{\circ}\text{C}$), Relative humidity (%) and PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$) were recorded daily (throughout the experimental period) at experimental field by automatic data logger (Fig. 1). The average irradiance, average air temperatures and relative humidity during experimental time period were $1600\text{--}1750 \mu\text{mol m}^{-2} \text{s}^{-1}$, $32\text{--}42^{\circ}\text{C}$ and $55\text{--}60\%$ respectively. These experimental and environmental conditions were almost constant throughout the experimental period. Further, all mulberry genotypes in both the plots were grown under field conditions with regular watering for one year till the plants reached a height of around 200–250 cm. After one year, all mulberry genotypes belonging to three different groups, grown in two different plots were divided into well-watered (WW) and water stressed (WS) plots; where WW plot was irrigated every two days, while WS plots were exposed to natural drought by complete water withholding for 25 days. In order to take photosynthetic and hydraulic conductivity measurements in all the genotypes belonging to a particular group; drought stress was applied with three days interval between each group. After completion of drought stress, plants were irrigated regularly for one week to perform recovery (REC) studies. Daily measurements of leaf water status and photosynthetic parameters were recorded after re-watering to know the complete recovery of the plant. Experiments were carried out for two consecutive years (2016 and 2017), covering two summer seasons. All the measurements were recorded for three consecutive days at each representative time point in four ($n = 4$) plants per genotype by covering all the genotypes of a particular group at 0th day, 25th days after stress (DAS) and 7th day after re-watering (REC) to avoid experimental errors.

2.2. Measurement of leaf and stem water status

Leaf water status was estimated by measuring the midday leaf water potential (ψ_L ; MPa) in randomly chosen young and mature leaves by using psychrometer (PSY; ICT International, Australia) at 11:00–13:00hr. Similarly xylem water potential (ψ_x ; MPa) was also measured by using psychrometer (Dixon and Downy 2013) by installing instrument on to the stem at a height of 100 cm from base. PSY was equipped with thermocouple probe to measure the water potential gradient. Thermocouple probe chamber was fixed at the exposed surface of leaf and stem xylem, to record ψ_{mid} and ψ_x for every 5 min. interval of time.

For leaf relative water content (LRWC), fresh leaf discs (1.5 cm^2) were collected by using leaf borer from control, drought stressed and re-hydrated plants, weighed immediately for fresh weight (FW), then re-hydrated by immersing in distilled water for 24 h at 4°C followed by oven-drying for 24 h at 105°C . LRWC (%) was calculated as

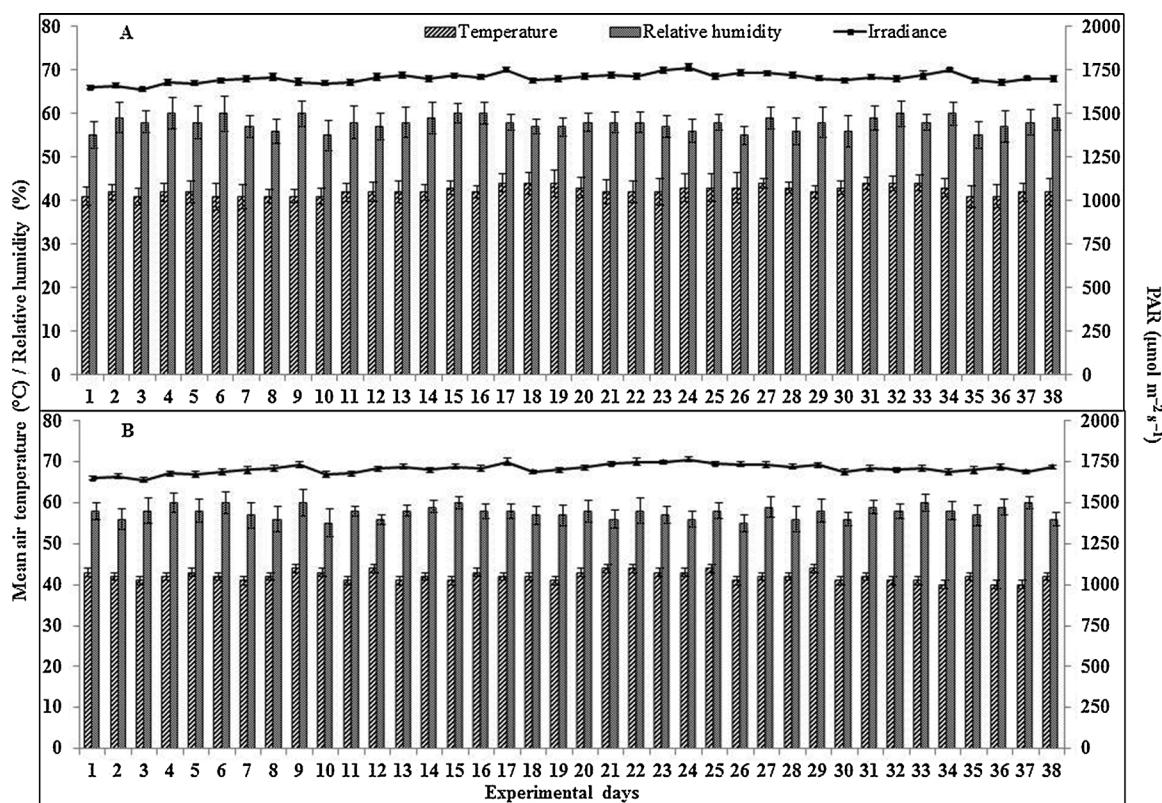


Fig. 1. Changes in local climate conditions at experimental site, air temperature ($^{\circ}\text{C}$), Relative humidity (%) and PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$) of (A) season-1 and (B) season-2 during experimental period.

Table 1

Leaf moisture content (LMC) and leaf relative water content (LRWC) under different water treatments in the one year old leaves of mulberry genotypes grown under field conditions. Data represented are the average \pm SE ($n = 4$) with Least significance difference values.

Genotypes	LMC (%)			LRWC (%)		
	WW	WS	REC	WW	WS	REC
S13	76.3 \pm 1.1	70.4 \pm 1.2 ^(0.724)	76.5 \pm 2.3 ^{ns}	84.8 \pm 2.3	72.3 \pm 1.6 ^(0.976)	83.7 \pm 1.9 ^{ns}
S34	75.3 \pm 0.9	70.1 \pm 1.3 ^(0.727)	75.2 \pm 1.3 ^{ns}	85.1 \pm 1.6	71.8 \pm 0.9 ^(1.39)	84.6 \pm 2.5 ^{ns}
V1	76.1 \pm 2.3	71.5 \pm 1.1 ^(1.067)	75.8 \pm 1.9 ^{ns}	86.4 \pm 2.5	71.8 \pm 1.2 ^(1.41)	85.1 \pm 1.8 ^{ns}
S1	76.8 \pm 1.3	69.5 \pm 2.1 ^(0.913)	76.4 \pm 2.3 ^{ns}	85.4 \pm 1.3	72.4 \pm 2.1 ^(0.828)	84.1 \pm 1.8 ^{ns}
S1635	71.6 \pm 1.5	66.5 \pm 1.9 ^(1.079)	70.3 \pm 2.4 ^{ns}	83.4 \pm 1.9	66.1 \pm 2.7 ^(1.38)	83.2 \pm 2.3 ^{ns}
MR2	72.5 \pm 1.8	65.9 \pm 2.5 ^(0.953)	73.1 \pm 1.6 ^{ns}	82.9 \pm 1.6	70.3 \pm 1.3 ^(1.105)	82.1 \pm 2.1 ^{ns}
KL	73.1 \pm 0.9	66.6 \pm 1.1 ^(0.954)	72.8 \pm 2.1 ^{ns}	81.6 \pm 2.1	69.4 \pm 2.1 ^(1.164)	82.5 \pm 2.2 ^{ns}
ML	73.5 \pm 2.3	67.8 \pm 1.7 ^(1.42)	73.1 \pm 1.7 ^{ns}	82.2 \pm 1.1	71.1 \pm 1.4 ^(0.968)	83.3 \pm 1.9 ^{ns}
PNG	73.5 \pm 2.1	63.1 \pm 1.2 ^(1.067)	70.9 \pm 2.5 ^(1.231)	83.4 \pm 1.8	64.9 \pm 2.6 ^(1.323)	80.5 \pm 1.7 ^(1.109)
DD	72.9 \pm 2.5	65.1 \pm 2.1 ^(1.071)	69.5 \pm 1.8 ^(1.189)	83.1 \pm 2.1	66.4 \pm 0.9 ^(1.124)	78.9 \pm 2.6 ^(0.983)
S36	74.3 \pm 1.8	64.4 \pm 0.9 ^(1.066)	71.6 \pm 2.1 ^(1.164)	85.6 \pm 0.9	67.1 \pm 1.4 ^(1.271)	81.9 \pm 1.3 ^(1.514)
K2	73.1 \pm 1.4	63.4 \pm 1.6 ^(1.853)	74.1 \pm 1.8 ^{ns}	82.4 \pm 1.8	67.9 \pm 2.3 ^(1.156)	80.9 \pm 2.1 ^(1.217)

$$\left[\frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \right] \times 100.$$

Where, FW is the fresh weight of leaf discs, TW is the turgid weight after re-hydrating the discs for 24 h, and DW is the dry weight of oven-dried discs.

Leaf moisture content (LMC) (%) was calculated as $\left[\frac{\text{FW} - \text{DW}}{\text{FW}} \right] \times 100$.

2.3. Leaf gas exchange measurements

Photosynthetic leaf gas exchange parameters (net photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO_2 (C_i), and transpiration rate (E)) were measured 25 DAS in all genotypes as well as controls in between 9:30:00 - 11:30hr by using a portal infrared $\text{CO}_2/\text{H}_2\text{O}$ gas analyzer (IRGA) (ADC Bioscientific Ltd. U.K.). IRGA was equipped with a broad leaf chamber (LCpro-32070, UK), a PAR sensor

(silicon based sensor, LCpro-32070) and a leaf thermistor probe (ADC, M.PLC-011). All measurements were performed on fully matured and light exposed third or fourth leaves from upper canopy, which were used previously for the measurement of leaf water status. The following conditions were maintained throughout the experiment; saturating photo synthetically active radiation (PAR) of $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$ supplied by a LED light source (LCpro Lamp 32070- Broad, ADC Bioscientific Ltd. UK) attached to leaf chamber, air temperature of $25\text{--}26^{\circ}\text{C}$ and relative humidity of $55\text{--}60\%$, CO_2 concentration $390 \mu\text{mol mol}^{-1}$ for both control and treated plants. Leaf was placed in the leaf chamber and acclimatized for 2–3 minutes before taking the readings.

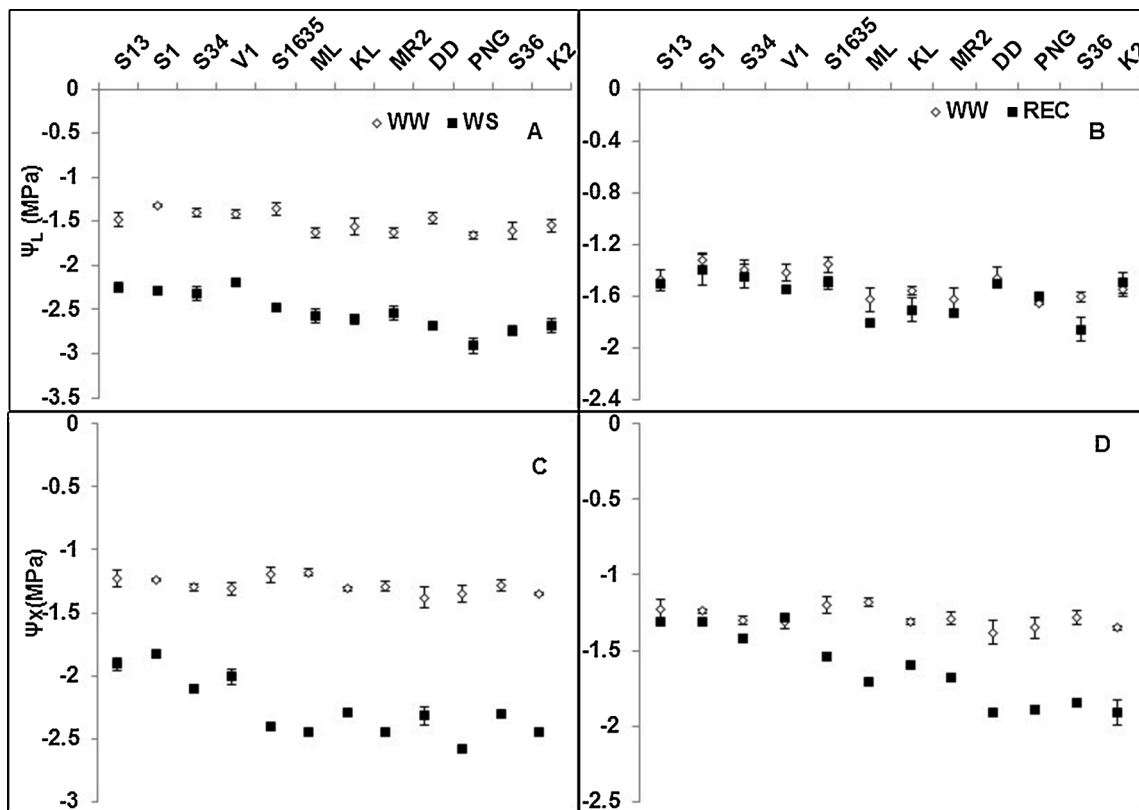


Fig. 2. (A, B) Midday leaf water potential (ψ_{md}) and (C, D) xylem water potential (ψ_x) in well-watered (WW), water stressed (WS) and recovered (REC) plants of different mulberry genotypes. Data presented are means \pm SE (n = 4).

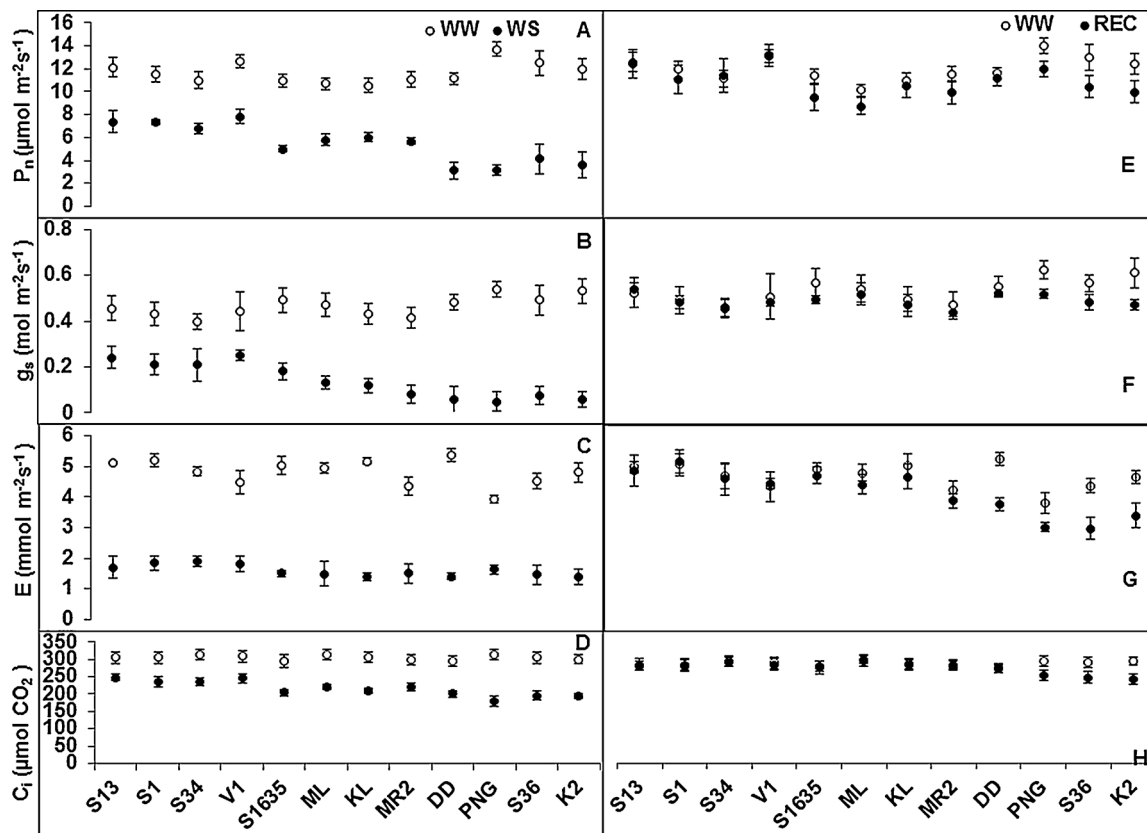


Fig. 3. Changes in (A, E) photosynthetic rates (P_n), (B, F) stomatal conductance (g_s), (C, G) leaf transpiration rate (E), (D, H) intracellular CO_2 concentration (C_i) in twelve mulberry genotypes at severe drought stress (25 days after stress) and 7 days after rehydration (recovery). The values presented are means (n = 4) and vertical bars represents \pm SE (n = 4).

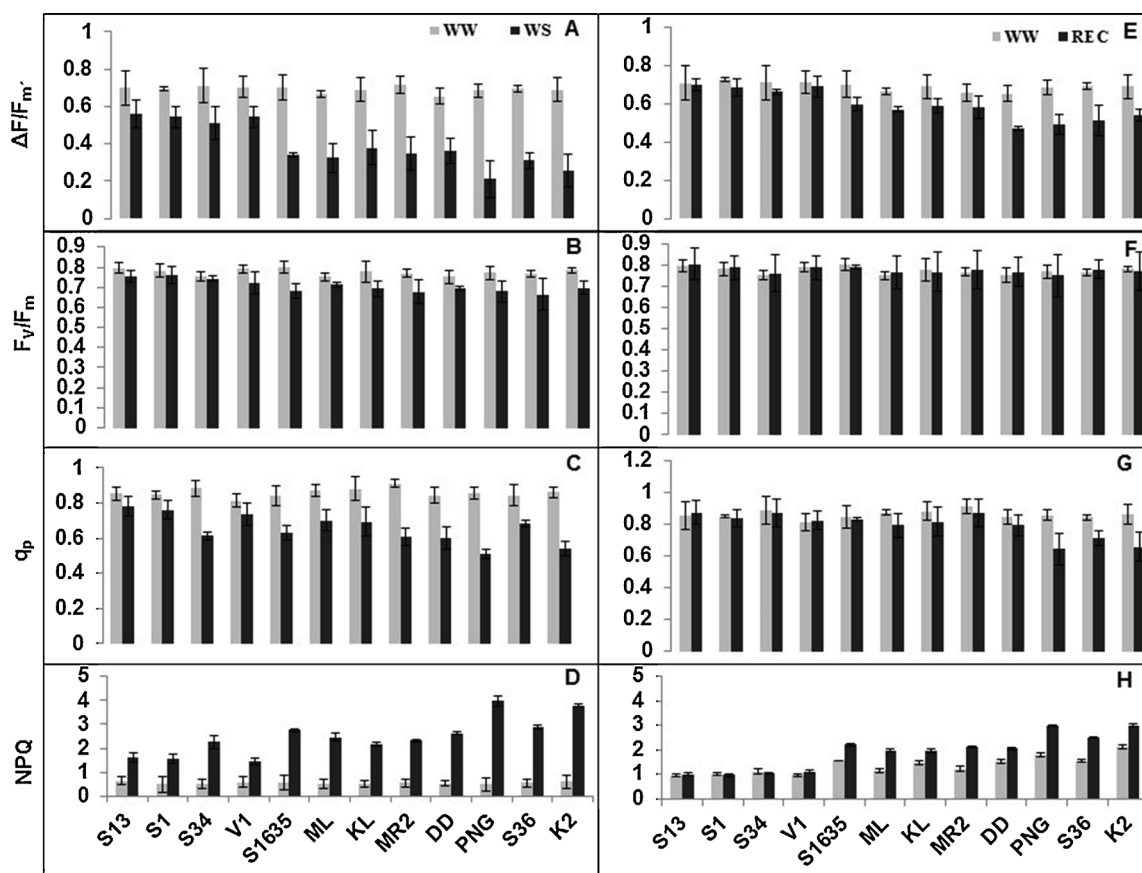


Fig. 4. Changes in (A, E) effective quantum efficiency of PSII ($\Delta F/F_m'$), (B, F) maximum quantum efficiency of PSII photochemistry (F_v/F_m), (C, G) photochemical (q_p) and (D, H) non-photochemical quenching (NPQ) in 12 mulberry genotypes at drought stress and recovery. The values presented are means ($n = 4$) and vertical bars represents \pm SE ($n = 4$).

2.4. Chlorophyll a fluorescence

Chlorophyll *a* (Chl *a*) fluorescence variables were measured on same leaves which were used previously for leaf gas exchange measurements by using MINI- version of imaging PAM (Heinz Walz GmbH, Effeltrich, Germany). For dark adapted parameters, leaves were adapted in dark for 20 min to evaluate the maximal photochemical efficiency [$(F_m - F_0)/F_m = F_v/F_m$] of photosystem-II. While effective quantum yield [$(F_m' - F)/F_m' = \Delta F/F_m'$] was measured under natural light conditions. Measurements were taken on adaxial surface of leaves and high light flash of $4000 \mu\text{mol m}^{-2} \text{s}^{-1}$ was used for 0.8 s duration to measure the saturated fluorescence values. Photochemical [$(F_m' - F_s)/(F_m' - F_0) = q_p$], and non-photochemical [$(F_m - F_m')/F_m'$] fluorescence quenching were also calculated (Maxwell and Johnson, 2000; Rascher et al., 2000).

2.5. Xylem sap flow and Stem hydraulic conductance

Stem hydraulic conductivity (K_s) was measured by using hydraulic conductivity meter (HCM, ICT International, Australia). HCM consists of two psychrometers (PSYs) (Dixon and Downy 2013) along with sap flow meter (SFM) (Burgess and Downey 2014). Pair of PSYs was installed on the stem and the distance between the two PSYs (up and down) was uniformly maintained for all genotypes. Both PSYs records the Ψ_x values at two positions of the stem (in and out). To obtain the sap flow rate (F ; kg/hr) SFM was installed on the stem exactly in between the two PSYs. Transport efficiency of plant was measured through the xylem sap flow rate (F , kg/hr) through SFM which utilizes the heat ratio method (HRM) (Burgess et al. 2001), SFM was equipped with three needles having two sensors, which measure the heat pulse

velocity by attaining downstream and upstream sapwood temperature following the release of a heat pulse. Heat pulse velocity was converted to sap flow rate based on the stem wood traits (Vandegheuchte and Steppe, 2013). SFM was set to record the measurements between 11:00-13:00hr for every 10 min. interval. Stem hydraulic conductivity was calculated as:

$$K_s = [F * L / (\Psi_{in} - \Psi_{out})] \text{ (kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}\text{)},$$

Where F is sap flow rate (kg/sec), L is distance between two PSY (m), Ψ_{in} is water potential at inlet (MPa) and Ψ_{out} is water potential at outlet (MPa) (Pivovarov et al., 2014).

2.6. Plant growth and biomass yield

Plant biomass was evaluated after one year for WW, WS as well as REC by destructive harvest of four representative plants per genotype. These plants were used to measure the height of the plant number of branches per plant was recorded before harvesting. Plant height was measured from the ground to the canopy top by using measuring tapes. Plant shoots were coppiced above the ground level and components (leaves, branches and stems) were separated and immediately weighed in the field to get total leaf fresh biomass (LFBM), stem fresh biomass (SFBM) including primary + axillary branches and above ground total fresh biomass (TFBM).

2.7. Statistical analysis

In all our experiments plants were arranged in random block design with four replications of each genotype ($n = 4$) for each treatment. Total data represented as mean \pm SE. Data obtained were analyzed by analysis of variance (ANOVA), mean values of photosynthetic

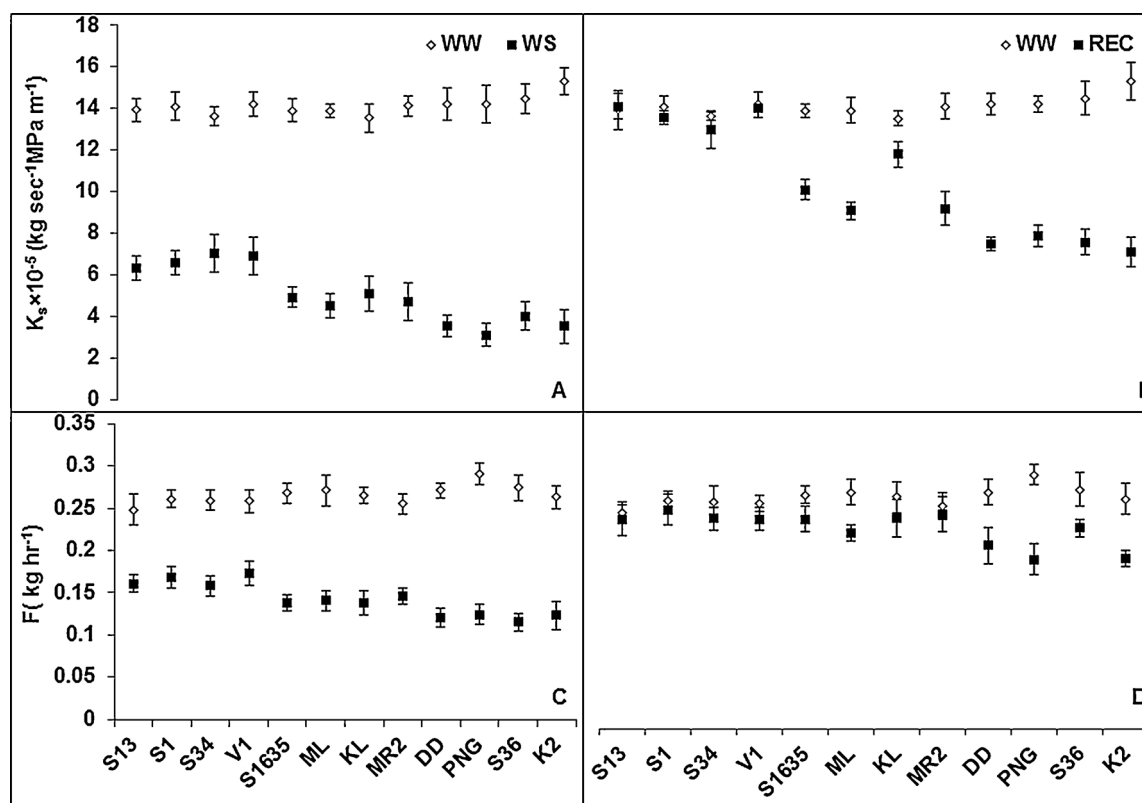


Fig. 5. (A, B) Stem hydraulic conductance (K_s) and (C, D) sap flow rate (F) measured in 12 mulberry genotypes grown under control conditions or subjected to drought stress followed by re-watering. Values are means \pm SE (n = 4).

physiology, plant water status and biomass were tested for significant difference between genotypes by using Fisher least significance difference method with statistical software Sigma Plot11.0 and a $P < 0.1$ was considered as significant.

3. Results

3.1. Plant water status

LRWC and LMC varied significantly among the mulberry genotypes in both water regimes WW and WS (Table 1). S13, V1, S1 and S34 maintained higher leaf water status (LRWC and LMC) under severe drought stress conditions. During severe drought stress, minimum LMC was observed in PNG (63.1%) followed by K2 (63.4%) while, maximum LMC was maintained by V1 (71.5%) and S13 (70.4%). However, S13, S1 and V1 showed higher LRWC (72.3%, 72.4% and 71.8%), while maximum reduction in LRWC was recorded in PNG (64.9%) during severe drought stress. Similarly, plant water status was also assessed by measuring midday leaf (ψ_L) and xylem water potential (ψ_x) after subjecting to drought stress (Fig. 2). All genotypes maintained their ψ_L and ψ_x consistently (between -1 to -1.6 MPa) throughout the experiment under WW conditions, whereas the drought stressed plants showed significant reduction in ψ_L as well as in ψ_x (Fig. 2A). Comparatively higher values of ψ_L were recorded in V1 (-2.19 ± 0.15 MPa), S13 (-2.25 ± 0.3 MPa) and S34 (-2.28 ± 0.24 MPa) (Fig. 2A). However, drought induced reduction in ψ_x was observed in all genotypes, maximum reduction in ψ_L and ψ_x was observed mainly in PNG and K2 (Fig. 2C). After 7 days of REC, all 12 genotypes recovered their ψ_L almost similar to that of WW plants (Fig. 2B). However, ψ_x was restored only in S13, V1, S1 and S34 genotypes while the rest failed to recover completely (Fig. 2D).

3.2. Gas exchange parameters

Compared to WW plants, P_n reduced in all genotypes during severe drought conditions. Maximum reduction of P_n was observed in PNG (78.51%), DD (72%), followed by K2 (69%) and S36 (67%), while relatively less reduction of P_n was recorded in V1 (38%), S13 (39%) and S34 (40%) during WS condition (Fig. 3A). Under control conditions, g_s did not vary significantly among the genotypes. After 25 days of WS, significant reduction in g_s occurred in all genotypes with maximum reduction of g_s was observed in PNG (91.12%) and K2 (88.9%) genotypes. Minimum inhibition of g_s was noted in V1 (43.31%) and S34 (46%) (Fig. 3B). Stomatal closure caused significant reduction in both E as well as intercellular CO_2 concentration (C_i , $\mu\text{mol } CO_2$) (Fig. 3C, D) started after 15 DAS in all genotypes with respect to their controls. After 25 DAS V1, S13, S1 and S34 genotypes maintained significantly higher E and C_i respectively (Fig. 3C, D). S13, V1, DD, S34, S1 and Kollegal Local (KL) genotypes restored P_n during REC almost similar to that of the control plants (Fig. 3E). E recovered in all genotypes, while partial recovery was recorded in DD, PNG, S36 and K2 (Fig. 3F). Also genotypes recovered their g_s and C_i values upon REC except K2, S36 and PNG (Fig. 3G and H).

3.3. Chlorophyll a fluorescence

All genotypes showed reduction in $\Delta F/F_m'$ varying in the range of 19–69% after WS (Fig. 4A). S13, V1, S34 and S1 showed $\Delta F/F_m'$ value similar to control plants during 7 REC (Fig. 4E). Maximum quantum efficiency of PSII (F_v/F_m) did not show variation among the genotypes under control conditions, but with increasing stress severity (25 DAS) F_v/F_m reduced in all 12 genotypes (Fig. 4B). Maximum reduction in F_v/F_m was observed at 25 DAS in S1635, S36 followed by MR2 and low inhibition was observed in S1, S13 and V1. All genotypes showed full recovery of F_v/F_m values after rehydration (Fig. 4F). Photochemical

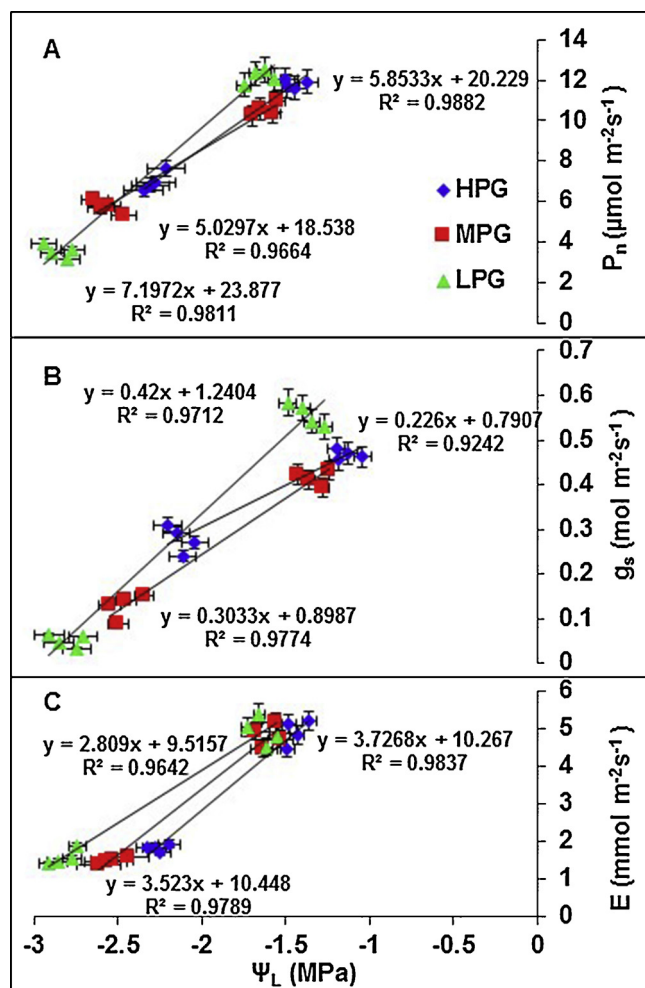


Fig. 6. Relationship between midday leaf water potential (Ψ_L) and (A) photosynthetic rates (P_n), (B) stomatal conductance (g_s) and (C) transpiration rate (E) during the period of drought stress. Each point is an average of 10 values \pm SE ($n = 4$).

quenching (q_p) was inhibited significantly at severe drought stress in all genotypes with respect to controls (Fig. 4C). Maximum reduction of q_p was recorded in PNG (40%) and K2 (37%) genotypes with respect to WW plants. All genotypes recovered the q_p values almost equal to control plants except K2, PNG and S36 (Fig. 4G). Non photochemical quenching (NPQ) also varied significantly among the genotypes at both control and water deficit conditions (Fig. 4D). NPQ increased in all genotypes during severe water stress, but maximum enhancement was recorded up to 120% in PNG. NPQ was found to be reduced in all genotypes upon rehydration but, S13, S34 and S1 recovered NPQ almost similar to their WW counterparts while the other genotypes could not reduce NPQ to such extent (Fig. 4H).

3.4. Plant water conductivity

Stem hydraulic conductance (K_s) and xylem sap flow rate (F) remained same in WW plants throughout the experiment in all genotypes. All genotypes showed significant reduction in F and K_s with increasing water stress severity (Fig. 5). However, stressed V1, S13, S1 and S34 genotypes showed a less reduction in both K_s and F , while maximum reduction was noticed in PNG, K2 and DD. During WS, K_s was reduced by 54% in V1, S13, S1 and S34, while it was reduced by 75% in PNG followed by K2 and DD (Fig. 5A). Similarly, F was reduced from 0.278 kg/hr to 0.178 kg/hr in drought stressed V1, S13, S1 and S34 genotypes, while PNG, K2 and DD genotypes exhibited maximum

reduction in F from 0.289 kg/hr to 0.131 kg/hr (Fig. 5B). After REC, V1, S13, S1 and S34 genotypes recovered their K_s and F values similar to control plants, whereas other genotypes showed only partial recovery in upon rehydration (Fig. 5C).

3.5. Correlation between hydraulic dynamics and photosynthetic parameters

Plant hydraulic dynamics (K_s , F , Ψ_L) were strongly correlated with photosynthetic characteristics (P_n , g_s , E) as shown in Fig. 6 and 7. Leaf gas exchange variables declined rapidly as plant water status decreased. Higher rates of P_n , g_s and E were recorded during control conditions when plant water status was high. Both plant water transport capacity and photosynthetic parameters decreased significantly with increasing water stress severity in all genotypes. PNG, K2, S36 and DD genotypes showed higher restriction of water transport, corresponding to the reduction in photosynthetic physiology under severe drought stress. However, V1, S13, S1 and S34 genotypes maintained better photosynthetic performance along with hydraulic dynamics when compared to other genotypes.

3.6. Above ground biomass

Four plants were harvested for growth and biomass yield measurements from control, treated as well as recovery. Morphological changes after WS treatment in field grown mulberry genotypes were shown in Fig. S1. Upon drought treatment, plant height as well as biomass yields varied significantly among genotypes with respect to their controls (Fig. 8). S13, V1, S1 and S34 maintained significantly higher above ground biomass even under severe drought stress, when compared to other genotypes. We recorded higher biomass in S1 followed by V1, S13 and S34 with respect to other mulberry genotypes under control and drought as well as during recovery. Under well water conditions, total fresh biomass (TFBM) ranged from 3.1 kg (V1) to 2.78 kg (K2), whereas TFBM was ranged from 2.21 kg (S1) to 1.21 kg (DD) under drought stress conditions. However, leaf fresh biomass (LFBM) and stem fresh biomass (SFBM) were reduced during drought stress compared to controls (Fig. 8).

4. Discussion

The current study was aimed at screening and selection of better performing mulberry genotypes based on their stem hydraulic conductance (K_s), sap flow (F) and photosynthetic responses to natural drought stress followed by re-watering. We selected a total of 12 mulberry genotypes that are well established under uniform climatic conditions and subjected them to progressive drought stress followed by re-watering. Based on their photosynthetic performance, hydraulic dynamics and above ground biomass during drought stress and recovery, the 12 mulberry genotypes were characterized into three functional groups including high performance group (HPG including S13, V1, S1 and S34), average performance group (APG including ML, KL, S1635 and MR2) and poor performance group (PPG including K2, S36, DD and PNG).

Plant water status is a key parameter which acts as a major regulatory switch for both physiological and molecular responses during low water regimes. WS induces hydraulic signals from root to leaves via xylem, to exhibit the symptoms of drought at both roots as well as shoot level (Sengupta et al., 2013). In mulberry, water stress caused significant reduction in plant water status (LRWC, LMC, Ψ_L and Ψ_x) in all three groups of mulberry genotypes, wherein HPG genotypes were able to maintain relatively higher and stable plant water status (LRWC, LMC, Ψ_L and Ψ_x) when compared to other groups (APG and PPG) even under stress conditions (Table 2). The hydrated leaves of HPG plants facilitated better carbon assimilation, plant growth and development under prolonged dry environments. This was in accordance with

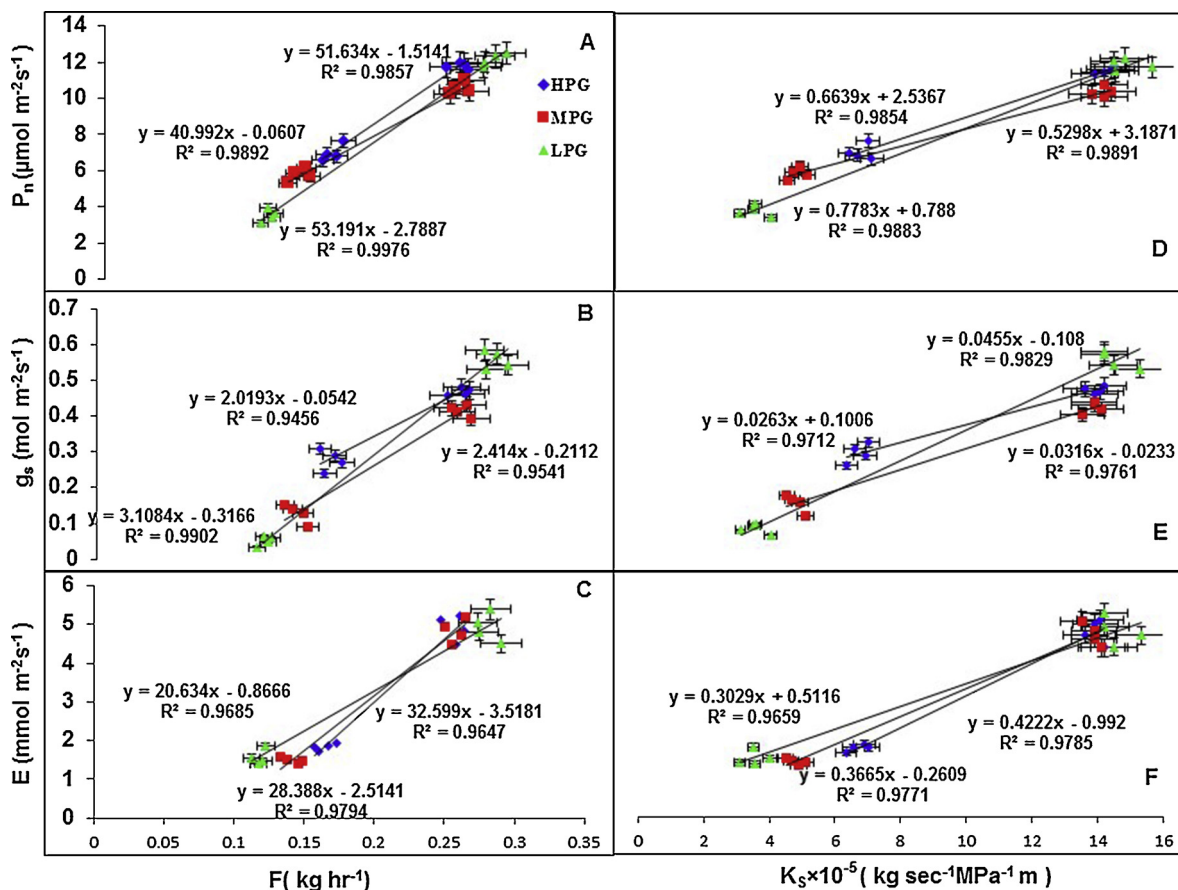


Fig. 7. Relationship between stem hydraulic conductance (K_s) and xylem sap flow rate (F) versus (A) photosynthetic rates (P_n), (B) stomatal conductance (g_s), and (C) transpiration rate (E) during the period of drought stress. Data presented are an average of 10 values \pm SE ($n = 4$).

previous reports showing a correlation between root: shoot ratio of drought tolerant plants and leaf water status under water deficit conditions (Guha et al., 2010a). Reduced leaf water status in drought stressed plants ultimately induces negative effects on leaf gas exchange parameters, leading to reduced C_i which may further leads to reduction in ribulose-1, 5-bisphosphate carboxylase (RUBISCO) activity as well as carboxylation efficiency (Gomes et al., 2008; Chaitanya et al., 2003). In consistent with above results, drought stress induced significant reduction in photosynthetic parameters in all three groups (Table 2). However, HPG genotypes exhibited better photosynthetic parameters during WS compared to APG and PPG demonstrating leaf turgor loss proportionally influenced the photosynthetic performance and tolerance in mulberry (Reddy et al., 2017). Our results depicts that HPG genotypes showed better P_n associated with higher rates of C_i which in turn favors Rubisco's carboxylation, and less carbon starvation as well as drought related symptoms during WS conditions when compared to APG and PPG.

Maintenance of better photosynthetic efficiency in HPG during WS was further supported by chlorophyll a fluorescence parameters, which indicated better photosystem-II (PS-II) efficiency during severe WS conditions. In general, increasing drought intensity leads to gradual reduction in PS-II efficiency due to structural modifications of thylakoid membrane (Oukarroum et al., 2007). F_v/F_m is a widely used parameter to evaluate the photosynthetic performance of a plant species under different environmental conditions (Sreeharsha et al., 2015). In the present study, we noticed that reduction in F_v/F_m occurred negligibly in all mulberry genotypes after 25DAS, but there is a significant reduction in the $\Delta F/F_m$ under drought. The reduction in $\Delta F/F_m$ might be due to closed PS-II reaction centers causing reduced light harvesting and energy transduction suggesting that severe drought stress negatively

affects PS-II efficiency. However, HPG genotypes showed higher $\Delta F/F_m$ values during even after 25DAS indicating the presence of more number of opened PS-II reaction centers when compared to APG and PPG genotypes. The reduction in $\Delta F/F_m$ values in PPG genotypes under WS was significantly higher, suggesting that maximum proportion of the absorbed light was not utilized in photochemical quenching (q_p), due to the presence of closed PS-II reaction centers, but rather was dissipated in the form of heat energy leading to significant increase in NPQ. Lower NPQ values in HPG at 25 DAS, indicating that most of absorbed light was efficiently utilized in q_p when compared with APG and PPG under drought stress. Maximum reduction of F_v/F_m and $\Delta F/F_m$ in PPG during WS attributing that severe drought stress was negatively affecting the PS-II efficiency in mulberry genotypes. Our results from chlorophyll a fluorescence measurements demonstrate that HPG mulberry genotypes exhibited better PS-II efficiency and less photo oxidative damage to thylakoid membranes even under prolonged drought conditions with respect to APG as well as PPG.

In addition to above, plant water status and photosynthetic performance are directly/indirectly associated with the whole plant hydraulic conductivity through soil-root-shoot-leaf continuum. Stem hydraulic conductance (K_s) and xylem sap flow (F) together determines the efficiency of water transport from roots to leaves and also regulates the gas exchange physiology and biomass yields of plants under different environmental stress conditions (Costa et al., 2004). In the present study, WS induced significant limitations in hydraulic conductivity (including K_s and F) in all the 3 groups demonstrating constrained water transport from soil to atmosphere. However, PPG genotypes showed higher restriction of water supply to leaves with reduced K_s and F values compared to APG and HPG inferring that PPG perceived early drought symptoms due to reduced plant hydraulics resulting in lower

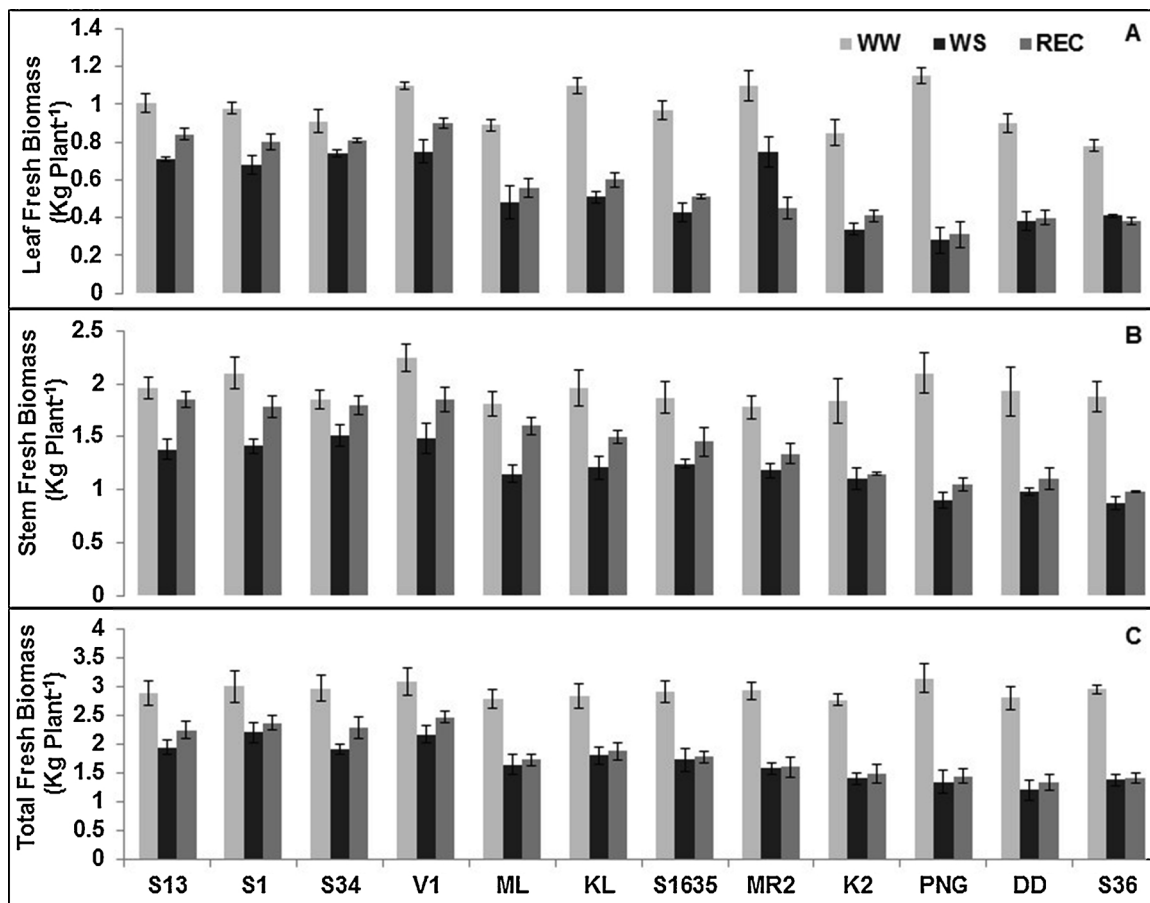


Fig. 8. Changes in above ground biomass patterns in 12 mulberry genotypes subjected to severe drought stress and rehydration. (A) Total leaf fresh biomass (TLFB), (B) Total stem fresh biomass (TSFB), (C) Total fresh biomass (TFBM). Values represented are the mean \pm SE (n = 4).

Table 2

Changes in photosynthetic leaf gas exchange characteristics, hydraulic dynamics, chlorophyll *a* fluorescence parameters and destructive biomass yields among the genotypes under drought stress. Values are least significance difference and statistical significance of *(P < 0.05), ***(P < 0.01), ****(P < 0.001) and ns (non-significant).

	Parameter	WW			WS			REC		
		HPG \times APG	APG \times LPG	HPG \times LP	HPG \times APG	APG \times LPG	HPG \times LPG	HPG \times APG	APG \times LPG	HPG \times LPG
Photosynthetic physiology	P _n	ns	1.34**	1.062**	0.766**	0.792***	0.784***	0.709**	0.853*	0.965***
	g _s	ns	0.0571*	0.0481**	0.0559**	0.0515*	0.108***	0.0345**	0.0323**	ns
	E	ns	ns	ns	0.125***	0.033***	0.108***	0.162**	0.197***	0.203***
	C _i	ns	6.579**	8.283**	12.346***	14.01***	13.435***	ns	7.834**	7.169***
Hydraulic dynamics	K _s	ns	0.33***	0.339***	0.493***	0.56**	0.595***	1.136**	1.087**	0.66***
	F	0.01**	0.0095***	0.0154**	0.0175**	0.0103***	0.0115***	0.0142**	0.0132**	0.013***
	Ψ _x	ns	0.0532***	0.0586**	0.171***	0.101**	0.212**	0.069***	0.118***	0.109***
	Ψ _L	0.0915***	0.0826**	0.087***	0.174**	0.0962***	0.109**01*	0.082***	ns	0.151**
Chlorophyll <i>a</i> fluorescence	ΔF/F _m '	ns	ns	ns	0.0372***	ns	0.0834***	ns	ns	ns
	F _v /F _m	ns	ns	ns	0.0289**	ns	0.0284**	0.024***	0.039**	0.041***
	q _p	ns	ns	ns	0.0738*	ns	0.104**	ns	0.0926*	0.088**
	NPQ	0.0457**	0.0473**	0.0456**	0.0331***	0.873*	0.82**	0.166***	0.188***	0.137***
Biomass	LFBM	ns	0.102**	0.115*	0.0496***	0.0754**	0.0789***	0.096***	0.0966**	0.077***
	SFBM	ns	ns	ns	0.0877***	0.0989***	0.146***	0.139***	0.159***	0.098***
	TFBM	ns	ns	ns	0.225**	0.163**	0.218***	0.185***	0.16***	0.146***

photosynthetic performance under drought stress. Maintenance of better K_s and F in HPG even under extended WS (25DAS), attributing that these genotypes are less vulnerable to hydraulic failure and plant mortality compared to PPG. We also observed that changes in leaf gas exchange parameters (P_n, g_s, and E) were strongly correlated with leaf water status which in turn were driven by stem xylem water transport efficiency (K_s and F). Thus, recorded higher rates of P_n, g_s and E in HPG followed by APG and PPG under prolonged drought conditions may

possibly be associated with better hydraulic conductivity (K_s and F). Previous studies have demonstrated that early stomatal closer during extended drought conditions induce early hydraulic failure as well as plant mortality (Costa et al., 2004; Brodrigg and Holbrook, 2003; Choat et al., 2007). In the present study, mulberry genotypes belonging to HPG showed augmented g_s, facilitate better CO₂ acquisition even at 25 DAS followed by APG and PPG cultivars may possibly associated with better hydraulic conductivity and delayed drought related symptoms or

vice versa. Further, we observed that better water relations in HPG under water deficit environments were associated with better rooting behavior as reported in our previous study (Guha et al., 2010b). It was previously reported that changes in plant water transport efficiency and photosynthetic performance during severe drought stress proportionally affects plant growth, development and biomass yields (Guha and Reddy, 2013). In this study, we observed better photosynthetic performance and hydraulic conductivity in HPG mulberry genotypes under prolonged drought conditions should be linked with their superior above ground biomass yields as compared with APG as well as PPG.

In the present study, we also observed that there was a significant difference in recovery from drought stress among the genotypes belonging to three different groups which in turn suggest that fast recovery from drought stress might be associated with drought adaptation mechanisms in perennial tree species. Complete recovery in leaf gas exchange physiology after re-watering depends on the intensity of drought stress and between species as well as genotypes of the same species (Sapeta et al., 2013). Further, leaf physiological functions can be completely recovered at a faster rate after a moderate stress; but it may take days to weeks to recover from severe drought stress and sometimes never completely recovered (Chaves et al., 2011). In our study, LRWC and ψ_1 recovered to that of control level after rehydration in all three groups. Early recovery of water transport efficiency indicates fast restoration of drought induced damage and formation of new xylem tissue (Martorell et al., 2014). In woody plants, rehydration after drought stress recovers hydraulic capacity completely by refilling the embolised xylem vessels, which in turn linked with restoring the photosynthetic capacity to normal levels (Brodribb and Cochard, 2009; Chen et al., 2010; Martorell et al., 2014). In this study, we observed complete recovery in leaf water status, which might be strongly associated with the fast recovery of K_s and F values in HPG mulberry genotypes compared to APG and PPG. Based on our results, we postulates that the recovery of photosynthetic physiology may directly/indirectly related with plant hydraulic dynamics due to the inter dependence of stomatal behavior and transpiration rates with xylem water transport efficiency. Our results from recovery studies also showed that P_n , g_s , E and C_i were restored within four days after rehydration in HPG genotypes; PPG genotypes showed only partial recovery of g_s and C_i but not P_n and E even after seven days after REC ascribing significant differences in efficiency of water transport. In consistent with photosynthetic leaf gas exchange parameters, $\Delta F/F_m'$ was completely recovered in HPG genotypes, whereas in PPG $\Delta F/F_m'$ was recovered incompletely at 7 days of REC suggesting that leaf photochemical damage was more in PPG at 25DAS. Our results from drought and recovery studies infer that HPG mulberry genotypes, exhibited increased tolerance for drought and early recovery, and thus were considered as DT. PPG genotypes, showed poor tolerance and slow recovery which were considered as drought susceptible (DS) while APG genotypes exhibited medium tolerance and recovery and were considered as moderate tolerant (MT).

5. Conclusions

Based on our results, we concluded that HPG mulberry genotypes exhibited high efficiency in water transport during WS and REC should linked with greater DT capacity even under prolonged drought conditions as compared with APG and PPG mulberry genotypes. There was a stringent positive correlation between photosynthetic performance and hydraulic dynamics which were collectively linked with superior biomass yields in HPG genotypes during drought and recovery. These results clearly demonstrated that rapid screening based on changes in hydraulic traits along with photosynthetic leaf gas exchange characteristics would be a way to identify efficient DT mulberry genotypes suitable for present as well as future climate change scenarios.

Authorship contributions

Please indicate the specific contributions made by each author (list the authors' initials followed by their surnames, e.g., Y.L. Cheung). The name of each author must appear at least once in each of the three categories below.

Category 1

Conception and design of study: **K.S. Reddy, K.M. Sekhar, A. R. Reddy**

Acquisition of data: **K.S. Reddy, K.M. Sekhar, R.V. Sreeharsha**
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Category 2

Drafting the manuscript: **K.S. Reddy, K.M. Sekhar, R.V. Sreeharsha, A. R. Reddy**

Revising the manuscript critically for important intellectual content: **K.S. Reddy, K.M. Sekhar, R.V. Sreeharsha, A. R. Reddy**

Category 3

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Acknowledgments

Senior Research Fellowship-P from DST-Inspire (Department of Science and Technology, Government of India) Government of India to Kanubothula Sitarami Reddy gratefully acknowledged. R.V. S is thankful to UGC, New Delhi for fellowship. Thanks to Department of Science and Technology, Government of India, for financial assistance (Grant SERB/SR/SO/PS/86/2010) to Attipalli R. Reddy. Plant germplasm was provided by Regional Sericulture Research Stations (Anantapur and Salem, India).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.envexpbot.2018.10.038>.

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