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Photosynthetic gas exchange, chlorophyll fluorescence, antioxidant enzymes, and growth responses of *Jatropha curcas* during soil flooding

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Abstract: The response of chlorophyll fluorescence, photosynthetic CO_2 assimilation (PN), stomatal conductance (gs), electrolyte leakage, and transpiration (E) was observed in *Jatropha curcas* seedlings subjected to soil flooding. A strong reduction in growth, leaf-area expansion (64%), and stomatal conductance (45%) impaired photosynthetic CO_2 assimilation (66%), which eventually reduced biomass yield. The ratio between variable-to-initial chlorophyll fluorescence (Fv/Fo) and the maximum quantum yield efficiency of the photosystem II (Fv/Fm) was used to explore damage associated with the functioning of the photosynthetic apparatus. A strong, non-linear correlation between physiological parameters and soil flooding duration was found. Our study primarily revealed consequences of epigenetics, i.e. stagnant soil flooding, which affected growth, development, and performance of *Jatropha curcas* significantly. The activities of catalase (CAT), ascorbate peroxidase (APx), glutathione reductase (GR), and glutathione peroxidase (GPx) in leaves increased, implying an integrated pathway involving CAT, APx, GR, and GPx for protection against the detrimental effects of reactive oxygen species (ROS) during soil flooding.

Key words: Biomass, flooding, photosynthesis, physic nut, relief, stress

1. Introduction

Soil flooding is a major abiotic stress that imposes restriction in gaseous diffusion, i.e. oxygen and carbon dioxide in plants. The slow rate of gas diffusion in water limits the oxygen supply (Visser and Voesenek, 2004; Tan et al., 2010; Balakhnina et al., 2012). Soil flooding due to excess rains or seepage from large reservoirs may damage crops completely as it causes a dramatic impact on biochemical activities viz., aerobic respiration and photosynthesis in stagnant or slow-moving water (Armstrong and Drew, 2002; Islam et al., 2008; Jackson, 2008; Else et al., 2009). The soil is considered flooded if free-standing water on its surface is ~20% higher than the field capacity (Aggarwal et al., 2006). This leads to insufficient supplies of oxygen to the root cells. Consequently, shoot cells and the fundamental requirements of the plant's life may become injurious for cellular functioning. As soil flooding results in major changes in the soil environment and physical status of the soil, the breakdown of large aggregates into smaller particles occurs (Pociecha et al., 2008), which poses a severe threat to the survival of terrestrial plants.

The most important consequences of flooding are reduction in water and nutrient uptake and disturbance of plant respiratory metabolism (Dat et al., 2004). As a result, O_2 deprivation induces several physiological and biochemical changes. Oxygen is an essential substrate for respiratory metabolism, passes rapidly through membranes to all compartments of the cell, and acts as a substrate or cofactor in many biochemical reactions in the primary and secondary metabolism of plants (Holmberg et al., 1997). The adverse effects of soil flooding are inhibition of leaf growth, reduction in shoot and root growth and whole plant biomass (Pociecha et al., 2008), changes in biomass partitioning, and promotion of overall plant senescence followed by mortality. The shoot growth is reduced because flooding affects leaf-area expansion and induces premature leaf senescence and abscission (Kozlowski, 1997; Mielke et al., 2003). The chlorophyll fluorescence may become impaired along with functioning of the photosynthetic apparatus in vivo by soil flooding. The reduction in plant biomass becomes directly correlated with net carbon assimilation regulated by the stomatal and non-stomatal limitations of photosynthesis

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(Mielke et al., 2003; Pociecha et al., 2008). The shortage of oxygen in the rhizosphere becomes detrimental for the development of root systems, and the root may die (Drew, 1997). The roots are major sensory organs for detecting stressful conditions in the soil. Root growth gets reduced mainly due to lack of available O_2 for root respiration. The soil phytotoxins also inhibit root formation and promote root decay. Higher levels of antioxidant enzymes viz., catalase, peroxidase, glutathione reductase, and ascorbate peroxidase were found to be important for survival under oxidative stress in many plants (Tan et al., 2010; Baloğlu et al., 2012; Saeidnejad et al., 2013).

Jatropha curcas L. is a multipurpose plant with many potential attributes. It is a sub-tropical plant grown in low-to-high-rainfall areas that can be used to reclaim lands and also as a commercial crop to provide employment in rural areas. The plant produces useful products viz., seeds, from which oil can be extracted (~35%). It has similar properties to palm oil and can be used as a substitute for kerosene and diesel. By 2008, *Jatropha curcas* had already been planted over an estimated 900,000 ha globally; an overwhelming 85% of plantations are in Asia, 13% in Africa, and the remaining 2% in Latin America. It is expected that by 2015 *Jatropha curcas* may be planted on 12.8 million ha worldwide (Kant and Wu, 2011) in order to blend fossil diesel with biofuel across the world under the climate change campaign to mitigate greenhouse gases (GHGs). Generally, flowering season, number of flowering events, and male-to-female flower ratio in *Jatropha curcas* depend upon soil fertility, available moisture, and temperature to affect the production of seeds. In drier zones, it exhibits one major flowering flush, while it acquires flowers episodically in humid areas. This logic has promoted *Jatropha curcas* cultivation on a large scale in the Tarai regions of Uttar Pradesh (India), which often experiences soil flooding during the rainy season (July–September). Apart from this, understanding the response of *Jatropha curcas* to soil flooding is also required for designing growth models. Therefore, our study aims to reveal the influence of this epigenetic factor on phenotypic, physiological, and biochemical characteristics in order to correlate plant performance.

2. Materials and methods

2.1. Plant material and growth conditions

The 45-day-old seedlings of *Jatropha curcas* were raised from stem cuttings (~18–20 cm length) in earthen pots (~30 cm diameter and 40 cm depth) filled with fertile soil in an open area at the College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), India. These seedlings were subjected to soil flooding. A standing water level ~5 cm in height was maintained from the soil surface throughout the experimental period, while the control seedlings were raised by irrigating exactly up to field capacity. The environmental variables, i.e. temperature, relative humidity, and sunshine, at the experimental site are shown in Figure 1.

2.2. Leaf gas exchange measurements

The photosynthetic CO_2 assimilation (P_N), stomatal conductance (*g*s), and transpiration (E) were measured by an open infrared gas analyzer (CIRAS-1 IRGA, PP System, England) under natural PPFDs (~1500 μmol m^{-2} s^{-1}) in the morning (0900–1000) to avoid the high temperature and low humidity effects of the afternoon. All measurements were taken on mature and fully expanded leaves (6th position). Leaf-chlorophyll content or soil plant analysis development (SPAD) value was measured (Tan et al., 2008) by using a chlorophyll meter (SPAD-502, Minolta, Japan). Transient chlorophyll fluorescence indicated primary reactions of photosynthesis as a useful tool for reading photosynthetic efficiency of PS II (Krause and Weis, 1991), because changes in chlorophyll fluorescence (Kautsky transient) can reveal the status of the photochemical activities of PS II and the plastoquinone pool (Liang et al., 2007). Chlorophyll fluorescence was assessed by Handy plant efficiency analyzer (Handy, PEA, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK). The initial (Fo), maximum (Fm), and variable (Fv = Fm – Fo) fluorescence; Fv/Fo ratio; and maximum quantum efficiency of the PS II (Fv/Fm) were measured by using dark-adapted (30 min) leaves.

2.3. Electrolyte leakage

Membrane injury, a measure of cell membrane leakage, was determined (Crane and Davis, 1987). The leaf and root pieces (1 g each) were placed in test tubes (15 mL of deionized water), capped tightly, and stirred (3 h, 25–30 °C) to monitor conductivity (EC_1) using a conductivity meter (Hanna Instruments Inc., Woonsocket, RI, USA). Afterwards, the samples were frozen (–20 °C, 12 h), boiled (1 h), cooled to room temperature to re-monitor their conductivity (EC_2) and calculate electrolyte leakage (EC), as stated below:

$$\text{electrolyte leakage (\%)} = (EC_1/EC_2) \times 100.$$

2.4. Enzyme extract preparation

Plant leaves were freshly harvested after specific treatment intervals. They were cut into pieces, ground to powder using liquid nitrogen, lyophilized, and kept in a freezer –20 °C) for enzymatic activities. Lyophilized leaf powder (0.5 g) was added to a test tube containing ice-cold extraction buffer (5 mL) (100 mM potassium phosphate, pH 7.0; 0.1 mM EDTA), filtered, and centrifuged (16,000 × *g*, 15 min, 4 °C). The supernatant fraction was used as crude extract for the assay of enzymatic activities. All operations were carried out at 0–4 °C.

2.5. Antioxidant enzymatic activities assays

The catalase (CAT) activity was measured with minor modifications (Beers and Sizer, 1952). The reaction mixture (1.5 mL) consisted of phosphate buffer (100 mM, pH 7.0), EDTA (0.1 µM), H_2O_2 (20 mM), and crude enzyme extract (50 µL). The enzyme activity was monitored spectrophotometrically (A_{240nm}), quantified by its molar extraction coefficient (36 M^{-1} cm^{-1}), and the results were expressed as µmol H_2O_2 min^{-1} g^{-1} FM.

The reaction mixture (1.5 mL) for ascorbate peroxidase (APx) enzyme activity contained phosphate buffer (50 mM, pH 6.0), EDTA (0.1 µM), ascorbate (0.5 mM), H_2O_2 (1 mM), and crude enzyme extract (50 µL). The reaction was started by adding H_2O_2, ascorbate oxidation was measured spectrophotometrically (A_{290nm}) for 2 min, quantified by using the molar extinction coefficient for ascorbate (2.8 mM^{-1} cm^{-1}), and the result was expressed in µmol H_2O_2 min^{-1} g^{-1} FM (Nakano and Asada, 1981). Glutathione peroxidase (GPx) reaction mixture (2 mL) contained phosphate buffer (100 mM, pH 7.0), EDTA (0.1 µM), guaiacol (5 mM), H_2O_2 (15 mM), and crude enzyme extract (50 µL). The addition of enzyme extract started the reaction. An increase in absorbance (A_{470nm}) was recorded for 2 min. Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient (26.6 mM^{-1} cm^{-1}) and expressed as µmol H_2O_2 min^{-1} g^{-1} FM (Urbanek et al., 1981). Glutathione reductase (GR) activity was measured as described by Foyer and Halliwell (1976), with minor modifications. The reaction mixture (1 mL) consisted of phosphate buffer (100 mM, pH 7.8), EDTA (0.1 µM), NADPH (0.05 mM), GSSG (3 mM), and crude enzyme extract (50 µL). The reaction was started by adding GSSG and NADPH and oxidation was monitored (A_{340nm}) for 2 min and expressed as µmol NADPH min^{-1} mg^{-1} FM by using the molar extinction coefficient for NADPH (6.2 mM^{-1} cm^{-1}).

2.6. Growth parameters

Growth parameters were recorded during the 8 weeks of the study, i.e. 4 weeks each for soil flooding and recovery. All observations related to soil flooding and recovery were recorded weekly. The soil flooding treatment was terminated after 4 weeks. Subsequently, the recovery was allowed (4 weeks) by draining out the excess water from the pots. During recovery, careful irrigation exactly up to field capacity was maintained, similar to the control seedling treatment. Plant height and stem diameter were measured (~15 cm above the soil surface) by caliper. The leaf area was estimated by Leaf-Area Meter (CI-202, CID Inc., USA). The biomass yield was assessed by harvesting, washing, and oven drying plants (70 ± 2 °C, 48 h) to achieve constant dry weight.

2.7. Equation-model description

Apart from the major aim of our study, we also calculated growth responses to develop a mathematical equation associated with any value of time. As field and laboratory experiences show, under soil flooding stress physiological responses were adversely affected, and once the stress was over the impaired responses showed recovery. It is hypothesized that the rate of change (% loss or gain) of physiological response is directly proportional to the escalating time period and mathematically may be expressed as:

$$\frac{dR}{dT} \propto T \tag{1}$$

where

R = photosynthetic response and

T = time under stress or after stress.

To generalize Eq. (1) an escalation constant should be introduced. Accordingly, Eq. (1) takes the following form:

$$\frac{dR}{dT} \propto (T+\lambda) \tag{2}$$

where

λ = escalation constant and

$T + \lambda$ = escalation time.

By replacing the proportionality sign by introducing another constant one can write the above equation as:

$$\frac{dR}{dT} = \alpha(T+\lambda) \tag{3}$$

where α is the proportionality constant. Separating the variables of Eq. (3) it may be expressed as:

$$dR = \alpha(T+\lambda)dT \tag{4}$$

Integrating Eq. (4), one will obtain the solutions as:

$$R_t = \frac{\alpha}{2} T^2 + \alpha \lambda T + \beta \tag{5}$$

where β is the integration constant. This can be evaluated by substituting the initial conditions in Eq. (5), i.e. $T = 0$, $R_t = R_0$:

$$R_0 = \frac{\alpha}{2} \times 0 + \alpha \lambda \times 0 + \beta \;\Rightarrow\; \beta = R_0 \tag{6}$$

Eq. (5) can now be written as:

$$R_t = \frac{\alpha}{2} T^2 + \alpha \lambda T + R_0 \tag{7}$$

where R_0 is initial photosynthetic response. Eq. (7) is a quadratic equation and is non-linear in nature.

For validation of the above hypothesis or equation, the data of physiological responses viz., photosynthesis, transpiration, and stomatal conductance were fitted to Eq. (7).

2.8. Statistical analysis

The experimental design was completely randomized. Photosynthetic and growth parameters were analyzed independently for each observation using t-test and standard error of means.

3. Results

The epigenetic variables were recorded throughout the experimentation period (Figure 1). The 4-week soil flooding treatment duration drastically reduced plant height (21%), stem diameter (25%), and root length (67%), while the long-term recovery process (28 days) could restore these losses only ~3%, 7%, and 8% (Figure 2A–C). The soil flooding also impaired leaf number (58%), leaf-area expansion (64%), and leaf mass per unit (38%), and a subsequent recovery of 4 weeks could recover these losses ~8%, 20%, and 36%, respectively (Figure 2D–F).

The short-term soil flooding (7 days) reduced P_N (27%), E (38%), and *g*s (24%), and further loss in these values continued with the continuation of soil flooding (4 weeks), nearly 66%, 67%, and 45% (Figure 3A–C). Afterwards, the 4-week recovery process partially restored the reduced values of P_N, E, and *g*s by ~14%, 13%, and 10%. The values of chlorophyll fluorescence, i.e. Fv/Fm and Fv/Fo, decreased ~17% and 42% due to flooding (Figure 3D, E). These losses recovered about 4% and 19% upon removal of soil flooding, i.e. after the 28 day recovery process. The data of P_N, E, and *g*s fit well within the derived equation, and their respective values of R^2 were 1.000, 0.998, and 1.000 for soil flooding and 0.990, 0.999, and 0.974 for recovery (Table 1). The plant height, stem diameter, root length, leaf number, leaf-area expansion, and specific leaf weight also fit well in the derived equation with R^2 values ranging from 0.981 to 0.999 for soil flooding and 0.953 to 0.999 for recovery (data not shown).

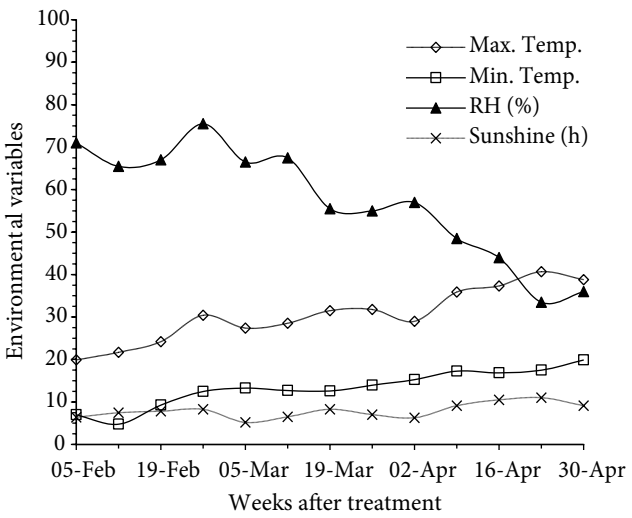

Figure 1. Variation in temperature (°C), relative humidity (%), and sunshine hours (h) at experimental site.

The soil flooding (28 days) raised electrolyte leakage (EC) values about 66% and 23% higher in *Jatropha curcas* seedlings as compared to control for root and leaf tissues (Figure 4A, B). Upon removal of soil flooding, the impaired values recovered nearly to the level of control plants, which took about 4 weeks. The SPAD values and photosynthetic pigments (chl a + b) declined ~18% and 48%, while a 4-week recovery process allowed restoration of these impaired values to 12% and 9% (Figure 4C, D).

The stress-inducible enzyme activities were higher for ascorbate peroxidase (55%), glutathione peroxidase (20%), catalase (15%), and glutathione reductase (51%) and were correlated well with soil flooding stress (Figure 5A–D). Upon recovery, these up-regulated enzymatic activities decreased nearly 40%, 15%, 13%, and 40%, respectively.

Plant biomass characteristics viz., root dry mass (Rdm), stem dry mass (Sdm), leaf dry mass (Ldm), and total dry biomass (Tdm) were significantly affected by 4 weeks of soil flooding treatment, showing impaired root-to-shoot mass ratio and harvest index (Table 2). Thus, the overall loss in these values was ~65%, 36%, 45%, 46%, 41%, and 10%, respectively, as influenced by flooding. The subsequent recovery process of 4 weeks triggered restoration for Rdm, Sdm, Ldm, Tdm, root-to-shoot mass ratio, and harvest index by nearly 20%, 16%, 24%, 18%, 6%, and 5%, while the values shown in the parentheses indicate the remaining unrecovered status of these characteristics, respectively (Table 2). Hence, these plants showed a tendency to recover gradually, but could not reach the level of normal plants.

4. Discussion

The 4-week soil flooding treatment negatively influenced vegetative growth, development, and biomass in *Jatropha curcas* (Figure 2, Table 2). Consequently, impaired plant height (21%), stem diameter (25%), leaf number (58%), leaf-area expansion (64%), specific leaf weight (38%), and root length (67%) were found after soil flooding of up to 4 weeks. Similar trends have been reported in several crop plants (Shi et al., 2007; Pociecha et al., 2008; Bai et al., 2010). The loss in root growth occurred due to soil flooding which created anaerobic conditions in the soil, i.e., hypoxia followed by anoxia (Armstrong and Drew, 2002). Soil flooding also enhances the CO_2, ethylene, Mn^{2+}, Fe^{2+}, S^{2-}, and carboxylic acids (McKee and McKevlin, 1993; Greenway et al., 2006) associated with loss in growth and development because of growth regulators due to increase

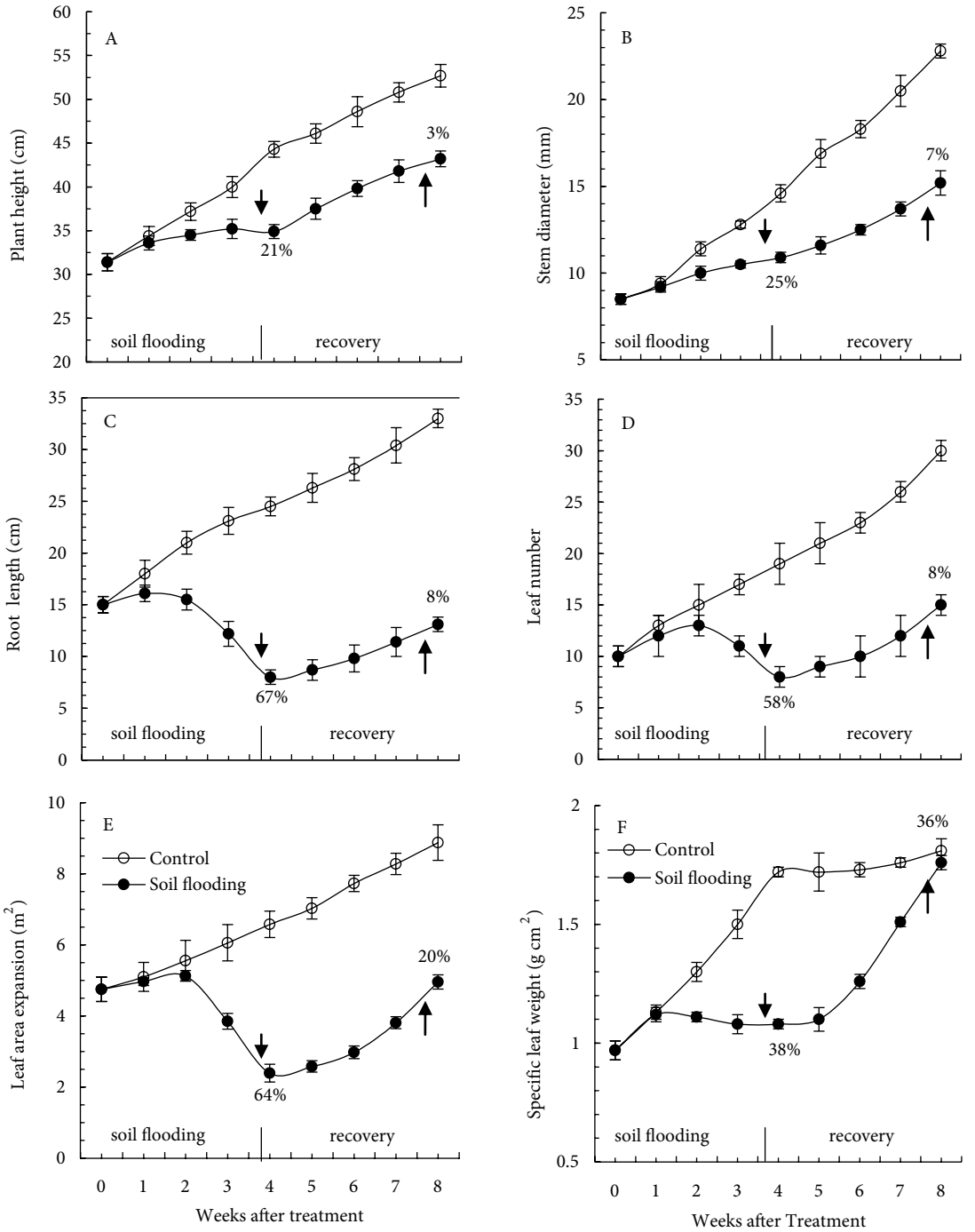

Figure 2. Influence of soil flooding and its withdraw on growth responses in *Jatropha curcas*. PH- plant height (A), SD- stem diameter (B), RL- root length (C), LN- leaf number (D), LA- leaf-area expansion (E), and SLW- specific leaf weight (F) during soil flooding and recovery. The soil flooding was maintained ~5 cm above the soil surface throughout; afterwards, soil flooding was withdrawn to allow recovery process by maintaining soil water content up to field capacity. Values are means (±SE) of at least 7–10 independent observations.

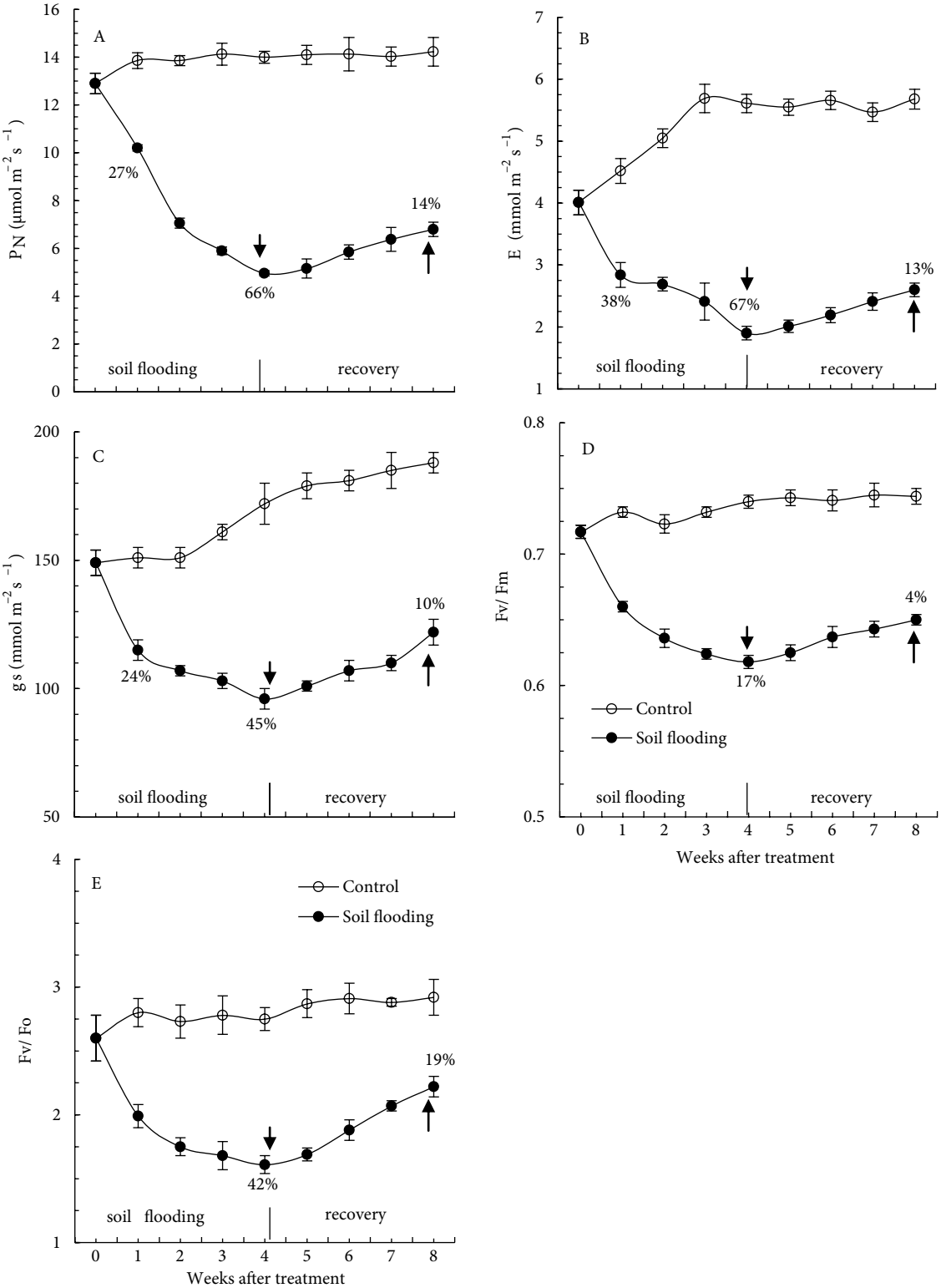

Figure 3. Influence of soil flooding and its withdraw on physiological responses in *Jatropha curcas*. P_N- photosynthetic CO_2 assimilation (A), E- transpiration (B), *g*s- stomatal conductance (C), Fv/Fm- chlorophyll fluorescence variable per maximum yield PS II (D), and Fv/Fo- variable to initial chlorophyll fluorescence (E) during soil flooding and recovery. The soil flooding was maintained ~5 cm above the soil surface throughout; afterwards, soil flooding was withdrawn to allow recovery process by maintaining soil water content up to field capacity. Values are means (±SE) of at least 7–10 independent observations.

Table 1. Regression analysis of photosynthetic CO_2 assimilation rate (P_N), transpiration rate (E), and stomatal conductance (*g*s) during soil flooding and recovery.

Parameters	Soil flooding (4 weeks)	R^2	Recovery (4 weeks)	R^2
P_N	$-0.0322T^2 + 2.4666T + 22.755$	1.000	$-0.0379T^2 - 3.1332T + 66.176$	0.990
E	$-0.0053T^2 + 1.5814T + 26.200$	0.998	$0.0149T^2 - 2.5048T + 82.470$	0.999
*g*s	$0.0146T^2 + 0.4529T + 19.903$	1.000	$-0.0107T^2 - 1.6429T + 56.568$	0.974

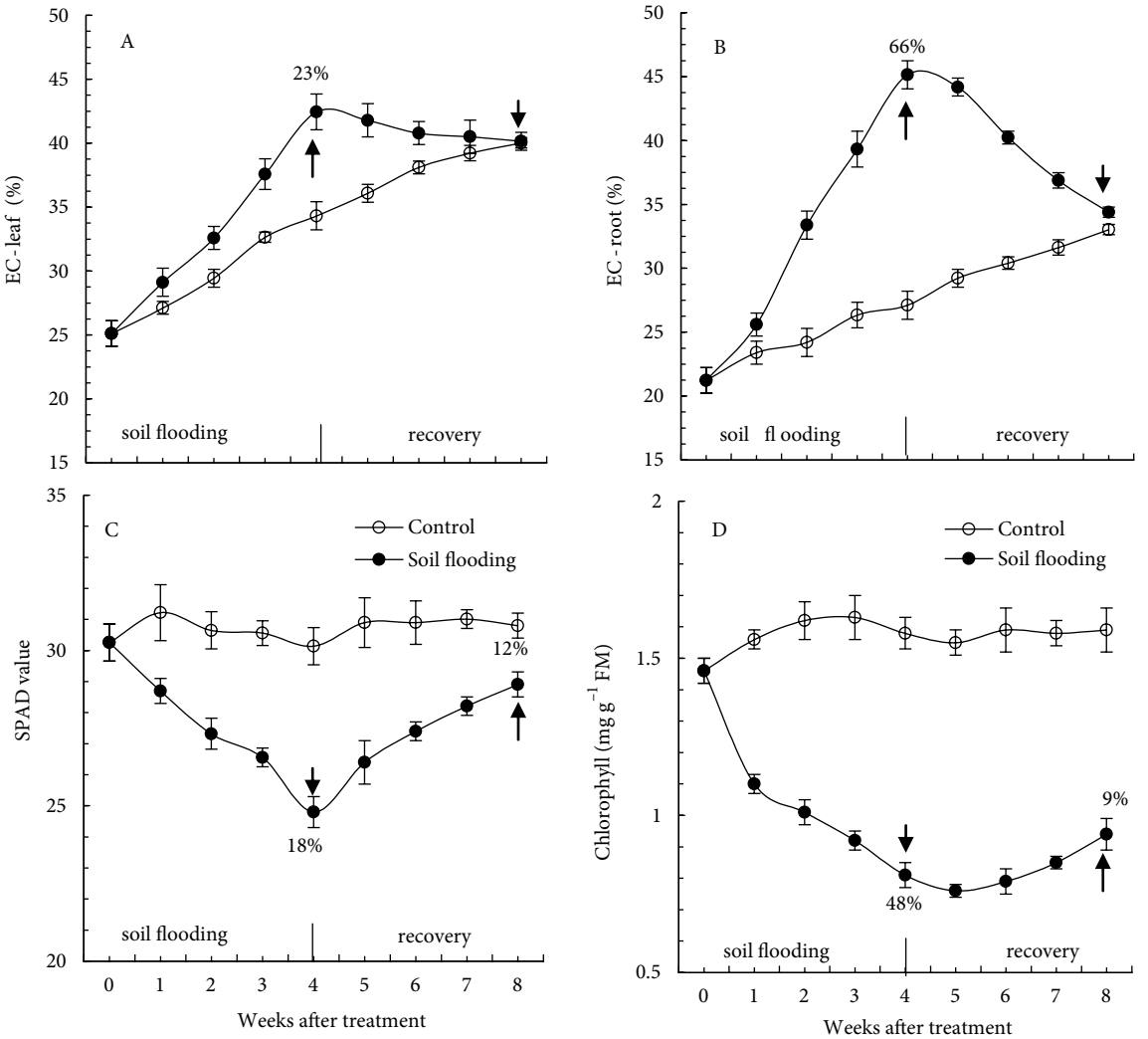

Figure 4. Influence of soil flooding and its withdraw on physiological responses in *Jatropha curcas*. EC- leaf and root electrolyte leakage (A,B), SPAD values (C), and total chlorophyll (D) during soil flooding and recovery. The soil flooding was maintained ~5 cm above the soil surface throughout; afterwards, soil flooding was withdrawn to allow recovery process by maintaining soil water content up to field capacity. Values are means (±SE) of at least 7–10 independent observations.

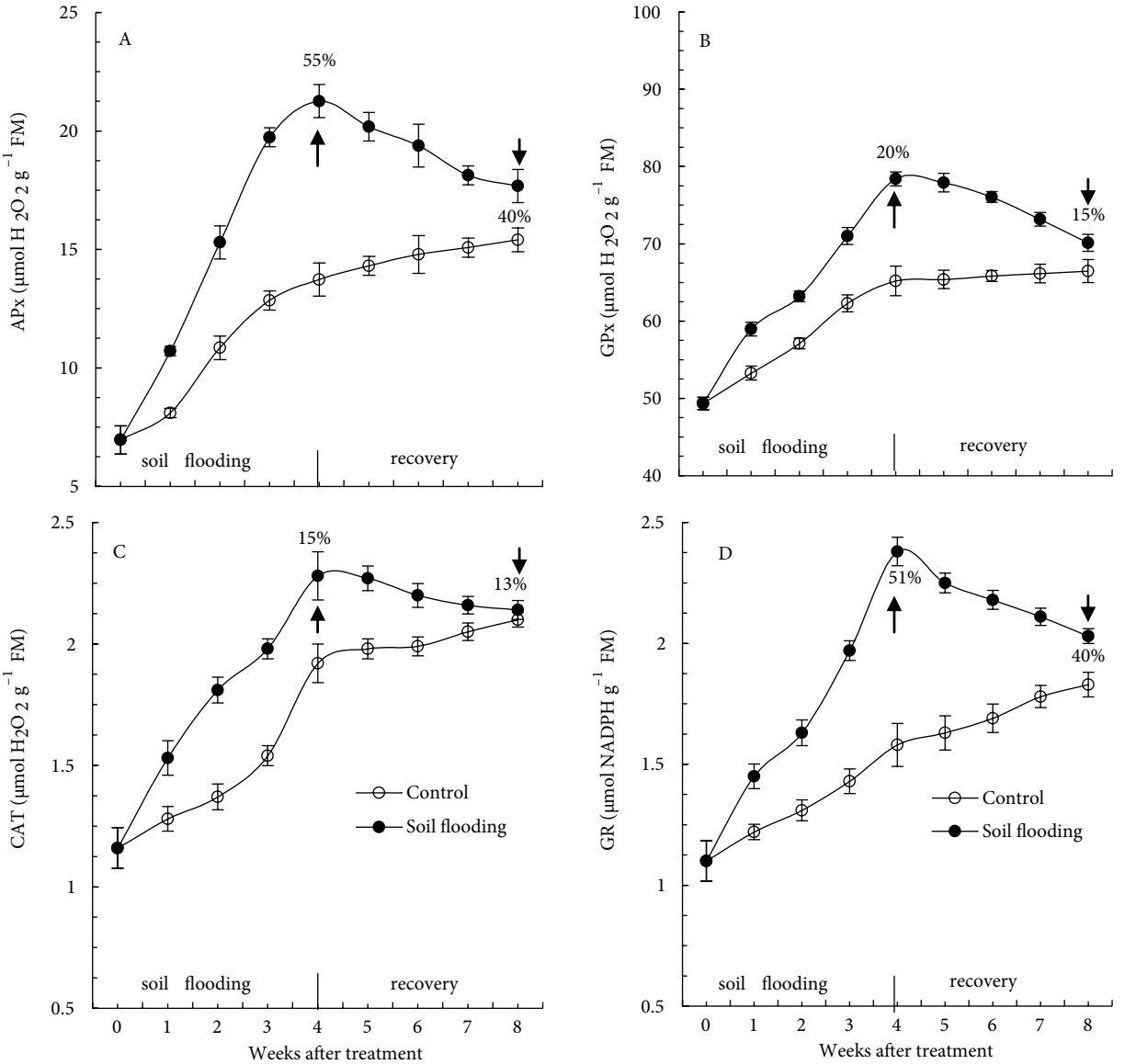

Figure 5. Influence of soil flooding and its withdraw on biochemical responses in *Jatropha curcas*. APx- ascorbate peroxidase (A), GPx- glutathione peroxidase (B), CAT- catalase (C), and GR- glutathione reductase (D) during soil flooding and recovery. The soil flooding was maintained ~5 cm above the soil surface throughout; afterwards, soil flooding was withdrawn to allow recovery process by maintaining soil water content up to field capacity. Values are means (±SE) of at least 3–5 independent observations.

in ethylene, which cause the onset the programmed cell death, affecting older leaves significantly. Thus, the reduction in leaf number and leaf-area expansion occurred as a result of flooding acclimation strategy (Pociecha et al., 2008). The loss in biomass and limited leaf-area expansion appeared to be related to slow metabolic activities of roots experiencing hypoxia (Mielke et al., 2003; Yiu et al., 2011) and impaired photosynthetic CO_2 assimilation regulated by the source-sink phenomenon linked to xylem and phloem (Bai et al., 2010).

The 4-week soil flooding also reduced photosynthetic CO_2 assimilation (66%), maximum quantum yield efficiency of the PS II (17%), stomatal conductance (45%), and transpiration (67%), as shown in Figure 3. The loss in stomatal dynamics during flooding causes imbalanced gaseous exchange. Thus, soil flooding influences loss in cellular oxygen, intercellular CO_2 availability, and photosynthetic CO_2 assimilation (Pociecha et al., 2008), which triggers internal CO_2 deficiency along with loss in transpiration (de Souza et al., 2011; Verma et al.,

Table 2. Effect of soil flooding on growth and biomass, i.e., root dry mass (Rdm), stem dry mass (Sdm), leaf dry mass (Ldm), total dry mass (Tdm), root mass ratio (R:S ratio), and harvest index (HI) in *Jatropha curcas*. The soil flooding was maintained ~5 cm above the soil surface throughout; afterwards, soil flooding was withdrawn to allow recovery process by maintaining soil water content up to field capacity. Values are means (±SE) of at least 7–10 independent observations. Values within the parentheses indicate specific unrecovered status.

Characteristics	Control	Soil flooding (4 weeks)	Loss (%)	Recovery (4 weeks)	Recovered (%)
Rdm (g)	8.10 ± 0.33	2.90 ± 0.18	65.2	4.18 ± 0.23	20.0 (44.2)
Sdm (g)	13.80 ± 0.35	8.87 ± 0.41	35.8	10.69 ± 0.30	15.8 (20.0)
Ldm (g)	11.50 ± 0.63	6.28 ± 0.40	45.4	7.62 ± 0.56	24.1 (21.3)
Tdm (g)	33.40 ± 0.98	18.05 ± 0.48	45.9	23.01 ± 0.39	18.4 (27.5)
R:S ratio	0.32 ± 0.05	0.19 ± 0.02	40.6	0.26 ± 0.004	5.8 (34.8)
HI	0.92 ± 0.007	0.83 ± 0.005	9.8	0.87 ± 0.005	5.4 (4.4)

2012). Apart from this, impaired stomatal conductance is correlated with the decrease in root permeability and root hydraulic conductivity (Mielke et al., 2003), which promotes rapid stomatal closure as a flooding tolerance mechanism (Pociecha et al., 2008). Chlorophyll fluorescence is an efficient tool for detecting changes in functioning of the photosynthetic apparatus during soil flooding (Mielke et al., 2003; Pociecha et al., 2008). Fv/Fo has a high power of discernment under the influence of any stress. A decrease in the Fv/Fm and Fv/Fo ratios suggests loss in photosynthesis due to damage to the photosynthetic apparatus (Tan et al., 2008), while 4-week soil flooding up-regulated leakage of bio-membranes in leaves (23%) and roots (66%) with down-regulation of soil plant analysis development (18%) and photosynthetic pigments (48%) (Figures 4A–D). Thus, while soil flooding drastically affected leaf as well as root membranes, the roots were more severely damaged than the leaves (Anilsulthian et al., 2003). The degradation of chlorophyll proceeded intensively through chlorosis in the leaves (Figures 4C,D), specifically those located near the flooded roots, as a measure of flooding tolerance (Pociecha et al., 2008).

An over-expression of stress-associated enzymes viz., ascorbate peroxidase, glutathione peroxidase, catalase, and glutathione reductase was found throughout the 4-week soil flooding treatment (Figure 5). Consequently, enzymes, i.e. APx, GPx, CAT, and GR, enhanced their levels up to 55%, 20%, 15%, and 51%, respectively, similar to Bai et al. (2010) and Sairam et al. (2011). In contrast to our findings, inhibition of GR, APx, CAT, and SOD activities occurred in corn leaves under prolonged soil flooding (Yan et al., 1996). The involvement of oxidative stress in soil flooding induces damage, and antioxidant response is an indicator of flooding tolerance or sensitivity (Arbona et al., 2008). The enhanced stress induces activities of these enzymes in seedlings subjected to soil flooding to protect them from the stress (Liu et al., 2006; Arbona et al., 2008; Bailey-Serres and Voesenek, 2008). Several enzymes (superoxide dismutase, catalase, peroxidase, and glutathione peroxidase) scavenging ROS and low molecular mass antioxidants (ascorbate, glutathione, phenolics, and tocopherols) extend tolerance against stress (Noctor and Foyer, 1998; Blokhina et al., 2003). The 4-week restoration process could induce expression of desired proteins inadequately involved in regulating various physiological and biochemical metabolic activities. Hence, the flood-treated *Jatropha curcas* plants could not reach normalcy, as shown by the control plants, i.e. plants grown by irrigating up to field capacity.

The coefficients of determination (R^2) for each set of data, i.e. photosynthesis, transpiration, and stomatal conductance with or without soil flooding stress were in the range of 0.974–1.000 (Table 1). This means that the derived equation model explains the variation in physiological responses under both conditions almost 100%. Our hypothesis explains the rate of change in either loss or gain (%) of physiological responses upon subjecting *Jatropha curcas* to soil flooding and after soil flooding, i.e. during the recovery process. This may be directly proportional to the escalating time period for predicting the losses or gains in other plants or crops in similar situations, which can also be verified. In conclusion, photosynthetic responses of *Jatropha curcas* during soil flooding may be useful for evaluating the level of flood tolerance. An integrated pathway implying CAT, APx, GPx, and GR activities extended protection against the detrimental effects of ROS during flood stress.

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