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ORIGINAL ARTICLE

Interactive effects of rice residue and water stress on growth and metabolism of wheat seedlings

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In the present study effects of rice residue with and without water stress were studied on *Triticum aestivum* L. cv. Shatabadi. The mixture of residue and garden soil in 1:1 ratio was considered as 50% (R_1) and only decomposed residue as 100% (R_2). Garden soil was taken as control. Twenty five seeds were sown in each experimental trays filled with soil mixture according to the treatments. Trays were arranged in two groups. After 15 days one set was subjected to water stress (WS) by withholding water supply for 3 days. Morphological and biochemical parameters of 18 days old seedlings were recorded. Seedling height decreased in all treatments. A gradual decrease in relative water content, pigment and protein contents of wheat seedlings were observed. Sugar and proline contents increased in treatments. An increase in malondialdehyde (MDA) content and antioxidative enzyme activities was recorded. Elevation in catalase activity was observed in all treatments except in plants with water deficit. Ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) activities increased when residue mixed with soil but decreased in seedlings under the combined influence of the residue and water stress. Higher amount of MDA and lower activities of APX and GPX reflected the oxidative damage in seedlings under combined treatments. Rice residue inhibited growth of wheat seedlings. Water stress intensified the effects of residue.

Key words: allelochemicals, antioxidants, lipid peroxidation, proline, rice residue, water stress.

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Key words: allelochemicals, antioxidants, lipid peroxidation, proline, rice residue, water stress.

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; DW, dry weight; FW, fresh weight; GPX, guaiacol peroxidase; H_2O_2, hydrogen peroxide; $HgCl_2$, mercuric chloride; LP, lipid peroxidation; MDA, malondialdehyde; NBT, nitroblue tetrazolium; ROS, reactive oxygen species; RWC, relative water content, WS, water stress; SOD, superoxide dismutase; TCA, trichloroacetic acid; TW, turgid weight.

Lambers *et al*. (1998) described allelopathy as "suppression of plant growth due to release of chemicals from another species". The growth and development of plants are influenced by allelochemicals and physical interferences. Kumar and Goh (2000) defined plant residue as plant or plant parts left in field for decomposition after harvesting of crop. Retention and incorporation of stubbles and plant debris in cropping system have been adopted by farmers. Stubble retention may be beneficial in improving soil structure and water infiltration but negative aspects cannot be ignored. A variety of organic and inorganic compounds are released in soil from crop residues. In aerobic condition these compounds are digested rapidly by microbial activity but in anaerobic conditions volatile fatty acids and other organic acids accumulate. Accumulation of phytotoxic compounds results in soil sickness causing decline in quality and yield of crops. There is risk of phytotoxic effects of chemicals released due to decomposition. Allelochemicals are released into the soil from the living and dead plant tissue and debris. A wide range of natural compounds are synthesized in the plants and released in the soil (Wink, 1999). Allelochemicals in soil are exposed to physiochemical and biochemical processes and may serve as carbon skeleton for production of new toxins by microorganisms (Blum *et al*., 1995) or they interact synergistically resulting further inhibition of plant growth.

A wide array of compounds including phenolic acids, cytokinins, alkyl resorcinols, momilactones B, flavonoids and steroids has been reported from the root exudates of rice seedlings. The phytotoxic effect of rice straw and stubble on the successive crop has been evaluated (Chung *et al*., 2001). The soil amended with decomposed rice residue contain higher concentration of momilactone B and several phenolic acids such p-hydroxybenzoic acid, p-coumaric, ferulic, syringic and vanillic acids which were responsible for inhibition of plant growth in successive crops (Kong *et al*., 2006).

In natural environmental conditions plants growth and development are not only influenced by soil but are also affected by abiotic factors like drought, heat, heavy metal, etc. Drought is one of the most limiting factors for plant growth (Anjum *et al*., 2003). Plants subjected to water stress suffer from reduced biomass (Specht *et al*., 2001), chlorophyll content (Massacci *et al*., 2008) and relative water content (Lawlor and Cornic, 2002). Drought inhibits various processes such as photosynthesis, respiration, transpiration, nutrient metabolism and biosynthesis of protein, carbohydrates and growth promoters (Bray, 1997)

Various metabolic processes in different plant organs result in production of reactive oxygen species (ROS) as byproduct (Foyer and Harbinson, 1994). ROS production is increased in response to plant stimuli due to pathogen attack, hormones signaling, polar growth and gravitropism (Mori and Schroeder, 2004). In steady state the ROS are continuously scavenged by antioxidant defense components (Navrot *et al*., 2007). The imbalance in any cell

compartment between ROS production and antioxidant defense causes oxidative stress resulting into oxidative damage (Karuppanapandian and Manoharan, 2008). The reports on combined effects of rice residue and water stress are scanty. The aim of present study was to investigate the effect of rice residue on morphological and biochemical properties of water stressed wheat seedlings.

MATERIALS AND METHODS

Plant materials and chemicals

Certified and pure line seeds of wheat (*Triticum aestivum* L. cv. Shatabadi) were procured from a seed agency in Allahabad, Uttar Pradesh, India.

Stress treatment

Rice plants were grown in Roxburgh Botanical Garden, Department of Botany, University of Allahabad, Allahabad (24°47' and 50°47' N latitude; 81°91' and 82°21' E longitude; 78 m above sea level). The harvested plants and debris were air dried in shade and chopped into 1-2 cm long pieces. The plant debris was mixed in field soil in 6 gm debris/kg soil and water was added to promote decomposition. The mixture was covered with tarpaulin and incubated for 15 days. The plant residue amended soil was taken as 100% (R_2). The mixture of residue and garden soil in 1:1 ratio was considered as 50% (R_1). Garden soil was taken as control. Experimental trays (length 30 cm; width 30 cm; height 10 cm) were each filled with soil mixture according to the treatments. The wheat seeds were surface-sterilized with 0.01% $HgCl_2$ solution for 1 min and washed for three times and then soaked in distilled water. Sterilized seeds of uniform size were sown at equal distance at the rate of 25 seeds per experimental tray. Seedlings were maintained in the growth chamber (temperature: 28±2°C; photoperiod: 18h; humidity: 65±5% and photon flux density: 240 µmol $m^{-2}s^{-1}$). The experiment was performed in triplicate. The trays with fifteen days old seedlings were grouped into two sets and one set was subjected to water stress by withholding water supply for 3 days while other set was regularly irrigated with water as and when required. Eighteen days old seedlings were harvested for biophysical and biochemical analyses. The seedling height was recorded.

Relative water content

The first fully expanded leaves were sampled and cut into discs of uniform size. Fresh weight (FW) of 10 discs from each treatment and control was recorded and then they were immediately floated in double distilled water at 25°C in dark. After 24 h turgid weight (TW) of discs was recorded and then oven dried at 80°C for dry weight (DW). Relative water content (RWC) was calculated following Bars and Weatherly (1962).

RWC (%) = (FW-DW) / (TW-DW) X 100

Sugar content

Sugar content was determined following method of Hedge and Hofreiter (1962). Plant leaves (100 mg) were homogenised in 5 mL 95% (v/v) ethanol. The homogenate was centrifuged at 4000 *g* for 15 min. The supernatant (0.1mL) was mixed with 0.9 mL distilled water and 4 mL anthrone solution (0.2% Anthrone and concentrated H_2SO_4). The reaction mixture was boiled in water bath for 15 min. Absorbance was recorded at 620 nm. The amount of

total soluble sugar was calculated using standard curve obtained from glucose as reference.

Protein and pigment content

The protein content was determined following Lowry *et al*. (1951). The amount of protein was calculated using standard curve obtained from bovine serum albumin as reference. The pigments viz. chlorophyll a, chlorophyll b and carotenoids from the leaves of experimental plants were extracted with 80% acetone and estimated following Lichtenthaler (1987).

Free proline content

Free proline content was determined following the method described by Bates *et al*. (1973). Plant leaves (250 mg) were homogenised in 3% (w/v) sulphosalicylic acid and centrifuged at 4000 *g* for 15 min. The supernatant was mixed with acid- ninhydrin reagent prepared by mixing 1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL of 6 M orthophosphoric acid and acetic acid. The reaction mixture was boiled for 1h and extracted with 4mL toluene. The absorbance of chromophore containing toluene was determined at 520 nm. Amount of free proline was expressed in term of µmol g^{-1}FW.

Lipid peroxidation

Lipid peroxidation was measured by estimating the malondialdehyde content (MDA) following Heath and Packer (1968). Wheat leaves (200 mg) were homogenised in 5 mL of 0.01% w/v of trichloroacetic acid (TCA) and centrifuged at 10,000 *g* for 10 min. One mL of supernatant was mixed with 4 mL of 0.5% (w/v) thiobarbituric acid (TBA) prepared in 20% TCA. The mixture was heated in water bath at 95° C for 30 min followed by quick cooling and centrifuged at 10,000 *g* for 10 minutes. The absorbance of supernatant was recorded at 532 nm and corrected by subtracting the non-specific absorbance at 600 nm. MDA content was determined by using extinction coefficient of 155 $mM^{-1}cm^{-1}$ and expressed as µmol g^{-1} FW.

Preparation of antioxidant enzymes extract

Enzyme extract was prepared by homogenising 500 mg of plant leaves in 10 mL of sodium phosphate buffer (0.1M, pH 7.0, 1% PVP). The homogenate was filtered through cheese cloth and centrifuged at 15,000 *g* for 30 min in cooling centrifuge (Remi instruments C 24). The supernatant was collected, stored at 4° C and used as enzyme extract for determining the activities of superoxide dismutase, catalase, ascorbate peroxidase and guaiacol peroxidase

Assay of antioxidant enzymes

The superoxide dismutase (EC 1.15.1.1) was assayed according to the method of Beyer and Fridovich (1987) by measuring the activity of superoxide dismutase required to inhibit photochemical reduction of nitroblue tetrazolium (NBT). The 4 mL reaction mixture consisted of 20 mM methionine, 0.15 mM ethylene diamine-tetra acetic acid (EDTA), 0.12 mM NBT and 0.5 ml supernatant. Riboflavin 11.96 µM was administered at the end. Test tubes were exposed to fluorescent lamps for 30 min and identical un-illuminated assay mixture served as blank. The absorbance was recorded at 560 nm. One unit of enzyme was expressed as the amount of enzyme which caused 50% inhibition of NBT

reduction.

Catalase (EC1.11.1.6) activity was assayed following Cakmak and Marschner (1992). Assay mixture in a total volume of 2 mL contained 25 mM sodium phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.2 mL enzyme extract. The activity was measured by determining the rate of disappearance of H_2O_2 per min at 240 nm and calculated using an extinction coefficient of 39.4 $mM^{-1}cm^{-1}$ and expressed as enzyme unit $g^{-1}FW$. One unit of catalase was determined as the amount of enzyme required to oxidize 1µM $H_2O_2min^{-1}$.

Ascorbate peroxidase (EC1.11.1.11) was assayed on the method based on Nakano and Asada (1981). Assay mixture (2 mL) contained 25 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H_2O_2 and 0.2 mL enzyme extract. The absorbance was recorded for 1 min at 290 nm and calculated using extinction coefficient of 2.8 $mM^{-1}cm^{-1}$. Enzyme specific activity was measured as enzyme unit $g^{-1}FW$ as the amount of enzyme required to oxidise 1µM ascorbate min^{-1}.

Guaiacol peroxidase (EC 1.11.1.7) was assayed following Hemeda and Klein (1990). The reaction mixture (2mL) consisted of 25 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.05% guaiacol, 1.0 mM H_2O_2 and 0.2 mL of enzyme extract. The increase in absorbance due to oxidation of guaiacol was monitored at 470 nm. The enzyme activity was calculated using extinction coefficient of 26.6 $mM^{-1}cm^{-1}$ and expressed as enzyme unit $g^{-1}FW$.

Statistical analysis

Statistical significance was assessed at the P< 0.05 level using one way ANOVA and means were separated by Duncan's multiple range test (P<0.05) with the help of SPSS 10 software. Mean (± SE) were calculated from 3 replicates.

RESULTS

Seedlings height, relative water content and sugar content

The growth of wheat seedlings was measured in terms of seedlings height. A significant decrease in seedling height was observed under all treatments. Plants under water stress (WS) recorded 1.27 folds decrease in height over control. A gradual decline in seedling height was observed in water stressed seedlings grown in residue with maximum 32% inhibition in R_2+WS. The relative water content (RWC) of wheat leaves was affected in response to water stress and residue treatments. A significant ($p<0.05$) decrease was observed in RWC of R_1 and R_2 treatments. However, higher reduction in RWC was observed in R+WS and WS treatments with maximum decrease of 1.52 times in R_2+WS. The amount of total soluble sugar was affected in response to the treatments. Plants under water stress recorded significant 46% increase in amount of sugar. R_1 and R_2 treatments resulted in increased sugar content. The sugar content further increased in combined treatments when compared with seedlings grown in soil amended with residue (Table 1).

Protein and pigment contents

The inhibitory effect of residue and water stress on protein and pigment contents of the seedlings was observed. The amount of protein and chlorophyll was significantly less in seedlings grown in soil amended

with residue. The chlorophyll and protein content decreased when amount of residue increased. Water stress caused reduction in pigment and protein content significantly. In combined treatments (R+WS), pigment content decreased with maximum 40.4% inhibition in R_2+WS. However, protein content was slightly elevated in combined treatments when compared to R_1 and R_2 (Table 1; Figure 1).

Proline content

A drastic increase in the amount of free proline was recorded in seedlings grown in water stress. Water stressed seedlings expressed maximum level of proline. Proline was not significantly altered in seedlings grown in residue. The combined treatment of residue and water stress caused elevation in proline content but was lower than that recorded in WS seedlings (Table 2).

Lipid peroxidation

The lipid peroxidation (LP) was measured in terms of MDA content. The amount of MDA increased in water stressed seedlings grown in soil incorporated with residue. Higher amount of residue (R_2) caused 24% increase in MDA content. The combined treatments (R+WS) elevated MDA content significantly with 102.48% elevation in R_2+WS. The residue intensified the adverse effect of water stress (Table 2).

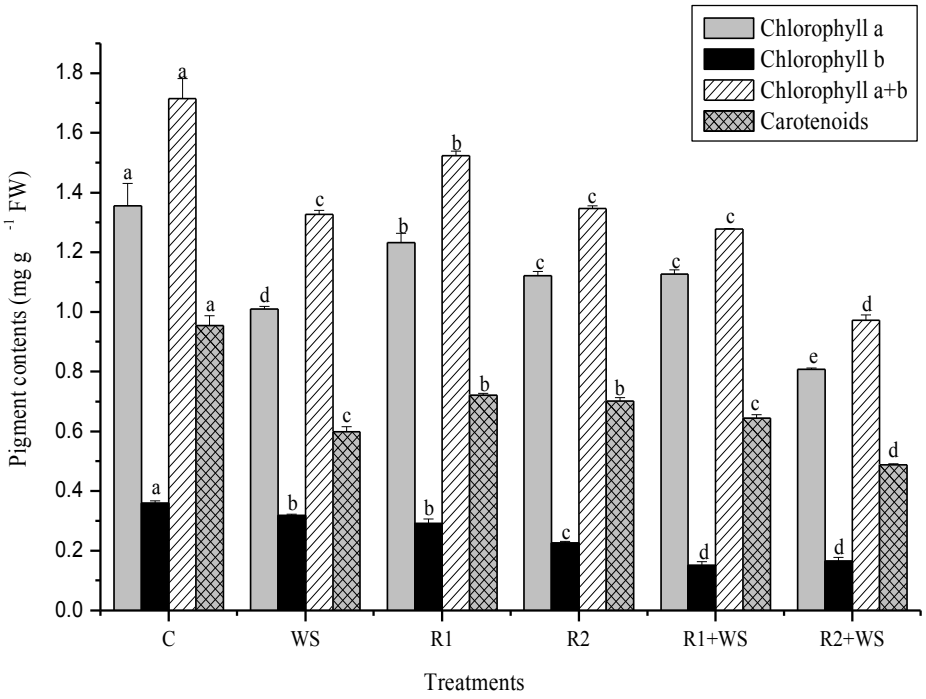

Figure 1. Interactive effects of rice residue and water stress on total pigment content of *Triticum aestivum* L. Mean±SE values followed by same letters are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. C= control, WS = water stress, R_1= 50% residue, R_2= 100% residue amount.

Table 1: Interactive effects of rice residue and water stress on seedling height, relative water content (RWC), protein and sugar content of *Triticum aestivum* L.

TREATMENT	SEEDLING HEIGHT (cm)	RWC (%)	SUGAR (mg/g FW)	PROTEIN (mg/g FW)
C	8.65±0.259a	69.81±1.615a	46.07±1.418e	26.52±0.682a
WS	6.80±0.404b	48.01±2.126d	67.18±1.072a	18.81±1.984d
R_1	7.40±0.115b	61.61±1.037b	49.86±1.214d	22.34±0.887bc
R_2	6.95±0.086b	58.30±0.301bc	54.09±1.593c	19.86±0.541cd
R_1+WS	7.00±0.230b	55.49±1.819c	59.54±1.119b	24.48±0.588ab
R_2+WS	5.90±0.230c	45.95±0.675d	63.95±0.732a	20.82±0.985cd

Mean±SE values followed by same letters are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. C= control, WS = water stress, R_1= 50% residue, R_2= 100% residue amount.

Table 2: Interactive effects of rice residue and water stress on proline content, lipid peroxidation and antioxidant enzyme activity of *Triticum aestivum* L.

TREATMENT	PROLINE (mg g^{-1} FW)	LP (n mol g^{-1} FW)	SOD (EU g^{-1} FW)	CAT (EU g^{-1} FW)	APX (EU g^{-1} FW)	GPX (EU g^{-1} FW)
C	0.316±0.053d	25.01±1.583d	60.89±2.151d	0.543±0.055c	0.560±0.035c	1.215±0.118a
WS	1.439±0.158a	34.25±3.142c	73.43±2.009c	0.403±0.010d	1.091±0.194a	0.574±0.145d
R_1	0.441±0.108cd	28.60±1.056cd	71.13±2.601c	0.637±0.057bc	0.783±0.011bc	0.899±0.114bc
R_2	0.611±0.098cd	31.05±1.448c	84.98±2.135b	0.747±0.028ab	0.976±0.027ab	1.014±0.053ab
R_1+WS	0.715±0.098bc	44.41±0.898b	89.65±1.150b	0.755±0.026ab	0.698±0.038bc	0.872±0.017bcd
R_2+WS	0.989±0.138b	50.62±1.457a	97.53±1.145a	0.871±0.033a	0.416±0.056d	0.705±0.019cd

Mean±SE values followed by same letters are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. C= control, WS = water stress, R_1= 50% residue, R_2= 100% residue amount.

Antioxidant enzyme activities

The activities of ROS scavenging enzymes enhanced in wheat seedlings under stress. The gradual increase in SOD activity corresponded to LP. The combined (R+WS) treatments recorded higher activity of SOD than R_1 and R_2. Maximum increase of 60 and 40% was observed in R_2+WS in comparison to control and WS respectively. CAT activity recorded a gradual increase in all treatments except WS when compared to control. Highest CAT activity was observed in R_2+WS. The activity of APX increased in the wheat seedlings grown in residue with higher activity in R_2. Maximum increase of 95% was recorded in WS. The APX activity decreased in combined treatments when compared with R_1 and R_2 treatments. The activity of APX was lowest in R_2+WS as it decreased by 26% in comparison to control. GPX activity corresponded to APX with maximum in R_2+WS treatment (Table 2).

DISCUSSION

In natural conditions plants are generally subjected to more than one type of stresses. The present study demonstrates the phytotoxic influence of rice residue and water stress on growth and metabolism of wheat seedlings. The decomposed plant residue has potential secondary metabolites/allelochemicals which are released into soil rhizosphere. The decomposing products have shown toxic effects on seedlings causing stunted plant growth, inadequate nutrient uptake and chlorosis.

Biotic and abiotic stresses induce the production of allelochemicals in plants. Gershenson (1984) reported that water stress enhanced the biosynthesis of secondary metabolites. Our results illustrate reduction in seedling height under both stresses. Plant residues have been found to suppress the growth of other plants (Guenzi and McCalla, 1962). Water stress intensified the effects of allelochemicals. Rice residue suppresses growth of successive crops (Chung *et al*., 2001). Physiological processes like cell wall extension, protein synthesis, enzymatic activities and plant water relations are affected by allelochemicals (Rice, 1984; Baziramkenga *et al*., 1997). Drought stress is an established limiting factor at initial phase of plant growth. Cell expansion and elongation may be suppressed due to low turgor pressure caused by water deficit. The allelochemicals released from residue and water stress caused reduction in growth, resulting in decreased photosynthetic area.

RWC of seedlings decreased significantly under water stress as well as allelochemical stress. Phenolic compounds are known to cause water deficiency in plants (Barkosky and Einhellig, 2003; Hussain and Regiosa, 2011). Water stress decreased the relative water content of plant leaves (Sánchez-Blanco *et al*., 2002). The phenolic compounds and water stress primarily affect root cell membrane by changing configurations, causing ion efflux and inhibition of water and nutrient uptake (Baziramkenga *et al*., 1995) and thus alter the plant water relations. Lower water content is the cause of sugar accumulation in plants under stress (Kameli and Losel, 1993). The soluble sugar content increased in response to all treatments. The elevated sugar level observed in stressed seedlings can be explained by less utilization of sugar for growth of seedlings subjected to stress (Asgharipour *et al*., 2011). Soluble sugars act as osmoprotectant causing stabilization of cell membrane and maintenance of turgor pressure (Gupta and Kaur, 2005).

Incorporation of certain amino acids in protein is inhibited by phenolic acids resulting into decreased rate of protein synthesis (Baziramkenga *et al*., 1997). Elevated ROS production causes oxidative damage leading to protein degradation. The slight increase of protein in R_2+WS in comparison to WS may be due to accumulation of some proteins induced during water stress (Pelah *et al*., 1997). Total chlorophyll and carotenoid contents were found to decline in plant grown in residue and water deficit condition. Reduction in chlorophyll synthesis (Yang, 2002) or elevation in chlorophyll degradation (Kanchan and Jayachandra, 1980) has been reported in response to allelochemicals. Water stress caused greater decrease in chlorophyll and carotenoid of wheat seedlings in combined treatments and thus elevated the inhibitory effect of the allelochemical. Carotenoids stabilize and protect the lipid phase of the thylakoid membrane, and quench the excited triplet state of chlorophyll and singlet oxygen (Farooq *et al*., 2009). Thus carotenoids protect chlorophyll from photooxidative destruction. Decreased amount of carotenoids reflects the level of oxidative damage resulting in degradation of chlorophyll in wheat plants due to stress.

It is clear from our data that stress caused accumulation of proline. Water stress resulted in greater accumulation of proline in plants in comparison to plants grown in residue. Reddy *et al*. (2004) correlated proline accumulation to drought as it acted as osmolyte as well as compatible solute. It plays protective role in adaptation of plant cell against water deficit (Ueda *et al*., 2008). Proline accumulation may be a part of stress signals and thus effecting adaptive response of plants under stress (Maggio *et al*., 2002).

Lipid peroxidation increased significantly in treatments. Lipid peroxidation of membranes is considered as indicator of oxidative stress (Smirnoff, 1993). Membrane lipids are sensitive to ROS produced under stress. The ROS production along with H_2O_2 is triggered under stressful condition due to environmental stresses such as water stress and allelopathic stress. Biotic and abiotic stresses disrupt the equilibrium between ROS production and their degradation by the defense system of plants. Oxidative damage is caused by accumulation of superoxide and hydroxyl radicals. The production of superoxide radicals increased under allelochemicals and water deficiency which caused lipid peroxidation resulting in membrane damage (Halliwell and Gutteridge, 1999). The ROS generated are removed with the help of antioxidant enzymes viz. SOD, CAT, APX and GPX. Allelochemicals induced SOD activities in maize (Singh *et al*., 2009) and mung (Singh *et al*., 2010). Drought stress in presence of allelochemicals enhanced SOD activities in maize (Singh *et al*., 2009). SOD converts free oxygen radical to H_2O_2. H_2O_2 accumulation is toxic to cells. Toxic H_2O_2 is detoxified by CAT, APX and GPX. Madhusudhan (2003) reported that APX and GPX are responsible for conversion of H_2O_2 to H_2O using ascorbate and guaiacol as electron donor to decompose H_2O_2. Thus increased CAT, APX and GPX activities in seedlings grown in soil with residue reflect the plant struggle to adapt under stressful conditions. However, reduced activities of antioxidant enzymes in combined stresses reflect that the seedling were not able to cope with the environmental stresses.

CONCLUSIONS

It can be concluded from the present study that rice residue and water stress in single and combined treatments affected the growth and relative water content of wheat seedlings. All treatments caused degradation of pigment content or inhibition of biosynthesis. Water stress influenced sugar and protein contents. Rice residue and water stress caused oxidative stress which was evident from elevated MDA and proline content. Activities of antioxidant enzymes increased due to exposure of water stressed seedlings to residue. Simultaneous exposure of wheat seedlings to residue and water stress suppressed the antioxidant enzyme systems. Activities of antioxidant defense system against oxidative stress broaden the understanding of impact of two different stresses on wheat plants. The plants were not able to overcome the damage caused by the two stresses in combination thus reflecting the detrimental nature of allelopathic and drought stresses. Stubble retention in agricultural crop land may cause some problems. The monotype of crop

rotations in fields where the load of crop residues is heavy may experience adverse effect.

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