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Effects of rice residues and water deficit on growth and metabolism of *Triticum aestivum* L.

NIMISHA AMIST, N. B. SINGH* and KAVITA YADAV

Plant Physiology Laboratory, Department of Botany,
University of Allahabad, Allahabad-211002, India.
E. Mail: nbsingh.au@gmail.com

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ABSTRACT

We investigated the morphological and physiological processes of wheat seedlings under the influence of rice residue with and without water stress. Phenolic contents increased in the rice residues amended soil but decreased by activated charcoal (AC). Seedling emergence decreased with increase in dose of rice residue. Wheat seedlings height, dry weight, relative water content and pigment content decreased when grown in residue amended soil with and without water stress. The soil incorporated residues significantly decreased the protein content but water deficit alone and with residue incorporated increased the protein content. Higher level of sugar and proline contents was recorded in the water stressed seedlings in comparison to control and activated charcoal treated seedlings. Electrolyte leakage and malondialdehyde contents increased in the stressed seedlings as compared with that recorded in control and activated charcoal treatments. A concentration dependant increase in activities of antioxidant enzymes viz. superoxide dismutase, catalase, ascorbate and guaiacol peroxidases was observed in the seedlings grown in soil amended with rice residue while combined stresses resulted in gradual decrease. This study helped in obtaining an insight into crop interaction with combined biotic and abiotic stresses and thus may provide a futuristic goal to maintain sustainable agriculture.

Keywords: Activated charcoal, allelochemicals, antioxidants, electrolyte leakage, lipid peroxidation, reactive oxygen species, water deficit.

INTRODUCTION

Monocultures of high yielding crops are common in modern agro-ecosystems, however, monoculture has adverse effects like accumulation of residue of synthetic agrochemicals, less diversity and development of resistant pests harmful to crops. Sustainable agro-ecosystems can be attained by maintaining balance between the crop diversity, space and time. Plants are the storehouse of multiple chemicals synthesized as secondary metabolites, which influences the physiological functions and thus play important role in plant-plant interactions. Lambers *et al.* (30) described allelopathy as "suppression of plant growth due to release of chemicals from another species". Plants produce both primary and secondary metabolites and these are released into environment. Secondary metabolites released from the plants are called allelochemicals, which play

*Correspondence author.

major role in plant defence mechanism. The allelochemicals exert negative influences on the neighbouring plants. Recently the retention and incorporation of stubbles and plant debris in cropping system have been adopted by farmers. Stubble retention may be beneficial to improve the soil structure and water infiltration but their negative aspects cannot be ignored viz., phytotoxic effects of chemicals released from stubbles decomposition (9,10). Decomposed plant litter has positive and negative impacts on germination and growth of same species and other plants species. Allelochemicals released during the decomposition of plant debris (9,10), effects the cell division, cell differentiation, water status, photosynthesis, enzyme function, signal transduction as gene expression (51).

In field conditions crops experience various combination of abiotic stresses (39). The abiotic stresses (drought, high salinity, cold and heat) decrease the crop survival rate, biomass production and crops yield up to 70% (62). Drought or water deficit stress is major environmental stress on plants and adversely affects most plant functions. Water deficit influences various processes viz., closure of stomatal aperture translocation, nutrient metabolism, ion uptake and altered protein synthesis and carbohydrates, resulting into retarded growth, reduced biomass (58), decreased relative water content (32), decreased chlorophyll content and grain yields.

Rice and wheat are major crops grown worldwide. In cropping systems rice is followed by wheat. Rice-wheat cropping system (RWS) is practiced in China and states like Uttar Pradesh, Punjab, Bengal in Indian subcontinent and south-east Asia (44). Alsaadawi *et al*., (2) has already shown the allelopathic inhibition of rice by wheat residue. Wheat is sown in same field, 1-2 weeks after the rice harvest. Underground plant parts, leaf litter and debris of rice are left in the soil to decompose. Thus the wheat plants are exposed to synergistic effects of water deficit and allelopathy. Little information is available about the combined effects of crop residues allelopathy and water deficit. Hence, this study aimed to explore the impact of water deficit and rice crop residues on succeeding wheat crop.

MATERIALS AND METHODS

The seeds of test crops viz., rice (*Oryza sativa* cv. Pusa Basmati 1) were procured from Prayag Seed Agency, Allahabad and wheat (*Triticum aestivum* cv. Deva/K-9107) from Crop Research Farm, Mauranipur, Jhansi, Uttar Pradesh. To study the effects of allelopathy and water deficit on growth and metabolism of recipient plants, the allelopathic stress was mediated through incorporation of rice residues at various doses in garden soil. Water deficit was induced by withholding the water supply for 6 days in wheat seedlings.

Rice residue

Rice crop was raised in experimental plots, Department of Botany, University of Allahabad, Allahabad ($24^{o}47'$ and $50^{o}47'$ N latitude; $81^{o}91'$ and $82^{o}21'$E longitude; 78m above sea level) from July to September in 2010. Nursery of rice was raised from 15 June, 2010 to 6th July, 2010. The 21 days old rice seedlings were transplanted in plots on 7th July, 2010. The mature rice plants were harvested on 30th September, 2010. The healthy and disease free, mature donor rice plants were collected from field with earheads and the

grains were removed. The plants were washed to remove dirt and dust and air dried in shade for 48 h. The whole plants were chopped into 1-2 cm long pieces. The chopped plant pieces were mixed with garden soil at 8 g rice debris/kg soil and placed in large pits lined with tarpaulin to prevent leaching of extracts from the decomposing residues into the soil. Residue was covered with tarpaulin to avoid excessive water from rains. The soil with rice residue was irrigated with water till saturation, to promote the decomposition of rice residues and decomposition was allowed for 15 days.

Wheat crop

The experimental treatments consisted of 3- factors: (i). Rice residue rates: 5 (0,2,4,6,8 g/kg soil) and (ii). Water deficits: 3 levels (No water deficit, water deficit, water deficit + rice residue), (iii). Activated charcoal: 2 (Control, Activated charcoal). Activated charcoal was added at 50g/kg soil i.e. 5% (w/w). The experiment was done in glasshouse at optimum temperature (20-25°C) with natural sunlight. The rice plant residue was incorporated in soil at 0,2,4,6 and 8 g/kg soil. The control was garden soil alone. Additionally, 8 g rice residue/kg soil was mixed with 5% activated charcoal (w/w) i.e. 50g/kg soil.

Experimental trays (30 x 30 x 10 cms (length, width, height) were filled with soil mixture as per treatments. The wheat seeds were surface-sterilized with 0.01% $HgCl_2$ solution for 1 min and washed thrice with double distilled water and then soaked in distilled water for 3 h. The seeds were sown at equal distance in 5-rows in each tray. The row to row and plant to plant spacing was 5 cm. Three replicates were taken. Wheat seeds were sown in rice residue amended soil on October 18, 2010 and were harvested on 8th November 2010 for biophysical parameters and biochemical analyses. After 15- days, pots for each treatment were grouped into two sets and one set was subjected to severe water deficit by withholding water supply for 6 days and the other set was regularly irrigated with water as required**.** To measure the biophysical parameters [seedling height, dry weight and relative water content (RWC)], 21-days old seedlings were harvested. As the first fully expanded leaves are mature and metabolically active, hence, were sampled for biochemical analyses.

Seedling emergence and plant growth

Seedling emergence was recorded every 24 h upto 6 days and Emergence rate was calculated as under:

$$ER = \sum (\text{number of seedlings emerged}) / (\text{number of days})$$

Plant height of test plants was measured with measuring tape. Twenty one days old seedlings were harvested and their height were measured from root-shoot transition zone to the tip of young leaf emerged from top of the seedlings.

Seedlings biomass was recorded based on dry weight (mg/plant). Twenty one days old seedlings were harvested and kept in oven at 80°C. After 48 h plant materials were removed from oven and dry weight was recorded.

I. Relative water content: The stressed and non stressed plant leaves were cut into discs of uniform size. Fresh weight (FW) of 10 discs from each treatment was recorded and then

they were immediately floated in double distilled water at 25ºC in dark. After 24 h turgid weight (TW) of discs was measured and then oven dried at 80ºC for dry weight (DW). Relative water content (RWC) was calculated as per Bars and Weatherly (3).

$$RWC\ (\%) = (FW\text{-}DW) / (TW\text{-}DW)\ X\ 100$$

II. Sugar content: Total soluble sugar was quantified as per Hedge and Hofreiter (22). Plant leaves (100 mg) were taken and homogenised in 5 mL 95% 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. 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.

III. Protein and pigment content: The protein content was determined following Lowry *et al.* (36). The amount of protein was calculated using standard curve obtained from bovine serum albumin as reference. The pigments (chlorophyll a, chlorophyll b and carotenoids) from leaves of experimental plants were extracted with 80% acetone and estimated following the method of Lichtenthaler (35).

IV. Free proline content: Proline content was determined as per the modified method of Bates *et al.* (4). Plant leaves (250 mg) were homogenised in 3% 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 1 h and extracted with 4 mL 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.

V. Determination of electrolyte leakage: Fresh leaves were harvested (100 mg) from stressed and control seedlings. They were cut into small pieces and then immersed in 10 mL of deionized water and kept for 24 h at 10 °C. Electrical conductivity was measured and then the leaf tissues were autoclaved for 15 min, cooled to 25°C, and the electrical conductivity was measured for the second time. Electrolyte leakage was evaluated as the percentage injury index following the formula of Sullivan (59):

$$EL= [1-(1-T_1 /T_2)/ (1-C\ 1/C_2)]\ x\ 100\%,$$

Where, C_1 and C_2: Measured conductivity in control samples before and after autoclaving, respectively; T_1 and T_2: Conductivity measurements of water-stressed samples before and after autoclaving, respectively.

VI. Lipid peroxidation: Lipid peroxidation was measured by estimating the malondialdehyde content (MDA) following Heath and Packer (21). 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% thiobarbituric acid (TBA) prepared in 20% TCA. The mixture was heated in water bath at 95º 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.

VII. Preparation of antioxidant enzymes extract: Enzyme extract was prepared by homogenising 500 mg plant leaves from each treatment 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 4º C and used as enzyme extract for determining the activities of superoxide dismutase, catalase, ascorbate peroxidase and guaiacol peroxidase.

VIII. Assay of antioxidant enzymes

(i). The superoxide dismutase (EC 1.15.1.1) was assayed according to the method of Beyer and Fridovich (7) by measuring the activity of superoxide dismutase 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 an identical un-illuminated assay mixture served as blank. The absorbance was measured at 560 nm. One unit of enzyme was measured as the amount of enzyme which caused 50% inhibition of NBT reduction.

(ii). **Catalase activity (EC1.11.1.6):** It was assayed following Cakmak and Marschner (12). Assay mixture in a total volume of 2 ml contained 25 mM 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 $H_2O_2min^{-1}$.

(iii). **Ascorbate peroxidase (EC1.11.1.11):** It was assayed as per the method of Nakano and Asada (41). Assay mixture (2 ml) contained 25 mM 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 read for 1 min at 290 nm (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 oxidized 1µM ascorbate min^{-1}.

(iv). **Guaiacol peroxidase (EC 1.11.1.7):** It was assayed following Hameda and Klein (23). The reaction mixture (2 ml) 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.

IX. Total phenolic contents in soil: The amount of total phenolics in soil was determined using Folin-ciocalteu reagent as per the method of Swain and Hillis (60).

Statistical analysis: Treatments were arranged in randomized block design with three replicates. Statistical significance was assessed at $P< 0.05$ level. Data were statistically analyzed using analysis of variance (ANOVA) by using SPSS (Ver.10; SPSS Inc., Chicago, IL, USA). The treatment means were analyzed by Duncan's multiple range test (DMRT) at $P< 0.05$.

RESULTS AND DISCUSSION

The phytotoxic activity of substances released from plant residues is affected by environmental factors [physicochemical properties of soil, microbiological inactivation and activation (46)]. The behavior of allelochemicals in soil is governed not only by the physicochemical properties but also by the soil organic matter and organisms. However, induction of stress due to any environmental factor increases the production and / or release of phenolic compounds. The concentration of allelochemicals in soil water also has key role in allelopathic activity, because receiver plants can directly absorb the allelochemicals from the soil solution. Gershenson (18) has reviewed effects of water deficit on the secondary metabolites and reported that water deficit increased the concentration of secondary metabolites thus increasing the toxicity of allelochemicals.

Effects of activated charcoal on phenolic content

The soil amended with rice plant debris have higher amount of phenolic content. Activated charcoal exhibited counteracting effects on phenolic contents in crop residues. Activated charcoal decreased the level of phenolics significantly (Figure 1). Decrease in amount of phenolic acids in soil due to absorption by activated charcoal presents strong evidence in favour of allelopathic interactions among plants. In present study, activated charcoal when mixed with residue caused increase in seedling emergence, seedling height and dry weight. Protein and sugar contents were found equal to control. Lower level of EL, LP and proline was recorded, indicating the plants tolerance to stress. Antioxidant enzyme activities were also lower in activated charcoal treated seedlings. Higher level of phenolics was found in rice residue amended soil. Activated charcoal decreased the level of phenolics significantly thus decreasing the toxicity of residue and indicating that residue phytotoxicity was due to phenolic acids. Several studies have reported that activated charcoal mitigates the effects of allelochemicals (29,31).

Seedling emergence

The rice residues at all doses significantly ($P<0.05$) decreased in seedling emergence and emergence rate. Maximum inhibition (41.59%) in seedling emergence occurred with 8 g residue/kg soil. However, incorporation of activated charcoal with residue decreased the negative impact of rice residue as only 3.3% of inhibition was observed than control (Figure 2). Germination and seedling establishment depend on external factors (42).Germination is resumption of metabolic activities and elongation and expansion of embryonic cells which ruptured seed coat and results in extrusion of radicle. An indirect relation between lower germination rate and allelopathic inhibition may be due

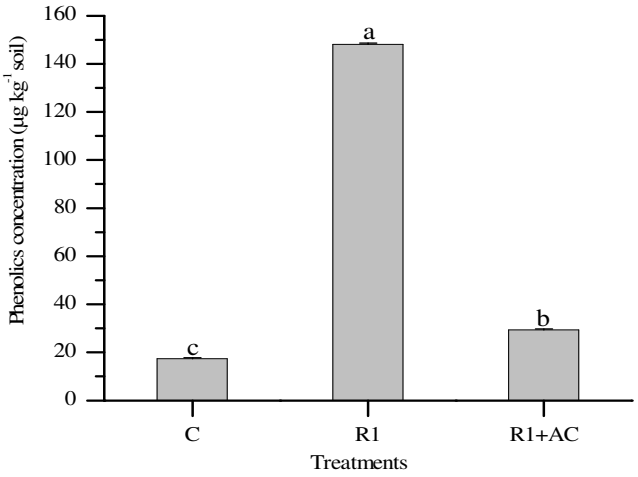

Figure 1. Phenolic concentrations of decomposed rice residues incorporated soil with and without activated charcoal. Mean values followed by same letter within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. C = control, R1 = decomposed rice residue, R1+AC = decomposed rice residue with 5% (w/w) activated charcoal.

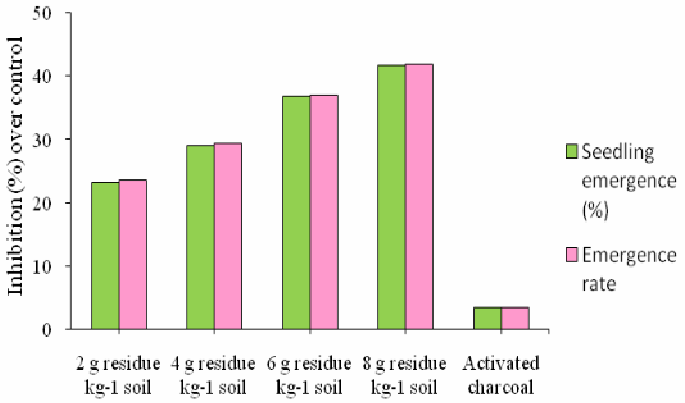

Figure 2. Effects of rice residue and activated charcoal on seedling emergence and emergence rate of wheat seeds. 2, 4, 6, 8 g rice residue kg^{-1} soil, Activated charcoal = 8 g rice residue kg^{-1} soil mixed with 5% (w/w) activated charcoal.

to the consequence of inhibition in water uptake (61). The inhibition in germination may be due to disruption of activity of metabolic enzymes involved in glycolysis and pentose phosphate pathway (63).

Plant height and dry weight

Rice residue with and without water deficit influenced the seedling height of wheat. Allelopathic effects of rice residue doses significantly ($P<0.05$) decreased the seedling height. Seedling height was inhibited by 34.7and 39.8% in treatments with 6 and

8g residue/kg soil respectively. However, only 8.28% inhibition was recorded in seedling height under activated charcoal treatment. The plants subjected to severe water deficit showed 22.9% decreased in height over control. The combined treatments (residue with water deficit) decreased height. Dry weight (DW) of wheat seedlings was decreased under both stresses. Residue incorporation in soil caused significant reduction in DW of seedlings. The combined residue and water deficit treatments further reduced the biomass (Figure 3). The 8 g residue/kg soil treatment caused maximum decrease (31.71%) in DW. Seedling growth is more sensitive to allelochemicals than seed germination (14). Our results illustrated the in seedling height decreased in response to treatments with residue and water stress. Allelochemicals alters the plant metabolic processes thus leading to reduced plant growth. Chung *et al*. (13) reported that the phytotoxicity of water extracts from different parts of rice plant as well as straw residues was inhibitory to the seed germination and seedling growth of *Echinochloa crus-galli*. Relatively small amount of wheat residue caused inhibition of root and seedling growth (2). Inhibitory effects of low doses of rice residue in soil showed the toxicity of rice residue in present study. Intensified process of lignin synthesis and its deposition decreases cell wall extensibility resulting into decreased growth (16). Low turgor pressure as a result of water deficit decreases the cell expansion and cell growth thus affecting the elongation and expansion of plant growth (47). Inhibition of cell expansion due to allelochemicals and water deficit may be possible reasons behind severe decrease in seedling height. Singh *et al*. (53, 54) reported decrease in growth under combined effect of allelochemicals and water stress. Dry weight of plants is an indicator of plant growth and development. A significant decrease in dry biomass accumulation in chickpea was observed in response to *Chenopodium murale* residue (5). Plant productivity under water deficit is strongly related to the dry matter partitioning and temporary biomass distribution (26).

Relative water content and sugar content

All concentrations of rice residue and water deficit in single and combined treatments significantly decreased the relative water content (RWC). Plants under water deficit recorded 43.49% RWC i.e. a reduction of 53.81% when compared with control. 38.07% inhibition was observed in treatments with 8 g residue/kg soil. However, combination of stresses caused greater water deficit in seedlings with maximum 59.25% reduction in combined treatments of 8 g residue/kg soil and water deficit. The effect of combined stresses was more severe than water deficit and residue treatments separately. Total soluble sugar content was variably affected under all stresses. A significant increase (142.0%) in amount of sugar was observed in plants under water deficit. Sugar content increased in all concentrations of residue and the maximum stimulation (56.99%) was with 8 g residue/kg soil. Plants under combined treatments showed significant increase in amount of sugar than control with maximum increase (217.03%) in 8 g residue/kg soil and water deficit treatment (Table 1).

Leaf relative water content is considered as a better indicator of water status than water potential (49). Certain phenolic acids decreased the water content in plants (24).Water deficit decreased the relative water content of plant leaves (45). Decreased RWC under the joint action of allelochemicals and water deficit has been observed in maize (53) and mungbean (54). Phenolic acids in contact with the root cell membrane

Table 1. Interactive effects of rice residue and water deficit on relative water content, sugar and protein contents of wheat seedlings

Treatments (g residue/kg soil)	RWC (%)	Sugar (mg g^{-1} FW)	Protein (mg g^{-1} FW)
Control	94.16a	22.42h	23.17cd
Water deficit	43.49h	54.25c	25.56b
2	66.29c	25.41g	21.78e
4	64.96c	27.29fg	19.35f
6	60.53d	28.39f	16.73g
8	58.31e	35.19e	14.77h
Activated charcoal	90.99b	23.05h	22.70de
2 +water deficit	48.66f	41.69d	21.63e
4 +water deficit	46.26g	53.31c	24.11c
6 +water deficit	41.18i	63.39b	25.92b
8 +water deficit	38.37j	71.07a	27.71a

Mean values followed by same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. C= control, water deficit = 6 days withholding water supply, 2, 4, 6, 8 g rice residue kg^{-1} soil, Activated charcoal = 8 g rice residue kg^{-1} soil mixed with 5% (w/w) activated charcoal.

leads to depolarization, an efflux of ions, and a reduction of hydraulic conductivity, water uptake and net nutrients uptake (24). Accumulation of compatible solutes in plants causes resistance to various stresses by preventing water loss and maintaining cell turgor pressure. Protective role of soluble sugars against stresses has been suggested. Lower water content and growth inhibition (27) may be the reasons behind accumulation of soluble sugars. The hydroxyl groups of sugars substitute for water in maintaining hydrophilic interactions in membranes and proteins during dehydration through hydrogen bonding (34).

Protein and pigment content

The residue incorporation caused significant decrease in protein content. Maximum decrease (36.21%) was recorded in A_4 treatment. WS significantly increased (10.33%) the protein content in seedlings. The combined treatments caused gradual increase in protein content with highest increase (19.63%) in treatments with 8 g residue/kg soil and water deficit (Table 1). Highest amount of total chlorophyll was observed in plants in control**.** Total chlorophyll content was inhibited by 62.2% in seedlings under water deficit. A gradual decrease in chlorophyll content in wheat seedlings was observed with increase in residue concentrations as highest decrease (66%) was recorded under 8 g residue/kg soil treatment**.** Incorporation of activated charcoal elevated the chlorophyll content but was lower than control. The seedlings grown in residue incorporated soil when subjected to water stress, further increase in the inhibition of chlorophyll content was recorded. The amount of carotenoids decreased considerably under all stress treatments. Carotenoids followed the trends of chlorophyll. Eight g residue/kg soil treatment recorded lowest amount of carotenoids. Water deficit decreased the amount of carotenoids with inhibition of 76.59%. The water deficit intensified the impact of residue on carotenoid content (Figure 4).

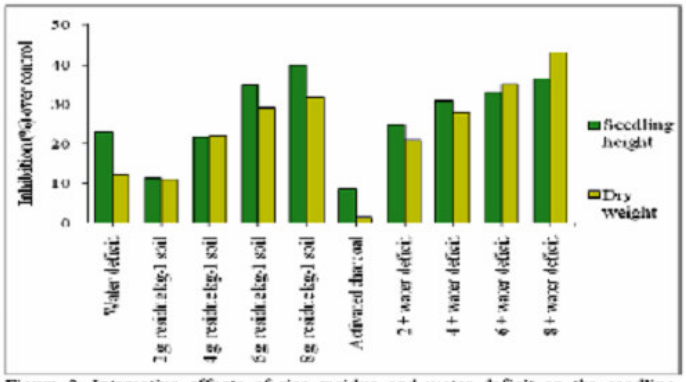

Figure 3. Interactive effects of rice residue and water deficit on the seedling height and dry weight of wheat seedlings. where : water deficit = 6 days withholding water supply, 2, 4, 6, 8 g rice residue kg^{-1} soil, Activated charcoal = 8 g rice residue kg^{-1} soil mixed with 5% (w/w) activated charcoal.

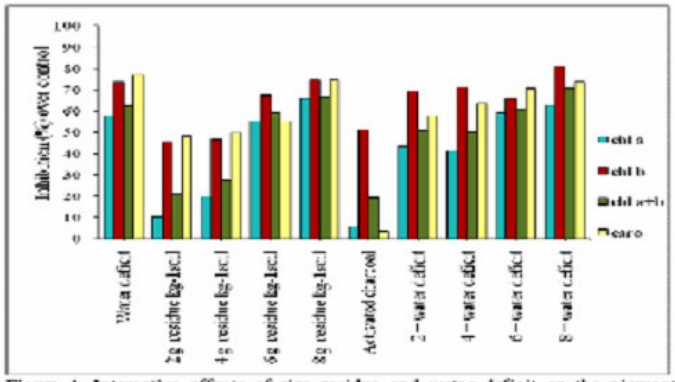

Figure 4. Interactive effects of rice residue and water deficit on the pigment content of wheat seedlings. where water deficit = 6 days withholding water supply, 2, 4, 6, 8 g rice residue kg^{-1} soil, Activated charcoal = 8 g rice residue kg^{-1} soil mixed with 5% (w/w) activated charcoal.

Table 2. Interactive effects of rice residue and water deficit on the proline content, electrolyte leakage, malondialdehyde (MDA) content and antioxidant enzyme activities of wheat seedlings

Treatments (g residue/kg soil)	Proline (mg g^{-1} FW)	Electrolyte Leakage (%)	MDA (n mol g^{-1} FW)	SOD (EU g^{-1} FW)	CAT (EU g^{-1} FW)	APX (EU g^{-1} FW)	POD (EU g^{-1} FW)
Control	0.139i	7.94g	5.76h	8.55h	0.138f	0.280f	1.73ef
Water deficit	4.241b	56.18ef	30.61de	85.31a	0.655a	1.191b	3.39cd
2	0.350hi	53.89f	25.13g	34.75ef	0.434b	1.030b	2.07e
4	0.631gh	57.32e	28.47ef	38.37d	0.385bc	1.071b	4.75ab
6	0.888f	65.02d	34.07c	43.40c	0.282cde	1.150b	2.59de
8	0.959f	76.96b	42.75b	53.64b	0.222def	1.392a	3.87bc
Activated charcoal	0.145i	8.44g	5.37h	9.65h	0.142f	0.348ef	0.99f
2 +water deficit	1.415e	56.53e	27.02fg	53.77b	0.358bc	0.467de	3.29cd
4 +water deficit	2.712d	63.01d	32.48cd	35.84de	0.319bcd	0.673c	5.01a
6 +water deficit	3.353c	72.41c	44.16b	32.22f	0.241def	0.600cd	4.74ab
8 +water deficit	5.437a	83.66a	49.84a	19.36g	0.197ef	0.555cd	4.92a

Mean values followed by same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. Control, water deficit = 6 days withholding water and Activated charcoal = 8 g rice residue kg^{-1} soil mixed with 5% (w/w) activated charcoal.

Decrease in pigment content and impairment of metabolic processes due to stresses might reduce the protein content. BOA (2-Benzoxazolinone) is degradation product of DIBOA caused reduction in protein concentration in cucumber (11) and mung bean (6). Increased protein content in the seedlings subjected to WS and combined residue and water deficit was also observed. Increased protein may be due to protective role of accumulated sugar in preventing protein denaturation or through induction of certain specific proteins in response to stresses. These proteins are referred as dehydrins and are synthesized by cells in response to environmental influences [drought, salinity or freezing] that causes dehydration (19). The allelochemicals stress increases the proteins content (28). Stimulation of biosynthesis or inhibition of degradation of protein may be the possible reason for increase in protein content in allelopathic stress (50). Effects of allelochemicals on pigment content have been studied on maize (50,52), tomato (55), mung bean (54) and wheat. Allelopathic compounds might interfere with the synthesis of porphyrin, precursors of chlorophyll biosynthesis. Diminution of chlorophyll under allelochemical stress could be owing to the inhibition of chlorophyll biosynthesis or the stimulation of degradation or both (6). The reduction in chlorophyll content and decreased photosynthetic rate under water deficit has been reported in different plants (25). Decreased amount of carotenoids reflected the level of oxidative damage resulting in degradation of chlorophyll in plants due to stress. Singh *et al.* reported reduction in pigment content in maize (53) and mungbean (54) in response to combined treatment of allelopathins and water stress.

Proline content

Amount of endogenous free proline increased significantly in response to different concentrations of rice residue with and without water stress. Maximum increase (2951.6%) over control was observed in water deficit. Low elevation in proline content was noticed in residue treated seedlings with highest (590.5%) in 8 g residue/kg soil. Combined residue and water deficit treatments elevated the proline content significantly highest in 8 g residue/kg soil and water deficit treatment (Table 2). Accumulation of proline, protects the cell by balancing the osmotic potential of cytosol with vacuole and the external environment when exposed to stress (17). Proline along with functioning as osmotic adjustment mediator (40) also acts as free radical scavenger (1) and a redox potential buffer. The extensive accumulation of ROS during stress and their contribution to damage induced by water deficit and other stresses are capable of inducing proline accumulation (57).

Electrolyte leakage and lipid peroxidation

Electrolyte leakage (EL) is an indicator of stress. Significant increase in EL level was observed in the seedling in response to residue treatments. Maximum 869.27% increase in EL was recorded in treatments with 8 g residue/kg soil. 607.55% stimulation in EL was recorded in water stressed seedlings. Water deficit further increased the membrane damage. Water deficit when combined with residue increased the EL upto 953.65% in treatment of 8 g residue/kg soil and water deficit. However, plants relieved from severe water deficit exhibited decline in EL percentage but was still higher than control. Lipid peroxidation (LP) was measured in terms of MDA content which increased significantly under all treatments. MDA content increased by 431.42% in water deficit. A gradual

increase in MDA content was observed in response to residue with higher doses causing more elevation. Inhibition of 6.75% was observed in activated charcoal. Induction of water deficit further elevated the level of MDA in wheat seedlings (Table 2).

Cell membranes are the first targets of several plant stresses and the maintenance of their integrity and stability under stress conditions is major component of tolerance in plants. Plant membranes subjected to environmental stresses change with the increase in permeability and loss of integrity (8). Allelochemical and water deficit mediated increase in EL has been reported by several workers (43,52) and correlated to sensitivity of crops to stress. Damage to membrane permeability may be due to peroxidation of polyunsaturated fatty acids in biomembranes resulting into formation of byproducts like malondialdehyde. LP is the process correlated to oxidative damage and, hence, is regarded as an indicator of oxidative stress (48). Free radicals causes peroxidation of membranes lipid and lipid peroxidation, measures the level of stress induced damage at cellular level.

Antioxidant enzyme activities

In response to rice residue and water stress, the activities of ROS scavenging enzyme varied in wheat seedlings. Activity of superoxide dismutase (SOD) in all treatments was diverse in comparison to control as well as to water deficit. Maximum 897.77% increase in SOD activity was observed in water stressed seedlings. A concentration dependant increase in SOD activity was observed in the seedlings grown in soil amended with rice residue. However, SOD activity declined in combined (residue and water deficit) treatments with only 126.43% stimulation in treatments with 8 g residue/kg soil and water deficit. Catalase activity was parallel to that SOD. Although, significant increase was observed in all treatments in comparison to control. The CAT activity decreased gradually in seedlings grown in residue amended soil with lowest activity in treatments with 8 g residue/kg soil but activity was still higher than control. Similar pattern was observed in residue and water deficit treatments. A significant ($P < 0.05$) effect of all stresses on ascorbate peroxidase (APX) and guaiacol peroxidase (POD) activities was seen. Under water stress, APX and POD activities increased over control respectively. The antioxidant enzyme activities were lower in AC treated seedlings (Table 2).

Modulation of antioxidative enzyme activities is important to evaluate the responses of plants to environmental stresses. Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (POD) work synergistically in scavenging ROS species in plants under stress. SOD activity increased under allelochemicals in tomato (37) and maize (50). In combined treatments, amended rice residue and water deficit decreased the SOD activity than single allelopathic stress treatments. Lower SOD activity due to stress has been reported (56). Levels of SOD can be correlated to plants sensitivity to stress. It is important that H_2O_2 be scavenged rapidly by the antioxidative defence system to water and oxygen (20). It is reduced to water and molecular oxygen by CAT in peroxisome, APX in cytosol and chloroplast and POD in cell wall (8). In comparison to control, CAT activity was elevated but stimulation was lower than APX and POD. Lower stimulation of CAT may be due to the inactivation and degradation under severe stress. APX and POD are most common peroxidases, which convert the H_2O_2 to H_2O using ascorbate and guaiacol as electron donors in decomposition process (38). Zhang *et al.* (64) reported increased POD under drought in maize. Since POD mainly functions in cell wall so apart from scavenging H_2O_2, POD are involved in

lignin biosynthesis and modulating cell wall properties during plant growth (33). Decreased plant growth and increased POD activity can be associated as POD restricts cell expansion, which in turn causes growth limitation due to lignin deposition (15).

CONCLUSIONS

This investigation showed that residue and water deficit resulted in stressful conditions in test crops causing oxidative burst requiring activation of defense system. This study helped in getting an insight into crop reaction to combination of biotic and abiotic stresses and thus providing a futuristic goal to help in maintaining sustainable agriculture. Involvement and activities of enzymatic and non enzymatic antioxidant defense systems broadened our understandings about the impact of two stresses on the test crops. Under combined treatments, toxicity increased. The level of toxicity decreased due to addition of activated charcoal as it reduced the phenolics content. This study gave clues about the allelopathy and water deficit interactions. There is a need to conduct this study under field conditions to fully understand these interactions.

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