

**Physiological and biochemical effects of salicylic  
acid in rice (*Oryza sativa* L.)  
under cadmium stress**

**By**

**DUONG HOANG SON  
(2005BS126M)**

*Thesis submitted to the Chaudhary Charan Singh  
Haryana Agricultural University in partial fulfilment  
of the requirements for the degree of*

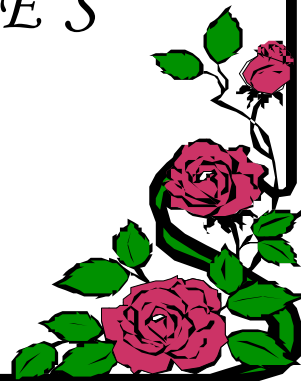
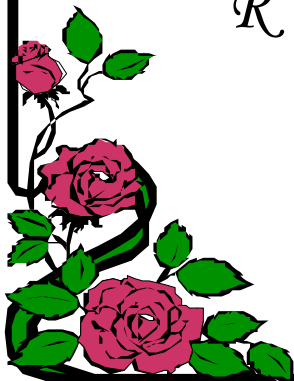
**Master of Science  
in  
Plant Physiology**



**2007**

**College of Basic Science and Humanities  
CCS Haryana Agricultural University  
Hisar - 125 004 (Haryana)**

DEDICATED TO  
MY BLOVED  
PARENTS,  
MY LOVING WIFE  
AND  
MY INTIMATE  
RELATIVES



## **CERTIFICATE – I**

This is to certify that this thesis entitled, **“Physiological and biochemical effects of salicylic acid in rice (*Oryza sativa* L.) under cadmium stress”**, submitted for the degree of **Master of Science** in the subject of **Plant Physiology** of the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Mr. Duong Hoang Son** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

**[Dr. A. L. Patel]**

Major Advisor

Professor Department of Botany & Plant  
Physiology

CCS Haryana Agricultural University  
Hisar – 125004, Haryana

## **CERTIFICATE – II**

This is to certify that this thesis entitled, **“Physiological and biochemical effects of salicylic acid in rice (*Oryza sativa* L.) under cadmium stress”**, submitted by **Mr. Duong Hoang Son** to the Chaudhary Charan Singh Haryana Agricultural University, in partial fulfillment of the requirements for the degree of **Master of Science**, in the subject of **Plant Physiology**, has been approved by the Student’s Advisory Committee after an oral examination on the same.

**MAJOR ADVISOR**  
**[DR. A. L. PATEL]**  
Major Advisor

**HEAD OF THE DEPARTMENT**

**DEAN, POSTGRADUATE STUDIES**

# Acknowledgement

---

*I would like to express my deep heart with the gratefulness and respect to teachers, friends, people who contributed to my success during two years of accumulation in the study and research which led to release of this M.Sc. thesis.*

*First for all, I take great pride and privilege to express my profound sense of gratitude and infinite indebtedness to my esteemed major advisor Dr. A. L. Patel, Professor, Department of Botany and Plant Physiology, for his wise planning, peerless guidance, untiring and bountiful help, constructive criticism and affectionate encouragement during the entire period of investigation.*

*My special thanks to the learned members of my advisory committee, Dr. K.D. Sharma, Associate Scientist, Department of Botany and Plant Physiology, Dr. Anoop Singh, Professor, Department of Soil Science, Dr. Rajender Singh, Associate Professor, Department of Statistics and Mathematics, Dr. D.S. Choopra, Professor, Department of Biochemistry, for their valuable suggestions, timely and precious help.*

*It is my profound privilege to express my sincere thanks to Dr. Swaraj Kumari, Professor, Department of Botany and Plant Physiology, for her expert technical guidance, emphatic help, timely advice, intellectual stimulation and critical amendments made during the course of investigation.*

*I am highly thankful to Dr. C.L. Goswami, Senior Plant Physiologist, Dr. M.S. Kuhad, Professor, Dr. H.R. Dhingra, Professor and Head, Dr. Sunita Sheokand, Associate Professor, Dr. A.S. Nandwal, Dr. R. Angrish, Dr. K.S. Datta, Professor of Department of Botany and Plant Physiology and Dr. S.L. Verma, Professor of Soil Science at Kaul for their valuable suggestions and providing me necessary facilities and support for the conduct of the present study.*

*My sincere thanks are also to the faculty members for their constant assistance in this academic endeavor. Thanks are also extended to all laboratory, non-teaching and staffs of Botany and Plant Physiology and Dr. Vinod Unvi of central lab for their help during this study.*

*I am shorts of word to express my whole hearted thanks to friend Mr. Asuwani, Mr. Chandan. Without their valuable helps, it would have been very difficulty to accomplish this project.*

*I duly acknowledge the timely and constant co-operation and love received from my Indian and Vietnamese friends Ms. Anita, Mr. Duc, Ms. Deepika, Ms. Kavita, Mr. Khang, Mr. Khiem, Mr. Joosep, Mr. Gioi, Mr. Huy, Mr. Hieu, Mrs. Hanh, Mrs. Hang, Mr. Hung, Mr. Neel, Ms. Pooja, Mr. Phong, Ms. Sukham, Mr. Tam, Mr. Thanh, Mr. Thang, Mr. Thu, Ms. Thuan, Mrs. Trang, Ms. Truc, Mr. Tuan and other for their encouragement and valuable help.*

*I extend my sincere thanks to the Vietnamese and Indian governments, ICCR and HAU for providing financial support and encouragements in completion of my studies.*

*No words of mine can value the all around help and encouragement to me by the leaders and scientists of the Cuu Long Delta Rice Research Institute. Ministry of Agriculture and Rural Development Vietnam.*

*There is no world to express my sentiments for my beloved parents for their ever ending love and immense blessings and I am sincerely grateful to all the other family members particularly, my brothers-sisters-in-law, for their continuous encouragement.*

*The lack of vocabulary utterly fails me to express the stupendous weight of my heart felt gratitude to my better half Quynh Mai, for her constant inspiration, encouragement and moral support during my post graduation.*

Place: Hisar

**(Duong Hoang Son)**

Date: 20 July 2007

# CONTENTS

CHAPTER	DESCRIPTION	PAGE(s)
I	INTRODUCTION	1-4
II	REVIEW OF LITERATURE	5-25
III	MATERIAL AND METHODS	26-43
IV	RESULTS	44-68
V	DISCUSSION	69-84
VI	SUMMARY	85-88
	REFERENCES	i-xii
	APPENDIX	I

## LIST OF TABLES

TABLE NO.	Description
1	Effect of Salicylic acid on plant height, number of tillers and leaf area at flowering of rice under different levels of cadmium.
2	Effect of Salicylic acid on dry weight of stem and leaf at harvest of rice under different levels of cadmium.
3	Effect of Salicylic acid on dry weight of panicle and total shoot at harvest of rice under different levels of cadmium.
4	Effect of Salicylic acid on water potential, osmotic potential and relative water content at flowering of rice under different levels of cadmium.
5	Effect of Salicylic acid on photosynthetic rate, transpiration rate and stomatal conductance content at flowering of rice under different levels of cadmium.
6	Effect of salicylic acid on total chlorophyll content and total soluble carbohydrate content at flowering of rice under different levels of cadmium
7	Effect of salicylic acid on total soluble protein, free amino acids and free proline content at flowering of rice under different levels of cadmium
8	Effect of salicylic acid on malondialdehyde content and membrane injury at flowering of rice under different levels of cadmium
9	Effect of salicylic acid on cadmium concentration in stem and leaf at harvest of rice under different levels of cadmium
10	Effect of salicylic acid on cadmium concentration in panicle and in shoot at harvest of rice under different levels of cadmium

- 11 Effect of salicylic acid on number of panicles per plant and number of spikelets per panicle at harvest of rice under different levels of cadmium
  - 12 Effect of salicylic acid on filled grain percentage and 1000-grain weight at harvest of rice under different levels of cadmium
  - 13 Effect of salicylic acid on grain yield ( $\text{g plant}^{-1}$ ) at harvest of rice under different levels of cadmium
  - 14 Effect of salicylic acid on starch, amylose content and cadmium concentration in brown rice at harvest of rice under different levels of cadmium
-

---

**CHAPTER – I***Introduction*

---

---

Cadmium (Cd) is an important and widespread heavy metal, released into the environment by power stations, heating systems, metal industries, waste incinerators, urban traffic, cement factories, and agricultural fertilizers. Cadmium is a highly toxic and persistent environmental poison for plants and animals (Toppi and Gabbrilli, 1999). Cadmium penetrates the root via cortical tissues and reaches the xylem by apoplastic or symplastic pathways (Salt *et al.*, 1995) where it forms complexes with several ligands, organic acids and phytochelatins (Sanden *et al.*, 1994). The entry of Cd into the food chain is of concern as it causes chronic health problems in human (Wagner, 1993; Staessen *et al.*, 1999).

In Haryana, average Cd content in sewage irrigation water had been reported to be  $76 \mu\text{g L}^{-1}$  (Yadav *et al.*, 2003). At Hisar, sewage water and non-sewage water contain similar concentration of cadmium ( $0.1\text{mg g}^{-1}$ ) and Cd concentration in different crops like cereal, pulses, oil seeds, fodder,

---

vegetable receiving sewage water irrigation varied from 3.25 to 4.88  $\mu\text{g g}^{-1}$  (Malik *et al.*, 2003).

Cadmium is not essential for plants growth, though it is readily taken up by many plant species and affect plant metabolism in many ways (Wagner, 1993; Pietrini *et al.*, 2005). Cadmium reduces growth, yield and yield attributes; reduces root, stem, leaf and panicle dry matter production (Liu *et al.*, 2007); reduces chlorophyll concentration (Hsu and Kao, 2003 and Chien *et al.*, 2001) photosynthesis, transpiration and stomatal conductance and alters water relations of plants (Vassilev *et al.* (1997). Cadmium alters carbohydrates (Moya *et al.*, 1993), proteins, amino acids (Hsu and Kao, 2003) particularly proline (Nover, 1989) metabolism. In many plants, Cd enhances the level of lipid peroxidation and membrane damage (Choudhury and Panda, 2004). Cadmium accumulation in crops including rice depends on the Cd concentration present in soil (Arao and Ishikawa, 2006).

Salicylic acid has been defined as a new potential plant hormone (Raskin, 1992) and its application protects against oxidative damages (Lankindale and Huang, 2004). Salicylic acid (SA) is an important signal compound and endogenous growth regulator involved in local and endemic disease resistance in plants (Alvarez, 2000). Salicylic acid

regulates different physiological processes, including plant growth and development, ion uptake and photosynthesis (Popova et al., 1997). Salicylic acid regulates cadmium induced oxidative damage (Choudhary and Panda, 2004).

Rice is a staple food in most Asian countries. In India, rice is the most important and extensively grown food crop, occupying 43.5 million hectares and its production is 85.5 million tones per year. In Haryana, rice is grown on 0.837 million hectares which is equal to 23.8% of its net sown area (ICAR, 2006). Cd toxicity in rice, leads to loss in seedling vigor; causes stunted growth, decreases the activities of many key hydrolytic enzymes and induces synthesis of proline and certain novel proteins (Shah and Dubey 1998). Cadmium is easily taken up by the plant and accumulation in plant tissues and grains might lead to food chain transfer to humans (Gupta and Gupta, 1998). Rice is one of the greatest sources of dietary intake of Cd. Choudhary and Panda, (2004) reported that SA application partially alleviated the oxidative stress in rice roots.

The present investigations were aimed to reveal some of the “physiological and biochemical effects of salicylic acid in rice (*Oryza sativa* L.) under cadmium stress” with the following objectives:

**Objectives**

To study the effect of cadmium stress on some physiological and biochemical parameters in rice.

To study the effects of salicylic acid on some physiological and biochemical parameters under cadmium stress in rice.

**CHAPTER – II**

## *Review of Literature*

---

---

An attempt has been made to review the available literature on “Physiological and Biochemical effect of Salicylic acid on rice cv. (*Oryza sativa* L.) under cadmium stress”. Since the information on rice is very limited on salicylic acid therefore, the literature available on some other crops is also reviewed here under the following heads:

### **2.1 Growth studies**

Growth is one of the best indices for evaluating plant response to environment stress. Cd adversely affects rice growth and metabolism (Shah and Dubey, 1995; 1997), but some authors have indicated that rice has a strong physiological tolerance to Cd (Kawada and Suzuki, 1998 and Wang, 2002). The growth inhibiting effect of Cd on plant height, tillering, leaf area and dry mass accumulation has been observed in rice by Liu *et al.* (2007).

### 2.1.1 Plant height

Cadmium stress causes reduction in plant height in rice (Liu *et al.*, 2007; Hassan *et al.*, 2005; Moya *et al.*, 1993). Liu *et al.* (2007) in pot experiments showed that plant height was significantly reduced under high level of soil Cd stress (100 mg kg<sup>-1</sup>). Hassan *et al.* (2005) observed that two cv. of rice plants (Bing 97252 and Xiushui 63) exposed to four levels of Cd (0; 0.1; 1.0; 5.0  $\mu$ mol) in hydroponic culture, showed a dramatic influence on plant height. The exposure 0 to 5.0  $\mu$ mol Cd reduced the plant height in cv. Bing 97252 by 16.3% and 17.8% at tillering and booting stages, respectively; the reduction in Xiushui 63 was 33.0% and 32.2%, respectively. Moya *et al.* (1993) reported that six day old rice seedlings grown for 10 days in nutrient solution with Cd (0.01 and 0.1 mM) showed reduction in shoot length. Cadmium stress has been shown to generate oxidative stress (Somaskhekaraiyah *et al.*, 1992) and leading to growth inhibition in plants (Clysters and Van Assche, 1985).

Salicylic acid has been defined as a new potential plant hormone (Raskin, 1992) and its application protects against oxidative damages (Larkindale and Huang, 2004). He *et al.* (2005) reported that when Kentucky bluegrass was sprayed with five levels of SA (0, 0.1, 0.25, 1, 1.5 mM), the increase in plant height was significantly higher in 0.25

mmol SA spray than those for other SA treatment or water spray control.

### **2.1.2 Number of tillers**

Cadmium stress causes reduction in number of tillers per plant in rice (Liu *et al.*, 2007). Liu *et al.* (2007) conducted pot experiments and showed that number of tiller per plant was significantly reduced under high level of soil Cd stress (100 mg kg<sup>-1</sup>). Vassilev (2002) found that application of Cd 25 mg kg<sup>-1</sup> soil and higher concentration significantly decreased the total tillers in barley plant. Cadmium stress has been shown to generate oxidative stress (Somaskhekaraiyah *et al.*, 1992) and leading to growth inhibition in plants (Clysters and Van Assche, 1985).

### **2.1.3 Leaf area**

Leaf growth, an important attribute of plant growth and productivity, is sensitive to Cd stress (Vassilev and Yordanav, 1997). Cadmium stress causes reduction in leaf area per plant in rice (Liu *et al.*, 2007). They conducted pot experiments and showed that leaf area was significantly reduced under high level of soil Cd stress (100 mg kg<sup>-1</sup>). Samiulla *et al.* (2007) observed that application of Cd(NO<sub>3</sub>)<sub>2</sub>

(100 mg kg<sup>-1</sup> soil) reduced the leaf area as compared to control in wheat (cv. PBW343 and WH542). Vassilev *et al.* (1998) observed that when barley plants were grown in nutrient solution with 54 µM Cd leaf area was reduced by 64% as compared to control. Cadmium stress has been shown to generate oxidative stress (Somaskhekaraiiah *et al.*, 1992) and leading to growth inhibition in plants (Clysters and Vanassche, 1985). Negative effect of Cd on leaf area could be related with disorders in water supply to the plant (Barcelo *et al.*, 1988). Barcelo *et al.* (1988) suggested that reduced cell turgor potential and cell-wall elasticity led to the formation of small cells and intracellular space in Cd-treated plants.

Khan *et al.* (2003) reported that foliar spray of SA enhanced the leaf area of corn.

#### **2.1.4 Dry weight of plant**

Growth of cell as well as whole plant is drastically inhibited due to Cd toxicity (Prasad, 1995). Liu *et al.* (2007) in pot experiments on rice showed that plant height, numbers of tillers, leaf area, and dry matter accumulation of root, straw and total plant were significantly reduced under high level of soil Cd stress (100 mg kg<sup>-1</sup>). Hsu *et al.* (2006) reported that application of 0.5 µM Cd to hydroponically

grown rice cv. Taichung Native 1 under three different temperatures (35/30°C; 25/20°C and 15/13°C day/night) reduced shoot and root dry weight in parallel to temperature increases.

Khan *et al.* (2003) reported that foliar spray of SA enhanced the plant dry mass of corn.

## **2.2 Plant water relations**

Cadmium reduces water uptake, transport and transpiration (Vassilev *et al.*, 1997; Costa *et al.*, 1994) which causes disturbed water balance in plants.

### **2.2.1 Water potential**

Vassilev *et al.* (1997) reported that barley plant grown in water culture supplied with 0.54  $\mu\text{M}$  Cd showed 500 kPa lower water potential as compared to control value (-230 kPa). Costa and Morel (1994) observed that in hydroponically grown lettuce supplied with 0, 0.01, 0.1, 1.0 and 100  $\mu\text{M}$   $\text{CdCl}_2$  reduced plant water potential with the increase in Cd concentration in the nutrient solution.

Poschenrieder *et al.* (1989) reported that bean seedlings grown in nutrient solution supplied with 3  $\mu\text{M}$  Cd

showed decline in water potential as compared to control only after 120 h exposure.

Tari *et al.* (2002) observed that tomato plants grown in hydroponic culture with SA applied showed increase in water potential of leaves as compared to without SA applied control.

### **2.2.2 Osmotic potential**

Poschenrieder *et al.* (1989) reported that bean seedlings grown in nutrient solution supplied with 3  $\mu\text{M}$  Cd showed decline in osmotic potential as compared to control after 48 h exposure.

### **2.2.3 Relative water content**

Vassilev *et al.* (1997) reported that barley plant grown in water culture supplied with 0.54  $\mu\text{M}$  Cd showed 2.0% reduction in relative water content over control (96.22%).

Costa *et al.* (1994) observed that in hydroponically grown lettuce supplied with 0, 0.01, 0.1, 1.0 and 100  $\mu\text{M}$   $\text{CdCl}_2$  decreased relative water content with the increase in Cd concentration in the nutrient solution.

Poschenrieder *et al.* (1989) reported that bean seedlings grown in nutrient solution supplied with 3  $\mu\text{M}$  Cd showed decline in relative water content as compared to control after 48 h exposure.

Pena *et al.* (2006) reported that in hydroponically grown sunflower supplied with 100, 200 and 300  $\mu\text{M}$   $\text{CdCl}_2$  showed reduction in relative water content by 200 and 300  $\mu\text{M}$   $\text{CdCl}_2$  to the extent of 11% and 14%, respectively respect to control plants.

## **2.3 Gaseous exchange**

Stomatal opening, transpiration, and photosynthesis have been reported to be affected by cadmium in nutrient solutions and soil (Toppi and Gabrielli, 1999).

### **2.3.1 Photosynthesis rate**

Cadmium is an effective inhibitor of photosynthesis in plants (Vassilev and Yordanov, 1997).

Moya *et al.* (1993) observed that six day old rice seedlings grown for 10 days in nutrient solution with Cd (0.1 mM) showed reduction in net photosynthesis.

Samiulla *et al.* (2007) observed that application of  $\text{Cd}(\text{NO}_3)_2$  ( $100 \text{ mg kg}^{-1}$  soil) reduced the photosynthetic rate as compared to control in wheat (cv. PBW343 and WH542).

Khan *et al.* (2003) reported that foliar spray of SA enhanced the photosynthetic rate of corn. Wang and Li (2007) also reported that spray of  $100 \mu\text{M}$  SA increased the net photosynthesis rate as compared to those sprayed with water.

Chandra and Bhatt (1998) observed that spray application of SA (0.02%) decreased the net photosynthetic rate in IFC 8401, 8402 and in IGFRI 450, whereas it increased in IFC 902 and Bundel 2-8503 genotypes of cowpea.

On the other hand, Janda (2000) suggested a decrease in net photosynthesis, stomatal conductivity and transpiration after 1 day of benzoic acid or aspirin (SA analogue) treatment at  $20/22^\circ\text{C}$  in maize. Similarly, Bohra and Gardi, (2003) reported that net photosynthetic rate decreased in Ber plant sprayed with 100 and  $500 \text{ mg L}^{-1}$  SA as compared to control. This inhibitory effect on net photosynthetic rate was coupled with higher stomatal resistance and low transpiration rate.

### 2.3.2 Transpiration

Hsu *et al.* (2006) observed that Cd decreased transpiration rate in rice seedlings grown at low, medium and high temperatures, respectively, to 53%, 29%, and 13% of control.

Vassilev *et al.* (1997) reported that barley plant grown in water culture supplied with 0.54  $\mu\text{M}$  Cd decreased transpiration rate by 18% over control.

Khan *et al.* (2003) reported that foliar spray of SA enhanced the transpiration rate of corn.

On the other hand, Janda (2000) suggested a decrease in net photosynthesis, stomatal conductivity and transpiration after 1 day of benzoic acid or aspirin (SA analogue) treatment at 22/20°C in maize.

### 2.3.3 Stomatal conductance

Vassilev *et al.* (1997) reported that barley plant grown in water culture supplied with 0.54  $\mu\text{M}$  Cd showed increased stomatal resistance by 79% over control.

Samiullah *et al.* (2007) observed that pot grown wheat cultivars (PBW343 and WH542) supplied with 100 mg  $\text{Cd}(\text{NO}_3)_2 \text{ kg}^{-1}$  soil reduced the carbonic anhydrase activity, stomatal conductance, photosynthetic rate and

photosynthetic water use efficiency over control. They suggested that mesophyll and stomatal processes contributed to the decrease in photosynthetic rate due to Cd.

Khan *et al.* (2003) reported that foliar spray of SA enhanced the stomatal conductance of maize

On the other hand, Janda (2000) suggested a decrease in net photosynthesis, stomatal conductivity and transpiration of maize with 0.5 mM aspirin (SA analogue) treatment at 22/20°C.

## **2.4 Biochemical estimations**

Plants exposed to Cd showed a series of physiological disorders, such as decreases in chlorophyll, sugar and protein contents, reduction in photosynthetic carbon assimilation and changes in related enzymes activity, and a drop in yield (Satyakala, 1997).

### **2.4.1 Chlorophyll content**

Storbart *et al.* (1985) showed that Cd is a potent inhibitor of Chl biosynthesis.

Hsu and Kao (2003) observed that when seedlings of TN1 and TNG67 rice cultivars were treated with 0.5 mM CdCl<sub>2</sub> for 7 days, there was decrease in chlorophyll content in leaves which was more pronounced in TN1 than in TNG67.

Chien *et al.* (2001) observed that chlorophyll content in CdCl<sub>2</sub>-treated detached rice (Taichung Native 1) leaves was lower than that in water treated controls.

The total Chl content was reduced by 30% to 60% in leaves of plants that were grown in the presence of 50 and 100 µM Cd, respectively. The reduction was less pronounced in Chl a than in Chl b; the ratio of Chl a to Chl b tend to increase with increasing Cd concentration (Pietrini *et al.*, 2003).

Apart from inhibition of biosynthetic enzymes of Chl formation, the increased activity of lipoxygenase may also contribute to the decreased level of Chl with Cd treatment on dark grown seedlings suggesting the inhibition of Chl synthesis by Cd achieved by reaction with constituent biosynthetic enzymes as well as peroxide-mediated degradation (Somashekaraiah *et al.*, 1992).

With the increase in concentration of SA, Chl content decreased in both wheat and moong seedlings. Chla/b ratio decreased only in wheat and remained constant in moong. Increase in the xanthophylls pool and de-epoxidation rate indicates that SA may create oxidative stress; the degree of which is different in various plants (Moharekar *et al.*, 2003). The lowest quality of photosynthesis pigment (Chlorophyll a/b and carotenoids) was observed in ber plant treated with

100 mg/L SA spray (3.5750 mg/g FW) (Bohra and Gardi, 2003).

Chandra and Bhatt (1998) observed that spray application of SA (0.02%) decreased the chlorophyll content in IFC 8402 and in IGFRI 450, whereas it increased in IFC 8401, IFC 902 and Bundel 2-8503 genotypes of cowpea.

#### **2.4.2 Total soluble carbohydrate**

Moya *et al.* (1993) observed that six day old rice seedlings grown for 5 or 10 days in nutrient solution with Cd (0.1 mM) showed higher level of soluble sugars as compared to control at both the stages.

Verma and Dubey (2001) observed a steady increase in the content of total sugars in roots and shoots with increasing concentrations of Cd(NO<sub>3</sub>)<sub>2</sub> (0, 100 and 500 µM) in nutrient solution with advancement of age (5 to 20 day old) of rice (cv. Ratna and Jaya) grown in sand.

Chandra and Bhatt (1998) observed that spray application of SA (0.02%) increased the total soluble carbohydrate in cowpea.

In Indian mustard, spray of SA (25 and 50 mg L<sup>-1</sup>) increased the total sugar content and reduced the starch

content in leaves as compared to water spray control (Sangha and Atwal, 2004).

Spray of SA (100 and 500 mg L<sup>-1</sup>) decreased the total soluble sugar content in ber plants cv. Tikadi and cv. Gola, (Bohra and Gardi, 2003; 2005).

### **2.4.3 Total soluble protein**

Hsu and Kao (2003) observed that when rice seedlings of TN1 and TNG67 were treated with 0.5 mM CdCl<sub>2</sub> for 7 days, there was decrease in protein content in leaves which was more pronounced in TN1 than in TNG67.

Chien *et al.* (2001) observed that protein content in CdCl<sub>2</sub>-treated detached rice (Taichung Native 1) leaves was decreased as compared to water treated controls. Hsu *et al.* (2006) observed that Cd increased protease activity and amino content in nontolerance lines but not in tolerant lines of rice.

Total protein content, in both roots and shoots of barley, was reduced in response to applied Cd in the culture solution. The highest concentration of Cd decreased the protein content of shoots by 31% and 60% in the roots (Boussama *et al.*, 1999).

Increasing the concentration of SA spray (100 and 500 mg/L) reduced the soluble protein content in (cv Tikadi and cv. Gola) ber plants (Bohra and Gardi, 2003; 2005).

#### **2.4.4 Free amino acids**

Hsu and Kao (2003) observed that when rice seedlings of TN1 and TNG67 were treated with 0.5 mM CdCl<sub>2</sub>, there was increase in total amino acids content and protease activity in second leaf of TN1 (non-tolerant) while it decreased in TNG67 (tolerant), suggesting that proteolysis or accumulation of amino acids might play some role in regulating Cd toxicity.

Lesko and Simon-Sarkadi (2002) observed that Cd stress caused the accumulation of total free amino acids both in shoots and roots of wheat seedlings. They reported that the ratio of total free amino acid in shoots increased 1.5 times at higher Cd concentration.

#### **2.4.5 Proline content**

Among amino acids, proline responds most sensitively to stress conditions (Nover, 1989). Accumulation of free proline in response to heavy metal exposure seems to be wide-spread phenomena among plants. The precise

mechanism and the functional significance of proline accumulation in plants under heavy metal stress have not been elucidated till date (Schat *et al.*, 1997).

Cd is known to deteriorate the plant water balance and lead to proline accumulation in plants decreasing water potential of the plants and functional significance of proline accumulation would lie in its contribution to water balance (Barcelo and Poschenrieder, 1990; Costa and Morel, 1994). On the other hand, Kastori *et al.*, (1992), observed proline accumulation in metal exposed isolated leaf discs suggesting that this was due to metal uptake per se, rather than due to water deficit stress. Cadmium stress has been shown to increase in proline content in rice (roots) by Chen and Kao. (1995) and in barley leaf by Vassilev *et al.* (1998).

Schat *et al.*, (1997), brilliantly demonstrated that proline accumulation under Cd stress, in leaves of tolerant and non-tolerant ecotypes of *Silene vulgaris*, is a consequence of a Cd imposed water deficit stress. Proline accumulation did not occur until metal imposed growth reduction was apparent or proline accumulation can at most alleviate, but not prevent Cd stress, irrespective of the level of Cd exposure.

Costa and Morel, (1994), concluded that [<sup>14</sup>C] proline was not actively synthesized suggesting that Cd did not affect proline synthesis but more probably causing inhibition of

proline oxidation. The increased content of proline with elevated Cd concentrations was found. It could be explained by an active proteolysis of cell wall glycoprotein (Costa and Spitz, 1997).

SA application contributed accumulation of proline under stress through maintaining an enhanced level of ABA in wheat seedling (Sakhabutdinava *et al.*, 2003). Spraying with SA retarded the accumulation of proline in *Vicia faba* plant (Tammam, 2003). Spray of 500 mg L<sup>-1</sup> SA recorded the highest proline content (1.042 mM g<sup>-1</sup> FW) in ber cv. Tikadi, but in cultivar Gola treated with 100 and 500 mg/L SA sprays show decreased proline content (Bohra and Gardi, 2003; 2005).

#### **2.4.6 Malondialdehyde content**

The level of lipid peroxidation in plant leaves was quantified by determining the content of malondialdehyde (MDA), a break down product of lipid peroxidation. Lipid peroxidation is a free radical mediated process (Foyer *et al.*, 1997).

Chien *et al.* (2001) observed that MDA content in CdCl<sub>2</sub>-treated detached rice (Taichung Native 1) leaves was greater than that in water treated controls.

In bean, concentration of MDA was significantly increased with Cd treatment in all the organs (roots, stems, leaves) of plant. They suggested that Cd toxicities induced oxidative stress by increased accumulation of lipid peroxidation products, in all organs of stress seedling (Chaoui *et al.*, 1997).

In sunflower leaves exposed to Cd, it was shown that Cd induced oxidative stress is mediated by ROS since low level of lipid peroxidation, prevention of decrease in GSH content and increase in lipoxygenase activity were observed in the presence of free radical scavengers (Gallego *et al.*, 1996). Treatment of Cd showed a very high increase in lipoxygenase activity (247%). LOX activity increased by Cd suggests higher lipolytic activity on the membrane bound fatty acids causing propagation of lipid peroxidation (Aravind and Prasad, 2003).

Salicylic acid inhibited lipid peroxidation in the presence of Cd and increase the active of Catalase and ascorbate peroxidase. This indicated SA alleviates Cd toxicity not at the level of antioxidant defense but affecting other mechanisms of Cd detoxification (Metwally *et al.*, 2003). In rice roots, Cd applied to SA free plants increased the level of lipid peroxidation, hydrogen peroxide content and superoxide radical production. SA treatment decreased the toxic level of Cd manifested by lower lipid peroxidation, lesser production

of hydrogen peroxide and reduction in generation of superoxide radical (Choudhury and Panda, 2004).

#### **2.4.7 Membrane injury**

Choudhury and Panda (2004) observed that the membrane integrity loss was higher by 177.8% and 194.9% in SA free plants as compared to 165.4% and 185% in SA (100  $\mu\text{M}$ ) primed ones at 100  $\mu\text{M}$  and 1000  $\mu\text{M}$  of Cd application in rice roots.

Mishra and Choudhuri (1999) observed that presence of 100  $\mu\text{M}$  SA in growing medium of rice ameliorated the increased leakage of electrolytes caused by heavy metal stress.

#### **2.5 Cadmium concentration in plant parts**

Cadmium though, a non-essential element, is readily taken up and accumulated in various parts of plants; Cd is believed to penetrate the root through the cortical tissue. As soon as Cd enters the roots, it can reach the xylem through an apoplastic and symplastic pathway (Toppi and Gabrielli, 1999; and Salt *et al.*, 1995). Normally Cd ions are mainly retained in the roots, and only small amounts are transported to the shoot, Cd accumulation was found

approximately 10 times higher in root than shoot. Up to 98% of the accumulated total Cd is retained by the soybean roots (Zhao *et al.*, 2005).

Hsu and Kao (2003) observed that when rice seedlings of TN1 and TNG67 were treated with 0.5 mM CdCl<sub>2</sub> for 7 days, there was increase in Cd content in leaves of TN1 while Cd content in leaves of TNG67 remain unchanged as compared to control.

Chien *et al.* (2001) observed that Cd content in CdCl<sub>2</sub>-treated detached rice (Taichung Native 1) leaves was greater than that in water treated controls.

Cadmium uptake rate of barley plant increased slowly before the booting stage, then increased sharply during the 70-100 days period, and after that Cd uptake rate tended to slow dramatically. The dry weight also decreased after booting stage (Wu *et al.*, 2005).

Cd accumulation in root, shoot, leaves, seed of various crops including rice is related with Cd concentration in solution and soil. So, the degree to which plant parts are able to take up Cd depends on its concentration in solution and soil (Arao and Ishikawa, 2006).

Salicylic acid does not decrease Cd uptake, but changes its distribution in plant organs depending on the concentration of added Cd, this indicated the influenced of SA on the alleviation of toxic effects of Cd is probably

indirect, through a development of general anti stress response of the soybean seedlings (Drazic and Mihailovi, 2005).

## **2.6 Yield and its attributes**

It was reported that plants exposed to Cd showed a series of physiological disorders, such as decreases in chlorophyll, sugar and protein contents, reduction in photosynthetic carbon assimilation and changes in related enzymes activity, and a drop in yield (Satyakala, 1997).

Grain yield of rice depends on the number of panicles, grains per panicle, filled grain percentage and grain weight (Yoshida, 1972). The grain number per panicle was affected more than the other three yield components by soil Cd treatment (Liu *et al.*, 2007).

Liu *et al.* (2007) observed that application of Cd (100 mg kg<sup>-1</sup> soil) reduced yield characteristics (number of panicle per plant, number of spikelets per panicle, filled grain percentage, 1000-grain weight and grain yield per plant). Spikelets number per panicle was affected more than the other three yield components by soil Cd treatment.

## 2.7 Quality of brown rice

Literature on the effect Cd and SA on brown rice quality starch and amylose is not available. However, Dewan and Dhingra (2004) have reported that when pea plants were exposed to 0, 2.5, 5.0, 7.5 and 10 mM of Cd approximately 15 days prior to initiation of flowering reduced the starch content in physiologically matured seeds with increasing concentrations of Cd.

Liu *et al.* (2005) in a pot trail with 52 rice cultivars of different types collected from different origins exposed to 100 mg kg<sup>-1</sup> soil showed that the concentration of Cd in brown rice ranged from 0.22 to 2.86 mg kg<sup>-1</sup>. Cheng *et al.* (2006) observed that average Cd concentration in grains of *Japonica* rice genotypes varied from 0.068 to 0.185 mg kg<sup>-1</sup>.

Cadmium concentration in rice grain is primarily dependent on shoot Cd concentration, which in turn is determined by Cd translocation from roots to shoots (Cheng *et al.*, 2004).

Cadmium accumulation in crops including rice depends on the Cd concentration present in solution and soil (Arao and Ishikawa, 2006).

**CHAPTER – III**

---

---

*Materials and  
Methods*

The present investigation was conducted on “Physiological and biochemical effects of salicylic acid in rice (*Oryza sativa* L.) under cadmium stress” in the screen house, Department of Botany and Plant Physiology, CCS HAU, Hisar during *Kharif*, 2006. The seeds of cv. HKR 99-60 were obtained from Regional Rice Station, Kaul, CCS HAU, Hisar.

**3.1. GROWING OF RICE**

The seeds were surface sterilized with 2.5% sodium hypochlorite for 15 min and washed extensively with distilled water. These seeds were then germinated in Petri plates with wetted Whatman No 1 filter paper for 48 h at 37°C under dark condition. For salicylic acid (SA) treatment, seeds were soaked with 100 µM SA for a period of 16 h and then germinated as described above. Uniformly germinated seeds were selected and 10 seeds were sown in polythene lined earthen pots containing 6 kg sandy loam soil at a uniform depth of 2.5 cm. Fifteen days of sowing (DAS), three plants of uniform seedling were retained in each pot. At regular

interval of three weeks, the plants were supplied with equal volume of Hoagland nutrient solution (1940) as modified by Went (1957).

Plants were irrigated with tap water and it was maintained under flooding conditions (5 cm depth) throughout the growth period, except for 2 days plants were grown under saturating condition just before cadmium (as  $\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$ ) application (30 DAS). SA spray (100  $\mu\text{M}$ ) treatment was applied at boot stage of rice.

### 3.2. TREATMENT

Treatment	Treatment description	Abbreviation
Salicylic acid treatment	Either as seed treatment or spray treatment	T
i)	0 $\mu\text{M}$ SA seed treatment	Control
ii)	100 $\mu\text{M}$ SA seed treatment	SA Seed
iii)	0 $\mu\text{M}$ SA spray treatment	Water spray
iv)	100 $\mu\text{M}$ SA spray treatment	SA spray
Cadmium application	Soil drenching	Cd
i)	0 $\mu\text{M}$ Cd	0
ii)	250 $\mu\text{M}$ Cd	250
iii)	500 $\mu\text{M}$ Cd	500

Number of treatments consisted of the combination of a) and b) mentioned above (i.e. = 12)

<b>a) Replication</b>	:	3
<b>b) Total number of pots in one set</b>	:	36
<b>c) Number of sets</b>	:	2

(One set was used for different observations/estimations at flowering stage and another set was used at harvest).

### **3.3. STATISTICAL ANALYSIS**

To calculate critical different (C.D) factorial completely randomized design was used at 5 percent level of significance.

### **3.4. OBSERVATIONS RECORDED**

The following observations were recorded:

#### **3.4.1. GROWTH PARAMETERS**

Plant height, number of tillers and leaf area per plant were recorded at flowering stage, and dry weights of plant parts at harvest.

##### **a) Plant height**

Plant height of three plants from each pot was measured from the ground level up to the longest leaf at flowering.

**b) Number of tillers**

Numbers of tillers of plants were counted at flowering and the number of panicles at harvest and their mean values were computed and expressed as their number per plant.

**c) Leaf area**

All the leaf blades were cut out and used to measure leaf area at flowering using a Portable Leaf Area Meter (Model Li 3000, Li COR USA) and expressed as cm<sup>2</sup> per plant.

**d) Dry weight of above ground plant parts**

Three plants were cut at ground level and separated into stem, leaves and panicles at harvest. These plant parts were oven dried at 65°C to a constant weight and then weighed with the help of electronic balance.

**3.4.2 PLANT AND WATER RELATIONS**

Water potential, osmotic potential and relative water content were recorded in flag leaf at flowering.

**a) Water potential ( $\Psi_w$ )**

Water potential of flag leaf was measured with the help of pressure chamber (Model 3005, Soil Moisture Equipment Corporation, Santa Barbara, CA, USA), between 10 AM to 12:00 noon. The flag leaf was cut from the plant

with the help of sharp edged knife and sealed in the pressure chamber one at a time with the cut end protruding outside and the pressure was developed till the sap just appeared at the cut end from xylem tissue. This gives the water potential in (-) bar which expressed in megapascal (-MPa).

**b) Osmotic potential ( $\Psi_s$ )**

The osmotic potential was measured by taking three or four flag leaves from the plant from which leaves were taken to measure  $\Psi_w$  earlier. The airtight syringes were used to keep the leaves samples in deep freezer at  $-15^{\circ}\text{C}$ . The sap from these leaf tissues was extracted on filter paper discs and  $\Psi_s$  measured using Vapor Pressure Osmometer (Model 5100-B, Wescor Inc. Logan, Utah, USA). The osmometer was calibrated by using Osmolality Reference Standards of Sodium Chloride (Wescor mc, USA). The readings of osmometer, thus, obtained in miliosmoles per kg were converted to molality and finally to (-) bars with the help of calibration curve which was expressed in (-) MPa by using the following conversion factor.

$$40 \text{ Osmol} = -1 \text{ bar}$$

$$-10 \text{ bar} = -1\text{MPa}$$

**c) Relative water content (RWC, %)**

The flag leaves were detached from the plants between 10:00 and 12:00 noon, which were quickly sealed in

polythene bags and transported to the laboratory on ice box. The leaves were weighed immediately to determine the fresh weight. The leaves were then floated on distilled water for 3 h at room temperature in diffused light. The turgid leaves were weighed and dried in oven at 65°C for 72 h till a constant dry weight was obtained. These three weights were used to calculate RWC (%) according to the formula given by Weatherley (1950).

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{dry weight}} \times 100$$

### **3.4.3 GASEOUS EXCHANGE STUDIES**

#### **3.4.3.1 Photosynthetic rate, transpiration rate and stomatal conductance**

Photosynthetic rate, transpiration rate and stomatal conductance were measured by Portable Infra Red Gas Analyser (IRGA) (CIRAS-1, PP system, England) between 10.00 am to 12.00 noon at flowering stage of plant.

#### **3.4.4. BIOCHEMICAL PARAMETERS**

The following biochemical estimations were performed in flag leaf at flowering stage.

##### **a) Total chlorophyll content**

Total chlorophyll content was extracted according to the method of Hiscox and Israelstam (1979) using dimethyl sulfoxide (DMSO) as solvent.

### **Reagent**

- i) Dimethyl sulfoxide (DMSO)

### **Procedure**

Flag leaf was detached; cleaned with filter paper; cut into pieces and 200 mg sample was placed in a test tube containing 5 ml of DMSO. Such Test tubes were placed in an oven at 60°C for about 2 h. Then the extract was cooled to room temperature and the absorbance was read at 649 and 665 nm on a computer added spectrophotometer (UV-VIS spectrophotometer -117; Systronic, India) using DMSO as blank.

Calculations for chlorophylls were made according to Welburn (1994).

$$\text{Chl 'a' } (\mu\text{g/ml}) = 12.19 A_{665} - 3.45 A_{649}$$

$$\text{Chl 'b' } (\mu\text{g/ml}) = 21.99 A_{649} - 3.32 A_{665}$$

$$\text{Total chlorophyll} = \text{Chl 'a'} + \text{Chl 'b'}$$

The data was expressed as mg g<sup>-1</sup> FW of sample.

### **b) Total soluble carbohydrate**

Total soluble carbohydrates were determined with the method of Yemm and Willis (1954).

### **Extraction**

Extraction was done by modified method of Barnett and Naylor (1966). For each sample, 200 mg of plant leaf was homogenized in 5 ml of 80% (v/v) ethanol. The homogenate was refluxed for 15 min on a water bath at 60°C and centrifuged for 10 min at 5000 rpm. This procedure of refluxing and centrifuging was done three times with residue and collect supernatant was decanted in a test tube each time. The supernatants were pooled together and volume was made 5 ml.

### **Reagents**

- i) Anthrone reagent: 0.4 per cent anthrone in concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>).

### **Procedure**

1ml of ethanol extract was taken in a test tube and mixed with 4 ml of anthrone reagent. The mixture was then heated in a water bath for 10 minutes. After cooling, absorbance was recorded at a wavelength of 620 nm against the reagent blank. Standard curve was prepared using graded concentration of glucose and the data were expressed as mg g<sup>-1</sup> FW of the tissue.

### **c) Total soluble proteins**

Total soluble proteins in leaves were estimated using Bradford's reagents (Bradford, 1976).

### **Extraction**

A 200 mg sample of frozen leaves was ground in 4 ml phosphate buffer (0.1 M, pH 7.0) containing 10% (w/v) insoluble PVP in a glass pestle and mortar and centrifuged at 10,000 g for 15 min at 4 °C. To one ml of supernatant added equal volume of 20% TCA (trichloroacetic acid) to precipitate the proteins and incubated for 1 h at 4°C. Again centrifuged at 10,000 g for 15 min. Supernatant was discarded and pellet was dissolved in 2 ml of 1M NaOH and incubated at room temperature for 1 h.

### **Reagents**

- i) Bradford reagent: Dissolved 100 mg Coomassie Brilliant Blue G-250 (CBBG-250) in 50 ml ethanol. To this solution added 100 ml of 85% o-phosphoric acid. The concentrated solution was diluted to 1 liter and then filtered through Whatman No. 1 filter paper. This reagent was stored in dark bottle at room temperature.
- ii) 1 M NaOH (w/v): Dissolved 4 g NaOH in 100 ml distilled water.

### **Procedure**

Each sample was diluted five times with 1M NaOH before estimation. To 0.1 ml of this diluted extract, 5 ml Bradford reagent was added. Mixed thoroughly and measured absorbance at 595 nm.

Standard curve was prepared using graded concentration of bovine serum albumin (1 mg ml<sup>-1</sup> 1 M NaOH) solution.

**d) Total free amino acids**

Total free amino acid was determined following the method of Yemm and Cocking (1955).

**Reagents**

- i) 0.01M KCN (0.1682g KCN was dissolved in 250 ml of 60% ethanol.
- ii) KCN–Acetone (50ml of 0.01M KCN was diluted to 250 ml with acetone.
- iii) Acetone-Ninhydrin (5% solution of ninhydrin was prepared in acetone).
- iv) KCN–Acetone–ninhydrine (50ml of acetone-ninhydrine solution was mixed with 250 ml of KCN-acetone solution).
- v) 0.2M citrate buffer (21.008g of citric acid was dissolved in 200 ml of distilled water and mixed with 200 ml of 1N NaOH, pH 5.0 and volume made to 500 ml.

**Extraction**

0.2 g of leaf sample was homogenized with 5 ml ethanol (80%, v/v), refluxed for 15 minutes on a steam bath and centrifuged. The supernatant was decanted in a test tube. The residue was further refluxed with 5 ml of 80% ethanol and supernatant was collected. The residue was refluxed third time with 5 ml of 80% ethanol and supernatant was collected. The pooled supernatant was evaporated to dryness in a boiling water bath. Then total

volume of extract was made to 10 ml with distilled water. The extract was used for estimation of total free amino acid.

### **Procedure**

To 0.1 ml of aliquot, 1 ml of ninhydrin solution was added in a test tube and final volume was made to 2 ml with distilled water. These test tubes were heated on a boiling water bath for 20 minutes. 5 ml of distilled water was added while the tube was still on the water bath and mixed thoroughly. After 15 minutes of boiling, tubes were cooled under running tap water and then reading of absorbance at 570 nm was made using the reagent blank as reference.

Leucine solutions of different concentrations (10 to 100  $\mu\text{g ml}^{-1}$ ) were prepared for standard curve. With the help of standard curve the concentration of free amino acids (equivalent to leucine) was determined and expressed as free amino acids content in  $\mu\text{g g}^{-1}$  FW of sample.

### **e) Proline content**

Proline content was estimated by using the method of Bates *et al.* (1973).

### **Reagents**

- i) 3 % aqueous suiphosalicylic acid (w/v)
- ii) Acid ninhydrin (prepared by dissolving 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6.0 M o-phosphoric acid)

iii) Toluene

### **Extraction**

A 200 mg of fresh leaves were separately homogenized in 5 ml of 3 % sulphosalicylic acid and then centrifuged at 5000 rpm for 15 minutes and supernatant was taken for proline estimation.

### **Procedure**

Two ml of supernatant was taken and 2.0 ml acid ninhydrin was added. This mixture was then kept in boiling water bath for 1 h at 100 °C and thereafter reaction was terminated by keeping the tubes in ice-bath. Then 4.0 ml of toluene was added. After vigorous shaking, the upper organic phase was taken after attainment of room temperature and its absorbance was recorded at 520 nm by using toluene as blank.

A standard curve was prepared by using graded concentration of proline 3% sulphosalicylic acid.

The proline content was expressed as  $\mu\text{M g}^{-1}$  FW.

$$\mu\text{moles per g tissues} = \frac{\mu\text{g proline/mL} \times \text{mL toluene}}{115.5} \times \frac{5}{\text{g sample}}$$

### **f) Malodialdehyde content**

The level of lipid peroxidation in plant leaves was quantified by determining the content of malondialdehyde

(MDA), a break down product of lipid peroxidation. MDA was measured by thiobarbituric acid (TBA) reaction with minor modifications of the method of Heath and Packer (1968).

### **Extraction**

Three hundred mg of leaves were homogenized separately with 5 ml of 0.1 % TCA. The homogenate was centrifuged at 8000 x g for 15 min. The supernatant was then directly used for the assay.

### **Reagents**

- i) 0.1 % Trichloroacetic acid (TCA)
- ii) 20 % TCA containing 0.5 % thiobarbituric acid (TBA)

### **Procedure**

To a 1 ml aliquot of the supernatant, 4 ml of 20% (w/v) trichloroacetic acid containing 0.5% (w/v) thiobarbituric acid was added. The mixture was heated at 95°C for 30 minutes and cooled immediately and then centrifuge at 10000g for 10 min. The absorption of the supernatant was read at 532 nm. The value was corrected for non-specific absorption at 600 nm. The concentration of MDA was calculated using the extinction coefficient of 155 m<sup>mol</sup><sup>-1</sup> l<sup>-1</sup> cm<sup>-1</sup> (Heath and Packer *et al.*, 1968).

### **g) Membrane injury**

Membrane injury index was measured as percent proportion of ion leakage in to the external aqueous medium

to the total ion concentration of the stressed tissue as measured by the EC of the external medium (Sullivan and Ross, 1979).

### **Procedure**

The 200 mg of leaf were kept in 20 ml vials containing 10 ml de-ionized water at 27°C. After 5 h, the electrical conductivity (EC) of the surrounding solution was measured and designated as EC1. Then the samples were kept in boiling water bath for 50 min to achieve total killing of the tissue. After cooling, the EC of the solution was again measured and designated as EC2. The membrane injury index was calculated as follows:

$$\text{Membrane Injury (\%)} = \frac{\text{EC1}}{\text{EC2}} \times 100$$

### **3.4.5. CADMIUM CONCENTRATION IN PLANT PARTS**

Cadmium content in different plant parts were estimated at harvest. 200 mg of dried samples of stem, and leaf, and 1 g of samples of husk and brown rice were separately digested in 20 ml of diacid mixture (perchloric acid and nitric acid, 1:9). Resulting digested solution was cooled to room temperature and its volume was made to 25 ml with distilled water and cadmium content was determined by Atomic Absorption Spectrophotometer (Perkinelmer analyst 100), using cadmium chloride solution as standard.

### **3.5. YIELD ATTRIBUTES**

#### **a) Number of panicles per plant**

Total numbers of panicles from three plants per replication (i.e. per pot) were counted and the average number of panicles per plant was calculated.

#### **b) Number of spikelets per panicle**

Number of spikelets from three plants per replication were counted and divided by their number of panicles, and expressed them as number of spikelets per panicle.

#### **c) Filled grain percentage**

Spikelets from three plants per replication were separated into filled and unfilled grains and counted them with the help of grain counter. The percentage of filled grains was calculated.

#### **d) 1000-grain weight**

Weight of filled grains were divided by the number of filled grains and multiplied by 1000 to obtain 1000-grain weight.

### **3.6. YIELD**

Filled grains were weighed for each sample and expressed as grain yield per plant.

### 3.7. QUALITY PARAMETER

Starch content, amylose content and Cd concentration in brown rice (dehulled rice grain) were recorded.

#### a) Starch content in brown rice

Starch was estimated by following method of Hassid and Neufeld (1964).

##### Reagent

- i) Anthrone reagent: 0.4 per cent anthrone in concentrated sulphuric acid ( $H_2SO_4$ ).
- ii) 80% Ethanol
- iii) 26% Perchloric acid

##### Extraction

Brown rice sample of 200 mg was homogenized with 5 ml of 80% ethanol. The homogenate was refluxed for 15 min on a water bath at 60°C and centrifuged for 10 min at 5000 rpm. This procedure of refluxing and centrifuging was done three times with residue and supernatant was discarded each time to remove sugar and residue was retained. The residue was suspended in 5 ml of chilled 26 % (v/v) perchloric and stirred for 5 minutes. It was allowed to stand overnight and centrifuged at 5000 rpm for 15 minutes. Next morning the residue was again extracted twice with 26% (v/v) perchloric acid. The supernatant was pooled and

volume made to 100 ml with distilled water. An aliquot portion was suitably diluted to give a total concentration approximately equal to 50 mg ml<sup>-1</sup>.

### **Procedure**

0.1 ml of the supernatant was taken and the volume was made to 1 ml with distilled water. Then 4 ml of anthrone reagent was added to it and the mixture was heated on a boiling water bath at 60°C for 10 minutes. It was cooled rapidly and the absorbance at 625 nm against reagent blank was recorded. Glucose (10-100 µg ml<sup>-1</sup>) was used as standard. Glucose content in the samples was estimated using the standard graph.

Starch content was then calculated using the following formula:

$$\text{Starch content} = \text{glucose content} \times 0.9$$

### **b) Amylose content in brown rice**

Amylose was estimated by following method of Thayumanavan and Sadasivam (1984).

#### **Reagents**

- i) 1N NaOH
- ii) 0.1% Phenolphthalein indicator
- iii) Distilled ethanol
- iv) Iodine reagent: Dissolve 1g of iodine and 10g of KI in water and make up to 500 ml.

- v) Standard amylose: 100 mg amylose in 10 ml of 1N NaOH and make up to 100 ml with water (1mg/ml)

### **Extraction**

100 mg of powdered brown rice was homogenized with 1ml of distilled ethanol and 10 ml of 1N NaOH was added to it; kept as such for overnight and the volume was made up to 100 ml.

### **Procedure**

To 2.5 ml of extract, 20 ml of water was added and three drops of phenolphthalein indicator were added and mixed thoroughly. 0.1N HCl was added drop by drop until the pink colour just disappeared. Then, 1 ml of iodine reagent was added and the final volume was made up to 50 ml and the absorbance was read at 590 nm against the reagent blank.

The amount of amylose in the sample was calculated using the standard curve prepared from amylose (range 0.2-1 mg ml<sup>-1</sup>) against a blank.

**Calculation:** Absorbance corresponds to 2.5 ml of the test solution = x mg amylose.

Therefore,

$$100 \text{ ml contains} = \frac{x}{2.5} \times 100 \text{ mg amylose} = \% \text{ amylose}$$

### **c) Cadmium concentration in brown rice**

Cadmium concentration in brown rice was estimated as described under section 3.5.5.

---

## CHAPTER – IV

# *Results*

---

---

The results of the experiment conducted during Kharif, 2006 under screen house on “Physiological and biochemical effects of salicylic acid in rice (*Oryza sativa* L.) under cadmium stress” are presented below.

### **4.1 GROWTH PARAMETERS**

#### **4.1.1 Plant height**

The data for plant height at flowering is presented in Table 1. Cadmium (Cd) application significantly reduced the plant height. Reductions in plant height were 9.6% and 18.5% with applied Cd concentration of 250  $\mu$ M and 500  $\mu$ M as compared to control (no Cd and no SA applied) value (86.8 cm), respectively.

Application of salicylic acid (SA) slightly increased the plant height. Such an increase was relatively more in SA spray treatment (3%) than in SA seed treatment (2%) over control. There was non significant difference between plant height with water spray treatment and control.

**Table 1. Effect of salicylic acid on plant height, number of tillers and leaf area at flowering of rice under different levels of cadmium**

Treatment (T)	Plant height (cm)				Tillers (No. plant <sup>-1</sup> )				Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )			
	Cd concentration (μM)				Cd concentration (μM)				Cd concentration (μM)			
	0	250	500	Mean	0	250	500	Mean	0	250	500	Mean
Control	86.8	78.5	70.7	78.7	5.78	5.11	4.33	5.07	199.3	148.1	135.4	161.0
SA seed	88.2	82.2	73.0	81.1	6.11	5.66	4.56	5.44	224.3	173.4	159.2	185.6
Water spray	86.5	78.3	71.2	78.7	5.78	5.18	4.44	5.13	200.2	153.4	138.8	164.1
SA spray	89.3	83.3	74.2	82.3	6.89	5.67	4.76	5.77	229.9	183.7	164.7	192.8
Mean	87.7	80.6	72.3		6.14	5.40	4.52		213.4	164.6	149.5	
CD (0.05 p)	T	0.43			T	0.18			T	1.09		
	Cd	0.37			Cd	0.15			Cd	0.94		
	T x Cd	0.75			T x Cd	0.31			T x Cd	1.89		

Interaction of SA with Cd was significant. SA treatment counteracted the Cd induced reduction in height. SA spray treatment proved more effective than SA seed treatment. The SA spray treatment increased the plant height from 78.5 cm to 83.3 cm under 250  $\mu\text{M}$  Cd and from 70.7 cm to 74.2 cm under 500  $\mu\text{M}$  Cd applied to soil.

#### **4.1.2 Number of tillers per plant**

The data for number of tillers per plant at flowering is presented in the Table 1, which shows that increasing the levels of Cd from 250  $\mu\text{M}$  to 500  $\mu\text{M}$  caused significant reduction in number of tillers by 11.6% to 25.1% as compared to control value (5.78), respectively.

Application of SA significantly increased the number of tillers per plant. Increase in the number of tillers was considerably more in SA spray treatment (19.2%) than in SA seed treatment (5.7%) as compared to control as well as water spray treatment.

Interaction effect of SA with Cd on number of tillers per plant was significant. SA treatment counteracted the Cd induced reduction in number of tillers per plant. SA spray treatment proved more effective than SA seed treatment. SA spray treatment increased the number of tillers per plant from 5.11 to 5.67 under 250  $\mu\text{M}$  Cd and from 4.33 to 4.76 under 500  $\mu\text{M}$  Cd.

### 4.1.3 Leaf area per plant

Table 1 depicts the leaf area per plant at flowering, which shows that under increasing levels of Cd from 250  $\mu\text{M}$  to 500  $\mu\text{M}$ , significantly reduced the leaf area by 25.7% to 32% as compared to control (199.3  $\text{cm}^2 \text{ plant}^{-1}$ ), respectively.

Application of SA brought significant increase in the leaf area per plant. Enhancement in the leaf area was relatively more in SA spray treatment (15.4%) than in SA seed treatment (12.6%) over control. Leaf area under water spray (200.2  $\text{cm}^2 \text{ plant}^{-1}$ ) and control treatments were at par.

Interaction effect of SA with Cd on leaf area per plant was significant. SA treatment counteracted the Cd-induced reduction in leaf area per plant. SA spray treatment proved more effective than SA seed treatment. SA spray treatment increased the leaf area per plant from 148.1  $\text{cm}^2 \text{ plant}^{-1}$  to 183.7  $\text{cm}^2 \text{ plant}^{-1}$  under 250  $\mu\text{M}$  Cd and from 135.4  $\text{cm}^2 \text{ plant}^{-1}$  to 164.7  $\text{cm}^2 \text{ plant}^{-1}$  under 500  $\mu\text{M}$  Cd.

### 4.1.4 Dry weight of stem per plant

Dry weight of stem per plant at harvest is presented in Table 2. Increasing levels of Cd from 250  $\mu\text{M}$  to 500  $\mu\text{M}$  significantly reduced dry weight of stem by 15.4% to 31.2% as compared to control (5.89  $\text{g plant}^{-1}$ ), respectively.

Application of SA significantly increased dry weight of stem per plant. Increase in dry weight of stem was considerably more in SA seed treatment (10.4%) than in SA

**Table 2. Effect of salicylic acid on dry weight of stem and leaf at harvest of rice under different levels of cadmium**

Treatment (T)	Dry weight of stem (g plant <sup>-1</sup> )				Dry weight of leaf (g plant <sup>-1</sup> )			
	Cd concentration (μM)				Cd concentration (μM)			
	0	250	500	Mean	0	250	500	Mean
Control	5.89	4.98	4.05	4.97	3.04	2.57	2.09	2.57
SA seed	6.50	5.81	4.42	5.58	3.33	2.98	2.30	2.88
Water spray	6.02	5.00	4.16	5.06	3.11	2.58	2.15	2.61
SA spray	6.33	5.63	4.41	5.46	3.34	2.99	2.34	2.89
Mean	6.18	5.36	4.26		3.21	2.78	2.22	
CD (0.05 p)	T	0.030			T	0.038		
	Cd	0.027			Cd	0.033		
	T x Cd	0.053			T x Cd	0.066		

spray treatment (7.5%) over control. Dry weight of stem per plant both under water spray (6.02 g plant<sup>-1</sup>) and control treatments were at par.

Interaction effect of SA with Cd on dry weight of stem per plant was significant. SA treatment counteracted the Cd induced reduction in dry weight of stem per plant. SA seed treatment proved more effective than SA spray treatment. SA seed treatment increased the dry weight of stem per plant from 4.98 g plant<sup>-1</sup> to 5.81 g plant<sup>-1</sup> under 250 µM Cd and from 4.05 g plant<sup>-1</sup> to 4.42 g plant<sup>-1</sup> under 500 µM Cd applied to soil.

#### **4.1.5 Dry weight of leaf per plant**

The data on dry weight of leaf per plant at harvest is presented in Table 2. Application of increasing levels of Cd from 250 µM and 500 µM brought significant reduction in dry weight of leaf by 15.4% and 31.2% as compared to control (3.04 g plant<sup>-1</sup>), respectively.

Application of SA significantly increased the dry weight of leaf per plant. Increase in dry weight of leaf was more in SA spray treatment (9.9%) than in SA seed treatment (9.5%) over control. Dry weight of leaf per plant under water spray and control treatments were at par.

Interaction effect of SA with Cd on dry weight of leaf per plant was significant. SA treatment counteracted the Cd induced reduction in dry weight of leaf per plant. SA spray

treatment proved more effective than SA seed treatment. SA spray treatment increased the dry weight of leaf per plant from 2.57 g plant<sup>-1</sup> to 2.99 g plant<sup>-1</sup> under 250 µM Cd and from 2.09 g plant<sup>-1</sup> to 2.34 g plant<sup>-1</sup> under 500 µM Cd applied through soil.

#### **4.1.6 Dry weight of panicles per plant**

The data on dry weight of panicles per plant at harvest is presented in Table 3, which shows that increasing levels of applied Cd from 250 µM to 500 µM caused significant reduction in dry weight of panicles by 15.4% and 31.2% as compared to control (8.27 g plant<sup>-1</sup>), respectively.

Application of SA significantly increased the dry weight of panicles per plant. Increase in dry weight of panicles per plant was considerably more with SA spray treatment (11.1%) than in SA seed treatment (9.7%) over control. Dry weight of panicles per plant under water spray and control treatments were at par.

Interaction effect of SA with Cd on dry weight of panicles per plant was significant. SA treatment counteracted the Cd-induced reduction in dry weight of panicles per plant. SA spray treatment proved more effective than SA seed treatment. SA spray treatment increased the dry weight of panicles per plant from 7.0 g plant<sup>-1</sup> to 8.17 g plant<sup>-1</sup> under 250 µM Cd and from 5.7 g plant<sup>-1</sup> to 6.35 g plant<sup>-1</sup> under 500 µM Cd applied to soil.

**Table 3. Effect of salicylic acid on dry weight of panicle and total shoot at harvest of rice under different levels of cadmium**

Treatment (T)	Dry weight of panicle (g plant <sup>-1</sup> )				Total dry weight of shoot (g plant <sup>-1</sup> )			
	Cd concentration (μM)				Cd concentration (μM)			
	0	250	500	Mean	0	250	500	Mean
Control	8.27	7.00	5.70	6.99	17.20	14.55	11.84	14.53
SA seed	9.07	8.13	6.22	7.81	18.90	16.92	12.94	16.25
Water spray	8.47	7.03	5.85	7.12	17.60	14.62	12.16	14.79
SA spray	9.19	8.17	6.35	7.90	18.86	16.79	13.10	16.25
Mean	8.75	7.58	6.03		18.14	15.72	12.51	
CD (0.05 p)	T	0.071			T	0.085		
	Cd	0.061			Cd	0.073		
	T x Cd	0.122			T x Cd	0.147		

#### **4.1.7 Total dry weight of shoot**

Table 3 shows that under increasing level of Cd, 250  $\mu\text{M}$  and 500  $\mu\text{M}$ , caused significant reduction in total dry weight of shoot by 15.4% and 31.2% as compared to control (17.2 g plant<sup>-1</sup>), respectively.

Application of SA increased the total dry weight of shoot. Enhancement in dry weight of shoot was slightly more with SA seed treatment (9.9%) than in SA spray treatment (9.7%) over control. Dry weight of plant under water spray and control treatments were at par.

Interaction effect of SA with Cd on total dry weight of shoot was significant. SA treatment counteracted the Cd induced reduction in dry weight of plant. SA seed treatment proved more effective than SA spray treatment. SA seed treatment increased the total dry weight of shoot from 14.55 g plant<sup>-1</sup> to 16.92 g plant<sup>-1</sup> under 250  $\mu\text{M}$  Cd and from 11.84 g plant<sup>-1</sup> to 12.94 g plant<sup>-1</sup> under 500  $\mu\text{M}$  Cd applied to soil.

## **4.2 PLANT WATER RELATIONS**

### **4.2.1 Leaf water potential**

Table 4 depicts the water potential ( $\Psi_w$ ) of leaf at flowering which declined progressively from -1.03 MPa in control to -1.36 MPa under 250  $\mu\text{M}$  Cd and -1.45 MPa under 500  $\mu\text{M}$  Cd applied.

**Table 4. Effect of salicylic acid on water potential, osmotic potential and relative water content at flowering of rice under different levels of cadmium**

Treatment (T)	Water potential (-MPa)				Osmotic potential (-MPa)				Relative water content (%)			
	Cd concentration ( $\mu\text{M}$ )				Cd concentration ( $\mu\text{M}$ )				Cd concentration ( $\mu\text{M}$ )			
	0	250	500	Mean	0	250	500	Mean	0	250	500	Mean
Control	1.03	1.36	1.45	1.28	1.25	1.56	1.63	1.48	86.82	79.34	69.68	78.61
SA seed	0.74	1.17	1.32	1.08	1.00	1.41	1.55	1.32	90.96	83.53	72.15	82.21
Water spray	1.00	1.35	1.43	1.26	1.22	1.55	1.61	1.46	86.47	79.23	69.53	78.41
SA spray	0.73	1.15	1.30	1.06	0.99	1.40	1.54	1.31	92.08	85.30	74.21	83.86
Mean	0.88	1.26	1.38		1.12	1.48	1.58		89.08	81.85	71.39	
CD (0.05 p)	T		0.033		T		0.048		T		0.513	
	Cd		0.029		Cd		0.041		Cd		0.444	
	T x Cd		0.058		T x Cd		0.082		T x Cd		0.889	

Application of SA significantly improved the  $\Psi_w$  of leaf. The  $\Psi_w$  was slightly higher in SA spray treatment (-0.73 MPa) than in SA seed treatment (-0.74 MPa). The difference in  $\Psi_w$  of leaf under water spray and control treatments were non significant.

Interaction effect of SA with Cd on  $\Psi_w$  of leaf was significant. SA treatment counteracted the Cd-induced reduction in  $\Psi_w$  of leaf. SA spray treatment proved more effective than SA seed treatment. The treatment of SA spray enhanced the  $\Psi_w$  of leaf from -1.36 MPa to -1.15 MPa under 250  $\mu\text{M}$  Cd and from -1.45 MPa to -1.30 MPa under 500  $\mu\text{M}$  Cd applied.

#### **4.2.2 Leaf osmotic potential**

Table 4 depicts the osmotic potential ( $\Psi_s$ ) of leaf at flowering which declined progressively from -1.25 MPa in control to -1.56 MPa under 250  $\mu\text{M}$  Cd and -1.63 MPa under 500  $\mu\text{M}$  Cd applied.

Application of SA significantly improved the  $\Psi_s$  of leaf. The  $\Psi_s$  was slightly higher in SA spray treatment (-0.99 MPa) than in SA seed treatment (-1.00 MPa). The  $\Psi_s$  of leaf under water spray (-1.22 MPa) and control (-1.25 MPa) treatments were at par.

Interaction effect of SA with Cd on  $\Psi_s$  of leaf was significant. SA treatment counteracted the Cd-induced reduction in  $\Psi_s$  of leaf. SA spray treatment proved more

effective than SA seed treatment. The treatment of SA spray enhanced the  $\Psi_s$  of leaf from -1.56 MPa to -1.40 MPa under 250  $\mu\text{M}$  Cd and from -1.63 MPa to -1.54 MPa under 500  $\mu\text{M}$  Cd applied.

#### **4.2.3 Relative water content**

Table 4 indicates that relative water content (RWC) of leaf at flowering which significantly decreased from 86.82% in control to 79.34% under 250  $\mu\text{M}$  Cd and 69.68% under 500  $\mu\text{M}$  Cd applied.

Application of SA significantly increased the RWC of leaf. Increase in RWC of leaf was more in SA spray treatment (92.08%) than in SA seed treatment (90.96%). RWC of leaf under water spray and control treatments were at par.

Interaction effect of SA with Cd on RWC of leaf was significant. SA treatment improved RWC of leaf under Cd stress. SA spray treatment improved RWC of leaf more effectively than SA seed treatment. The treatment of SA spray enhanced the RWC of leaf from 79.34% to 85.30% under 250  $\mu\text{M}$  Cd and from 69.68% to 74.21% under 500  $\mu\text{M}$  Cd applied.

### **4.3 GASEOUS EXCHANGE STUDIES**

#### **4.3.1 Photosynthetic rate**

Table 5 shows that under increasing level of Cd, 250  $\mu\text{M}$  and 500  $\mu\text{M}$ , significantly decreased the

photosynthetic rate of leaf at flowering by 15.3% and 35.1% as compared to control ( $26.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), respectively.

Application of SA increased the photosynthetic rate of leaf. Increase in photosynthetic rate was more in SA spray treatment (9.4%) than in SA seed treatment (8.1%) over control. Photosynthetic rate of leaf under water spray and control treatments were at par.

Interaction effect of SA with Cd on photosynthetic rate of leaf was significant. SA treatment counteracted the Cd induced reduction in photosynthetic rate of leaf. SA spray treatment improved photosynthetic rate of leaf more effectively than SA seed treatment. The SA spray treatment enhancement effect on photosynthetic rate was more under  $250 \mu\text{M Cd}$  (from  $22.3 \mu\text{mol m}^{-2} \text{s}^{-1}$  to  $25.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) than under  $500 \mu\text{M Cd}$  (from  $17.1 \mu\text{mol m}^{-2} \text{s}^{-1}$  to  $19.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ ).

#### **4.3.2 Transpiration rate**

Table 5 shows that under increasing level of Cd,  $250 \mu\text{M}$  and  $500 \mu\text{M}$ , significantly reduced the transpiration rate of leaf by 16.9% and 36.3% as compared to control ( $6.45 \text{mmol m}^{-2} \text{s}^{-1}$ ), respectively.

Application of SA increased the transpiration rate of leaf. Increase in transpiration rate was relatively more in SA spray treatment (7.3%) than in SA seed treatment (6%) over

**Table 5. Effect of salicylic acid on photosynthetic rate, transpiration rate and stomatal conductance at flowering of rice under different levels of cadmium**

Treatment (T)	Photosynthetic rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )				Transpiration rate ( $\text{mmol m}^{-2} \text{s}^{-1}$ )				Stomatal conductance ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )			
	Cd concentration ( $\mu\text{M}$ )				Cd concentration ( $\mu\text{M}$ )				Cd concentration ( $\mu\text{M}$ )			
	0	250	500	Mean	0	250	500	Mean	0	250	500	Mean
Control	26.3	22.3	17.1	21.9	6.45	5.36	4.11	5.31	419.0	338.7	259.7	339.2
SA seed	28.5	24.5	19.0	24.0	6.84	5.89	4.56	5.76	432.4	372.2	288.6	364.4
Water spray	26.5	22.7	17.0	22.1	6.37	5.45	4.07	5.30	422.5	344.8	257.5	341.6
SA spray	28.8	25.1	19.2	24.4	6.92	6.20	4.60	5.91	437.5	381.3	290.9	369.9
Mean	27.5	23.7	18.1		6.65	5.73	4.34		427.9	359.2	274.2	
CD (0.05 p)	T		0.15		T		0.091		T		6.54	
	Cd		0.13		Cd		0.079		Cd		5.67	
	T x Cd		0.26		T x Cd		0.158		T x Cd		11.34	

control. Transpiration rate of leaf under water spray and control treatments were at par.

Interaction effect of SA with Cd on transpiration rate of leaf was significant. SA treatment counteracted the Cd-induced reduction in transpiration rate of leaf. SA spray treatment improved transpiration rate of leaf more effectively than SA seed treatment. The SA spray treatment enhancement effect on transpiration rate was more under 250  $\mu\text{M}$  Cd (from 5.36  $\text{mmol m}^{-2} \text{s}^{-1}$  to 6.20  $\text{mmol m}^{-2} \text{s}^{-1}$ ) than under 500  $\mu\text{M}$  Cd (from 4.11  $\text{mmol m}^{-2} \text{s}^{-1}$  to 4.60  $\text{mmol m}^{-2} \text{s}^{-1}$ ).

#### **4.3.3 Stomatal conductance**

Table 5 shows that under increasing level of Cd, 250  $\mu\text{M}$  and 500  $\mu\text{M}$ , significantly reduced the stomatal conductance of leaf to the extent of 19.2% and 38% as compared to control (419.0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), respectively.

Application of SA increased the stomatal conductance. Increase in stomatal conductance was slightly higher in SA spray treatment (4.4%) than in SA seed treatment (3.2%) over control. Stomatal conductance of leaf under water spray and control treatments were at par.

Interaction effect of SA with Cd on stomatal conductance of leaf was significant. SA treatment counteracted the Cd induced reduction in stomatal

conductance of leaf. Treatment with SA spray had edge over SA seed treatment in regulating stomatal conductance.

The SA spray treatment enhancement effect on stomatal conductance was slightly more under 250  $\mu\text{M}$  Cd (from 338.7  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to 381.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) than under 500  $\mu\text{M}$  Cd (from 259.7  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to 290.9  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ).

#### **4.4 BIOCHEMICAL ESTIMATIONS**

##### **4.4.1 Total chlorophyll content**

Table 6 shows that under increasing level of Cd, 250  $\mu\text{M}$  and 500  $\mu\text{M}$ , significantly reduced the total chlorophyll content by 19.3% and 37.6% as compared to control (5.80 mg  $\text{g}^{-1}\text{FW}$ ), respectively.

Application of SA significantly increased the total chlorophyll content. Increase in chlorophyll content was slightly more in SA spray treatment (3.2%) than in SA seed treatment (3.0%) over control. Chlorophyll content of leaf under water spray and control treatments were similar.

Interaction effect of SA with Cd on total chlorophyll content of leaf was significant. SA treatments counteracted the Cd induced reduction in chlorophyll content. SA spray treatment was better than SA seed treatment. The SA spray treatment enhancement effect on total chlorophyll content in leaf was slightly more under 250  $\mu\text{M}$  Cd (from 4.69 mg  $\text{g}^{-1}\text{FW}$

**Table 6. Effect of salicylic acid on total chlorophyll content and total soluble carbohydrate content at flowering of rice under different levels of cadmium**

Treatment (T)	Total chlorophyll content (mg g <sup>-1</sup> FW)				Total soluble carbohydrate (mg g <sup>-1</sup> FW)			
	Cd concentration (μM)				Cd concentration (μM)			
	0	250	500	Mean	0	250	500	Mean
Control	5.80	4.69	3.62	4.70	56.2	47.7	39.3	47.7
SA seed	5.98	4.96	3.85	4.93	57.7	48.7	40.0	48.8
Water spray	5.80	4.70	3.61	4.70	56.2	47.6	39.9	47.9
SA spray	5.99	4.99	3.89	4.96	58.6	49.9	41.9	50.1
Mean	5.89	4.83	3.74		57.2	48.5	40.3	
CD (0.05 p)	T	0.025			T	0.24		
	Cd	0.021			Cd	0.21		
	T x Cd	0.043			T x Cd	0.41		

to 4.99 mg g<sup>-1</sup>FW) than under 500 µM Cd (from 3.62 mg g<sup>-1</sup>FW to 3.89 mg g<sup>-1</sup>FW).

#### **4.4.2 Total soluble carbohydrate**

Table 6 shows that under increasing level of Cd, 250 µM and 500 µM, reduced total soluble carbohydrate by 15.1% and 30.0% as compared to control (56.2 mg g<sup>-1</sup>FW), respectively.

Application of SA increased the total soluble carbohydrate. Increase in total soluble carbohydrate was relatively more in SA spray treatment (4.3%) than in SA seed treatment (2.7%) over control. Total soluble carbohydrate of leaf under water spray and control treatments were at par.

Interaction effect of SA with Cd on total soluble carbohydrate of leaf was significant. SA treatment partially alleviated the Cd induced reduction in total soluble carbohydrate. SA spray treatment was more effective than SA seed treatment. The SA spray treatment enhancement effect on total soluble carbohydrate in leaf was slightly more under 250 µM Cd (from 47.7 mg g<sup>-1</sup>FW to 49.9 mg g<sup>-1</sup>FW) than under 500 µM Cd (from 39.3 mg g<sup>-1</sup>FW to 41.9 mg g<sup>-1</sup>FW).

#### **4.4.3 Total soluble protein**

Table 7 shows that under increasing level of Cd, 250 µM and 500 µM, significantly reduced the total soluble

protein in leaf by 29.6% and 36.8% as compared to control (26.52 mg g<sup>-1</sup>FW), respectively.

Application of SA increased the total soluble protein in leaf. Increase in total soluble protein was considerably more in SA spray treatment (13.5%) than in SA seed treatment (6.9%) over control. Total soluble protein in leaf under water spray and control treatments were at par.

Interaction effect of SA with Cd on total soluble protein in leaf was significant. SA treatment partially overcomes the Cd induced reduction in total soluble protein. SA spray treatment was more effective than SA seed treatment. The SA spray enhancement effect was more under 250 µM Cd (from 18.67 mg g<sup>-1</sup>FW to 24.77 mg g<sup>-1</sup>FW) than under 500 µM Cd (16.75 mg g<sup>-1</sup>FW to 18.67 mg g<sup>-1</sup>FW) as compared to control.

#### **4.4.4 Total free amino acid**

Table 7 shows that under increasing level of Cd, 250 µM and 500 µM, significantly increased the total free amino acid content in leaf by 32.9% and 61.6% as compared to control (9.33 µmol g<sup>-1</sup>FW), respectively.

Application of SA increased total free amino acid content in leaf. Increase in total free amino acid was slightly more in SA spray treatment (11.4%) than in SA seed treatment (11.2%) over control.

**Table 7. Effect of salicylic acid on total soluble protein, free amino acids and free proline content at flowering of rice under different levels of cadmium**

Treatment (T)	Total soluble protein (mg g <sup>-1</sup> FW)				Total free amino acids (μmol g <sup>-1</sup> FW)				Free proline (μmol g <sup>-1</sup> FW)			
	Cd concentration (μM)				Cd concentration (μM)				Cd concentration (μM)			
	0	250	500	Mean	0	250	500	Mean	0	250	500	Mean
Control	26.52	18.67	16.75	20.64	9.33	12.40	15.08	12.27	0.44	0.59	0.78	0.60
SA seed	28.35	20.45	18.23	22.34	10.37	14.58	16.26	13.73	0.42	0.56	0.74	0.58
Water spray	26.86	19.10	17.10	21.02	9.18	12.21	15.25	12.21	0.43	0.58	0.76	0.59
SA spray	30.09	24.77	18.67	24.51	10.39	14.65	16.68	13.90	0.40	0.54	0.71	0.55
Mean	27.96	20.75	17.68		9.82	13.46	15.82		0.42	0.57	0.75	
CD (0.05 p)	T		0.75		T		0.264		T		0.003	
	Cd		0.65		Cd		0.228		Cd		0.003	
	T x Cd		1.29		T x Cd		0.457		T x Cd		0.006	

Interaction effect of SA with Cd on total free amino acid content of leaf was significant. SA treatment accentuated the Cd induced increase in total free amino acid. SA spray treatment was slightly more effective than SA seed treatment. The ameliorating effect of SA spray was remarkably more under 250  $\mu\text{M}$  Cd (12.40  $\mu\text{mol g}^{-1}\text{FW}$  to 14.65  $\mu\text{mol g}^{-1}\text{FW}$ ) than under 500  $\mu\text{M}$  Cd (15.08  $\mu\text{mol g}^{-1}\text{FW}$  to 16.68  $\mu\text{mol g}^{-1}\text{FW}$ ).

#### 4.4.5 Proline content

Table 7 shows that under increasing level of Cd, 250  $\mu\text{M}$  and 500  $\mu\text{M}$ , resulted in a conspicuous accumulation of free proline in leaf to the extent of 34.0% and 77.0% as compared to control (0.44  $\mu\text{mol g}^{-1}\text{FW}$ ), respectively.

Application of SA decreased the free proline content in leaf. The decrease in free proline content was more in SA spray treatment (9.09%) than in SA seed treatment (4.55%) over control. The free proline content in leaf under water spray and control treatments were at par.

Interaction effect of SA with Cd on the free proline content in leaf was significant. SA treatment slightly decreased the free proline accumulation under Cd induced stress condition. However, the magnitude of free proline content still remained above control. SA spray treatment was more effective than SA seed treatment. The SA dwindled the free proline accumulation more under 500  $\mu\text{M}$  Cd (0.59  $\mu\text{mol}$

$\text{g}^{-1}\text{FW}$  to  $0.54 \mu\text{mol g}^{-1}\text{FW}$ ) as compared to  $250 \mu\text{M Cd}$  ( $0.78 \mu\text{mol g}^{-1}\text{FW}$  to  $0.71 \mu\text{mol g}^{-1}\text{FW}$ ).

#### **4.4.6 Malondialdehyde content**

Table 8 shows that under increasing level of Cd,  $250 \mu\text{M}$  and  $500 \mu\text{M}$ , significantly increased the malondialdehyde content in leaf by 42.7% and 78.7% as compared to control ( $63.8 \text{ nmol g}^{-1}\text{FW}$ ), respectively.

Application of SA decreased the malondialdehyde content. Decrease in malondialdehyde content was more pronounced in SA spray treatment (32.6%) than in SA seed treatment (30.3%) over control. Malondialdehyde content of leaf under water spray and control treatments were at par.

Interaction effect of SA with Cd on malondialdehyde content of leaf was significant. Treatment of SA counteracted the Cd induced increase in malondialdehyde content. SA spray treatment was more effective than SA seed treatment. The SA spray treatment decreased the malondialdehyde content more under  $250 \mu\text{M Cd}$  ( $91.0 \text{ nmol g}^{-1}\text{FW}$  to  $65.2 \text{ nmol g}^{-1}\text{FW}$ ) as compared to  $500 \mu\text{M Cd}$  ( $114.0 \text{ nmol g}^{-1}\text{FW}$  to  $104.7 \text{ nmol g}^{-1}\text{FW}$ ).

#### **4.4.7 Membrane injury**

The data for membrane injury in leaf at flowering is presented in Table 8. Increasing levels of Cd application significantly increased the membrane injury. The membrane

**Table 8. Effect of salicylic acid on malondialdehyde content and membrane injury at flowering of rice under different levels of cadmium**

Treatment (T)	MDA content (nM g <sup>-1</sup> FW)				Membrane injury (%)			
	Cd concentration (μM)				Cd concentration (μM)			
	0	250	500	Mean	0	250	500	Mean
Control	63.8	91.0	114.0	89.6	22.26	24.64	29.41	25.43
SA seed	44.4	66.5	107.0	72.6	18.16	20.32	26.30	21.60
Water spray	62.6	89.9	111.8	88.1	21.39	23.69	27.68	24.26
SA spray	43.0	65.2	104.7	71.0	16.52	19.26	24.28	20.02
Mean	53.5	78.2	109.4		19.58	21.98	26.92	
CD (0.05 p)	T	4.50			T	0.47		
	Cd	3.90			Cd	0.40		
	T x Cd	7.79			T x Cd	0.81		

injury in control was 22.26% which raised to 24.64% under 250  $\mu\text{M}$  Cd and 29.41% under 500  $\mu\text{M}$  Cd.

Application of SA decreased the membrane injury. Membrane injury was lower in SA spray treatment (16.52%) than in SA seed treatment (18.16%). Membrane injury of leaf both under water spray and control treatments were at par.

Interaction effect of SA with Cd on membrane injury of leaf was significant. SA treatment lowered the Cd induced injury to the membrane. Treatment of SA spray proved more effective than SA seed treatment. The SA spray treatment decreased the membrane injury percentage relatively more under 250  $\mu\text{M}$  Cd (24.64% to 19.26%) as compared to 500  $\mu\text{M}$  Cd (29.41% to 24.28%).

## **4.5 CADMIUM CONCENTRATION IN PLANT PARTS**

### **4.5.1 Cadmium concentration in stem**

The data for Cd concentration in stem at harvest is presented in the Table 9. Increasing levels of Cd application progressively increased Cd concentration in stem. The value of Cd concentration in control was 1.06  $\mu\text{g g}^{-1}\text{DW}$  and the elevated values were 5.58  $\mu\text{g g}^{-1}$  under 250  $\mu\text{M}$  Cd and 14.23  $\mu\text{g g}^{-1}\text{DW}$ .

Application of SA lowered down the Cd concentration in stem. The lowering effect in Cd concentration in stem was more in SA seed treatment (0.68  $\mu\text{g g}^{-1}\text{DW}$ ) than in SA spray treatment (0.72  $\mu\text{g g}^{-1}\text{DW}$ ) as compared to control (1.06  $\mu\text{g g}^{-1}\text{DW}$ )

**Table 9. Effect of salicylic acid on cadmium concentration in stem and leaf at harvest of rice under different levels of cadmium**

Treatment (T)	Cd concentration in stem ( $\mu\text{g g}^{-1}$ DW)				Cd concentration in leaf ( $\mu\text{g g}^{-1}$ DW)			
	Cd concentration ( $\mu\text{M}$ )				Cd concentration ( $\mu\text{M}$ )			
	0	250	500	Mean	0	250	500	Mean
Control	1.06	5.58	14.23	6.95	0.35	1.90	5.28	2.51
SA seed	0.68	3.23	7.88	3.93	0.25	1.24	3.32	1.60
Water spray	1.03	5.48	14.03	6.84	0.35	1.90	5.02	2.42
SA spray	0.72	3.57	9.39	4.56	0.23	1.13	2.78	1.38
Mean	0.87	4.46	11.38		0.30	1.54	4.10	
CD (0.05 p)	T	0.449			T	0.170		
	Cd	0.432			Cd	0.147		
	T x Cd	0.865			T x Cd	0.294		

$\text{g}^{-1}\text{DW}$ ). Cd concentration in stem under water spray and control treatments were at par.

Interaction effect of SA with Cd on Cd concentration in stem was significant. SA treatment lessened the Cd induced increase in Cd content in stem. In this regard, SA seed treatment was more effective than SA spray treatment. The SA seed treatment lowered down the values of Cd concentration in stem from  $5.58 \mu\text{g g}^{-1}\text{DW}$  to  $3.23 \mu\text{g g}^{-1}\text{DW}$  under  $250 \mu\text{M Cd}$  and from  $14.23 \mu\text{g g}^{-1}\text{DW}$  to  $7.88 \mu\text{g g}^{-1}\text{DW}$  under  $500 \mu\text{M Cd}$  applied to soil.

#### **4.5.2 Cadmium concentration in leaf**

The data for Cd concentration in leaf at harvest is presented in the Table 9. Increasing levels of Cd application progressively increased Cd concentration in leaf. Cd concentration in control was  $0.35 \mu\text{g g}^{-1}\text{DW}$  which was elevated to  $1.90 \mu\text{g g}^{-1}\text{DW}$  under  $250 \mu\text{M Cd}$  and  $5.28 \mu\text{g g}^{-1}\text{DW}$  under  $500 \mu\text{M Cd}$ .

Application of SA lowered down the Cd concentration in leaf. The lowering effect in Cd concentration in leaf was more in SA spray treatment ( $0.23 \mu\text{g g}^{-1}\text{DW}$ ) than in SA seed treatment ( $0.25 \mu\text{g g}^{-1}\text{DW}$ ) as compared to control ( $0.35 \mu\text{g g}^{-1}\text{DW}$ ). Cd concentration in leaf under water spray and control treatments were at par.

Interaction effect of SA with Cd on Cd concentration in leaf was significant. SA treatment lessened the Cd induced increase in Cd content in leaf. In this regard, SA spray treatment was more effective than SA seed treatment. The SA seed treatment lowered down the values of Cd concentration in leaf from 1.90  $\mu\text{g g}^{-1}\text{DW}$  to 1.13  $\mu\text{g g}^{-1}\text{DW}$  under 250  $\mu\text{M}$  Cd and from 5.28  $\mu\text{g g}^{-1}\text{DW}$  to 2.78  $\mu\text{g g}^{-1}\text{DW}$  under 500  $\mu\text{M}$  Cd applications to soil.

#### **4.5.3 Cadmium concentration in panicle**

The data for Cd concentration in panicle at harvest is presented in the Table 10. Increasing levels of Cd application progressively increased Cd concentration in panicle. Cd concentration in control was 0.11  $\mu\text{g g}^{-1}\text{DW}$  which was elevated to 0.27  $\mu\text{g g}^{-1}$  under 250  $\mu\text{M}$  Cd and 0.96  $\mu\text{g g}^{-1}\text{DW}$  under 500  $\mu\text{M}$  Cd.

Application of SA lowered down the Cd concentration in panicle. The lowering effect in Cd concentration in panicle was more in SA spray treatment (0.07  $\mu\text{g g}^{-1}\text{DW}$ ) than in SA seed treatment (0.08  $\mu\text{g g}^{-1}\text{DW}$ ) as compared to control (0.11  $\mu\text{g g}^{-1}\text{DW}$ ). Cd concentration in panicle under water spray and control treatments were at par.

Interaction effect of SA with Cd on Cd concentration in panicle was significant. SA treatment lessened the Cd induced increase in Cd content in panicle. In this regard, SA spray treatment was more effective than SA seed treatment.

**Table 10. Effect of salicylic acid on cadmium concentration in panicle and in shoot at harvest of rice under different levels of cadmium**

Treatment (T)	Cd concentration in panicle ( $\mu\text{g g}^{-1}$ DW)				Cd concentration in shoot ( $\mu\text{g g}^{-1}$ DW)			
	Cd concentration ( $\mu\text{M}$ )				Cd concentration ( $\mu\text{M}$ )			
	0	250	500	Mean	0	250	500	Mean
Control	0.11	0.27	0.96	0.45	0.48	2.37	6.26	3.04
SA seed	0.08	0.18	0.63	0.30	0.32	1.41	3.58	1.77
Water spray	0.11	0.26	0.91	0.42	0.46	2.33	6.13	2.97
SA spray	0.07	0.17	0.55	0.26	0.32	1.48	3.93	1.91
Mean	0.09	0.22	0.76		0.39	1.90	4.97	
CD (0.05 p)	T	0.029			T	0.235		
	Cd	0.025			Cd	0.204		
	T x Cd	0.050			T x Cd	0.407		

The SA spray treatment lowered down the values of Cd concentration in panicle from 0.27  $\mu\text{g g}^{-1}\text{DW}$  to 0.17  $\mu\text{g g}^{-1}\text{DW}$  under 250  $\mu\text{M Cd}$  and from 0.96  $\mu\text{g g}^{-1}\text{DW}$  to 0.55  $\mu\text{g g}^{-1}\text{DW}$  under 500  $\mu\text{M Cd}$  applied to soil.

#### **4.5.3 Cadmium concentration in shoot**

The data for Cd concentration in shoot at harvest is presented in the Table 10. Increasing levels of Cd application progressively increased Cd concentration in shoot. Cd concentration in control was 0.48  $\mu\text{g g}^{-1}\text{DW}$  which was elevated to 2.37  $\mu\text{g g}^{-1}$  under 250  $\mu\text{M Cd}$  and 6.26  $\mu\text{g g}^{-1}\text{DW}$  under 500  $\mu\text{M Cd}$ .

Application of SA lowered down the Cd concentration in shoot. The values of Cd concentration in shoot was lowered down from 0.48  $\mu\text{g g}^{-1}\text{DW}$  in control to 0.32  $\mu\text{g g}^{-1}\text{DW}$  in SA seed treatment as well as in SA spray treatment. Cd concentration in shoot under water spray and control treatments were at par.

Interaction effect of SA with Cd on Cd concentration in shoot was significant. SA treatment lessened the Cd induced increase in Cd content in shoot. In this regard, SA seed treatment was more effective than SA spray treatment. The SA seed treatment lowered down the values of Cd concentration in shoot from 2.37  $\mu\text{g g}^{-1}\text{DW}$  to 1.41  $\mu\text{g g}^{-1}\text{DW}$  under 250  $\mu\text{M Cd}$  and from 6.26  $\mu\text{g g}^{-1}\text{DW}$  to 3.58  $\mu\text{g g}^{-1}\text{DW}$  under 500  $\mu\text{M Cd}$  applied to soil.

## **4.6 YIELD ATTRIBUTES**

### **4.6.1 Number of panicles per plant**

Table 11 shows that under increasing level of Cd, 250  $\mu\text{M}$  and 500  $\mu\text{M}$ , decrease the number of panicles per plant to extent of 13.7% and 29.4% as compared to control value (5.67), respectively.

Application of SA increased the number of panicles per plant. Increase number of panicles per plant was more in SA seed treatment (7.8%) than in SA spray treatment (5.9%) over control. The number of panicles per plant under both water spray and control treatments were at par.

SA treatments ameliorated the number of panicles per plant under Cd stress. SA seed treatment was more effective than SA spray treatment in mitigating the detrimental effect of Cd. The SA seed treatment enhancement effect on number of panicles per plant was slightly more under 250  $\mu\text{M}$  Cd (from 4.89 to 5.66) than under 500  $\mu\text{M}$  Cd (from 4.00 to 4.33).

### **4.6.2 Number of spikelets per panicle**

Table 11 shows that under increasing level of Cd, 250  $\mu\text{M}$  and 500  $\mu\text{M}$ , decreased the number of spikelets per panicle by 8.5% and 16.2% as compared to control value (73.90), respectively.

**Table 11. Effect of salicylic acid on number of panicles per plant and number of spikelets per panicle at harvest of rice under different levels of cadmium**

Treatment (T)	Panicles (No. plant <sup>-1</sup> )				Spikelets (No. panicle <sup>-1</sup> )			
	Cd concentration ( $\mu$ M)				Cd concentration ( $\mu$ M)			
	0	250	500	Mean	0	250	500	Mean
Control	5.67	4.89	4.00	4.85	73.90	67.62	61.92	67.81
SA seed	6.11	5.56	4.33	5.33	77.03	71.69	66.10	71.61
Water spray	5.78	4.89	4.11	4.93	73.05	67.85	63.34	68.08
SA spray	6.00	5.44	4.33	5.26	80.56	75.14	70.95	75.55
Mean	5.89	5.20	4.19		76.14	70.57	65.58	
CD (0.05 p)	T	0.101			T	0.452		
	Cd	0.087			Cd	0.391		
	T x Cd	0.174			T x Cd	0.782		

Application of SA significantly increased the number of spikelets per panicle. Increased number of spikelets per panicles was more in SA spray treatment (9.0%) than in SA seed treatment (4.2%) over control. The number of spikelets per panicle under both water spray and control treatments were at par.

SA treatment counteracted the Cd induced decrease in number of spikelets per panicle. SA spray treatment was more effective than SA seed treatment. The SA spray treatment enhancement effect on number of spikelets per panicle was more under 250  $\mu\text{M}$  Cd (from 67.62 to 75.14) than under 500  $\mu\text{M}$  Cd (from 61.92 to 70.95).

#### **4.6.3 Filled grain percentage**

The data for filled grain percentage is presented in Table 12. Increasing levels of Cd application significantly decreased the filled grain percentage. The filled grain percentage in control was 81.11% which declined to 80.38% under 250  $\mu\text{M}$  Cd and 79.50% under 500  $\mu\text{M}$  Cd.

Application of SA significantly decreased the filled grain percentage. The filled grain percentage was similar in SA spray treatment (81.59%) and in SA seed treatment. Filled grain percentage both under water spray and control treatments were also at par.

Interaction effect of SA with Cd on filled grain percentage was non significant.

**Table 12. Effect of salicylic acid on filled grain percentage and 1000-grain weight at harvest of rice under different levels of cadmium**

Treatment (T)	Filled grain (%)				1000-grain weight (g)			
	Cd concentration ( $\mu\text{M}$ )				Cd concentration ( $\mu\text{M}$ )			
	0	250	500	Mean	0	250	500	Mean
Control	81.11	80.38	79.50	80.33	20.46	20.28	20.16	20.30
SA seed	81.59	80.92	80.02	80.84	20.63	20.44	20.30	20.46
Water spray	81.20	80.51	79.64	80.45	20.46	20.29	20.16	20.30
SA spray	81.59	81.01	80.09	80.89	20.64	20.46	20.31	20.47
Mean	81.37	80.70	79.81		20.55	20.37	20.23	
CD (0.05 p)	T	0.348			T	0.087		
	Cd	0.295			Cd	0.075		
	T x Cd	NS			T x Cd	NS		

#### **4.6.4 1000 grain weight**

Table 12 shows that under increasing level of Cd, 250  $\mu\text{M}$  and 500  $\mu\text{M}$ , decreased 1000 grain weight by 0.88% and 1.47% as compared to control value (20.46 g), respectively.

Application of SA marginally increased the 1000 grain weight. Increase 1000 grain weight was very slight in SA spray treatment (0.88%) than in SA seed treatment (0.83%) over control. The 1000 grain weight under both water spray and control treatments were at par.

Interaction effect of SA with Cd on 1000 grain weight was non significant.

### **4.7 Yield**

#### **4.7.1 Grain yield per plant**

The data for grain yield per plant is presented in Table 13.

Application of Cd 250  $\mu\text{M}$  and 500  $\mu\text{M}$  resulted in significant decline in grain yield per plant to the extent of 22.4% and 42.9%, respectively as compared to control value (6.95 g plant<sup>-1</sup>).

Application of SA has ameliorating effect on grain yield per plant under Cd stress. Treatment with SA spray has edge over SA seed treatment in improving the grain yield per plant to the extent of 17.1% and 14.0%, respectively over

**Table 13. Effect of salicylic acid on grain yield (g plant<sup>-1</sup>) at harvest of rice under different levels of cadmium**

Treatment (T)	Cd concentration ( $\mu\text{M}$ )			
	0	250	500	Mean
Control	6.95	5.39	3.97	5.44
SA seed	7.92	6.59	4.65	6.39
Water spray	7.01	5.42	4.18	5.54
SA spray	8.14	6.78	5.00	6.64
Mean	7.51	6.04	4.45	
CD (0.05p)	T	0.074		
	Cd	0.064		
	T x Cd	0.129		

control. The grain yield per plant under both water spray and control treatments were at par.

SA treatment partially alleviated the Cd induced decline in the grain yield per plant. SA spray treatment proved more effective than SA seed treatment.

SA spray treatment enhanced the grain yield per plant more under 250  $\mu\text{M}$  Cd (from 5.39 g plant<sup>-1</sup> to 6.78 g plant<sup>-1</sup>) than under 500  $\mu\text{M}$  Cd (from 3.97 g plant<sup>-1</sup> to 5.00 g plant<sup>-1</sup>) applied to soil.

## **4.8 QUALITY OF BROWN RICE**

### **4.8.1 Starch content in brown rice**

The data for starch content in brown rice is presented in Table 14. Increasing levels of Cd application significantly decreased the starch content in brown rice. The starch content in brown rice in control was 83.01% which declined to 82.18% under 250  $\mu\text{M}$  Cd and 81.35% under 500  $\mu\text{M}$  Cd.

Application of SA significantly increased the starch content in brown rice. The starch content in brown rice was slightly higher in SA spray treatment (83.90%) than in SA seed treatment (83.68%). Starch content in brown rice both under water spray and control treatments were at par.

Interaction effect of SA with Cd on starch content in brown rice was non significant.

**Table 14. Effect of salicylic acid on starch, amylose content and cadmium concentration in brown rice at harvest of rice under different levels of cadmium**

Treatment (T)	Starch content (%)				Amylose content (%)				Cd concentration in brown rice ( $\mu\text{g g}^{-1}$ DW)			
	Cd concentration ( $\mu\text{M}$ )				Cd concentration ( $\mu\text{M}$ )				Cd concentration ( $\mu\text{M}$ )			
	0	250	500	Mean	0	250	500	Mean	0	250	500	Mean
Control	83.01	82.18	81.35	82.18	23.38	23.26	22.84	23.16	0.14	0.38	1.57	0.69
SA seed	83.68	82.82	82.00	82.83	23.49	23.39	23.27	23.38	0.10	0.24	0.97	0.44
Water spray	82.97	82.14	81.31	82.14	23.37	23.23	22.79	23.13	0.14	0.36	1.46	0.65
SA spray	83.90	82.87	82.22	83.00	23.48	23.36	23.20	23.35	0.09	0.22	0.81	0.37
Mean	83.39	82.50	81.72		23.43	23.31	23.02		0.12	0.30	1.20	
CD (0.05 p)	T		0.426		T		0.198		T		0.033	
	Cd		0.369		Cd		0.172		Cd		0.028	
	T x Cd		NS		T x Cd		NS		T x Cd		0.056	

#### **4.8.2 Amylose content in grain**

The data for amylose content in brown rice is presented in Table 14. Increasing levels of Cd application significantly decreased the amylose content in brown rice. The amylose content in brown rice in control was 23.38% which declined to 23.26% under 250  $\mu\text{M}$  Cd and 22.84% under 500  $\mu\text{M}$  Cd.

Application of SA significantly increased the amylose content in brown rice. The amylose contents in brown rice both in SA spray treatment as well as in SA seed treatment were non significantly different. Similarly, amylose contents in brown rice both under water spray and control treatments were also at par.

Interaction effect of SA with Cd on amylose content in brown rice was non significant.

#### **4.8.3 Cadmium content in brown rice**

The data for Cd concentration in brown rice is presented in the Table 14. Increasing levels of Cd application progressively increased Cd concentration in brown rice. Cd concentration in control was 0.14  $\mu\text{g g}^{-1}\text{DW}$  which was elevated to 0.38  $\mu\text{g g}^{-1}$  under 250  $\mu\text{M}$  Cd and 1.57  $\mu\text{g g}^{-1}\text{DW}$  under 500  $\mu\text{M}$  Cd.

Application of SA lowered down the Cd concentration in brown rice. The values of Cd concentration in brown rice was lowered down from 0.14  $\mu\text{g g}^{-1}\text{DW}$  in control to 0.09  $\mu\text{g}$

---

$\text{g}^{-1}\text{DW}$  in SA spray treatment and  $0.10 \mu\text{g g}^{-1}\text{DW}$  in SA seed treatment. Cd concentration in brown rice under water spray and control treatments were at par.

Interaction effect of SA with Cd on Cd concentration in brown rice was significant. SA treatment lessened the Cd induced increase in Cd content in brown rice. In this regard, SA spray treatment was more effective than SA spray treatment. The SA spray treatment lowered down the values of Cd concentration in brown rice from  $0.38 \mu\text{g g}^{-1}\text{DW}$  to  $0.22 \mu\text{g g}^{-1}\text{DW}$  under  $250 \mu\text{M}$  Cd and from  $1.57 \mu\text{g g}^{-1}\text{DW}$  to  $0.81 \mu\text{g g}^{-1}\text{DW}$  under  $500 \mu\text{M}$  Cd applied to soil.

**CHAPTER –V***Discussion*

The present investigation was undertaken on “physiological and biochemical effects of salicylic acid on rice (*Oryza sativa* L.) under cadmium stress”. In this chapter, an attempt has been made to offer explanations and experimental evidences for the observed variations in the experimental results. The results obtained in the present study are discussed herewith under the following heads:

- Growth
- Plant water relations
- Gaseous exchange studies
- Biochemical estimations
- Cadmium concentration
- Yield and its attributes
- Quality of brown rice

**5.1 Growth**

Growth is one of the best indices for evaluating plant response to environment stress. Cadmium adversely affects rice growth and metabolism (Shah and Dubey, 1995; 1997). In the present work, when the plants were exposed to 250  $\mu\text{M}$

to 500  $\mu\text{M Cd kg}^{-1}$  soil, a significant decrease in plant height (Table 1) of rice plant was observed. This accorded with earlier reports in rice (Liu *et al.*, 2007; Hassan *et al.*, 2005; Moya and Picazo, 1993). The decrease in plant height may be due reduction in cell division and cell expansion which are turgor driven physiological processes adversely affected by Cd stress (Somaskhekaraiyah *et al.*, 1992). Barcelo *et al.* (1988) suggested that reduced cell turgor potential and cell wall elasticity led to formation of small cells and intercellular space area under Cd treated plant. SA application significantly increased the plant height (Table 1). SA spray treatment proved better than SA seed treatment in partially improving plant height under Cd stress. Improvement in plant height might be due to higher values of RWC (Table 4) with the SA application. Such an increase in plant height has also been observed with the application of 0.25 mM SA in Kentucky bluegrass (He *et al.*, 2005).

Number of tillers per plant is another important vegetative growth parameter of plant affected by Cd stress. In the present study, when the plants were exposed to 250  $\mu\text{M}$  to 500  $\mu\text{M Cd kg}^{-1}$  soil, a significant decrease in number of tillers per plant (Table 1) of rice plant was observed. This accorded with earlier reports in rice (Liu *et al.*, 2007). The decrease in number of tillers per plant may be due reduction in cell division and cell expansion which are turgor driven

physiological processes adversely affected by Cd stress (Somaskhekaraiyah *et al.*, 1992). Barcelo *et al.* (1988) suggested that reduced cell turgor potential and cell wall elasticity led to formation of small cells and intercellular space area under Cd treated plant. Application of Salicylic acid significantly increased the number of tillers per plant (Table 1). SA spray treatment proved better than SA seed treatment in partially improving the number of tillers per plant under Cd stress. Improvement in number of tillers per plant might be due to higher values of RWC (Table 4) and photosynthetic rate (Table 5) with the SA application.

Leaf growth, an important attribute of plant growth and productivity, is sensitive to Cd stress. In the present study, when the plants were exposed to 250  $\mu\text{M}$  to 500  $\mu\text{M}$  Cd  $\text{kg}^{-1}$  soil, a significant decrease in leaf area per plant (Table 1) of rice plant was observed. This accorded with earlier reports in rice (Liu *et al.*, 2007). The decrease in leaf area per plant may be due reduction in cell division and cell expansion which are turgor driven physiological processes adversely affected by Cd stress (Somaskhekaraiyah *et al.*, 1992). Barcelo *et al.* (1988) suggested that reduced cell turgor potential and cell wall elasticity led to formation of small cells and intercellular space area under Cd treated plant. SA application significantly increased the leaf area per plant (Table 1). Such increase in leaf area has been

reported earlier in maize (Khan *et al.*, 2003). SA spray treatment proved better than SA seed treatment in partially improving the leaf area per plant under Cd stress. Improvement in leaf area per plant might be due to higher values of RWC (Table 4) and photosynthetic rate (Table 5) with the SA application.

In addition to plant height, number of tillers and leaf area, other growth parameters viz. dry weight of shoot (Table 3) and its components: stem, leaf and panicles weight (Table 2 and 3) showed significant decrease with the increasing levels Cd (250  $\mu\text{M}$  to 500  $\mu\text{M}$  Cd  $\text{kg}^{-1}$  soil) stress. This accorded with earlier reports in rice (Liu *et al.*, 2007; Hsu *et al.* (2006). Liu *et al.* (2007) in pot experiments on rice showed that plant height, numbers of tillers, leaf area, and dry matter accumulation of root, straw and total plant were significantly reduced under high level of soil Cd stress (100  $\text{mg kg}^{-1}$ ). SA application significantly increased the total dry weight of shoot as well as their components (dry weights of stem, leaf and panicle) at harvest. Increase in dry weight of maize has been reported with SA application (Khan *et al.*, 2003). SA spray treatment being better than seed treatment in partially improving the total dry weight of shoot as well as their components under Cd stress. Improvement in dry weight of shoot and its components: stem, leaf and panicles weight might be due to higher values of plant height (Table

1), numbers of tillers (Table 1), leaf area (Table 1), plant water status (Table 4) and photosynthetic rate (Table 5) with the SA application.

## 5.2 Plant water relations

Cadmium reduces water uptake, transport and transpiration (Vassilev *et al.*, 1997) which causes disturbed water balance in plants. Data presented in Table 4 showed decrease in water potential ( $\Psi_w$ ) when the plants were exposed to 250  $\mu\text{M}$  to 500  $\mu\text{M}$  Cd  $\text{kg}^{-1}$  soil. Decreased  $\Psi_w$  under Cd stress has been reported earlier in barley by (Vassilev *et al.*, 1997); in lettuce by (Costa and More, 1994 and in bean by (Poschenrieder *et al.*, 1989). Application of SA significantly improved the  $\Psi_w$  of leaf over control. The  $\Psi_w$  was slightly higher in SA spray treatment than in SA seed treatment. Such an increase in  $\Psi_w$  with SA application in tomato has been observed by Tari *et al.* (2002).

Data presented in Table 4 showed decrease in osmotic potential when the plants were exposed to 250  $\mu\text{M}$  to 500  $\mu\text{M}$  Cd  $\text{kg}^{-1}$  soil. Decreased osmotic potential under Cd stress has been reported earlier in barley by (Vassilev *et al.*, 1997); in white lupin by (Costa and Morel, 1994; Costa *et al.*, 1997); in bean by (Poschenrieder *et al.*, 1989) and in sunflower by Pena *et al.* (2006). Application of SA significantly improved the  $\Psi_s$  of leaf over control. The  $\Psi_s$  was

slightly higher in SA spray treatment than in SA seed treatment.

The data presented in Table 4 showed decrease in relative water content when the plants were exposed to 250  $\mu\text{M}$  to 500  $\mu\text{M}$   $\text{Cd kg}^{-1}$  soil. Decreased relative water content under Cd stress has been reported earlier in barley by (Vassilev *et al.*, 1997); in lettuce by (Costa and Morel, 1994; in bean by (Poschenrieder *et al.*, 1989) and in sunflower by Pena *et al* (2006). Application of SA significantly improved the RWC of leaf over control. The RWC was slightly higher in SA spray treatment than in SA seed treatment.

### 5.3 Gaseous exchange studies

Cadmium inhibits the photosynthetic rate in plants (Vassilev *et al.*, 1997). In the present work, the data presented in Table 5 showed decrease in photosynthetic rate, when the plants were exposed to 250  $\mu\text{M}$  to 500  $\mu\text{M}$   $\text{Cd kg}^{-1}$  soil. Similar results have been reported earlier in rice by Moya *et al.* (1993) and in wheat by Samiulla *et al.* (2007). The reduction in photosynthesis under Cd stress was mainly due to the decreased  $\Psi_w$  (Table 4) and RWC (Table 4) which led to loss of leaf turgor and ultimately decreased stomatal conductance (Table 5). Another factor contributing to photosynthetic inhibition under Cd stress may be decreased chlorophyll content (Table 6) and reduced leaf area (Table 1).

Application of SA increased the photosynthetic rate of leaf. Increase in photosynthetic rate was more in SA spray treatment than in SA seed treatment over control. Similar results have been obtained in corn with the application of SA (Khan *et al.*, 2003). Higher photosynthetic rate of SA spray treatment could be attributed to its higher  $\Psi_w$  (Table 4), RWC (Table 4), stomatal conductance (Table 5) and chlorophyll content (Table 6). Chandra and Bhatt (1998) reported that high chlorophyll content in cowpea which showed a good correlation with the net photosynthetic rate after the spray application of SA. Contrary to this, Janda (2000) observed a decrease in net photosynthesis, stomatal conductivity and transpiration after 1 day of benzoic acid or aspirin (SA analogue) treatment at 22/20°C in maize.

In the present work, the data presented in Table 5 showed decrease in transpiration rate when the plants were exposed to 250  $\mu\text{M}$  to 500  $\mu\text{M}$  Cd  $\text{kg}^{-1}$  soil. Similar results have been reported earlier in rice by Hsu *et al.* (1993) and in barley by Vassilev *et al.* (1997). The reduction in rate of transpiration under Cd stress was mainly due to the decreased  $\Psi_w$  (Table 4) and RWC (Table 4) which led to loss of leaf turgor and ultimately decreased stomatal conductance (Table 5). SA spray treatment in general showed more transpiration rate than SA seed treatment. Similar results have been

obtained in corn with the application of SA (Khan *et al.*, 2003). The higher transpiration rate of SA spray treatment could be attributed to its higher  $\Psi_w$  (Table 4), RWC (Table 4) and stomatal conductance (Table 5). Contrary to this, Janda (2000) observed a decrease in transpiration after 1 day of benzoic acid or aspirin (SA analogue) treatment at 22/20°C in maize.

In the present work, the data presented in Table 5 showed decrease in stomatal conductance when the plants were exposed to 250  $\mu\text{M}$  to 500  $\mu\text{M}$  Cd  $\text{kg}^{-1}$  soil. Similar results have been reported earlier in barley by Vassilev *et al.* (1997); in wheat by Samiulla *et al.* (2007). The reduction in stomatal conductance under Cd stress was mainly due to the decreased  $\Psi_w$  (Table 4) and RWC (Table 4) which led to loss of leaf turgor and ultimately decreased stomatal conductance. Application of SA increased the stomatal conductance. Increase in stomatal conductance was slightly higher in SA spray treatment than in SA seed treatment over control. Similar results have been obtained in corn with the application of SA (Khan *et al.*, 2003). The higher stomatal conductance of SA spray treatment could be attributed to its higher  $\Psi_w$  (Table 4), RWC (Table 4). Contrary to this, Janda (2000) observed a decrease in net photosynthesis, stomatal

conductivity and transpiration after 1 day of benzoic acid or aspirin (SA analogue) treatment at 22/20°C in maize.

#### **5.4 Biochemical estimations**

Plants exposed to Cd showed a series of physiological disorders, such as decreases in chlorophyll, sugar and protein contents, reduction in photosynthetic carbon assimilation and changes in related enzymes activity, and a drop in yield (Satyakala, 1997). In the present study, various biochemical estimations like chlorophyll, total soluble carbohydrate, total soluble protein, total free amino acids, free proline, malondialdehyde content and membrane injury percentage were performed to understand the biochemical effect of SA under the Cd stress.

Data presented in Table 6 showed decrease in total chlorophyll content when exposed to Cd stress. Decreased total chlorophyll content under Cd stress has been reported earlier in rice by Hsu and Kao (2003), Chien *et al.* (2001). The decreased chlorophyll content under Cd stress may be due to decreased synthesis (Storbart *et al.*, 1985) and increased degradation of chlorophyll (Somashekaraiah *et al.*, 1992). SA spray, showed higher chlorophyll content than the SA seed treatment. Similar observations have also been made by Chandra and Bhatt (1998) who have reported that high

chlorophyll content in cowpea which showed a good correlation with the net photosynthetic rate after the spray application of SA.

Carbohydrates (total soluble carbohydrate) are other important biochemical metabolites estimated under Cd stress. Carbohydrates are the building block of plant and are important source of energy for various physiological and biochemical processes. Total soluble carbohydrate (Table 6) decreased under Cd stress. Such decrease of total soluble carbohydrate under Cd stress have been reported in rice by Moya *et al.* (1993). The decreased level of total soluble carbohydrate resulted from the decreased hydrolysis of starch, as Cd stress inhibits amylase activity (Verma and Dubey, 2001). SA spray treatment in general revealed more total soluble carbohydrate than SA seed treatment even under Cd stress. Increase in total soluble carbohydrate content and reduction in starch content in leaves of Indian mustard has been shown with the spray of 25 and 50 mg L<sup>-1</sup> SA (Sangha and Atwal, 2004). Whereas, Spray of SA (100 and 500 mg L<sup>-1</sup>) decreased the total soluble carbohydrate content in ber plants cv. Tikadi and cv. Gola, (Bohra and Gardi, 2003; 2005).

Data presented in Table 7 showed decrease in total soluble protein under Cd stress. Such decrease in total soluble protein under Cd stress has been reported earlier in rice by (Hsu *et al.*, 2006), Hsu and Kao (2003) and Chien *et al.* (2001). Hsu *et al.* (2006) observed that Cd increased protease activity and amino acid content in non tolerant lines but not in tolerant lines, suggesting that proteolysis or accumulation of amino acids might play some role in regulating Cd toxicity. SA spray treatment had more total soluble protein than SA seed treatment even under Cd stressed plants. Contrary to this, reduced level of soluble protein has been observed in ber with increasing concentration of SA spray (Bohra and Gardi, 2003; 2005).

Data presented in Table 7 showed increase in total free amino acids when plants were exposed to Cd stress. Similar results have been reported in rice by Hsu and Kao (2003). SA spray treatment showed higher amount of total free amino acid than seed treatment of SA even under Cd stress.

In the present study, a conspicuous accumulation of free proline (Table 7) and total free amino acids (Table 7) accompanied with appreciable depletion of total soluble protein (Table 7) was observed under Cd stress. Proline is an

index of stress resistance and its accumulation contributes to maintain proper balance between extracellular and intracellular osmolarity under stress. High accumulation of proline helps maintaining turgor that is necessary for cell expansion. The increased concentration of proline in response to Cd stress showed a positive relationship with decreased water potential of leaf (Table 4). The possible way of proline accumulation might be either due to increased proteolysis or due to decreased protein synthesis (Table 7). Such changes in proline level were also reported in rice (roots) by Chen and Kao (1995) and in barley by Vassilev *et al.* (1998). SA spray treatment in general attained less proline content than seed treatment of SA even under Cd stress. Tammam (2003) reported that spraying with SA retarded the accumulation of proline in *Vicia faba* plant.. While, Bohra and Gardi (2003; 2005) observed that response of SA spray (500 mg L<sup>-1</sup>) on proline accumulation was depending upon cultivars of ber plant used. Cultivar 'Tikadi' accumulated proline while 'Gola' decreased the accumulation.

The data presented in Table 8 showed increase in Malondialdehyde content in Cd stressed rice leaves was observed to be greater than that in control. This showed that Cd-induced toxicity in rice leaves was linked to lipid

peroxidation. Lipid peroxidation is a free radical mediated process (Foyer *et al.*, 1997). The striking increase in lipid peroxidation in Cd-treated plant may a reflection of oxidative damage to the plant (Chien *et al.*, 2001). SA spray treatment in general showed less lipid peroxidation than seed treatment of SA even under Cd stress. Application of SA partially prevented the increase in lipid peroxidation (Table 8) as well as the decrease in chlorophyll (Table 6) and protein contents (Table 7). Lowering effect of SA on lipid peroxidation has been reported in rice by Choudhury and Panda (2004).

The results on membrane injury percentage (Table 8) reveal that it increased under Cd stress. Similar results of increase in membrane injury have also been reported in rice roots by Choudhury and Panda (2004). SA spray treatment in general showed less membrane injury than SA seed treatment even under Cd stress. Lowering effect of SA on membrane injury has been reported in rice by Mishra and Choudhuri (1999).

### **5.5 Cadmium concentration in plant parts**

The data presented in Table 9 reveal that increasing levels of Cd stress significantly increased the concentration of Cd in shoot and its components (Stem, leaf and panicle) at harvest. Increased Cd concentration under Cd stress has

been reported in rice by Chien *et al.* (2004) and Hsu and Kao (2003). SA application significantly lowered down Cd concentration of shoot and its components (Stem, leaf and panicle) even under Cd stress. SA spray treatment was better than seed treatment in lowering down the Cd-induced elevation on Cd concentration of shoot and its components. However, Drazic and Mihailovi (2005) have reported SA does not decrease Cd uptake of the soybean seedlings, but changes its distribution in plant organs depending on the concentration of added Cd, indicating that the influence of SA on the alleviation of toxic effects of Cd is probably indirect, through a development of general anti stress response.

## **5.6 Yield and its attributes**

It was reported that plants exposed to Cd showed a series of physiological disorders, such as decreases in chlorophyll, sugar and protein contents, reduction in photosynthetic carbon assimilation and changes in related enzymes activity, and a drop in yield (Satyakala, 1997). In the present study, Cd stress (250  $\mu\text{M}$  to 500  $\mu\text{M}$  Cd  $\text{kg}^{-1}$  soil) adversely affected the yield (Table 13) and its components: number of panicles per plant (Table 11), number of spikelets per panicle (Table 11), filled grain percentage (Table 12) and 1000-grain weight (Table 12) over control. Out of four yield

components rice, number of panicles per plant and number of spikelets per panicle were more sensitive yield components responsible for decrease in grain yield than, filled grain percentage and 1000-grain weight under Cd stress. Such results have also been obtained in rice by Liu *et al.* (2007). They have reported that grain number per panicle was the main cause of the loss in grain yield under Cd stress.

Cadmium stress also reduces the source strength by reducing photosynthesis (Table 5) thus decreasing the translocation of metabolites contributing toward yield. Grain yield and its components were higher in SA treated plants. SA spray treatment being better than SA seed treatment partially alleviated the grain yield more under Lower level (250 mM) Cd stress than under higher level (500 mM) Cd stress. It may be due to better growing environment in this treatment which is clearly reflected in higher plant water status (Table 4) and higher growth parameters (Table 1-3).

### **5.7 Quality of brown rice**

In the present study, the content of starch and amylose (Table 14) significantly decreased in brown rice with the increase in levels of Cd stress. Decrease in starch content in pea seed have been reported under Cd stress by Dewan and Dhingra (2004). Application of SA slightly increased the starch and amylose content in brown rice.

Cadmium stress significantly increased the concentration of Cd in brown rice. Similar increase in Cd concentration in brown rice has been reported by Arao and Ishikawa, (2006) and Cheng *et al.* (2006). Application of SA slightly decreased the Cd concentration in brown rice even under Cd stressed plants.

**CHAPTER –VI**

---

---

*Summary and  
Conclusions*

The present investigation on “Physiological and biochemical effects of salicylic acid in rice (*Oryza sativa* L.) under cadmium stress” was conducted during Kharif 2006 in the screen house, Department of Botany and Plant Physiology, Chaudhary Charan Singh, Haryana Agricultural University, Hisar. The plants were grown in polythene lined earthen pots containing 6 kg of sandy loam soil. The experiment was laid out in factorial completely randomized design with three replications. The treatments consisted of four levels of salicylic acid (SA) application (0  $\mu\text{M}$  SA seed treatment as control, 100  $\mu\text{M}$  SA seed treatment, 0  $\mu\text{M}$  SA spray as water spray at booting stage and 100  $\mu\text{M}$  SA spray at booting stage) and three levels of cadmium (Cd) application as  $\text{CdCl}_2 \cdot \text{H}_2\text{O}$  through soil at 30 days after sowing viz. 0, 250 and 500  $\mu\text{M kg}^{-1}$  soil. The results achieved are summarized as follows:

The increasing levels of Cd stress significantly reduced the plant height, number of tillers and leaf area at flowering. SA application significantly increased the values of these parameters. SA spray treatment proved better than SA seed

treatment in partially improving these parameters under Cd stress.

Cd stress significantly reduced the total dry weight of shoot as well as their components (dry weights of stem, leaf and panicle) at harvest. SA application significantly increased these parameters. SA spray treatment being better than seed treatment in partially improving the total dry weight of shoot as well as their components under Cd stress.

Cd stress significantly reduced the plant water relation parameters viz.  $\Psi_w$ ,  $\Psi_s$ , and RWC of leaf at flowering. SA application increased all the above parameters. SA spray treatment being better than seed treatment partially improved these parameters under Cd stress.

Remarkable decrease in gaseous exchange studies i. e. rate of photosynthesis, transpiration and stomatal conductance at flowering occurred under Cd stress. SA application showed a significant improvement on the above parameters. Improvements on these parameters were slightly higher in SA spray treatment than in seed treatment under Cd stress.

Cd stress significantly reduced the content of total chlorophyll, total soluble carbohydrate and total soluble protein in leaf at flowering. SA application significantly increased the content of these parameters. The improvements on these parameters were slightly more with SA spray treatment than with seed treatment under Cd stress.

Cd stress significantly increased the contents of total free amino acids, free proline, malondialdehyde (lipid peroxidation) and percentage of membrane injury in leaf at

flowering. SA application significantly increased the free amino acids. SA spray treatment was slightly better than seed treatment under Cd stress. However, free proline content, lipid peroxidation and percentage of membrane injury decreased with SA application. SA spray treatment was better than seed treatment in lowering down the Cd-induced elevation on free proline content, lipid peroxidation and percentage of membrane injury.

Increasing levels of Cd stress significantly increased the concentration of Cd in shoot and its components (Stem, leaf and panicle) at harvest. SA application significantly lowered down Cd concentration of the above parameters. SA spray treatment was better than seed treatment in lowering down the Cd-induced elevation on these parameters.

Cd stress adversely affected the yield and its components (Number of panicles per plant, number of spikelets per panicle, filled grain percentage and 1000-grain weight) over control. Number of panicles per plant and number of spikelets per panicle were more sensitive yield components responsible for decrease in grain yield. Likewise, filled grain percentage and 1000-grain weight also got reduced under Cd stress. Grain yield and its components were higher in SA treated plants. SA spray being better than SA seed treatment partially alleviated the grain yield more under Lower level (250 mM) Cd stress than under Higher level (500 mM) Cd stress.

The content of starch and amylose significantly decreased in brown rice with the increase in levels of Cd stress. Application of SA slightly increased the starch and amylose content in brown rice.

Cd stress significantly increased the concentration of Cd in brown rice. Application of SA slightly decreased them in brown rice even under Cd stressed plants.

### **Conclusion**

Considering the response of above parameters it can be concluded that Cd stress reduced the morpho-physiological and biochemical characters leading to reduced grain yield to the extent of 42.9 per cent. The reduction in grain yield was mainly due to reduction in yield components viz. number of panicles per plant, number of spikelets per panicle, filled grain percentage and 1000-grain weight. Application of SA, particularly as spray, partially improved the physiological and biochemical processes under Cd stress and thereby exhibited more growth and yield as compared to control as well as water spray treatment.

Cd stress decreased the starch and amylose content in brown rice, but increased the Cd concentration in it. SA application slightly improved the starch and amylose content while decreasing the Cd concentration in brown rice.

## References

---

- Alvarez, A.L. 2000. Salicylic acid in machinery of hypersensitive cell death and disease resistance. *Plant Mol. Biol.* **44**: 419-442.
- Arao, T. and Ishikawa, S. 2006. Genotypic differences in Cadmium concentration and distribution of soybean and rice. *J. Agri Res Qual* **40**(1): 21-30.
- Aravind, P and Prasad, M.N.V. 2003. Zinc alleviates cadmium induced oxidative stress in *Ceratophyllum demersum* L: a free floating freshwater macrophyte. *Plant Physiol and Biochem.* **41**: 391-397.
- Barcelo, J., Poschenrieder, C. 1990. Plant water relations as affected by heavy metal stress: a review. *J. Plant Nutr.* **13**: 1-37.
- Barcelo, J., Vazque, M., Poschenrieder, C. 1988. Structural and unstructural disorders in cadmium treated bush bean plants (*Phaseolus vulgaris* L.). *New Phytol.* **108**: 37-48.
- Barnett N.M. and Naylor, A.W. 1966. Amino acid and protein metabolism in Bermuda grass during water stress. *Plant Physiol.* **41**: 1222-1230.
- Bates, L.S., Waidren, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water stress studies. *Plant and Soil.* **39**: 205-207.
- Bohra, S.P and Gardi, B.R. 2003. Effect of plant growth regulator on pigment composition, photosynthesis and some biochemical aspect in ber (*Zizyphus maritiana* cv. Tikadi). *South Indian Horti.* **51**: 129-133.

- Bohra, S.P and Gardi, B.R. 2005. Effect of plant growth on photosynthesis and some biochemical parameters in ber (*Zizyphus maritiana* cv. Gola). *Indian. J. Horti.* **62**: 296-267.
- Boussama, N., Ouariti, O., Ghorbal, M.H. 1999. Changes in growth and nitrogen assimilation in barley seedlings under cadmium stress. *J. Plant. Nutr.* **22**: 731-752.
- Bradford, M.M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **72**: 248-254.
- Chakravarty, B and Srivastava, S. 1997. Effects of genotype and explant during in vitro response to cadmium stress and variation in protein and proline contents in linseed. *Annual of Botany.* **79**: 487-491.
- Chandra, A and Bhatt. 1998. Biochemical and Physiological response to salicylic acid in relation to the systemic acquired resistance. *Photosynthetica.* **35**: 255-258.
- Chaoui, A., Mazhoudi, S., Ghorbal, M.H. and Ferjani, E.E. 1997. Cadmium and zinc induction of lipid peroxidase and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). *Plant Sci.* **127**: 139-147.
- Chaudhuri, S and Panda, S.K. 2004. Role of salicylic acid in regulating cadmium induced oxidative stress in (*Oryza sativa* L.) root. *30*: 95-110.
- Chen, S.L and Kao, C.H. 1995. Cd induced changes in proline level and peroxidase activity in root of rice seedling. *Plant. Grow. Regul.* **17**: 67-71.
- Cheng, W., Gouping, Z., Haigen, Y., Dominy, P., Runvi, W. 2004. The different on growth and fours microelement concentration between two rice genotypes differing in grain cadmium accumulating capacity. *Agricultural Science in China* **3 (6)**: 416-424.

- Cheng, W., Gouping, Z., Haigen, Y., Wei, W., Min, X. 2006. Genotypic and environmental variation in cadmium, chromium, arsenic, nikel, and lead concentrations in rice grains. *J. Zejiang. Univ. Scien.* **7**(7): 565-571.
- Chien, H.F., Wang, J.W., Liu, C.C., Kao, C.H. 2001. Cadmium toxicity of rice leaves is mediated through lipid peroxidation. *Plant. Grow. Regul.* **33**: 205-213.
- Choudhury, S and Panda, S.K. 2004. Role of salicylic acid in regulating cadmium induced oxidative stress in *Oryza sativa* L. roots. *Bulg Plant Physiol.* **30**: 95-110.
- Clyjster, H., Van Assche, F. 1985. Inhibition of photosynthesis by heavy metals. *Photosynth. Res.* **7**: 31-40.
- Costa, G. and Morel, J.L. 1994. Water relations, gas exchange and amino acid content in Cd-treated lettuce. *Plant Physiol and Biochem.* **32**: 561-570.
- Costa, G. and Spitz, E. 1997. Influence of Cadmium on soluble carbohydrates, free amino acids, protein content of in vitro cultured *Lupinus albus*. *Plant Sci.* **128**: 131-140.
- Costa, G., Michaut, J. C., Guckert, A. 1997. Amino acids exuded from axenic roots of lettuce and white lupin seedlings exposed to different cadmium concentration. *J. Plant. Nutr.* **20**: 883-900.
- Costa, G., Michaut, J., Morel, J.L. 1994. Influence of Cadmium on water relations and gas exchanges, in phosphorus deficient *Lupinus albus* L. *Plant Physiol Biochem.* **32**: 105-114.
- Dewan, M and Dhingra, H.R. 2004. Cadmium partitioning and seed quality in two varieties of pea and their hybrid as influenced by rhizospheric cadmium. *Indian J. Plant Physiol.* **9**: 15-20.

- Drazic, G and Mihailovic, N. 2005. Modification of Cadmium toxicity in soybean seedlings by salicylic acid. *Plant Sci.* **168**: 511-257.
- Foyer, C.H., Lopez-Delgado, H., Dat, J.F., Scott, I.M. 1997. Hydrogen peroxide and glutathione associated mechanism of acclamatory stress tolerance and signaling. *Physiol. Plant.* **100**: 241-254.
- Gallego, S.M., Benavides, M.P. and Tomaro, M.L. 1996. Effect of heavy ion excess on sunflower leaves: evidence for involvement of oxidative stress. *Plant Sci.* **121**: 151-159.
- Gupta, U.C. and Gupta, S.C. 1998. Trace element toxicity relationships to crop production and livestock and human health: Implications for management. *Commun. Soil Sci. Plant Anal.* **29**: 1491-1522.
- Hassan, J.M., Shao, G., Zhang, G. 2005. Influence of Cadmium toxicity on growth and antioxidant enzyme activity in rice cultivars with different grain Cadmium accumulation. *J. Plant. Nutr.* **28**: 1259-1270.
- Hassid, W.Z. and Neufeld, B.F. 1964. Quantitative determination of starch in plant tissue. *Methods in Carbohydrate Chem.* **4**: 33.
- He, Y., Liu, Y., Cao, W., Huai, M., Xu, B., Huang, B. 2005. Effect of Salicylic acid on heat tolerance associated with antioxidant metabolism in Kentucky bluegrass. *Crop. Sci. Society of American.* **45**: 988-995.
- Heath, R.L. and Packer, L. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **125**: 189-198.
- Hiscox, J.D. and Isrealstam, G.F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* **57**: 1332-1334.
- Hsu, Y.T and Kao, C.H., 2003. Changes in protein and amino acid contents in two cultivars of rice seedlings with

- different apparent tolerance to cadmium. *Plant Growth Regul.* **40**: 147-155.
- Hsu, Y.T., Kuo, M.C., Kao, C.H. 2006. Cadmium induced ammonium ion accumulation of rice seedling at high temperature is mediated through abscisic acid. *Plant Soil.* **287**: 267-277.
- ICAR (2006) Handbook of Agriculture. Directorate of Information and Publications of Agriculture, Indian Council of Agricultural Research, New Delhi. p. 1346 biochemistry of fatty acid peroxidation. Arch. Biochem. Biophys., 125, 189-198. in root tip cells of garlic. *Biol Plant.* **47**(1), 79-83.
- Janda, T., Szalai, G., Antunovis, Z., Horvath, E., Paldi, E. 2000. Effect of benzoic acid and aspirin on chilling tolerance and photosynthesis in young maize plants. *Maydica*, **45**: 29-33.
- Janda, T., Szalai, G., Tari, I., Paldi, E. 1999 Hydroponic treatment with salicylic acid decreases the effect of chilling in maize (*Zea mays* L.) plants. *Planta.* **208**: 175-180.
- Kastory, R., Petrovic, M., Petroic, N. 1992. Effect of excess lead, cadmium, copper and zinc on water relation in sunflower. *Plant. Nutr.* **15**: 2427-2439.
- Kawada, T and Suzuki, S. 1998. A review on the cadmium content of rice, daily cadmium intake and accumulation in the kidneys. *J. Occup. Health.* **40**: 264-269.
- Khan, W., Prithviraj, B., Smith, D. 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. *Plant. Physiol.* **160**: 485-492.
- Lankindale, I and Hoang, B. 2004. Thermotolerance and antioxidant systems in *Agrostis stolonifera*: Involvement of salicylic acid, abscisic acid, calcium, hydrogen peroxide and ethylene. *J. Plant Physiol.* **128**: 682-695.

- Lesko, K and Sarkadi, L.S. 2002. Effect of Cadmium and titanium ascorbate stress on biological active compounds in wheat seedlings. *J. Plant. Nutr.* **25**: 2571-2581.
- Liu, J., Qingsen, Z., Cai, G., Xu, J., Yang, J and Wang, D., Qian, M. 2007. Effect of cadmium on the growth, dry matter accumulation and grain yield of different rice cultivars. *J. Sci Fd. Agri.* **87**: 1088-1095.
- Liu, J., Qingsen, Z., Zhang, Z., Xu, J., Yang, J and Wong, M.H. 2005. Variations in cadmium accumulation among rice cultivars and types and the selection of cultivars for reducing cadmium in the diet. *J. Sci Fd. Agri.* **85**: 147-153.
- Liu, W and Yang, Y.S. 2007. Impact assessment of cadmium contamination on rice (*Oryza sativa* L.) seedlings at molecular and population levels using multiple biomarkers. *Geophysical Research Abstracts.* **9**: 505-508.
- Maechiol, L., Leita, L., Martin, M., Peterssotti, A. 1996. Physiological responses of two soybeen cultivars to cadmium. *J. Environ. Qual.* **25**: 562-566.
- Malik, D., Sheoran, I.S., Singh, R. 1992. Carbon metabolism in leaves of cadmium treated wheat seedlings. *Plant Physiol. Biochem.* **30**(2): 223-229.
- Metwally, A., Finkemeier, I., Georgi, M., Dietz, K.J. 2003. Salicylic acid alleviates the cadmium toxicity in barley seedlings. *Plant Physiol.* **132**: 272-281.
- Mishra, A and Chaudhuri. 1999. Effect of salicylic acid on heavy metal induced membrane deterioration mediated by lipoxygenase in rice. *Biol. Plant.* **42**: 409-415.
- Moharekar, S.T., Lokhande, S.D., Hara, T., Tanaka, R., Tanaka, A., Chavan, P.D. 2003. Effect of salicylic acid on chlorophyll and carotenoid contents of wheat and moong seedlings. *Photosynthetica.* **41**: 315-317.

- Moya, J.L., Ros, R., Picazo, I. 1993. Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants. *Photosynthesis Res.* **36**: 75-80.
- Novel, L. Heat shock and other stress response system of plant. Novel, L., Newman, D., Schaff, K. Eds. 1989. Springer Verlag, NewYork.
- Nover, L. Heavy metal stress. 1989. In heat shock and other stress response system of plants; Nover, L., Neumann, D., Schaff, K., Eds. Springer Verlag: NewYork
- Pena, L.B., Pasquini, A.L., Tomaro, M.L., Gallego, S.M. 2006. Proteolytic system in sunflower (*Helianthus annuus* L.) leaves under cadmium stress. *Plant Sci.* **171**: 531-537.
- Pietrini, F., Iannelli, M.A., Montanari, R., Bianconi, D., Massacci, A. 2005. Cadmium interaction with thiols and photosynthesis in higher plants. *Avances in Plant Physiol.* **8**: 313-326.
- Pietrini, F., Iannelli, M.A., Pasqualini, S., Massacci, A. 2003. Interaction of cadmium with glutathione and photosynthesis in developing leaves and chloroplasts of *Phragmites australis* (Cav.) Trin. Ex Steudel. *Plant Physiol.* **133**. 829-837.
- Popova, L., Pancheva, T., Uzunova, A. 1997. Salicylic acid: properties biosynthesis and physiological role. *Bulg. J. Plant. Physiol.* **23**: 85-89.
- Poschenrieder, C., Gunse, B., Barcelo, J. 1989. Influence of cadmium on water relations, stomatal resistance, and abscisic acid content in expanding bean leaves. *Plant Physiol.* **90**: 1365-1371.
- Prasad, M.N.V. 1995. Cadmium toxicity and tolerance in vascular plants. *Environ Exp Bot.* **35**: 525-545
- Raskin, I. 1992. Role of salicylic acid in plants. *Annu. Rev. Plant. Physiol. Plant Mol. Biol.* **43**: 439-463.

- Sadasivam, S. and Manickam, A. 1992. In: *Biochemical Methods for Agricultural Sciences*, Wiley Eastern Limited, New Dehli. 12-13.
- Sakhabutdinava, A.R., Fatkhutdinova, D.R. Bezrukova, M.V., Shakirova, F.M. 2003. Salicylic acid prevents the damaging ation of stress factors on wheat plants. *Bulg. J. Plant. Physiol.* 314-319.
- Sullivan, C.Y. and Ross, W.M. 1979. In: *Stress Physiology in Crop Plant*. Ed. Mussel, H. and Staples, R.C. John Wiley and Sons, New York.
- Salt, D.E., Prince, R.C., Pickering, I.J., Raskin, I. 1995. Mechanism of cadmium mobility and accumulation in Indian mustard. *Plant Physiol.* **109**:1427-1433.
- Samiullah, N.A., Khan, N.A., Nazar, R., Ahmad, I. 2007. Physiological basis for reduced photosynthesis and growth of Cadmium-treated wheat cultivars differing in yield potential. *Food Agr Eviron.* **5**(1): 375-377.
- Sangha, M. K and Atwal, A. K. 2004. Effect of salicylic spray on sugar metabolised in *B. juncea* leaves infected with *A. brassicae*. *Cruciferae Newsletter.* **25**: 75-76.
- Satyakala, G. 1997. Sudies on the effect of heavy metal pollution on *Pistia stratiotes* (water lettuce). *Indian J. Environ. Health.* **39**: 1-7.
- Schat, H., Sharma, S.S., Vooijs, R. 1997. Heavy metal induced accumulation of free praline in a metal tolerant and a non tolerant ecotype of *Silene vulgaris*. *Physiol Plant.* **101**: 477-482.
- Shah, K. and Dubey, R.S. 1995. Effects of Cadmium on RNA level as well as activity and molecular forms of ribonuclease in growing rice seedlings. *Plant Physio. Biochem.* **33**: 577-584.
- Shah, K. and Dubey, R.S. 1997. Effects of Cadmium on proline accumulation and ribonuclease activity in rice seedlings

- role of proline as a possible enzyme protectant. *Bio; Plant.* **40**: 121-130.
- Shah, K. and Dubey, R.S. 1998. Cadmium elevates level of protein, amino acids and alters activity of proteolytic enzymes in germinating rice seeds. *Acta Physiol Plant.* **20**: 189-196.
- Somashekaraiah, B.V., Padmaja, K., Prasad, A.R.K. 1992. Phytotoxicity of cadmium ions on germinating seedlings of mungbean (*Phaseolus vulgaris*): Involvement of lipid peroxidation in chlorophyll degradation. *Physiol Plant.* **85**: 85-89.
- Staessen, J.A., Roels, H.A., Emelianov, D., Kuznetsova, T., Thijs, L., Vangronsveld, J. and Fagard, R. 1999. Environmental exposure to cadmium, forearm bone density, and risk of fractures: *Prospective population study.* *Lancet.* **353**: 1140-1144.
- Storbart, A.K., Griffiths, W.T., Bukkhar, I.A., Sherwood, R.P. 1985. The effect of Cd<sup>2+</sup> on the biosynthesis of chlorophyll in leaves of barley. *Physiol Plant.* **63**: 293-298.
- Tammam, A.A. 2003. Response of *Vicia faba* plants to the interactive effect of sodium chloride salinity and salicylic acid treatment. *Acta. Agro. Hung.* **51**: 239-248.
- Tari, I., Csiszar, J., Szalai, G., Horvath, F., Pecsvaradi, A., Kiss, G., Szepesi, A., Szabo, M., Erdei, L. 2002. Acclimation of tomato plants to salinity stress after a salicylic acid pre-treatment. *Acta Biologica Szegediensis.* **46**: 55-56.
- Theymoli, B. and Sadasivam, S. 1954. *Plant. Foods. Hum. Nutr.* **37**: 41.
- Toppi, D.L.S., Gabbrielli, R. (1999). Response to cadmium in higher plants. *Environ Expt. Bot.* **41**: 105-130.

- Vassilev, A. 2002. Physiological and agroecological aspects of cadmium interactions with barley plants. *J. Central Euro Agri.* **16**: 65-74.
- Vassilev, A., Berova, M., Zlatev, Z. 1998. Influence of Cd<sup>2+</sup> on growth, chlorophyll content, and water relations in young barley plants. *Biol Plant.* **41**: 601-606.
- Vassilev, A., Yordanov, T., Tsonev. 1997. Effects of Cd<sup>2+</sup> on the physiological state and photosynthetic activity of young barley plants. *Photosynthetica.* **34**: 293-302.
- Verma, S and Dubey, R.S. 2001. Effect of cadmium on soluble sugars and enzymes of their metabolism in rice. *Biol Plant.* **44**: 117-123.
- Wagner, G.J. 1993. Accumulation of cadmium in crop plants and its consequences to human health. *Advance in Agronomy.* **51**: 173-210.
- Wang, K. R. 2002. Tolerance of cultivated plants to cadmium and their utilization in polluted farmland soils. *Acta Biotechnol.* **22**: 189-198.
- Wang, L.J. and Li, S.H. 2007. Effect of salicylic acid on distribution of <sup>14</sup>C assimilation and photosynthesis in young grape plants under heat stress. *Acta. Horti.* **738**: 71-104.
- Weatherley, P.E. 1950. Studies on the water relations of the cotton plant. I. The field measurement of water deficit in leaves. *New Phytol.* **40**: 81-97.
- Went, F.W. 1957. Experimental control of plant growth. The Ronal Press Company, New York. pp. 79.
- Wu, F., Dong, J., Chen, F., Zhang, G. 2005. Response of Cadmium uptake in different barley genotypes to cadmium level. *J. Plant. Nutr.* **28**: 2201-2209.
- Yadav, R.K., Chaturved, R.K., Dubey, S.K., Joshi, P.K., Minhas, P.S. 2003. *Indian J. Agri. Sci.* **73**: 249-255.

- 
- Yemm, E.W. and Cocking, E.C. 1955. The determination of amino acids with ninhydrin. *Analyst*. **80**: 209-213
- Yemm, E.W. and Willis, A.J. 1954. The estimation of carbohydrates in plant extract by anthrone. *Biochem. J.* **57**: 508-514.
- Yoshida, S. 1972. Physiological aspect of grain yield. *Ann. Rev. Plant. Physiology*. **23**: 437-464.
- Zhao, Q.Z., Zhu, Y.G., Smith, S.E., Smith, F.A. 2005. Cadmium uptake by winter Wheat seedlings in response to interactions between phosphorous and zinc supply in soil. *J. Plant. Nutr.* **28**: 1569-1580.

## APPENDIX - I

### Physico-chemical characteristics of the soil

Parameter	Values
pH (1:2)	7.7
EC (1:2) (dS m <sup>-1</sup> )	0.2
OC (%)	0.2
CEC (cmol kg <sup>-1</sup> )	11.6
Sand (%)	78.0
Silt (%)	9.7
Clay (%)	12.3
Textural class	Sandy loam
Available nitrogen (kg ha <sup>-1</sup> )	80.5
Available phosphorus (kg ha <sup>-1</sup> )	15.1
Available potassium (kg ha <sup>-1</sup> )	150.0

## ABSTRACT

Title of thesis : **Physiological and biochemical effect of salicylic acid on rice (*Oryza sativa* L.) under cadmium stress**

Full name of degree holder : **DUONG HOANG SON**

Admission no. : **2005 BS 126M**

Title of degree : Master of Science

Name and address of major advisor : Dr. A.L. Patel  
Professor  
Deptt. of Botany and Plant Physiology  
CCSHAU, Hisar-125004, Haryana,  
India.

Degree awarding University : CCS Haryana Agricultural University,  
Hisar-125004, Haryana, India.

Year of award of degree : 2007

Major subject : Plant Physiology

Total no. of pages in the thesis : 88 + xi (ref.) + I

No. of words in the abstract : 391 (Approx.)

Key words: Salicylic acid, Cadmium, *Oryza sativa*, electrolyte leakage, lipid peroxidation, transpiration, water potential.

The present study entitled “Physiological and biochemical effects of salicylic acid in rice (*Oryza sativa* L.) under cadmium stress” was conducted during *Kharif* 2006 in pots in the screen house, Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar. The experiment was laid out in factorial completely randomized design with three replications. The treatments consisted of four levels of SA (0  $\mu\text{M}$  as control, 100  $\mu\text{M}$  seed treatment, 0  $\mu\text{M}$  as water spray at booting stage and 100  $\mu\text{M}$  spray at booting stage) and three levels of Cd viz. 0, 250 and 500  $\mu\text{M kg}^{-1}$  soil applied after 30 days of sowing.

Growth of rice plant in terms of plant height, number of tillers and leaf area at flowering, and total dry weight of shoot and its component (stem, leaf and panicle) at harvest decreased under Cd stress. SA spray

treatment proved better than SA seed treatment in partially improving these parameters under Cd stress.

Cd stress significantly reduced the  $\Psi_w$ ,  $\Psi_s$ , RWC, rates of photosynthesis, transpiration and stomatal conductance, and contents of total chlorophyll, total soluble carbohydrate and total soluble protein in leaf at flowering. SA spray treatment being better than seed treatment partially improved these parameters under Cd stress.

Considerable increase in the contents of total free amino acids, free proline, malondialdehyde (lipid peroxidation) and percentage of membrane injury in leaf at flowering, and the Cd concentration in shoot and its components (Stem, leaf and panicle) at harvest were observed under Cd stress. SA spray treatment being more effective than seed treatment counteracted the Cd-induced elevation on these parameters

Cd stress adversely affected the yield and its components number of panicles per plant, number of spikelets per panicle, filled grain percentage and 1000-grain weight) over control. Among these, number of panicles per plant and number of spikelets per panicle were more sensitive yield components responsible for decrease in grain yield under Cd stress. Grain yield and its components were higher in SA treated plants. SA spray being better than SA seed treatment partially alleviated the grain yield more under 250 mM of Cd than under 500 mM of Cd.

In brown rice, starch and amylose content decreased but Cd concentration increased under Cd stress. Application of SA decreased the Cd concentration and showed partial improvement in starch and amylose content in brown rice under Cd stress.

**MAJOR ADVISOR**

**SIGNATURE OF STUDENT**

**HEAD OF THE DEPARTMENT**