Phytochemistry 108 (2014) 57–66

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Exogenous application of methyl jasmonate lowers the effect of cadmium-induced oxidative injury in rice seedlings

Indra Singh [a], Kavita Shah [b,*]

[a] Department of Bioinformatics, MMV, Banaras Hindu University, Varanasi 221 005, India
[b] Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi 221 005, India

ARTICLE INFO

Article history:
Received 18 June 2014
Received in revised form 7 September 2014
Available online 6 October 2014

Keywords:
Antioxidant
Cadmium
Free radical
Methyl jasmonate
Rice

ABSTRACT

Rice seedlings grown under 50 μM cadmium alone or in combination with 5 μM methyl jasmonate were investigated for Cd-induced oxidative injury at 3, 7 and 10 days of treatment. MeJA treatments alone did not have any significant change in antioxidant enzyme activities or levels of H_2O_2 and $O_2^{\cdot-}$ in roots/shoots, as compared to controls during 3–10 days. The Cd-stressed plants When supplemented with exogenous MeJA revealed significant and consistent changes in activities of antioxidant enzymes CAT, SOD, POD and GR paralleled with an increased GSH-pools than that in plants subjected to Cd-stress alone. Synthesis of GSH driven by increasing demand for GSH in response to Cd-induced oxidative stress in rice was evident. Increased activity of LOX under Cd-stress was noted. Results suggest enhanced Cd-tolerance, lowered Cd^{2+} uptake, an improved membrane integrity and 'switching on' of the JA-biosynthesis by LOX in the Cd-stressed rice roots/shoots exposed to MeJA. Exposure to MeJA improved antioxidant response and accumulation of antioxidants which perhaps lowered the Cd-induced oxidative stress in rice. It is this switching on/off of the JA-biosynthesis and ROS mediated signal transduction pathway involving glutathione homeostasis via GR which helps MeJA to mitigate Cd-induced oxidative injury in rice.

1. Introduction

Cadmium, a non-essential toxic heavy metal, affects cellular processes via membrane damage, altered electron transport, enzyme activation/inhibition and DNA alteration (Smeets et al., 2005). Oxygen is essential for the existence of aerobic life, but toxic reactive oxygen species (ROS), which include the superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), and hydrogen peroxide (H_2O_2), are generated in all aerobic cells during metabolic processes (Noctor and Foyer, 1998). Injury caused by these ROS, known as oxidative stress, is one of the major damaging factors in plants exposed to environmental stresses. An increased production of ROS which are potentially harmful for the cell components, is a common outcome of cadmium exposure (Shah et al., 2001; Sanità diToppi et al., 2007; Sharma and Dietz, 2009; Rai et al., 2013). ROS are produced in a controlled manner through normal metabolic processes in aerobic organisms (Gratão et al., 2005), as signaling molecules in pathogen, programmed cell death and abiotic stress responses (Desikan et al., 2001; Mittler, 2002). Stressful conditions cause an imbalance in the steady-state level of ROS in plants (Foyer and Noctor, 2005; Sharma and Dietz, 2009). The over-accumulation of ROS induces oxidative processes like membrane lipid peroxidation, protein oxidation, enzyme inhibition, DNA and RNA damage that lead to cell damage (Gratão et al., 2005; Møller et al., 2007). In order to avoid the deleterious effects of ROS, several efficient antioxidant mechanisms comprising both of enzymatic components such as ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD) or guaiacol peroxidase (POD) and non-enzymatic components such as ascorbic acid (AA) or glutathione (GSH) come to rescue of plant cells (Gratão et al., 2005; Shah et al., 2013; Singh and Shah, 2014).

Jasmonates (JA) are a class of plant hormones that mediate various aspects of gene and metabolic regulation, stress responses, reproduction, defense and cell communication (Soares et al., 2010). Jasmonic acid and its volatile methyl ester, methyl jasmonate (MeJA), are a class of cyclopentanone compounds, regarded as endogenous regulators that play important roles for regulating the stress response, plant growth and development (Creelman and Mullet, 1997). In recent research, MeJA has been reported to reduce the development of chilling injury symptoms in a number of horticultural crops, including mango, sweet pepper and tomato fruit. These phytohormones induce the production of a wide array of direct and indirect chemicals such as pathogenesis-related and cellular protection molecules, including proteins involved in detoxification and redox balance, proteinase inhibitors, antimicrobial secondary metabolites, antioxidants and toxins (Farmer and

* Corresponding author. Tel.: +91 542 6701086, mobile: +91 9450955423.
E-mail address: kavitashah69@gmail.com (K. Shah).

http://dx.doi.org/10.1016/j.phytochem.2014.09.007
0031-9422/

Ryan 1990; Farmer, 2007; Cao et al., 2009; Soares et al., 2010; Guo et al., 2013; Zhou et al., 2013; Gill et al., 2013).

Plants continuously sense and assess the level of ROS and reprogram their gene expression to respond to the changing conditions in the environment (Xiang and Oliver, 1998; Soares et al., 2010). The variations in antioxidant activity within the cell can modify ROS content leading into death or acclimatization response. Soares et al. (2010) reported a 33% increase in ROS 1 h after treatment with MeJA in *Ricinus communis* with decreased activities of CAT and POD in these plants. Accumulation and synthesis of stress-specific defense related protein in Cd-stressed plants are well established (Shah and Dubey, 1998). Increased level of heat-shock protein, defensin, etc. is reported widely under stress conditions in plants (Gill and Tuteja, 2012; Gill et al., 2013).

Recently plant annexins (Ca^{2+} and phospholipid binding proteins) have been reported to be induced by heavy metals and jasmonic acid in *Zea mays* (Zhou et al., 2013). The annexin proteins are implicated in plant growth, development and stress responses and these were observed to be regulated by jasmonic acid. Expression of lipoxygenase (LOX) genes in plants is regulated throughout development (Bell et al., 1995). JA biosynthesis gene LOX2 and few other genes of the pathway are activated upon wounding and water deficit resulting in increased jasmonate levels under abiotic stress (Sasaki et al., 2001; Stenzel et al., 2003).

Therefore, this study aims to explore the effect of exogenously applied methyl jasmonate on Cd-induced oxidative injury in rice plants. A detailed study of the relationship between ROS accumulation and antioxidant enzyme activities in roots and shoots of Cd-stressed rice (*Oryza sativa* L.) plants in absence or presence of methyl jasmonate for different time durations is carried out. The activity of LOX, a JA-biosynthetic enzyme is also studied.

2. Results

2.1. Evaluation of growth and physiological parameters of rice seedlings exposed to Cd^{2+} and/or MeJA in the growth medium

Exposure of 50 μM Cd^{2+} resulted in decrease in shoot/root length and fresh weight of rice throughout growth period as seen in Table 1. Cadmium stress caused reduction in length by 10%, 22% and 24% at 3, 7 and 10 days respectively in shoots and 13%–25% in roots of rice seedlings (Table 1). Application of exogenous MeJA alone caused a slight decrease in the root/shoot length of rice plants with increasing days of treatment with almost 100% restoration in growth of rice seedlings at day 10 as reflected by the length and weight of rice seedlings exposed to a combination of Cd^{2+} + MeJA (Table 1).

Cadmium led to a loss of 46% cell viability in rice plants at day 7 of the growth period. Application of MeJA alone did not induce significant change in cell viability as compared to controls whereas Cd^{2+} + MeJA treatments resulted in only 27% loss in cell viability that accounted for almost 45% restoration of cells in rice seedlings in the latter at day 7 (Table 1).

Control or MeJA treated plants had less electrolyte leakage (EL) than Cd-treatments alone. Plants exposed to cadmium had a significantly increased EL at 3 and 10 days of exposure as compared to control plants. Cd^{2+} + MeJA treatments had significant lowering of EL values by 43%–76% during 3–10 days by growth (Table 1).

2.2. Effect of exogenous MeJA on cadmium content

The amount or uptake of Cd^{2+} present in roots and shoots of rice seedlings as estimated by Atomic Absorption Spectrophotometer (AAS) is represented in Table 1. Amount of Cd^{2+} increased in both roots and shoots with increasing exposure, the values being higher

Table 1
Effect of exogenous application of 5 μM methyl jasmonate (MeJA) on cadmium (Cd)-stressed rice seedlings at increasing days of growth. Values are mean of three replicates ± SE.

Treatments →		Days of growth											
		3				7				10			
		Control		50 μM Cd		Control		50 μM Cd		Control		50 μM Cd	
Parameter	↓	−JA	+JA	−JA	+JA	−JA	+JA	−JA	+JA	−JA	+JA	−JA	+JA
Length (cm)	Shoot	3.41 ± 0.34	2.91 ± 0.31	3.06 ± 0.33	2.96 ± 0.43	8.24 ± 0.028	5.88 ± 0.70	6.40* ± 0.67	5.23 ± 0.36	12.91* ± 0.50	11.70 ± 0.44	9.87 ± 0.61	9.90* ± 11.65
	Root	5.96 ± 0.20	4.60 ± 0.80	5.23 ± 0.35	3.66 ± 0.04	6.50 ± 0.40	4.80 ± 0.38	05.70 ± 0.35	4.73 ± 0.25	7.95 ± 0.55	5.30 ± 0.45	6.00 ± 0.77	5.90* ± 0.65
Fresh weight (g)		0.06 ± 0.003	0.042 ± 0.0029	0.042 ± 0.0070	0.19 ± 0.006	0.15 ± 0.005	0.13 ± 0.004	0.11 ± 0.0034	0.14* ± 0.004	0.17 ± 0.002	0.20 ± 0.004	0.14 ± 0.002	0.19* ± 0.005
Electrolyte leakage (millisiemens cm^{-1})	Leaf	22.2 ± 1.99	21.03 ± 0.83	47.57 ± 1.79	37.97* ± 1.53	24.27 ± 0.54	22.10 ± 0.67	33.70 ± 0.61	23.31 ± 0.68	33.02 ± 1.80	30.08 ± 1.40	69.00* ± 3.10	43.51 ± 1.90
Cell viability (%)	Leaf	0.270 ± 0.0036	0.28 ± 0.0062	0.20 ± 0.001	0.21 ±.001	0.22 ± 0.001	0.37 ± 0.016	0.12 ± 0.0006	0.18 ± 0.009	0.25 ± 0.12	0.26 ± 0.012	0.14 ± 0.006	0.19 ± 0.045
Cd content ($\mu g(g\ FW)^{-1}$)	Shoot	–	–	46.32* ± 1.90	29.95 ± 1.20	–	–	55.00* ± 2.60	36.32 ± 1.20	–	–	61.95* ± 2.91	40.97* ± 1.90
	Root	–	–	74.40* ± 3.20	53.85 ± 2.50	–	–	89.45* ± 4.3	60.32 ± 3.01	–	–	173.00* ± 7.9	69.40* ± 3.31
Amount of jasmonate derivatives ($ng(g\ FW)^{-1}$)	Shoot	240.00 ± 11.70	1111.00* ± 49.55	865.00* ± 41.2	1210.00 ± 57.5	484.00 ± 24.2	2352.00* ± 96.95	961.00 ± 45.05	2423.00* ± 118.05	1764.00 ± 76.20	1974.00* ± 94.71	3957.00 ± 184.7	4010.00* ± 156.5
	Root	110.00 ± 4.20	1095.00* ± 49.75	657.00* ± 32.05	660.00 ± 29.2	346.00 ± 14.3	1760.00* ± 71.88	815.00 ± 37.75	1490.00 ± 62.5	952.00 ± 32.65	1911.00* ± 82.55	1614.00* ± 69.70	1984.00* ± 86.20

* Indicates statistically significant change in treatments relative to control plants ($P \leqslant 0.05$).