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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

ROLE OF MANCOZEB IN PROTEIN METABOLISM DURING GERMINATION OF PADDY CULTIVARS

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ABSTRACT

Interaction of plants to abiotic stress is complex and involve various physiological and biochemical responses. A common response of plants to fungicidal stress is the accumulation of proteins and amino acids. Amino acids and other soluble nitrogenous compounds play an essential role in plant metabolism. Hence the present work was carried out to study the effect of different concentrations of mancozeb on protein metabolism during the germination of paddy cultivars. The seeds were soaked in different concentrations of mancozeb and control was maintained. Seed treatment with mancozeb suppressed the protein content but significantly increased the protease activity, free amino acids and proline content in relation to progressive concentrations of fungicide. Thus mancozeb act as modulator and endow plants with capacity to adapt to stressful condition by biological and physiological adjustment at cellular level.

Keywords: Germination, Total protein, Free amino acids, Proline, Protease.

1. INTRODUCTION

Plant diseases can be controlled by the application of chemical products like fungicides, bactericides, nematicides etc [1]. However seed treatment with fungicide at varying concentrations alters the enzyme activity and disrupts physiological balances [2]. This may impose osmotic stress and alteration in gene expression in plants. All these modifications leads to the accumulation or depletion of certain metabolites, alterations in the behavior of many enzymes, overall changes in protein synthesis and synthesis of new sets of novel proteins. Under stress conditions lysosomes breakdown resulting in increased level of several hydrolytic enzymes [3] which results in the accumulation of free amino acids and proline. Overproduction of proline functions as molecular chaperons and stabilizes the structure of proteins, buffers cytosolic pH and maintains cell redox status [4].

2. MATERIAL AND METHODS

2.1. Collection of seed samples and treatment

Paddy seeds (Jaya, Jyothi, and IR-64 cultivars) were procured from VC Farm, University of Agricultural Sciences, Mandya, Karnataka. Seeds were surface sterilized with 0.1% mercuric chloride for 10 minutes and repeatedly washed with distilled water for 4-5 times to remove the excess mercuric chloride. Seeds of uniform size were selected and soaked for 24 hours in distilled water (control) and with different concentrations (mg/g) of mancozeb-1mg, 3mg, 6mg, 9mg and 12mg/g of the seeds. The germination studies were carried out according to the "Between paper" method recommended by International Seed Testing Association [5]. The seeds were allowed to germinate for 14 days and then processed for further studies. Three sets in each concentration were maintained along with the control for comparison.

2.2. Preparation of crude extract

About 1g of rice seedlings treated with different concentrations of the mancozeb and untreated seedlings were homogenized in ice cold saline. The homogenate was centrifuged at 10,000 rpm for 10 minutes and the supernatant was used for further analysis.

2.3. Biochemical Studies

The total protein content of rice seedlings treated with different doses of fungicide was estimated as per the

method of Lowry *et al.,* [6]. The activity of proteases was determined following the procedure of Kunitz [7]. Estimation of total free amino acids was carried out as described in Moore and Stein [8]. Proline accumulation in fresh leaves of rice seedlings was estimated according to the method of Bates *et al.,* [9].

2.4. Statistical analysis

The data obtained were subjected to analysis of variance using SPSS package version 20.0. The data are expressed as the mean analyzed by two way analysis of variance (ANOVA) and Scheffee test was used as the test of significance.

3. RESULTS AND DISCUSSION

3.1. Effect of mancozeb on total protein

The total protein content in control and treated seedlings is presented in table 1. In the present study, the protein content of Jaya, Jyothi, and IR-64 cultivars treated with 1mg, 3mg, 6mg, 9mg and 12mg concentrations was significantly reduced as compared to the control and all the fungicide treated contained more or less the same amount of protein with slight variation and within the treated, higher concentration showed maximum amount of protein. The two-way ANOVA for the mean values of the protein content showed that Jyothi cultivar was found to be highly significant compared to Jaya and IR-64 cultivars. A decreased level of the total protein content may be due to degradation of proteins as well as the overall inhibition in protein synthesis under stress [10]. The present work also is in line with the findings of Uzma Majid *et al.,* [11], Jagatheeswari and Ranganathan [12], Rangwala Tasneem *et al.,* [13], and Bahar and Cuneyt [14].

3.2. Effect of mancozeb on protease activity

During seed germination, protease plays an important role in the mobilization of stored proteins as free amino acids. Free amino acids are required for building necessary proteins and enzymes for embryo development. In the present work enhanced protease activity was found in Jaya, Jyothi and IR-64 cultivars treated with different concentrations of mancozeb. In all the concentrations protease activity was more or less the same without much variation (Table-2). Increased protease activity in stressed plants appears to be of adaptive significance, because it leads to the accumulation of free amino acids [15]. Increased levels of free amino acids together with organic acids and quaternary ammonium compounds serve as compatible cytoplasmic solutes to maintain the osmotic balance [16]. Further Chibi Fatiha and Sayah Fouad [17] reported increased protease activity in *Lycopersicum esculentum* seedlings treated with endosulfan.

Table 1: Effect of mancozeb on total protein content (mg g^{-1}) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentrations of fungicide (mg/g)					Mean
			1	3	6	9	12	
Mancozeb	Jaya	2.650	2.070	2.250	2.250	2.150	2.500	2.311^{b}
	Jyothi	3.071	2.651	2.821	2.251	2.822	2.822	2.740^{a}
	IR-64	2.250	2.001	1.900	1.400	2.151	1.751	1.908^{c}
	Mean	2.657^{a}	2.241^{e}	2.323^{d}	1.967^{f}	2.374^{b}	2.357^{c}	2.320
	F value	Variety =1908192.662			Concentration = 274917.491			
		Variety * Concentration = 62447.971						

*Means followed by the same letter within a column/row are not significantly different as indicated by Scheffe ($P \leq 0.05$). **Significant at $P \leq 0.01$.*

Table 2: Effect of mancozeb on protease activity (10^{-4} mM of tyrosine liberated $g^{-1}min^{-1}$) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentrations of fungicide (mg/g)					Mean
			1	3	6	9	12	
Mancozeb	Jaya	0.10	0.11	0.11	0.15	0.13	0.11	0.10^{c}
	Jyothi	0.10	0.12	0.12	0.11	0.25	0.11	0.29^{b}
	IR-64	0.62	0.69	0.60	0.68	0.69	0.64	0.65^{a}
	Mean	0.26^{d}	0.31^{b}	0.28^{c}	0.30^{b}	0.67^{a}	0.28^{c}	0.35
	F value	Variety =56361.757			Concentration = 8984.723			
		Variety * Concentration = 7842.43						

*Means followed by the same letter within a column/row are not significantly different as indicated by Scheffe ($P \leq 0.05$). **Significant at $P \leq 0.01$*

3.3. Effect of mancozeb on total free aminoacids

Plants in response to environmental stress accumulate amino acids and has a protective effect and thus maintains homeostasis [15]. In the present study the free amino acids content in Jaya cultivar was found to be maximum at 6 mg and 12 mg concentration and it was reduced over the control at 1,3 and 9mg concentrations (Table 3). In Jyothi cultivar as compared to control the free amino acids content increased at all treatments. In IR-64 cultivar the free amino acids increased up to 9 mg concentration and decreased at 12mg concentration with 0.291 mg/g. Similar to our work Gabriel *et al.,* [18] also found treatment with hexaconazole and tebuconazole fungicide increases amino acids content in okra (*Abelmoschus esculentus L.*) plant under drought stress. Shanmugam *et al.,* [19] and Kavina *et al.,* [20] also reported increased free amino acids content in *Mentha piperita Linn* and *Basella alba Linn* treated with difenoconazole and propiconazole.

3.4. Effect of mancozeb on proline

During abiotic stress, proline accumulates in large quantities and plays an important role in maintaining cellular homeostasis. It also act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation and trigger specific gene expression which is very much essential for plants to recover from stress [21].

In the present study the proline content was found to be increased progressively in Jaya, Jyothi and IR-64 cultivars at all studied concentrations of mancozeb (Table 4). As compared to untreated seedlings, the proline content increased in all the cultivars and found to be maximum at 12 mg concentration.

The two-way ANOVA for the mean values of the proline showed that Jyothi cultivar was found to be highly significant compared to IR-64 and Jaya cultivars.

Du *et al.,* [22] reported increased proline content in rice cultivars treated with 1, 2, 4, trichloro benzene. Similarly Zhang *et al.,* [23] stated that omethoate stress in wheat increases proline content. Bordjiba and Ketif [24] also found increased proline content in *Triticum durum* treated with hexaconazole, bromuconazole and fluazifop-p-butyl. Our results were also in accordance with Vidya sagar *et al.,* [25], Manzoor Ashrafi and Pandit [26] and Wu *et al.,* [27].

Table 3: Effect of mancozeb on total free aminoacids (mg g^{-1}) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentrations of fungicide (mg/g)					Mean
			1	3	6	9	12	
Mancozeb	Jaya	3.720	0.720	1.080	4.360	3.640	3.921	2.907^{a}
	Jyothi	0.896	1.328	1.536	1.600	1.040	1.121	1.253^{b}
	IR-64	0.391	0.458	0.433	0.499	0.558	0.291	0.438^{c}
	Mean	1.669^{d}	0.835^{f}	1.016^{e}	2.153^{a}	1.746^{c}	1.778^{b}	1.533
	F value	Variety =28470859			Concentration = 2272271.696			
		Variety * Concentration = 273321.759						

*Means followed by the same letter within a column/row are not significantly different as indicated by Scheffe (P ≤ 0.05). **Significant at P ≤ 0.01.*

Table 4: Effect of mancozeb on proline content (µmoles g^{-1}) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentrations of fungicide (mg/g)					Mean
			1	3	6	9	12	
Mancozeb	Jaya	2.47	2.96	3.95	4.45	5.19	7.66	4.45^{c}
	Jyothi	5.07	6.43	7.42	7.91	8.90	11.13	7.81^{a}
	IR-64	3.71	6.43	6.67	7.42	7.91	8.65	6.80^{b}
	Mean	3.75^{f}	5.27^{e}	6.01^{d}	6.59^{c}	7.33^{b}	9.15^{a}	6.35
	F value	Variety =65617868**			Concentration = 37303161**			
		Variety * Concentration = 1147612**						

*Means followed by the same letter within a column/row are not significantly different as indicated by Scheffe (P ≤ 0.05). **Significant at P ≤ 0.01.*

4. CONCLUSION

In stress tolerance studies, seed treatment with different concentrations of fungicide induces changes in protein metabolism. Activation of metabolic processes in response to stress is manifested with increase in free amino acids, proline and protease activity. These stress specific components shows the internal tolerance mechanism of plants against the effect of toxicity of fungicides.

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