INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881; IMPACT FACTOR :6.514(2020); IC VALUE:5.16; ISI VALUE:2.286
Peer Reviewed and Refereed Journal: VOLUME:10, ISSUE:1(1), January :2021
Online Copy Available: www.ijmer.in

CARBENDAZIM INDUCED CHANGES IN PROTEIN METABOLISM DURING GERMINATION OF PADDY CULTIVARS

Anitha S R[1], Krishna K[2] and Savitha G[3]
[1]Assistant Professor and [2&3]Associate Professor
[1]Department of Biochemistry, SBRR Mahajana First Grade College,[2]Department of Botany, Yuvaraja's College, University of Mysoreand[3]Department of Chemistry, Maharani's Science College for Women,[1,2&3]Mysuru, Karnataka, India

Abstract

Amino acids play an important role in plant metabolism and development. In response to different stress, plants accumulate array of metabolites particularly amino acids. The present work is carried out to study the effect of different concentrations of carbendazim on protein metabolism during the germination of paddy cultivars. The seeds were soaked in different concentrations of carbendazim and control was maintained. Carbendazim treatment resulted in decreased free amino acids content in Jaya and IR-64 cultivars. Where as in Jyothi cultivar there was a considerable increase in free amino acids content and protease activity. Seed treatment with carbendazim also resulted in decreased protein content in Jyothi and IR-64 cultivars.However, a significant positive increase in proline content was found to occur in all treated seeds there by influencing adaptive response in plants against fungicidal stress.

Keywords: Carbendazim, Free Amino acids, Proline, Protease, Total Protein.

Introduction

Carbendazim is a systemic fungicide that control wide range of fungi at relatively low concentration. It is widely used in agriculture for pre and post harvest protection of crops against many fungal diseases. However, it has been reported that excessive dosage of fungicide may have negative impact on crop physiology (Anne Noelle Petit *et al.*,2012). Seed germination is marked by the biochemical changes of seed reserves which are considered as index of growth and development during seed germination and seedling growth (Bewely, 1997). The seed reserves get hydrolyzed and this is associated with change in cellular and bio molecular constituents such as carbohydrates, proteins and lipids. The rate at which changes occur varies from crop to crop and species to species (Rangwala Tasneem *et al.*, 2013). During germination process, storage proteins are hydrolyzed and amino acids are released (Lea and Joy 1983). Plants in response to environmental stress accumulate amino acids which has a protective effect in maintaining homeostasis (Aspinall and Paleg (1981). Proline is known to increase during seed germination in stressful environment. It maintains osmotic balance and the reactive oxygen species within normal range and there by prevents oxidative burst in plants. Thus, over production of proline may be part of stress signal influencing adaptive response in plants (Shamsul Hayat., *et al.*, 2012).

Materials and Methods

Collection of seed samples and treatment

Paddy seeds (Jaya, Jyothi and IR-64 cultivars) were procured from VC Farm, University of Agriculture Science, 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 carbendazim -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. 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.

Preparation of crude extract

About 1g of each rice seedlings treated with different concentrations of the carbendazim 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.

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.*, (1951). The activity of proteases was determined following the procedure of Kunitz (1947). Estimation of total free amino acids was carried out as described inMoore and Stein (1948). Proline accumulation in fresh leaves of rice seedlings was estimated according to the method of Bates *et al.*, (1973).

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 was used as the test of significance.

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881; IMPACT FACTOR :6.514(2020); IC VALUE:5.16; ISI VALUE:2.286
Peer Reviewed and Refereed Journal: VOLUME:10, ISSUE:1(1), January :2021
Online Copy Available: www.ijmer.in

Results and Discussion

Effect of carbendazim on Total protei

Protein content in control and treated seeds are presented in table 1. In the present study the protein content in Jaya cultivar was found to be increased over the control at all studied concentrations except at 3mg concentration. This may be due to mobilizations of storage nitrogen needed for the biosynthesis of new proteins (Amar and Reinlod, 1973). The present work also is in line with the findings of Shanmugam *et al.*, (2012) and Mishra *et al.*, (2015). Further Habiba *et al.*, (1992) and Radwan *et al.*, (2004) also suggested that prefenofos and primifos methyl application significantly increased the total protein in potatoes and green pepper respectively.

Significant decline in protein content was observed in Jyothi cultivar up to 9mg concentration but 12mg concentration showed a higher protein content with 5.86 mg/g. Even in IR-64 cultivar there was a sharp decline in the total protein at all studied concentrations. This may be due to inhibitory effect ofcarbendazim on the synthesis of protein. Reduced protein content may affect nitrogen metabolism. The toxicants produced by the application of systemic fungicides inhibit protein synthesis by binding to larger ribosomal subunit thus affecting the enzyme system and also inhibit the ATP and NADP formation (Person *et al.*, (1975). Similar observations were made by Manzoor Ashrafi and Goutam Pandit (2014).

Table 1: Effect of carbendazim on protein content (mgg^{-1}) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentrations of fungicide (mg/g)					Mean
			1	3	6	9	12	
Carbendazim	Jaya	1.634	2.307	1.442	3.076	2.696	3.269	2.404^b
	Jyothi	5.384	4.711	3.750	3.076	4.519	5.865	4.551^a
	IR-64	2.426	2.293	2.345	1.986	1.613	1.766	2.072^c
	Mean	3.148^b	3.104^c	2.512^f	2.713^e	2.942^d	3.633^a	3.009
	F value	Variety = 32597598.39			Concentration = 1362450.577			
		Variety * Concentration = 1909911.357						

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

Effect of carbendazim on protease activity

Protease is the hydrolytic enzymes synthesized during seed maturation and catalyzes the hydrolysis of protein to smaller peptides and amino acids (Taiz and Zeiger, 2003). The protease activity is related to the growth of seedlings (Sathaiah*and* Reddy (1985).

The protease activity in Jaya cultivars was fund to be reduced at all studied concentrations (Table 2). Reduction of protease activity may be due to reduced utilization of protein (Noogle and Fritz, 1989). Further inhibitory effect on the protease activity has also been observed in Spinach and Gaur treated with hexaconazole and triazophos (Kengar*et al.*, 2014).

In Jyothi and IR-64 cultivars the effect was reversed. The activity of protease was found to be increased at all concentrations. Treatment with 12 mg concentration in Jyothi cultivar and 6 mg concentration in IR-64 cultivars showed maximum activity of protease.

Effect of carbendazim on protease activity (10^{-4} mM of tyrosine liberatedg^{-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	
Carbendazim	Jaya	0.25	0.13	0.18	0.22	0.13	0.12	0.50^a
	Jyothi	0.16	0.18	0.16	0.20	0.17	0.21	0.18^c
	IR-64	0.15	0.16	0.16	0.71	0.16	0.19	0.25^b
	Mean	0.18^b	0.16^c	0.16^c	1.04^a	0.15^d	0.17^{bc}	0.31
	F value	Variety = 5897.407			Concentration = 13270.429			
		Variety * Concentration = 6456.029						

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

Increased protease activity may be attributed to protein catabolism (Dilley, 1970). Increased protease activity mobilizes the protein reserves and promotes the growth of the embryonic axis. Chandrashekar *et al*., (2011) also reported increased protease activity in *Solanum melongena* treated with endosulfan. Our results are consistent with Sabale and Mishra, (2000) who showed enhanced protease activity at lower doses of endosulfan and methyl parathion in *Sorghum bicolor*.

Effect of carbendazim on free amino acids

Aminoacids and other soluble nitrogenous compounds play an important role in plant metabolism and are primary product of inorganic nitrogen assimilation and precursor of protein and nucleic acids. Aminoacids accumulates in many plant species under a wide range of stress conditions such as water shortage, salinity, xenobiotics and extreme temperatures (Bordjiba and Ketif, 2009).

In Jaya cultivar free amino acids content was found to be increased at 3and 12 mg concentrations while at 1mg, 6mg, and 9mg concentrations there was decline in the free amino acids content (Table 3), where as In Jyothi cultivar the free amino acids were significantly more in seedlings treated with 1mg, 3mg, and 12mg concentrations. While a sharp decline of free amino acids content was observed at 6 mg and 9mg concentration. Similar to our results Shanmugam *et al.*, (2012) and Kavina*et al.*, (2011) also found increased free amino acids content in *Menta piperitaLinn*and Basella *albaLinn* treated with difenoconazole and propiconazole.

A reverse trend was observed in IR-64 cultivar where the free amino acids content was found to decrease at all studied concentrations. This may be due to inhibition of proteolytic enzyme (Moumit Roy Goswami *et al.*, (2013), inhibition of dipeptepidase activity (Tsay&Ashtion, 1971) and proteolytic enzyme (Ashton *et al.*, 1968). Mishra *et al.*, (2015) also reported decreased free amino acids in *Vigna radiata* L. seedlings treated with increased profenofos concentration.

Table 3: Effect of carbendazim on free aminoacids (mgg^{-1}) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentrations of fungicide (mg/g)					Mean
			1	3	6	9	12	
Carbendazim	Jaya	1.173	0.933	1.294	1.106	0.893	1.293	1.115^{a}
	Jyothi	0.283	0.483	0.424	0.066	0.116	0.458	0.305^{c}
	IR-64	0.549	0.349	0.416	0.408	0.458	0.341	0.420^{b}
	Mean	0.668^{c}	0.588^{d}	0.712^{a}	0.527^{e}	0.489^{f}	0.697^{b}	0.614
	F value	Variety = 321977.515			Concentration = 72613.809			
		Variety * Concentration = 59134.466						

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

Effect of carbendazim on proline content

Proline as a storage component serves as major source of energy and nitrogen required by the plants to overcome adverse environment stress. (Singh *et al.*, 1973). Proline is also known to accumulate in plants subjected to unfavorable environmental conditions (Aspinall and Paleg, 1981) or abiotic stress. During seed germination proline is involved in the edification of cell wall of young tissues (Hare and Cress, 1997). Accumulation of large quantities of proline is an adaptive response of plants to various biotic and abiotic stresses (Singh *et al.*, 1973).

The data obtained on the effect of fungicides on proline content in the rice cultivars showed increased proline content with increased concentrations of carbendazim. Over production of proline in plants in turn imparts stress tolerance by maintaining cell turgor and osmotic balance. During stress conditions accumulated proline functions as molecular chaperons and stabilize the structure of proteins and membranes. Its accumulation buffers cytosolic pH and maintain cell redox status (Shamsul Hayat *et al.*, 2012). Our results were also in accordance with Gabriel Rabert*et al.*, (2013), SemraKilie, (2015) and Souahi Hana et *al*., (2015).

Table 4: Effect of carbendazim on proline (µmolesg^{-1}) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentrations of fungicide (mg/g)					Mean
			1	3	6	9	12	
Carbendazim	Jaya	2.47	2.96	3.95	4.45	11.37	11.37	6.09^{a}
	Jyothi	2.96	3.21	3.46	4.20	4.94	6.18	4.16^{c}
	IR-64	2.47	3.46	3.95	4.45	6.18	6.18	4.45^{b}
	Mean	2.63^{f}	3.21^{e}	3.79^{d}	4.36^{c}	7.50^{b}	7.91^{a}	4.90
	F value	Variety = 1714201**			Concentration = 3980785**			
		Variety * Concentration = 749525**						

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

Conclusion

Activation of metabolic processes in response to chemical or fungicidal stress manifested in accumulation of proline. The study reveals the internal tolerance mechanism of rice cultivars in response to stress and adaptive role in mitigating the toxic effects of fungicides.

References

1. Anne Noelle Petit., Florence Fontaine., ParulVatsa., Christophe Clement and NathalieVaillantGaveau. (2012). Fungicide impacts on photosynthesis in crop plants. Photosynth. Res. 111: 315-326.
2. Bewely, J.D (1997). Seed germination and dormancy. Pl. Cell 9: 1055-1066.
3. Rangwala Tasneem., BafnaAngurbala and Maheshwari, R.S. (2013) Harmful effects of fungicides treatment on wheat (Triticum aestivum L.) seedlings. International Research Journal of Environmental Science. 2(8):1-5.
4. Lea, P. J and Joy, K. W (1983). Amino acids inter conversion in germinating seeds. In: Nozillo C, Lea P.J, Loewus F.A. Recent Advances in Phytochemistry: Mobilization of Reserves in Germination Volume 17, Plenum Press, New York, pp. 77-109.
5. Aspinall, D and Paleg, L.G., (1981). Proline accumulation: Physiological aspects: In: Paleg L.G and Aspinall D. (eds), The physiology and biochemistry of drought resistance in plants. Academic Press, Sydney, Australia, 205-241.
6. Shamsul Hayat., Qaiser Hayat., Mohammed Nasser Alyemeni., ArifShafiWani., John Pichtel and Aqil Ahmad. (2012). Role of proline under changing environments - A review. Plant signaling & Behavior. 7:11, 1456-1466.
7. ISTA, (2003). International Rules for Seed Testing Proceedings of the International Seed Testing Association, Seed Science Technology, 21:25-30.
8. Lowry, O.H., Rosebrough, N.J., Farr, A.L and Randall, R.J. (1951). Protein measurement with the Folin-phenol reagent. J.Biol Chem. 193: 265-275.
9. Kunitz, M. (1947). Isolation of a crystalline protein compound of trypsin and of soybean trypsin- Inhibitor. J. Gen. Physiol, 30 (4): 311-320.
10. Moore, S and Stein, W.H. (1948). Photometric method for use in the chromatography of amino acids. J. Biol. Chem. 176: 367-388.
11. Bates, L. S., Waldren, R. P and Teare, L. D. (1973). Rapid determination of free proline for water stress studies. Plant and Soil 39:205–207.
12. Amar L and Reinlod L. (1973). Loss of membrane transport ability in leaf cells and release of protein as a result of an osmotic shock. Plant Physiol,51:620-625
13. Shanmugam, M., Alagu Lakshmanan, G.M., Mathumathi, S and Panneerselvam, R. (2012). Effect of plant growth regulator fungicide and ABA on growth and biochemical properties of Basella alba Linn. International Journal of Research in plant Science. 2(4): 67-73.
14. Mishra, I.P., Sabat, G and Mohanty, B.K. (2015). Phytotoxicity of profenofos 50% EC (Curacron 50 EC) to Vigna radiata.L. seedlings: II. Studies on biochemical parameters. International Journal of Applied Sciences and Biotechnology. 3 (1): 101-105.
15. Habib, R. A., Ali, H. M and Ismail SMM. (1992). Biochemical effects of Profenofos residues in potatoes. Journal of Agriculture Food Chemistry.40: 1852-1855.
16. Radwan, M.A., Shiboob, M.H, Abu-Elamayem, M.M and Abdel-Aal, A. (2004). Residues of primiphos-methyl and profenofos on green pepper and eggplant fruits and their effects on some quality properties Emirates Journal of Agriculture Science, 16(1):32-42.
17. Person, C. D., Sambroski, J and Forysth, F.R. (1975). The effect of BZI on detached wheat leaves. Can. J. Bot., 180: 1294-1295.
18. Manzoor, A. Ashrafi and Goutham, K. Pandit. (2014). Effect of carbofuran on the formation of some biomolecules in brinjal (Solanum melongena L.) leaf. The Bioscan. 9(3): 959-963.
19. Taiz, L and Zeiger, E. (2003). Plant physiology.3rdedt, Panima publication corporation, New Delhi.
20. Sathaiah, V and Reddy.T.P. (1985). Seed protein profiles of castor (Ricinus communis L) and some jatropasps., Genet Agr. 39:35-43.
21. Noggle, G.R and Fritz, G.J (1989). Introductory Plant Physiology. Prentice Hall of India Private Ltd. New Delhi.
22. Kengar, Y.D., Kamble, A.B and Sabale, A.B. (2014). Effect of hexaconazole and triazophos on seed germination and growth parameters of spinach and guar. Scholars Research Library, Central European Journal of Experimental Biology. 5 (5):89-92.
23. Dilley, D.R. (1970). Enzymes in the Biochemistry of Fruits and their Products. Hulme, A.S (ed) Academic press, New York, pp-I: 197-207.
24. Chandra Shekar., Ramkrishna Prasad, V., Jagan Mohan Reddy, K and Chandrashekar, D. (2011). Pesticide induced alterations in physiological response in Solanum melangina (L). International Journal of Pharma and Bioscience, 2(1):374-383.
25. Sabale, A and Misal, B.N. (2000). Effect of endosulfan and methylparathion on hydrolytic enzymes in germinating seeds of jowar. J. Environ. Biol., 21(1): 29-35.
26. Bordjiba, O and Ketif, A. (2009). Effect of three pesticides (Hexaconazole, Bromuconazole and Fluazifop-p butyl) on some biochemical metabolites of the hard wheat: Triticum durum. Desf. Europ. J. Sci. Res., 260-268.
27. Moumit Roy Goswami., Priya Banerjee., SnehasiktaSwarnakar and Aniruddha Mukhopadhyay. (2013). Carbaryl mediated biochemical alterations in Egg plant. International Journal of Research in Environmental Science and Technology; 3(2): 51-57.
28. T say, R and Ashton, F.M. (1971). Effect of several herbicides on dipeptidase activity of squash cotyledons. Weed Sci., 19: 682-684.
29. Ashton, F. M., Penner, D and Hoffman, S. (1968). Effects of several herbicides on proteolytic activity of squash seedlings. Weed Sci., 16:169-171
30. Mishra, I.P., Sabat, G and Mohanty, B.K. (2015). Phytotoxicity of profenofos 50% EC (Curacron 50 EC) to Vigna radiata.L. seedlings: II. Studies on biochemical parameters. International Journal of Applied Sciences and Biotechnology. 3 (1): 101-105.
31. Hare, P. D and Cress, W. A. (1997). Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul., 21: 79-102.
32. Singh, T. N., Aspinall, D., Paleg, L. G and Boggers, S.F. (1973). Stress metabolism-II. Changes in proline concentration in excised plant tissue. Australian J. Biol. Sci. 26: 57-63.
33. SemraKilie., RagbetEzgiDurman and Yasemin Coskun. (2015) Morphological and physiological responses of maize seeds grown under increasing concentrations of Chlorantraniliprole insecticide. Pak.J.Environ Stud. 24(3):1069-1075.
34. Souahi Hana., Meksem Amara Leial., GraraNedjoud and Djebar Mohamed Reda. (2015). Physiology and biochemistry effects of herbicides sekator and zoom on two varieties of wheat (Waha and HD) in semi-arid region. Annual Research & Review in Biology. 5(5):449-459.