

Original Research Article

Metalaxyl induced changes in Protein metabolism during germination of Maize

Abstract

The present work was carried out to investigate the effect of fungicide, metalaxyl as a seed treatment on the protein metabolism of maize during early germination. The study was carried out for 7 days after soaking the seeds in different concentrations of metalaxyl and a control was maintained. Biochemical analyses of whole seedlings were done up to 7 days. Changes in the amount of total soluble protein, total free amino acids, and the protease activity were performed using standard methods. The results indicate that protein content was maximum on 4th and 6th day of germination in both control and treated seeds. Metalaxyl treatment resulted in the decreased protein content (20-50% inhibition) over the untreated in a dose dependent manner till the 4th day of germination. But as the growth proceeded a proportionate increase in the protein content was observed on the 6th day of germination in fungicide treated seeds compared to the control. The specific activity of protease was decreased by 46%, 81%, 88%, 97%, and 100% with 1.5mg, 3mg, 4.5mg, 6mg and 7mg concentration of Metalaxyl on 3rd day of germination when compared to the control. An increase in the total free amino acids occurred during the germination and maximum free amino acids content was observed on the 5th day. While, Metalaxyl treatment resulted in the dose dependent depletion of free amino acids content. A significant increase in proline content was found to occur in treated seeds. From the overall findings, the present study gives an insight into a protective effect of the system with an increased production of proline and decreased protease activity, free amino acids and at the same time a higher protein content during later stages of germination for a particular concentration which may be due to the synthesis of novel proteins as a defense mechanism indicating the dual role of metalaxyl.

Keywords: *Metalaxyl; germination; protease; proteins; free amino acids; proline.*

1. INTRODUCTION

The mobilization of seed storage proteins represents one of the most important post-germination events in the growth and development of seedling. Proteolytic enzymes play a central role in the biochemical mechanism of germination [1]. During germination period, the storage proteins are degraded by a variety of proteases which convert the insoluble storage proteins in to soluble peptides and these peptides are further hydrolyzed to free amino acids. These free amino acids are mobilized to the embryonic axis to support its growth and also to provide energy [2]. Downey mildew of maize (*Zea mays.L*) caused by *Peronosclerospora* is one of the most destructive diseases of this crop in the tropical Asian countries [3]. Integrated approaches are used to manage the disease including crop rotation, planting resistant cultivars and the application of fungicides like mancozeb and Metalaxyl. Metalaxyl compounds have been widely used for the control of Downey mildew in a number of crops. Metalaxyl (methyl *N*-(methoxyacetyl)-*N*-(2, 6-

48 xylyl)-DL-alaninate) is a systemic fungicide acts by suppressing sporangial formation, mycelia growth and 49 establishment of new infection [4]. Much of our knowledge of reserve mobilization and its control 50 processes during germination is very well understood. In contrast, available information on metalaxyl is 51 limited to its effect on the pathogen and not much data is available on the effect of metalaxyl in protein 52 metabolism of maize seeds. Hence, the present work is an attempt for furthering our knowledge towards 53 better understanding of the effect of metalaxyl in germinating maize seeds focusing on the protein 54 metabolic changes during germination.

55 2. MATERIAL AND METHODS

57 2.1 Collection of seeds and Treatments

58 Maize seeds were procured from VC farm, University of Agriculture Science, Mandya, Karnataka. All the 59 chemicals were purchased from SLR and MERCK and the chemicals were of analytical grade. Seeds 60 were surface sterilized with 0.1% mercuric chloride for 10 minutes and repeatedly washed with distilled 61 water for 4-5 times. Seeds of uniform size were selected and soaked for 24 hours in distilled water 62 (control) and with different concentrations(mg/g) of metalaxyl, 1.5, 3, 4.5, 6 and 7mg/gm of the seeds(1:5 63 weight/volume) for 24 hours. Five seeds in triplicate were placed on petridish with 8-10 layer of soaked 64 filter paper and incubated at 25 °C both in light and dark condition. Uniform seedlings were selected and 65 processed for further studies. Everyday filter paper was wetted with 10ml of distilled water. [5] 66

67 2.2 Preparation of crude extract

68 Around 1-2 gram of seedling treated with different concentrations of the metalaxyl and the untreated 69 seeds were taken each day, (up to 7 days) homogenized in ice cold saline (5ml) using pestle and mortar. 70 The solution was centrifuged for 10 minutes and supernatant were used for further analysis.

71 2.3 Biochemical studies

72 Protein was estimated as described by Lowry et al. [6] using BSA as standard. The activity of Protease 73 was determined following the procedure of Kunitz [7]. Total free amino acids were extracted and 74 determined following the method of Sugano et al [8]. Free proline content was estimated following the 75 method of Bates et al [9]. 76

77 2.4 Statistical Analysis

78 The data are expressed as the mean $\pm$ SEM analyzed by one-way analysis of variance (ANOVA) and 79 Dunnett's *t*-test was used as the test of significance. P value < 0.05 was considered as the minimum level 80 of significance. All statistical tests were carried out using SPSS statistical software. 81 82

83 3. RESULTS AND DISCUSSION

85 3.1 Total Protein

86 The protein content during different days of germination in control and fungicide treated seedlings is 87 presented in Table-1. The protein content was maximum on 4th and 6th day of germination in both control 88 and treated seeds. Metalaxyl treatment resulted in the decreased protein content (20-50% inhibition) over 89 the untreated in a dose dependent manner till the 4th day of germination. The treated seeds showed 90 similar pattern of protein content on different days of germination. However an increase in the protein 91 content was seen on the 6th day of germination in treated seeds. 92 93

Table 1- Effect of Metalaxyl on the Total Protein (mg/g) in the seedlings of Maize.

Concentration of Metalaxyl (mg/g)	Seedling age in days							
	0	1	2	3	4	5	6	7
Control	12.6 ±0.9	24.2±1	26.69±1. 4	10.75±0 .6	42.48±2. 1	29.52±1. 4	57±3.1	50.65±1
1.5	10.7±0. 7	22.82±1	24.45±1. 5	9.21±0. 3	34.09±1. 4	21.54±1. 2	76.4±2	60.49±1. 9
3.0	13.1±0. 5	25.43±1. 2	25.91±1. 2	9.71±0. 4	30.8±1.2	33.15±1. 5	96.06±2. 4	31.96±1. 1
4.5	34.4±1. 2	27.61±1	25.69±0. 4	2.71±0. 1	26.78±0. 9	33.69±1. 6	67.51±2. 3	52.4±2.1
6.0	12.6±1	25.18±1. 1	12.43±0. 3	12.14±0 .6	20.16±0. 5	17.87±0. 9	63.19±2. 3	22.33±0. 8
7.0	10±0.5	21.12±0. 9	23.15±1. 1	7.65±0. 3	25.45±0. 7	15.91±0. 7	47.52±1. 2	31.21±0. 9

In the present study the protein content in germinated seeds were found to be increased ,due to the mobilization of storage nitrogen for the production of protein needed for the development of the young seedling and the observation was well in agreement with the studies conducted in germinated seeds [10]. A decline in the protein content was observed in treated seeds till the 4th day in a dose dependent fashion. The decrease in the protein content in fungicide treated maize may be due to osmotic shock effect by the fungicides which results in the release of protein and loss of membrane transport ability [11]. It has been suggested that the toxicant produced in the treated seeds may inhibit the protein synthesis by binding to the larger ribosomal subunit inducing change in the enzyme system ceasing ATP and NADP formation .The results of our studies were in parallel, as the concentration of fungicide increases, the amount of protein gradually decreased. But as the growth proceeded a proportionate increase in the protein content was observed on the 6th day of germination in fungicide treated seeds compared to the control. The enhanced protein content may be attributed to the fact that as growth proceeds, Metalaxyl may induce the expression of many defense related genes in plants which results in the synthesis of novel proteins. The results of our study correlates with the recent proteomic analysis which revealed the occurrence of novel proteins in several plant species under heavy metal stress [12].

3.2 Activity of Protease

Protease is a hydrolytic enzyme which acts on proteinaceous substance to produce amino acid and amides. The specific activities of protease during germination of maize seeds treated with Metalaxyl are shown in Fig 1. Both control and treated seeds showed increasing specific activity up to 3rd day of germination and decreased then onwards. Specific activity of protease in maize seedling at different concentration of Metalaxyl on 3rd day were found to be 27, 9.1, 6, 1.6 and 0.3 ×10⁻⁷ mM whereas in control

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the activity is found to be 50×10^{-7} mM . The specific activity was decreased by 46%, 81%, 88%, 97%, and 100% with 1.5mg, 3mg, 4.5mg, 6mg and 7mg concentration of Metalaxyl on 3rd day of germination when compared to the control.

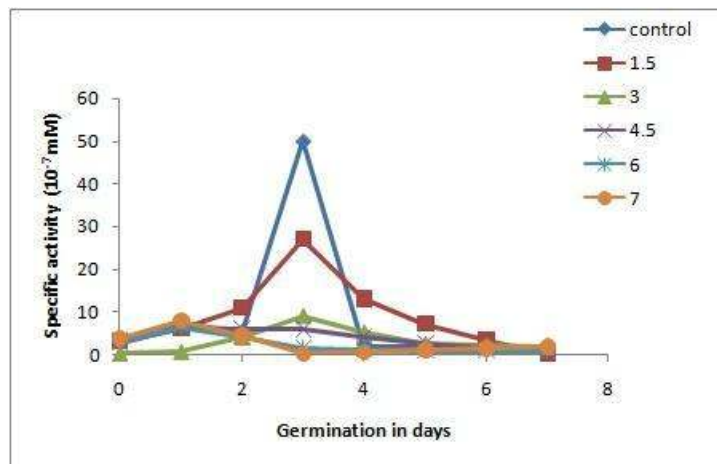


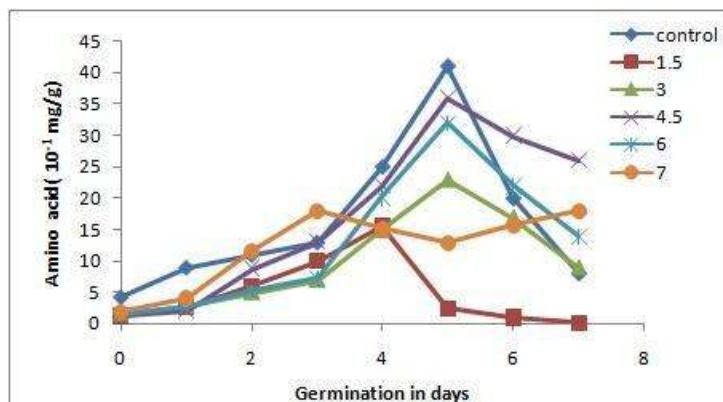
Fig.1. Effect of Metalaxyl on the Specific activity of Protease in the seedlings of Maize

*Specific activity of Protease is expressed as 10^{-7} mM of proteins degraded/mg of protein/min

Increasing in proteolytic activity with concomitant reserve protein depletion agrees with the findings of earlier studies on other seeds; *Phaseolus vulgaris* [13], *Lupinus albus* [14] *Vicia sativa* [15] and *Macrotyloma uniflorum* [16]. The result from our study is not parallel with the above data as there is a dose dependent inhibition of protease activity in Metalaxyl treated maize seeds on 3rd day of germination. Impairment of proteasome functionality and decreased protease activities seems to be a common feature involved in metal toxicity in plants [17].

3.3 Free amino acid

The amino acid content in control and treated seeds during different days of germination is presented in the Fig.2. A gradual increase in the amino acid content is seen and the maximum content of amino acid (41×10^{-1}) was seen on the 5th day in control seed and a gradual decrease was seen till the 7th day of germination. The free amino acid content was decreased effectively with the increasing concentration of fungicides with the maximum inhibition was seen with the highest concentration (7mg/g). Only 30% of free amino acids were present in that concentration. An increase in the total free amino acids occurred during the germination and maximum free amino acids content was observed on the 5th day While, metalaxyl treatment resulted in the dose dependent depletion of free amino acids content. There was no change in the pattern of amino acid profile on different days of germination in both treated and control seeds. These result suggests, the protective effect of amino acids against unfavorable condition, increasing the tendency of the system to maintain the homeostasis.



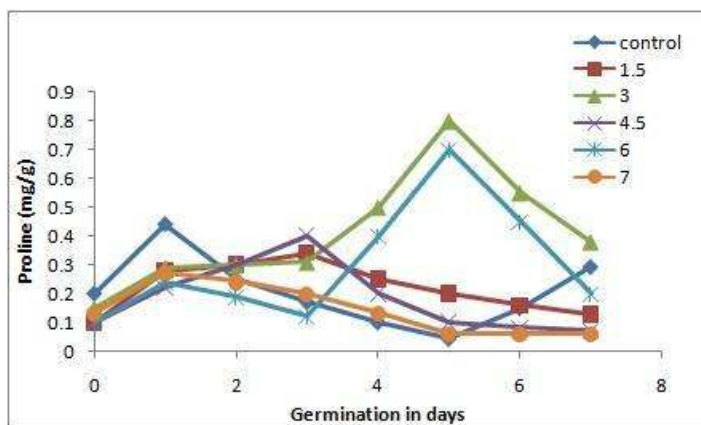
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149 **Fig.2. Effect of Metalaxyl on the Amino acid content (10^{-1} mg/g) in the seedlings of Maize**

150 3.4 Proline content

151 Proline content in control and treated seeds are presented in the Fig.3. The proline content increased in
 152 treated seeds and maximum proline content was seen with 3mg/g and in 6mg/g concentration of
 153 Metalaxyl (0.797mg/g, 0.705mg) on 5th day of germination and decreased thereafter. The proline content
 154 was lowest in control (0.043mg/g) on 5th day and in treated (7mg/g) and it was 0.06mg/g on 7th day. A
 155 significant increase in proline content was found to occur in treated seeds.



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158 **Fig.3. Effect of Metalaxyl on the Proline content (mg/g) in the seedlings of Maize**

159 Proline is considered as an antioxidant free radical scavenging and biochemical indicator of stress. It is
 160 considered as a metabolic measures of abiotic stress [18]. In the present work the stress caused by
 161 metalaxyl resulted in an increase in proline level. In general water stress has been known to increase

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free proline in leaves[19]. Plant growing in stress condition need to produce specific proteins having higher proline content (hydroxyproline rich glycopeptide or proline rich glycopeptides) [20]. The high content of free proline inhibits proline biosynthesis in plants growing under heavy metal contamination [21]. Effect of this change is subsequently contributed to higher production of glutamic acid which stressed plants need to create phytochelatins[22].

4. CONCLUSION

From the overall findings, the present study gives an insight into a protective effect of the system with an increased production of proline and decreased protease activity, free amino acids and at the same time a higher protein content during later stages of germination for a particular concentration which may be due to the synthesis of novel proteins as a defense mechanism indicating the dual role of metalaxyl. The biosynthesis of these proteins depends on the supply of free amino acids. This is correlated with the fact that there is a depletion of free amino acids in a dose dependant manner. Proline which is considered to be the biochemical indicator of stress, found to increase significantly in metalaxyl treated seeds. This study has helped us in understanding the changes in protein metabolism during germination process in control and in the fungicide treatment. Further work is in progress in investigating the increased level of protein in maize during germination in treated with different concentrations, which may help us in furthering our knowledge on the mechanism and its regulation.

REFERENCES

1. Muntz K, Belozersky MA, Dunaevsky YE, Schlereth A, Tiedemann J. Stored proteinases and the initiation of storage protein mobilization in seeds during germination and seedling growth. *Journal of Experimental Botany*. 2001; 52:1741–1752.
2. Bewley JD, Black M. *Physiology and Biochemistry of Seeds in Relation to Germination*. Vol. I. Springer – Verlag, Berlin. 1983.
3. Bonde MR. Epidemiology of downy mildew diseases of maize, sorghum and pearl millet. *Tropical Pest Management*. 1982; 28(1):49-60.
4. Fisher DJ, Hayes AL. Mode of action of the systemic fungicides furalaxyl, metalaxyl and ofurace. *Pestic. Sci*. 1982; 13:330–339. DOI: 10.1002/ps.2780130316
5. ISTA. International rules for seed testing. In: Daper SR.(Ed), Rules 2003. International seed testing association, Zurich, Switzerland, 1-520
6. Lowry, O.H., Rosenbrought, N.J., Farr, F.L. and Randall, R.J. 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem*. 193: 265-275.
7. Kunitz, M. (1947) *J. Gen. Physiol.*, 30, 311.
8. Sugano N, Tanaka T, Yamamoto E, Nishi A (1975) Phenylalanine ammonia lyase in carrot cells in suspension cultures. *Phytochemistry* 14:2435–2440
9. Bates, L.S., Waldran, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil* 39: 205-207.
10. Gernah DI, Ariahe CC, Ingbian EK. Effects of malting and lactic acid fermentation on some chemicals and functional properties of maize. *Am.J.Food. Technol*. 2011;6:404-412
11. Amar L, Reinhold L. Loss of membrane transport ability in leaf cells and release of protein as a result of an osmotic shock. *Plant Physiol*. 1973;51:620-625

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- 203 12. Gianazza E, Wait R, Sozzi A, et al. Growth and protein profile changes in *Lepidium sativum* L.
204 plantlets exposed to cadmium. Environmental and Experimental Botany. 2007; 59:179–187.
205 (cross reference)
- 206 13. Taneyama M, Okamoto T, Yamauchi D, Minamikawa T. Development of endopeptidase activity in
207 cotyledons of *Vigna mungo* seedling: Effects of exogenously applied end products and plant
208 hormones. Plant Cell and Physiology. 1996; 37:19–26.
- 209 14. Ferreira RB, Malo TS, Teixeira AN. Catabolism of the seed storage proteins from *Lupinus albus*:
210 Fate of globulins during germination and seedling growth. Australian Journal of Plant Physiology.
211 1995; 22:373–381.
- 212 15. Schlereth A, Standhardt D, Mock HP, Muntz K. Stored cysteine proteinases start globulin
213 breakdown in protein bodies of embryonic axis and cotyledons of germinating vetch (*Vicia sativa*
214 L.) seeds. Planta, 2001; 212:718–727.
- 215 16. Rajeswari J, Ramakrishna Rao P. Storage protein degradation in germinating
216 horse gram seeds. Indian J Plant Physiol. 2002; 7:314–320.
- 217 17. Wang C, Tian Y, Wang X, Geng J, Jiang J, Yu H, Wang C. Lead-contaminated soil induced
218 oxidative stress, defense response and its indicative biomarkers in roots of *Vicia faba* seedlings.
219 Ecotoxicology. 2010 Aug; 19(6):1130–9.
- 220 18. Andurwulan N, Fardiaz D, Wattimenia GA, Setty K. Antioxidant activity associated with lipid and
221 phenolic mobilization during seed germination of *Pangium edule Reinw.* J Agric Food Chem.
222 1999; 47:3158–63.
- 223 19. Stewart CK, Lee JA. The role of proline accumulation in halophytes. Planta 1974; 120:1279–289.
- 224 20. Ueda A, Yamamoto-Yamane Y, Takabe T. Salt stress enhances proline utilization in the apical
225 region of barley roots. Biochemical and Biophysical Research Communication. 2007; 355:61–66.
- 226 21. Štefl M, Vašáková L. Allosteric regulation of proline-inhibitable glutamate kinase from winter-
227 wheat leaves by L-proline, adenosine-diphosphate and low temperatures. Collection of
228 Czechoslovak Chemical Communications. 1982; 47:360–369.
- 229 22. Pavlíková D, Pavlík M, Staszková L, Motyka V, Száková J, Tlustoš P, Balík J. Glutamate kinase
230 as a potential biomarker of heavy metal stress in plants. Ecotoxicology and Environmental Safety.
231 2008; 70:223–230.
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