



Effects of salicylic acid on biochemical defense mechanisms of maize (*Zea Mays* L.) stressed with fluconazole

M Vennila¹, R Velayutham^{1*}, V Ambikapathy², Shijila Rani A S³

¹ Department of Botany, M.R. Govt. Arts College (Affiliated to Bharathidasan University, Trichy), Mannargudi, Tamil Nadu, India

² Department of Botany, A.V.V.M Sri Pushpam College (Auto) (Affiliated to Bharathidasan University, Trichy), Poondi, Thanjavur, Tamil Nadu, India

³ Department of Microbiology, Marudupandiyar College (Affiliated to Bharathidasan University, Trichy), Thanjavur, Tamil Nadu, India

Abstract

Salicylic acid (AS) may modulate several physiological and biochemical processes in plants, and may contribute to improve crop yield. The present study evaluated the physiological and biochemical mechanisms through which exogenous salicylic acid (SA) mitigates fluconazole fungicide stress in maize. The experiments conducted to the biochemical effect of salicylic acid and fluconazole in maize by pot culture method. Every 30 days interval to evaluate the amino acid, carbohydrate, protein and reducing sugar content in treated pots. The maximum amino acid content recorded in pot 2 (Foliar spray of Salicylic acid and Fluconazole @ 20mg/L) 90 days interval as 97.39 ± 0.06 mg/kg and carbohydrates, protein and reducing sugar are recorded in pot 3 (Foliar spray of Salicylic acid and Fluconazole @ 30mg/L) than compared to control.

Keywords: maize seeds, salicylic acid, fluconazole

Introduction

Maize (*Zea mays* L.) is the third most important cereal crop after wheat and rice all over the world (Sleper and Poehlman, 2006) [25]. There are five species of genus *Zea* and only 1 species "mays" is cultivated, remaining species are wild grasses (Ellensskog *et al.*, 2007). Maize is consumed directly as food at different growth stages (baby corn to mature grain) (Australian Government, 2008) [1]. It provides bulk of raw materials for the livestock and many agro-allied industries in the world (Bello *et al.*, 2010; Randjelovic *et al.*, 2011) [3, 22]. It is a major source of food for both human and animals through the year (Xu *et al.*, 2004) [29]. The environmental stresses such as drought, temperature, salinity, air pollution, heavy metals, pesticides and soil pH are major limiting factors in crop production because, they affects almost all plant functions (Hernandez *et al.*, 2001) [13].

It contains Vitamins A, B, E and also some important nutrients for metabolism (Orhun, 2013) [21]. In maize seed the carbohydrate is present in high amount than the other chemical components. In carbohydrate the amount of starch is 72 to 73 percent in the maize kernel and the amount of other carbohydrates such as glucose, sucrose and fructose are 1 to 3 percent in the maize kernel (FAO., 1993; Iken *et al.*, 2002; Barikmo *et al.*, 2004; Orhun, 2013) [9, 16, 2, 21]. Salicylic acid (SA) is a phenolic compound resulting from the secondary metabolism (Hayat *et al.*, 2010) [12], present in vegetable tissues (Raskin, 1992) [23]. Salicylic acid regulates plant growth and may increase crop yield, when low concentrations are supply exogenously (Rivas San Vicente and Plasencia, 2011) [24]. This compound may increase length, weight, perimeter, and root area (Gutierrez Coronado *et al.*, 1998; Larque Saavedra *et al.*, 2010) [11],

may modify its morphology (Echevarria Machado *et al.*, 2007) [6], and may also increase stalk's fresh and dry biomass (Villanueva Couoh *et al.*, 2009) [28], while improving the nutrient status of the plant (Khan *et al.*, 2010) [18].

The fungicides play a major role in the management of the plant diseases. The fungicides were always in the attention of scientists, regardless of their origin, concentration or usage are toxic both for the environment and human health, causing controversy and discussion worldwide for years (Horotan and Oancea, 2013) [14].

Some fungicides inhibit respiration in the mitochondria by blocking electron transport chain in complex III (cytochrome bc1 complex) (Venancio *et al.*, 2003) [27]. Fungicide often leads to serious environmental problems, including human health. Therefore it is necessary to minimize the chemicals used to control plant disease (Chourasiya *et al.*, 2014) [4].

Materials and Methods

Collection of *Zea mays* seed

For the present investigation, seeds of *Zea mays* were procured from Department of Agricultural Thanjavur, Tamilnadu, India. Collected seeds are identical in size and shape was segregated manually by visual appearance. The collected seeds were washed and surface sterilized into of 0.1% HgCl₂ for 10 minutes and three times rinsed into sterile distilled water.

Collection of chemicals

Salicylic acid chemical were purchased from Techno Scientific, Thanjavur and fluconazole fungicide was collected from local market, Thanjavur.

Pot culture experiments

The pot culture experiments was carried out Indian Biotrack Research Institute, Thanjavur, Tamilnadu, India

Treatment combination

T1-Control

T2-Foliar spray of Salicylic acid and Fluconazole @ 10mg/L

T3-Foliar spray of Salicylic acid and Fluconazole @ 20mg/L

T4-Foliar spray of Salicylic acid and Fluconazole @ 30mg/L

Estimation of total carbohydrate (Dubois *et al.*, 1956)^[5]

100 mg of sample were taken in a boiling tube. Then hydrolysed by keeping it in a boiling water bath for three hours with 5 ml of 2.5N HCl and cooled in room temperature. It was neutralized with solid sodium carbonate until effervescence ceased. The volume was made up to 100 ml and centrifuged. Then collected the supernatant and 1ml aliquot was taken for analysis. The standards were prepared by taking 0.2 ml to 1 ml of working standards. All tubes were made up to 1 ml with distilled water and 4 ml anthrone reagent was added to all the tubes. A blank were prepared without sample and same quantity of reagents were added. Then boiled for 8minutes following cooled rapidly and the development of green colour were read at 630nm. A standard graph was prepared and the amount of carbohydrate present in the sample was calculated.

Estimation of total protein (Lowry *et al.*, 1951)^[19]

100 mg of fresh leaves were treated with reagent A and centrifuged at 10,000 x g for 10 minutes. The resulting pellet were suspended in copper alkaline reagent and boiled for 30 minutes. Then cooled and centrifuged to eliminate light scattering materials. The supernatant were made up to a known volume. To the 0.1 ml of supernatant add 0.9 ml of distilled water then 5 ml of alkaline reagent (i.e., 2% alkaline Na₂CO₃ and CuSO₄-Na-K tartrate solution) were added and allowed to stand for 10 minutes at room temperature. Finally 0.5 ml of Folin's phenol reagent was added. The absorbance was measured after 30 minutes at 660 nm in spectronic 20 against blank. The amount of proteins was calculated with a standard graph prepared by using Bovine Serum Albumin.

Estimation of total amino acids (Jayaraman, 1981)^[17]

100 mg of fresh leaves were homogenized with 80 % ethanol in a pestle and mortar. The homogenate were centrifuged at 5000 x g and the clear supernatant was made

up to a known volume. From this 1 ml were pipette out in to a test tube and diluted with 4 ml distilled water. To this 1 ml of ninhydrin reagent were added and kept in boiling water bath for 15 minutes. Then the test tubes were cooled and 1 ml of 50 % ethanol was added. The purple colour development was measured in spectronic 20 at 540 nm. Standard graph were made using a mixture of glycine.

Estimation of reducing sugar

100 mg of fresh leaves were homogenized with 80 % ethanol in a pestle and mortar. The homogenate were centrifuged at 5000 x g and the clear supernatant was made up to a known volume. From this 1 ml were pipette out in to a test tube and 1ml of 0.05 M acetate buffer (pH 4, 8) and mixed. Added 3 mL DNS reagent to all the test tubes and mixed well. Place the tubes in boiling water for 5 minutes. Cool the tubes to room temperature and measure the absorbance at 540 nm.

Result and Discussion

In the present investigation, the treated plant were harvested in every interval of 30, 60 and 90th days and analyzed for biochemical and mineral contents. The maximum amount of aminoacid (92.25±0.06, 95.85±0.06 and 97.39±0.06 mg/100g), carbohydrates (84.04±0.00, 86.02±0.00 and 90.36±0.56mg/100g), protein (85.65±0.03, 89.18±0.03 and 92.32±0.06mg/100g) and reducing sugar (74.05±0.36, 76.32±0.33 and 83.63±0.33mg/100g) was measured in T3 (Foliar spray of Salicylic acid and Fluconazole @ 20mg/L) of 30, 60 and 90th day intervals than compared to other treatment (Table 1). The number of days increasing the biochemical content also increasing order. It might be due to salicylic acid stimulation of physiological processes that were reflected on improving vegetative growth that followed by active translocation of the photosynthesis products from source to sink in maize. This finding was agreement with the results of Hussain *et al.* (2009)^[15]. Somayyeh Bayat and Ali Sepehri (2012)^[26] suggested that the exogenous application of SA and Paclobutrazol (PBZ) may reduce adverse effects of drought in maize. During this study, spraying of SA showed profound influence on seed yield. Applying low concentrations of salicylic acid to increases the yield and quality of pepper (*Capsicum annum*), (*Capsicum chinense*), tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), and papaya (*Carica papaya*) (Martin Mex *et al.*, 2013)^[20]. Salicylic Acid on Physiological Indices and Defense Mechanisms of Maize (*Zea Mays* L. Giza 2) Stressed with Cadmium (El Dakak and Hassan, 2020)^[17].

Table 1: Analysis of biochemical parameters treated and untreated maize plants

Different treatment	Days	Quantity (mg/kg)			
		Amino acid	Carbohydrates	Protein	Reducing Sugar
T1	30	45.01±0.29	85.56±0.00	85.61±0.56	63.12±0.33
	60	48.21±0.33	89.12±0.01	96.54±0.56	65.20±0.56
	90	50.06±0.33	92.31±0.08	99.81±0.88	71.35±0.56
T2	30	90.03±0.65	90.03±0.33	91.85±0.33	75.12±0.08
	60	92.45±0.89	94.10±0.33	95.03±0.33	79.65±0.08
	90	96.63±0.30	99.03±0.45	97.59±0.88	81.36±0.08
T3	30	92.25±0.06	84.04±0.00	85.65±0.03	74.05±0.36
	60	95.85±0.06	86.02±0.00	89.18±0.03	76.32±0.33
	90	97.39±0.06	90.36±0.56	92.32±0.06	83.63±0.33
T4	30	52.05±0.57	76.63±0.59	79.63±0.33	69.06±0.45
	60	59.62±0.57	83.21±0.50	81.02±0.33	71.26±0.49
	90	64.10±0.88	87.25±0.33	86.56±0.56	79.03±0.88

The values are expressed in terms of (Mean ± Standard deviation)

T1-Control, T2-Foliar spray of Salicylic acid and Fluconazole @ 10mg/L, T3-Foliar spray of Salicylic acid and Fluconazole @ 20mg/L, T4-Foliar spray of Salicylic acid and Fluconazole @ 30mg/L

Acknowledgment

The authors extend sincere thanks to the principal, M.R. Govt. Arts College, Mannargudi and the Director, Indian Biotrack Research Institute, Thanjavur for the laboratory facilities provided.

Reference

1. Australian Government, Department of Health and Ageing, office of the gene technology regulator. "Biology of *Zea mays* L. spp mays (maize or corn), 2008, 1
<http://www.ogtr.gov.au>.
2. Barikmo I, Ouattara F, Oshaug A. Protein, carbohydrate and fibre in cereals from Mali how to fit there sults in a food composition table and database. *J Food Comp Anal*,2004;17:291-300.
3. Bello OB, Abdulmaliq SY, Ige SA, Mahamood J, Oluleye F, Azeez MA *et al*. Evaluation of early and late/intermediate maize varieties for grain yield potential and adaptation to a southern Guinea savanna agro-ecology of Nigeria. *J Agri Sci*,2010;2(3):42-51.
4. Chourasiya S, Khillare PS, Jyethi DS. Health risk assessment of organochlorine pesticide exposure through dietary intake of vegetables grown in the periurban sites of Delhi, India. *Environmental Science and Pollution Research*,2014;22(8):5793-5806.
5. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. *Anal. Chem*,1956;28:350-356.
6. Echevarria Machado IRM, Escobedo GM, Larque Saavedra A. Responses of transformed *Catharanthus roseus* roots to femtomolar concentrations of salicylic acid. *Plant Physiol. Biochem*,2007;45:501-507.
7. El Dakak RA, Hassan IA. The Alleviative Effects of Salicylic Acid on Physiological Indices and Defense Mechanisms of Maize (*Zea Mays* L. Giza 2) Stressed with Cadmium. *Environmental Processes*, 2020, 1-21.
8. Ellneskog-Staam P, Henry LC, Merker A. "Chromosome C-banding of the teosinte *Zea nicaraguensis* and comparison to other *Zea* species." *Hereditas*,2007;144:96-101.
9. FAO. Maize in human nutrition. Rome, 1993.
10. Fernandez-Hernandez A, Mateos R, Garcia-Mesa JA, Beltran G, Fernandez-Escobar R. Determination of mineral elements in fresh olive fruits by flame atomic spectrometry *Spanish Journal of Agricultural Research*,2010;8(4):1183-1190.
11. Gutierrez Coronado MA, Trejo-Lopez C, Larque Saavedra A. Effects of salicylic acid on the growth of roots and shoots in soybean. *Plant Physiol. Biochem*,1998;36:563-565.
12. Hayat Q, Hayat S, Irfan M, Ahmad A. Effect of exogenous salicylic acid under changing environment: a review. *Environmental and Experimental Botany*,2010;68:14-25.
13. Hernandez JA, Ferrer MA, Jimenez A, Barcelo AR, Sevilla F. Antioxidant system and O₂-/H₂O₂ production in the apoplast of *Pisum sativum* L. leaves: its relation with NaCl-induced necrotic lesions in minor veins. *Plant Physiology*,2001;127:817-831.
14. Horotan A, Oancea S. Effects of fungicide and acetylsalicylic acid treatments on the physiological and enzymatic activity in tomato (*Lycopersicon esculentum* Mill.), *Acta Universitatis Cibiniensis Series E: Food Technology*,2013;17:13-26.
15. Hussain M, Malik MA, Farooq M, Khan MB, Akram M, Saleem MF. Exogenous glycinebetaine and salicylic acid application improves water relations, allometry and quality of hybrid sunflower under water deficit conditions. *Journal of Agronomy and Crop Science*,2009;195:98-109.
16. Iken JE, Amusa NA, Obatolu VO. Nutrient composition and weight evaluation of some newly developed maize varieties in Nigeria. *J of Food Tech in Africa*,2002;7:27-29.
17. Jayaraman J. Calorimetric estimation of aminoacids. In: *Laboratory Manual in Biochemistry Wiley Eastern Ltd., New Delhi*, 1981, 64.
18. Khan NA, Syeed S, Masood A, Nazar R, Iqbal N. Application of salicylic acid increases contents of nutrients and antioxidative metabolism in mung bean and alleviates adverse effects of salinity stress. *Int. J. Plant Biol*,2010;1:1-8.
19. Lowry OH, Roseberough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J.Biol.Chem*,1951;193:265.
20. Martin Mex R, Nexticapan Garcez A, Larque Saavedra A. Potential benefits of salicylic acid in food production. In: Hayat S., A. Ahmad, and M. N. Alyemeni (eds). *Salicylic Acid*. Springer publishers, Dordtrech, The Netherlands, 2013, 299-213.
21. Orhun GE. Maize for life. *Int J of Food Sci and Nutri Eng*,2013;3(2):13-16.
22. Randjelovic V, Prodanovic S, Tomic Z, Simic A. Genotype x year effect on grain yield and nutritive values of maize (*Zea mays* L.). *J Anim Vet Sci Adv*,2011;10(7):835-840.
23. Raskin I. Role of salicylic acid in plants. *Annu. Rev. Plant physiol. Plant Mol. Biol*,1992;43:439-463.
24. Rivas San Vicente M, Plasencia J. Salicylic acid beyond: its role in plant growth and development. *J. Exp. Bot*,2011;1:1-18.
25. Sleper DA, Poehlman JM. *Breeding Field Crops*. 5th Ed. Blackwell Pub, 2006, 277-281.
26. Somayyeh Bayat, Ali Sepehri. Paclbutrazol and Salicylic Acid Application Ameliorates the Negative Effect of Water Stress on Growth and Yield of Maize Plants. *Journal of Research in Agricultural Science*,2012;8(2):127-139.
27. Venancio WS, Marco Antonio TRE, Begliomini NL de Souza. Physiological effects of strobilurin fungicides on plants. *Publ. UEPG Exact Soil Sci., Agr. Sci. Eng., Ponta Grossa*,2003;9(3):59-68.
28. Villanueva Couoh EG, Alcantar-Gonzalez P, Sanchez-Garcia M, Soria-Fregoso YA, Larque Saavedra. Effect del ácido salicílico y dimetilsulfóxido en la floración de *Chrysanthemum morifolium* (Ramat) Kitamura en Yucatán. *Rev. Chapingo Ser. Hortic*,2009;15:25-31.
29. Xu N, Yrle K, Miller PO, Cheilch N. Co-regulation of ear growth and internode elongation in corn. *Plant Growth Regulation*,2004;44:231-241.