

## Effect of Foliar Application of Methyl Salicylate (MeSA) on Antioxidant Ability Depicting the Role in Allelopathic Potential of Rice Genotypes

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The present investigation was conducted in the Norman E. Borlaug Crop Research Centre in G. B. Pant University of Agriculture and Technology, Pant nagar, Uttarakhand, with an objective to elucidate the role of MeSA on antioxidant activity which is significantly correlated with increase in allelopathic and yield potential of rice genotypes. For the study two allelopathic (UPR 2962-6-2-1 and Govind) and another non-allelopathic (UPR 2992-17-3-1) rice genotypes were undertaken and thereafter dealt with MeSA solution at diverse amount (1 mM, 2 mM, and 3 mM). Exogenous treatment of rice (*Oryza sativa* L.) with MeSA improved its allelopathic probability and led to buildup of additional phenolics, and an increase in gene transcription of enzymatic activities (SOD)

Increasing phenolic content and enzymes activities without compromising the yield of the crop plants is necessary for increasing the competitiveness of the genotypes. Antioxidant activity was measured at different growth stages. In the present investigation superoxide dismutase (SOD) was found to be 10-50% enhanced in the rice plants with the application of MeSA. Treatment with MeSA has resulted in increase in antioxidant activity which can be suggested to improve the competitive ability of the genotypes and amongst all the treatments 0.2 mM dose of MeSA has responded best.

From our study it is highlighted that the resilient capacity and plasticity of these contrasting genotypes is different in response to MeSA treatments which can further be evaluated and moreover antioxidant activity can be presupposed to be applied as suitable trait in rice weed interaction for sustainable agriculture.

*Key words: Allelopathy, MeSA, Phytotoxicity*

Rice (*Oryza sativa* L.) is one of the most significant grain yields in the world, and its allelopathy has involved great attention since (Dilday *et al.*, 1989) established that some varieties have allelopathic capacity against one or more paddy weeds. Salicylic acid is a famous inducer of plant scientifically developed resistance in plant–pathogen interactions, as a consequences, there is resistance to pathogens and is categorized by the initiation of defense-associated gene expression and production of phenylpropanoids, phytoalexins, and pathogenesis-related proteins (Mettraux *et al.*, 2001; Durrant and Dong, 2004; De Vos *et al.*, 2005). Salicylic acid encourages the production of secondary metabolites in plants (Taguchi *et al.*, 2001). The main idea of this paper is to highlight the significance of enzymatic activity which was enhanced under foliar application of elicitor compound, and at the same time to validate its role in allelopathic potential of rice genotypes.

#### **Application of MeSA on cell protective enzymes**

SOD plays a very important role in scavenging reactive oxygen species which would otherwise result in membrane lipid peroxidation, that causes destruction of membrane system (programmed cell death) (Ding *et al.*, 2007). Hong *et al.* (2007) have reported higher levels of activities of protective enzymes SOD and POD as well as root physiological capability in PI312777 genotype of rice. The reverse was true in barnyardgrass, which showed decreased activities for all defensive enzymes consisting of SOD, POD, and CAT when co-cultured with SA-mediated allelopathic rice plants, implying that the plant chemical defense in rice has taken effect in the conquest of the target weed.

In present study, activity of cell protective enzyme, superoxide dismutase (SOD) was found to be 10-50% enhanced in the rice plants with the application of MeSA. The SOD activity of MeSA treated rice plants was enhanced suggesting its possible role in the increased phenolic acid array of treated plants, in protecting the host plant against phenolic activity. Similar report is evident in my previous studies, where the use of elicitors in laboratory experiment in effective

concentrations has improved the allelopathic capability of the crops, as well as their profit and suppleness (Patni *et al.*, 2019)

Increasing phenolic content and enzymes activities without compromising the yield of the crop plants is necessary for increasing the competitiveness of the genotypes. Generally, plants treated with salicylates or methyl jasmonate had higher yield in many plant species, cultivated either in greenhouse or in open conditions (Abdel-Wahed *et al.*, 2006; Iqbal and Ashraf, 2006; Farouk and Osman, 2008).

SA augmented the flowers production, pods/plant and seed harvest of soybean (Gutierrez-Coronado *et al.*, 1998); enhanced wheat growth (Shakirova *et al.*, 2003) and maize growth (Shehata *et al.*, 2001; Abdel-Wahed *et al.*, 2006; El-Mergawi and Abdel-Wahed *et al.*, 2007). Moreover, in Plum quality parameters, for example weight, total phenolics (including anthocyanins, in BS), total carotenoids, and antioxidant activity, in both hydrophilic and lipophilic compounds were discovered to be at higher levels in plums from SA and MeSA-treated trees than in those from control trees (Esplá *et al.*, 2017)

In current analysis, we tried the overhead theory by applying MeSA to rice exogenously and by visualizing changes in the allelopathic potential of rice aqueous extracts, as well as in enzymatic activities of SOD which plays a very important role in scavenging reactive oxygen species.

## **MATERIALS AND METHODS**

The experimentation was conducted in a randomized block design (RBD) with complete randomization of three treatments (0.1 mM MeSA, 0.2 mM MeSA, 0.3 mM MeSA and control). Foliar application of MeSA was recorded threefold at 5, 15 and 30 days in field study after transplanting. Weeding was not done in this experiment during the period of study. The superoxide dismutase activity (SOD activity) was estimated *in vitro* in the freshly harvested leaves by using the method described by Gianopolitis and Reis (1977).

### **Extraction**

For the extraction of superoxide dismutase 0.2 g leaf sample was taken in an ice cooled mortar and pestle to

which 4 ml ice cold extraction buffer was added. Then the mixture was homogenized and the homogenate was filtered through muslin cloth. The filtrate was then

centrifuged for 15 min at 16000 g at 4 °C. Then the supernatant obtained was used as crude enzyme extract for quantification of enzyme activity.

**Table (1):** Effect of methyl salicylate (MeSA) on SOD (U/mg FW) activity of three rice genotypes.

DAT	Rice Genotypes				
	Treatments	UPR-2962-6-2-1	Govind	UPR-2992-17-3-1	Mean
15	Control	3.77	3.78	2.03	3.19
	0.1 mM MeSA	2.49	3.54	2.48	2.84
	0.2 mM MeSA	2.98	3.17	3.07	3.07
	0.3 mM MeSA	3.20	2.11	3.38	2.90
	Mean	3.11	3.15	2.74	3.00
	CD (p< 0.05)	0.34			
	SEm ±	0.11			
30	Control	2.87	3.22	2.84	2.98
	0.1 mM MeSA	4.79	4.44	3.88	4.37
	0.2 mM MeSA	5.11	3.70	4.27	4.36
	0.3 mM MeSA	4.82	5.25	4.73	4.93
	Mean	4.40	4.15	3.93	4.16
	CD (p< 0.05)	0.16			
	SEm ±	0.56			
45	Control	1.50	4.30	2.12	2.64
	0.1 mM MeSA	4.72	5.94	6.09	5.58
	0.2 mM MeSA	5.63	4.44	4.54	4.87
	0.3 mM MeSA	5.71	4.66	6.11	5.49
	Mean	4.39	4.83	4.72	4.65
	CD (p< 0.05)	0.18			
	SEm ±	0.62			
60	Control	1.87	3.58	2.30	2.58
	0.1 mM MeSA	4.76	6.24	5.86	5.62
	0.2 mM MeSA	5.26	4.60	4.54	4.80
	0.3 mM MeSA	5.62	4.65	5.90	5.39
	Mean	4.38	4.77	4.65	4.60
	CD (p< 0.05)	0.17			
	SEm ±	0.17			
75	Control	1.29	1.62	1.59	1.50
	0.1 mM MeSA	1.65	1.85	1.76	1.75
	0.2 mM MeSA	1.87	2.09	2.24	2.07
	0.3 mM MeSA	1.61	1.77	1.61	1.66
	Mean	1.61	1.83	1.80	1.75
	CD (p< 0.05)	0.11			
	SEm ±	0.39			

### Estimation

To 1.5 ml reaction mixture 50 µl of enzyme extract was added and the tubes were shaken and were illuminated with two 20W fluorescent tubes. The reaction was allowed to proceed for 15 minutes. Then the light was switched off and the tubes were covered with a black cloth and the absorbance was recorded at 560 nm. Along with the reaction tubes one control and one reference tube was also set up. In reference tube everything was added, and the tube was immediately covered a blank cloth and in control everything was added except control. Reference tube served as blank for taking readings.

A unit of SOD activity is demarcated as the quantity of enzyme mandatory to trigger 50% inhibition of NBT photoreduction rate and is expressed as U mg<sup>-1</sup> of fresh weight.

### Statistical analysis:

The statistical investigation for all the factors was done using analysis of variance for split plot design with means being tested at  $P > 0.05$  using an STPR software.

## RESULTS

Superoxide dismutase activity of three rice genotypes as affected by different doses of MeSA, at different stages of growth (15, 30, 45, 60 and 75 DAT) is presented in (table 1).

The action of cell protecting enzyme superoxide dismutase was boosted in rice genotypes with the exogenous application of diverse concentration (0.1, 0.2, 0.3 mM) of MeSA.

SOD activity in plants treated with 0.1 mM MeSA increased by 31.8% at 30 DAT, 52% at 45 DAT, 54% at 60 DAT and 14% at 75 DAT, while an increase of 31.6% at 30 DAT, 45% at 45 DAT, 46% at 60 DAT and 27% at harvest was noted in the plants treated with 0.2 mM MeSA and an increase of 39% at 30 DAT, 51% at 45 DAT, 52% at 60 DAT and 9% at 75 DAT was recorded in the plants treated with 0.3 mM MeSA. Compared to all the treatments SOD activity peaked at 0.3 mM MeSA at 30 DAT, 0.1 mM MeSA at 45 and 60 DAT, while, it peaked at 0.2 mM MeSA at harvest. Amongst the

genotypes, SOD activity increased from 15 to 60 DAT and declined thereafter. Maximum SOD activity was noted in the genotype govind (4.83) followed by UPR-2992-17-3-1 (4.72) and lowest activity was found in UPR-2962-6-2-1 (4.38) at 45 DAT, similar trend for genotypes was found at 60 and 75 DAT. However, at 15 and 30 DAT trend was as follows: Govind > UPR-2962-6-2-1 > UPR-2992-17-3-1.

## DISCUSSION

The activity of cell protective enzyme superoxide dismutase, was enhanced in rice genotypes with the exogenous application of MeSA. SOD activity peaked at concentration 0.1 mM at 45, 60 DAT, and peaked at 0.2 mM at harvest and peaked at 0.3 mM at 30 DAT. If the trend of increasing dependence on heavy use of chemicals for weed control is to be reversed, more alternative strategies against weeds have to be developed. The possibility of incorporating traits into improved cultivars would enhance the competitive ability of the crops, and thereby reduce or delay the need for applying herbicides (Olofsdotter et al., 2001).

Results obtained in the present investigation and their comparison with the earlier observations has made it clear that application of MeSA to rice have increased both the antioxidant activity and competitive ability and resulted in a higher yield.

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