

Effect Of Foliar Application Of Seaweed Based Panchagavya On The Antioxidant Enzymes In Crop Plants

Sangeetha, V and Thevanathan, R*

Post Graduate and Research Department of Botany, Presidency College,
Chennai-5, Tamil Nadu, India. sangeethadotv@gmail.com

*Centre for Advanced Study in Botany, University of Madras (Guindy campus),
Chennai-25, Tamil Nadu, India. thevanathan@gmail.com.

ABSTRACT - A modified formulation of panchagavya, amended with three seaweed extract (*Sargassum wightii*) has been investigated for its effect on the antioxidant enzymes namely, SOD, GR and GPx in the leaves of the seedlings of the pulses, *Vigna radiata*, *Vigna mungo*, *Arachis hypogaea*, *Cyamopsis tetragonoloba*, *Lablab purpureus*, *Cicer arietinum* and the cereal *Oryza sativa* var. *ponni*. The seaweed based panchagavya formulation increased the levels of all the three enzymes in the experimental plants when used as a foliar spray. The spray was highly effective at 3% level. [The Journal of American Science. 2010;6(2):185-188]. (ISSN 1545-1003).

Key words: Panchagavya, Seaweed, SOD, GR, GPx, pulses, cereal.

INTRODUCTION

Panchagavya, a Vedic formulation of the five products of cow is used as a foliar application to boost yield of crop plants and to restrict the incidence of common diseases. This traditional panchagavya formulation is now being used by some farmers in organic farming with some modifications (Natarajan, 2002). Similarly, in the recent past, concentrated liquid preparations of brown seaweeds have been shown to exhibit biostimulant and biofertilizer properties (Bukhare and Untawale, 1978; Albertz *et al.*, 1983; Kannan and Tamilselvan, 1990; Crouch and van Staden, 1992; Verkeij, 1992; Immanuel and Subramaniam, 1999; Thevanathan *et al.*, 2005). However, foliar application of panchagavya in combination with seaweed extract has not been tried by any. We have earlier shown that panchagavya amended with seaweed extract has biofertilizer potential in increasing the yield of some crop plants (Sangeetha, 2009). In this paper, we present the results of an investigation on the effect of the foliar application of seaweed based panchagavya on the levels of the antioxidant enzymes namely, superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx).

MATERIALS AND METHODS

Seeds of the pulses *Vigna radiata*, *Vigna mungo*, *Arachis hypogaea*, *Cyamopsis tetragonoloba*, *Lablab purpureus*, *Cicer arietinum* and the cereal *Oryza sativa* var. *ponni* were surface sterilized with 1.0% mercuric chloride, washed

several times in running water, soaked overnight in sterile water and allowed to germinate in dark. Germinating seeds were implanted in soil preparations kept in pots of the size 5.2" tall and 3.5" radius. Seedlings raised in sterilized garden soil were used as control. The developing seedlings were sprayed with seaweed based panchagavya in desired concentrations (1.0%, 2.0% and 3.0% in sterile water) at intervals of 7 days for 3 times. A day after the third spray, the leaves were harvested and crude enzyme preparations were made to assay the enzymes SOD, GR and GPx.

Extraction of cell-free enzymes

Freshly harvested leaves were rinsed in ice-cold, sterile water and homogenized with 5.0 mL of ice-cold Marsden's buffer, pH 7.4 containing 50.0 mM MOPS [3-(N-morpholino)propanesulfonic acid], 2.0 mM EDTA, 50.0 mM ascorbic acid, 0.5 mM dithiothreitol, CaCl₂ (0.2 g L⁻¹), TWEEN 80 (1.0 mL L⁻¹) and insoluble polyvinyl pyrrolidone (PVP) (100 g L⁻¹) (pretreated according to Loomis, 1974). The homogenate was strained through three layers of cheese cloth and centrifuged at 7000 x g for 15 minutes. The supernatant was collected and centrifuged at 20,000 x g for 30 minutes. The supernatant (crude extract) thus obtained was treated with Sephadex G-25 and dialysed overnight. The dialysate was centrifuged at 20,000 x g for 30 minutes. The resulting clear supernatant was used as the enzyme extract (Thevanathan, 1980). The entire operation was carried out at 4°C. The extraction

procedure is same for all the enzymes, unless otherwise mentioned.

Enzyme assay

1. Superoxide dismutase (SOD; E.C. 1.15.1.1): Superoxide dismutase was assayed following the method of Mishra and Fridovich (1972). The reaction mixture contained 50.0 μ L of the crude enzyme extract, 2.5 mL of carbonate – bicarbonate buffer (0.05 M, pH 10.2), 500 μ L of EDTA solution (11.4 mg in 30 mL of buffer) and 50.0 μ L of water. The reaction was initiated by the addition of 200.0 μ L of epinephrine (7.5 mg in 7.5 mL of buffer) and the increase in absorbance at 480 nm was measured for 2 minutes at every 15 sec. interval in a Shimadzu UV Spectrometer. Simultaneously, 100% autoxidation of epinephrine to adrenochrome was performed without enzyme and used as control. The enzyme activity is expressed as units mg^{-1} protein. One unit of enzyme activity is defined as the enzyme required to cause 50% inhibition of epinephrine autoxidation.

2. Glutathione reductase (GR; E.C.1.11.1.9): Glutathione reductase activity was measured by the method of Dubler *et al.* (1981). The reaction mixture containing 2.2 mL Tris buffer (pH 7.6), 0.5 mL GSSG (14.14 mg oxidized glutathione dissolved in 14.0 mL of d_2O) and 0.1 mL NAD(P)H (20.004 mg in 3.0 mL of d_2O) was made up to 3.0 mL with water. Reaction was initiated by the addition of 0.1 mL of the enzyme extract and the change in O.D at 340nm was monitored for 2 minutes at 30.0 sec. intervals. The enzyme activity is expressed as n moles of GSSG utilized min^{-1} mg^{-1} protein.

3. Glutathione peroxidase (GPx; E.C.1.11.1.12): GPx activity was determined following the method of Rotruck *et al.* (1973). To 1.0 mL of phosphate buffer (0.1 M, pH 7.4) taken in a tube, 0.5 mL Sodium azide solution (29.25 mg in 15.0 mL of buffer), 0.5 mL of EDTA solution (50.4 mg in 15.0 mL of buffer), and 100.0 μ L of the enzyme were added and mixed well. To this mixture, 0.5 mL glutathione (36.75 mg in 15.0 mL of buffer) was added and incubated at 37°C for 10 minutes, followed by the addition of 1.0 mL of hydrogen peroxide (freshly prepared by mixing 240 mL of hydrogen peroxide in 40.0 mL of buffer). The control contained all the reagents except the enzyme. After the incubation period, aliquots (1.0 mL) of the samples (both test and control) were taken in a tube to which 2.0 mL of Meta phosphoric acid and 1.0 mL of DTNB (5, 5'- dithio-bis-2-

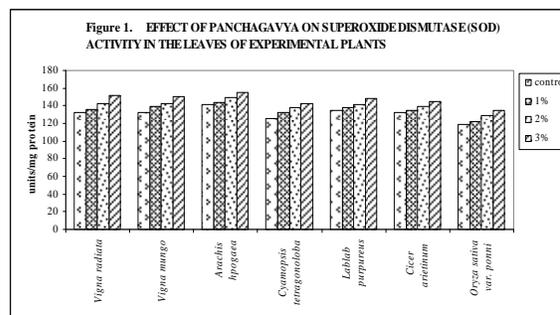
nitrobenzoic acid) reagent were added. The absorbance was then read at 412 nm in a Spectrophotometer. The enzyme activity is expressed as n moles of GSH oxidized min^{-1} mg^{-1} protein.

RESULTS

Foliar application of the panchagavya amended with seaweed extract increased the activities of all the three enzymes of antioxidation.

a. Superoxide dismutase (SOD; E.C. 1.15.1.1)

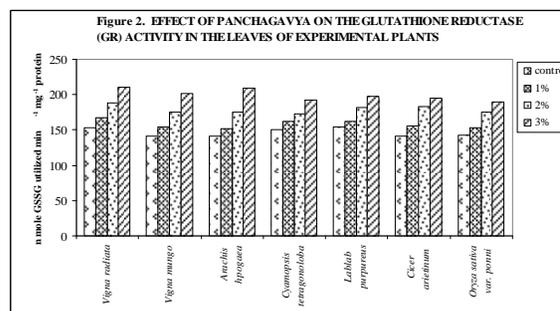
Levels of SOD in the experimental plants ranged between 119 and 141 units / mg protein (Figure 1). Maximum activity was found in *Arachis hypogea* while minimum activity was recorded in the leaves of *Oryza sativa*.



Spraying the seedlings with panchagavya resulted in an increase in the superoxide dismutase activity in all the experimental plants. Increasing the concentration of panchagavya from 1% to 3% concomitantly increased the levels of the enzyme in all the seedlings. Seedlings that received 3% panchagavya spray exhibited 9 to 14% more activity for the enzyme than their respective controls.

b. Glutathione reductase (GR; E.C.1.11.1.9)

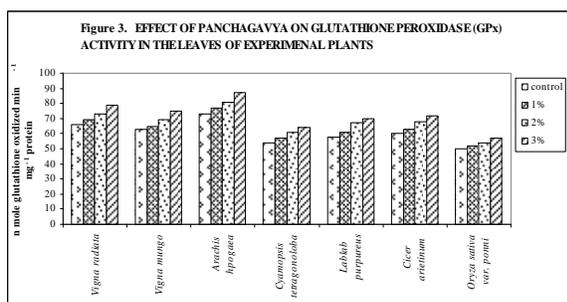
High levels of glutathione reductase were detected in the seedlings of all experimental and control seedlings and the levels were in the range of 141 to 153 n moles GSSG utilized min^{-1} mg^{-1} protein (Figure 2).



Seedlings that received the spray treatment with 1% panchagavya increased the levels of the enzyme by about 5 to 9% and the effect was high in the pulses *Vigna radiata* and *Cicer arietinum*. A linear relationship existed between the levels of GR in the seedlings and the concentration of panchagavya treatment given. Increase in the levels of the enzyme in seedlings that received 3% panchagavya was 27 to 48% more than that of the respective control seedlings. *Arachis hypogea* recorded the highest activity for the enzyme for 3% panchagavya treatment.

c. Glutathione peroxidase (GPx; E.C.1.11.1.12)

Arachis hypogea recorded the highest activity (73 n moles glutathione oxidized $\text{min}^{-1} \text{mg}^{-1}$ protein) among the experimental plants while *Cyamopsis tetragonoloba* registered the lowest (54 n moles glutathione oxidized $\text{min}^{-1} \text{mg}^{-1}$ protein) (Figure 3).



All experimental seedlings except *Oryza sativa* showed significant levels of increase in glutathione peroxidase activity in response to spray treatment with panchagavya. In rice, the quantum of increase in the levels of the enzyme was low. Nevertheless, the foliar application was able to influence the activity of this enzyme in rice also. Increasing the concentration of panchagavya resulted in concomitant increases in the activity of glutathione peroxidase of the seedlings exhibiting positive correlation between the two. Seedlings that received 3% panchagavya treatment recorded 12 to 20% more activity for the enzyme than that of the respective control seedlings (Figure 3).

DISCUSSION

Production of reactive oxygen species (ROS) or the superoxide radical is of common occurrence in biological systems either through exposure to ionizing radiations and xenobiotics or through normal metabolic reactions. Reactive oxygen species mediated reactions in plants can inactivate enzymes and interfere with the integrity of membranes, DNA strands and many other

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macromolecules (Bowler *et al.*, 1992; Mehdy, 1994). This would in turn lead to a decrease in the yield potential of crop plants primarily through pathological conditions. Organisms have evolved mechanisms that can scavenge the superoxide radicals in cells to ameliorate the damage caused by ROS mediated pathological conditions either through specific enzymes or antioxidants. Enzymes mediated scavenging of reactive radicals is one such mechanism observed in plants. Three enzymes namely, superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx) play a key role in scavenging these reactive radicals. The effect of panchagavya as a foliar spray on these three antioxidant enzymes in the leaves of the experimental plants has been investigated in the present study.

Glutathione is a naturally occurring tripeptide and is a major source of intracellular non-protein thiol group (Meister and Anderson, 1983). It serves an important role in antioxidant defense as it scavenges free radicals produced by oxidation or radiation and protects cells against a variety of endogenous and exogenous toxic agents (Brehe and Burch, 1976; Cook *et al.*, 1991; Colvin *et al.*, 1993). Glutathione exists as reduced glutathione (GSH) and oxidized glutathione (GSSG). In the reduced state, the amino acid residue cysteine of glutathione provides reducing equivalents (protons and electrons) to other unstable molecules such as reactive oxygen species. As a result, glutathione itself becomes reactive to form GSSG by reacting with another glutathione. Under such conditions, the enzyme glutathione reductase plays a key role in regenerating reduced glutathione (GSH) from GSSG. Glutathione is a cofactor for the enzyme glutathione peroxidase which by its peroxidase activity protects cells from oxidative damage. Glutathione peroxidase plays a major role by reducing lipid hydroperoxides to their corresponding alcohols in addition to reducing free hydrogen peroxide to water (Rotruck *et al.*, 1973).

Spraying the seedlings with seaweed based panchagavya increased the activities of all the three antioxidant enzymes namely; superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx) in the leaves of all the experimental plants (Figures 1, 2 and 3). A linear relationship existed between the levels of these three enzymes and the concentration of panchagavya used in the foliar spray. Seedlings that received 3% panchagavya increased the activity of SOD by 9 – 14% (Figure 1), GR by 27 – 48% (Figure 2) and GPx by 12 – 20% (Figure 3). The effect of panchagavya was more pronounced on the glutathione reductase levels as compared to the

other two enzymes. Induction in the levels of these three enzymes by the foliar spray of panchagavya indicates a better defense mechanism in the plants that received the treatment against ROS mediated pathological conditions and this could be construed as a positive aspect of panchagavya in promoting the yield potential of crop plants.

CONCLUSION

The study revealed that foliar application of panchagavya amended with seaweed extract to the experimental pulse and rice seedlings induced the activities of the antioxidant enzymes in all the seedlings. The spray was effective at a concentration of 3% and needed at least three applications at an interval of seven days each. Of the three enzymes studied, GR exhibited highest response to the treatment.

CORRESPONDING AUTHOR

Thevanathan, R.
Professor, Centre for Advanced Study in Botany,
University of Madras (Guindy campus),
Chennai-25, Tamil Nadu, India.
thevanathan@gmail.com.

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