

Aim 11

To find out the Total Peroxidase Activity in the Plant material

Introduction

Peroxidase catalyses the oxidation of phenolics and certain other substances using oxygen derived from H_2O_2 . H_2O_2 is formed in small quantities in cells. Peroxidase functions first forming H_2O_2 from NADH and O_2 . Next, they remove a hydrogen atom from each of the substrate and combine the two H atoms with one H_2O_2 to release H_2O molecule as by-product. Guaiacol is the substance and it intensifies the activity of peroxidase (& its isoenzyme). Guaiacol is a phenolic compound. Phenols on oxidation give quinines. Peroxidases are the active only in the presence of H_2O_2 and then the activity is observed.

Requirements

Plant material, H_2O_2 , K_2HPO_4 , Na_2HPO_4 , Guaiacol, test tube, test tube stand etc.

Reagents

Preparation of phosphate buffer: 61.1 mg of Na_2HPO_4 and 38.9 mg of KH_2PO_4 were dissolved in small amount of distilled water and finally volume was raised to 100 ml and pH was maintained at 7.

Preparation of Guaiacol: 20 mM of Guaiacol solution is prepared by dissolving 0.22 ml of Guaiacol in 100 ml of distilled water.

Preparation of H_2O_2 : 10 ml of H_2O_2 was prepared by dissolving 0.4 ml of H_2O_2 in 9.6 ml of distilled H_2O .

Preparation of Enzyme extract: 100 mg of plant sample was taken and grinded in 10 ml of phosphate buffer at pH 7. This was then centrifuged at 5000 rpm. The residue was discarded and the filtrate was taken as enzyme extract.

Procedure

1. Take the clean test tubes and mark them 1 to 4.
2. 1st test tube is taken as control and other three for the experimental setup.
3. In the control test tube, 4 ml of H₂O and 2 ml of phosphate buffer was added along with 2 ml of enzyme extract.
4. In other three test tubes, 2 ml of enzyme extract, 2 ml of phosphate buffer, 2 ml of Guaiacol and 2 ml of H₂O₂ was taken. Brown colour solution was formed.
5. The test tubes were shaken well and absorbance was recorded at 420 nm.

Precautions

1. pH of a buffer solution should be 7.
2. Wash the apparatus before and after use with distilled water.