**MATERIAL AND METHODS**

**Plant Material** - Seeds of uniform size of barley (*Hordeum vulgare* var. IB 65) were taken and cut in half on equatorial axis and the embryo-containing-halves discarded. The endosperm halves, thus obtained, were surface sterilized for 15 minutes in 1% sodium hypochlorite (a commercial liquid bleach called Clorox, diluted 1:4 with distilled water), rinsed several times in sterile distilled water and preincubated in sterile distilled water in a conical flask at 4-5°C for 3 days. Half seeds, preincubated for 3 days, were employed as experimental material in all experiments, except where otherwise indicated.

**Incubation of Half Seeds** - After preincubation, the half seeds were again surface sterilized with 1% sodium hypochlorite, rinsed thoroughly with sterile distilled water. Fifteen half seeds were transferred aseptically to 25 ml conical flasks, each containing 5 ml of test solution (incubation medium). In order to ensure against microbial growth, 20 μg/ml streptomycin was added to each flask. The pH of the incubation medium was adjusted to 7.0. All incubations were carried out at a constant temperature of 25±1°C in a B.O.D. incubator. Treatments of half seeds with various hormones and other compounds have been described in
respective tables and figures. At the end of the incubation period, the incubation media were decanted into glass tubes and stored in ice for measurement of peroxidase activity in the medium.

**Enzyme Extraction** - The incubated half seeds were rinsed with distilled water, dried on a blotting paper and ice-chilled till homogenized in 6 ml of sodium phosphate buffer 0.1 M; pH 7.0 (6 ml buffer/15 half seeds) in mortar and pestle at 4°C. The homogenate was centrifuged at 20,000Xg for 30 minutes at 2°C. The resulting supernatant fraction (referred to as extract) was used for enzyme assay and gel electrophoresis.

**Enzyme Assay** - Activity of the enzyme peroxidase (E.C. 1.11.1.7.) was assayed both in medium and extract by recording change in absorbance at 610 nm at 15 seconds intervals after adding 50 μl, 100 μl or 200 μl of sample supernatant (extract) or incubation medium to 5 ml of assay mixture at 25°C. The assay mixture contained 2.4 x 10⁻² M benzidine, 7 x 10⁻² M acetic acid, 3 x 10⁻² M hydrogen peroxide, pH 3.8. Benzidine solution was prepared by dissolving 1 g of benzidine in 9 ml of glacial acetic acid at 50°C and adding 36 ml of distilled water to it. One unit of enzyme activity is
arbitrarily defined as change in absorbance of 0.1 at 610 nm per 15 seconds (1 unit = 0.1 A₆₁₀·15sec⁻¹). Peroxidase activity is expressed as units of enzyme per fifteen half seeds.

**Gel Electrophoresis** - Isozymes of peroxidase were separated by polyacrylamide gel electrophoresis at 4°C. Samples (100 μl), prepared by mixing extract and glycerol (2:1 ratio) were layered on top of the gels. The anionic and cationic isozymes were resolved by the methods of Davis (1964) and Reisfeld et al. (1962), respectively. The tray buffer for anionic isozymes was tris-glycine (pH 8.3) and for cationic isozymes β-alanine-glacial acetic acid buffer (pH 4.5) was used. A current of 4mA per gel tube was employed. After completion of the run, gels were taken out and stained by first dipping in benzidine-acetic acid mixture (2.4 x 10⁻⁴ M and 7 x 10⁻³ M, respectively) for 10 minutes and then in hydrogen peroxide (3 x 10⁻² M) for 2 minutes. Dark blue bands which later turned brown, were obtained. Gels were rinsed with distilled water and stored in 7% acetic acid.

**Filter-Sterilization of GA Solution** - Gibberellic acid solutions were routinely sterilized with the help of millipore filter assembly (filter pore size 0.25 μ).
just before giving each treatment.

**Light Sources** - White light was obtained through two cool white fluorescent tube lights. Red light was obtained from two 100 watt tungsten lamps wrapped with two layers of red cellophane paper (emission maxima 650 nm). The intensity of red light reaching the plant material was 500 μW/cm². The light from a 300 watt reflector lamp was filtered through 8 cm of continuously flowing tap water and a CBS-750 (Carolina Biological Supply Company) filter (emission maxima 750 nm) to obtain far-red light. The intensity of the far-red light at the level of the flasks was 140 μW/cm². Temperature during all these treatments was maintained at 25±1°C.

**Chemicals** - All inorganic chemicals were of reagent grade and were obtained from British Drug House, Bombay, India or E. Merck, Darmstadt, Germany. Indoleacetic acid was obtained from Nutritional Biochemical Inc., Cleveland, Ohio, U.S.A., gibberellic acid, kinetin, cis-trans abscisic acid, theophylline, cycloheximide, cyclic 3', 5'-adenosine monophosphate, N₆,₀²'-dibutyryl cyclic adenosine monophosphate, puromycin, 6-methylpurine, chloramphenicol, streptomycin sulphate, antimycin A, 2,4-dinitrophenol, cordycepi
(3'-deoxyadenosine) were obtained from Sigma Chemical Company, St. Louis, M.O., U.S.A., actinomycin D from Calbiochem, Los Angeles, California, U.S.A., and benzidine from Reanal, Budapest, Hungary.