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Short Communication

Foldscopebased methods to detect in-tissue antioxidant activity and secondary metabolites in pollen and stomata of Lantana camara

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Abstract

Foldscope is the ultra-affordable, paper microscope designed to be extremely portable, durable, and to give optical quality similar to conventional research microscopes. However it's potential as in tool in the area of agricultural sciences is still not known. In this study, how foldscope can be used as an efficient tool to detect in –tissue activities of antioxidants and secondary metabolites in invasive alien plant *Lantana camara*.Foldcsope images revealed viable stained areas in the respective seeds, indicating live activities. Based on data the use of foldscope was recommended.

Keywords: Foldscope, pollens, stomata, Lantana

Foldscope, is an paper based, portable microscope with magnification power of 140 X. it was invented by Prakash lab at Stanford University of America in 2014 [1]. Thereafter, Department of Biotechnology (DBT), Government of India and Prakash Lab at Stanford University, USA earlier signed an agreement to bring the Foldscope to India to encourage curiosity in science.

Invasive Alien Species are basically non-native plant species that displace native species and pose adverse effects to environment, ecosystem, economy and human health by diminishing the growth of native plants and also possess higher stress tolerance. They are either introduced to a new environment from their natural habitat by sudden or accidental escape. *Lantana camara* (belongs to *Verbenaceae*), is an ornamental and noxious weed as it harms other plants, crop productivity, animals, organisms and others [2]. Stomata and pollen represents are two important organs present in plants, that are challenged by unfavourable conditions including biotic and abiotic stress like high and low temperature, light, nutrients, etc. Fluctuations in all these factors have large impact on plant performance during their regular life cycle. Stomata mainly helps in exchange of gases and water balance between the environment and intracellular spaces of plants which results in changing environment around the plant species and involved in adaptation of plants under adverse abiotic conditions. Pollen grains contain the male reproductive cells which need to be transferred to the female reproductive structure of the plant for further fertilisation process.However the

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role of foldscope as an efficient tool in plant biology is not known worldwide so far. With this background, the present study was carried out to assess the usefulness of portable foldscope for in–situ detection of some antioxidant metabolites in pollens and stomata of *Lantanacamara*. Due to its less cost, environmental friendly, small size, and portable in nature, can be widely used even by Plant Biologists in the field conditions.

MATERIALS AND METHODS

Plant materials

Leaves and flowers of *Lantanacamara* were washed with distilled water for further analysis. Epidermis from leaves and pollens from flowers was then peeled out carefully from fully expanded leaves at reproductive stage, immediately prior to each experiment.

Image detection

Foldscope was used in current study, was supplied by Department of Biotechnology (DBT), Government of India. Thin section of tissues were taken on glass slides and covered with transparent cello tape/ cover slip. The slide was inserted into the foldscope in such a way that sample side was close to lens of foldcope. A LED light supplied with foldscope instruments was used a light source. The clear images under foldscope for each sample were photographed using cell phone camera (Samsung, Galaxy Tab A) by adjusting zoom and focusing of camera and foldscope.

In-situ localisation of ROS and antioxidants

H_2O_2 content

The tissue samples were incubated in 1 mg/ml of 3,3'-Diaminobenzidne (DAB)-HCl ,pH 3.8 for 6-7 hours at room temperature. H_2O_2 was visualized as deposits of dark Brown insoluble formazan compounds.

O^{2-} content

Plant tissues were incubated in 6mM Nitro Blue Tetrazolium (NBT) in 10mM Tris-HCl buffer, pH 7.4 at room temperature for 24 h. O^{2-} was visualized as deposits of dark blue insoluble formazan compounds.

Glutathione (GSH) content

The samples were incubated in reaction mixture containing 50 mMTris–HCl buffer (pH 7.0) and 0.3 mM 5,5´-dithiobis(2-nitrobenzoic acid (DTNB) in a total volume of 5 ml at 37°C for 24 hours. The activity can be visualized as yellow colored stain in the tissue.

Ascorbate (AsA) content

The tissue samples were incubated in reaction mixture contained 2 ml of 2% Na-molybdate, 2 ml of 0.15 N H_2SO_4, 1 ml of 1.5 mM Na_2HPO_4 bringing the total volume to 5 ml with buffer for 24 hours. The activity can be visualized as yellow colored stain in the tissue.

Thioredoxinreductase (TRx.R) activity

Plant tissues were incubated in assay mixture contained 50 mMTris-HCl (pH 7.0), 1 mM EDTA, 0.2 mM NADPH, 0.2 mg/mL bovine serum albumin and 5 mM 5,5´(dithiobis-(2-nitrobenzoic) acid at 25°C in a total volume of 5 mL for 24 hours. The activity can be visualized as yellow colored stain in the tissue.

NADPH Oxidase activity

Tissues were incubated in dark for 24 hours in a reaction mixture solution containing 50 mMTris-HCl buffer (pH 7.4), 0.2 mM NBT, 0.1 mM $MgCl_2$ and 1 mM $CaCl_2$, 0.2 mM NADPH for 3-4 hours and the appearance of blue formazancolor was monitored.

Catalase (CAT) activity

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The tissues were immersed in 50 mM K-PO4, pH 7.0 for 20 min and then in 0.03% H_2O_2 solution in H_2O (1/1000 dilution of 30% stock) for 30 min. The tissue was rinsed twice with H_2O and then incubated in a mixture (1:1) of freshly prepared 2% $K_3Fe(CN)_6$ and 2% $FeCl_3$ for 15 minutes. The tissue became greenish-blue while zones of catalase activity were yellow.

Superoxide dismutase (SOD) activity

The tissues were first soaked in 10 ml of 1.23 mM NBT for 15 min, briefly washed with water, then soaked in the dark in 10 ml of 100 mM potassium phosphate buffer (pH 7.0) containing 28 mM TEMED and 2.8 x 10^{-2}mM riboflavin for another 4 hours. The reaction was stopped by briefly washing the tissue with water and illuminating it under white light to initiate the photochemical reaction. The regions of SOD activity appeared as colourless areas on a purple background.

Peroxidase (POD) activity

The tissue was washed three times in 50 mM sodium acetate buffer (pH 5.0). Peroxidase activity was visualised by incubating the tissue in 50 ml of a solution containing 50 mM sodium acetate buffer (pH 5.0), 330 µl of guaiacol and 330 µl of 6.6 % H2O2 for 20 minutes. The tissue was incubated at room temperature in the dark until reddish brown bands appeared.

Monodehydroascorbatereductase (MDAR) activity

The plant tissue was immersed in a solution of 1.3 mM NADH, 1.2 mM 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide and 0.06 mM 2,6-dichlorophenol-indophenol in 0.25 M potassium phosphate buffer (pH 8.0) for 2 hours in the dark. The spots appeared dark on a blue background.

Histo-chemical detection of secondary metabolites

Lignin Detection (Potassium permanganate)

Samples were flooded with a solution of 1% $KMnO_4$ for 4 hours and rinsed with water. Precipitated $KMnO_4$ appears very dark and is claimed to be quite specific for lignin.

SuberinDetection (Gentian Violet)

Samples of plant tissues were flooded for 4 hours with a solution of 1% (w/v) gentian violet in 70% ethanol plus 1 or 2 drops of concentrated ammonium hydroxide. The tissues were rinsed with 9% HCl for 2 min or until clear. The sample were then flooded with 95% ethanol for 10 min and rinsed with ethanol and water. Suberized areas stain blue or purple.

Phenolics and Tannins Detection (Ferric chloride)

A few drops of 10% (w/v) ferric chloride solution were added to the tissue for upto 4 hours. A blue-green precipitate will develop in the presence of phenolic compounds and tannins.

Lipids Detection (Nile Blue)

Tissues were incubated in 10 ml of saturated aqueous solution of Nile blue sulfate containing 1 ml of 0.5% H_2SO_4 for 4 hours. Lipid stain blue.

Cellulose or hemicellulose Detection (Iodide-sulphuric)

The tissues were treated with upto 1 ml of potassium tri-iodide solution for 3 hours. A cover slip was added and a drop of 65% H_2SO_4 introduced and allowed to diffuse under the glass. Cellulose or hemicellulose stains dark blue, lignin stains orange to yellow.

Cellulose or hemicellulose Detection (Zinc-chloride-iodide)

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The samples were treated with a few drops of chloro-iodide (50 g of zinc chloride plus 16 g of potassium iodide in 17 ml of water) for 4 hours. Large amounts of cellulose or hemicellulose stain blue. Lignin, cutin, suberin or chitin stain yellow to orange and may mask cellulose.

Suberin and Cutin Detection (Sudan IV)

Several drops of a saturated solution of Sudan IV in 70% ethanol were added to the tissues for 4 hours following by quick rinses in three changes of 50% ethanol. Lipid containing material such as suberin and cutin stain pink to orange.

Callose Detection (Aniline Blue)

Five to ten drops of 0.5% (w/v) aniline blue in 50% ethanol were placed on the sample for 4-8 h and rinsed with 50% ethanol. Callose deposits were stained blue.

RESULT AND DISCUSSION

Foldscope based in-tissue activity analysis was carried out to detect antioxidants and secondary metabolites in pollens and stomata of *lantanacamara*. As compared to control (unstained) conditions, In-situ localisation and histochemical staining both in pollens and stomata indicated the activity stained area of various antioxidant enzymes (including glutathione, NADPH oxidase, SOD, POD, MDAR etc.) and secondary metabolites (including lignin, lipid, cellulose and hemicellulose, etc.) in pollens and stomata when incubated in respected staining solutions (Fig1 and 2).

Fig. 1

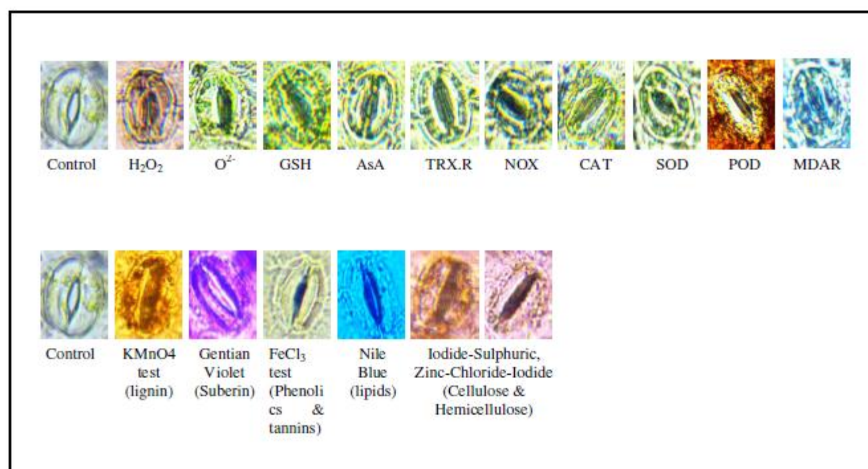

Fig.1 Representative figures of stomata showing positive results for in-tissue localisation of various antioxidant enzymes and secondary metabolites as compared to control (unstained) conditions.

Many investigators reported that increase in activity of antioxidant enzymes and level of secondary metabolites in stressful conditions are the major factors that lead to stress tolerance in various plant species under various adverse abiotic conditions like high and low temperature, drought, salinity, etc. [3].

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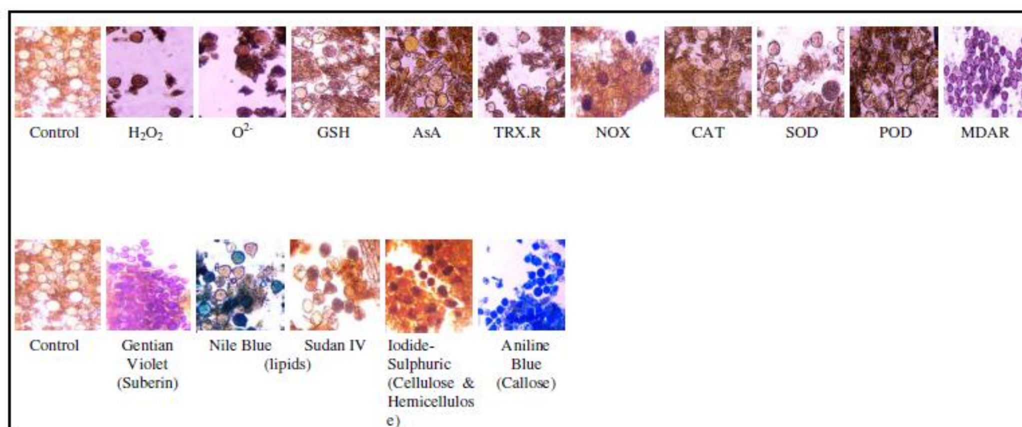

Fig.2 Representative figures of pollen showing positive results for in-tissue localisation of various antioxidant enzymes and secondary metabolites as compared to control (unstained) conditions.

Based on this data it was concluded that foldcope can be a cheap, portable and powerful efficient biological tool to evaluate plant biology based studies. Further studies are underway to find out effect of various abiotic stresses and plant hormones like ABA on enzymatic activities and metabolites in stomata and pollens of *Lantanacamara*.

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Conflict of interest: No

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