3. HISTOCHEMISTRY OF ENZYMES IN KNOT

3(a). **Enzyme Succinate Dehydrogenase:**

3(a)-i. *A. indica*:

**Clean wood** - The enzyme SDH was localized as granules, discrete groups or amorphous mass in rays (Fig. 3.1 A). In some ray cells they formed a coat of fine granules around the starch grains (Fig. 3.1 A). In some cases they lined up the pits of lateral walls of ray cells. Axial parenchyma showed poor SDH localization in comparison to that in rays (Fig. 3.1 B). In axial parenchyma SDH granules formed discrete groups and some of them encased the starch grains.
In fibre cells the enzyme SDH occurred as granules and rod-shaped bodies (Fig. 3.1 C). Of all the cell types of CW the fibres were the poorest in SDH content. As in case of the metabolites, parenchyma cells contiguous to vessels exhibited higher SDH activity than the non-contiguous ones (Fig. 3.1 B). At the pits on walls of vessels also SDH activity was localized.

Knot wood - The general pattern and forms of SDH manifestation in all the four regions of KW were almost identical to that in clean wood. However, the tendency of staining reaction varied among various cell types within each region, as well as in various regions. Nonetheless, in each region only rays showed the highest intensity of the enzyme activity. Some of the ray cells of BKR were found to be completely filled with granules of reacted SDH (Fig. 3.1 D-F).

M. indica:

The general distribution and forms of SDH activity were essentially the same as in A. indica. Hence, the duplication of description has been avoided. However, in each region of KW and in CW the major sites of the enzyme activity were ray cells followed by axial parenchyma.
cells (Fig. 3.2 A). In BKR axial parenchyma contiguous to vessels showed high enzyme activity. The starch grains in these cells were enveloped by SDH, whereas those away from the vessels were devoid of SHD film around starch grains. The fibres of BKR showed SDH as an amorphous and rod-shaped bodies. In addition to this, the starch grains of fibres were also coated by SDH. Rod-shaped bodies of reacted SDH were localized in lumen and pits of vessels of BKR (Fig. 3.2 B).

In CKR (Fig. 3.2 C), AbKR and AdKR all the axial parenchyma cells (unlike in BKR) and ray cells had their starch grains enclothed by a film of SHD (Fig. 3.2 D-F). Lumen of fibres of AbKR was completely filled with fine granules of reacted SDH (Fig. 3.3 A). Inspite of similarity in general distributional pattern of SDH in various regions of KW, the general intensity of its activity varied from that in A. indica.

3(a)-iii. S. saman.

Enzyme SDH was localized in the ray cells of CW in various forms, i.e. as clumps of granules present in the centre of the cellular lumen, discrete groups of granules, scattered granules and as a coat around starch grains. The
rays rich in starch content were poor in SDH (Fig. 3.3 B). In axial parenchyma (Fig. 3.3 C) and fibres (Fig. 3.3 D) the pattern of SDH distribution was basically the same as described earlier.

In KW also the forms and distribution of SDH were the same as in CW. Crystalliferous cells of BKR had the SDH around the starch grains as well as in discrete groups of granules in the cellular lumen (Fig. 3.3 E). Rays and axial parenchyma, however, were rich in SDH in comparison to other cell types. In axial parenchyma of AbKR and AdKR the SDH activity was restricted around the starch grains only. Unlike the common pattern of SDH activity in various regions of KW, in AdKR its maximum activity was found in fibres, followed by that in rays and the least in axial parenchyma (Fig. 3.3 F). The distributional pattern of SDH activity in various regions, and various cell types in each of them is as under.

i. *A. indica* :

a) CW < KW
b) BKR > CKR > AdKR > AbKR
c) CW: RAYS > APC > FIBRES
d) BKR: RAYS > APC > FIBRES
e) CKR: RAYS = APC > FIBRES 
f) AdKR: RAYS > APC > FIBRES 
g) AbKR: RAYS > APC > FIBRES 

M. indica:
a) CW < KW 
b) AbKR > AdKR > BKR > CKR 
c) CW: RAYS > APC > FIBRES 
d) BKR: RAYS = APC > FIBRES 
e) CKR: RAYS > APC > FIBRES 
f) AdKR: RAYS = APC > FIBRES 
g) AbKR: RAYS = APC > FIBRES 

S. saman:
a) CW < KW 
b) BKR > CKR > AdKR > AbKR 
c) CW: RAYS > APC > FIBRES 
d) BKR: RAYS > APC > FIBRES 
e) CKR: RAYS > APC > FIBRES 
f) AdKR: RAYS > APC < FIBRES 
g) AbKR: RAYS > APC < FIBRES
3(b). Enzyme Peroxidase:

3(b)-i. A. indica:

Intense peroxidase activity was localized in the pits and walls of vessels in CW. Rays and axial parenchyma cells contiguous to vessels showed granular peroxidase (Fig. 3.4 A). Some fibres showed feeble peroxidase activity.

Peroxidase was localized in all the four regions of KW, as a film lining the walls of vessels, rays, axial parenchyma cells and pits of vessels. In the cellular lumen of some rays and axial parenchyma cells it was localized as granular or amorphous mass (Fig. 3.4 B-E).

3(b)-ii. M. indica:

Peroxidase activity was localized in rays and a few axial parenchyma (Fig. 3.4 F) and G-fibres of CW. G-fibres showed uniform and intense staining for the enzyme (Fig. 3.5 A). The enzymatic activity in most of the rays was seen in a granular form, while in a few it was in the form of an amorphous mass (Fig. 3.4 F).

Moderate to intense peroxidase was localized at the pits of vessels and on the inner surface of walls of all cell types in KW (Fig. 3.5 C, E and F). Some rays and
axial parenchyma showed granular or amorphous form of reacted peroxidase (Fig. 3.5 E,F). A few fibres in BKR and most of the fibres in AdKR showed amorphous peroxidase (Fig. 3.5 B and D). The CKR showed an intense peroxidase activity (Fig. 3.5 C). The rays and axial parenchyma cells of AbKR showed the maximum activity (Fig. 3.5 E, F).

3(b)-iii. S. saman:

G-fibres in CW were the major sites of intense enzyme activity (Fig. 3.6 A). Some rays and axial parenchyma cells non-contiguous to vessels showed amorphous mass (Fig. 3.6 B) indicating the presence of the enzyme, whereas the living cells contiguous to vessels showed granular form of reacted peroxidase. The enzyme was also localized along the walls of crystalliferous cells. The pits of a few rays and axial parenchyma contiguous to vessels were also sites of enzyme activity. All the four regions of knot showed intense to moderate enzyme activity along the walls of rays and axial parenchyma. Granular or amorphous forms of reacted enzyme were found in some rays, axial parenchyma and fibres (Fig. 3.6 C).

The region of CKR just adjacent to AbKR showed high peroxidase activity in fibres and along the walls of living
cells. Its region adjoining AdKR had amorphous reacted peroxidase in some rays, axial parenchyma and a few fibres (Fig. 3.6 D).

In AdKR and AbKR ray cells were seen to have the maximum enzyme activity (Fig. 3.6 E,F).

The distributional pattern of the enzyme peroxidase in various regions, and various cell types in each species is as under.

1. **A. indica**
   a) CW < KW
   b) AdKR > AbKR > BKR > CKR
   c) CW: RAYS = APC < FIBRES
   d) BKR: RAYS = APC > FIBRES
   e) CKR: RAYS = APC = FIBRES
   f) AdKR: RAYS > APC < FIBRES
   g) AbKR: RAYS > APC < FIBRES

2. **M. indica**
   a) CW < KW
   b) CKR > AdKR > AbKR > BKR
   c) CW: RAYS > APC < FIBRES
   d) BKR: RAYS = APC < FIBRES
   e) CKR: RAYS = APC > FIBRES
f) AdKR: RAYS > APC > FIBRES

g) AbKR: RAYS = APC > FIBRES

III. FIBER

a) CW < KW

b) AdKR > AbKR > BKR > CKR

c) CW: RAYS > APC < FIBRES

d) BKR: RAYS = APC > FIBRES

e) CKR: RAYS = APC > FIBRES

f) AdKR: RAYS > APC > FIBRES

g) AbKR: RAYS > APC < FIBRES