CELL MEMBRANE INJURY IN FLAG LEAF OF WHEAT BY BROWN RUST (*PUCCINIA RECONDITA ROB. EX. DESM. F. SP. TRITICI*) AT DIFFERENT NITROGEN LEVELS

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Appreciable alterations in cell membrane injury by *Puccinia recondita* was observed to be higher in inoculated plants as compared to noninoculated ones at flag leaf stage of wheat. These changes were very much evident in both cultivars viz. susceptible Lalbahadur as well as in resistant Raj 3765 at 72, 96, and 120h of progressive rust infection supplemented with different nitrogen levels (No, N30, N90).

**Keywords:** Brown rust; Cell membrane injury; Nitrogen fertilizer.

**Introduction**

Brown rust (*Puccinia recondita* Rob. Ex. Desm. F. Sp. tritici) is one of the most wide spread disease of wheat (*Triticum aestivum*) in the world<sup>1</sup>. During infection the resistant host plants defends itself against potential pathogen by means of a number of physical and chemical factors, which may already present in the host or may be produced in response to infection.

Alterations in plant water relations due to pathogenic interference has been known since long<sup>2</sup>. Increased permeability of susceptible host may also be due to the fungal toxins<sup>3</sup>. Cell wall defense structures involve morphological changes invaded by the pathogen. The outer layer of the cell wall of epidermal cells in contact with incompatible pathogen swells and produces an amorphous, fibrillar material that surrounds and traps the pathogen and prevents them from multiplying. Cell wall thicken in response to several pathogens by producing a type of cellulosic material. This material however is often infused with phenolic substances that are cross-linked and further increase its resistance to penetration. Thus during the infection process a series of structural features appear on the host cell wall and the tissues, biochemical and molecular reactions take place in exchange of series of signals, messages and dialogues between host and the pathogen<sup>4</sup>.

Nitrogen plays an important role in the enhancement of wheat production and higher dosages are often recommended to increase wheat yield of the high yielding varieties. 50% of the nitrogen through urea reduced the blast disease severity to a maximum extent and produced maximum yield<sup>5</sup>. However, application of nitrogen is also known to modify the plant susceptibility to many pathogens. This remains the nature of the present studies.

**Materials and Method**

**Raising of crop:** The crop was raised in earthen pots (height 30cm, diameter 20 cm) filled with sterile coarse sand (pH 8.3) in polythene bags. Two wheat varieties viz. LalBahadur (susceptible) and Raj 3765 (resistant) were taken for conducting studies during the usual winter season (December- March).

**Nitrogen fertilizer:** The pots were supplemented by different doses of urea (with 46.5% N) so as to give 0 (no urea), 30 and 90 kg of Nitrogen per hectare of field area calculated on the basis of the weight of the sand at the time of sowing.

**Rust inoculation:** The flag leaves were inoculated artificially by rubbing uredospore powder of the brown rust on the lower surface of the leaves. The rust inoculum was the mixture of races 12-2, 77-2, 77-5 and 104-2 and was procured from Regional Research Station of the Directorate of Wheat Research, Flowerdale, Shimla (India). Upon inoculation the plants were covered with polythene bags (40x 80 cm) to ensure enough humid environment.

**Sampling:** Upon rust inoculation, the polythene bags were removed after 24h and the leaf samples were collected 0 (just before inoculation), 72, 96 and 120h after attempted inoculation.

**Estimation of the cell membrane injury:** Leaves of healthy and infected wheat plant from resistant and susceptible varieties were taken for following physiological studies at different intervals (0h, 72h, 96h, and 120h). The studies were confined to flag leaf only, keeping in view of its major contribution towards economical yield.

Cell membrane injury was estimated by the method described by Sullivan<sup>6</sup>. Six leaves were taken per replication for detecting the cell membrane injury. Conductivity Bridge of normal and autoclaved tissues measured the Electrical Conductance (EC). The percent injury of tissues was calculated by the following formula:

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\text{Percent Injury in Normal Tissue} = \frac{\text{Conductivity of the Tissue}}{\text{Total Conductivity}} \times 100
\]
Percent Injury in Stressed Tissue = Conductivity of the Tissue / Total Conductivity x 100
Percent Uninjured Tissue = 100 - Percent Injury
Percent Membrane Injury = 100 – [(Percent Uninjured Stressed Tissue / (Percent Uninjured Normal Tissue) x 100)

Results and Discussion

The cell membrane injury was observed to be higher in inoculated plants as compared to noninoculated ones. These changes were very much evident in both CVs. Viz. susceptible Lal Bahadur as well as resistant Raj. 3765 supplemented with different nitrogen levels (N0, N30, and N90).

The cell membrane injury was slightly higher at lower nitrogen level (i.e. N30) in the susceptible CV. Lal Bahadur as compared to N90 level. However, the resistant CV. Raj. 3765, the cell membrane injury was observed to be higher at early hours of inoculation i.e. 72h, 96h and later on decreased at 120h of inoculation, particularly at N90 level. The recovery in cell membrane injury was observed in Raj. 3765 at 96h and 120h supplemented with respective nitrogen levels.

An increase in cell membrane injury was associated with disease development. These results indicate that increased Electrolyte leakages are one of the initial responses of wheat to infection by *Puccinia recondita* at flag leaf stage. The increase in cell membrane injury at lower nitrogen levels (i.e. N0, N30) in susceptible CV. Lal Bahadur may be due to the different physiological interactions. The lower nitrogen levels might be assimilating easily and providing nitrogen for growing pathogen. The utilization of nitrogen and carbon are interdependent and competitors in the sense both require energy for assimilation of CO₂ and nitrogen. Application of nitrogen in plants increases the synthesis of proteins, as the nitrogen is the main constituents of protein biosynthesis, which ultimately transformed into important constituent of cell membranes. The plant cell wall acts as a barrier to penetration by fungal pathogens to overcome this. These include the secretion of a range of plant cell wall degrading enzymes (Depolymerases) and the production of the fungal toxins such as oxalic acid by fungal pathogens. Another developed strategy that could have potential to reduce pathogen infection is immunomodulation, the expression of genes encoding antibodies or platibodies that could bind to pathogen virulence products.

In the resistant var. Raj 3765, lower values of cell

![Graph 1](image1.png)

% Cell membrane injury in susceptible CV. Lal Bahadur

![Graph 2](image2.png)

% Cell membrane injury in resistant CV. Raj. 3765.

Fig. 1. Cell membrane injury (%) by brown leaf rust (*Puccinia recondita*) in flag leaf stage of wheat at different nitrogen levels.
membrane injury at later stages of inoculation supplemented with N0, N30, and N90 levels, may be due to the genotypic resistant factors, imparting resistance to plants by way of providing strong membrane bound proteins in cell membranes. Plants do not naturally produce antibodies against their biochemical defenses are inactive until they are mobilized by some signal transmitted from an attacking pathogen. Induced resistance is at first localized around the point of plant necrosis caused by infection by the pathogen or by the chemical and it is then called local acquired resistance. Subsequently resistance spreads systemically and develops in distal untreated parts acts nonspecifically throughout the plants and is called systemic acquired resistance (SAR). This type of resistance acts nonspecifically through out the plants and reduces the severity of disease caused by all classes of pathogens including normally virulent ones. Wheelar and Black showed that susceptible Oat tissue treated with Victorin, lost electrolytes more rapidly than the control. Physical or chemical changes induced by the presence of pathogen could be of basic plant pathological significance.

In general, integrity of plasma membrane is a very important factor for host survival. Irreversible membrane damage is a crucial point in cell metabolism and is directly associated with hypersensitive response.

Above findings indicate that changes in membrane permeability occur early in the disease process and therefore, could affect the development of the pathogen at this crucial stage of pathogenesis. Impairment of membrane permeability would greatly influence the normal physiological functioning of the host cells. Our findings further agree with the fact that the permeability properties of cell membranes are fundamental to normal functioning of the cell.

References