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STUDIES ON SEED TREATMENTS AND HISTOCHEMICAL CHARACTERS OF WATER BARRIER IN SEED COAT OF *LEUCAENA GLAUCA* (L.) BENTH.

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Effect of mechanical and chemical scarification have been investigated by various treatments on the seeds of *Leucaena glauca* (L.) Benth. Scarification by mechanical abrasion on the sand paper was the only way in breaking seed coat-imposed dormancy. A strong barrier to water entry seemed to be located in the upper most part of the palisade cells. Histochemical investigations on the seed coat proved the presence of pectin as a water barrier.

Keywords: Dormancy; *Leucaena glauca*; Palisade cells; Scarification; Water barrier.

Introduction

Leucaena glauca (L.) Benth. though is a useful leguminous tree species in afforestation, soil conservation and supply of fuel and fodder etc., but its natural regeneration by seed takes a long period of 4 to 5 months. In laboratory for its qualitative evaluation by seed germination it creates a problem to the researchers and tree breeders due to its high seed-coat dormancy. This is due to hardness or impermeability of seed coat and is called asseed coat-imposed dormancy. The testa is generally responsible for this type of dormancy. Inhibitors of different chemical classes have been found for impermeability of seed coat to water such as fats, tannin, wax, lignin, subrin, callose, cutin, pectin and phenolics[1-7]. These water barriers usually thought to be present in the palisade layer of seed coat[7]. The present investigation on *Leucaena glauca* seeds, deals with the seed treatments against the water barriers present in the seed coat and their histochemistry.

Material and Methods

Seeds of *Leucaena glauca* were randomly collected from the trees growing in three different soil of Lucknow i.e. (i) Garden soil, from garden campus of National Botanical Research Institute (ii) Road soil from road side area of Chinhut (iii) Clay soil from Banthara Research Station and then mixed together. A fixed number of 50 seeds was sown in the earthen pot filled with field and road soil (3:1) and kept under open field condition for natural germination. For petridish culture random samples of seeds were taken for each seed lot and treated with following physio-chemical treatments before sowing:-

1. *Pre-chilling;* Seeds were kept in between two pieces of moist filter paper for 4 week at 3° C for chilling and transferred into petridishes for germination at laboratory condition (25±2° C).
2. *Hot water treatment*: Seeds were soaked in water at 60° C±1° C for 10, 20, 30, 40, 50 & 60 minutes.

3. *Absolute alcohol treatment*: Seeds were soaked in absolute alcohol for 24 and 48 hours at laboratory temperature.
4. *Concentrated sulphuric acid treatment*: Seeds were dipped in concentrated 95% sulphuric acid for 20, 30, 40, 60 & 80 seconds and then washed thoroughly before sowing.
5. *Scarification by sand paper*: Seeds were rubbed against the sand paper for 15 to 20 second.

After the above treatments 50 seeds of each treatment were sown in seperate sterilized glass petridishes on moist filter paper lined with a layer of wet cotton bed at laboratory condition. The experiments were replicated twice for each culture.

To locate the presence of water barrier whole seeds were soaked in an aqueous solution (0.5%) of crystal violet[8] for five days and then seeds were sectioned by microtome at 12µm.

For macerations, small pieces were treated with 2% HCl at 60° C for over night. Gentle pressure on the macerated pieces released pallisade cells. Macerated pieces were treated with periodic acid Schiff's reagent (PAS) for total insoluble polysaccharides, Sudan Black B for lipids, Phloroglucinol-HCl for ligin and ruthenium red for pectin[9,10].

Observations

Germination tests revealed that pre-chilling and absolute alcohol treatment had no effect on the seeds, very little effect was observed in seeds soaked in water for 48 hours at 60° C±1° C which was insignificant for germination studies. Concentrated sulphuric acid treatment made cracks on the seed surface which caused severe damage (30 out of 50) to the embryo.

Scarification of seed by mechanical abrasion against sand paper was found to be the most effective treatment in breaking the seed coat imposed dormancy of *Leucaena glauca*. Subsequent immersion of scarified seeds in water caused immediate imbibition and successive90% germination. Seedling survival was recorded 100%. On the basis of seed coat analysis it was found that seed coat is internally lined with a thick layer of mucilage which helps in germination. When the seed is scarified, water enters through the wound and causes mucilage capable of swelling which exerts a pressure on the outer part of the seed coat and to start the process of germination.

Seeds collected from the trees growing in three different type of soil showed similar response towards each physio-chemical treatment.

Untreated seeds sown in earthen pots showed 20% germination after 5 months of sowing which was much lower than the scarified against sand paper. Gradual microbial decomposition of seed coat was the factor in breaking dormancy under natural conditions. After sactioning of stained seeds (with 0.5% crystal violet aqueous solution) it was seen that the stain stopped on the superficial part of the seed coat. It confirmed the presence of water barrier on the superficial part of the palisade cells.

During histochemical investigations, treatment with periodic acid-Schiff's reagent (PAS) confirmed the presence of insoluble polysaccharides. During further

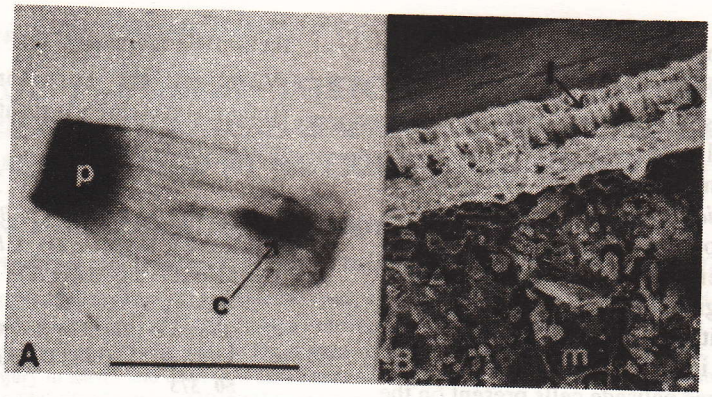

Fig.1 *Leucaena glauca* (L) Benth. **A.** macerated palisade cell showing dark stained band of pectin. Bar = 100 µm. **B.** SEM photograph of seed coat (x 200). c = cell lumina, p = band of pecting, I = light line, m = mucilage lining.

analysis of polysaccharides upperpart of the palisade cells showed affinity with ruthenium red only, by exhibiting a dark red colour band, which confirmed the presence of pectin (Fig. 1). Treatment with hydroxylamine-Ferric chloride solution[11] also confirmed the presence of pectin.

SEM photograph showed prominent seed coat structure (Fig. IB). A florescent continuous line (light line) paralled to the surface of palisade cells was observed. Pallisade cells showed conspicuous thickening and a lumen in the middle of the radial portion (Fig. 1A & B).

Discussion

All the seeds of *Leucaena glauca* do not imbibe water and germinate due to seed coat impermeability to water. These seeds when kept in water for over six or seven months they do not swell. Neither immersion for a limited time in boiling water, in lipid solvent like acetone or ethanol nor in conc.H_2 SO_4 permeabilized the seeds. In this study only mechanical abrasion of the seeds against the sand paper for a few seconds was more effective in breaking the seed coat-imposed dormancy. Although sulphuric acid has also broken the seed coat, but a pronounced injury to the embryo inhibited the germination.

Water impermeabillity, in Leguminosae generally occurs due to the palisade layer. The different reasons of its water imperviousness were described by many workers in different plants. Martin[12] for *Melilotus alba* and Cavanagh[13] for *Acacia*. Serrato-Valenti *et al.*[7] for *Prosopis juliflora* suggested that some physical or chemical nature of light line is responsible for seed coat dormancy. Corner[14] mentioned that water imperviousness takes place due to the contraction of the walls of palisade cells during maturation of seed. Some water proofing substances on the testa such as lignin, tannin, suberin, wax, pectins, lipids and phenolic compounds also prevent water entry[4,15] . For *Leucaena glauca* our observations also suggest that barrier to water entry in the seed lies on the

superficial part of the palisade cells, which seemed to be pectin.

In some cases size and thickening of the pallisade cells affects water imperviousness as reported by Egley and Paul[16] for *Sida spionsa*. They demonstrated two types of palisade-cells. The palisade cells present on the water permeable chalazal region were normally tall, lightly lignified with large cell lumina. Light line was present in the distal one third of palisade cells, whereas palisade cells present on the impermeable region were short heavily lignified, tightly packed, light line was present near the top of these cells. However, in *L. glauca* it was observed thatall the palisade cells were of an equal size (not taller) more in thickening, heavily lignified and tightly packed with small cell lumina (Fig. 1A). Light line was visible on the distal one third of the palisade cells (Fig. 1B).

Finally it was concluded that a greater amount of pectin in the outer part of the palisade cells was responsible for water impermeability in *Leucaena glauca* seed. Presence of light line and thickening of the palisade cells in the seeds could be another factor for water impermeability.

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References

1. Reeve R M 1946a *Am. J. Bot.* **33** 191
2. Reeve R M 1946b, *Am. J. Bot.* **33** 806
3. Marbach I and Mayer A M 1974, *Plant Physiol* **54** 817
4. Rolston P 1978, *Bot. Rev.* **44** 365
5. Slattery H D, Atwell B J, Kuo J 1982, *Ann. Bot.* **50** 373
6. Rangaswamy N S, nandkumar L 1985, *Bot. Gaz.* **146** (4)
7. Serrato-valenti G, Modenesi P, Rot-Michelozzi, G, Bevilacqua L 1986, *Acta. Bot. Nerrl.* **35** (4)
8. Jannerette C A 1979, *Seed Sci. & Technol* **7** 347
9. Jensen W A 1962, *Botanical Histochemistry* Freeman & Co., San Francisco, 408.
10. Heslop-Harrison Y, Heslop-Harrison J 1981, *Ann. Bot.* **47** 293
11. Reeve R M 1959, *Stain Tech.* B **34** 209
12. Martin J N 1922, *Proc. Iowa. Acad. Sci.* **29** 345
13. Cavanagh A K 1980, *Proc. Roy. Soc. Victoria* **91** 161
14. Corner E J H 1951, *Phytomorphology* **1** 117
15. Ewerker I, Marbach and mayer A M 1979, *Ann. Bot.* **43** 765
16. Egley G H, Paul N, Lax A R 1986, *Physiologia Plantarum* **67** 320