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Drifts in Protein and RNA as Influenced by Rifampicin during Seed Germination in *Pinus kesiya* L. Royle. Ex-Gord.

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ABSTRACT

Effect of Rifampicin — a metabolic inhibitor on the contents of total soluble proteins, and RNA during imbibition, subsequent seed germination and seedling emergence has been studied in embryonal and extra-embryonal parts of *Pinus kesiya*.

Key words : *Pinus kesiya*, Rifampicin, quantitative changes in proteins and RNA.

INTRODUCTION

Seeds of *Pinus kesiya* — a tree endemic to North-Eastern Indian hills and Burma hills (altitude 1400 to 2000 m) along 91^0-93^0E longitude and $24.^05'$-$25^0.5'$ N latitude are small, brownish in colour with membranous wings. Each seed comprises an embryo enclosed in endosperm (megagametophyte) and the covering armour of integuments.

The studies available in literature on the germination of seeds and the growth of the seedling of gymnospermous trees are very scarce. Whatsoever is available is either of the metabolic drifts in the seed system as a whole or otherwise from different parts of the seeds through *in vitro* studies.

With a view to understanding the changes in contents of macromolecular components as such, as well as under the influence of rifampicin under natural *(in vivo)* conditions of growth and developmental process of seed germination and seedling establishment, we conducted the present studies on intact seeds in different parts of seeds under different stages of germination.

In the present investigation, we have presented our work on the effect of rifampicin on the contents of total soluble proteins and RNA in different seed parts (viz., embryonal and extra-embryonal parts) and under different stages (viz., 0 hr, 24 hr after start of treatment i.e. imbibition stage, radicle emergence and plumule emergence) of *P. kesiya* seed germination.

MATERIAL AND METHODS

The seeds of *P. kesiya* collected from a single tree from North-Eastern Himalaya after subjecting to cleaning were used for following experiments.

GERMINATION TRIAL

Fifty uniform, healthy, viable (tested by Tetrazolium Choride Method) seeds selected for each treatment. After soaking the seeds in 30 ml. of water or rifampicin (for control or treatment) for 24 hr. they were subjected to germination trial on Whatman filter paper underlined with a thin cotton wad and moistened

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with respective liquid under controlled conditions of temperature (30±2.0°c) and humidity (92%±1%) in a seed germinator. With the aid of hand lens the protrusion of radicle, (taken as an index of the start of germination), was noticed at 12hr. interval till the completion of the experiment.

PREPARATION OF SAMPLES

The seeds were separated microsurgically into embryonal and extra-embryonal parts under aseptic conditions during different stages of germination. The samples were ground in acetone, freed of pigments, water and phenols and finally air dried. Contents of total soluble proteins and RNA were estimated by the methods of Lowry *et al.* (1951), and Mezbaum (1939), respectively.

RESULTS

In water treated control the content of soluble protein with the advancement of germination whereas decreased in embryonal axis, increased in the extra-embryonal part. The trend of changes in total soluble protein content in embryonal part after imbibition stage, in treated seeds was almost the same to that of control. Thus, after imbibition, the content gradually decreased with advancement of germination, thereby showing minimum comparative amount at the plumule initiation stage. However, in treated samples the content during imbibition increased. (Fig. 1a)

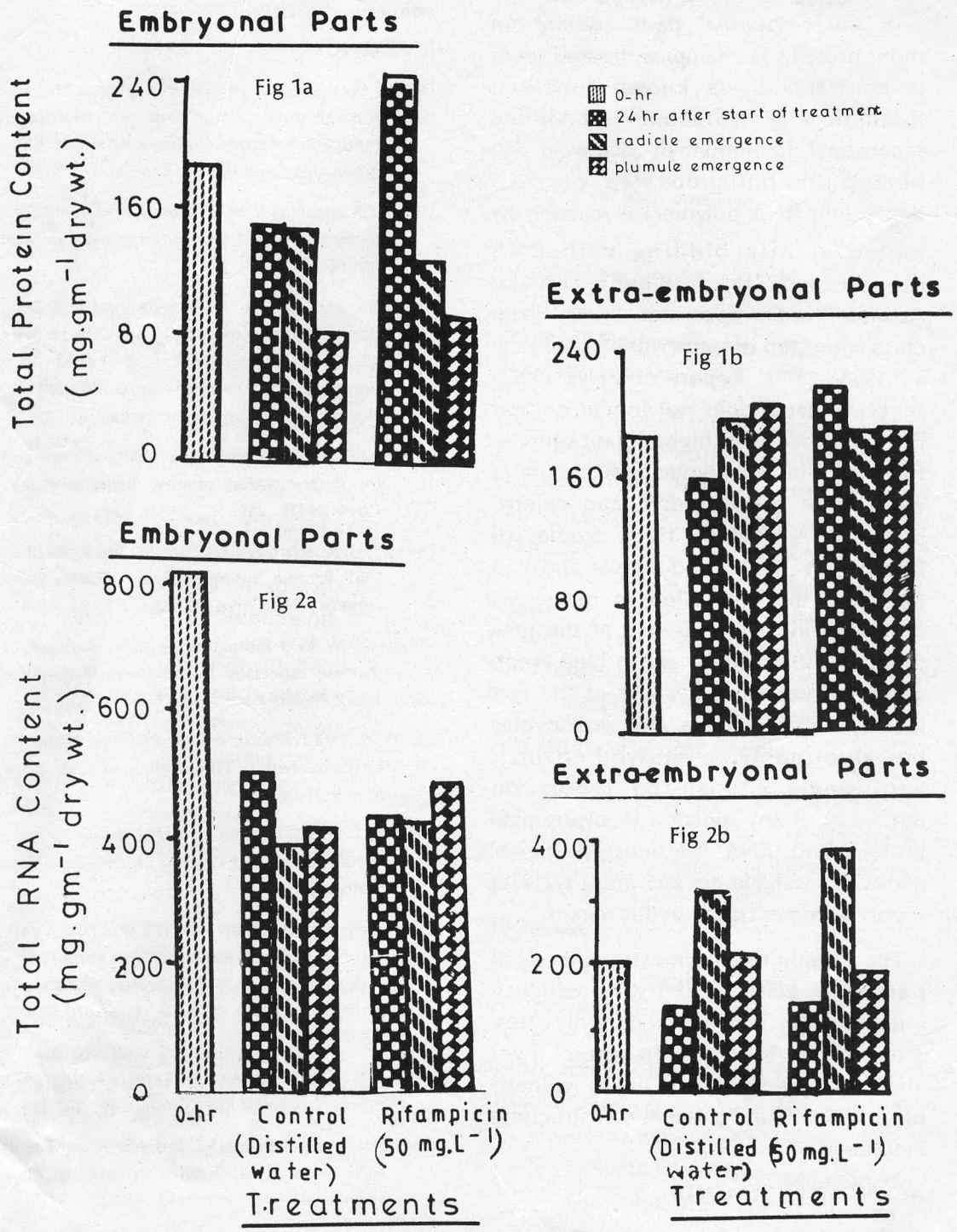
In extra-embryonal part minimum content was noticed at imbibition stage in control, whereas maximum at this stage in sample treated with rifampicin (Fig. 1b)

The embryonal part was found to be about four times rich in RNA content compared to extra-embryonal part. During active germination especially radicle emergence, the content of RNA whereas decreased in embryo, it increased in extra-embryonal part. The content in the embryonal parts during germination stages in treated samples was low compared to ungerminated, presoaked seeds. It decreased during imbibition and following radicle emergence stage, and increased during the plumule emergence in untreated as well as rifampicin treated seeds (Fig. 2a).

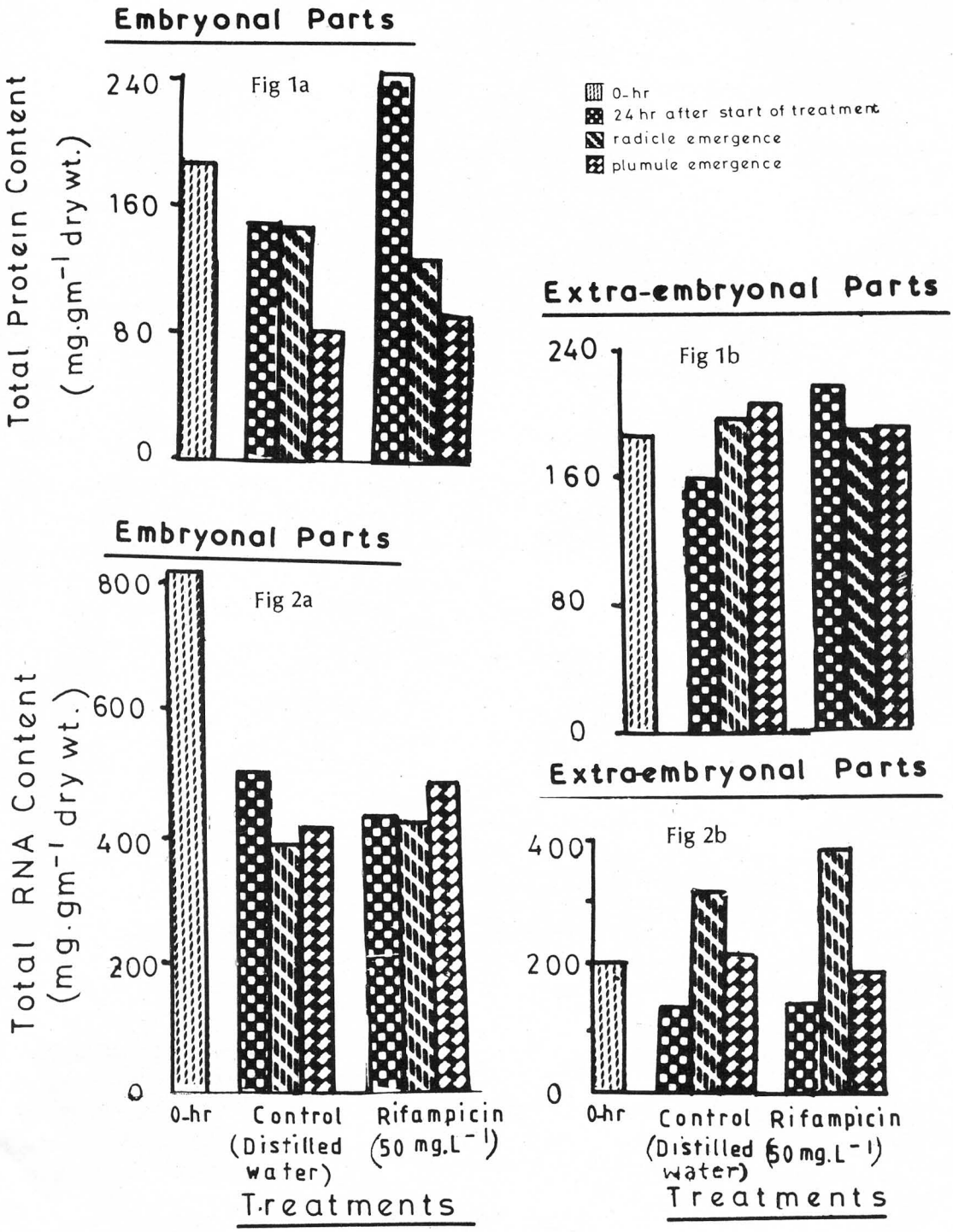
In the extra-embryonal zone, the contents of RNA did not change considerably during germination except that after a little fall during imbibition, it increased during radicle emergence stage (Fig. 2b).

DISCUSSION

The decrease in the RNA content in different parts during imbibition does not mean that no protein synthesis is involved during early stages of germination.It has been alleged by Walbot *et al.* (1974) and later by Spiegel and Marcus (1975) that newly synthesized RNA is not essential for protein synthesis. That means pre-formed mRNA were handy in the addition of new proteins to the system at the early stages. However, increase in the content during radicle emergence in the extra-embryonal parts when correlated with corresponding increase in protein content suggests that 'de novo' synthesized RNA during the most active phase of germination is responsible for new protein synthesis and



Effect of Rifampicin on total Protein and RNA content in Embryonal and Extra-Embroynal parts of germinating *Pinus Kesiya* L. seeds



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these proteins are not translocated to the embryonal parts for its elongation.

In the embryonal parts addition of more proteins in rifampicin treated seeds is contrary to its known function. Rifampicin (a hydrazone-3-(4-Methyl piperazinyl iminomethyl) rifamycin SV) blocks the initiation step of DNA dependent RNA polymerase reaction by noncovalently binding with RNA polymerase. It, however, unlike Actinomycin-D, does not affect chain elongation step of transcription (wehrli at al. 1968, 1971). Report of Polya (1973) suggests that it does not inhibit nuclear RNA polymerase in higher plant sources. However, in eukaryotic systems, it is effective in mitochondrial and chloroplastic RNA (Richter 1978). Studies of Dikshit et al. (1979) and Grover and Puri (1979) on the promotion of heterocyst differentiation which is one of the new protein synthesizing event in blue green algae, is interesting because of the fact that cyanobacteria are also prokaryotes but autotrophic (photosynthesizing). Surprisingly enough no report on inhibition of any function involving new protein and RNA synthesis in higher plants is available so far. Polya's (1973) report becomes handy in this regard.

The stored proteins in extra-embryonal part are also hydrolysed during imbibition in untreated control cases. Then little increase in their level afterwards points out 'de novo' synthesis of proteins during stages of structural increment.

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