

Influence of pre-treatment of some seeds on the rhizosphere microflora of the seedlings

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As early as in 1904, Duggeli reported that healthy seeds contain specific bacterial flora in respect of number and species. Since then, innumerable reports have appeared in the literature on the variations in the quality and quantity of microflora carried by the seeds of different plant species, both healthy and diseased (Chranowska 1965) Bloomberg 1966, Ordin 1966, Jackson and Press 1967). At present many plant diseases are known to be transmitted through internally and/or externally seed-borne infections which cause pre-emergence and/or post emergence rot, systemic infections of the seedlings spreading upto plant maturity, etc. To eradicate the pathogenic organisms carried by the seed both physical and chemical agents have been tried; hot-water treatment has been recommended to eliminate the internally borne infections of certain organisms; treatments with organic and inorganic substances to eliminate the pathogens on the surface of the seed have also been recommended; more recently certain antibiotic substances have been recommended for treatment against internally and externally borne organisms, particularly bacterial pathogens. These recommendations have come into wide use all over the world.

The role of the normal flora of micro-organisms on seed germination and subsequent plant growth has been investigated by a few workers. It has been shown that certain organisms cause beneficial effects to the seeds because of their certain biochemical characteristics. Some workers have also reported on the excretions from the seed coat at the time of germination, which greatly influence the quality and quantity of the microflora on the seed and in the immediate vicinity (Picci 1959 a, b, Flentje 1959, Vagnerova, Macura and Catska 1960, Pearson and Parkinson 1961 Schroth and Snyder 1961, Schroth, Toussoun, and Snyder 1963; Schroth and Cook 1964, Stanek and Lasik 1965, Parkinson 1965, Cook and Flentje 1967). Secretions from the seed of certain antimicrobial substances have also been reported by

some workers (Osborn 1943, Ivanovics and Horvath 1947, Osborn and Harper 1951, Frenczy 1956, Ark and Thompson 1958, Jacobs and Dadd 1959, Garber and Houston 1959, Thompson 1960, Bowen 1961).

For the past two decades, attempts have been made by several workers, particularly in U. S. S. R., to treat the seeds with some bacteria like *Azotobacter* and *Phosphobacterium*, to favourably influence the growth of the seedlings and very encouraging results have been obtained. However, the reasons for beneficial effects due to bacterization of the seed have not been investigated in detail.

Some workers have reported that the organic tissues, including the seed coats, when added to soil form an excellent medium for colonization by certain groups of soil micro-organisms. There are reasons to believe that certain micro-organisms utilize the nutrients present in the added organic tissues and produce anitibiotic substances which are inhibitory to other organisms in the vicinity, including the pathoganic ones (Wright 1954, 1955, 1956a, b, c, Grossbard 1948, 1952, Lockwood 1960, Krassilnikov, Kuchaeva and Skryabin 1959, Lingappa and Lockwood 1962, Lockwood and Lingappa 1963, Lloyd, Neverorke and Lockwood 1965).

The changes in the normal seed-microflora due to various chemical and physical seed treatments have been reported by some workers (Sowell 1965, Batra and Bajaj 1966, Maude 1966, Rao and Durgapal 1966).

It has been established through the studies of some workers that the rhizosphere effect is initiated in the seedlings within a few days after germination and the rhizosphere organisms in the initial stages are mostly related to the seed-microflora, thereby indicating that the seedborne organisms are carried to the rhizosphere (Borodulina 1941, Wallace and Lochhead 1951, Rovira 1956, Rouatt 1959). Patterson (1959) and Parkinson, Taylor and Pearson (1963), however, found that the seed-microflora did not materially alter the rhizosphere microflora. Bacterization of seed and its effect on rhizosphere microflora has also been studied by some workers (Jensen 1942, Federov and Tepper 1945, Maska and Fabian 1952, Cooper 1959). The results obtained by these authors, however, are inconclusive as to the added bacterium establishing in the rhizospheres.

In our laboratory we have been interested during the past five years to examine the possibility of altering the rhizosphere microflora through pre-treatment of the seeds. In the present paper certain principles involved and the interpretations of the results reported earlier are presented.

INFLUENCE OF PRE-TREATMENT OF SOME SEEDS ON THE RHIZOSPHERE
MICROFLORA OF THE SEEDLINGS

EXPERIMENTAL

Bacterization and plant growth: Rangaswami and Mahadeviah (1963) reported on the general increase in the rate of plant growth when rice seeds were pre-treated with *Azotobacter chroococcum*. Such an effect was significantly more when the pre-treated seed was sown in semi-dry soil as compared to water-logged soil. Neelakantan (1964) examined the effect of bacterilization with *A. chroococcum* on the germination, rate of growth and the wet and dry weights of rice, sorghum, bajra, ragi, setaria, bhendi, tomato and amaranthus. There was an increase of more than 10 per cent. germination of the pre-treated seeds than the respective controls of bajra, ragi and tomato, whereas in the other crops there were no large changes. The seedlings arising from the treated seeds grew more rapidly for about 8-10 days after sowing and they were tall, vigorous and darker green as compared to the respective controls. The increased growth rate of the treated plants over control was statistically significant at various stages in sorghum, and to a lesser extent in rice, bajra, ragi, setaria, and amaranthus, and not significant in bhendi and tomato. There were varying increases in dry weights of treated plants over the control, at the end of 60 days after sowing; the percentage increase in rice was 8.7, sorghum 34.7, bajra 6.7, ragi 17.0, setaria 10.6, bhendi 1.2, tomato 5.1 and amaranthus 6.3. Of these, the increase in the dry weights of rice, sorghum and ragi were significant. Similar results were obtained with some crop plants by Gopalakrishnamurthy, Mahadevan and Rangaswami (1967).

Seed coat exudates and seed and soil microflora: In another study Balasubramanian and Rangaswami (1967a) found the germination percentages of sorghum, sunnhemp and tomato seeds when sown in normal and steam-sterilized soils varied considerably. Also, the average shoot lengths and dry weights of the seedlings arising out of the seeds sown in the normal and steam-sterilized soils varied significantly, thereby indicating the role of soil micro-organisms in influencing germination of seed and subsequent growth of the seedlings. The same authors (1967b) reported on the presence of certain chemical inhibitors of fungal spores in the seed coats of sorghum, ragi and tomato. The seed coat leachates from the three varieties of seeds caused delay in germination of spores of *Helminthosporium oryzae* Breda de Haan, malformation and subsequent lysis of germ-tubes of the fungus and considerable inhibition of germination ranging upto 80 per cent. These studies indicated certain types of inter-relationships between the seed-microflora and the seed that might exist at the time of seed germination.

Seed treatment and rhizosphere microflora : In another series of experiments the possibility of seed-microflora entering the rhizosphere region of the plants was examined. Neelakantan (1964) found that when seeds of rice were treated with *A. chroococcum* at the rate of about 7,000 cells/g of seed and sown in sterile soil the bacterial population increased upto about 20 million/g rhizosphere sample in 50 days and declined thereafter and when sown in normal unsterile soil it increased to 12.3 million/g of rhizosphere sample in 50 days and declined later on. In the case of bhendi when about 16,000 cells/g of seed were added, the rhizosphere population increased to 7.5 million/g in 60 days in sterile soil and 10.8 million/g in 40 days in normal unsterile soil. Such treatments also materially altered the quantities of bacteria, actinomycetes and fungi in the rhizosphere. Gopalakrishnamurthy, Mahadevan and Rangaswami (1967) studied the changes in the rhizosphere microflora of rice plants at different stages of growth, when the studies were pre-treated with *A. chroococcum*, *Phosphobacterium* sp. and *Helminthosporium oryzae*, alone and in different combinations. When the seeds were treated with either. *A. chroococcum* or *Phosphobacterium* sp. the respective bacteria readily got established in the rhizosphere and multiplied in number upto 70-80 days, whereas the pathogen, *H. oryzae*, when added to the seed increased only slightly.

In a further attempt to alter the rhizosphere microflora through seed-treatments, cotton seeds were treated with concentrated sulphuric acid, Agrosan GN, and streptomycin sulphate and the rhizosphere microflora was studied both quantitatively and qualitatively (Prasad and Rangaswami 1967). When untreated cotton seeds were sown in sterile soil there was readily colonisation of the rhizosphere by the epiphytic microflora by about the fourth day ; when sown in unsterile soil the epiphytic flora continued to be predominant for some time and in about 15 days it declined, giving place to slow colonization of the region by the soil microflora. The quantity and quality of the bacteria, actinomycetes and fungi establishing in the rhizosphere of seedlings arising from the different pre-treated seeds varied significantly.

DISCUSSION

The works of Picci (1959a, b) and others brought to light the influence of seed on the seed-microflora and the soil microflora in the vicinity of germinating seed, which influence is termed 'spermosphere effect'. That the spermosphere effect would vary with plant

INFLUENCE OF PRE-TREATMENT OF SOME SEEDS ON THE RHIZOSPHERE
MICROFLORA OF THE SEEDLINGS

species, soil and various other environmental factors is to be recognized in analogy with "the rhizosphere effect". When the seeds of crop plants are treated in different ways, we are interfering with the normal relationships between seed, its microflora and the spermosphere effect. For nearly a century agriculturists have been advised to pre-treat the seeds with chemicals to get rid of the pathogens. Such treatments not only kill the pathogens but also most other organisms carried by the seed. That these treatments bring in changes in the quantity and quality of the micro-organisms establishing in the rhizosphere of certain plants at various stages of growth has been brought out through our studies.

Bacterization of seed to obtain better seed germination, plant growth and the resultant crop yield has become an approved practice in many parts of U. S. S. R. The results obtained in this country also substantiate these results (Sankaram 1963, Sundara Rao and Sinha 1963, Sundara Rao, Bajpal Sharma and Subbiah 1963, Rangaswami and Mahadeviah 1963, Neelakantan and Rangaswami 1965, 1967, Gopalakrishnamurthy, Mahadevan and Rangaswami (1967). Furthermore, our studies also indicate that by pre-treating the seeds with *A. chroococcum* and *Phosphobacterium* sp., the organisms are transported to the rhizosphere region, where they materially alter the quantity and quality of the microflora.

There are also many reports in the literature on the beneficial effects by way of better seed germination, rapid growth, robust stand and increased wet and dry weights of seedlings through pre-treating the seeds with certain chemicals, particularly organo mercurials (Cristensen and Stakman 1935, Jamalainen 1962, Clark 1963, Kukin 1963). There are also some reports on the adverse effect of such treatments on the seed germination (Dempsey and Chandler 1963, Timonin 1964). In the light of the more recent observations made by the various workers on the role of seed-microflora and spermosphere microflora on seed germination and subsequent growth of the seedling, re-examination of some of the results reported by earlier workers to understand the role of various groups of micro-organisms appears necessary.

SUMMARY

The various internally and externally borne seed microflora greatly influence the percentage germination and subsequent growth of the plant. The different physical and chemical as well as microbial treatments given to the seed not only alter the effect of the normal flora on the seed but also cause certain beneficial effects to the plant growth. The normal microflora of the seed is also carried to the

rhizosphere of young seedlings of certain plant species. The various physical and chemical pre-treatments given to the seed therefore alter the rhizosphere microflora, and this has been confirmed by the earlier works of the author and his associates. It is suggested that more work in this direction is required to examine the possibility of altering the rhizosphere microflora in desired lines through pre-treatment of the seeds with different chemicals and micro-organisms.

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INFLUENCE OF PRE-TREATMENT OF SOME SEEDS ON THE RHIZOSPHERE
MICROFLORA OF THE SEEDLINGS

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