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Comparison of Epiphyllous Microfungi on two Cultivars of Soybean

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The distribution of microfungi on two cultivars of *Glycine max*, viz. cv Ankur and cv Bragg were studied throughout the growing period of plants. Higher fungal population/cm^2 was found in cv Bragg as compared to that in cv Ankur. The total fungal population gradually increased with the age of the plant. Certain fungi were common to both cultivars whereas others were isolated either from one or the other cultivar. Some fungi appeared during seedling to 60-day-old stage of plant and others occurred when plants were 75–130 and 60–105-day-old. However, some fungi were isolated throughout the growing period of plant. Young leaves of both the cultivars harboured more fungi than old leaves.

Key Words: Epiphyllous, Epiphytic, Colonizers, Leachates, Distribution, Plant age, Senescence

Introduction

The aerial surfaces of higher plants growing under natural conditions are usually covered with varied populations of microorganisms. The quantitative and specific composition of epiphytic microflora depends on plant species, climatic conditions and the habitat. Some microbes succeed in colonizing a wide variety of plant species, whereas others are highly selective in their host range. A third group of microorganisms has no specificity to any plant, and seems to lie on plant surfaces by accident.

The nature of aboveground parts of plants is the most important in determining the types of epiphytic microbes (Last 1955, Kerling 1964, Sinha 1971, di Menna 1971, Leben 1971, Sharma & Mukerji 1974, Dickinson 1976).

The present study was carried on two cultivars of *Glycine max* viz. cv Ankur and cv Bragg to see whether the fungi which colonized the young leaves continued multiplying on the gradually expanding leaves or a new set of colonizers succeeded the previous ones in a definite sequence. The aim was to find out how far one group of colonizers was responsible to facilitate the growth of succeeding group.

Materials and Methods

The experiments were carried out in the Botanical garden of the Hindu College, Delhi. The seeds of *Glycine max* (L.) Merrill cvs Ankur and Bragg, were procured from the Genetics Division, IARI, New Delhi and were grown under natural conditions. The experimental beds were located away from the trees to avoid contamination from other sources. Two types of leaves viz. young and old (fully expanded), were chosen for the study. Leaf samples were collected at random between 9.00 to 10.00 am at fortnightly intervals throughout the growing period of the crop. First collection was made when the plants were one-month old. One sample comprised five leaflets from five plants of a particular cultivar.

Qualitative and quantitative studies were done using dilution plate technique (Sharma et al. 1974) and moist chamber technique (Keyworth 1951). The dilution plates and the moist chambers were incubated at room temperature in laboratory conditions. Different fungi were isolated and grown on Czapek's Dox Yeast agar for identification. Rosebengal and streptopenicillin were added to the medium as bactericides. Environmental characteristics viz., atmospheric temperature, relative humidity and *p*H of leaf leachates were recorded using standard instruments.

The quantitative estimation of fungi was done on the basis of number of colonies/cm^2 of leaves and on the frequency percentage basis using Tresner et al.'s (1954) formula:

$$\text{Percentage frequency} = \frac{\text{Number of samples of occurrence}}{\text{Total number of samples}} \times 100$$

Observations

The variations in atmospheric temperature, relative humidity and *p*H of leaf surface washings were recorded during July 1, 1978 to December 7, 1978 at fortnightly intervals (figure 1). The maximum temperature was recorded on September 7, 1978 and the minimum on November 7, 1978. Maximum relative humidity was 95% and minimum 60%. In the cv Bragg, the *p*H of leaf surface washings ranged between 6.30 and 6.75, and 6.52 and 7.05 in old and young leaves respectively. However, in the cv Ankur it ranged between 6.80 and 7.02, and 7.05 and 7.84 respectively.

The fungal population/cm^2 of leaves was higher in cv Bragg than in cv Ankur (figure 1). The maximum fungal population/cm^2 of leaf surface was isolated in the collection made on October 22, 1978 in cv Bragg while that on October 7, 1978 in cv Ankur. However, the lowest fungal population was recorded at seedling stage in both cultivars. In general, total fungal population on leaf surfaces gradually increased with the age of the plants, followed by a gradual decrease until the plants underwent senescence. The data indicated that young leaves of both cultivars were more densely populated than the old ones. *Fusarium moniliforme* showed highest population in cv Ankur while *Aspergillus nidulans* in cv Bragg. Other fungi in a decreasing order were *Aspergillus fumigatus*, *A. flavus* and *Fusarium oxysporum* in cv Ankur, whereas *Aspergillus flavus*, *A. fumigatus*, and *A. niger* in cv Bragg.

Qualitatively, more fungal species were associated with cv Bragg than with cv Ankur. While certain fungi were common to both the cultivars, [*Acremonium indicum*, *Acrophialophora nainiana*, *Alternaria* spp., *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, Black sterile mycelium, *Candida albicans*, *Chaetomium* spp., *Cladosporium* spp., *Colletotrichum* spp., *Curvularia* spp.,

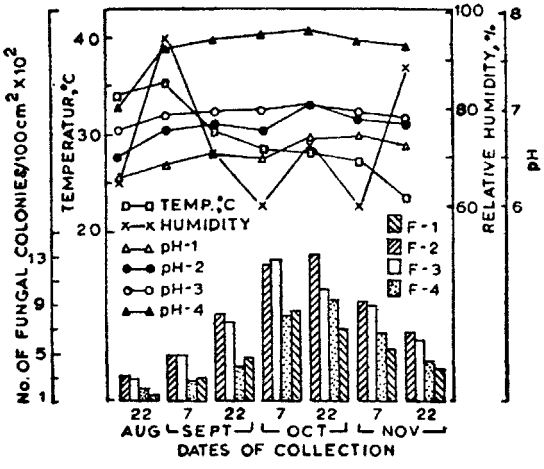

Figure 1 Epiphyllous mycoflora in relation to temperature, relative humidity and *p*H of leaf surface washings. *F-1*, Mycoflora from old leaves of *G. max* cv Ankur; *F-2*, Mycoflora from young leaves of *G. max* cv Bragg; *F-3*, Mycoflora from young leaves of *G. max* cv Ankur; *F-4*, Mycoflora from old leaves of *G. max* cv Bragg
*p*H-*1*, *p*H of surface washings of old leaves from *G. max* cv Bragg; *p*H-2, *p*H of surface washings of young leaves from *G. max* cv Bragg; *p*H-*3*, *p*H of surface washings of old leaves from *G. max* cv Ankur; *p*H-*4*, *p*H of surface washings of young leaves from *G. max* cv Ankur

Drechslera spp., *Fusarium moniliforme*, *F. oxysporum*, *Fusidium* spp., *Humicola* spp., *Memnoniella* spp., *Mycogone* spp., Myxomycetes, *Neurospora* spp. (*Monilia* stage), *Penicillium* spp., *Phoma* spp., *Rhizoctonia solani*, *Rhizopus* spp., *Stachybotrys* spp., *Starkeyomyces* spp., *Thielavia* spp., *Trichoderma* spp., *Trichothecium* spp., *Volutella* spp.,and White sterile mycelium]. The others occurred either in cv Bragg [*Choanephora* spp., *Coprinus* spp., *Nigrospora* spp., and *Sordaria* spp.] or in cv Ankur [*Cephalosporium* spp., *Melanospora* spp., *Paecilomyces* spp., and *Sporotrichum* spp.]

It was interesting that the sterile mycelial forms sporulated highly on cv Bragg than on cv Ankur. Some fungi were recorded only in the dilution plate studies,—*Aspergillus* spp., *Nigrospora* spp., *Paecilomyces* spp., and *Penicillium* spp., while others only in the moist chambers—*Colletotrichum* spp., *Coprinus* spp., *Melanospora* spp., *Physarum* spp., and *Sordaria* spp. *Nigrospora* and *Sporotrichum* species occurred from seedling to 60-day-old stage of plants. However, *Chaetomium* spp., *Cephalosporium* spp., *Phoma* spp., Myxomycetes, *Trichothecium* spp., and *Volutella* spp. occurred on 75–30-day-old plants. Some fungi occurred when the plants were 60–105 days old—*Acremonium indicum*, *Coprinus* spp., and *Sordaria* spp. A large number of fungi were isolated throughout the growing period of the plants. The important ones are: *Alternaria*, *Aspergillus*, Black sterile mycelium, *Cladosporium*, *Curvularia*, *Drechslera*, *Fusarium*, *Humicola*, *Rhizoctonia*, *Rhizopus*, *Neurospora*, *Penicillium*, *Stachybotrys*, *Starkeyomyces*, *Thielavia*, *Trichoderma*, and white sterile mycelium. Interestingly enough the total number of *Humicola* spp. colonies/cm² on both cultivars exhibited a gradual increase starting from seedling stage until the initiation of flowering and fruiting on the plants. Subsequently, its populations gradually decreased till the plants underwent senescence, following which the fungi once again exhibited a tendency to increase until death of the plants.

Discussion

The differences in the epiphytic microflora of the two cultivars observed by us, might be due to variations in the morphology of leaf; nature and quantity of leaf leachates; and climatic factors such as atmospheric temperature, relative humidity and rain.

Crosse (1963) observed that the population of *Pseudomonas morsprunorum*, a bacterium, was higher in the Napolian variety

than in Roundel variety of cherry. Sinha (1971) too found variance in the distribution of microorganisms in four solanceous species. Our findings on the increase in population of microfungi depending on the age of plants are similar to those obtained by Last (1955) on wheat, Kerling (1958) on *Beta vulgaris*, Sharma (1974) on *Sesamum orientale* and *Gossypium hirsutum*, and Kumar and Gupta (1976) on three varieties of potato. Kerling (1964), however, suggested that the metabolites released by host gradually increased with the age of leaves, which might have led to an increase of microbial population density. Ruinen (1970) too reported that the phylloplane mycoflora increased during leaf maturation and subsequently decreased during senescence.

The higher fungal population in young leaves as compared to the old ones in both the cultivars, can be surmised due to leaching of more substances (favourable for fungal growth) in the former than in the latter. Sharma and Mukerji (1974) reported that the population of *Candida albicans* was higher on young leaves of *Sesamum orientale*. Contrary to this, are various reports of lower fungal populations from young leaves and higher fungal populations on mature plant organs after senescence (Last 1955, Kerling 1958, Sinha 1971, Sharma 1974, Kumar & Gupta 1976).

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