NODULATION OF SOYBEAN AS INFLUENCED BY THE LIQUID ENDOSPERM OF COCONUT

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Introduction

The liquid endosperm of coconut (coconut water) is known to contain sugars and growth-promoting substances (Ramakrishnan *et. al.*, 1957; Tulecke *et. al.*, 1961). Recently, it has been demonstrated that the liquid endosperm has a thermostable principle which is active in stimulating the formation of nodules on excised roots of bean inoculated with *Rhizobium phaseoli* (Schaffer and Alexander, 1966, 1967 *a* and 1967*b*). However, it has not been shown whether stimulation of nodulation could also occur *in situ* in soil when coconut water is applied to seed along with the inoculum. It is relevant to mention that sugars in the growth medium has also been shown to increase nodulation in legumes (van Schreven, 1956).

An experiment was designed to find out if coconut water and sucrose had any effect on nodulation of soybean in unsterilised soil. In as much as fungi associated with nodules and other fungi in soil as well have been shown to inhibit or stimulate the growth of *Rhizobium* spp. besides solubilising tricalcium phosphate (Subba-Rao and Vasantha, 1965; Subba-Rao and Bajpai, 1965; Chhonkar and Subba-Rao, 1966, 1967; Sethi and Subba-Rao, 1968 *a*, 1968 *b*) the fungal flora of nodules of soybean in different treatments were also analysed to determine whether the addition of coconut water or sucrose had in any way influenced the composition of fungi occurring on nodules.

Materials and Methods

Delhi soil (pH 7·6) sieved through 2 mm sieve was used in the pot culture experiment. Soybean seed (var. Bragg) was inoculated by pelleting the seed with Indian peat based culture of (Iswaran *et al.*, 1969) *Rhizobium japonicum* SB 16. The treatments were: (1) coconut water, (2) 10% sucrose solution pelleted on seeds according to the method of Hastings (1962) and the series excluding both the coconut water and sugar serving as controls.

Nodular fungi were isolated from nodules collected at random from plants. The nodules were washed with sterile water using the technique of Harley and Waid (1955). After 20 washings under aseptic conditions, the nodules were plated on Martin's Rose Bengal agar medium incorporated with streptomycin (Martin, 1950). After 96 hour incubation, colonies of fungi appearing on the nodules were isolated and identified.

Dialysed culture filtrates of the fungi grown on Richard's synthetic medium were tested for inhibitory/stimulatory activity towards *Rhizobium japonicum* by the standard filter-paper disc method.

The phosphate solubilising ability of the fungal isolates was evaluated by growing the cultures on Richard's synthetic medium as modified by Chhonkar and Subba-Rao (1967).

Results and Discussion

Coconut water had a beneficial influence on the number and volume of nodules. The yields after 8 weeks in terms of dry weight were not different between the sugar and coconut water treated plants (Table I).

Table I

Effect of coconut water and sucrose on soybean (var. Bragg) inoculated with R. japonicum *at the end of the* 8 *weeks (Average of triplicates)*

Readings	Un-inoculated	Inoculated with peat based *R. japonicum* containing 10% sucrose solution	Inoculated with peat based *R. japonicum* in coconut water
No. of nodules	29	125	165
Mean vol./nodule	0·0094	0·0107	0·0122
Nodule status*	+	++	+++
Dry wt./plant (g)	5·3	7·6	7·8

* + Average; ++ Good; +++ Very good

It was interesting to observe that all the nodules from coconut water-treated plants yielded only *Rhizopus arrhizus* while more than one species of fungi were isolated from the nodules of remaining treatments (Table II).

TABLE II

Incidence of nodular fungi (from 24 nodules for each treatment)

Treatment	No. of nodules yielding fungi				
	Rhizopus arrhizus	*Aspergillus niger*	*Fusarium merismoides*	*Rhizopus* sp.	*Gliocladium fimbriatum*
Uninoculated	5	..	..	6	1
Inoculated with peat based culture containing 10% sucrose	..	9	4	..	..
Inoculated with peat based culture in neat coconut water	24	..	..	..	..

The culture filtrate of *R. arrhizus* accelerated the growth of *R. japonicum in vitro*. In keeping with this observation, it was also found that *R. arrhizus* could not grow on Richard's synthetic medium, but could do so only in coconut water or 0·25% peptone amended Richard's medium. The remaining species of fungi did not inhibit or stimulate the growth of *R. japonicum* but had the ability to solubilise tricalcium phosphate *in vitro*. The results obtained in this study confirm the stimulatory effects of coconut water on nodulation.

The fungal flora of washed nodules is an index of the nature of fungi colonizing the rhizoplane of legume roots. The shift in the qualitative nature of fungal colonization of the cortical cells of the nodules in the case of coconut water-treated plants by *Rhizopus arrhizus* is indicative of the role of coconut-water in altering the pattern of fungi on the rhizoplane. The significance of *R. arrhizus* may probably lie in its ability to augment the inoculum potential of *Rhizobium* in the rhizosphere, a factor conducive for the establishment and survival of *Rhizobium japonicum*.

Summary and Conclusions

The liquid endosperm of coconut showed a stimulatory effect on the number and volume of nodules of soybean (var. Bragg) inoculated with peat based culture of *Rhizobium japonicum* S.B. 16. Coconut water stimulated the growths of *Rhizopus arrhizus* on nodules which, in its turn, was shown to increase the growth of *R. japonicum*.

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