method followed by Sundara Rao and Sinha[2].

Only shaking brought into solution about 7.5 μg P per 50 ml broth in control with Jhabua rockphosphate (without microorganisms). Phosphorus of all the rockphosphates included in the study was vulnerable to microbial solubilization (table 1). Gaur *et al*[1] also reported that due to the production of organic acids, these microbes can solubilize the insoluble phosphates. Solubilization was maximum in Jordan rockphosphate which could be attributed to its greater reactivity[5] and better support to the growth of inoculated rockphosphate bacteria found in the study.

Among all the P-solubilizers studied, *Aspergillus awamorii* was the most efficient solubilizer and gave 180 μg P per 50 ml broth while *P. striata* was the least efficient giving only 28 μg P per 50 ml broth. Sundara Rao and Sinha[2] and Gaur *et al*[1] also found that P-solubilizing capacity of fungus is more than bacteria in broth under laboratory conditions. Interestingly, a combination of $S_1 + S_2$ solubilized more P than individually but when these bacteria were mixed with fungus, phosphorus released was always less than that solubilized by the fungus alone, though it was more than the capacity of the bacteria alone. Bacterial culture, when grown with fungus, reduced its P solubilizing capacity probably by producing antifungal substances (figure 1). Kundu and Gaur[6] also reported that *Pseudomonas striata* when grown with *Aspergillus awamori* or *Bacillus polymyxa* inhibited their growth and this effect was more pronounced on *A. awamori*. Critical examination of the results revealed no correlation between the degree of solubilization and change in pH. Results reported elsewhere[7–10] also corroborate the above findings. However, results showing increased solubilization with increased acidity have also been reported in the literature[2,11].

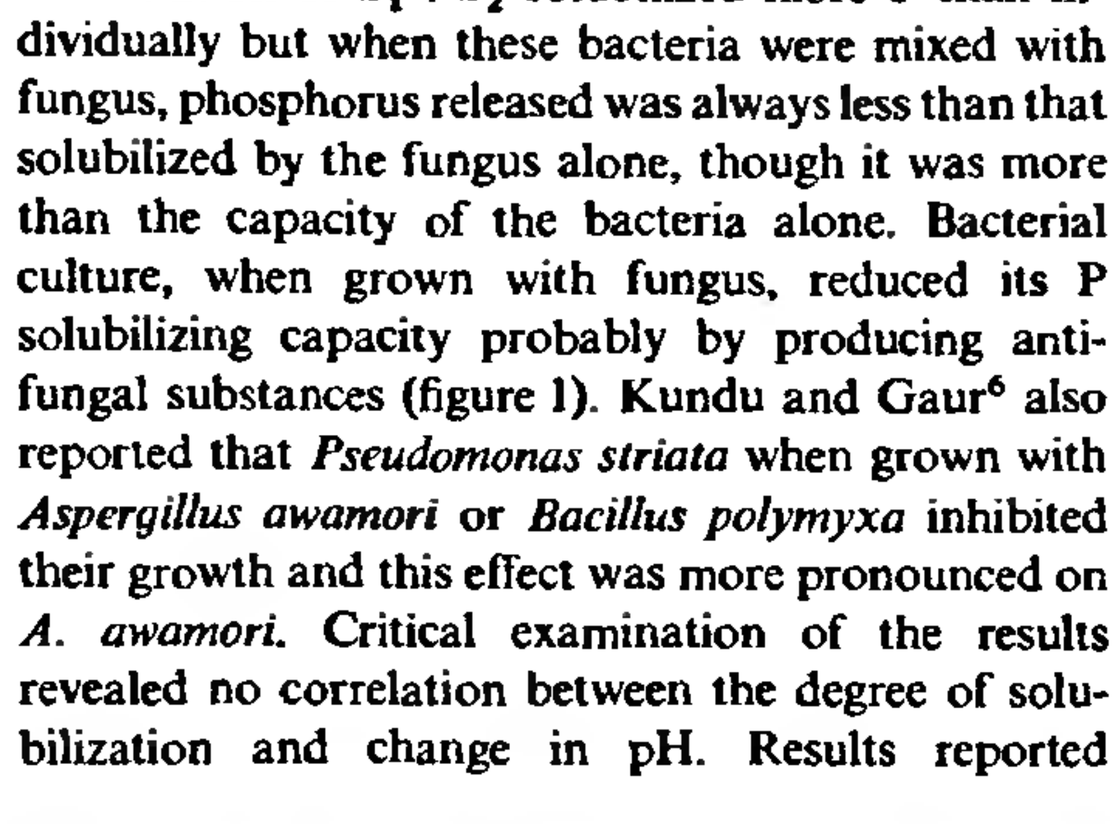

Figure 1. Inhibitory action of *Bacillus polymyxa* (S_2) on *Aspergillus awamori* (S_3)

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CYTOLOGICAL STUDIES ON DIPLOID, AUTOTETRAPLOID AND AUTOTRIPLOID *SOLANUM SISYMBRIFOLIUM*

P. B. KIRTI*, K. V. MOORTY**
and B. G. S. RAO
Department of Botany, Andhra University, Waltair 530003, India.
*Present Address * A. I. C. S. I. P-I. A. R. I-R. S, Rajendranagar, Hyderabad 500030, India.*
*** C. S. R. Sarma College, Ongole (A.P), India.*

CYTOLOGICAL studies on induced autopolyploids form an important aspect of chromosome behaviour and such studies are rare in spinous solanums, which are of importance in medicines as well as vegetables[1]. With a view to obtaining such information autotetraploids were produced in some of the species, and our observations on diploid ($2n = 24$), autotriploid ($2n = 36$) and autotetraploid ($2n = 48$) *Solanum sisymbrifolium* Lam. are reported here. The triploid

was obtained by crossing the autotetraploid as female with the diploid.

Autotetraploidy was induced by treating actively growing stem tips with 0.2% aqueous colchicine solution. Propionic carmine schedule was used in cytological analyses.

Chromosome association in the diploid was ^{12}II with ring bivalents predominating. At the triploid level, chromosome associations ranged from 11_{III}· 1_{II}·1_I to 3_{III}·9_{II}· 9_I. At the tetraploid level, the variation in chromosome associations was much higher ranging from 8_{IV}· 8_{II} to 1_{III}· 22_{II}· 1_I per nucleus. Kinds of chromosome association and their frequencies in the triploid and the tetraploid are given in tables 1 and 2. The pattern of distribution of quadrivalents and bivalents in the tetraploid fitted well with the principle of binomial distribution of Hall[2] and the chi-square value (6.24, table value 19.68) is not significant indicating that the distribution of chiasmata in all the sets of chromosomes was random and there was no preferential pairing in any set of chromosomes in the tetraploid.

The tetraploid had a mean chiasma frequency significantly lower than the doubled diploid value (38.00/nu, diploid value 21.12/nu, doubled value 42.24, t value 17.75** for 149 degrees of freedom). The autotriploid had shown a marginal increase in chiasma frequency over the diploid (24.56 as against 21.12/nu in the diploid). In the triploid, low chiasma frequency reflected in the predominance of low chiasmate trivalents (chain and 'Y' types, table 3) even though their mean frequency per nucleus was high (7.30). The half chiasma value for quadrivalents in the tetraploid (1.86) was comparable with the half chiasma value for the bivalents in the diploid (1.76) indicating that the effect of reduced chiasma frequency is not much reflected in the types of quadrivalent configurations. However, the half chiasma values for trivalents (1.61) and bivalents (1.55) in the tetraploid were less than the value for bivalents in the diploid (1.76) thereby suggesting that the lower chiasma frequency resulted in high incidence of low chiasmate trivalents and rod bivalents probably by breaking the association of four into a trivalent and a univalent, or two bivalents, generally rods.

In the autotetraploid, quadrivalent configurations 11 and 17 were frequent. Types 12, 13, 15, 16 and 18 also occurred, but to a lesser extent[3] (table 3). Types 14, 19 and 20 were absent altogether, because they require

Table 1 *Frequencies of various chromosome associations in autotetraploid*

Chromosome Assocations				Diakinesis	
IV	III	II	I	No. of cells	% Frequency
8		8		1	1.96
7	1	8	1	1	1.96
6	3	6	3	1	1.96
6	2	8	2	2	3.92
5	4	6	4	2	3.92
5	3	8	3	2	3.92
5	2	10	2	3	5.90
5	1	12	1	3	5.90
4	4	8	4	1	1.96
4	3	10	3	1	1.96
4	2	12	2	4	7.80
4	1	14	1	3	5.90
4		16		1	1.96
3	4	10	4	1	1.96
3	3	12	3	6	11.80
3	2	14	2	5	9 80
3	1	16	1	1	1.96
2	4	12	4	3	5.90
2	3	14	3	4	7.80
2	2	16	2	2	3.92
2	1	18	1	1	1.96
2		20		2	3.92
	1	22	1	1	1.96
				Total 51	

Table 2 *Frequencies of the various chromosome associations in the autotriploid*

Chromosome associations				
III	II	I	No. of cells	% Frequency
11	1	1	3	6
10	2	2	3	6
9	3	3	8	16
8	4	4	9	18
7	5	5	10	20
6	6	6	9	18
5	7	7	3	6
4	8	8	3	6
3	9	9	2	4
			50	

Table 3 *Types and frequencies of higher chromosome configurations observed at diakinesis in autotriploid and autotetraploid*

	Trivalent types*			Quadrivalent types*						
	7	8	9	11	12	13	15	16	17	18
Autotriploid	166	73	125	—	—	—	—	—	—	—
Autotetraploid	36	29	47	57	7	2	5	18	82	15

*cf. Darlington[3]

at least two partner exchanges, one on each side of the centromere with chiasmata at appropriate places. These types would be rare in organisms having chromosomes with submedian centromeres.

Jackson and Casey[4] postulated that 2/3rds of chromosomes should be associated as quadrivalents and 1/3rd as bivalents in autotetraploids. Timmis and Rees[5] calculated the same as 50% each in bivalents and quadrivalents and explained the higher than expected frequency of bivalents on the basis of positioning of chromosomes in pairs prior to pachytene. Avivi[6,7] conceived of the existence of low pairing genes in *Triticum longissimum* to explain the predominance of bivalents. Dewey[8] argued that the nonexistence of two chiasmata per bivalent at the diploid level would lead to the prevalence of bivalents at the tetraploid level.

Chromosome segregation at anaphase-I in the autotetraploid was regular (24:24) in about 40% of the PMCs. Laggards numbering 2–4 were observed in the remaining PMCs. In the triploid no single PMC with 12:24 distribution was observed. The chromosome distribution was highly irregular. This triploid was completely sterile. In the tetraploid, pollen fertility was about 45%. Several explanations are offered to explain the occurrence of sterility in ploypoids. Irregular chromosome distribution resulting from multivalent formation[9] holds good for the present autotetraploid since the fertility could be correlated with the chromosome distribution at anaphase-I.

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INDUCTION OF GROWTH IN FROZEN EMBRYOS OF COCONUT AND OVULES OF CITRUS

Y. P. S. BAJAJ
Tissue Culture Laboratory, Punjab Agricultural University, Ludhiana 141004, India.

THE storage of seeds is the customary method for conservation and international exchange of germplasm. However, in a number of tree species and plantation crops the seeds are recalcitrant[1]. Such seeds are sensitive to changes in humidity and temperature, and thus cannot be preserved under ordinary conditions for long periods, due to degeneration of embryos. In such cases, the germplasm could possibly be conserved through the cryopreservation of embryos or their segments[2,3]. In this communication survival of frozen embryos of two tree species (coconut and citrus), whose seeds are short-lived, is reported.

The immature embryos (1–1.5 cm) of West Coast Tall cultivar of coconut (*Cocos nucifera L.*) removed from nuts and stored for one month were partially dehydrated, and were cut into transverse halves. They were treated with a cryoprotectant solution (7% dimethylsulfoxide and 7% sucrose in Murashige and Skoogs' liquid medium[4]), blotted dry, then wrapped in a single layer of sterile aluminium foil[5], and frozen by gradually lowering into the liquid nitrogen cylinder, or were suddenly dropped in it and kept for five minutes. The frozen material was then thawed in warm water (35–40°C), washed and cultured on MS + 2,4-D (0.2 mg/l) + NAA (0.5 mg/l) + kinetin (0.1 mg/l). Likewise, the young ovules taken from the unripe fruits of *Citrus* sps. were subjected to the same protocol, and cultured on a medium supplemented with casein hydrolysate (CH)[6].

The retrieved coconut embryos and their segments in cultures showed a lag period of upto 4 months without showing any sign of growth. However, in some of the cultures, the embryos subsequently showed an overall swelling and elongation. The embryo segment at the cut end underwent sparse proliferation (figure 1, table 1), which at places turned brown.

Entire young ovules, and the micropylar half of the split citrus ovules showed a survival of 28.8 and 24.3% respectively (table 1). The retrieved material, like the controls[7] when cultured on a medium containing CH (500 mg/l), proliferated to form pseudobulbils[6]. Figure 2 shows the shoots obtained from the frozen ovules.