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EFFECT OF OXYTETRACYCLINE ON SEED GERMINATION, EARLY SEEDLING GROWTH AND SOME BIOCHEMICAL CHANGES IN GERMINATING SEEDS OF SOYBEAN

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The results of the present study indicate that, relatively, lower concentrations of oxytetracycline enhanced seed germination, seedling growth, hydrolysis of buffer-soluble proteins, starch and nucleic acids in the cotyledons and promoted the translocation of hydrolysed products to the growing embryonic axis. On the contrary, the higher concentrations of oxytetracycline suppressed seed germination, seedling growth, hydrolysis of buffer-soluble proteins, starch and nucleic acids and also prevented the translocation of hydrolysed products to the embryonic axis.

Key words : Oxytetracycline, soybean, seed germination, biochemical changes.

A perusal of the available literature reveals that there are conflicting reports with regard to the effect of antibiotics on seed germination and seedling growth (Raza, 1981, Bose *et al.*, 1982; Jayam and Janardhanan, 1984; Maragathavalli and Janardhanan, 1986). The effect of antibiotics on the hydrolysis of reserve substances and the translocation of hydrolysed products to the growing embryonic axis of germinating seeds is meagre (Rao *et al.*, 1975). Therefore, in the present investigation, an attempt was made to elucidate the effect of oxytetracycline on seed germination, seedling growth and some biochemical changes associated with seed germination in soybean.

MATERIALS AND METHODS

Seeds of *Glycine max* (L.) Merr. var. Co.1 (soybean) were procured from Tamil Nadu Agricultural University, Coimbatore. Healthy seeds more or less of the same weight (125 ± 5 mg) were divided into batches of thirty each and were surface sterilized with 0.1% $HgCl_2$ for 1-2 min then rinsed repeatedly with distilled water and soaked in beakers containing equal volume of different concentrations (0.1, 1, 10 and 100 µg/ml) of oxytetracycline solutions. One batch of seeds was kept as control by soaking them in distilled water. The solutions were decanted after 24 h and the imbibed seeds were washed with distilled water repeatedly and were allowed to germinate in germination towels at room temperature (27 ± 2°C). The germinating seeds of 5-day and 7-day-old seedlings were taken for calculating seed germination percentage and seedling growth parameters viz., the length of the primary root, the length of epicotyl, the length of hypocotyl and the number of lateral roots formed. Employing the 't' test the significant differences of different seedling growth parameters of the treated seeds with that of the control were statistically evaluated at p = 0.05 and 0.01 levels. The germinating seeds of 3-day, 5-day and 7-day-old seedlings were taken up for biochemical studies. From the above samples the cotyledons and embryonic axes were separted for the analysis of the following biochemical substances.

Three to five pairs of cotyledons or five to three embryonic axes were homogenised to a paste in precooled mortar with pestle and the paste was extracted with 5-8 ml of 0.1M. Phosphate buffer (pH 7.0). The homogenate was centrifuged at 5000 x g for 20 min at 4°C in a refrigerated centrifuge. The supernatant containing buffer-soluble proteins was collected and measured. Suitable aliquot of buffer-soluble proteins was pipetted into a centrifuge tube kept in an ice bucket. To the above aliquot equal amount of cold 20% TCA was added and the mixture was left undisturbed in the centrifuge tube for 30 min. Later the mixture was centrifuged at 5000 x g for 10 min. The supernatant was discarded and the precipitated proteins were dissolved in a known volume of 0.1N NaOH. The protein content was estimated using Lowry *et al* (1951) method.

Total free amino acids were extracted in hot 80% ethanol for 15 min over a boiling waterbath. After cooling, the homogenate was cleared by centrifugation at 5000 x g for 10 min. The pellet was extracted once more with hot 80% ethanol as above and centrifuged at 5000 x g for 10 min. Both the supernatants were pooled together. The volume was reduced to 1-2 ml by

Table 1 : Effect of Different Concentrations of Oxytetracycline on Seed Gerimnation and Early Seedling Growth in Germinating Seeds of Soybean (*Glycine max* L.) Merr. var. Co-1.

S. No.	Concentration (mg/ml)	Days after germination	Seed germination	Root length (cm)	Epicotyl length (cm)	Hypocotyl length (cm)	Number of lateral roots formed
1	0	5	76	8.21±0.82	--	6.82±1.12	17±2.11
	(control)	7	76	13.67±1.31	2.56±0.42	9.60±0.82	26±3.11
2	0.1	5	80	14.62±1.18*	--	11.81±1.53*	25±3.13*
		7	80	20.63±1.77*	4.82±0.62**	14.76±0.69*	36±5.50*
3	1	5	79	10.76±1.22	--	8.17±1.10	20±2.97
		7	79	17.41±1.31	4.13±0.32*	12.43±1.36	33±5.13
4	10	5	77	9.33±0.91	--	7.21±1.43	20±2.31
		7	77	14.53±1.57	3.27±0.23	9.89±0.85	23±4.11
5	100	5	75	8.09±1.25	--	6.38±1.07	13±2.51
		7	75	11.24±.64	1.5±0.69	8.68±0.71	19±2.37

± - Denotes the standard error
* - Denotes the significant differences from the control at p=0.05 level
** - Denotes the significant differences from the control at p=0.01 level
-- - Epicotly not differentiated.

evaporation and diluted to a known volume with distilled water. The total free amino acid content was estimated by Rosen (1957) method using leucine as a standard in a spectrophotometer at 540 nm.

Starch content of the cotyledons and embryonic axes were extracted and determined using glucose as a standard in spectrophotometer at 625 nm (Clegg, 1956).

The total soluble carbohydrate content of different samples were determined following the anthrone reagent method (Yemm & Willis 1954) using glucose as a standard in a spectrophotometer at 620 nm.

RNA and DNA contents of the cotyledons and embryonic axes were extracted following the method of Jayaraman, (1981). The RNA content of different samples were estimated by orcinol reagent method using yeast RNA as a standard in a spectrophotometer at 665 nm (Jayaraman, 1981). DNA content of the cotyledons and embryonic axes were estimated by diphenylamine method using calf-thymus DNA as a standard in a spectrophotometer at 595 nm (Jayaraman, 1981).

RESULTS AND DISCUSSION

The results of the present study reveal that the lowest concentration of oxytetracycline (0.1 µg/ml) slightly promoted seed germination and significantly enhanced early seedling growth compared to control samples. In contrast, the highest, concentration of oxytetracycline (100 µg/ml) suppressed seed germination and early seedling growth (Table, 1). The antibiotic-stimulated seed germination may be attributed to the prevention of certain germination inhibiting process and/or by overcoming the inhibitory processes of seed germination (Brain, 1957). In the present study also lower concentrations of oxytetracycline stimulated seedling growth in soybean which might be due to an increase in the endogenous levels of gibberellin and cytokinin-like substances as in almond and willow (Van Staden *et al.*, 1980); mungbean (Mukherji & Wareing, 1983) or increased cytokinin activity as in mungbean (Biswas & Nagar, 1988). During the course of seed germination various storage reserves are hydrolysed and the hydrolysed products are translocated to the growing embryonic axis (Bewley & Black, 1985). In general, the content of buffer-soluble proteins decreased in the cotyledons of germinating seeds treated with lower concentrations of oxytetracycline with concomitant increase in the build up of buffer-soluble proteins in the embryonic axes. On the contrary, the content of buffer-soluble proteins remain more or less unaffected in the cotyledons of germinating seeds treated with higher concentrations (10 and 100 µg/ml)

of oxytetracycline with concomitant decrease in build up of buffer-soluble proteins in the embryonic axes when compared to control samples (Table 2). The above mentioned results suggest that, perhaps, the lower concentrations of oxytetracycline (0.1 and 1 µg/ml) might directly or indirectly promote hydrolysis

Table 2 :Effect of oxytetracyline on levels of buffer-soluble proteins (in mg per pair of cotyledons/one embryonal axis)*

S. No.	Concentration µg/ml	Days after soaking: 3 Day Cotyledons	3 Day Embryonal axis	5 Day Cotyledons	5 Day Embryonal axis	7 Day Cotyledons	7 Day Embryonal axis
1.	Control	30.40	6.61	15.80	9.82	10.75	15.64
2.	0.1	28.38	7.59	13.88	10.25	8.27	17.41
3.	1.0	29.45	7.27	15.09	9.91	10.19	16.00
4.	10	31.47	5.12	16.21	8.27	11.98	14.58
5.	100	33.25	3.27	17.43	6.56	11.51	14.61

*Mean of triplicate experiments

and depletion of buffer-soluble proteins from cotyledons to the embryonic axes whereas higher concentrations (10 and 100 µg/ml) of the antibiotic might suppress hydrolysis and depletion of buffer-soluble proteins from cotyledons to the embryonic axes. The total free amino acid content slightly increased in the cotyledons of germinating seeds treated with the lower concentration of antibiotic employed. It might be due to increased proteolysis accompanied by increased translocation to the growing embryonic axis. On the contrary, the total free amino acid content increased in the cotyledons of germinating seeds subsequently treated with, relatively, higher concentrations of oxytetracycline (1, 10 and 100 µg/ml). It might be due to a block in the translocation of hydrolysed protein products from cotyledons to the embryonic axes (Table 3). The findings of the present study suggest the trend that, in general, the lower concentrations of oxytetracycline also directly or indirectly stimulated starch hydrolysis in cotyledons and subsequent translocation of sugars to the growing embryonic axes, where they are rapidly utilised in respiratory metabolism resulting in reduction in their content. The rapid hydrolysis and depletion of starch in the cotyledons and translocation and utilisation of sugars in embryonic axes of germinating seeds can be correlated with an overall increase in seedling growth manifested in the form of increase in the length of root, shoot and formation of more number of lateral roots. On the otherhand,

Table 3: Effect of oxytetracyline on the levels of total free aminoacids (in mg per pair of cotyledons/one embryonal axis)*

S. No.	Concentration µg/ml	Days after soaking: 3 Day Cotyledons	3 Day Embryonal axis	5 Day Cotyledons	5 Day Embryonal axis	7 Day Cotyledons	7 Day Embryonal axis
1.	Control	3.81	1.31	5.04	3.21	4.06	5.19
2.	0.1	4.03	1.67	5.44	4.31	3.41	5.92
3.	1.0	3.86	1.42	5.71	4.09	3.92	5.84
4.	10	3.71	1.40	4.94	3.10	4.01	5.08
5.	100	3.91	1.18	4.76	3.13	4.38	4.72

*Mean of triplicate experiments.

higher concentrations (1, 10 and 100 µg/ml) of oxytetracycline inhibited the hydrolysis and depletion of starch in cotyledons and utilisation of translocated sugars in the growing embryonic axes (Tables 4 and 5). The germinating seeds treated with the following concentrations, 1, 10 and 100 µg/ml of oxytetracycline inhibited the various growth parameters of the seedlings which might be due to the reduced utilisation of translocated sugars and respiratory activity. Earlier studies of Rao *et al.* (1975) suggest that chloramphenicol affects the hydrolysis of starch to reducing sugars, thus reducing the availability of substrate for respiration consequently, affecting seedling growth.

Table 4 : Effect of oxytetracycline on levels of starch (in mg per pair of cotyledons/one embryonal axis)*

S. No.	Concentration µg/ml	Days after soaking: 3 Day Cotyledons	3 Day Embryonal axis	5 Day Cotyledons	5 Day Embryonal axis	7 Day Cotyledons	7 Day Embryonal axis
1.	Control	4.68	0.81	4.05	1.17	3.58	0.93
2.	0.1	3.24	0.91	3.01	1.32	2.67	1.05
3.	1.0	4.28	0.78	4.04	1.16	3.45	1.13
4.	10	5.19	0.64	4.53	1.04	3.67	0.91
5.	100	5.41	0.62	4.54	0.78	3.71	0.74

*Mean of triplicate experiments

The RNA content of cotyledons obtained from germinating seeds treated with lower concentrations of oxytetracycline (0.1 and 1 µg/ml) decreased with corresponding increase in embryonic axes. However, the RNA content of the cotyledons of germinating seeds treated with higher concentrations (10 and 100

µg/ml) more or less remained unaffected resulting in a decrease in content in embryonic axes. The findings of the present study reveals that lower concentrations of oxytetracycline (0.1 and 1 µg/ml) might induce rapid hydrolysis and translocation of nucleic acids from the cotyledons to the embryonic axes where there is a rapid synthesis and accumulation of RNA and DNA. On the contrary, higher concentrations of oxytetracycline suppressed not only the hydrolysis of RNA and DNA but also translocation of nucleotides to embryonic axes compared to control, thus limiting the availability of nucleotides for RNA and DNA syntheses in embryonic axes (Tables 6 & 7).

Table 5: Effect of oxytetracycline on levels of total soluble carbohydrates (in mg per pair of cotyledons/one embryonal axis)*

S. No.	Concentration µg/ml	Days after soaking: 3 Day Cotyledons	3 Day Embryonal axis	5 Day Cotyledons	5 Day Embryonal axis	7 Day Cotyledons	7 Day Embryonal axis
1.	Control	26.01	4.65	20.45	6.98	10.91	12.18
2.	0.1	25.25	6.15	19.78	8.53	11.25	12.20
3.	1.0	25.62	5.79	19.57	8.19	11.17	12.03
4.	10	26.11	4.27	20.57	6.23	12.28	10.39
5.	100	26.67	3.67	22.52	5.01	13.01	10.25

* Mean of triplicate experiments

Table 6: Effect of oxytetracycline on levels of RNA (in mg per pair of cotyledons/one embryonal axis)*

S. No.	Concentration µg/ml	Days after soaking: 3 Day Cotyledons	3 Day Embryonal axis	5 Day Cotyledons	5 Day Embryonal axis	7 Day Cotyledons	7 Day Embryonal axis
1.	Contorl	12.89	1.62	8.72	3.63	5.45	2.57
2.	0.1	12.45	1.94	8.14	3.91	4.89	2.88
3.	1.0	12.62	1.82	8.67	3.65	5.43	2.66
4.	10	13.02	1.56	9.06	3.47	5.61	2.26
5.	100	13.26	1.31	9.11	3.12	5.61	2.21

* Mean of triplicate experiments

In the 5 day-old-seedlings of both control as well as different experimental samples, the cotyledons as well as embryonic axes exhibited slight increase in the content of DNA. As it is assumed that the cotyledonary cells are not undergoing cell division, the cause for increase in DNA content is not apparent. Nonetheless, the slight increase in content of DNA in the cotyledons might have been contributed by the subcellular organelles which develop during the peak period of germination (Cherry, 1963).

Table 7: Effect of oxytetracycline on levels of DNA (in mg per pair of cotyledons/one embryonal axis)*

S. No.	Concentration µg/ml	Days after soaking: 3 Day Cotyledons	3 Day Embryonal axis	5 Day Cotyledons	5 Day Embryonal axis	7 Day Cotyledons	7 Day Embryonal axis
1.	Control	0.90	0.25	0.98	0.53	0.87	0.52
2.	0.1	0.88	0.27	0.94	0.56	0.85	0.54
3.	1.0	0.92	0.25	0.96	0.53	0.89	0.51
4.	10	0.97	0.19	1.06	0.43	0.96	0.41
5.	100	0.96	0.18	1.10	0.42	0.96	0.41

* Mean of triplicate experiments

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