EFFECT OF CHLORAMPHENICOL ON SOME BIOCHEMICAL CHANGES IN EARLY STAGES OF RAGI (ELEUSINE CORACANA (L) GAERTN) SEED GERMINATION

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The results of the present study indicate that lower concentration of chloramphenicol promoted hydrolysis of buffer-soluble proteins, starch and nucleic acids in the cotyledons with concomitant increase of their contents in the embryonal axis of 3-day old germinating soybean seeds when compared to control. On the country, the higher concentrations of chloramphenicol suppressed hydrolysis of the above mentioned substances in the germinating seed cotyledons.

INTRODUCTION

Our knowledge on the effect of antibiotics on hydrolysis of reserve substances in the storage tissues and the translocation of hydrolysed products to the growing embryonal axis during seed germination in meagre (Rao *et al.* 1975). Therefore, in the present study, an attempt was made to understand the effect of different concentrations of chloramphenicol on certain biochemical changes during the early stage (3-day old) of Ragi seed germination.

MATERIALS AND METHODS

Seeds of Eleusine Coracana (L) Gaertn were procured from O.U.A.T, Bhubaneswar. Healthy seeds more or less of the same weight 0.25 $\pm$ 1 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 volumes of different concentrations (0.1, 1, 10 and 100 μg/ml) of chloramphenicol solution. 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 $\pm$ 2°C). The germinating seeds of 3-day old were taken for biochemical studies. From the above samples, the cotyledons and embryonal axis were separated and kept in shaved ice until they were used for extraction of the various biomolecules.

Extraction and estimation of buffer-soluble proteins

Buffer-soluble proteins of the cotyledons and embryonal axes were extracted following the method of Thambidural and Janardhanan (1990) and estimated according to the method of Lowry *et al.* (1951) using bovine serum albumin fraction *V* as a standard in a spectrophotometer at 540 nm.

Extraction and estimation of starch

Starch from the cotyledons and embryonal axes was extracted and determined according to the method of Clegg (1956) using glucose as a standard in a spectrophotometer at 625 nm.

Extraction and estimation of total soluble carbohydrates

Total soluble carbohydrates of different samples were extracted as has been described elsewhere (Thambidurai and Janardhanan 1990) and estimated following the method of Yemm and Willis (1954) using glucose as a standard in a spectrophotometer at 620 nm.

Extraction and estimation of RNA and DNA

RNA and DNA contents of the cotyledons and embryonal axes were extracted following the method of Jayaraman (1981). The RNA content of different samples was estimated by orcinol reagent method of Jayaraman (1981). The RNA content of different samples was estimated by orcinol reagent method of Jayaraman (1981) using yeast RNA as a standard in a spectrophotometer at 665 nm. The DNA content of different samples was estimated by diphenylamine method of Jayaraman (3) using calf-thymus DNA as a standard in a spectrophotometer at 595 nm.

RESULTS AND DISCUSSION

During the course of seed germination, various storage reserves are hydrolysed and the hydrolysed products are translocated to the growing embryonal axis (Bewlly and Black 1985). The content of buffer-soluble proteins decreased in the cotyledons of germinating seeds treated with lower concentrations of chloramphenicol (0.1 and 1 μg/ml) with concomitant increase in the build up of buffer-soluble proteins in the embryonal axes (Table 1). On the other hand, the content of buffer-soluble proteins remains more or less unaffected in the cotyledons of germinating seeds treated with 10 μg/ml) of chloramphenicol suppressed the hydrolysis of buffer-soluble proteins. The accumulation of buffer-soluble proteins in the embryonal axes of seeds treated with higher concentrations of chloramphenicol (10 and 100 μg/ml) decreased when compared with control samples. Conceivably lower concentrations of chloramphenicol (0.1 and 1 μg/ml) directly or indirectly promoted hydrolysis of buffer-soluble proteins in the cotyledons of 3-day old germinating soybean seeds. The content of total free amino acids slightly increased in both the cotyledons and embryonal axes of early germinating seeds treated with the lowest concentration of chloramphenicol (0.1 μg/ml) which might be due to increased proteolysis in the cotyledons accompanied by increased translocation to the growing embryonal axis. Apparently lower concentrations of chloramphenicol (0.1 and 1.0 μg/ml) also, directly or indirectly, stimulated starch hydrolysis in cotyledons, whereas the higher concentrations (10 and 100 μg/ml) inhibited starch hydrolysis in the cotyledons of 3-day old germinating soybean seeds. While the content of soluble carbohydrates increased in the embryonal axes treated with lower concentrations, the content of starch more or less remained unaffected in the early stage of seed germination. In general, the higher concentrations of chloramphenicol suppressed starch accumulation in the embryonal axes of the germinating seeds. Earlier, chloramphenicol has been shown to inhibit hydrolysis of starch

to reducing sugars, thus reducing the availability of substrate for respiration, consequently affecting seedling growth (Rao *et al*. 1975). The RNA content of cotyledons obtained from germinating seeds treated with the lowest concentration (0.1 μg/ml) of chloramphenicol decreased with corresponding increase in embryonal axes. However, the RNA content of cotyledons obtained from germinating seeds treated with higher concentrations (10 and 100 μg/ml) of chloramphenicol remained unaffected. On the other hand, RNA content decreased in the embryonal axes treated with higher concentrations of chloramphenicol. The DNA content of cotyledons obtained from germinating seeds treated with lower concentrations of chloramphenicol (0.1 and 1 μg/ml) decreased with corresponding increase in the embryonal axes. While the higher concentrations (10 and 100 μg/ml) of chloramphenicol decreased the DNA content in embryonal axes, they lightly increased DNA content in the cotyledons. Lower concentrations of chloramphenicol (0.1 and 1 μg/ml) seemed to induce rapid synthesis and accumulated of RNA and DNA in embryonal axes; while higher concentrations seemed to suppress the hydrolysis of nucleic acids in the cotyledons of germinating seeds.

Table 1. Effect of different concentrations of chloramphenicol on levels of biomolecules in the 3-day old germinating Ragi seed cotyledons and embryonal axis.

Sl. No.	Chlor-amphenicol (μg/ml)	Part of the seedling	Buffer soluble proteins	Total free amino acids	Total soluble carbo-dydrates	Starch	RNA	RNA
1.	Control	Cotyledon (mg/pair)	30.40 ±0.270	3.84 ±0.540	26.04 ±0.365	4.682 ±0.136	13.09 ±175	0.98 ±0.016
		Embryonal axis (mg)	6.64 ±0.368	1.34 ±0.043	4.66 ±0.244	0.84 ±0.016	1.63 ±0.046	0.26 ±0.007
2.	0.1	Cotyledon (mg/pair)	28.46 ±0.218	3.98 ±0.135	25.78 ±0.309	4.01 ±0.093	12.32 ±0.043	0.75 ±0.029
		Embryonal axis (mg)	7.36 ±0.387	1.87 ±0.456	5.58 ±0.273	0.89 ±0.015	1.84 ±0.026	0.392 ±0.013
3.	1	Cotyledon (mg/pair)	28.76 ±0.416	4.28 ±0.080	25.96 ±0.301	4.26 ±0.043	12.44 ±0.063	0.782 ±0.02
		Embryonal axis (mg)	8.50 ±0.315	1.16 ±0.085	5.42 ±0.319	0.99 ±0.013	1.98±1 ±0.034	0.381 ±0.016
4.	10	Cotyledon (mg/pair)	30.95 ±0.665	4.29 ±0.049	25.56 ±0.168	4.97 ±0.063	13.28 ±0.133	0.93 ±0.015
		Embryonal axis (mg)	5.28 ±0.258	0.985 ±0.010	4.79 ±0.168	0.78 ±0.013	1.13 ±0.038	0.261 ±0.016
5.	100	Cotyledon (mg/pair)	38.43 ±0.383	3.56 ±0.045	27.28 ±0.216	5.58 ±0.015	13.44 ±0.049	1.063 ±0.050
		Embryonal axis (mg)	3.38 ±0.269	0.98 ±0.013	3.38 ±0.124	0.395 ±0.014	0.982 ±0.019	0.161 ±0.029

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