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EFFECT OF SOME TRACE ELEMENTS ON THE NUCLEIC ACIDS OF GERMINATING WHEAT SEEDS

M. Khurshid Alam Khan and Miss Kaniz Fizza

Pharmaceutical and Fine Chemicals Reserach Division, PCSIR Laboratories, Karachi-39

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It is well established that trace elements play a decisive role in the metabolism and growth of plants [1]. Trace elements like molybdenum and manganese are essential for nitrogen metabolism, nitrogen fixation, carbondioxide assimilation and carbohydrate breakdown. Zinc is involved in the formation of growth hormones, promotion of protein synthesis, grain maturation, etc. Iron is involved in chlorophyll synthesis, oxidation-reduction and respiration. Copper acts as catalyst for respiration and is a constituent of enzymes. Cobalt is essential for nitrogen fixation and is also a component of vitamin B_{12}.

The exact mechanism of growth promoting effect of trace elements is not fully understood and it is suggested that some of them may be effective through co-enzymes or may act as cofactors for certain enzymes. Furthermore, copper, iron and molybdenum are capable of acting as "electron carriers" in enzyme systems which carry out oxidation-reduction reactions in plants.

Many studies are reported in the literature concerning the effect of trace elements on the physiology of growing plants. As for example, it is reported that a pre-sowing treatment of corn and wheat seeds with solutions of manganese sulphate, zinc sulphate and boric acid result in an appreciaable increase of protease, amylase and lipase activity [2]. Moreover, under the influence of manganese treatment a sharp, increase in catalase activity was also observed [2]. As regards nucleic acid metabolism, it is reported, specially with reference to plant nutrition, that in sugar and corn leaves RNA/DNA content was lower in the later stages of growth on substrates with lower amount of NKP fertilizers [3].

Since nucleic acids play a controlling role in cell proliferation as well as the translation and storage of genetic information therefore the effects of trace elements on the matabolism of nucleic acids in germinating seed appeared to be of interest. The present paper describes the effect of some trace elements on the nucleic acids of germinating seeds of *Triticum aestivum Linn* (wheat).

MATERIALS AND METHODS

Locally available wheat seeds were grown on sterilized cotton in petri dishes in the dark at the ambient temperature. Controls without using trace elements were run simultaneously for all experiments. The solutions of salts containing non-toxic levels of trace element were prepared in distilled water [3], and contained manganese sulphate (20 ppm), ferrous sulphate (200 ppm), copper sulphate (30 ppm), ammonium molybdate (25 ppm), cobalt chloride (0.05 ppm) and zinc sulphate (20 ppm).

Nucleic acids estimation. Single germinating seeds of controls as well as of trace element treated samples including the germinated portion were taken out and placed in a test tube containing 1 ml of cold methanol. The total nucleic acids were estimated according to the published methods [4, 5]. The samples were homogenised in cold methanol in a small mortar and pestle. The insoluble pellet was washed with cold methanol, cold 0.2 N perchloric acid and again with cold methanol maintaining a temperature of 0-4° during these operations. The insoluble pellet was defatted with ethanol: ether (2:1 v/v) at 50° for 30 min. The total nucleic acids were then extracted with 5 % perchloric acid (6 ml) at 70° for 40 min. Absorbency difference at 260 and 290 nm of the supernatant was measured and was referred to a standard curve obtained by similarly treated yeast RNA to estimate total nucleic acids.

The DNA was estimated by a colorimetric method [6] using diphenylamine. A standard curve made by using calf thymus DNA was used for determining the DNA concentration, RNA was estimated by subtracting the DNA concentration from that of total nucleic acids. The spec-

Table 1. The effect of trace elements on total nucleic acids (TNA) ribonucleic acids (RNA) and deoxyribonucleic acids (DNA) of germinating wheat seeds.

Days of growth	Trace element	Nucleic acids (μg) control			Nucleic acids (μg) with trace element		
		TNA	DNA	RNA	TNA	DNA	RNA
1.	Manganese	50	20	30	91	28.5	62.5
2.		66	40	20	91	28.5	62.5
3.		76	28.5	47.5	91	20.0	71.0
4.		80	17.0	63.0	102	20.0	82.0
7.		91	11.5	79.5	112	17	95.0
1.	Iron	48	20	28	50	20	30
2.		64	44	20	98	32.5	65.5
3.		76	17	59	88	11.5	76.5
4.		80	11.5	68.5	116	11.5	104.5
7.		91	11.5	79.5	120	11.5	108.5
1.	Copper	48	30	18	52	39.5	12.5
2.		66	39.5	26.5	120	28.5	91.5
3.		76.0	20.0	56.0	154	20.0	148.5
4.		82.0	11.5	70.5	194	11.5	165.5
7.		91.0	17.0	74.0	206	17.0	186.0
1.	Molybdenum	52	20	32	60	46	14
2.		65	40	25	92	39.5	52.5
3.		76	26	50	103	28.5	74.5
4.		80	19.5	60.5	108	6.5	101.5
7.		91	11.5	79.5	114	11.5	102.5
1.	Cobalt	50	20	30	60	28.5	31.5
2.		66	20	46	88	39.5	48.5
3.		78	20	58	94	28.5	65.5
4.		82	17	65	160	17.0	143.0
7.		91	11.5	79.5	202	17.0	185.0
1.	Zinc	50	20	30	30	28.5	1.5
2.		66	20	46	43	39.5	3.5
3.		78	20	58	50	40.0	10.0
4.		82	17	65	23	20.0	3.0
7.		91	11	79	19	17.0	2.0

trophotometric determinations were carried out on a UNICAM SP500 Spectrophotometer.

The experiments were carried out three times and no appreciable quantitative variation of nucleic acid values was observed.

RESULTS AND DISCUSSION

The methods used to determine the total nucleic acids and DNA in our studies have been used by previous workers [4, 6] and are reliable and sensitive up to microgramme

amounts of nucleic acids from a single seed. In order to eliminate the possibility of error due to the presence of proteins, it was made certain that the ratio of optical density at 260 nm to that 280 nm was greater than 1.8 indicating the absence of proteinous contaminants [7]. All experiments with trace elements as well as with blanks were run three times, and no appreciable difference between the values of RNA and DNA was observed.

The results obtained for the quantitative determination of RNA and DNA during germination of wheat seeds at different intervals using non-toxic levels of trace-elements are shown graphically in Fig. 1, together with controls. The quantitative, values obtained for total nucleic acids (TNA), deoxyribonucleic acid (DNA) and ribonucleic acids (RNA) in the presence as well as absence of trace elements are included in Table 1.

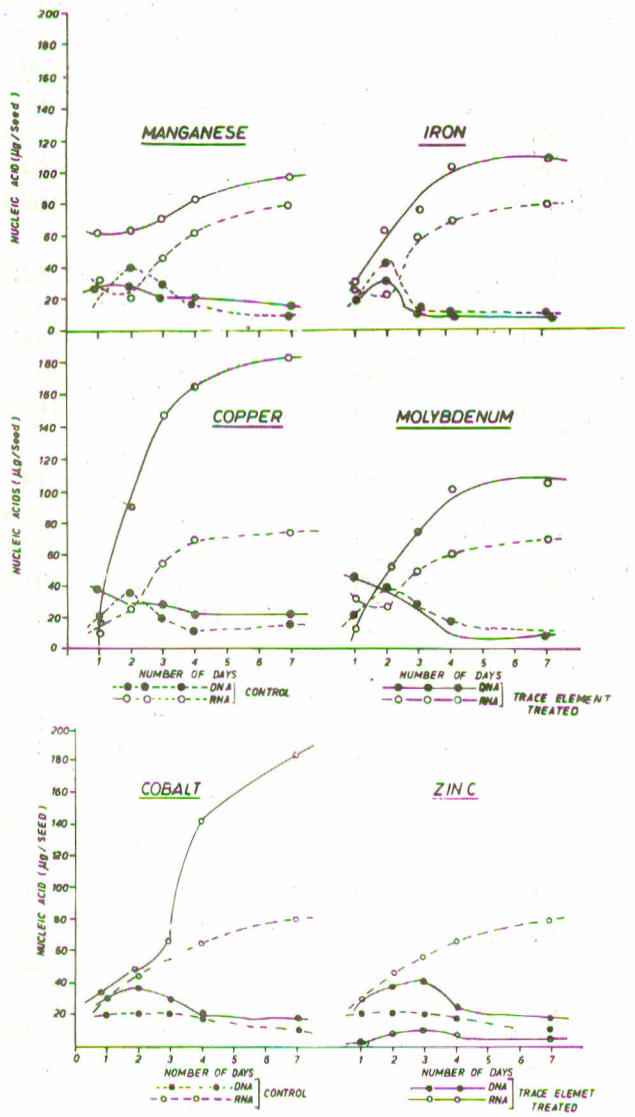

Fig. 1. Effect of trace element on the quantity of RNA and DNA in growing wheat seeds.

An examination of the data clearly reveals that except for zinc, all trace elements used by us show a pronounced stimulation of RNA synthesis as compared to controls. The DNA synthesis, however, has remained at a fairly constant level, as compared to controls. This is in agreement with the findings of other workers [5]. At the initial stage of germination i.e. up to about 4 days the RNA synthesis is at a maximum level and levels off after about 7 days of germination. The use of manganese, iron and molybdenum stimulates RNA synthesis moderately while copper and cobalt exhibits unusually strong RNA synthesis stimulation as compared to controls. The effect of zinc is rather unusual, it inhibits RNA synthesis even after using lower limits of non-toxic amounts i.e. 20 ppm. It is just possible that for the seeds used by us this low level of zinc may produce toxicity.

The exact mechanism of RNA synthesis stimulation during the germination of wheat seeds cannot be ascertained from this study. It is plausible that some of these trace elements may catalyse the activity of RNA polymerising enzyme i.e. RNA polymerase. Further work on the effect of these trace elements on the RNA polymerase activity will probably reveal the specific effect of these trace elements on the germination of seeds.

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