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Copper Deficiency-Induced Changes in Wheat Anther

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Copper deficiency retarded the development of ears, anthers and pollen grains in wheat. In plants that showed severe foliar symptoms of copper deficiency florets failed to open and anthers which were largely empty failed to dehisce. The pollen grains produced by copper-deficient plants were of smaller size, lacked dense cytoplasmic contents and failed to stain with iodine solution. *In vitro* germination of pollen grains was markedly reduced by copper deficiency. The pollen-bearing anthers of copper-deficient plants showed marked decreases in catalase, peroxidase and cytochrome oxidase activities and marked increase in ribonuclease and aldolase activities.

Key Words: Cu deficiency, Wheat, Anther development

Introduction

Lohnis (1937, 1940) reported that plants raised in boron-deficient medium produced abnormal anthers and abortive pollen grains. Kibalenko and Sidorshina (1971) found pollen grains of sugarbeet plants grown in boron-deficient soils to be smaller in size and poor in viability. More recently, Graham (1976) reported that pollen grains of wheat plants grown on copper-deficient soils were abortive even in plants that failed to exhibit visible symptoms of copper deficiency. In this paper we describe some morphological and physiological changes accompanying development and germination of pollen in wheat plants subjected to copper deficiency.

Materials and Methods

Wheat (*Triticum aestivum* L.), var. UP 262, plants were raised in refined sand culture (Agarwala & Sharma 1976) at normal (0.065 ppm) and deficient (0.013 ppm) levels of copper supply. At the time of heading, study was made of length and pollen-producing capacity of anthers, size of pollen grains, *in vitro* germination of pollen and activities of some enzymes—catalase, peroxidase, cytochrome-c oxidase, ribonuclease, acid phosphatase and aldolase—in anthers containing mature pollen grains. Samples for all these studies were drawn from the

oldest florets of the spikelets borne in the middle portion of the spikes.

At the time of anther dehiscence, pollen-producing capacity of anthers was determined by the method of Kapoor and Nair (1974). Intact anthers, picked at the time of anthesis, were dissected in 15 drops of 80% glycerine. The suspension was thoroughly mixed and the number of pollen grains counted under the microscope in five separate drops taken on a clean glass slide. For measurement of pollen size, freshly liberated pollen grains were suspended in glycerine-jelly (Stanley & Linskens 1974) and diameter of pollen grains along the polar axis was measured using an ocular micrometer. As a measure of viability, the germination of the pollen grains was tested *in vitro* using the hanging drop technique with the growth medium comprising 0.8 M sucrose, 0.2 M glucose, 0.01% boric acid, 0.03% calcium nitrate, 0.01% potassium nitrate, 0.02% magnesium sulphate and 0.01 mM manganese sulphate. The cultures were maintained for one hour at 20 ± 1°C and 100% relative humidity. Percentage germination was worked out by counting the total number of germinated and ungerminated pollen grains after mounting them in 50% glycerine. Only those pollen grains, which produced tubes longer than the average diameter of pollen grains, were considered germinated.

The activities of catalase, peroxidase, ribonuclease, acid phosphatase and aldolase were assayed in crude homogenates of pollen-bearing anthers made in glass distilled water in a chilled pestle and mortar at 4 C according to the procedure described earlier (Agarwala et al. 1965). Cytochrome c oxidase was assayed in mitochondria-rich preparation (Pierpoint 1959). Two grammes of pollen-bearing anthers were ground in 10 ml of chilled grinding medium containing 0.4 M Sucrose, 0.01 M K_2HPO_4, 5 mM EDTA 0.02 M sodium citrate and 0.05 M Tris-HCl buffer *p*H 7.7, strained through two-fold muslin cloth and centrifuged in cold at 3000 × g for 10 min. The supernatant was re-centrifuged at 15,000 × g for 30 min. The residue was suspended in 10 ml of washing medium containing 0.4 M sucrose, 0.005 M K_2HPO_4, 0.0025 M EDTA, 0.005 M sodium citrate and 0.025 M Tris-HCl buffer *p*H 7.3 and again centrifuged at 15,000 × g for 30 minutes. The residue (mitochndrial pellet) thus obtained was suspended in 3 ml of the washing medium using a teflon/glass homogeniser and centrifuged at 6000 × g for 20 min. The supernatant was used for assay of cytochrome c oxidase activity by an adaptation of the method of Cooperstein and Lezarow (1951). The reaction mixture contained 1 ml of 0.1 M phosphate buffer *p*H 7.5, 0.1 ml of 1% digitonin, 0.45 ml (equivalent to 0.045 μ moles) reduced cytochrome c and 1.35 ml water. The reaction was started by adding 0.1 ml enzyme in a spectrophotometer cuvette and ΔOD_{540} was measured at 15 sec intervals for 60 sec. Enzyme activity has been expressed as μ moles cytochrome c oxidised/min by calculating the initial rate of reaction using the expression described by Yonetani (1962). For expressing the enzyme activities/mg protein, soluble protein concentration of the enzyme preparations was estimated by the method of Lowry et al. (1951).

The entire data have been analysed statistically and tested for significance at $P=0.05$. Data for length and pollen producing capacity of anthers and size and germination of pollen grains has been subjected to confidence interval estimation and the confidence interval of the mean ($t \times S.E.$) are provided along with the mean values. The data for assay of enzyme activities has been put to analysis of variance and the L.S.D. values at $P=0.05$ are given along with the data.

Results

Plants grown at low (0.013 ppm) copper

supply showed retarded growth and developed foliar symptoms of copper deficiency after the initiation of the floral primordia, at six weeks of growth. At the time of anthesis, copper-deficient plants showed chlorosis and necrosis of margins and apical half of the lamina of the young leaves. Anthesis in copper-deficient plants was delayed by 10 days. Copper deficiency also reduced the size of the ears, the extent of the effect being related to the severity of the foliar symptoms induced by the deficiency. In plants showing more severe foliar symptoms florets failed to open and their lemma and palaea turned papery and necrotic.

In copper-deficient plants, the size (length) and pollen-producing capacity of the anthers and pollen size were markedly reduced (table 1). The cellular contents of pollen grains from copper-deficient plants were sparce and shrunken (figure 1A, B). In general, such pollen grains also failed to give a positive test for starch. In plants that exhibited more severe foliar symptoms of copper deficiency, the anthers failed to dehisce and pollen development was more severely retarded. Pollen germination *in vitro* was markedly reduced by copper deficiency. Germination was inhibited even in pollen grains of plants that did not show apparent abnormality.

Activities of all the three oxido-reductases assayed-catalase, peroxidase, and cytochrome c oxidase—showed a marked decrease in the pollen-bearing anthers of copper deficient plants. Amongst these three enzymes, the effect of copper deficiency was most marked on cytochrome c oxidase. The specific activities of aldolase and ribonuclease were higher in copper-deficient than in normal

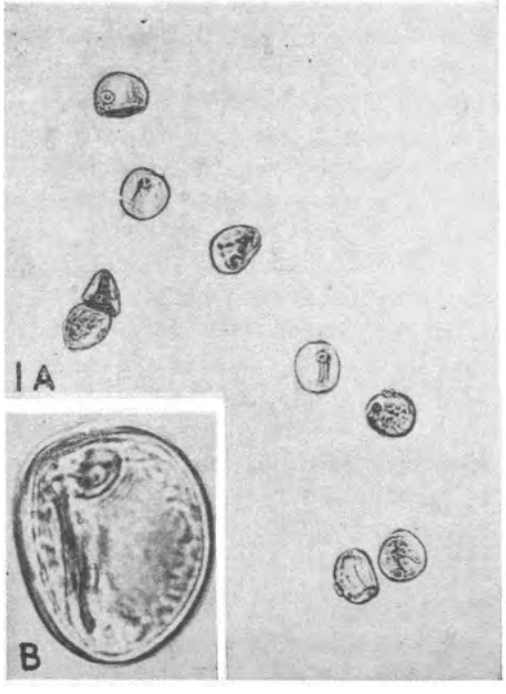

Figure 1 Pollen grains of copper deficient wheat plants. *A* (×200), *B* (×800)

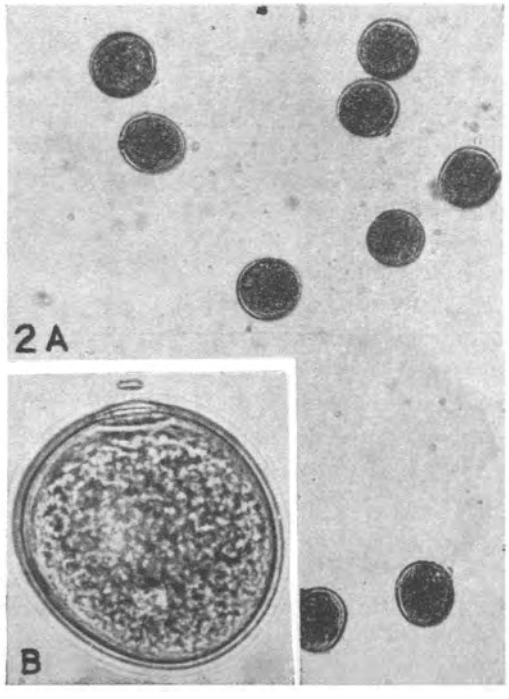

Figure 2 Pollen grains of normal wheat plants. *A* (× 200), *B* (× 800)

Table 1 *Effect of copper deficiency on length and pollen-producing capacity of anthers and size and viability of pollen grains in wheat* (Triticum aestivum L. var. U P. 262)

Copper supply (ppm)	Anther length (mm)	Pollen-producing capacity[1] (grains anther)	Pollen diameter (μm)	*In vitro* germination[2] (%)
0.065	3.54±0.11	2617±186	52.40±0.25 (600)	53.0±6.1
0.013	2.09±0 23	2076±161	45.92±0.41 (508)	7.1±3.5

[1]Mean of 20 determinations; [2]Mean of 10 measurements from 10 culture replicates

Figures in parentheses denote the number of pollen grains measured

Table 2 *Effect of copper deficiency on specific activities of certain enzymes in mature anthers of wheat* (Triticum aestivum L. var. U P. 262) *plants grown in sand culture*

Copper supply (ppm)	Enzyme activities*					
	Catalase (μmoles H_2O_2 split/mg protein/5 mt	Peroxidase Units/mg protein	Ribonuclease Units/mg protein	Acid phosphatase (μg Pi liberated/mg protein/20 mt)	Aldolase(μg P solubilised/mg protein/20 mt)	Cytochrome-c oxidase (μ moles cyt.-c oxidised /mg protein/mt)
0.065	507	108	146	149	137	0.153
0.013	349	66	167	129	246	0.088
LSD (P=0.05)	33	5	9	9	8	0.029

*Mean of duplicate estimations

anthers but acid phosphatase activity of the anthers was decreased by copper deficiency (table 2).

Discussion

As reported here, impaired development and reduction in size of pollen grains has also been reported by Graham (1975) in copper-deficient wheat and by Kibalenko and Sidorshina (1971) in boron-deficient sugarbeet plants. Fewer and shrivelled cytoplasmic contents that did not stain with iodine solution are suggestive of poor starch accumulation

in the pollen grains that may contribute to reduced viability of pollen grains as has been observed here and by Graham (1975) in copper-deficient wheat plants.

Poor germination in pollen grains of copper-deficient plants associated with decreases in the activities of phosphatase and the three oxido-reductases, more so in cytochrome c oxidase, for which copper is a constituent part, and increases in the activities of ribonuclease and aldolase would suggest poor metabolism as a cause of reduced viability in copper deficient pollen grains. It is likely that low activity of cytochrome-c oxidase in copper-deficient plants retards respiratory oxygen uptake which serves as a source of energy for the growth of the pollen tube. Stanley and Linskens (1974) have earlier suggested that depressed activities of catalase, peroxidase and cytochrome oxidase indicate impaired pollen metabolism and hence reduction in pollen viability.

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