

## Effect of choline chloride on the photosynthesis in wheat

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**Summary** Choline chloride can increase the activities of Hill reaction and ATPase in isolated chloroplast from wheat leaves. It can also increase the activities of ribulose-1,5-bisphosphate carboxylase (RuBPCase EC 4. 1. 1. 39) and glyceraldehyde-3-phosphate dehydrogenase (G-3-PDH EC 1. 2. 1. 13) with the method of vacuum infiltration. But higher concentrations of choline chloride tends to inhibit the activities of these enzymes. As a result of choline chloride treatment the amounts of both protein and chlorophyll can be increased, and the photosynthetic rate can be raised, too. The above data suggest that the choline chloride may act on the enzymes involved in light reaction and dark reaction in the photosynthesis.

**Key words** choline chloride, wheat, photosynthesis

### Introduction

Choline chloride was taken as a feed additive for domestic fowls and animals as early as in 1940s. In 1985 Farkas et al. discovered that choline chloride could be transformed into phosphatidyl choline. Standlander (1982) also reported that the amount of phosphatidyl choline in yeast increased when choline chloride was put into the yeast culture medium. Kates et al. (1975) reported that phosphatidyl choline was one of major components in membrane system of plant cells, and for total phospholipids, 27% in chloroplast, 62% in thylakoid, 24% in lamella were phosphatidyl choline.

In the recent years we discovered that when wheat was treated with 200-500 ppm of choline chloride, the yield was increased by 11.8-19.2% compared with that of control. Therefore, these physiological processes were studied in order to provide the basis for yield increase.

### Materials and methods

The leaves of winter wheat (*Triticum aestivum*) taken from the experimental field at different development stages were sprayed with different concentrations of choline chloride 10 days before assay.

#### Enzyme extraction

Wheat leaves were put in a precooled masher, in which 0.5 M phosphate buffer (pH 7.4) was added for stamping. The fresh homogenate was centrifuged at 11000 g for 15 min, and the settleings were removed. The supernatant was fractionated with solid  $(\text{NH}_4)_2\text{SO}_4$ . The settleings in 35-55% saturations were collected. The protein fraction was dissolved in a few ml of 0.05 M phosphate buffer (pH 7.4). Its supernatant was chromatographed on sephedex G-50 and G-200, and washed with 0.05 M phosphate buffer, then the supernatant was fractionated with solid  $(\text{NH}_4)_2\text{SO}_4$  to 60% saturation and centrifuged at 11000 g for 10 min. The settled protein was dissolved in a few ml of 0.01 M Tris-HCl buffer, and then

the homogenate was chromatographed on sephedex G-25. So the protein fraction obtained was the purified RuBPCase.

#### *Assay of RuBPCase activity*

The RuBPCase activity was assayed with the spectrophotometric methods in a coupled system with NADH. The standard assay mixture contained 90mM Tris-HCl buffer, 5mM ATP (Sigma), 25 mM RuBP (Sigma), 3mM NADH (Serva), 50mM phosphocreatine (Sigma), 20 mM NaHCO<sub>3</sub>, creatine phosphate kinase, phosphoglyceric phosphokinase, phosphoglycerol dehydrogenase (Sigma) and 0.1 ml RuBPCase. The rate was measured when the reaction was in steady-state. The other rates and contents were measured with routine methods (Feng Fusheng, 1984, 1986; Wu Guangyao, 1980; Arnon DI, 1949; Fork DC, 1977).

### Results

The activity of ATPase in wheat leaves increased when leaves were treated with choline chloride at 200 and 500ppm (Tab. 1).

**Table 1** Effect of choline chloride on the activity of ATPase in wheat leaves

Concentration of choline chloride (ppm)	ATP activity ( $\mu\text{mol pi/mg chl}\cdot\text{hr}$ )	Relative activity
0(control)	54.492	100
200	89.243	164
500	104.224	191

Hill reaction activity increased in different degree when the leaves of wheat growing at every development stages were treated with different concentrations of choline chloride. The highest activity was obtained if the wheat leaves were treated with 200 ppm choline chloride (Tab. 1).

**Table 2** Effect of choline chloride on the activity of Hill reaction in isolated chloroplast from wheat leaves

Concentration of choline chloride (ppm)	Hill reaction activity ( $\mu\text{mol DCIP/mg chl}\cdot\text{min}$ )							
	A	%	B	%	C	%	D	%
0(control)	212.8	100	233.4	100	248.8	100	241.4	100
50	232.6	109.3	236.2	101.1	260.2	104.5	252.2	104.4
100	244.3	114.8	246.4	105.6	272.9	109.6	282.1	116.8
200	260.3	122.3	282.2	123.4	312.8	125.7	302.2	125.1
500	242.4	113.9	272.2	116.8	302.4	121.5	298.6	123.6

A;Seedling stage; B;Grainfilling stage; C;Flowering stage; D;Booting stage

RuBPCase activity increased when leaves were treated with choline chloride with the method of vacuum infiltration. It was increased to 1.7 times compared with that of control (Fig. 1). When leaves of wheat developing at every stages were treated with different concentrations of choline chloride, the activity of RuBPCase increased in different degrees. The 200 and 500 ppm concentration of choline chloride could act most obviously. RuBPCase activity was more sensitive to choline chloride in flowering and grainfilling stages of wheat than in booting and seedling stages (Fig. 2). The activity of G-3-PDH increased when the wheat leaves were treated with higher concentrations of choline chloride at these four

stages. However, if the concentrations were beyond 500 ppm, its activity declined (Fig. 3). The contents of protein, chlorophyll and soluble sugar increased significantly as a result of choline chloride treatment at these four development stages (Data not on).

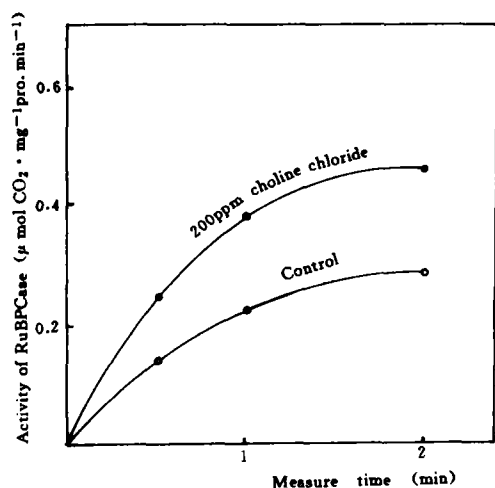


Fig. 1. Effect of choline chloride on the activity of RuBPCase in wheat leaves

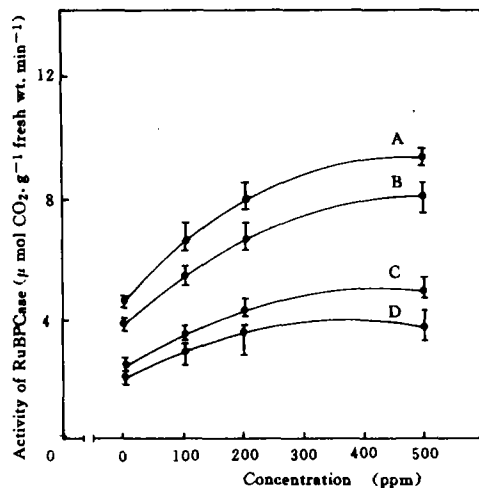


Fig. 2. Effect of choline chloride on the activity of RuBPCase in wheat leaves at different developmental stages

A: Flowering stage, B: Grainfilling stage, C: Booting stage, D: Seedling stage.

As for the photosynthetic rate, it could increase when leaves were treated with different concentrations of choline chloride at flowering stage, and the highest rate was obtained

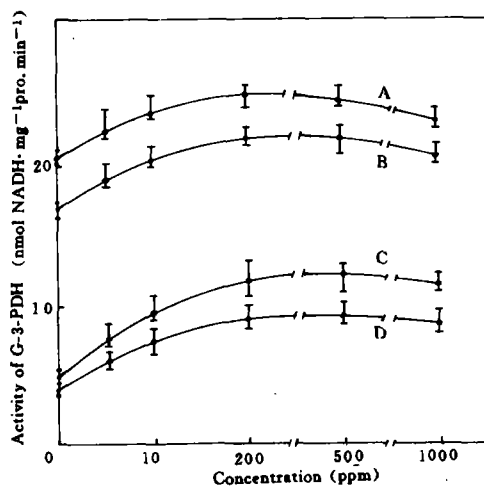


Fig. 3. Effect of choline chloride on the activity of G-3-PDH at different developmental stages

A: Flowering stage, B: Grainfilling stage, C: Booting stage, C: Seedling stage.

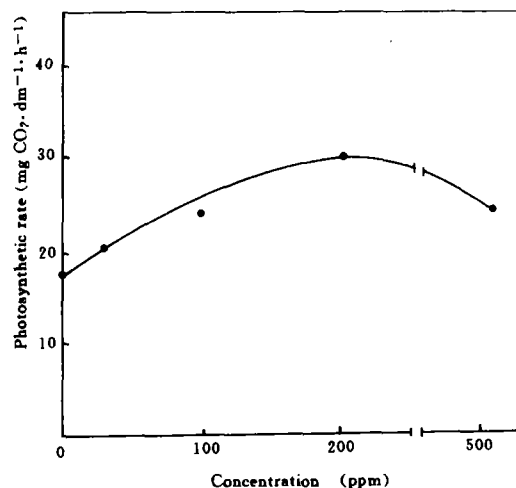


Fig. 4. Effect of different concentrations of choline chloride on photosynthetic rate in wheat leaves at flowering stage

when the choline chloride concentration came to 200 ppm. If the concentrations increased from 200 to 500 ppm or more, on the contrary, the rate declined (Fig. 4).

## Discussion

Choline chloride can increase the activities of ATPase (Tab. 1), RuBPCase (Figs 1, 2) and G-3-PDH (Fig. 3). The mechanism may be as follows: Choline chloride is transformed into phosphatidyl choline, and the later as a component of lamella and thylakoid membranes of chloroplast provides a hydrophobic surroundings for membrane-binding enzymes such as ATPase, RuBPCase and G-3-PDH. The increase of Hill reaction may be the result of joining of phosphatidyl choline in lamella and thylakoid membranes. The increases of activities of enzymes and Hill reaction lead to the raise of photosynthetic rate and the increase of contents of protein, chlorophyll and soluble sugar in leaves.

## Reference

- Ames BN. (1966) Assay of inorganic phosphate, total phosphate and phosphatases, In: Nneufeld EE, Ginsbury V (eds). Academic Press, New York, Methods in Enzymol., Vol. VIII, p. 115
- Arnon DI. (1949) Copper enzymes in isolated chloroplast polyphenoloxidase in *Beta vulgaris*, *Plant Physiol*, (24): 1—5
- Colleman R. (1973) Membrane-Bound enzymes and membrane ultrastructure. *Biochim Biophys Acta*, (300): 1—30
- Cronan JE Jr et al. (1975) Physical Properties of Membrane Lipids; Biological relevance and regulation. *Bacteriol. Reviews*, 39(3): 232—256
- Farkas T. (1985) Absciscic acid-related changes in composition and physical state of membrane in bean leaves, *J Plant Physiol*, 118(4): 373—379
- Feng Fusheng et al. (1984) Variation of the activity of glyceraldehyde-3-phosphate dehydrogenase in chloroplast from wheat plant grown at different nitrogen levels. *Plant Physiology Communication*, (4): 25—27 (in Chinese)
- Feng Fusheng et al. (1986) Variation of the activities of ribulose-1,5 bisphosphate carboxylase and nitrate reductase in leaves from wheat plant grown at different nitrogen levels. *Plant Physiology Communication*, (6): 20—22 (in Chinese)
- Fork DC. (1977) Hill reaction with DCIP. *The Science of Photobiology*, : 365—366
- Grau CR et al. (1943) The utilization of the sulfur amino acid by the chick. *J Nutrition*, (25): 631—640
- James A, Carrick MCW. (1944) The supplementary value of choline and methionine in a corn and soybean oil meal chick ration. *Poultry Sci*, (23): 279—246
- Tang Zhangcheng. (1981) Plant phosphatlipids and its function in plant, *Plant Physiology Communication*, (3): 8—14 (in Chinese)
- Wu Guangyao et al. (1980) The light activation of ribulose-1,5-bisphosphate carboxylase. *Acta Phytophysiologica Sinica*, (6): 277—281 (in Chinese)