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The activity and content of ribulose-1,5-bisphosphate carboxylase/oxygenase in wheat plants as affected by water stress and kartolin-4

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Abstract

The carboxylating activity and content of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO, EC 4.1.1.39), and other soluble proteins in young seedlings and mature leaves of Lutescens-758, a drought-sensitive cultivar of soft spring wheat *Triticum aestivum* L., were studied under the conditions of drought and subsequent rehydration. Seedlings and mature plants preliminarily treated with the cytokinin-like compound kartolin-4 were compared to untreated plants. Drought-induced decrease in RuBPCO activity should be attributed not only to proteolytic decomposition of the enzyme protein itself but also to a partial inhibition of its catalytic activity. The decrease in RuBPCO activity was larger than that in RuBPCO content. Water stress induced a marked decrease in the soluble protein content. Kartolin-4 increased the resistance to drought.

Additional key words: cytokinin; drought; proteins; *Triticum aestivum*.

Introduction

Dark and light photosynthetic reactions depend to a different extent on the duration and severity of drought. Soil drought impairs the pigment content in chloroplasts, operation of photosystems, efficiency of carboxylation and photosynthetic carbon assimilation (see reviews of Šesták and Pospíšilová 1986, Zholkevich *et al.* 1989, Tarchevskiĭ 1993, Chernyad'ev 1997). As the drought becomes more severe, there is a trend toward deceleration of the export of photoassimilates (Gamaleĭ 1994) and inhibition of photosynthesis (Nilsen 1992, Muthuchelian *et al.* 1994, 1997, Fedina and Popova 1996, Chernyad'ev 1997). The drought-induced ATP deficiency causes an uncoupling of dark reactions to the light ones and also inhibits the biosynthesis of reserve (starch) and transport (sucrose) photosynthates (Tarchevskiĭ 1993).

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The literature contains conflicting opinions of the water stress effects on the pivotal photosynthetic enzyme RuBPCO. Its activity either remained unchanged, or increased, slightly decreased, even fell sharply (for a review see Chernyad'ev 1997). The loss of the O_2-evolving thylakoid polypeptides (33, 23, 17 kDa), and the large (55 kDa) and small (15 kDa) subunits of RuBPCO was found in water-stressed seedlings of *Erythrina variegata* Lam. (Muthuchelian *et al.* 1997).

Adaptation to drought and other unfavourable factors is accompanied by structural and functional changes in the photosynthetic apparatus, the leading role in the process being played by cytokinins and other phytohormones. A cytokinin-induced stimulation of synthesis of the most important proteins increases the plant resistance to various stress factors, including water stress (Kulaeva 1982, Kislyakova *et al.* 1989, Chernyad'ev 1997). Cytokinins, among others 6-benzylaminopurine (BAP) or 6-furfurylaminopurine, affect the net photosynthetic rate (P_N) (Chernyad'ev *et al.* 1986, Uprety and Tomar 1993, Muthuchelian *et al.* 1994), photochemical activity (Doushkova *et al.* 1989, Soeda *et al.* 1990, Muthuchelian *et al.* 1994), and the carboxylating activity and content of RuBPCO (Lerbs *et al.* 1984, Chernyad'ev 1994).

Compounds with a high cytokinin activity produced in Russia include rather novel ones that are called kartolins (Baskakov 1988). They create a group of four similar compounds with a similarly effective cytokinin-like protection against water stress as well as other plant stresses. In contrast to many natural and synthetic cytokinins, kartolins have a narrower range of physiological cytokinin-like activities (Kulaeva 1985, Baskakov 1988). For example, they do not activate the cell growth in etiolated leaves, development of excised cotyledons, chloroplast biogenesis, *etc.* The kartolins affect the P_N, activities of RuBPCO and other photosynthetic enzymes, pigment system, and ultrastructure of leaf plastids in crop plants exposed to drought (Kulaeva 1985, Baskakov 1988, Kislyakova *et al.* 1989, Chernyad'ev 1997).

The purpose of present work was to find and explain the protective effects of kartolin-4 (oxycarbam), a recently synthesized member of the kartolin group, on the carboxylating activity and content of RuBPCO and other soluble proteins in water-stressed seedlings and adult plants of wheat.

Materials and methods

Seedlings: Dry seeds of Lutescens-758, the drought-sensitive cultivar of soft spring wheat *Triticum aestivum* L., were sterilized in 0.02 % $KMnO_4$ for 30 min, washed by distilled water, and divided into two parts. They were then kept for 4 h either in aerated distilled water (control seeds) or in aerated solution of kartolin-4 (100 g m^{-3}). The seeds were then placed on glass plates covered with moistened filter paper and allowed to germinate for two days in trays. The trays were covered with glass and kept in the dark. Etiolated seedlings were transferred to a greenhouse and grown at 14-h photoperiod (irradiation with hydrogen lamps, 50 W m^{-2}, at 22 °C and 70 % relative humidity). Initial samples were taken after 5 d of growing. The remaining

plants were deprived of water for 1 d, and then the second sample was taken. After a following 1-d rehydration, the third sample was taken.

Plants were grown in 5-kg-containers (10 plants per container) with soil (50 %), sand (25 %), peat (25 %), and [mg kg^{-1}] 75 N, 46 P, and 42 K. At the stage of tillering, the plants were sprayed with kartolin-4 solution (0.04 mg per plant). Control plants were sprayed with water. Soil moisture in the containers was maintained at 70 % of full water capacity (FWC) by daily watering. Two weeks after the treatment with kartolin-4, half of the experimental plants was exposed to soil drought for two weeks. During the drought, the soil humidity decreased to 20 % of FWC. After termination of drought, mass of these containers was brought to the mass of control containers with water, and the plants continued to grow up to grain production under normal watering (70 % of FWC).

In all variants the subflag leaves from the main shoots were sampled. The first leaf sample for analyses was collected before the drought imposition, the second sample was collected immediately after drought period, and the third one was collected one week after the watering was resumed.

Soluble proteins were assayed by the method of Bradford (1976). Proteins were extracted from the leaves with an isolation medium containing 0.05 M Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM dithiothreitol, and 0.01 M saccharose. Plant samples (500 mg) were ground in a mortar in 5 cm^3 of the medium and then centrifuged (20 000×*g*, 20 min). The resulting supernatant was decanted, the pellet was washed with 5 cm^3 of the isolation medium, and centrifuged again. The supernatants were combined and assayed for soluble proteins.

Cell-free preparations for determining the carboxylating activity were obtained by our method (Chernyad'ev 1994). In addition to the components listed above, the isolation medium contained 2 % insoluble polyvinylpyrrolidone. After filtration through a dense gauze, the homogenates were centrifuged at 20 000×*g* for 30 min. All manipulations were carried out at 2-4 °C.

RuBPCO carboxylating activities were assayed radiometrically by a standard method (Chernyad'ev 1994). Gel electrophoresis in 7.5 % polyacrylamide gel (PAGE) was used for quantitative determination of RuBPCO content (Monakhova *et al.* 1987). After electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250, and then subjected to scanning densitometry. The area of RuBPCO peak was used for evaluating the relative content of this enzyme in the total pool of leaf soluble proteins. In addition, the stained bands of RuBPCO and other leaf proteins were extracted and their absorbance was measured by spectrophotometry as described by Esen (1978). The molecular mass of RuBPCO [kDa] was established by comparison with a set of marker proteins: thyroglobulin (669), ferritin (440), catalase (232), lactate dehydrogenase (140), and bovine serum albumin (67).

Chlorophylls *a* and *b* were determined spectrophotometrically in 85 % acetone extracts (Monakhova *et al.* 1987).

Results and discussion

The carboxylating activities of RuBPCO in control and kartolin-4-treated seedlings or plants were similar before water stress imposition (Table 1). The RuBPCO activity in leaves of adult plants was higher than that in young plants.

Table 1. Effect of drought (measured before drought, after drought, and after reparation) on the activity of RuBPCO [mmol kg^{-1}(protein or f.m. or d.m. or chlorophyll) s^{-1}] in seedlings and leaves of wheat treated (K) and untreated (C) with kartolin-4. Means ± SE were calculated from 3 independent experiments with 4 determinations in each. Values in bracketts are in %. $^{*}p<0.05$.

Drought		Carboxylating activity per protein	per f.m.	per d.m.	per chlorophyll
	Seedlings				
Before	C	11.30±0.33 [100]	0.28±0.01 [100]	2.03±0.06 [100]	319.1±4.7 [100]
	K	10.70±1.33 [94.7]	0.27±0.01 [96.4]	1.80±0.02 [88.7]	269.1±4.7 [88.4]
After	C	6.30±0.32 [100]	0.18±0.02 [100]	0.79±0.01 [100]	138.6±2.1 [100]
	K	8.70±0.57 [138.1]*	0.26±0.01 [144.4]*	1.13±0.01 [143.0]*	195.5±2.9 [141.1]*
Repar.	C	7.00±0.51 [100]	0.12±0.01 [100]	0.77±0.01 [100]	156.8±2.4 [100]
	K	9.00±0.17 [128.6]*	0.16±0.01 [133.3]*	1.05±0.01 [136.4]*	189.9±2.8 [121.1]*
	Leaves				
Before	C	15.20±0.50 [100]	0.57±0.02 [100]	3.58±0.07 [100]	402.1±15.0 [100]
	K	15.00±0.67 [98.7]	0.60±0.02 [105.3]	3.90±0.08 [108.9]	421.7±13.8 [104.9]
After	C	8.30±0.33 [100]	0.28±0.02 [100]	1.31±0.06 [100]	198.2±7.3 [100]
	K	11.00±0.34 [132.5]*	0.44±0.01 [157.1]*	2.22±0.05 [169.5]*	286.7±9.5 [144.6]*
Repar.	C	12.50±0.50 [100]	0.39±0.01 [100]	1.66±0.05 [100]	304.6±10.0 [100]
	K	14.30±0.83 [114.4]	0.49±0.02 [125.6]*	2.76±0.06 [166.3]*	376.7±12.5 [123.7]*

In water-stressed seedlings or plants, RuBPCO activity per any unit decreased. However, the activity of RuBPCO remained higher in kartolin-4-treated samples than in the control ones. For example, the carboxylating activity of RuBPCO per soluble protein unit decreased by more than 40 % in control seedlings, whereas it was lower by 20 % in kartolin-4-treated seedlings. The RuBPCO activity was almost half-inhibited by water stress in leaves of untreated adult plants, whereas this effect did not exceed 25 % in kartolin-4-treated plants.

After rehydration, there was an increase in RuBPCO activity although the recovery of activity was not complete in both the control and kartolin-treated plants. However, the extent of RuBPCO recovery per unit of soluble protein induced by 7-d-long reparation was 95 % of the initial level in kartolin-treated adult plants and less than 80 % in control plants. In seedlings, the RuBPCO activity calculated per protein unit recovered to 85 % of the initial level in kartolin-treated but only to 60 % in control samples.

In order to study the mechanism of protective effects of kartolin-4 on RuBPCO during water stress, we determined the contents of RuBPCO protein and other

soluble proteins and compared these results with the enzyme activity. Isolation of soluble proteins from leaves and their separation by PAGE showed that wheat RuBPCO had a characteristic low electrophoretic mobility and was concentrated in the broadest band. The protein had a molecular mass of 510 kDa.

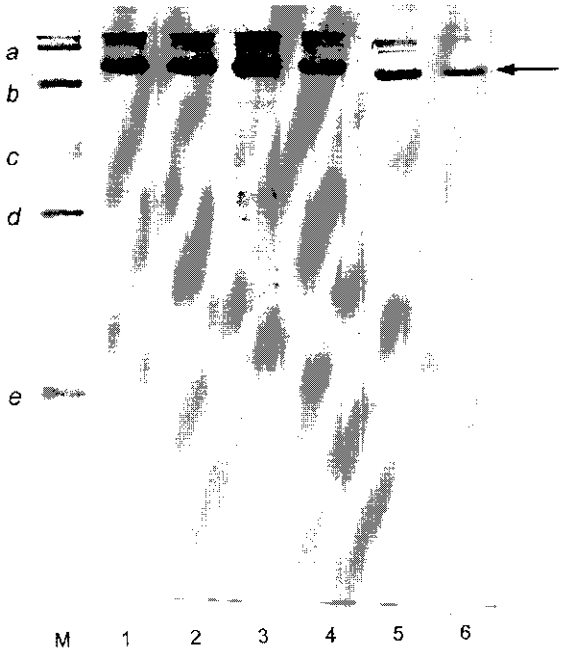

Fig. 1. PAGE of wheat seedling soluble proteins: M, marker proteins [kDa]: *a*, thyroglobulin, 669; *b*, ferritin, 440; *c*, catalase, 232; *d*, lactate dehydrogenase, 140; *e*, albumin, 67. Treatments: *1* - before water stress, kartolin-4; *2* - before water stress, control; *3* - after water stress, kartolin-4; *4* - after water stress, control; *5* - after rehydration, kartolin-4; *6* - after rehydration, control. The arrow indicates RuBPCO.

PAGE (Fig. 1) showed that control and kartolin-4-treated samples exhibited similar RuBPCO coloration. This was confirmed by gel scanning (Fig. 2) and direct spectrophotometry of the dye eluted from this zone. In kartolin-4-treated and control plants under normal water supply, the RuBPCO content and activity were similar. When the activity of RuBPCO was expressed per fresh and dry mass, the same regularity was found because kartolin-4-treated and control plants contained equal amounts of soluble proteins under normal conditions (Table 2).

Water stress produced only a small decrease in the content of RuBPCO protein in kartolin-4-treated plants, whereas a 30 % decrease was found in the control samples (Fig. 2). The activity of RuBPCO decreased by 20 and 40 % in these samples, respectively (Table 1).

The results of our earlier studies of the drought-induced changes in ultrastructure of barley chloroplasts (Kislyakova *et al.* 1989) suggest that the plastids in untreated (control) plants are more degraded than the plastids in plants treated with kartolin-4.

In control plants, stromal proteins including RuBPCO were released from degraded organelles and subjected to proteolysis. The chloroplast membranes from leaves of kartolin-4-treated plants were more intact, and RuBPCO activity was less inhibited.

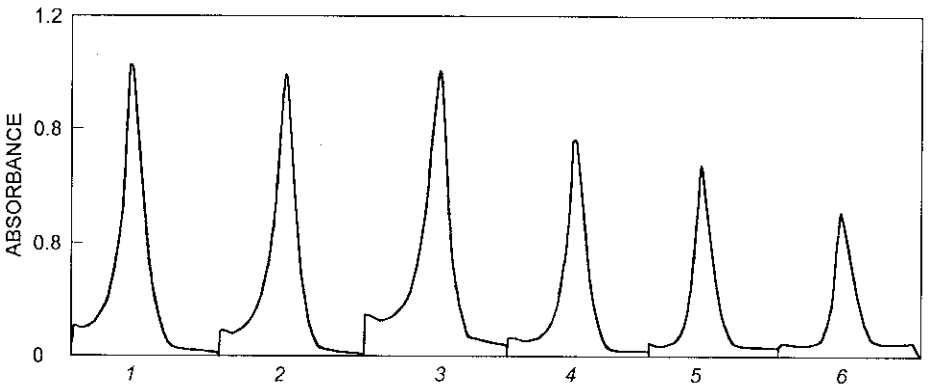

Fig. 2. Peaks corresponding to the gel scanning bands of RuBPCO. For treatments, see Fig. 1.

Table 2. Effect of drought (measured before drought, after drought, and after reparation) on the content of soluble proteins [g kg^{-1}(d.m.) s^{-1}] in seedlings and leaves of wheat treated (K) and untreated (C) with kartolin-4. Means ± SE were calculated from 3 independent experiments with 4 determinations in each. Values in brackets are in %. $^{*}p<0.05$.

		Seedlings	Leaves
Before	C	178.54±5.96 [100]	236.28±2.85 [100]
	K	177.81±8.18 [99.6]	230.07±6.30 [97.5]
After	C	123.56±2.96 [100]	159.67±3.62 [100]
	K	130.78±3.79 [105.8]	202.13±2.92 [126.6]*
Reparation	C	109.74±5.76 [100]	133.18±3.80 [100]
	K	116.70+5.13 [106.3]	192.35±2.69 [144.4]*

The results of extraction of soluble proteins (Table 2) indirectly support this suggestion: drought induced a significant decrease in the soluble protein content normalized per unit of dry mass in the seedlings and leaves of both treated and untreated plants. Water stress was reported to decrease the protein content in leaves of *Cajanus cajan* L. (Nandwal *et al.* 1991) and *Pisum sativum* L. (Fedina and Popova 1996). According to our results, drought-induced decrease in leaf protein content per unit of dry mass in kartolin-4-treated *T. aestivum* L. plants was about 12 %, while in untreated plants it was about 30 %. Under the same conditions, the RuBPCO carboxylating activity per unit dry mass decreased in experimental and control plants by *ca.* 40 and 60 %, respectively. In seedlings a sharper decrease in the RuBPCO activity was observed as compared to the not so evident decrease in RuBPCO content.

Our results show that the drought-induced decrease in RuBPCO activity should be attributed not only to the proteolytic decomposition of the enzyme protein itself, but

also to a partial inhibition of its catalytic efficiency. A significant (>30 %) decrease in the RuBPCO content was induced by a three-week drought in the leaves of *Mesembryanthemum cordifolium* L. (Wang *et al.* 1991). The RuBPCO activity in willow leaves under water stress declined although the enzyme protein content changed insignificantly (Vapaavuori 1986). In that work, the effect was explained by a modification of RuBPCO activity by various effectors (ADP, ATP, NADPH, P_i, *etc.*), which may either stimulate or inhibit the carboxylating activity. Because the Calvin cycle in C_3-plants critically depends on the ATP content, the ATP content in chloroplasts is significantly reduced under water stress (Tarchevskiĭ 1993). The higher rates of photophosphorylation in kartolin-treated plants during drought may provide an additional supply of ATP (Kislyakova *et al.* 1989). The reduction in RuBPCO activity in the leaves of *Citrus sinensis* L. (Osbeck) subjected to water stress was due to a decrease in RuBPCO content and partly to the reduced CO_2-Mg^{2+} enzyme activation (Vu and Yelenosky 1988).

UV-induced stress provoked by degradation of the ozone layer can impair the RuBPCO target, because the UV-induced covalent dimerization of one small and one large subunits of RuBPCO results in the formation and accumulation of 65 kDa polypeptide that interferes with the enzyme activity (Ferreira *et al.* 1996). The drought-induced control of RuBPCO activity possibly operates at the level of quaternary structure of the enzyme. However, this hypothesis should be tested in further studies.

Thus, our results indicate that water stress inhibits the carboxylating activity and decreases the RuBPCO content which is improved by kartolin-4. Our results confirm the protective role of kartolin-4 in photosynthesis of wheat plants subjected to water stress.

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