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Effects of Water Deficit on Photosynthesis in Wheat Plants

III. Effect on non-stomatal mediated photosynthesis and RuBP carboxylase content in different plant parts*

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Abstract : In order to explore the physiological basis of the difference in photosynthetic depression by soil water deficit, the non-stomatal mediated photosynthesis (NSP) and ribulose-1, 5-bisphosphate carboxylase (RuBPCase) content were compared among different parts of a wheat plant grown under soil water deficit condition. The depression of NSP due to water stress was larger in the lower leaf blades than in the upper ones, and in leaf blades than in ear, sheath and stem. The content of RuBPCase was decreased by soil water deficit to a larger extent in the lower leaf blades than in the upper ones, and in leaf blades than in stem and leaf sheath. But, in the ear, the extent of RuBPCase decrease was relatively large, compared with that of photosynthetic depression. The results here suggest a possibility that the difference among plant parts in photosynthetic depression by soil water deficit is partly attributed to the different sensitivity of photosynthetic apparatus to water stress.
Key words : Ear, Leaf position, Photosynthesis, RuBP carboxylase, *Triticum aestivum* L., Water stress, Wheat.

水欠乏がコムギの光合成に及ぼす影響 第3報 気孔の影響がない条件下の光合成速度および RuBPCase 含量に及ぼす土壌水分欠乏の影響：徐 会連・石井龍一・山岸 徹・玖村敦彦（東京大学農学部）
要 旨：本研究の前報まで，土壌水分欠乏による光合成の低下程度は植物体の部分によって異なることを報告した．本報では光合成機構に及ぼす土壌水分欠乏の影響を調べるために，気孔の影響のない条件下の光合成（Non-stomatal mediated photosynthesis, NSP）を，酸素電極法により測定した．NSP に及ぼす土壌水分欠乏の影響を部分別に見ると，老化が進んだ第3葉葉身が最も大きな影響を受け，穂への影響が最も小さかった．茎，葉鞘，止葉はほぼ両者の中間であった．つぎに，NSP と最も関連が深いと考えられる CO_2 固定酸素，RuBP カルボキシラーゼ（RuBPCase）含量を調べた．その結果，RuBPCase 含量の土壌水分欠乏による低下も，ほぼ NSP の傾向と対応していた．ただし，穂において，土壌水分欠乏による RuBPCase 含量の低下程度は光合成の低下程度に比べて，大きかった．
キーワード：RuBP カルボキシラーゼ，気孔，光合成，コムギ，土壌水分欠乏，穂，水ストレス，葉位．

It is reported by many researchers[2,5,15,17] that the effect of soil water deficit on photosynthesis is not only through stomata, but also through photosynthetic machinery.

Our previous papers[25,26] also showed that the difference between plant parts in photosynthetic depression by soil water deficit, was caused by the difference in the increase of stomatal and mesophyll CO_2 diffusion resistances.

In this paper, therefore, to elucidate the mechanism of different photosynthetic depression among plant parts by soil water deficit, non-stomatal photosynthesis (NSP) in each part of a water stressed plant was measured by the oxygen electrode method, in which the effect of stomata could be excluded. Furthermore, ribulose-1, 5-bisphosphate carboxylase (RuBPCase) content, which is considered to be the most important factor relating to NSP, was also examined in different plant parts.

Materials and Methods

1. Plant materials and application of soil water deficit

The seeds of wheat (*Triticum aestivum* L. cv. Asakaze-komugi) were sown in 1/2000 a Wagner pots in November 1986. When the seedlings were established, 10 plants were left per pot by thinning, and grown outdoor with sufficient water until the next spring. A plastic pipe with many small holes was inserted vertically into the soil in the pot, and water was

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supplied from the pipe. The holes contributed to the uniform distribution of water in the pot. When the plants reached the anthesis stage, the pots were transferred to an environment controlled growth cabinet in the Center of Environment Regulation System for Biology (CERES) of the University of Tokyo, where the temperature, relative humidity, and light intensity were maintained at 25/20°C (day/night), 75%, and 600 μmol PPFD m^{-2} s^{-1}, respectively. Two days after the transfer, the pots were grouped into two soil water regimes, i.e. 75% (control) and 40% (water stressed) of the field capacity. In the water stressed regime, when the soil water content decreased to 40% of the field capacity, the pots were watered to regain 45%. In the next evening, the soil water content decreased again to about 35%, and the pots were rewatered. This watering method led the water stressed plot to 40% of soil water content on a day average. In the control plot, watering was made in the manner as in the water stressed plot, except that the soil water content before rewatering was 65% and that after rewatering was 85%.

2. Measurement of non-stomatal mediated photosynthesis

Non-stomatal mediated photosynthesis was determined according to the method proposed by Ishii et al[10] on 8 days after the start of soil water deficit treatment. The reaction medium contained 50 mM HEPES (pH 7.2), 0.5 mM $CaCO_3$ and 20 mM $NaHCO_3$. Osmotic potential of the reaction medium was adjusted with sorbitol to −0.3 MPa, which was nearly the same water potential as in the control plant.

One cm^2 of the tissue was cut out of a leaf blade, stem, and leaf sheath, and one half of a spikelet was excised from the central part of a ear. The tissue was sliced into sections of about 1 mm^2 with razor blade. The sections were vacuum-infiltrated with reaction medium. After the O_2 concentration in the medium is stabilized, measurement of photosynthetic rate was started by turning the light on. Temperature of the reaction medium was maintained at 25°C by circulating the temperature controlled water in the external jacket of the reaction cup. The cup was illuminated by a projector lamp with the intensity of 900 μmol PPFD m^{-2} s^{-1}. The measurement was replicated 3 times for each plant part.

3. Determination of RuBPCase content

The RuBPCase content was measured 10 days after the soil water deficit treatment. RuBPCase was extracted using the method by Makino et al[11]. Fifteen cm^2 each of leaf blade, stem, and leaf sheath, as well as one whole ear was used for the extraction. The leaf blade area was calculated from the length and the mean width. The surface areas of stem and leaf sheath were calculated from the length and the mean diameter of a cylindrical section. The ear surface area was calculated from the length and the width of a ear, according to the method by Qiu et al[18]. The RuBPCase content was determined by the method of single radial immuno diffusion according to the method by Matsuhashi et al[12]. Specific antibodies against RuBPCase were supplied by Wada[23], and purified RuBPCase (Sigma) was used as a standard protein. A standard curve was plotted beforehand, and the concentration of enzyme was calculated from the regression line.

Results

The effects of soil water deficit on non-stomatal mediated photosynthesis (NSP) were shown in Table 1. It was shown that depression of NSP by soil water deficit treatment tended to be larger in the leaf blades than in the ear, the stem and the leaf sheath, and larger in the lower leaf blades than in the upper ones. This suggested that the photosynthetic apparatus was more sensitive to soil water deficit in the lower leaf blades than in the upper leaf blades, and in the leaf blades than in the ear, the stem and the leaf sheath. These results are consistent in the trend of changes in mesophyll conductance which was reported in the previous paper[26]. The results in present paper suggest that photosynthetic depression by soil water deficit in field conditions was, at least in part, attributed to NSP inhibition, and consequently that the difference among plant parts in photosynthetic depression was partly attributed to the differing sensitivities of NSP mechanism to soil water deficit.

NSP measured with oxygen electrode is considered to be the integrated activity of many photosynthetic processes. The most important factor related to NSP is the content and/or the activity of RuBPCase. Table 2

Table 1. The effect of soil water deficet on the non-stomatal mediated photosynthesis in the different parts of a wheat plant.

Pant part	Photosynthesis (μmol O_2 dm^{-2} h^{-1})	
	Control	Stressed
Flag leaf blade	337±4	283±5 (84)
2nd leaf blade	330±4	264±6 (80)
3rd leaf blade	317±6	238±8 (75)
Ear	390±8*	420±5 (103)*
Stem	163±4	137±5 (84)
Flag leaf sheath	217±5	182±7 (84)

Valus are mean of three measurements with standard errors.
*μmol O_2 (100 spikelets)$^{-1}$ h^{-1}.
Fingures in the parenthesis are relative values against control.

Table 2. RuBP carboxylase content in the different parts of a wheat plant in soil water dificit and control plots.

Pant part	RuBP carboxylase content (mg dm^{-2})	
	Control	Stressed
Flag leaf blade	26.0±2.1	21.8±2.0(84)
2nd leaf blade	22.1±2.3	17.2±1.7(78)
3rd leaf blade	24.0±2.6	15.5±1.7(65)
Ear	20.8±3.2	16.2±1.9(78)
Stem	28.4±2.0	24.2±2.8(85)
Flag leaf sheath	16.5±1.6	14.5±1.8(88)

Valus are mean of nine measurements with standard errors.
Fingures in the parenthesis are relative values against control.

shows the change of RuBPCase content in response to soil water deficit. RuBPCase contents in all plant parts were reduced by soil water deficit treatment. The decrease in RuBPCase content was larger in the lower leaves than in the upper ones, and in the leaf blades than in the ear, the stem and the leaf sheath. This trend in RuBPCase content was in parallel with that of NSP, suggesting that the photosynthetic decrease by soil water deficit seems to be attributed to the breakdown of RuBPCase. However, the decrease of RuBPCase content in the ear was more than that expected from the decrease of NSP. This implies that the drought resistance in the ear might be attributable to some other factors than RuBPCase content.

Discussion

As to the differences in the extent of drought resistance between plant parts, so far there have been a few papers[19,24] dealing only with leaf position. However, the differences between more plant parts including the stem, the leaf sheath and the ear have not been examined, and information regarding the mechanisms which cause these differences are still obscure.

Photosynthetic depression caused by soil water deficit has been usually considered as the result of stomatal closure[4,8]. The result of this paper, however, showed that soil water deficit could cause not only stomatal closure but also nonstomatal mediated photosynthetic depression, as suggested by many other researchers [1,3,7,9,16,21,27].

The difference between plant parts in nonstomatal mediated photosynthetic drought resistance, could be associated with the characters of mesophyll cells and chloroplasts in each part. Duan et al[6] reported that the upper leaf blades of wheat have more multi-linked mesophyll cells than the lower leaves. Further, Zuo and Duan[28] reported that the ultrastructures of chloroplasts varied with the differently positioned leaves, and that the chloroplast in the upper leaves contained more stacked grana lamellae and larger number of ribosome in the stroma, compared with those in the lower leaves. Therefore, the difference of NSP depression between the plant parts might be associated with these ultrastructural difference in mesophyll cells and chloroplasts of the plant part.

The present paper suggested that the difference of photosynthetic drought resistance between the plant parts could be attributed to the maintenance of RuBPCase content. At the same time, it suggested also that the drought resistance of photosynthetic mechanism in the ear might be associated with some factors other than RuBPCase content. Singal et al[20] examined the enzyme activities of CO_2 fixating metabolism in a flag leaf, and different parts of a ear in a wheat plant. They found that the glumes, pericarp and awn, in a ear, have the recapturing mechanism of respired CO_2 with PEP carboxylase. Furthermore, it

was reported in several papers[13,14,22] that PEP carboxylase was more drought tolerant than RuBPCase. The results obtained in this paper will be supported by the works above mentioned.

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