Studies on the Salt Tolerance of Crops
Variations in Electrophoretic Patterns of Water-Soluble Proteins of Saline Crops (2)※

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作物の耐塩生態型に関する研究
水溶性たん白質の Electrophoretic Pattern の変化 (2)

小　合　龍　夫

In the previous investigations in this series, the nature of saline injury to crops was studied and the author's attention had been focused on the causes for reduced development of toxicity symptoms resulting from nitrogen supply. Then the changes occurred in the nitrogen fractions in crops were traced at successive growth stages in relation to the variation in their salt tolerance with environment. The correlation observed between the changes in water-soluble proteins in crops and their salt tolerance suggested that the constituent bore some causal relationship to the mechanism of salt tolerance (1).

In this paper previous published data (2) on the differences between water-soluble proteins in crops were reevaluated and multiple forms of acid phosphatase were also investigated in extracts of wheat plants grown under saline conditions.

The electrophoretic multiplicity of water-soluble proteins extracted from crops can easily be studied by polyacrylamide gel as demonstrated in previous report (2)※※※. Analysis of changes in the enzymatic pattern may allow some insight into the physiological role of proteins in relation to salt tolerance and may reveal the physiological role and the significance of multiple forms of the enzyme.

The author is grateful to Professor K. Adachi of this laboratory for his helpful discussions during the course of this work. Thank is also due to Professor H. Hasegawa, Head of Crop Science Laboratory, University of Kyoto, for some valuable suggestions.

Materials and Methods

The wheat plants, cv. Nōrin No. 27 and Nōrin No. 20, were grown in sand and irrigated with a nutrient solution as shown previously (1).

The steps in the preparation of the dry extracts were all carried out under a temperature of 4 °C. The wheat leaves were homogenized in deionized water. After 2 centrifugations (15 min at 3500 × g and 30 min at 15000 × g), the clear supernatant was dialyzed against deionized water for 24 hr. The dialysate was lyophylized and the dry extracts were stored at −20 °C.

Protein separation was carried out with electrophoresis on polyacrylamide gel at pH 8.9 (0.15 M Tris-EDTA buffer). The samples consisted of 5—10 % solutions of the dry extracts in buffer. Protein bands were detected by staining with 1 % Amido Black 10 B

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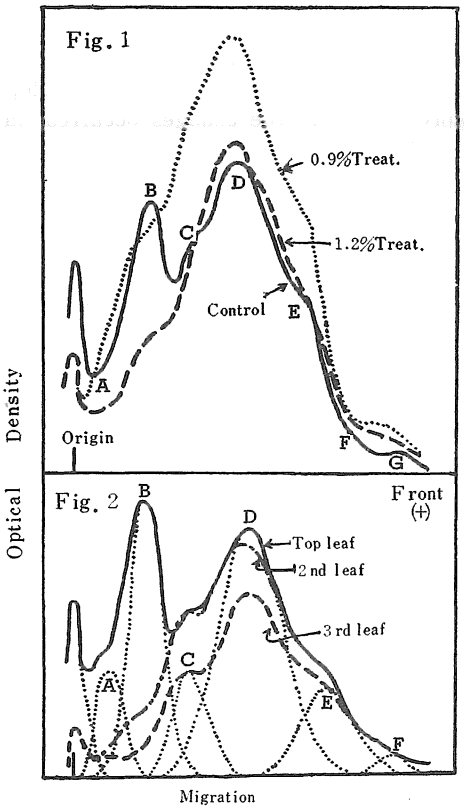
※※※ Correction for previous report in No. 14—A (1965) : Page 2 ; for pH 8.9 read pH 6.4

in a solvent of methanol-water-acetic acid (5 : 5 : 1) and destaining in this solvent (2). Diazonium dye method (3) was used for detection of acid phosphatase bands in the gels. After electrophoresis, the gels were incubated twice for 15 minutes in acetate buffer (pH5.1) and then incubated in reaction mixture (40 ml M/5 acetate buffer, pH 5.1; 40 mg Diazo Blue B ; 40 mg α-naphthylphosphate) until sharp bands developed, usually in 2—4 hours at 35 °C.

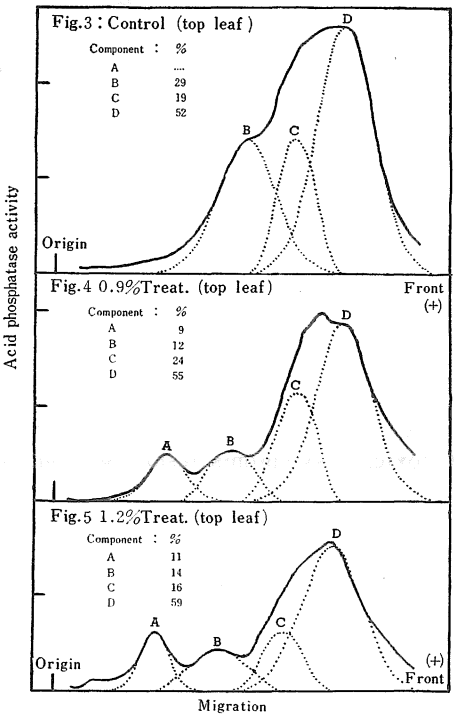
Results and Discussion

The extractable proteins of wheat leaves consisted of 8 or more fractions. Attention



Treat.	Component (%)						
	A	B	C	D	E	F	G
Fig. 1							
Cont.	5	24	10	36	19	4	2
0.3%	5	20	6	33	30	3	3
0.9%	6	16	11	38	25	2	2
1.2%	5	16	10	32	28	6	3
Fig. 2							
Top leaf	9	26	10	42	12	1	—

Fig. 1—2. Curves of optical density and migration velocity for the protein fractions in spectra of photo. 1—2.



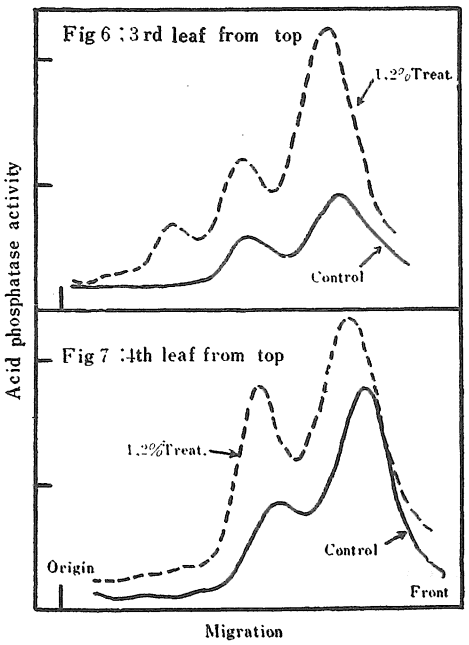


Fig. 3—7. Densitometer scanning of gels, stained for acid phosphatase with Diazo Blue B and α-naphthylphosphate. (Photo. 3—5)

was drawn to the great difference observed as to the level of specific fraction during early stages after the saline treatment as well as the observation of proteins from rice leaves (2). The response at the level of fraction-B (2 nd band) of proteins from the leaves (3 rd leaf from top) developed after saline treatment tended to decrease (fig. 1, photo. 1) and the level of fraction-E (5th band) tended to increase, even if toxicity symptoms in the leaves did not appear. In the saline leaves, however, the decrease of leaf-length resulted representatively from saline treatment was observed and the data on 2 nd leaf (cv. Nōrin No. 27) were as follows : Control leaf : 22.3 cm, 0.3% NaCl treat. leaf : 18.4 cm, 0.6% treat. : 16.7 cm, 0.9% treat. : 16.1 cm, and 1.2% treat. : 14.9 cm. This decrease at the level of fraction-B was also visible in lower leaves (obviously green) of control plants (fig. 2, photo. 2). In acid phosphatase composed of 4 active fractions (A, B, C, D) towards α-naphthylphosphate, fraction-A with low mobility in extracts from top leaves became very pronounced with saline treatment and the activity of fraction-B decreased during early stages after saline treatment (fig. 3, 4, 5, photo. 3), but this tendency was obscured in lower leaves, being obviously green (fig. 6, 7, photo. 4, 5). On the other hand both fraction-A and fraction-B activities decreased with aging of a leaf in control plants (photo. 6). Thus every change with saline treatment was accompanied by a change in acid phosphatase pattern, but whether an altered acid phosphatase pattern was responsible for disappearance or development of salt tolerance could not be determined yet. It would be of interest to investigate further, if there are any correlations between enzymatic pattern in different crops and their salt tolerance.

Summary

Wheat leaves contained 8 or more protein fractions and 4 acid phosphatase fractions. After the saline treatment, the level of fraction-B of proteins tended to decrease and acid phosphatase fraction-A in extracts from top leaves became very pronounced but the activity of fraction-B decreased during early stages. The correlation between an altered acid phosphatase pattern and salt tolerance could not be determined yet.

Literature Cited

1. Ogō, T. : Spec. Rep. Lab. Crop Sci. Shimane Agr. Coll. 1 : 1～105, 1964
2. Ogō, T. : Bull. Shimane Agr. Coll. 14 (A) : 1～2, 1966
3. Rudolph, K and Stahmann, M. A. : Plant Physiol. 41 : 389～394, 1966

摘　　要

本報は作物の耐塩生態条件の一つとして重視している水溶性たん白質画分の役割を解析することを目的とした一連の実験のうち，acid phosphatase pattern の追跡を試みたものである．酵素の multiple form については，いまだ充分の知見が得られていないので，その生物学的意義づけには多くの困難を伴う．しかし酵素 pattern から耐塩生態条件を見いだすことができれば，multiple form の生理的役割の解明に近づくことになるようにも考えている．

小麦葉身の水溶性たん白質は8個以上の fraction に分けられた．塩水下で出葉した葉身（上から第3葉位）では，葉身長の減少は顕著であったが，葉枯れが現われなくても，特定の band の動きが見られ，fraction-B の減少が目だった．しかし，この動きは age による動きとよく似ていた．acid phosphatase は4個以上の fraction に分けられ，抽出葉の pattern では，fraction-B の活性が塩水の処理とともに低下するのに対し，fraction-A の活性は高まるのがみられた．しかし，age の進んだ下位葉の pattern からは，このような動きをはっきり認めることはできなかった．一方 age による pattern の動きでは fraction-A および B の減少がみられ，老葉ほど著しかった．

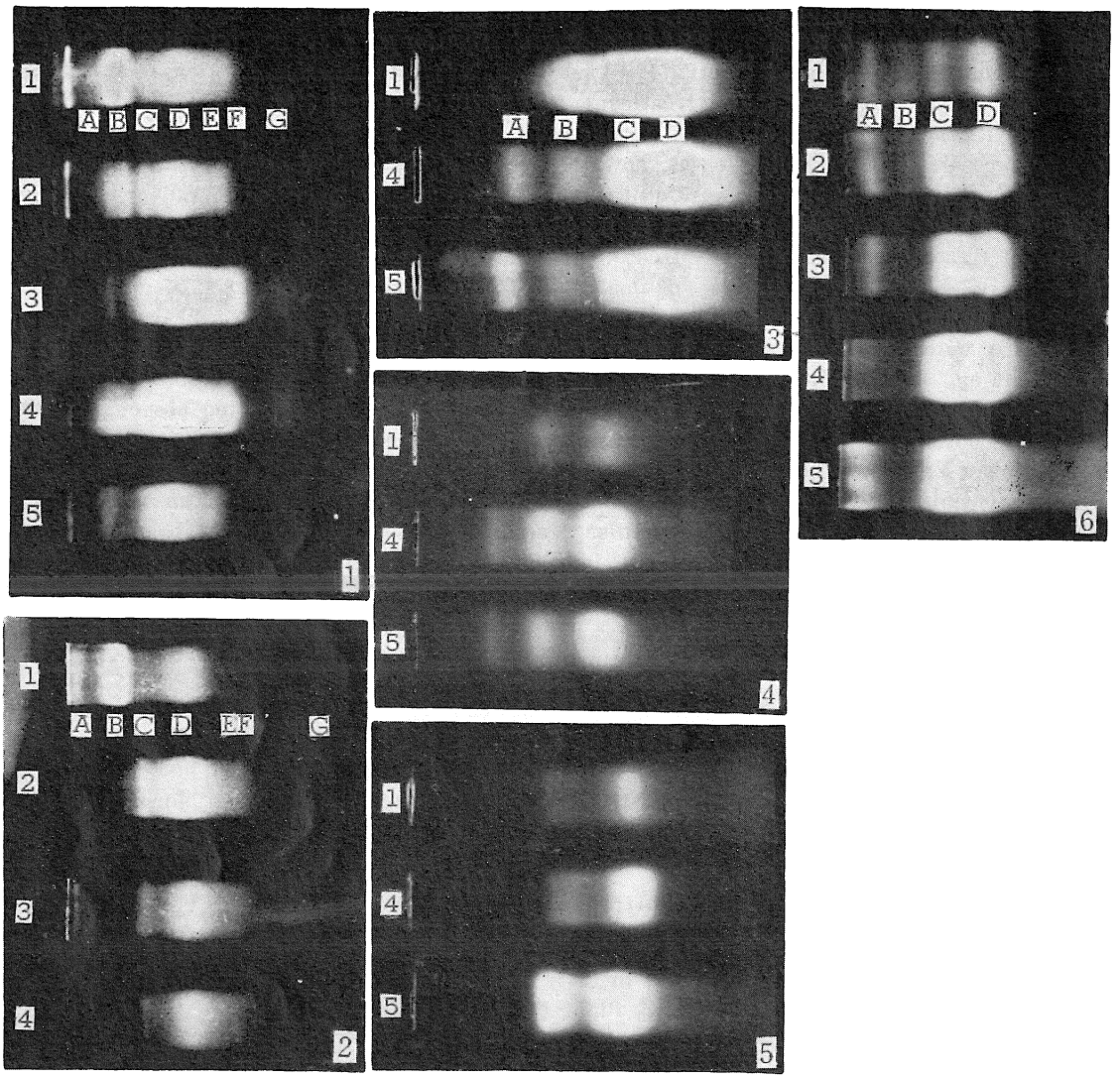


Photo. 1.　Electrophoresis of water-soluble proteins of wheat leaves. (3rd leaf from top, cv. Nōrin No. 27). 1) Control plant, 2) Plant treated by 0.3% NaCl soln. 3) By 0.6% soln. 4) By 0.9% soln. 5) By 1.2% soln.

Photo. 2.　Electrophoresis of water-soluble proteins of wheat leaves. (cv. Nōrin No. 20). 1) Top leaf in control plant, 2) 2nd leaf from top, 3) 3rd leaf, 4) 4th leaf

Photo. 3—5 : Acid phosphatase in wheat leaves (cv. Nōrin No. 27). Photo. 3 : Top leaf : 1), 4) and 5) are given in Photo. 1, Photo. 4 : 3rd leaf, Photo. 5 : 4th leaf

Photo. 6 : Acid phosphatase in wheat leaves given in photo. 2.