BREEDING FOR HIGH PROTEIN AND BALANCED AMINO ACID RICE VARIETIES

II. Application of dye-binding capacity method in rice grain[1)]

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The dye-binding capacity method was first used by Frankel-Conrat and Cooper (1944) as a simple and rapid method for qualitative and quantitative determinations of proteins. Principles of the chemical reations are as follows. An azosulfonic dye solution is mixed with protein-containing samples. The dye then binds with imidazol, quanidine, and ε-amino group of the proteins. These moeities constitute the functioning groups of the basic amino acids, i. e., histidine, arginine, and lysine. The amount of proteins is estimated colorimetrically. This method has been for the estimations of proteins in many crops as reviewed by Mossberg (1969). High correlation coefficients were found between the dye-binding capacity and nitrogen contents in barley, wheat, oats, rye, and corn. Recently, Osone and Takagi (1970), and the workers of International Rice Research Institute (1969) reported that dye-binding capacity method was also applicable for screening rice samples, although the correlation coefficients found were lower than those of other crops. The present work is undertaken to study the cause of the low correlation and to improve the efficiency of the method for rice analysis.

The interrelationship between nitrogen content and dye-binding capacity was studied by using 49 varieties carrying different protein contents. The varieties were grown in the 2nd crop season of 1969. The seed samples contained 13.5% moisture.

For measuring protein contents, samples of dehulled grains (0.1 gr./sample) were analyzed by the micro-Kjeldahl method. The percentage of nitrogen obtained for each sample was converted into protein content by multiplying with a factor of 5.95 (Cagampang 1966).

The dye-binding capacity method described by Mossberg (1969) and Osone and Takagi (1970) were modified in several places. The concentrated solution of Qrange G* was diluted to 1.3 gr./litre with citrate/phosphate buffer* (pH

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2.2). Samples were ground and homogenized in a blender (Fren Stein Lab. Model L Tape 2) for 10 minutes. The resulted particles were about 149 μ in in diameter (100 mesh). The dye-binding capacity value of samples increased according to the time of griding and approached constant after 8 minutes of grinding (Fig. 1). 75 mg of finely ground sample was mixed with 6 ml of dye solution in a 20 ml test tube. This ratio was determined according to the dye limiting test where a linear relationship was achieved between 25 mg to 125 mg of samples using 6 ml the dye solution (Fig. 2). The test tube used were

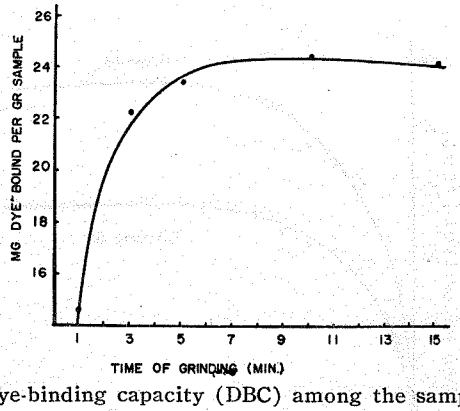

Fig. 1. Dye-binding capacity (DBC) among the samples after being ground by different times.

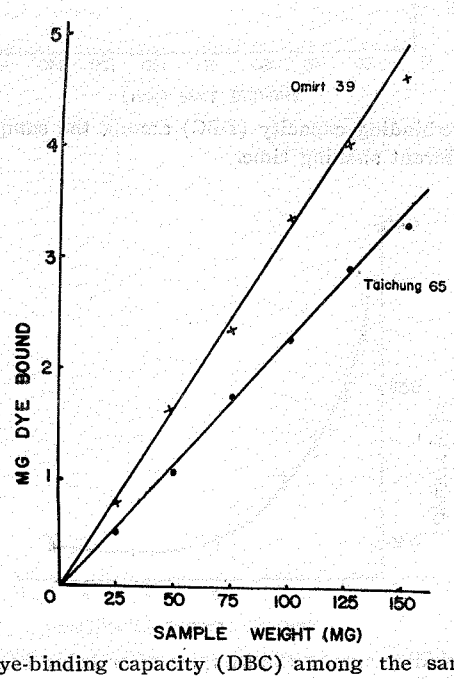

Fig. 2. Dye-binding capacity (DBC) among the samples with different weight.

pluged with stoppers and shaken horizontally for 80 minutes. The dye-binding capacity became constant when the sample was shaken over 60 minutes (Fig. 3). The mixture was centrifuged for 6 minutes at 1000xg. The turbidity became smallest and constant after 5 minutes centrifugations (Fig. 4). Optical density of the supernatant was obtained at 575 nm with a coloremeter (Bauch and Lamp Spectronic 20). Dilution was not necessary when optical desity was obtained at 575 nm with a long light path cuvette. The dye-binding capacity was determined according to the calibrated curves. Each micro-Keldahl determined according to the calibrated curves. Each micro-Keldahl determination

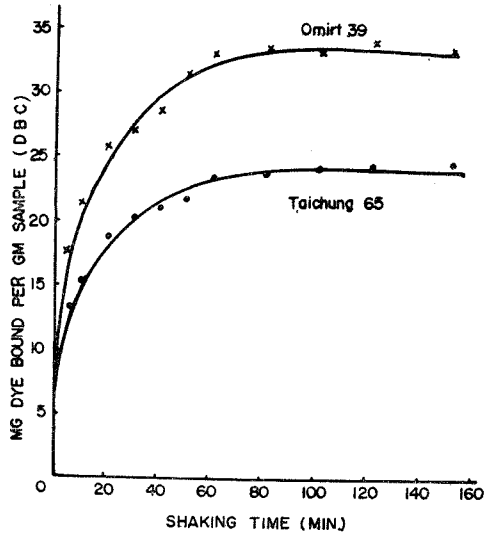

Fig. 3. Dye-binding capacity (DBC) among the samples under different shaking time.

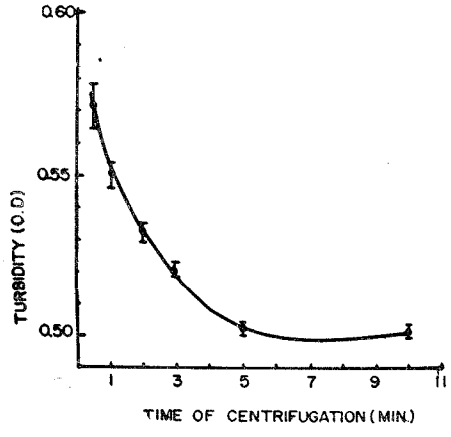

Fig. 4. Turbidity among the samples after being centrifugated by different times.

and the dye-binding capabity of a sample were obtained through three repetitions.

The aforementioned procedure for dye-binding capacity method differs from those of Mossberg (1969) and Osone and Takagi (1970) as listed in Table 2.

Both protein contents and dye-binding capacites of 49 varieties gave wide distributions (Table 1). However they are highly correlated. The correlation coefficient was 0.886 as shown in Fig. 5. The correlation coefficient is higher than that of Osone and Takagi ($r=0.65$) (1970). In the process of preparing this manuscript, we have come to realize that the International Rice Research Institute was able to achieve a correlation coefficient of 0.88 (1969) which is similar to the result of our modified method. However, detailed proceedures of their work are not available to us and therefore we have no way of comparing our techniques. The dye-binding capacity method modified here seems to be well suited for screening of high protein strains in rice.

Table 1. *Comparison of the conditions in DBC method established by three different workers*

	Materials used	Particle size (mesh)	Sample treatment	Dye solution concentration (%)	Sample applied (mg)	Dye solution applied (ml)	Sample of applied (mg) per mg dye
Mossberg (1969)	Wheat, barley, oat, rye, corn,	–	No	0.20	1,000	40	12,500
Osone (1970)	rice	60	dried 2hr. at 104°C	0.20	400	4	50,000
Chan (1971)	rice	100	No	0.13	75	6	9,615

	Shaking time (min)	Filtration	Dilution	Cuvette	Wave length (mμ)	Correlation coefficient between DBC and crude protein contents	No. of sample used
Mossberg (1969)	30–120	glass fibre	No	short light path (0.75 mm)	475	0.93–0.97	7–18
Osone (1970)	180	filter paper Whatman 44	200 folds	long light path	470	0.65	22
Chan (1971)	80	centrifuge 6 min 3000 rpm	No	long light path (11.67 mm)	575	0.886	49

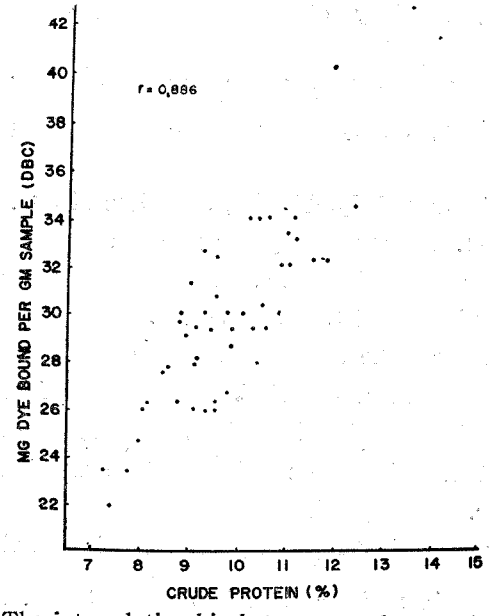

Fig. 5. The interrelationship between crude protein contents and dye-binding capacity (DBC) in 49 rice varieties.

水稻高蛋白質及氨基酸平衡性之育種

色素吸着法在稻米上的應用

詹孝元 周濤聲 朱耀源 李先聞

使用色素吸着法 (Dye-Binding Capacity Method) 和微量定氮法 (Micro-Kejldahl Method) 來分析 49 種不同的稻米品種中蛋白質之含量，二者結果相比較，有很高的相關性 ($r=0.886$)。

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