A RAPID DYE-BINDING METHOD OF SCREENING SINGLE GRAINS FOR PROTEIN CHARACTERISTICS

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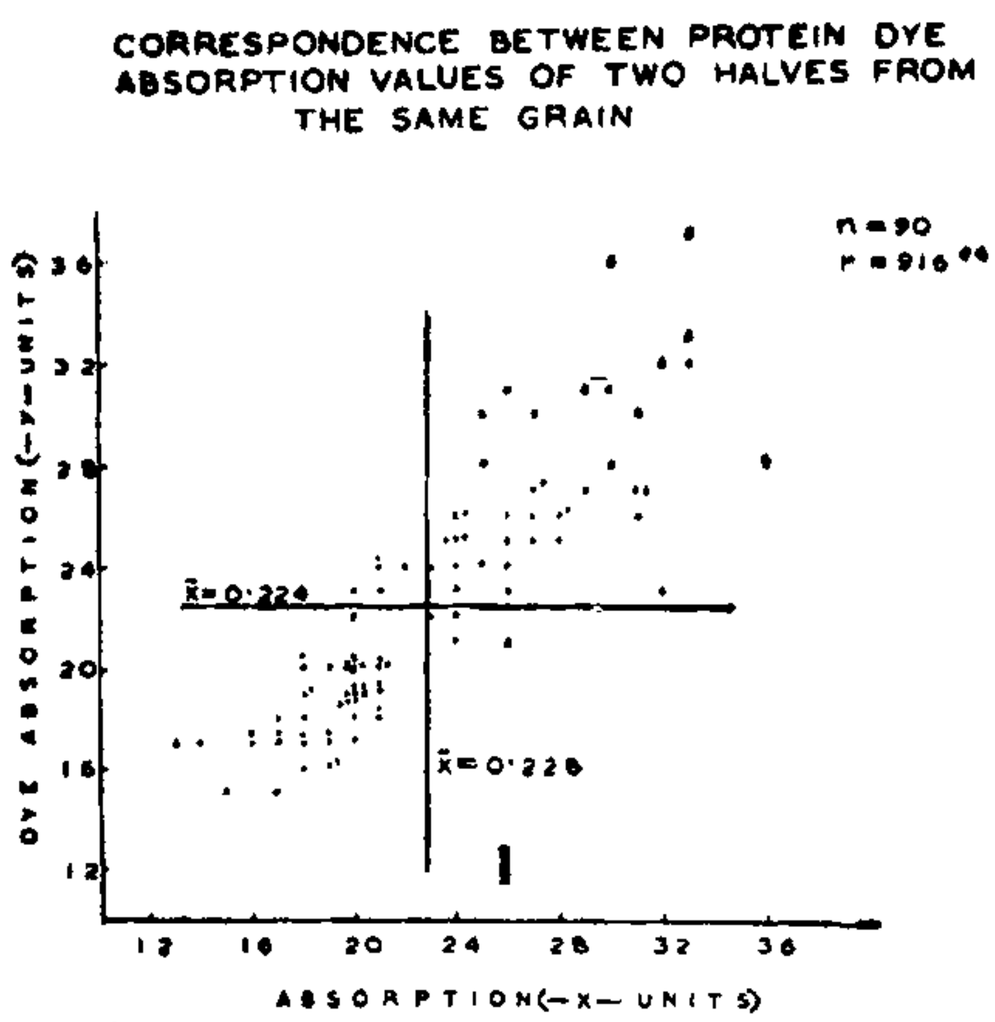
FOLLOWING the demonstration of Mertz, Bates and Nelson[1] that genetic manipulation of amino-acid balance is possible in maize, there has been much interest among geneticists and plant breeders in exploring the possibilities for upgrading the protein quality of the major food crops. This approach is of particular significance to fighting protein malnutrition in the developing countries since no special educational effort would be needed for improving nutrition if the basic staple has superior protein quality.[2] An important requisite for isolating genotypes with superior protein properties and *for making rapid selection advance in segregating* populations is the availability of rapid, reliable and non-destructive methods of assessing protein quality. Techniques involving the measurement of dye-binding capacity (DBC) have been reported to provide an estimate of the content of crude proteins,[3-5] basic amino-acids and lysine[7,8] in cereals. In a programme in our laboratory designed to identify genotypes in rice (*Oryza sativa* L.) with more protein and an altered pattern of distribution of proteins (usually, protein is concentrated in the aleurone and subaleurone layers of the rice kernel), we developed a micro-DBC method which is useful for estimating protein quality in single grains, without damaging the embryos. Though developed for rice, this technique was later found to give equally reliable results in *Zea mays, Triticum æstivum, Phaseolus aureus, Cajanus cajan, Sorghum vulgare* and *Pennisetum typhoides*. The technique, described below, may hence be of wide interest.

The germ, along with a small portion of endosperm, was cut and preserved in a refrigerator for raising seedlings later, if desired. The rest of the seed was gently crushed in a small mortar. A $12 \pm 0{\cdot}5$ mg. crushed sample was placed in a plastic mixing vial ($3{\cdot}0 \times 0{\cdot}5$ cm. of 0·65 ml. capacity) and two glass beads were introduced into it. The sample was further reduced to a fine powder by vigorously shaking the stoppered tubes on a dentist's amalgamator (Wig-L-bug) for one minute. A 0·50 ml. dye solution (Acilane orange G 'Bayer' 2·000 g., Citric acid 15·8400 g., Disodium-hydrogen phosphate 2·980 g. and Thymol 0·300 g./litre)[9] was introduced into the vial and it was again mixed for two minutes on the amalgamator. Five vials could be mixed successfully at a time. The tubes were centrifuged in lots of sixty-four at 3,500 r.p.m. The unabsorbed dye was diluted 200 times before reading the absorption at 470 mμ. Dilution could be avoided by using a special calorimeter having a short light path cuvette.[10] The absorbed dye was then computed by subtracting the absorption figures from the standard value (each new batch of dye was calibrated to 0·42 absorption units at 470 mμ) and further adjusted to express it uniformly on a 10 mg. sample basis. The bound dye could be best expressed as mM/g. of sample but for purposes of preliminary screening the absorption values as such were found to be quite satisfactory.

The reproducibility of the technique is remarkable provided the samples are stored under controlled conditions.[11,12] The duplicate analyses of one hundred seeds gave identical results. However, when transverse halves of the same grain were analysed separately, the differences detected between the halves were highly significant indicating the existence of intra-grain variation for protein (Fig. 1).

The effect of sample size was studied by analysing, in duplicate, twenty-five samples each of 7 mg., 7–10 mg. and 10–12 mg. weight from the same lot of seed. The results, summarised in Fig. 2 indicated that samples of 10–12 mg. class showed the least variation. The mean absorption value of this class ($0{\cdot}196 \pm 0{\cdot}054$) tallied closely with the mean of the large population (black rice in Fig. 3). However, in the case of protein-rich grains, *e.g.*, pulses, the sample size should not exceed 5 mg. The correspondence noted between the micro- and macro-DBC method is indicated in Table I.



FIGS. 1-3. Depicting the intar-grain variation for DBC. the effect of sample size and frequency distribution of DBC values in a cross between two rice strains, respectively.

TABLE I

Comparison of different methods of estimating the protein quality in 5 varieties of rice

Variety	Protein Content N×6·25	Protein Content On 500 mg. DBC basis	Protein Content On 10 mg. DBC basis	Lysine g./16 gm. N	DBC absorption value On 10 mg. sample	DBC absorption value On 500 mg. sample
1	8·5	8·6	8·1	3·35	0·18	0·19
2	10·0	10·4	9·7	4·00	0·21	0·22
3	9·2	9·2	9·2	3·89	0·20	0·20
4	8·6	8·1	7·5	3·01	0·17	0·18
5	9·8	9·2	9·2	3·73	0·20	0·20
Mean	9·25	9·10	8·74	3·596	0·192	0·198

The method was used successfully to study the protein quality of a large number of primitive cultivars of *Oryza sativa*. For example, where two parents and their hybrid were studied, the genetic differences in protein quality could be detected successfully (Fig. 3). Thus, this technique may be of value in both breeding and genetic experiments. DBC values and lysine content show a high positive correlation[13] and hence the technique may be of value in a preliminary screening for lysine content. This would help in restricting the number of samples that may be subjected to detailed lysine and amino-acid analysis using the standard methods.

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