

Rapid Test for Screening of Wheat Grains for Tyrosinase Activity

Y. P. ABROL, D. C. UPRETY, and S. TIKOO, Cereal Quality Laboratory, Division of Genetics, Indian Agricultural Research Institute, New Delhi, India

Presence of the enzyme tyrosinase in wheat grains was reported by Bertrand and Muttermilch (1), and its localization in bran has been shown by a number of investigators (2,3,4).

Studies from this laboratory have shown that darkening of whole-wheat-meal dough and subsequent browning of chapatties (unleavened pan-baked bread) made from dwarf wheats introduced recently into India is due to high tyrosinase activity (5,6).

In this communication, a simple and rapid test is reported which can be utilized by plant breeders to select material for low tyrosinase activity at early generation, thus making the wheat grains suitable for chapatti making.

Grain-coat pieces were peeled from the wheat grains with a razor blade. These pieces were placed on a slide and a drop of L-tyrosine solution (20 mg. per ml.) put on them. The time taken for dyeing and the color developed were noted.

To study the keeping quality of whole-wheat-meal dough, wheat grains were ground in a Labconco mill to pass through 40 mesh. Dough balls were wrapped in moist muslin cloth and placed in beakers which in turn were kept in an incubator at $32^{\circ}\pm 2^{\circ}\text{C}$.

Tyrosinase activity was assayed following the method of Horowitz et al. (7). DL-Dopa (3,4-dihydroxy phenylalanine) was used as substrate, and increase in absorbancy was read with a Spectronic 20 colorimeter. Protein was determined by the modified Biuret method (8).

The study revealed that the rate and intensity of the color developed differed with varieties. Accordingly, these were classified into four groups. To group A

TABLE I. COLOR REACTION OF GRAIN COAT, TYROSINE ACTIVITY, AND RATE OF WHOLE-MEAL DOUGH DARKENING AT $32^{\circ}\pm 2^{\circ}\text{C}$.

Wheat Varieties	Grain Coat Color Reaction	Tyrosinase Activity absorbance/mg. protein	Whole-Meal Dough Darkening time, hr.
Group A	20-30 min. (black)	0.40-0.30	2.00-3.00
Group B	1-2 hr. (reddish)	0.25-0.15	4.00-6.00
Group C	Slight color after 6-8 hr.	0.12-0.08	16.00
Group D	No coloration	0.00	No darkening till 24 hr.

belonged the strains or varieties whose grain coat developed reddish color within 5 to 10 min. and turned black in 20 to 30 min. Grain coats of group B developed reddish coloration in 1 to 2 hr. In group C were grains which gave a feeble color reaction after 6 to 8 hr., with no increase in intensity even when kept for 16 hr. In group D the grain coat developed no coloration.

Table I shows excellent correlation between the rapid (semi-quantitative) tyrosinase test, quantitative determination of tyrosinase activity, and darkening of whole-meal doughs. These findings show that the simple, rapid test described in this note can be utilized by plant breeders for the selection of dwarf wheat varieties.

Acknowledgment

The authors wish to thank H. K. Jain, Head of the Division of Genetics, Indian Agricultural Research Institute, New Delhi, for providing the facilities and encouraging the present studies.

Literature Cited

1. BERTRAND, G., and MUTTERMILCH, W. Sur l'existence d'une tyrosinase dans lesons de froment. *Compt. Rend.* 144: 1285 (1907).
2. MILNER, M., and GOULD, M. R. The quantitative determination of phenol oxidase activity in wheat varieties. *Cereal Chem.* 28: 473 (1951).
3. HONOLD, G. R., and STAHMANN, M. A. The oxidation-reduction enzymes of wheat. IV. Qualitative and quantitative investigations of the oxidases. *Cereal Chem.* 45: 99 (1968).
4. ABROL, Y. P. Keeping quality of whole wheat meal dough. Proc. 5th World Congress on Cereals and Bread, Dresden, Germany (1970).
5. ABROL, Y. P., and UPRETY, D. C. Studies on darkening of whole meal dough. *Current Sci.* 39: 421 (1970).
6. ABROL, Y. P., UPRETY, D. C., and RAM, A. Browning of *chapaties*. *J. Food Sci. Tech.* 8: 26 (1971).
7. HOROWITZ, N. H., FLING, H. L., MacLEOD, H. L., and SUEOKA, N. Genetic determination and enzymatic induction of tyrosinase in *Neurospora*. *J. Mol. Biol.* 2: 96 (1960).
8. WILLIAMS, P. C. The determination of proteins in whole wheat meal and flour by the biuret procedure. *J. Sci. Food Agr.* 12: 58 (1961).

[Received March 3, 1971. Accepted May 18, 1971].