

A Single Grain Screening Technique for Breeding Alkylresorcinol-Poor Rye

F. Hoffmann and G. Wenzel

Projektgruppen Haploide in der Pflanzenzüchtung, Max-Planck-Institut für Pflanzengenetik, Ladenburg (BRD)

Summary. A quick and simple semi-quantitative method of selecting single seeds of rye for their content of 5-alkyl-resorcinols is presented. The resorcinols are extracted from whole, intact caryopses with acetone. Under the conditions described the germination rate is hardly decreased. The single grain method offers especially striking advantages when selffertile inbred lines are used.

Key words: Alkylresorcinol - Rye - Seed - Extraction - Inbreeding

The use of rye as fattening-pasture is restricted, as higher additions in the fodder mixture cause growth inhibitions, specially in younger animals (v. Knie-riem 1900; Wieringa 1967). Wieringa succeeded in identifying the toxic principle as 5-alkyl-derivatives of resorcinol, which are present in rye grains in an average concentration of 0.1% (Musehold 1974; Mejbaum-Katzenellenbogen et al. 1975a). Lower concentrations of these compounds are also detectable in wheat (Wenkert et al. 1964) and Triticale (Munck 1972). As rye protein contains very valuable amino-acid compositions (Munck 1972) the selection of rye types, with an alkylresorcinol content comparable to wheat would be highly beneficial. The paper describes a step towards breeding of such toxin-free material, using single grain screening.

Alkylresorcinols are extracted from each grain in a sealed test tube with 0.2 ml acetone for 20 min. at room temperature. At the end of this incubation it is thoroughly shaken and 0.01 ml of the extract is pipetted onto a silica gel 60 plate (Merck 5721) which has been sprayed with an aqueous solution of fast blue B salt (0.5%, Merck 3191; Musehold 1974) shortly before or after spotting. The colour reaction immediately appearing and changing from a light rose to violet is highly correlated with the content of alkylresorcinol (Fig. 1). Other substances reacting with this reagent are below the detection level in this procedure. The remaining extract may be used for a quantitative analysis of the extracted resorcinols (Musehold 1973;

Mejbaum-Katzenellenbogen et al. 1975b). A method using high pressure liquid chromatography has been developed in our laboratory (unpublished). Grains showing hardly any colour reaction are immediately rinsed with water and sown. It is also possible to dry the grains again and to plant them after further storage.

Besides acetone other organic solvents have been tested, however, only grains extracted with acetone (even for 3 hrs at 50°C) and hexane showed a high percentage of germination. After 3 hrs of extraction at 50°C the residue contains negligible amounts of resorcinols. The proposed incubation time of 20 min. at room temperature results in a 70% extraction of the toxin, which is sufficient for a relative assay. Under these conditions the germination rate is hardly decreased: 194 grains from 200 of the anthocyanin-free variety 'Perolo' (von Lochow-Petkus, Bergen) and 183 from 200 of the anthocyanin-containing variety Kustro (v.L.-P.) germinated after extraction, whereas 196 and 199 grains of the untreated controls germinated. According to this germination capacity there was no difference between grains having a high level and those with low resorcinol content.

In testing the varieties 'Kustro' and 'Perolo' 5% of the grains gave spots comparable to wheat controls (Fig. 1). This demonstrates that the chance for selecting resorcinol-poor types in rye is quite good. It is, however, not yet proven that the absence of any negative effect during feeding of wheat is due to this low

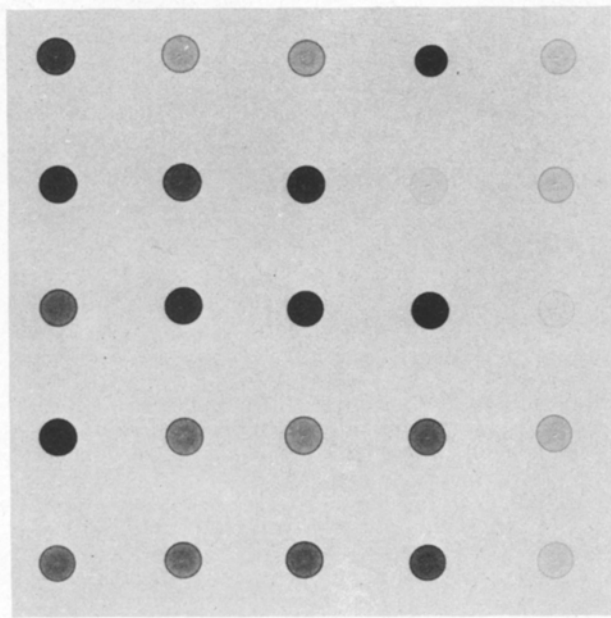


Fig. 1. Single grain acetone extract spots due to the reaction of alkylresorcinols with fast blue B salt. The upper row shows extracts from naturally alkylresorcinol-poor wheat grains, the four lower rows demonstrate the segregation of the resorcinol content in 20 grains of a commercial cultivar of rye taken at random

concentration of resorcinols or to a different mixture of homologues. Further the method described here compares the resorcinol content per grain without considering the size or the specific weight. As the alkylresorcinols are stored in the pericarp, very small grains (having a smaller surface) of low weight may give the impression of having lower alkylresorcinol. By a simple preselection for large grains with a specific weight of more than 1.3 g/cm^3 this can be easily overcome.

Besides the two varieties mentioned above, the results could be confirmed for a spring rye variety and other winter types, including F_1 hybrids and selffertile inbred lines. As the desired quality is se-

lected after flowering and fertilization, the single grain method offers especially striking advantages when selffertile inbred lines are used in breeding for toxin-free stocks. Consequently this method has been developed for the selection of variants in such material obtained from selffertile homodiploid lines produced via anther culture (Wenzel et al. 1977). This method is, however, not restricted to such material. It should also be possible to build up alkylresorcinol-free populations from normal heterozygous cultivars within a shorter time.

Acknowledgement

The technical assistance of G. Soiné is gratefully acknowledged.

Literature

- Knieriem v., W.: Der Roggen als Kraftfuttermittel. *Landwirt. Jahrb.* **29**, 483-523 (1900)
- Mejbaum-Katzenellenbogen, W.; Sikorski, A.; Tluscik, F.: Alkylresorcinols in rye (*Secale cereale* L.) grains II. Dependence of alkylresorcinols level on weight and specific weight of grains. *Acta Soc. Bot. Polon.* **44**, 597-606 (1975a)
- Mejbaum-Katzenellenbogen, W.; Tluscik, F.; Kozubek, A.; Sikorski, A.; Maresz, Z.: Alkylresorcinols in rye (*Secale cereale* L.) grains I. Micro-method for determination of alkyl derivatives of resorcinol in rye grain. *Acta Soc. Bot. Polon.* **44**, 479-489 (1975b)
- Munck, L.: Improvement of nutritional value in cereals. *Hereditas* **72**, 1-128 (1972)
- Musehold, J.: Zur quantitativen Bestimmung einer toxischen phenolartigen Substanz des Roggenkorns. *Z. Pflanzenzüchtg.* **69**, 102-106 (1973)
- Musehold, J.: Zur Methodik der Selektion auf 5-Alkyl-Resorcin-arme Pflanzen beim Roggen. *Z. Pflanzenzüchtg.* **71**, 124-129 (1974)
- Wenkert, E.; Loeser, E.M.; Mahapatra, S.N.; Schenker, F.; Wilson, E.M.: Wheat grain phenols. *J. Org. Chem.* **29**, 435-439 (1964)
- Wenzel, G.; Hoffmann, F.; Thomas, E.: Induction, regeneration and chromosome doubling of androgenetic haploid rye plants. in prep. (1977)
- Wieringa, G.W.: On the occurrence of growth inhibiting substances in rye. Wageningen: H. Veenman en Zonen N.V. 1967

Received May 23, 1977
Communicated by G. Melchers

Dr. F. Hoffmann
Dr. G. Wenzel
Projektgruppen Haploide in der Pflanzenzüchtung
Max-Planck-Institut für Pflanzengenetik
D-6802 Ladenburg-Rosenhof (BRD)