Transient NMR Selection Method in Plant Breeding

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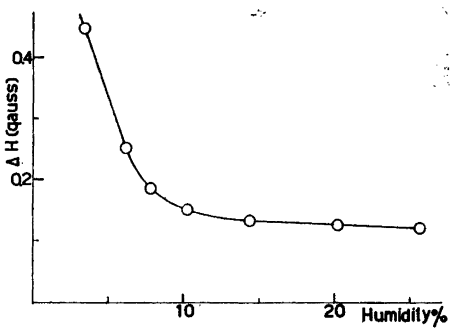

Fig. 1. Width of water PMR line as a function of water content in maize flour.

Plant breeding for the improvement of product quality and increase in nutritive value is becoming increasingly important. The factor limiting the possibility to select lines with wanted properties is often the lack of appropriate analytical methods permitting an accurate determination of a sufficiently large number of samples. Further, most conventional chemical methods are destructive and therefore do not allow the direct propagation of selected analysed individuals.

Recently Conway and Smith [1] introduced a wide line nuclear magnetic resonance (NMR) technique for nondestructive determination of oil content in multiple or single kernel samples of corn dried to below 5 % moisture. The heritability of variations in oil content of individual kernels within the same ear was demonstrated.[2]

In this note we describe the results obtained with an improved NMR technique exploiting transient effects in steady state detection. This method is by one to two orders of magnitude faster than the one described in the references given above [1,2] and, in view of its selectivity, it does not require drying of the kernel samples to suppress the signal of the bound water. It is the feeling of the authors that in view of the speed of this method new fields are opened in plant breeding and ecology as well as in biometrics.

The selectivity of this method is based on the different degree of mobility and the resulting differences in the spin-spin (T_2) and spin-lattice (T_1) relaxation times of the various hydrogen containing constituents of plant seeds. The proton magnetic resonance spectrum of a single kernel maize sample consists at room temperature of: (i) a relatively broad ($H_{max} = 10$ Gauss) component due to the hydrogen containing host substance (mainly carbohydrates and proteins); (ii) a much narrower component due to bound water, the width of which depends on the water content and which changes from about 0.3 Gauss at a 5 % water content to about 0.05 Gauss when the water content is increased to over 12 % (Fig. 1); and (iii) a still narrower component due to oil protons. Whereas the signal of the bound water significantly differs from that of liquid water the signal of the oil protons in corn and sunflower seeds with an oil content larger than 2 % is not very much broader than that of liquid oil.

The absorption signal of the host substance is so broad that it is essentially constant over the water and oil absorption region and hence does not interfere with the measurements of the latter two quantities. By a proper adjustment of the level of the resonant radio-frequency field as well as the sweep rate (a typical value being 400 mGauss/sec) and the amplitude and frequency of the magnetic field modulation, and by exploiting the resulting differences in the transient effects on the oil and water signal line shape, selective sensitivity to oil or water was obtained. The result can be understood on the basis of a numerical solution of the Bloch equations.[3] Calibration of the spectrometer in the "oil sensitive" mode of operation

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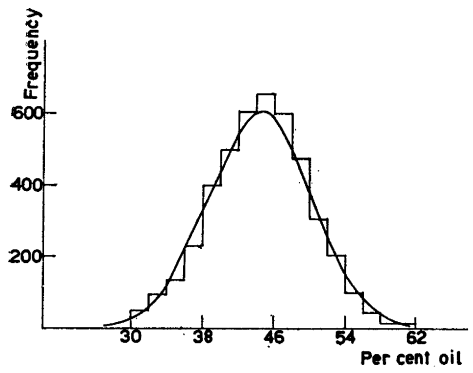

Fig. 2. Frequency distribution and theoretical normal distribution of percent oil in 4406 individual achenes of a sunflower variety (47 values < 29.5 %, *i.e.* $< \mathrm{m} - 2.5\ \sigma$, were excluded).

further showed that the peak value of the NMR signal is linearly related to the oil content of the corn seeds in case that the seeds contain 2 % or more of oil. The relative error in the oil determination was less than 1 % and the minimum quantity of oil which could be determined was 0.1 mg. The semiautomatic determination of the oil content in one seed together with the presentation of the result on a digital voltmeter takes about 5 to 10 sec. Details of the electronics will be published elsewhere. The method has been used now for five years in the Zemun Institute.

The frequency distribution of percent oil in single kernels of a sunflower variety is shown in Fig. 2. It may be noted that in this case where several thousand measurements of a biochemical property have been performed, good agreement with a normal distribution curve was obtained after elimination of a small number of extreme values ($< \mathrm{m} - 2.5\ \sigma$) probably representing immature seeds. In maize the single kernel differences in oil content within one and the same ear were found to be significantly heritable [4] in agreement with the results of other authors.[2] In a maize breeding programme the same increase in oil content has been reached in 4–5 generations as in 20–30 generations when conventional chemical methods were used.

As a continuation of this work, we are developing NMR and nuclear quadrupole resonance methods for a rapid nondestructive determination of the nitrogen content in seeds, within a plant breeding programme for higher protein content.

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Abscisin II as an Inhibitor of α-Amylase

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In 1961 Hemberg and Larsson [1] showed that the inhibitor β complex from resting potato tubers suppressed the activity of α-amylase but only insignificantly affected the activity of β-amylase. According to Cornforth *et al.*[2] the growth-inhibiting substance dormin, which is found in leaves of sycamore during early September when resting buds have been formed, is identical with the substance abscisin II. Cornforth *et al.*[3] have detected this substance in many different plant organs, including potato tubers. They are of the opinion that abscisin II causes the inhibitory activity found in the acid fraction of plant extracts.

Thomas *et al.*[4] find that the sycamore inhibitor inhibits the synthesis of α-amylase but seems to have no action on the activity of this enzyme. According to these findings