Analysis of water soluble and insoluble polysaccharides in kernels of different corns (*Zea mays* L.)

Upama Mishra[1], Sunit K. Tyagi[1], Raveendra N. Gadag[1,*], Kandasamy Elayaraja[2] and Himanshu Pathak[3]

[1]Division of Genetics,
[3]CESCRA, Indian Agricultural Research Institute,
New Delhi 110 012, India
[2]Directorate of Seed Research, Kushmaur, Mau 275 103, India

Phytoglycogen and starch components were assayed in twelve lines of fresh kernels belonging to different types of corns such as normal corn (field corn), sweet corn and quality protein maize (QPM). The technique, using single kernel and a series of digestions and dilutions, measured glucano lactone contents of soluble and insoluble components in the three groups of corn with distinct and diverse uses. Extensive variability was found among sweet corn, field corn and QPM for the water soluble and insoluble carbohydrate components and kernel weight, especially for genotypes belonging to different types of corn. Further, such differences are broadly related and comprehendible to distinct types which give an insight into the unique characteristics and their end use.

Keywords: Glucose-6-phosphate dehydrogenase, kernel, maize, phytoglycogen, starch.

CEREAL crops possess starch as a major biochemical component in grain. The structure and composition of starch are reasonably well studied in the endosperm of maize kernel. It is the key carbohydrate component in the foods of humans and livestock, and used profusely in many industries. This is especially applicable to maize, on account of different types and consequent diverse uses for direct human consumption, represented by specialty corns. Starch in maize kernel consists of amylose (linear unit), amylopectin (branched unit) and phytoglycogen, a highly branched and water-soluble polymer (WSP). Glucose units in amylose are arranged linearly, linked together by α-(1 $\rightarrow$ 4) glucosydic bonds while slightly branched forms of amylose have also been identified[1]. Amylopectin is a highly branched polymer of glucose having high molecular mass and constituting approximately 75% of granule mass. It is produced by the formation of α-(1 $\rightarrow$ 6) linkages between adjoining straight glucan chains. Amylopectin and phytoglycogen contain both linear chains of glucose joined together by α-(1 $\rightarrow$ 4) bonds, and branches involving α-(1 $\rightarrow$ 6) bonds with minor changes in proportion of branching. Branches account for about 10% of the bonds in phytoglycogen and around 5% in amylopectin. In the most commonly cultivated types (field corn), the amylose : amylopectin ratio is relatively stable and close to 20 : 80. However, the proportion may strongly be affected by specific mutations, leading to changes in content and composition of starch in grains. Consequently, these alterations in kernel characteristics confer such genotypes with specific properties, leading to speciality corns, such as high amylose corn, waxy corn, sweet corn, etc.

In general, the principal polysaccharide storage product in the endosperm is different or altered in many specialty corns. The genotypes with sugary (*su*) mutation are unique in accumulating water soluble polysaccharide phytoglycogen, an α-1,4-glucan that is more highly branched than the amylopectin components of maize starch[2]. A number of maize lines with endosperm mutations in the shrunken (*sh2*) and brittle (*bt*) genes accumulate as much as twofold the sucrose content of sugary (*su*), but phytoglycogen does not accumulate[3,4]. Hence, enhanced phytoglycogen in the WSP fraction of sweet corn endosperm is attributed to mutant gene sugary-1 (*su*1) on chromosome 4 resulting in creamy texture of the grain. It was reported that mutant genes dull (*du*) on chromosome 10, waxy (*wx*) on chromosome 9 and amylose-extender (*ae*) on chromosome 5 singly or in combination with *su*1, also exert significant effect on the WSP content of the endosperm[5], thereby affecting the overall sugar content.

To improve the nutritional quality of maize, QPM was developed after the discovery of opaque-2 (o_2) and floury-2 (f_2) mutants. QPM has the balanced amount of amino acids with high content of tryptophan, lysine and low content of isoleucine and leucine. The balanced proportion of all these essential amino acids in QPM genotypes improves the biological value of protein of the kernel.

Three enzymes namely *ADP glucose pyrophosphorylase*, *starch synthase* and *starch branching enzyme* are directly required for synthesis of starch, and a fourth enzyme (*isoamylase*; *glycogen 6-glucanohydrolase*) has been proposed to play an important role. Many studies reported similar mutations and consequent changes in many economically important cereals. For example, mutations of maize and rice (*sugary1* [*su1*]) and barley (*isa-1*) result in enhanced amount of phytoglycogen and reduced starch content[6–8]. The objective of this experiment was to extract and estimate starch and water soluble polysaccharides (WSP) from single kernel of different types of tropical corn genotypes on the basis of highly precise individual kernel assay, relating the differences to diverse uses and understanding their possible implications.

In this experiment, we used 12 different corn lines, viz. normal corn, sweet corn and QPM. Details of the biochemical assay included the following sequential steps. Each corn kernel after recording the weight was placed in a single 15 ml falcon tube containing 2 ml 0.3% sodium metabisulphite and 1% lactic acid (pH 3.8). Subsequently

*For correspondence. (e-mail: rn_gadag@yahoo.com)

capped tubes were incubated at 52°C for 24 h. The steep liquid was discarded in sink and the seed was rinsed with distilled water. The pericarp and embryo were carefully removed and discarded with forceps. The endosperm was placed in eppendorf tube and ground in 0.5 ml water with micro-pestle making it into a fine paste. The final volume was adjusted to 1 ml using distilled water. The contents were mixed well and 0.1 ml sample was transferred for starch analysis to second eppendorf tube. The remaining 0.9 ml extract was stored in –20°C to serve as a source for subsequent repeat assay if required. Water (0.4 ml) was added and centrifuged at 14,000 *g* in cold room for 5 min. Supernatant was transferred to second eppendorf and 0.5 ml water was added, mixed well and re-centrifuged. The two supernatants were pooled to get a soluble extract. Aliquots (50 µl) of soluble extract were transferred to each of four screw-capped eppendorf tubes. The pellet of the first eppendorf tube was resuspended in 1 ml water, which represented insoluble extract. The contents were mixed well before removing 50 µl aliquots of insoluble extract to each of four screw-capped eppendorf tubes. The volumes in each eppendorf tube were made-up to 0.5 ml with distilled water. The screw capped tubes with insoluble extracts were autoclaved to solubilize the starch.

For digestion, two of the replicate 0.5 ml aliquots were taken and the starch/WSP was digested to glucose by adding a 0.5 ml solution containing 100 mM sodium acetate (pH 5.2), 2 U α-amylase and 11.2 U amyloglucoside. To 0.5 ml of aliquot, added 0.5 ml of 100 mM sodium acetate (pH 5.2). No enzymes were added to these other two replicate samples (undigested controls). All tubes were incubated at 37°C for overnight. Immediately before assay, the samples were centrifuged for 5 min at 14,000 *g* and the supernatant was assayed.

For glucose assay, 500 µl solution containing 200 mM Bicine (pH 7.7), 10 mM $MgCl_2$, 50 µl 10 mM NADP, 50 µl 20 mM ATP, 2.5 U hexokinase, 30 µl extract and 365 µl H_2O was added to a 1 ml cuvette. OD was monitored at 340 nm, and the value was recorded after the readings were stable. This represented the initial OD (OD_1). 2.5 U G6PDH was added and mixed well with mini glass stirring stick. OD was monitored frequently as it was increasing rapidly and then slowly reached a stable value, which was recorded as the second value, OD_2. (Difference in OD/6.22 = µmol glucose in cuvette). For computation purposes, 1 µmol glucose equivalent to 162 µg starch was used as standard parameter.

Extensive variability was found among genotypes belonging to three groups, viz. comprising of sweet corn, field corn and QPM for the water soluble and insoluble carbohydrate components as well as kernel weight. Water soluble glucans in sweet corn varied from 16.54 to 59.55 mg/kernel whereas in field corn and QPM, it was 3.31 to 9.92 mg/kernel (Figure 1). Starch content in sweet corn was relatively less compared to field corn, ranging from 9.92 to 41.35 mg/kernel. In field corn, however, it is the major component, 66.16 mg/kernel. Interestingly, in QPM it was towards lower value (when compared to sweet corn as a group).

The principal difference between normal and *sugary-1* maize is that, *sugary-1* endosperms accumulate the highly branched, water soluble form of polysaccharide phytoglycogen[9].

In this experiment, it was found that the kernel of normal corn had highest dry weight compared to the other two types, belonging to specialty corn groups, viz. sweet corn and QPM (Figure 2). The present study supports the previous finding that, compared to normal kernels, *sugary-1* and QPM kernels have less dry weight[10,11]. This in turn is mainly related to biochemical components including type and amount of polysaccharides.

In maize kernels, changes brought about by specific mutations lead to altered usage pattern and direct human consumption. Mutations in the gene *sugary1* (*sul*) or *shrunken* (*sh2*) result in accumulation of water-soluble gluco-polysaccharide phytoglycogen, increased sucrose concentration and decreased concentration of amylopectin. Such genotypes also have great commercial importance in the form of utility as sweet corn. These types are suitable for direct human consumption at green ear stage (around 22 days after pollination), and contain lower starch in comparison to field corn. However, lines

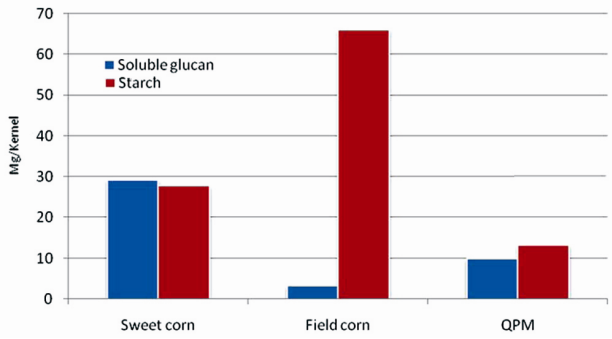

Figure 1. Comparison among sweet corn, normal corn and QPM for soluble and insoluble glucans.

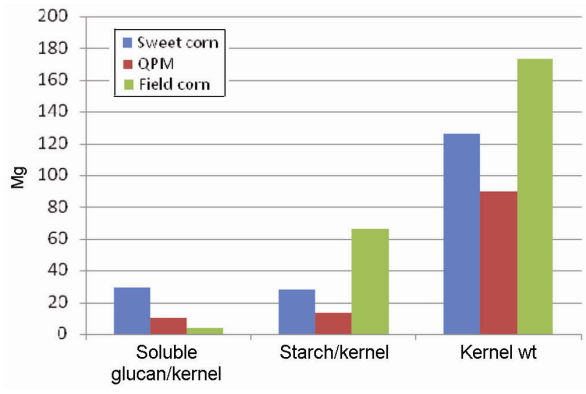

Figure 2. Comparison among sweet corn, normal corn and QPM for soluble and insoluble glucans and dry weight.

with distinct types of mutations (*su* or *sh2*) differ in terms of soluble glucan content, which is higher only in *su1* genotypes. Such differences are attributed to finer biochemical differentiation among sweet corns for soluble glucans (16.54 to 59.55 mg/kernel) and could be understood and comprehended in the context of earlier information relating to effects of different mutations. Hence, this technique could easily differentiate the sweet corn genotypes into *su1* (with higher value of soluble glucans) versus non-*su1* types (with lower value), even on the basis of individual kernel. Similar to field corn and sweet corn as a group, QPM also conformed to a characteristic range of values in terms of content and composition of soluble and insoluble polysaccharides. Results can be extrapolated and applied to other major cereals (wheat, rice barley, jowar, etc.), considering their common core pathway of starch metabolism[12,13]. Some insights into apparent variations and consequent specialized utilization are evident in crops like barley, sorghum and wheat.

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Importance and sensitivity of variables defining throw and flyrock in surface blasting by artificial neural network method

A. K. Raina[1,*] and V. M. S. R. Murthy[2]

[1]CSIR-Central Institute of Mining and Fuel Research, Regional Centre Unit-I, MECL Complex, Seminary Hills, Nagpur 440 006, India
[2]Department of Mining Engineering, Indian Institute of Technology (ISM), Dhanbad 826 015, India

Rock breakage by explosives is followed by throw or heaving the broken material and occasional flyrock. Heaving is a desired feature of blasting for efficient mucking. However, flyrock is a rock fragment that travels beyond the designated distance from a blast in surface mines, and poses a threat to adjacent habitats. Here, we decipher the importance and sensitivity of the variables and factors used to establish the predictive regime of throw with more emphasis on flyrock. The data collected were modelled using artificial neural network approach. The importance and sensitivity of variables and factors were delineated so that they are in tune with the rationale of the outcome of the blast. A combinatory approach was devised to arrive at minimal variables and factors to reduce the statistical redundancy, and to propose a rational predictive regime for throw and flyrock in surface mines.

Keywords: Artificial neural network, blasting, flyrock, throw, surface mines.

BLASTING is an integral part of excavation in mines and continues to be a major method of rock fragmentation due to the economy of operation. Blasting, in addition to fragmentation, is associated with throwing the muck generated, vibrations, air overpressure and flyrock. While fragmentation and throw are desired effects, flyrock is an undesirable outcome. Flyrock is a fragment of rock that travels greater distances than desired, in comparison to throw which is limited to a few multiples of bench height. Flyrock is not only a threat to nearby habitats, but poses a challenge to miners as all sorts of 'Objects of Concern' (OC)[1] are affected by it. Flyrock is one of the major causes of blast induced fatalities and accidents[2].

There are several reasons for flyrock which belong to the domain of rockmass including structural discontinuities[3], blast design and explosive variables. Several attempts were made by different authors to identify the reasons for flyrock and several equations have been proposed to predict flyrock distance. However, there is a disparity between cause of flyrock and the variables identified that have been used in prediction regime[4]. Such a disparity is reflected in Tables 1 and 2 and a comparison is shown in Figure 1.

*For correspondence. (e-mail: rainaji@gmail.com)