ESRF	**Experiment title: Novel** structures and properties of starches from mutant maizes obtained by breeding (double mutation) or genetic modification	**Experiment number:** LS 709
Beamline: ID 13	**Date of experiment: ,** from: 17-july-97 to: 18-july-97	**Date of report:** **September 10 1997**
Shifts:	**Local contact(s):** Christian Riekel	*Received at ESRF:* 31 AOUT 1998

Names and affiliations of applicants (* indicates experimentalists):
C. Gérard, INRA, Nantes *
A. Buléon, INRA, Nantes *
H. Chanzy, CERMAV, Grenoble *
R. Vuong, CERMAV, Nantes*

In the preceding experiments, technical difficulties (see report on SC 282 and 283) prevented us from doing a thorough mapping of the natural polymorphism present in pea starch. This was carried out during this beam time with some preliminary experiments on mutant starches showing specific structural ifeatures.

EXPERIMENTAL Samples of starch granules were dispersed in an aqueous solution (0.15% w/w) of polyacrylic acid. Drops of this suspension were deposited on collodion coated electron microscope with a large central cavity in order to prevent from undesirable scattering. The grids were photographed with optical microscopy and mounted on the microfocus beam line ID13. A 4 µm X-ray beam (λ = 0.92 Å) was produced by a tapered glass capillary. Background scattering was cut with a 10µm aperture positioned between the capillary exit and the sample. Individual granules were then scanned with steps of either 5 or 10 microns, after positionning into the beam with a microscope. The diffraction diagrams were recorded with a flux of 2.10^9 photons/sec/µm² per second for 16 second using an image intensified CCD camera Photomicrographs taken after the experiments showed clearly the areas that had been irradiated by the X-ray probe (figure 1).

RESULTS AND **PERSPECTIVES** - Using images intensified from the CCD readout, we were able to record a large number of meaningful diffraction patterns on pea starch. Most of them were characteristic of mixtures of A and B crystalline types present in the same 4 µm area within a single granule (Figure 2). The B type was proved to be more present in the center of the granule with a strong intensity for the specific 100 reflexion at 1.6nm. This reflexion is very sensitive to hydration which shows that our sample preparation technique is convenient even for hydrated substrates. No well oriented diagrams were obtained on contrary to those obtained in the preceding trials (SC282). This could be due to the larger beam used in this experiment. These results are of great interest for understanding of specific properties of C-type starches and their biosynthesis (especially the crystallization step). They show unambigously that a natural polymorphism may be present inside a single granule. Very preliminary results were also obtained on amylose-rich starches from maize which exhibit a pure B type (figure 3); a poor crystallinity and a weak radial orientation on the edges of granules. Further studies on specific single and double mutant starches should lead to a more complete description of the biosynthesis scheme and therefore to opimization of plants for production of starch with more specific applications in both food and non food applications

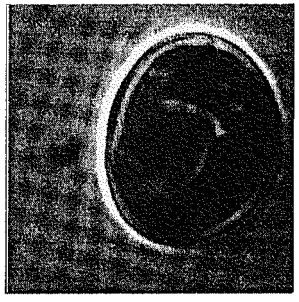

Figure 1 - Typical sample of pea starch after scanning with 5 micron steps. The imprint of the beam in the sample is associated with beam damage of the supporting collodion film.

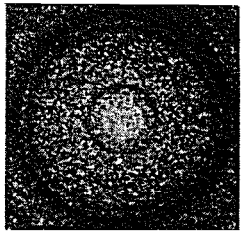

Figure 2 - C-type diffraction diagram recorded on the centre of the granule showing a strong 100 reflexion (B-type)

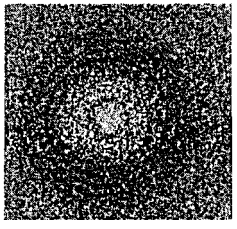

Figure 3 - typical diffraction diagram of amylose rich starch from maize (B-type, low crystallinity).