The mineral nutrient solution used in our wheat-ovary cultures was the same which we had specially elaborated for embryo culture[1], supplemented with 3% of sugar, 0·8% of agar, and with vitamins. An addition of 5% of the juice of maize in the milky stage benefited the differentiation.

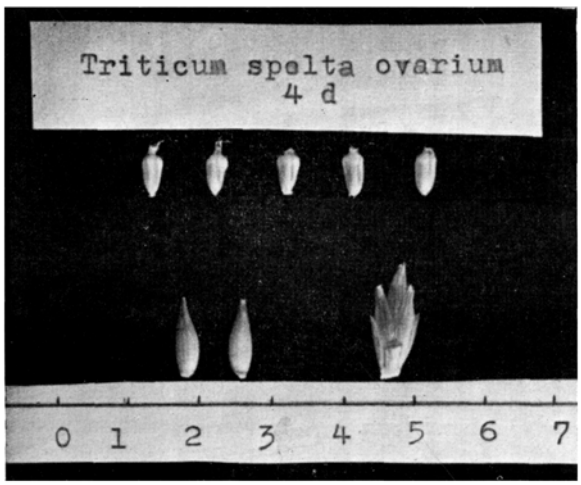

Fig. 1.–Wheat ovaries (upper row), flowers and spikelet (lower row) on the fourth day after anthesis when the culture was started.

After natural pollination occurred, the spikes were cut off, treated with a 10% calcium hypochlorite solution for 10–15 min, and washed thoroughly with sterile water. Ovaries, flowers, or spikelets with quite small rachillae, taken only from the middle third part of the spikes, were used for the cultures (fig. 1). The age of the ovaries was determined as beginning with the day on which the anthesis of the two main flowers of the spikelets was recorded. Development was the better the more of the rachillae and paleae remained attached to the ovaries. If ovaries deprived of all other maternal tissues were planted on the nutrient medium, the embryos seldom continued to develope. Ovaries enveloped by two paleae ordinarily promoted the differentiation of the embryo, even though the caryopsis grew but little in size. The endosperm discontinued developing normally, grew but feebly, and formed a glassy, jelly-like substance.

Having cultured the ovaries for 8–12 days, the embryos were excised and transferred to a fresh medium of the same composition, but supplemented with 0·5% of casein hydrolysate. They continued to develope, and a few days later germination began. From ovaries cultured for more than a fortnight a decrease in the number of viable embryos was observed. Later, the embryos germinated were planted in pots. They throve well developing into fairly normal plants.

In a single case, embryos capable of germinating were obtained even from in vitro cultivated ovaries excised on the second day after pollination. From ovaries excised on the fourth and sixth day, respectively, the embryos could be developed into seedlings quite regularly.

As far as we know this is the first instance reported of obtaining viable plants from *in vitro* cultures of wheat proembryos by subculturing embryos excised from ovary cultures.

G. RÉDEI and Mrs. G. RÉDEI

Institute of Genetics, Budapest, June 19, 1955.

[1] G. RÉDEI, B. GYÖRFFY, and Mrs. G. RÉDEI, Acta Bot. Acad. Sci. Hung. *2*, (1955) (in press).

Zusammenfassung

Es wurden 2–4 Tage nach dem Anthesis Weizenovarien *in vitro* in Kultur geführt. 8–10 Tage später wurden die Embryonen präpariert und wieder künstlich gezüchtet. Die so erhaltenen lebensfähigen Keimpflanzen entwickelten sich nach späterer Auspflanzung zu samenbringenden Pflanzen. Es ist uns kein Fall bekannt, wo aus Weizen Proembryonen *in vitro* Keimlinge gezogen wurden.

Role of Cystine Chelation in the Mechanism of Fusarium Wilt of Cotton

There has been much difference of opinion on the mechanism of pathological wilting of plants by pathogenic Fusaria[1]. GÄUMANN[2] and his school of workers working on the tomato wilt by *Fusarium oxysporum* f. *lycopersici* (SACC.) have attributed the wilt symptoms to an irreversible destruction of the osmotic pre-requisites for turgor by the systemic toxin lycomarasmin. WALKER[3] and his school of workers, who formerly advanced the vessel plugging theory against the systemic toxin theory, have subsequently reported typical wilt symptoms in tomato with fungal extracts rich in pectin methyl esterase activity and claimed that pectic enzymes could reproduce many of the wilt symptoms[4]. While it has been established that resistance or susceptibility to Fusarium wilts is only with reference to the fungus and not to the action of the active toxic principle[2], little is known of the mechanism of *in vivo* detoxication in the resistant varieties particularly in cotton.

Extensive chromatographic studies made by the author recently (employing the circular macro-[5] and micro-[6]techniques developed in this connection) on the distribution of α-amino constituents (positive to ninhydrine) in roots, shoots and leaves of susceptible and resistant varieties of cotton (Karunganni 2, Cambodia and Madras Uganda), have revealed the interesting fact that the resistant varieties of cotton, both the shoots and roots, particularly the latter contained considerable amounts of cystine besides the major constituents as asparagine, glutamine and other minor amino acids, while the susceptible ones did not reveal any cystine. It was therefore considered worthwhile to investigate if cystine was in any way related to resistance to wilt both in the presence and in absence of ferric iron, particularly in view of the earlier reports that the antibiotic activity of clavacin was due to its chelation to sulfhydryl group in plant saps[7].

Cut shoots of cotton (Susceptible variety Karunganni *2-Gossypium arboreum*) 10 to 12 days old, grown in normal garden soil were treated to neat dialysed culture filtrates of *Fusarium vasinfectum* Atk.[8] (grown in

[1] K. LAKSHMINARAYANAN, Proc. Indian Acad. Sci. *41B*, 132 (1955). – T. S. SADASIVAN and C. V. SUBRAMANIAN, J. Indian bot. Soc. *33*, 162 (1954). – C. V. SUBRAMANIAN, Curr. Sci. *24*, 144 (1955).

[2] E. GÄUMANN, Adv. Enzym. *11*, 401 (1951).

[3] R. P. SCHEFFER and J. C. WALKER, Phytopathology *43*, 116 (1953).

[4] S. S. GOTHOSKAR, R. P. SCHEFFER, J. C. WALKER, and M. A. STAHMANN, Phytopathology, *43*, 535 (1953). – N. N. WINSTEAD and J. C. WALKER, Phytopathology *44*, 153 (1954).

[5] K. LAKSHMINARAYANAN, Arch. Biochem. Biophys. *49*, 396 (1954).

[6] K. LAKSHMINARAYANAN, Arch. Biochem. Biophys *51*, 367 (1954).

[7] G. MIESCHER, Phytopath. Z. *16*, 369 (1950).

[8] R. KALYANASUNDARAM and K. LAKSHMINARAYANAN, Nature *171*, 1120 (1953).

Richard's medium for 8 weeks and sterilized by seitz filtration) prechelated with ferric chloride at the following levels of Fe''' for 24 h: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{5}–M. Fe''' was not toxic at levels lower than 10^{-3}M. and greatly increased the toxic symptoms as shrivelling, yellowing and vein clearing at the optimum level indicating that the active toxic principle of *F. vasinfectum* acts in a manner analogous to lycomarasmin on tomato by forming a chelate complex with Fe'''. Cystine was added to the chelate complex (at 10^{-3}M. Fe''' level) at the following levels both before and after chelation: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}M. Cystine was not toxic to the cut shoots at levels lower than 10^{-4}M. nor was the cystine-iron complex. Addition of cystine at the optimum level 10^{-4}M. to the toxin-iron complex before chelation gave effective protection to the cut shoots which indicated no toxic symptoms compared to the controls without cystine. A complete set of controls was maintained. Cut shoots pretreated to cystine at the optimum level for 24 h were able to overcome the toxic effects of the toxin-Fe''' complex. In the absence of added Fe''' cystine produced no significant beneficial effects in overcoming the toxicity. The results suggest that cystine present in the resistant varieties of cotton possibly chelates with the Fe''' available in the host and renders it unavailable for chelation with the toxin which seems to be a pre-requisite for toxigenic wilting of cotton. The availability of free ferric iron in the hosts for the *in vivo* formation of the toxic chelate would thus be an important factor in determining resistance or susceptibility to wilt.

Further work on the mechanism of cystine formation and its chelation is underway and is likely to throw more light on the mechanism of wilt resistance in cotton.

It is a pleasure to record my indebtedness to Professor T. S. SADASIVAN for guidance and to Dr. C. V. SUBRAMANIAN for his keen interest in the work. My thanks are also due to the Government of India for the award of a senior research scholarship.

K. LAKSHMINARAYANAN

University Botany Laboratory, Madras-5, India, June 30, 1955.

Zusammenfassung

Cystin verhindert *in vitro* in Gegenwart von Fe^{III}-Ionen bei sensiblen, abgeschnittenen Baumwollschösslingen eine Vergiftung durch Kulturfiltrat von *Fusarium vasinfectum*. Die mögliche Beteiligung von Cystin am Mechanismus der Welkeresistenz von Baumwolle wird diskutiert.

The Precipitation of Cytochrome C with a Lipidic Fraction from Tissues and Yeast

During some research work on the influence of lipidic extracts from baker yeast on the succinoxidase activity of homogenates and mitochondria from rat liver, it was observed that precipitate was formed almost immediately after the addition of cytochrome C. It seemed most likely that this precipitate contained the cytochrome C.

The system adapted in the experiment was: 0·20 ml of rat liver homogenate prepared in Potter-Elvehjem and diluted 1:10 with 0·25 M sucrose + 2 ml of 0·067 M Puffer Phosphate Ph 7·4 + 0·20 ml of $AlCl_3$ + 0·20 ml of $CaCl_2$ + 0·40 ml of Na succinate (final concentration: 0·125 M) + 1 mg of cytochrome C. Gas: O_2.

Following the above observation, I investigated the reason for this precipitation.

Material and Methods

The lipidic fraction from baker's yeast, and from rabbit's or rat's liver was prepared according to FAURE's technique[1]; a slight modification was introduced: 700 ml of sulphuric ether was added to 0·5 kg of baker yeast and shaken; then, 700 ml of 95% alcool was also added and shaken again; the material was then placed in water-bath at 37° for 12 h and then dried in a crystallizator at a temperature of 37°.

The dried material was treated with 100 ml of $CHCl_3$ at room temperature; the insoluble residue which consisted mainly of polysaccharides was then discarded by filtration. The filtrate was treated with 2 volumes of acetone, the resulting precipitate was separated by centrifugation at low speed, and then treated with ether. The fraction of precipitate which was soluble in ether contained mainly lecithines, cephaline and phosphatides (fraction I), whilst the insoluble fraction (fraction II) contained mainly cerebrosides and sphyngomyeline.

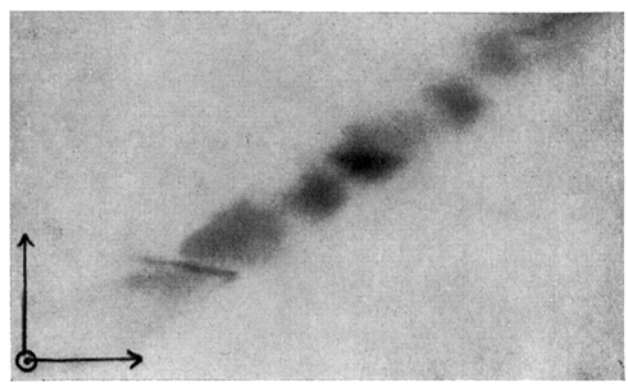

Fig. 1. – From right to left: Phenylalanine, leucine, alanine, threonine, glutamic acid, glycine, serine, aspartic acid, histidine, exonic basis.

Fraction I was the part which interested the investigator and was subjected to the following analyses:

(1) Paper electrophoresis (which was made with an Elphor apparatus): no migrating protein was detected.

(2) Paper chromatography: no free amino acids were found. After hydrolysis with 6 NHCl for 24 h and desalting with Consden apparatus, 6–10 amino acids (see Fig. 1) became evident.

Two preparations of cytochrome C were used: the first one was prepared from horse heart according to the method of KEILIN and HARTREE and contained 0·35% Fe; the second one was an electrophoretically purified preparation which was received through the courtesy of I.S.M.; its Fe content was 0·43%.

In a first experiment, 0·10 ml of a 10% suspension of lecithines prepared from baker yeast were added to a 1% solution of cytochrome C in distilled water. Almost immediately a dense red coloured precipitate was formed. The precipitate was separated by centrifugation. Nitrogen and P contents of the original lecithine emulsion and of the original cytochrome C solution as well as of both precipitate and supernatant fluid were determined. The results obtained are summarized in the Table:

[1] M. FAURE, *Techniques de Laboratoire* (Masson Publ., Paris 1947).