I am certain there are other workers doing research on soybean rust and/or its causal agent P. pachyrhizi. It would be greatly appreciated if you send me the names and addresses of these scientists.

Miscellaneous:

1) Rust pustules that I saw on leaves in both Java and Thailand were dark colored and did not look "powdery." I got the impression that they were being attacked by a bacterial hyperparasite. Does anyone have any information on this? Actually, it was frequently difficult for me to distinguish between bacterial pustule lesions and rust lesions in Java and Thailand. This was in marked contrast to the situation in Taiwan where the pustules were "reddish tan" and quite pulverulent in appearance.

2) In my notes I recorded, without further elaboration, that HY2217 from the Philippines carries resistance to rust. Any further information on this?

3) S. Moin Shah, Chief Pathologist, Department of Agriculture, Khatmandu, Nepal informed me that Uromyces vicae-fabae attacks soybeans in Nepal. Is there any other evidence for this?

Hope you find useful information in this summary. Please keep me informed on the soybean rust situation in your respective area of the world.

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1. Soybean tissue culture studies.

Tissue culture methods may benefit soybean breeders if whole plants can be differentiated from aneuploid, mutated, fused, or haploid cells. However, in order to realize this potential, it must be possible to derive plantlets from previously undifferentiated tissues — and ultimately from masses of callus cells. This report summarizes the information we obtained concerning adventitious budding from soybean tissues (Kimball and Bingham,

1973), early stages of embryo formation within masses of callus cells, and actual differentiation of plantlets from callus tissue.

'Dunn' was selected for initial studies, with other cultivars tested later. Modifications of Miller's (1965) and Gamborg's (1966, 1968) media were used for most studies. All experiments were carried out aseptically, using either semi-solid or liquid media. Cultures were kept under about five Klx continuous fluorescent light at 28 C. Several replications of each treatment were examined.

Initial studies indicated that from 2 to 8% of the uppermost hypocotyl sections and basipetal cotyledon sections produced buds from parenchymatous somatic cells, but root tips, root-hypocotyl transition zone sections, and cotyledon mid- and tip-sections did not respond. Bud differentiation was polarized, with shoots developing from the acropetal cut surface of the hypocotyl sections and from the basipetal cut surface of the basipetal portion of the cotyledons. Gamborg's medium (1966) with 15% coconut milk (or 0.5 mg/liter 2-isopentenyl adenine) and 0.5 mg/liter IAA; and Miller's medium (1965) with 0.5 mg/liter kinetin (or 2-iP) and 0.5 mg/liter IAA, were most conducive for such adventitious budding. Several cultivars produced adventitious buds (Table 1), hence the phenomenon is not overly variety-specific.

Generally, there is an inverse relationship between callus formation and adventitious budding, with 2,4-D causing the most callus but no budding, and IAA causing the least callus formation but the most bud differentiation. Addition of about 0.03 mg/liter 2,4-D to any of the above-mentioned media caused optimum callus and shoot development, but over 0.05 mg/liter 2,4-D inhibited bud formation.

Free cell cultures from 'Dunn' callus were also evaluated for morphogenetic trends. Hypocotyl and cotyledon sections were placed on Gamborg's B5 medium (1968) with 0.5 mg/liter IAA and kinetin for two weeks to form callus. The newly formed callus was then transferred to a liquid medium (Miller, 1965) with 0.5 mg/liter IAA and kinetin, and 1.0 mg/liter 2,4-D. After two weeks on a shaker table, the original callus had given rise to a cloudy suspension of free-floating cells. Microscopic examination of cell suspensions, four weeks in shake culture, revealed chains and clusters of

cells similar to filamentous and globular-phase proembryos observed in normal embryogenesis. Compact spheres of cells began to accumulate at the bottom of culture flasks after five to six weeks, often 500 to 600 from an initial 2mm hypocotyl section. Two weeks after these spheres began to accumulate, about 95% of them developed a single root, and soon recallused. Of the spheres that failed to develop rootlets, about 20% appeared to have the external morphology of proembryos (net: about 1% of spheres) including a cotyledonary-like cleft at one end and an elongated axis and having vascular differentiation within.

Similar results were obtained with the cultivated varieties 'Dunn', 'Wayne' and 'Hardee' and plant introductions of G. tabacina and G. ussuriensis (now designated G. soja). The low frequency of heart-phase proembryos probably reflects the inability of globular-phase proembryos to develop organized apical and leaf meristems when exposed to the high auxin levels necessary to produce cell suspensions.

Several different cultures have actually differentiated shoots from callus clumps, but at a low frequency. 'Dunn' embryos were cultured on Gamborg's B5 medium, the developing callus growth being subcultured on similar media after 15 weeks. Seven weeks later, the calli were broken up and subcultured again onto new B5 media. After another four weeks, leaf-like structures were noted on one of the subcultures — after a total of six months of culture on the B5 media. Shoots and plantlets have also developed from callus tissue of Glycine tabacina after culturing apices on the above-mentioned B5 medium. Roots developed after 7 weeks, followed by a cluster of five shoots that developed from a subsurface root or rhizome-like structure seven weeks later.

In summary, it is now known that soybean can produce adventitious buds from tissue sections; that under shake-culture, free cells can be induced to divide and organize into proembryoid-like structures; and that, under special conditions, adventitious shoots develop from masses of callus cells. The task at hand now is to refine the above mentioned techniques to produce consistent differentiation from callus.

Table 1

Differentiation of adventitious buds

Cultivar	Plant material cultured	
	Hypocotyl	Cotyledon
Dunn	+	+
SRF 307P	+	+
Verde	+	+
Corsoy	+	-
Ransom	+	-
Anoka	-	+
Dunfield* (T214)	-	+
Lincoln* (T212)	-	+
Richland* (T213)	-	+
Altona	-	-
Disoy	-	-
Hardee	-	-

*Tetraploids.

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