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THE ROLE OF BIOCHEMISTRY IN CROP IMPROVEMENT

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Modern plant breeding is a collective effort of a team of breeders, plant pathologists, entomologists, physiologists, biochemists, geneticists and statisticians to develop higher yielding, better adapted, disease and pest resistant and better quality crop varieties. What role do biochemists play in this team effort of plant improvement?

The biochemist can and does contribute to all aspects of crop improvement although currently his presence is most felt in the latter two-development of disease and pest resistant and better quality crops. This paper will deal with: (a) biochemical studies on plant-pathogen/plant-insect relationships; (b) investigation of nutritional and anti-nutritive substances in plants and their metabolism; and (c) how these studies aid in improving crops and thus help in the worldwide war against hunger and malnutrition.

Biochemical Basis of Resistance of Plants to Pathogens and Insects

Not so long ago in 1971 at the sixth congress of the European Association for Research in Plant Breeding, P.W. Brian of Cambridge University had this to say, "Considering how much we know of the genetics of the resistance of plants to invasions by pathogens, and considering how long plant breeders have been breeding plants with resistance to specific pathogens, it is remarkable how little we know about the biochemical basis of resistance (1)." A glaring example of how important it is to know the mechanism of a plant's resistance to pathogen or pest is illustrated by the following example. All species of cotton produce a dimeric sesquiterpene called gossypol (please see Table 1) which occurs in the seed and green plant parts of cotton and is toxic when fed to poultry and swine. To increase the feed value of the cottonseed for nonruminants, plant breeders bred strains of glandless cotton with low gossypol content, about 1/3 - 1/4 of normal cotton plant. This low gossypol cotton strain, however, turned out to be susceptible to known cotton insect pests, and at the same time, made the plant susceptible to insects not normally considered pests of cotton. Certainly, the time, effort and money spent on the breeding of the low gossypol cotton could have been devoted to some other endeavours if the role of gossypol was first studied.

Some biochemical explanations for plant resistance to pathogens and insects. In general, plant resistance may be due to: (a) structural features, (b) presence of a plant chemical such as feeding deterrents, repellants, or physiological toxins, or of a mechanism by which the plant host can inactivate the fungal toxin; or (c) the absence or low concentration of certain substances such

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as required nutrients, feeding stimulants and attractants. (a) and (b) could be preformed or inducible.

Much of the biochemical work on plant-pathogen interactions have been done on the following systems: cotton-*Verticillum albo-atrum* (2), cotton-*Rhizoctonia solani* (3), tomato-*Verticillum albo-atrum* (4), bean plants-*Colletotrichum lindemuthianum* (5, 6), and bean plants-*Pseudomonas phaseolicola* (5). The role of antimicrobial compounds called phytoalexins which occur in trace or small amounts in healthy plants but are synthesized extensively after infection has been shown in the above systems. In many cases, both susceptible and resistant varieties produce the same phytoalexins against a particular pathogen, the major difference being the greater rate of the infection - induced biochemical responses of the resistant variety (2, 5, 7).

Fewer plant-insect interactions have been studied: cucumber-cucumber beetle *(Crysolinadeae)* (8), cotton-cotton bollworm *(Heliothis* spp.*)* (9), and corn-European corn borer *(Ostrinia nubilalis)* (10).

Tables 1 and 2 show the structures of some of the phytoalexins produced by plants in response to infection by pathogens and insects.

Resistance of plants to certain disease may not only be due to the post-infectional substances called phytoalexins. The presence of specific substances in a healthy plant (preformed or pre-infectional substances) which can prevent growth of a potential pathogen has been cited to account for plant resistance although to a lesser degree. The concentration of the pre-infectional resistance factor in healthy plants is sufficient to inhibit the pathogen's growth and establishment in the plant. Recent studies have shown that the synthesis of pre-infectional toxins or inhibitors is also enchanced in cells when they are infected or chemically stressed, a behavior similar to that of phytoalexins (7).

A plant's structural features may also explain resistance. The form and/or composition of structures or tissues could prevent or diminish infection by and growth of the pathogen.

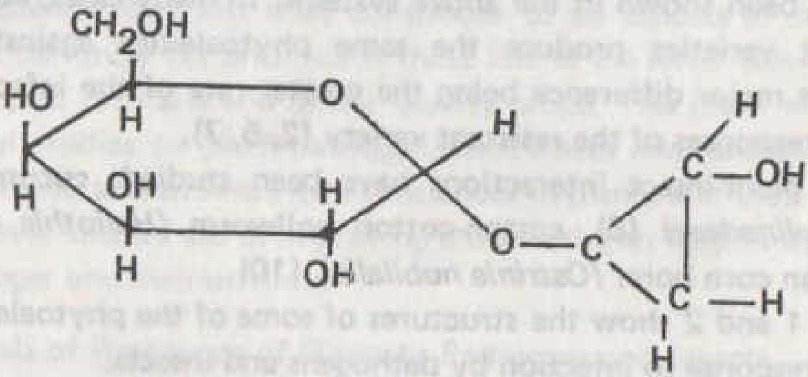
It is also possible that plant resistance may be due to the presence of more toxin receptors or sensitive sites in susceptible cells than in resistant cells or the more rapid inactivation of the toxin in resistant cell. Of course, an interplay of several modes of plant resistance to disease or insect cannot be discounted.

Plants may respond to pathogens by secreting lytic enzymes like glucanases and chitinase which could deter their colonization by microorganisms (11). Albersheim, *et al.* (12) have extracted proteins from cell walls of red kidney bean hypocotyls, tomato stems, and suspension-cultured sycamore cells, which can completely inhibit the activity of polygalacturonases secreted by fungal plant pathogens *C. lindemuthianum, C. oxysporum,* and *Sclerotium rolfsii.*

Phytotoxins produced by plant pathogens. Aside from pectic and other cell-wall degrading enzymes which pathogens secrete, they also produce toxins which can cause the symptoms of the disease in the absence of the pathogen

(13). The fungus *Helminthosporium victoriae* which causes a devastating blight disease of oats in north America produces a host-specific toxin called victorin. The chemical structure of victorin is yet to be established because of its instability upon purification. Victorin causes primarily immediate damage to the plasma membrane.

Another host specific plant toxin is helminthosporoside from the fungus *Helminthosporium sacchari.* As little as 5×10^{-12} moles causes symptoms on sugar cane leaves very similar to those caused by the pathogen.



Proposed structure of helminthosporoside,
2-hydroxycyclopropyl-α-D-galactopyranoside

Only a few other host specific toxins have been studied in detail and chemical studies are lacking. Phytotoxins which are products of bacteria and fungi that are specific in their parasitism, may be non-specific in that they have much the same effect on species resistant and susceptible to the pathogen that produces the toxin. This would suggest that other factors determine specificity of parasitism.

Applications in plant breeding. A knowledge of the biochemical basis of resistance of plants to a pathogen or insect could help in the more systematic planning and less arbitrary tactics in plant breeding programs. Practical applications are a direct result of such studies. (a) The objective chemical analysis for the resistant factor could be of great value to plant breeders because it would eliminate some of the variables in a selection program and could increase the effectiveness and efficiency for identifying resistant genotypes. The chemical analysis of DIMBOA (Please see structure in Table 2) has been reported to be used in selection of corn-borer resistant genotypes (10). After 3 cycles of inbreeding, 22 borer-resistant lines were selected by chemical analysis. Significantly, these 22 lines were all included in the 122 lines selected by the usual visual rating system. Some of the 122 lines may have other kinds of resistance factors.

(b) Host specific toxins have been used in breeding programs. Helminthosporoside, the toxin produced by *H. sacchari* against sugarcane to cause the eye spot disease has been used in selecting sugarcane seedlings resistant to said disease. Byther and Steiner (14) observed that a seedling reaction to spray application of toxin is indicative of its reaction as an adult plant. Hence, an early screen for eye spot susceptibility is now possible and can result in valuable savings in time and money. The host specific toxin victorin produced by *H. victoriae* has also been used successfully in screening oat seedlings resistant to Victoria blight.

(c) Phytoalexin production has been shown to be stimulated not only by infection by fungi but also by a wide variety of other agents such as spore-free germination fluids, heat-killed cells of the pathogen, heavy metal ions especially silver, mercury and copper. Metabolic inhibitors like iodoacetic acid and p-chloromercuribenzoate and antibiotics like actinomycin D and cycloheximide also stimulate phytoalexin production. This wide range of elicitors might suggest involvement of little host-elicitor specificity. However, as shown in the stimulation of phaseollin and pisatin formation in peas, there is a quantitative relationship of the dosage-response type between concentration of elicitor and concentration of phytoalexin. This suggests that elicitors also have a controlling influence on the rate of phytoalexin biosynthesis and its net accumulation in plant tissues (15).

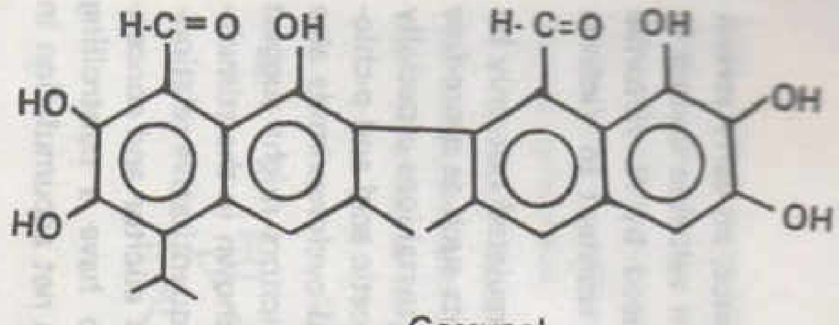
It has been suggested that harmless elicitors can be used to stimulate production of phytoalexins in plants and hence, make them resistant to the more harmful microorganisms. No large scale experimentation of this sort has been reported but the possibility of the usefulness of this technique exists.

IMPROVEMENT OF CROP QUALITY

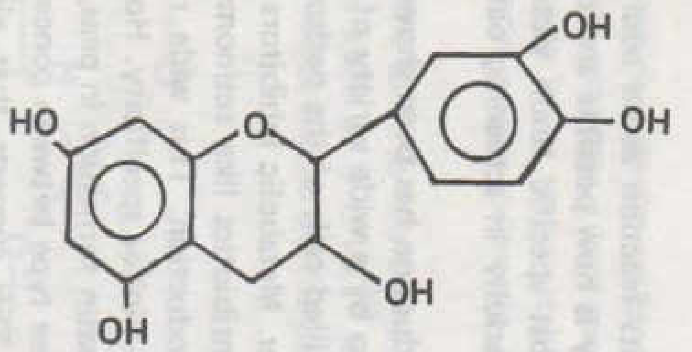
Studies on the biochemical constituents of crop produce, their nutritional values and metabolism. Again, with the current worldwide drive against hunger and malnutrition, plant breeders have directed their efforts in developing not only higher yielding, disease-resistant crops but also better quality, nutrition-wise, crops with the help of the biochemist. We are aware of the significant work done in the improvement of the lysine level in corn by the group of Mertz and Nelson at Purdue University in the 1960's. A systematic study of the different maize proteins showed that the alcohol-soluble component zein which consists of 52% of total protein has limiting amount of lysine. A search for maize with a lower zein and a higher lysine content resulted in the discovery of a strain homozygous for the recessive mutant gene, opaque-2 (o2) which had lower zein (30%) and had 4% lysine, twice that found in typical normal corn. Further surveys of mutant varieties showed that a strain homozygous for the recessive mutant gene, floury 2 (fl_2) had lower zein content and had a lysine content approximately twice that of normal and nearly as high as opaque-2. Decreasing the zein component and increasing the other types of proteins resulted in the overall increase in lysine content and improvement of the biological value of corn protein. This high lysine character in the opaque-2 and floury-2 genes has been successfully incorporated into corn varieties which are higher yielding, of

Table 1. Phytoalexins produced by plants in response to pathogenic infection

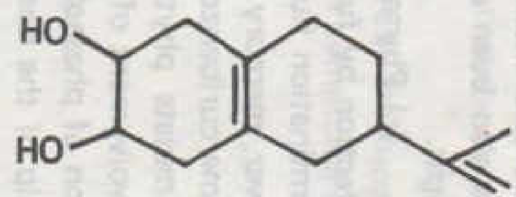
PLANT	MICROORGANISM	PHYTOALEXIN
Cotton	*Verticillium albo-atrum*	gossypol and gossypol-related compounds
Cotton	*Rhitozoctonia solani*	catechin and other polyphenols
tomato	*Verticillium albo-atrum*	rishitin



Gossypol

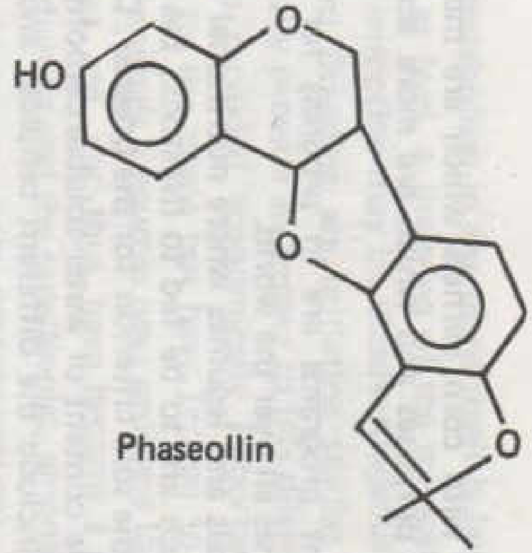


Catechin



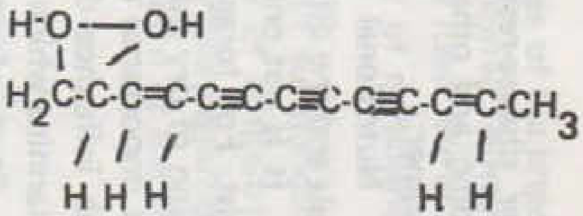
Rishitin

bean	*Colletotrichum*	phaseollin
plants	*lindomuthianum*	phaseollidin
		phaseollinisoflavan
		kievetone
	Pseudomonas phaseolicola	ditto
	tobacco necrosis virus	ditto



Phaseollin

safflower	*Phytophthora drechsleri*	safynol
		dehydrosafynol



Safynol

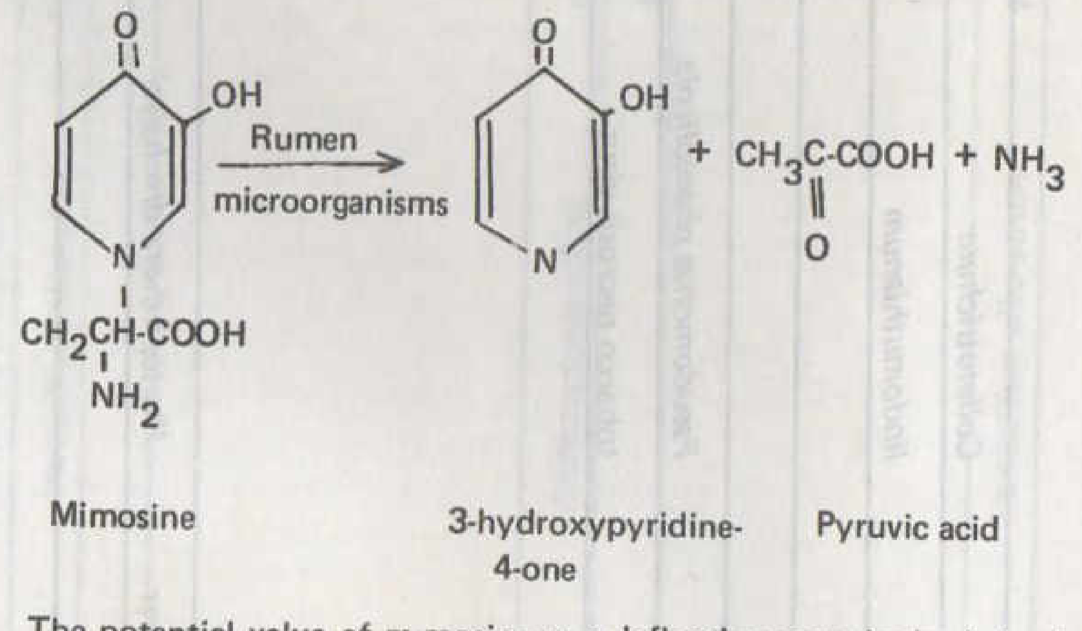
better disease-resistance and with corn kernels which are more suitable for grinding and other industrial purposes and which are more acceptable to the consumer.

Nutritional improvement programs are in progress for rice and other cereals and the different legumes all over the world.

It is not only with cereals and legumes where nutritional improvement is sought. For forage crops which are to be fed to livestock, the maximum production of dry matter is not the sole criterion for the breeder. Digestibility is of great importance as well as the content of water-soluble carbohydrates. Factors which influence digestibility include the different cellulose and hemicelluloses and tannins which have been shown to reduce protein digestibility.

Study of antinutritive or toxic constituents of plants and their metabolism. Some plants of economic importance have substances deleterious not to themselves but to animals which feed on them. An example mentioned before is gossypol which has been shown to be responsible for the resistance of cotton to most cotton pests. Intensive work is currently being done on the metabolism and toxicological effects of mimosine, a biologically active amino acid from Ipil-ipil *(Leucaena leucocephala)* at the CSIRO Tropical Crops and Pastures Division in Australia (16). *Leucaena* is used as feed for dairy cattle in northeast Queensland and for beef cattle in northern Australia and has also become popular in use here in the Philippines.

Research at CSIRO is concerned with the substances which may be responsible for the enlarged thyroid glands and poor weight gains of beef cattle and calves grazing at *Leucaena.* Their studies show that mimosine is metabolized into 3-hydroxypyridine-4-one (DHP) which is a potent goitrogen.



Mimosine 3-hydroxypyridine-4-one Pyruvic acid

The potential value of mimosine as a defleecing agent is also being investigated with emphasis on the excretion and metabolism of mimosine in sheep during defleecing with a single dose of mimosine (300-600 mg/kg body weight). Although researches on metabolism of mimosine in animals are on going, work on its role and metabolism in the plant itself is lacking. Because of the importance of Ipil-ipil as feed, a lowering of the mimosine level is desired. However, this lowering of mimosine may be disadvantageous to the plant itself. We at the Institute of Plant Breeding are initiating studies on the role of mimosine in ipil-ipil to hopefully provide an answer to this dilemma.

Nature has provided economically important crops with substances of unknown value to the plant but have proven to be toxic or of antinutritive value to man and animals. Legumes have protease inhibitors, phytohemaglutinins, flatullence factors, etc., and thus, have become a primary concern of the plant breeder, the biochemist and the nutritionist, among others.

IN CONCLUSION

We have enumerated above some instances of the biochemist's involvement in crop improvement. The list is getting longer with the advances in technology. In the near future we may find the following as routine techniques in the improvement of crops: (a) Use of specific enzyme activity as predictor of crop yield potential; (b) Use of seed isoenzyme patterns as a guide to effective response to specific *Rhizobium* strains for more efficient nitrogen fixation; (c) Use of biochemical parameters to predict seedling vigor and performance, photosynthetic efficiency, etc.; and (d) Use of biochemical parameters to screen for drought, pest, and disease resistance.

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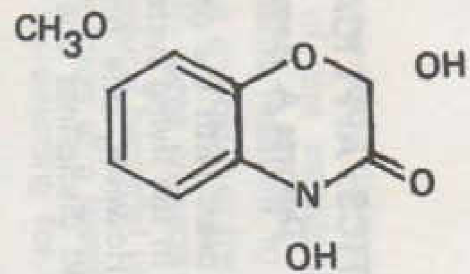
Table 2. Plant Chemicals against insects

PLANT	INSECT	SUBSTANCE
Cotton	Bollworn (Heliothis app.)	Gossypol
Corn	Cornborer *(Ostrinia nubilalis)*	DIMBOA 2,4-dihydroxy-7-methoxy-1,4-(2H)-benzoxazin-3-one
Cucumber	Cucumber beetle (Crysolinadeae)	Cucurbitacin (tetracylic terpenoids)



DIMBOA 2,4-dihydroxy-7methoxy-1,4-(2H)-benzoxazin-3-one