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July 1, 1969 - June 30, 1970

Regional Pulse Improvement Project
U. S. Agency for International Development
U. S. Department of Agriculture, ARS

Improvement of Food Legumes for the Trop

Plant Science Research Divisio
Federal Experiment Station
Mayaguez, Puerto Rico

Reference Center
Room 1656 NS

Summary

In 1969, The USDA/AID Regional Pulse Improvement Project was expanded to include research on the improvement of beans and cowpeas (southern peas) for use by developing countries in tropical Latin America. Dr. N. G. Vakili, plant pathologist-breeder, was assigned to this project in August 1969. Dr. Vakili is located at the Federal Experiment Station, Mayaguez, Puerto Rico. He cooperates with other members of the Station Staff including Dr. R. Alconero, Plant Pathologist, and Dr. Eric G. Stone, Agronomist. He also cooperates with staff members of the Agricultural Experiment Station, Puerto Rico.

Many underdeveloped tropical countries rely heavily on pulse crops as sources of dietary protein. This is particularly true in the American tropics. Although beans are native to the area and are widely grown, production falls far short of supplying local requirements in most Latin-American countries.

The Mayaguez station provides an excellent location for developing improved cultivars adapted to various tropical areas. Significant advantages at Mayaguez include the availability of highly diverse soil and climatic conditions; availability of a staff with a high level of technical competence combined with experience in tropical agriculture; and organizational affiliation with USDA staff and facilities at other locations with which cooperative projects can be conducted.

These advantages provide the opportunity for a high level of project achievement.

The prospect is excellent for producing bean and cowpea cultivars of great usefulness to many Latin American countries needing to increase their protein production.

A detailed report of progress in the form of exerpts from the Report of Activities of the Federal Experiment Station for the Semesters July 1 to December 31, 1969, and January 1 to June 30, 1970, will be provided upon request. A summary of research progress on bean (*Phaseolus vulgaris*) and cowpea (*Vigna sinensis*) improvement follows:

Beans (*Phaseolus vulgaris* L.)

1. Field Evaluation of Plant Introductions for Disease Resistance

A major objective in the improvement of beans for tropical countrie is the development of disease resistant germ plasm. Diseases are a major factor limiting yields of beans in all tropical countries. Frequently, the only practical disease control for growers in developing countries is the use of resistant varieties.

The foundations for the present investigation was laid in 1967 and 1968 by the staff of the USDA Federal Experiment Station in Puerto Rico, when all available USDA plant introductions, some 2,500 lines, were grown in Puerto Rico for evaluation. More than 200 promising lines were selected from these plantings, for more intensive evaluation.

Thus, when the AID funded program was initiated in September 1969, substantial information and germ plasm was at hand for the more intensive study permitted by the additional funding. During the period covered by the report, both this germ plasm as well as additional germ plasm was screened against virus, fungus, and bacterial diseases. In the Fall of 1969, a planting of 148 lines of single plant and six bulk selections (total 154) from 84 Plant Introduction accessions were planted in the field at Mayaguez and observed for reaction to diseases either with or without inoculation.

In the tropics, beans are attacked by a number of virus diseases, the most serious of which is the seed-borne virus, common bean mosaic (CBMV). The 154 lines and selections were inoculated twice in the field with CBMV using a spray technique. Of the 154 lines, 3 were immune, 24 highly resistant, and 30 moderately resistant. The remaining were moderately to highly susceptible. Five lines were segregating. Although field inoculations were not made, the following viruses were observed in the field planting: Bean Yellow mosaic, Golden Yellow mosaic, and a complex of viruses designated as Pseudo Curly Top-Witches Broom-Thick Leaf Dwarf. Some 30 lines were free from these viruses. Lines appearing to be resistant to CBMV in this test must, of course, be subjected to additional inoculation tests in the greenhouse to confirm this, and resistance to the other viruses must be confirmed by inoculation experiments where techniques are available. Thus, virus-resistant germ plasm has been identified but is not yet ready for distribution.

In addition to viruses, diseases caused by fungi and bacteria also are associated with low and erratic yields of beans in tropical areas. Those present in Puerto Rico included Common bacterial blight (*Xanthomonas phaseoli*), rust (*Uromyces phaseoli* var. *typica*), fusarium wilt (*Fusarium oxysporum* f. *phaseoli*), Rhizoctonia stem canker (*Pellicularia filamentosa*), southern blight (*Sclerotium rolfsis*), angular leaf spot (*Isoriopsis griseola*), and anthracnose (*Colletotricum lindemuthianium*).

Screening and observation for fungus and bacterial disease resistance was made in the same bean planting used for screening for resistance to viruses. Inoculations were made only with rust by shaking severely infected plants over the nursery. Of the 154 entries inoculated, 112 were resistant, and 42 were susceptible to rust. The predominance of rust resistance could be due to the fact that 123 of the lines were black-seeded - a characteristic frequently correlated with rust resistance.

Since the nursery was located on a site uniformly infested with the fusarium wilt organism, it was possible to screen entries for resistance. Sixty were resistant and 94 susceptible.

Common bacterial blight appeared in the field 1-1/2 to 2 months after imergence and spread rapidly due to rain and overhead irrigation. Of the 154 entries, 81 were immune, 4 resistant, 13 intermediate, 37 susceptible, and 19 were defoliated by rust or killed by fusarium wilt before bacterial blight readings could be made.

Observations also were made on other diseases present although actual systematic screening of entries for resistance were not made because of the lack of uniformity of infection.

As with virus diseases, the reaction of the selections to fungus and bacterial diseases must be reconfirmed by additional screening before they can be released to breeders as resistant germ plasm. It is possible that a new and very virulent strain of anthracnose is present in Puerto Rico.

Table 1 summarizes the reaction of the 154 selections to Common bean mosaic, rust, and fusarium wilt, the only diseases to which all entries were screened.

Table 1. Resistance of 154 P.I. entries to CBMV, rust and fusarium wilt

Response to Diseases			Number of
CBMV[1/]	Rust	Fus. wilt	entries
Res.	Res.	Res.	5
Sus.	Res.	Res.	30
Res.	Sus.	Res.	9
Res.	Res.	Sus.	35
Sus.	Sus.	Res.	16
Res.	Sus.	Sus.	8
Sus.	Res.	Sus.	42
Sus.	Sus.	Sus.	9
		Total	154

1/ Common Bean Mosaic Virus

In many crops, it has been necessary to obtain resistance to certain diseases from wild types. For this reason, wild types of beans, collected by plant explorers in the tropics, are being screened as sources of resistance. Eight such wild types from Mexico were planted on March 25, 1970, at Mayaguez and inoculated with disease organisms or observed for reaction to naturally-occurring diseases. Four Plant Introduction lines, selected for their high yielding characteristic, and one commercial variety were used as standards for comparison of performance Reaction to diseases ranged from susceptibility to immunity. In general, the wild types were susceptible to common bean mosaic virus and Cercospora leaf spot (Cercospora spp.), and resistant or immune to Bean Yellow mosaic, Common bacterial blight, rust, Angular leaf spot, Rhizoctonia stem canker and fusarium wilt. The resistance to Angular leaf spot is especially interesting since this is a very serious disease of beans in tropical countries.

Hybridization studies to produce improved beans for the tropics

Not all desirable attributes of agronomic performance, disease resistance, etc., are present in any one variety, breeding line, or selection. Therefore, it is necessary to produce hybrid progeny combining desirable traits of various strains of beans once germ plasm possessing the desirable characteristics has been identified. Such hybridization is a very laborious and time-consuming process, and the man-power on this project is extremely limited. For this reason, carpenter bees that frequent the bean flowers have been utilized for this purpose. Experiments indicate that the percent outcrossing resulting can be as high as 20%.

During the past year, 153 crosses have been made. Since parents were not isolated for this purpose, only the maternal line is known; but, knowledge of parentage is unnecessary if the desired combinations are secured. The apparent hybrids, mostly black and shiny seeded, have been put through screening trials for selection of the desired characteristics. Selection in some lines has reached F_4 progenies. Results of this work will be tabulated and presented in the next report.

Bean Virus Studies

As indicated earlier in this report, virus diseases limit bean production in most tropical (and temperate) countries. A variety of viruses produce numerous, diverse and confusing symptoms on bean plants. Basic to control of these diseases is the positive identification of specific viruses with specific syndromes, the determination of specific vectors, and the identification of reservoirs of weedy or alternate hosts that may contribute to epidemics.

Thus, considerable effort has been made to study and identify the viruses appearing in field plantings of beans, and some progress has been made in this difficult and time-consuming task.

Field symptoms of the virus diseases indicate that a number of viruses may be present. In an attempt to identify the viruses, inoculum (infected leaves) was obtained from plants showing the following symptoms: extreme stunting with leathery leaves, typical common bean mosaic virus symptoms, bright yellow mottle, blistering of the leaves, bean yellow mosaic symptoms, and bright yellow-mottle with thickened leaves. Seed of differential varieties for common bean mosaic plus seed of the plant introduction lines showing these symptoms in the field were planted in the greenhouse and inoculated mechanically with each of the virus sources mentioned above.

None of the differential varieties or lines inoculated responded with the same symptoms evidenced in the original plants. In other words, the inoculum from the dwarfed plant did not cause dwarfing in the plant inoculated. This suggests that more than one virus may be present in many plants. It suggests further that some can be mechanically transmitted, while others may not. Further tests are in progress to characterize the viruses.

One virus in complex that has been identified is Common bean mosaic. This virus is very important since it is seed-borne. A number of strains have been identified in various parts of the world. Preliminary studies with this virus indicate the presence of several strains in Puerto Rico, including some of which differ from any of the strains previously reported.

Cowpeas (Vigna sinensis)

The objective of the research on cowpeas (southern peas) is similar to that on beans, namely: the development of improved germ plasm for the tropical portions of Latin America. In fact, such germ plasm could have usefulness in tropical areas throughout much of the world. Major emphasis is on the development of desirable agronomic types and on disease and insect resistance. Initial efforts have involved screening Plant Introduction lines for desirable characteristics.

Approximately 500 Plant Introduction lines were planted at the Isabela farm of the Federal Experiment Station in January 1970. Twenty-five seeds of each line were planted in 20 foot rows, placed on 40 inch centers. Fertilizer was applied before sowing and as a side dressing 6 weeks after emergence. Soil was treated with an insecticide before planting and a month later. One month after planting, an invasion of cutworms eliminated 178 lines planted in a 70-foot wide belt and these had to be replanted. A contact and systemic insecticide was applied for up to 4 months following emergence. Agronomic data and reaction to natural infestations of diseases and insects were recorded.

Agronomic evaluation of cowpeas

Agronomic data were recorded with two objectives in mind: (1) early maturing bush types for cultivation in the tropical lowlands and (2) early vining types with prostrate and persistent growth habit for use as a cover crop in tropical highlands. Data were recorded on the following: (1) range of growth habit from compact, erect, bush-type growth to prostrate vines; (2) range of size from 12" or less in height or diameter to plants 30 inches tall or taller with a vine spread of 5 feet or more; (3) earliness, indicated by days to vining, flowering and mature pods and (4) mortality and defoliation as indicated by percentage of dead plants and percentage of foliage on surviving plants.

Of the approximately 500 lines tested, 36 had strong bush habit while 36 had strong prostrate habit with long vines. A relationship was observed between growth habit and length of survival; namely, prostrate viny types generally retained 70 to 100 percent of the foliage over a 6-month period whereas the bush early-maturing types had mortality of 70 to 100%. Except for a few notable exceptions, growth habit and also maturity appeared to be associated with susceptibility to anthracnose (*Colletotricum lindemuthianum*), in that generally bush type early maturing lines were susceptible while prostrate viny lines were resistant. However, it appears that senescence and not anthracnose was the cause of plant mortality in the early varieties.

Reaction of cowpeas to diseases

The planting used for agronomic evaluation also served for observation of reaction to diseases. Both virus and fungus diseases were present.

As in the case of beans, a number of viruses attack cowpeas in Puerto Rico and research is underway to provide positive identification, to study the extent to which these are seed-borne, and to determine the vector relationships. Preliminary results indicate two viruses were present, cowpea chlorotic mottle, and Southern bean mosaic. The former was widespread in the planting, but field inoculation will be necessary to determine the extent of resistance in the lines. In the case of mosaic, which is seed-borne in cowpeas, only a few scattered plants in the field displayed infection from the early seedling stage, and the disease spread very slowly, probably because of control of the vector with insecticides. A group of black seeded lines were inoculated in the field with diluted sap from mosaic infected plants. A number of these lines showed moderate to severe mosaic. Within the non-inoculated lines, eleven were classified as having no mosaic. In addition to southern bean mosaic virus, cucumber mosaic also may have been present.

The cowpea planting became naturally infected by the following fungus diseases: Powdery mildew (*Erysiphe polygoni*), Cercospora leaf spot (*Cercospora* spp.), and Anthracnose (*Colletotrichum lindemuthianum*).

Of the approximately 500 lines, 31 were recorded as resistant to powdery mildew and 11 were free from Cercospora leaf spot. Anthracnose was by far the most prevalent disease in the planting, and was the primary cause of extensive defoliation. No anthracnose lesions were observed in 63 lines.

Reaction of cowpeas to insects

In spite of application of contact and systemic insecticides, insects were prevalent in the planting, especially leaf miner (*Chrysocharis parksi*) which began to infest the seedlings from the time the first leaf expanded. The infestation continued and was brought under control whenever dimethoate, as systemic insecticide was applied at the rate of 1-1/2 pt/A and repeated at 6-7 day intervals. By late March, 4-1/2 months after sowing, infestation became severe and data were recorded indicating the total percentage of foliage infested by leaf miner. There were accessions with no more than 2-5% leaf infestation located adjacent to varieties which had 60-80% of the foliage tunneled. Not only was the percentage of the leaves infested low, but also the diameter of the tunnel was very small. This suggested that lack of uniform infestation was not due solely to preference for one variety over another by the insects. Sixty-seven accessions had less than 5% of the foliage tunnelled. Among these, 10 accessions were either free from infestation or displayed the narrow tunnel response.

Bean Leaf Beetle (*Cerotoma ruficornis*), were chewing the leaves to the point of reticulation. Up to six months after planting no accession has been observed which did not have its young leaves severely chewed by these beetles. Three accessions were only moderately damaged.

As indicated earlier, cutworms (*Laphygma frugiperda*) caused heavy seedling mortality, and were controlled by application of an insecticide. During the warm rainy season starting in early June, Caterpillers of the genera *Etiella* and *Fundula* and cowpea curculio caused heavy pod infestation.

Lines showing resistance to diseases or insects must undergo additional testing to be sure that they are truly resistant and not escapes, before they can be distributed to breeders as improved germ plasm.

Effect of photoperiod on growth habit of cowpeas

A study was initiated to determine the effect of photoperiod length on the growth habit of cowpea lines. In a preliminary planting made in the short photoperiod of the winter months the plants were indexed for growth habit ranging from low runner types to short bush types.

From the preliminary study, three runner types, three bush types, and three intermediate types were selected. These nine entries were planted in replicated tests under lights and under ambient conditions. The lighted area was set for a 14-1/2 hour photoperiod, which is longer than the longest day in Puerto Rico.

Results from this experiment are not yet available. However, it will be interesting to compare these two photoperiod responses with the short days of the winter season. Some variation is expected in the growth habit, especially in the two extremes of runner and bush, but a complete reversal in these two groups is not expected. This will give some idea of the variation to expect when selecting for certain growth habits in different seasons of the year.