Michigan State University
Dry Bean Breeding Program

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SUMMARY OF BREEDING PROGRAM

The dry bean breeding program at MSU is focused on the development of high yielding, upright, disease resistant cultivars with improved canning quality in eight commercial classes of dry bean. The research work is an ongoing investigation where genetic stocks at different stages of development exist in all commercial classes and new varieties are released after adequate testing. As different needs appear or as disease or quality problems arise, the program looks to address these by incorporating new genetic sources. The major focus for this current research is to develop high-yielding, anthracnose resistant navy and black bean varieties; identify high-yielding, disease-resistant bush and vine cranberry varieties; continuation of a study of root rot resistance in kidney beans; development of an upright, disease-resistant pink bean; combine genes for rust, virus, anthracnose and white mold resistance in pinto and great northern market classes to ensure the successful production of all new MSU varieties in Michigan.

OBJECTIVES

1) Continué to develop high-yielding, type-ll navy bean varieties exhibiting a range of maturities(90-100days), acceptable canning quality, tolerance to white mold and adequate levels of resistance to anthracnose.

2) Breed a light red kidney bean with the improved yield potential, early-season maturity coupled with resistance to Andean race 7 anthracnose and retain the canning quality of Chinook.

3) Produce a dark red kidney bean with the canning quality of Montcalm combined with higher yield potential, earlier maturity, additional anthracnose resistance and tolerance to root rots.

4) Develop an upright black bean with the same yield and maturity as T-39 combined with improved levels of anthracnose resistance from new Mesoamerican and Andean sources.

5) Improve the white mold tolerance and anthracnose resistance of bush cranberry beans.

6) Create a vine cranberry bean with better plant structure, earlier maturity, larger seed size and equivalent canning quality to the Michigan Improved Vine Cranberry variety.

7) Combine the rust, virus and anthracnose resistance in the next generation of upright pinto and great northern bean varieties.

8) Identify and develop lab/greenhouse screening methods to identify sources of resistance to white mold and Fusarium root rot, and incorpórate these resistance genes into current bean varieties.

RECENT ACCOMPLISHMENTS

Based on the combination of yield performance data, agronomic adaptation, disease resistance and canning quality, a new type-ll navy bean Mackinac was released. Mackinac is derived from a single cross with Avanti. It is similar to Avanti in maturity, canning quality and yield but possesses the Co-1 gene for resistance to anthracnose and the Ur-3 gene for resistance to rust to which Avanti is susceptible. Mackinac is more erectthan Avanti,

similar to Mayflower in architecture but is five days earlier in maturity than Mayflower and equivalent in yield to both Vista and Mayflower. Certified seed will be available in 1998.

In the dark red kidney (DRK) class, Red Hawk was released based on continued superior performance. Red Hawk matures earlier than Montcalm, exhibits better dry down at harvest, has excellent seed and canning quality traits and possesses the Co-1/Co-2 gene combination for resistance to anthracnose. Red Hawk lacks the level of common blight resistance present in Montcalm parent but has equivalent low levels of resistance to root rot as Montcalm. The breeding program plans to introduce improved levels of root rot resistance into the DRK class as soon as suitable sources of resistance are identified and characterized.

Afull season lightred kidney (LRK) line K94601 derived by selection from Chinook was released as Chinook 2000 based on continued consistent performance. Chinook2000 is identical to Chinook in agronomic traits but differs in possessing the Co-I/Co-2 gene combination which provides resistance to Andean race 7 anthracnose which attacked all LRK varieties in 1994. Currently no commercial LRK variety has this complete anthracnose resistance combination.

In the large white Alubia bean class, Beluga was released as the first variety in this market class. Beluga is similar in growth habit (type-I), full-season maturity, performance and agronomic and disease resistance to Montcalm. Since the canning quality of beans in this market class is critical and few lines meet the satisfactory standard, Beluga was released primarily for its consistent canning quality.

A high-yielding pinto breeding line P94207 with excellent large, fíat seed and resistance to rust (Ur-3/Ur-6 genes) and BCMV (I/bc-1^2 genes) was released as the variety Kodiak.The plant structure of Kodiak is weaker than other type-11 pinto varieties, such that it lodges under certain conditions but the combination of high yield and large seed size makes it very competitive with other vine pinto varieties. The combination oftwo resistance genes for rust and the protected I gene reaction to BCMV make Kodiak valuable in the inter-mountain areas where these diseases are endemic.

In the great northern (GN) class, an early-season, high-yielding GN breeding line G93414 with excellent bright white seed and resistance to rust (Ur-3 gene) and BCMV (I gene), was released as Glacier. The plant structure of Glacier is erect with excellent uniform dry down at 90 days after planting or three days earlier than Alpine. The superior agronomic traits of Glacier have been observed in locations outside Michigan and Glacier has topped GN triáis in Nebraska and Washington. Seed quality is excellent as compared with Alpine but it is smaller than Starlight.

SUMMARYOFGENETIC RESEARCH PROGRAM

Since anthracnose has become a serious disease problem in Michigan, less effort has been directed towards breeding for bean common mosaic virus (BCMV) and rust. Screening for resistance to BCMV was restricted to the greenhouse and the most virulent isolate of the virus (NL-3) was used to detect different resistance genes. This work has advanced rapidly and combined BCMV resistance (I plus bc-3 gene or I plus bc-2^2 gene or I plus bc-1^2 gene) is present in the majority of seed classes. Screening for rust is conducted annually in the field at Saginaw using race 53 which detects the resistance gene Ur-3 and has permitted the introgression of this gene into many breeding lines in all classes. The Ur-3 gene is still effective against the majority of rust races present in N. America. Todate in cooperation with Dr. Stavely, ARS Plant Pathologist, Beltsville, MD, we have released 11 navy, 13 pinto and 3 great northern lines with pyramiding resistance genes and the most recent releases also carry the I and bc-3 genes.

RECENT ACCOMPUSHMENT

The ¡nheritance of genetic resistance to bean anthracnose in genotypes, Catrachita and SEL 1360, derived from two anthracnose differential cultivars, AB 136 and G 2333, respectivety, is described. Segregation data from three different F2 populations and their respective F2:3 families indicated that a single dominant gene is responsible for the anthracnose resistance in Catrachita. In the test for allelism, Chisquare test confirmed that the single dominant resistance gene in Catrachita was situated at a different locus from previously characterized resistance genes: A, Are, Mexique 1, Mexique 2 and Mexique 3. tt is proposed that the single dominant resistance gene present in Catrachita be assigned the genetic symbol Co-6, Co for Colletotrichum and 6 corresponding to the sixth major anthracnose resistance gene characterized and reported in the literature. Segregation in the three F2 populations where SEL 1360 was used as the resistant parent, fitted a 3:1 (R-: rr) ratio and a 1:2:1 (RR: Rr: rr) ratio in the F2:3 families. Segregation data suggested that a single dominant gene was conditioning resistance to anthracnose in SEL 1360. The test for allelism involving SEL 1360 indicated that the single dominant gene in SEL 1360 is independent from A (Co-1), Are (Co-2), Mexique 1 (Co-3), and Mexique 2 (Co-4) genes. However, the dominant gene in SEL 1360 did not segregate independently from the resistance gene Mexique 3 in the differential cultivar TU, demonstrating that both dominant alíeles are located at the same locus. Deployment of major genes of Middle American origin, such as Co-6 and Mexique 3 (Co-5), in different combinations with other characterized genes of Andean origin is possible and should contribute to more durable anthracnose resistance in common bean.

Two independently assorting dominant genes conditioning resistance to bean anthracnose were identified in an F2 population derived from the highly resistant bean differential cultivar, G 2333. One gene was allelic to the Co-4 gene in the differential cultivar TO and was named Co-4^2, whereas the second gene was assigned the temporary ñame Co-7 until a complete characterization with other known resistance genes can be conducted. Two RAPD markers linked to the Co-4^2 alíele were identified. One RAPD, $OAS13_{950}$, co-segregated with no recombinants in two segregating populations of 143 F2 individuáis, whereas the second RAPD $OAL9_{740}$ mapped at 3.9 cM from the Co-4^2 alíele. Two 24-mer SCAR primers (SAS13), developed from the $OAS13_{950}$ RAPD marker, were dominant and polymorphic similar to the original RAPD, and supported the tight linkage between the marker(s) and the Co-4^2 alíele. The markers were present in germoplasm with known resistance alíeles at the Co-4 locus. The presence of the markers in two other differential cultivars, not previously characterized, and in four navy bean cultivars suggests the existence of a gene family for anthracnose resistance at or near the Co-4 locus. Since the Co-7 gene was present only in germplasm which also possessed the Co-4^2 and Co-5 genes, the SAS13 markers were used in combination with standard inoculation techniques to identify F3 lines in which the Co-7 gene was homozygous and the Co-4^2 alíele was absent. A similar strategy of marker assisted dissection is proposed to identify resistant lines in which the Co-5 gene is absent and the Co-7 gene is present by selecting against the OABS^ marker, shown previously to be linked to the Co-5 gene. These genes cannot be distinguished using traditional screening methods since all current races of the pathogen virulent to the Co-5 gene are avirulent to the Co-4^2 and Co7 genes.

Durability of resistance to anthracnose, could be improved if more than one resistance gene is incorporated into current bean cultivars. Markers linked to three independent resistance loci were identified using bulked segregant analysis and heterogeneous inbred populations. RAPD marker $OF10_{780}$cosegregated in repulsion-phase (1.9 ± 1.4 cM) with the Co-1 (A) alíele. A survey of diverse bean genotypes showed that the $OF10_{780}$ RAPD band could facilítate introgression of the Co-1 gene across the Andean and Middle American *Phaseolus* gene pools. RAPD marker $OAB3_{450}$

was linked in coupling-phase (5.9 ± 1.7 cM) to the Co-5 (Mexique 3) alíele. A coupling-phase ($OAH1_{780}$) and a repulsion-phase ($OAK20_{890}$) RAPD marker were linked to the Co-6 locus. These markers the Co-6 locus and mapped at 12.3 ± 1.9 from the Co-6 alíele and at 7.3 ± 1.5 cm from the Co-6 alíele, respectively. Coupling and repulsion-phase RAPD markers used as a codominant pair showed a higher selection efficiency (95 %), forthe identification of homozygous (Co-6 Co-6) F2 individuáis, compared to individual selection either for a coupling-phase (33 %) or against a repulsion-phase (92%) RAPD phenotype.

STRATEGIES DEPLOYED IN BREEDING FOR RESISTANCETO NEW RACES OFANTHRACNOSEIN MICHIGAN

Fourisolatesof C. *lindemuthianum,* pathogenic on previously resistant dry bean *(Phaseolus vulgaris)* cultivars, were collected in 1993 in Michigan and North Dakota from seeds produced in Michigan. Characterization of the isolates on two sets of differential dry bean cultivars demonstrated that three isolates were similar and were classified as race 73. These isolates resembled the alpha-Brazil race recently reported in Ontario.

Thefourth isolate was unique and was classified as race 7. This isolate resembled most closely the delta race identified in Ontario in 1976. This is the first report of the occurrence of either race 7 or 73 of C. *lindemuthianun* in Michigan. Although the origin of these races is unknown, race 73 appeared to have been present in Michigan State University bean breeding lines since 1991 but was not detected until 1993 when resistant cultivars showed typical anthranose symptoms. The presence of these races in Michigan threatens current commercial cultivars, since race 73 overcomes the Co-2 gene, while race 7 overcomes the Co-1 gene; both of which have been extensively used in the breeding program. The occurrence of these new races in North America challenges current breeding strategies of using single gene resistance to control anthracnose. Gene pyramiding using molecular markers as a disease resistance strategy is discussed, since the Co-1 /Co-2 gene combination affords resistance to both races.

A third race of anthracnose was identified in Michigan in 1996. Race 65, also known as epsilon, was found in commercial fields of T-39 and is the first report of this race in Michigan. Previous reports indicated its presence in Ontario. Race 65 does not differ greatly from race alpha (race 17) so both the Co-1 and Co-2 genes are effective as resistance sources. Given the concerns over anthracnose and the presence of new races, the program has identified three new sources of resistance. One source was identified in a red bean from Chiapas, México, the second in a small red bean from Honduras and the third in a pinto bean from México. The genes were confirmed to be single dominant genes and will be known as Co-4, Co-5, Co-6 and Co-7. All genes are effective against all North American races of anthracnose and are currently being incorporated into navy, black, pinto, great northern, kidney and cranberry bean classes. These genes are different than those previously used in the program and offer resistance to a greater number of races of anthracnose. In addition the breeding program has successfully identified six molecular markers known as RAPDs linked to four anthracnose resistance genes (work is underway with Co- 7 gene). Use will be made of these markers to facilítate the pyramiding of more than one anthracnose resistance gene into future bean varieties with the objective of improving the durability of anthracnose resistance genes. This is to avoid the problem that aróse as a result of the appearance of race 73 which attacked the previously resistant Co-2 gene in Blackhawk.

Durability of resistance to anthracnose could be improved if: (i) two major resistance genes were pyramided using marker assisted selection, (ii) different genes are deployed in different regions, and (iii) resistance genes from different gene pools are combined. Stepwise strategy used in breeding for resistance to anthracnose:

1) Combine the Co-1 and Co-2 genes into a single variety.

2) Incorpórate new Mesoamerican sources of resistance, Co-4, Co-5, Co-6, Co- 7 genes.

3) Identify Andean resistance genes in Mexican pintos and Kaboon.

4) Combine all resistance genes into single variety using linked markers.

SUMMARYOFPATHOLOGY RESEARCH

One hundred thirty eight isolates of *C. lindemuthianun* from Argentina, Brazil, Colombia, Costa Rica, the Dominican Republic, Honduras, México, Perú, and the United States were characterized in 41 races based on virulence to 12 differential bean cultivars. These 41 races were categorized into two groups: those found over a wide geographic area and those restricted to a singlecountry. Race 7,65, and 73 werewidespread. Race 73 was the most common (28%). Race 7 was found once in Argentina and México but at a higher frequency in the United States. Race 65 was found repeatedlyin Brazil and the U.S.Although 39 % of the races were detected repeatedly and three races were widespread, no race was isolated from both *P. vulgarisgene* pools. Pheneticanalyses showed no obvious patterns correlated with virulence clusters. No geographic pattern was evident. Molecular polymorphism generated by RAPDs confirmed the extensive variability in virulence of C. *lindemuthianun.* Virulence phenotypes were grouped into 15 clusters. The two largest clusters contained isolates from all the geographic regions sampled. Molecular polymorphism was observed among isolates from races 65 and 73 within and among countries, except among Brazilian isolates of race 65. The geneticdiversityof *C. lindemuthianumwas* greatest in México and Honduras. Our data suggest that *C. lindemuthianum* may not be highly structured to specific *Phaseoius* gene pools.

Thirty-four of the previous 41 races were inoculated on sixty-two bean cultivars from Brazil, the Dominican Republic, Honduras, México, and the United States. Bean genotypes clustered based on the gene pool origin of the resistance genes present, regardless of the actual gene pool ofthehostgenotype. Furthersub-groupsofcultivars based on overall level of resistance within each gene pool, were observed. Races of *C. lindemuthianum* with Middle American reaction showed broad virulence on germplasm from both gene pools, whereas races with Andean reaction showed high virulence only on Andean germplasm. The reduced virulence of Andean races on Middle American genotypes suggests selectíon of virulence factors congruent with diversity in P. *vulgaris* In addition, races of *C. lindemuthianum* were grouped according to specific gene pool (i.e. Middle American and Andean reaction groups) based on principal component analysis. However, the overlapping of specific races with races from different reaction groups might indícate that this group of isolates possesses factors of virulence to both host gene pools. Similar results from the phenetic analyses showed races grouped according to specific gene pool. Most races with Andean reaction were observed in the cluster B, except races 15 and 23, which clustered with Middle American races. Only races 38,39, and 47 from the Dominican Republic showed high similarity in both multivariate analyses and clustered based on geographic effect. Data based on virulence supports variability in *C. lindemuthianums\ruc\ureú* with diversity in *Phaseoius.*

Divergence among the same isolates of *C. lindemuthianum* was based on RFLP analysis and sequencing of the nuclear rDNA región of the two internal transcribed spacers (ÍTSI and ITS11) and the 5.8S rRNA gene. A reproducible 0.58kb fragment was amplified in 57 isolates. Races of *C. lindemuthianum* formed two groups based on RFLP-ITS analysis. Those races collected on bean genotypes from the Andean gene pool clustered predominantly into group I except race 23, whereas races collected on Middle American host genotypes were observed in both groups.

A bootstrap valué of 100% in parsimony analysis and 88% in the neighbor-joining analysis supported a monophyletic group formed by all isolates except race 31. Genetic distances among races of C. *lindemuthianun* ranged from 0.2% to 2.9%. Sequence homology analysis did not show a pattem parallel to a specific host reaction group ñor association with an obvious geographic distribution. Likewise, phenetic and parsimony analyses did not show polymorphism in the rDNA región linked to any specific factor. Molecular polymorphism among isolates of races 7,17,31, and 73 collected in different countries was demonstrated by RFLP-ITS analysis. Sequence homology of US regions of isolates of race 73 from México and the United States showed the greatest genetic distance valué among all isolates. These findings support a level of molecular variability within *C. lindemuthianun* greater than the variability previously characterized by virulence analysis.

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