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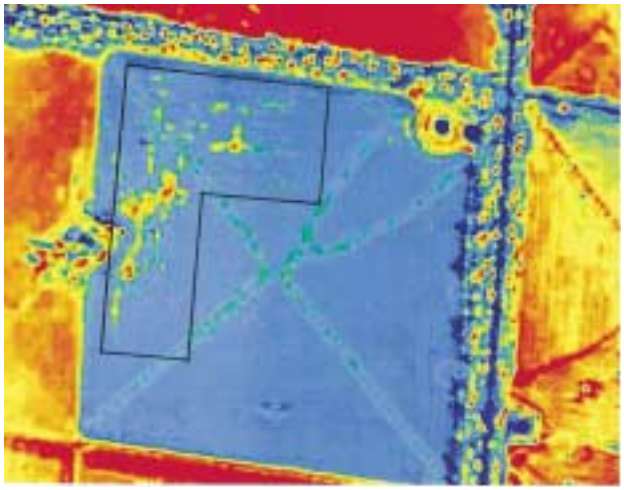

VOLUME 42, 2002

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Australian Journal of Experimental Agriculture, 2002, **42**, 481–490

Patterns of resistance to angular leaf spot, anthracnose and common bacterial blight in common bean germplasm

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Abstract. Diseases and insect pests are major causes of low yields of common bean (*Phaseolus vulgaris* L.) in Latin America and Africa. Anthracnose, angular leaf spot and common bacterial blight are widespread foliar diseases of common bean that also infect pods and seeds. One thousand and eighty-two accessions from a common bean core collection from the primary centres of origin were investigated for reaction to these three diseases. Angular leaf spot and common bacterial blight were evaluated in the field at Santander de Quilichao, Colombia, and anthracnose was evaluated in a screenhouse in Popayán, Colombia. By using the 15-group level from a hierarchical clustering procedure, it was found that 7 groups were formed with mainly Andean common bean accessions (Andean gene pool), 7 groups with mainly Middle American accessions (Middle American gene pool), while 1 group contained mixed accessions. Consistent with the theory of co-evolution, it was generally observed that accessions from the Andean gene pool were resistant to Middle American pathogen isolates causing anthracnoxe, while the Middle American accessions were resistant to pathogen isolates from the Andes. Different combinations of resistance patterns were found, and breeders can use this information to select a specific group of accessions on the basis of their need.

Additional keywords: CIAT, disease resistance, pattern analysis.

Introduction

Common bean is the most important food legume in the developing world. Latin America, the centre of origin for common bean, is the leading bean producer in the world. Evidence for existence of 2 major gene pools, namely Middle American and Andean, in the cultivated common bean is based on morphological traits (Singh *et al.* 1991*a*), seed proteins (Gepts and Bliss 1985), isozymes (Singh *et al.* 1991*b*) and DNA markers (Becerra Velasquez and Gepts 1994).

Diseases and insect pests are major causes of low yields in most bean-producing regions of Latin America and Africa (van Schoonhoven and Voysest 1989; Beebe and Pastor-Corrales 1991). Angular leaf spot (ALS), caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferr. (PG) is a yield-limiting disease of common bean. This disease occurs in tropical and subtropical countries of the Americas and Africa, especially in Argentina, Brazil and Burundi (Beebe and Pastor-Corrales 1991). ALS also occurs in Europe, Australia and Asia (Hill 1982) and North America (Correa-Victoria 1987). The ample virulence (race) diversity of the *Phaeoisariopsis griseola* often renders cultivars resistant in 1 location or year but susceptible in another location or year. Thus, the use of diverse host-resistance genes and the knowledge of the pathogen's diversity are essential in developing common bean cultivars with durable disease resistance. The genetic diversity of *P. griseola* has been studied using common bean differential cultivars (Pastor-Corrales and Jara 1995), isozymes (Correa-Victoria 1987), random amplified polymorphic DNA (RAPD) markers (Guzmán *et al.* 1995), and a combination of differential cultivars and RAPD markers (Maya *et al.* 1995; Pastor-Corrales and Jara 1995). Andean isolates of *P. griseola* were obtained in almost all cases from large-seeded common bean cultivars of Andean origin, and the Middle American isolates from small- and medium-seeded common bean cultivars of Middle American origin, where seed size was classified as per Singh *et al.* (1991*a*). The results of the virulence analyses also showed that the Andean isolates attacked only common bean cultivars of Andean origin. Conversely, the Middle American isolates displayed wider virulence diversity; they attacked a broader range of cultivars, preferring the Middle American cultivars but also attacking those of Andean origin (Maya *et al.* 1995; Pastor-Corrales and Jara 1995).

Anthracnose (ANT), caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Bri & Cav. (CL), is another destructive disease, especially in temperate and subtropical zones (Pastor-Corrales and Tu

 10.1071/EA01035 0816-1089/02/040481

1989). It causes complete yield loss in susceptible cultivars if seeds are infected and environmental conditions are favourable for pathogen infection and growth (Pachico 1989). The planting of resistant cultivars is considered to be the most viable option for controlling ANT (Singh *et al.* 1992). *Colletotrichum lindemuthianum* shows a pattern of co-evolution with common bean gene pools similar to that of *P. griseola*, in the sense that 2 pathogen populations exist attacking common bean accessions of either Middle American or Andean origin (Balardin and Kelly 1998).

Common bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye (Xcp), is another serious seed-borne disease of common bean. Saettler (1989) reported a reduction in seed yield and seed quality caused by Xcp. Resistant varieties are considered to be the best means of control of Xcp (Sanders and Schwartz 1980).

Much of the evidence leading to the theory of co-evolution of host with the pathogens for ALS, and to a certain degree with ANT, has been derived from sets of differential varieties. These genotypes are small and highly specialised samples of accessions that are distinguished by their interaction with pathogen isolates. They are not necessarily representative of the broader set of common bean germplasm. Furthermore, the different diseases have been studied individually. The objectives of this study were to use pattern analysis techniques to summarise the variation in the common bean core collection for the response to the 3 diseases and to see what insights such analyses would provide on the theory of co-evolution of host and pathogens.

Materials and methods

Accessions

The International Centre for Tropical Agriculture (CIAT), Cali, Colombia, holds the world's largest collection of common bean germplasm, about 30 000 accessions collected worldwide. Tohme *et al.* (1995) developed a core subset of 1441 accessions composed of a stratified random sample from the reserve collection. A further subset of 1082 accessions from the primary centres of diversity was evaluated for the pathogen isolates of ALS and ANT and seed-borne disease isolates of Xcp. In this paper, the terms Middle American and Andean refer to geographical origin and not genetic origin unless specified to the contrary. Accessions originating in Middle America included those from Mexico (398), Guatemala (85), Belise (4), Honduras (13), El Salvador (14), Nicaragua (13), and Costa Rica (21), while accessions from Andean America included those from Colombia (88), Ecuador (64), Peru (343), Bolivia (20), and Argentina (19).

All 1082 accessions were inoculated by mixtures of pathogen isolates that were previously classified as Andean or Middle American in origin. Because observations were on disease reactions, disease names were used rather than pathogen names. The disease reactions were defined with the suffices 'a' for Andean and 'm' for Middle American to identify the origin of the pathogen isolates. For example, ALS-a and ANT-a are the disease reactions for Andean pathogen isolates.

Field design and experimentation

Anthracnose was evaluated in a greenhouse in Popayán, Colombia (1700 m a.s.l., average temperature 18°C). Twenty seeds of each accession were planted in soil in raised beds and sufficient water was supplied to enable germination. Once seedlings were established, they were inoculated with mixtures of CL races obtained locally but which had been characterised as attacking either Andean (races 7 and 15) or Middle American (races 137, 521, 385 and 1545) common bean, as determined by using the differential varieties and race-numbering system proposed by Pastor-Corrales (1991). One set of 10 plants for each accession was used for inoculation. Beds were covered with a plastic tent to maintain humidity in order to favour disease development.

Angular leaf spot was evaluated at Santander de Quilichao, Colombia (1000 m a.s.l., average temperature 23°C, oxisol soil and rainfall 1770 mm/year). The crop was managed under rainfed conditions and supplemental irrigation applied as necessary. Lime was applied to raise pH from 4.5 to 5.8 and the fertiliser P was applied at 100 kg/ha. Separate plots of the germplasm were sprayed with inoculum consisting of local PG isolates that had been characterised as either Andean (race 63-0) or Middle American (races 5–47 and 31–47; Pastor-Corrales *et al.* 1998) in origin. Although contamination from other native isolates in the field cannot be totally avoided, in fact there is little disease development in the field in Santander de Quilichao in other plots where inoculation is not practiced. Therefore, we assume that there was little such contamination in the experimental plots reported here.

Common bacterial blight was evaluated in the same experiment station at Santander de Quilichao, Colombia. Unreplicated 3-m-long single-row field plots were inoculated 3 times by spraying foliage with a suspension of a locally obtained virulent isolate of Xcp (isolate Xcp 123).

Evaluation of ANT was performed 10 days after inoculation on a 1–9 scale. Foliar symptoms of ALS and CBB were also evaluated on a 1–9 scale, at 3 different dates, 35–55 days after planting. The scales were adapted from Pastor-Corrales (1991) according to van Schoonhoven and Pastor-Corrales (1987) (1, immune as plants showed no visible symptoms; 2, few isolated small portions are diseased; and 9, severely diseased or almost dead). For this analysis, the data collected at 55 days after planting were utilised. Other attributes [100-seed weight (SWt), days to maturity (M), protein concentration (PC) and phaseolin concentration (PhC)] were also recorded to aid in interpreting the genetic relationship underlying the disease reaction of the germplasm to isolates of the pathogens.

Analytical techniques

The correlation coefficients among disease reactions were calculated over the 1082 accessions. Pattern analysis, which is the joint use of ordination and clustering techniques (Williams and Lance 1958), enabled a simultaneous comparison of accessions over these disease reactions. An agglomerative, hierarchical grouping technique was used with squared Euclidean distance as the dissimilarity measure and incremental sum-of-squares as the sorting strategy following Ward's method (Ward 1963), as recommended by Eisemann (1981). The calculation of proximity measures required standardisation of the data because of sensitivity to differences in the variability of the input attributes. The clustering method summarised the patterns of diversity by assigning accessions to groups in such a way that the accessions within a group are similar to one another (Everitt 1988). The grouping level at which the data was summarised, was based on the proportion of total sum of squares retained among the groups (Byth *et al.* 1976), given that increasing the number of groups will always increase the amount of variation accounted for. Using this procedure, the dendrogram was truncated at a particular level and the percentage of resistant accessions to different diseases in each of the groups was calculated.

To display the relationships among accessions and disease reactions in the data, a biplot graphical representation from an ordination procedure was used (Good 1969; Gabriel 1971). The ordination method is a principal component analysis using singular value decomposition (Kempton 1984), which admits quantitative and/or categorical

attributes (Gower and Hand 1996). The relationships between the accessions and disease reactions were displayed using point-vector plots, with points representing accessions, and directional vectors representing the disease reactions. The angles between the vectors reflected the correlation structure among attributes. By drawing a perpendicular line from the accession points to the attribute vectors, the attribute measurements for the accessions can be compared with the average, which is represented by the origin. Individual accessions are often reported as having either measurements above average, close to average or below average for each of the attributes. Equal scaling of the component axes was needed for accurate projection of the points onto the attribute (disease) vectors.

Results

Correlation analysis

Most of the correlation coefficients between disease reactions (Table 1) were significantly ($P \leq 0.01$) different from zero, but the disease reactions of ALS-m and ANT-a were not correlated with CBB. The ANT and ALS reactions of accessions were significantly positively correlated when tested with pathogen isolates from the same origin, either Andean or Middle American, but were significantly negatively correlated with those from different origins.

Pattern analysis

It was subjectively determined that 15 accession groups adequately summarised the accession variation in that this number of groups explained 72% of the variability among accessions. Increasing the number of groups from 15 to 16 explained a little more than 72%, while decreasing from 15 to 14 explained 3% less. Of the 15 groups, groups G1–G7 were formed with mainly Andean accessions, groups G9–G15 with mainly Middle American accessions, while group G8 was composed of mixed accessions (Table 2). The groups were arranged in increasing order based on the mean score on the first principal component.

The first 3 principal components were considered to provide an adequate summary of the variation among the accessions and the disease reactions as they explained 39, 20, and 16%, respectively, of the variation. To simplify some of the plots, only group means were used to denote the accession locations. From the biplot for the first 2 principal components (Fig. 1), ALS-a and ANT-a responded similarly

Table 1. Correlation matrix for disease reactions of angular leaf spot (ALS) and anthracnose (ANT) caused by pathogen isolates of *Phaeoisariopsis griseola* (PG) and *Colletotrichum lindemuthianum* (CL), respectively, and seed-borne disease reaction of common bacterial blight (CBB) caused by *Xanthomonas campestris* pv. *phaseoli* over 1082 common bean accessions from the primary centres of origin in CIAT's core collection

a, isolate of Andean origin; m, isolate of Middle American origin

ANT-a	ALS-m	ANT-m	CBB	
ALS-a	0.44**	–0.23**	–0.45**	–0.16**
ANT-a		–0.26**	–0.20**	–0.03
ALS-m			0.22**	0.01
ANT-m				0.07*

** and * indicate significant ($P \leq 0.01$ and $P \leq 0.05$, respectively) correlation coefficients.

Table 2. Percentage of resistant (scores 1–3) and intermediate (scores 4–6) accessions to each of three diseases for each of 15 groups found in the cluster analysis of 1082 common bean accessions from the primary centres of origin in CIAT's core collection

ALS, angular leaf spot; ANT, anthracnose; CBB, common bacterial blight; a, isolate of Andean origin; m, isolate of Middle American origin

Group[A]	No. of acc.	No. of Andean acc.	Accessions resistant (intermediate) to different diseases (%) ALS-a	ANT-a	ALS-m	ANT-m	CBB
G1	75	72	26 (41)	0 (10)	75 (25)	81 (16)	0 (32)
G2	53	53	0 (53)	0 (13)	8 (41)	85 (9)	19 (81)
G3	129	117	0 (43)	1 (34)	0 (40)	83 (12)	0 (38)
G4	56	48	41 (31)	88 (12)	77 (23)	76 (20)	4 (58)
G5	23	19	17 (35)	96 (4)	0 (30)	87 (13)	44 (56)
G6	58	41	0 (47)	90 (10)	0 (36)	93 (7)	0 (60)
G7	44	37	82 (18)	0 (25)	0 (25)	100 (0)	0 (55)
G8	44	26	86 (14)	87 (13)	0 (100)	91 (9)	0 (36)
G9	112	30	76 (34)	93 (7)	0 (4)	93 (7)	0 (5)
G10	103	27	94 (6)	81 (17)	0 (9)	56 (44)	0 (74)
G11	25	5	100 (0)	96 (4)	80 (20)	0 (4)	0 (0)
G12	105	30	93 (7)	11 (62)	2 (13)	0 (8)	0 (36)
G13	33	4	73 (25)	85 (15)	0 (3)	6 (22)	45 (55)
G14	76	6	92 (8)	99 (1)	0 (13)	0 (18)	0 (100)
G15	146	13	86 (12)	98 (2)	0 (11)	0 (14)	0 (2)

[A]Groups G1–G7 are mainly Andean in origin and groups G9–G15 are mainly Middle American in origin, while group G8 is of mixed origin.

(as their vectors were in the same general direction), as did ALS-m and ANT-m (as their vectors were roughly in the opposite direction to ALS-a and ANT-a), while that for CBB was nearly perpendicular to both sets of vectors. These similar responses suggest grouping ALS-a and ANT-a together, ALS-m and ANT-m together and CBB alone, as was observed in the simple correlation structure of these attributes (Table 1). The opposing direction of vectors for ALS-a and ANT-a to ALS-m and ANT-m on the first principal component indicated that if ALS-a and ANT-a had high scores for a particular group of accessions then ALS-m and ANT-m would have low scores for that particular group.

From the biplot of the first and third principal components (Fig. 2), the vectors for ALS-a and ANT-a were broadly similar to what they were in Figure 1, while that for ANT-m was nearly perpendicular to those for ALS-m and CBB. This indicated somewhat independent responses for the 2 disease reactions to the isolates from the Middle American gene pool. The consistent relationship among traits within the Andean gene pool and the less-consistent relationship in the Middle American gene pool suggest some subtle contrast in the host–pathogen co-evolution in the 2 gene pools.

The groups occupied different positions in the reduced space, as can be seen somewhat more clearly by the presentation of the individual accession points in each group in Figures 3 and 4. Biplots indicated that the mainly Andean groups G1–G7 had generally above-average (or susceptible) scores on the ANT-a and ALS-a vectors, while the Middle American groups G9–G15 had generally above-average (or susceptible) scores for ANT-m and ALS-m. Accessions in group G8 were scattered around the origin, indicating that they had average disease responses. Groups G5 and G13 were unique in that their accessions showed more resistance to CBB, which is particularly well represented in the plot of the first and second components (Figs 1 and 3).

The general inference from the biplots is that the first principal component was associated with the difference in disease reaction of the accessions inoculated with isolates of Andean origin in comparison with those of Middle American origin. The second principal component was associated with susceptibility to CBB, while the third principal component separated the different disease reactions to isolates from Middle America, i.e. ALS-m and ANT-m.

On the basis of the severity of the symptoms of the diseases, the plants were reclassified as resistant, intermediate or susceptible according to Alzate-Marin *et al.* (1997). Plants with no or limited symptoms (scores 1–3) were said to be resistant phenotypes; plants graded 4–6 were considered to be intermediate, whereas plants graded 7–9 were considered to be susceptible. The percentage of resistant and intermediate accessions in each group is given in Table 2, with the balance to make up 100%, being the percentage susceptible. This gives another view of the resistance patterns already shown in Figures 3 and 4. For instance, it illustrates that most of the accessions in group G11 are resistant to ALS-a, ALS-m and ANT-a.

In the case of ANT, the major tendency was for accessions from a particular region to be susceptible to pathogen isolates for this disease from that region and resistant to the

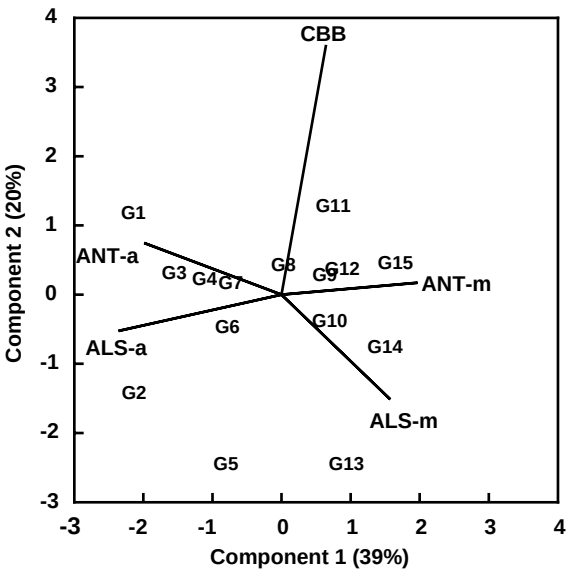

Figure 1. The biplot of the first and second component vectors from the principal component analysis of disease reactions to isolates of three pathogens measured on 1082 common bean accessions from the primary centres of origin in CIAT's core collection. Only the accession group means for G1–G15 are shown.

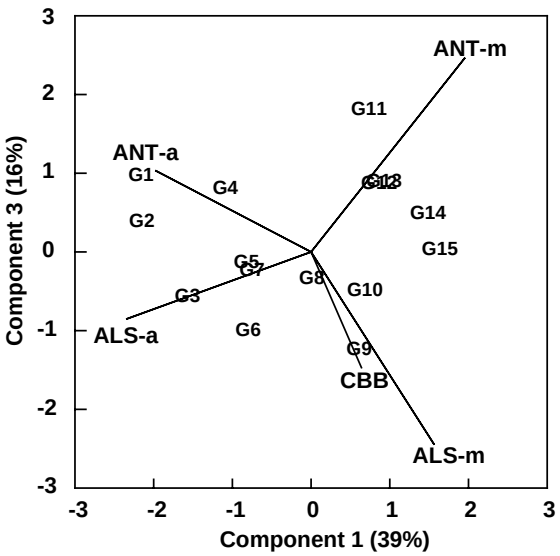

Figure 2. The biplot of the first and third component vectors from the principal component analysis of disease reactions to isolates of three pathogens measured on 1082 common bean accessions from the primary centres of origin in CIAT's core collection. Only the accession group means for G1–G15 are shown.

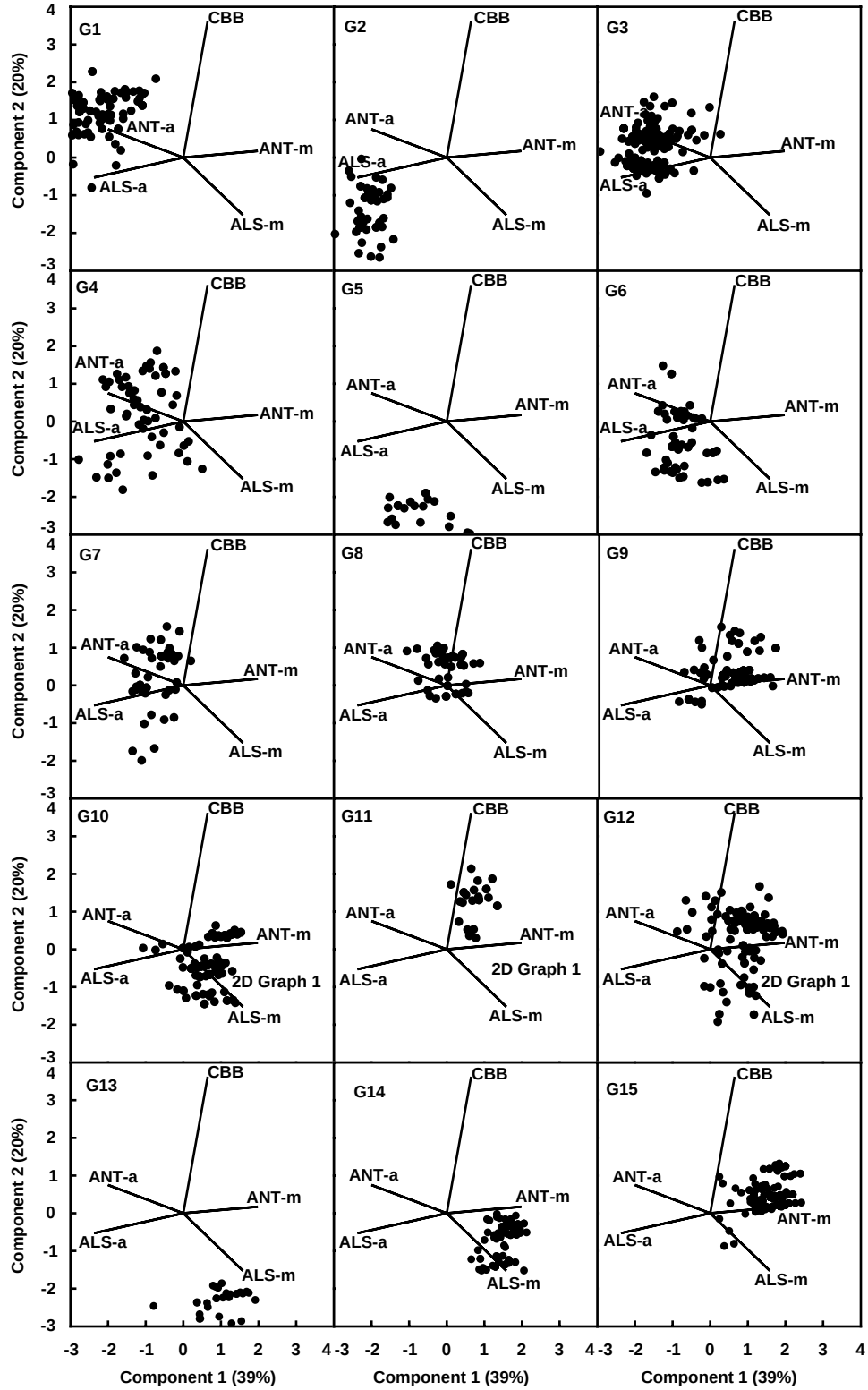

Figure 3. The biplot of the first and second component vectors from the principal component analysis of disease reactions to isolates of three pathogens measured on 1082 common bean accessions from the primary centres of origin in CIAT's core collection. The individual accession points in each group, G1–G15, are plotted with dot symbols.

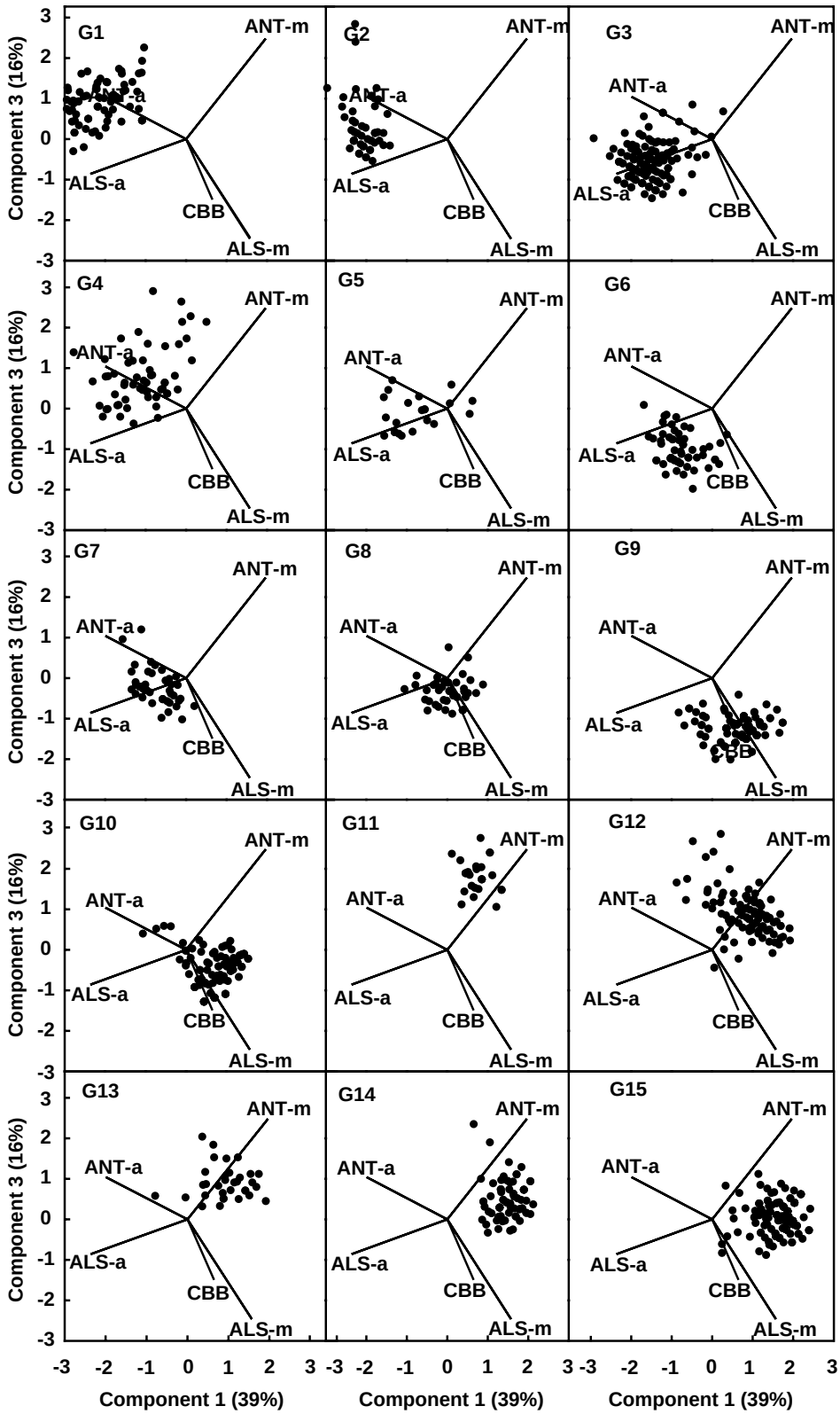

Figure 4. The biplot of the first and third component vectors from the principal component analysis of disease reactions to isolates of three pathogens measured on 1082 common bean accessions from the primary centres of origin in CIAT's core collection. The individual accession points in each group, G1–G15, are plotted with dot symbols.

isolates from another region, consistent with co-evolution of host and pathogen. In the groups composed of accessions of primarily Middle American origin, all were mainly susceptible to ALS-m (except for G11) and susceptible to ANT-m (except for G9 and G10), whereas they all were mainly resistant to ALS-a and ANT-a (except for G12 with mainly intermediate interactions). Most of the accessions were of intermediate resistance or susceptible to CBB, except for a reasonable proportion of those in groups G5 and G13 (as noted above) and a lesser proportion of those in G2, which were resistant.

None of the mainly Andean groups showed a higher percentage resistance to the Middle American ALS isolates than the Middle American group G11. Group G4 displayed moderate to high resistance to ALS and ANT isolates irrespective of the origin, while there were no accessions in G8 that were susceptible to these isolates.

The mean values for other attributes of seed weight, days to maturity, protein concentration and phaseolin concentration for each of the groups are also reported (Table 3). The results indicated that the groups formed from mainly Andean accessions were large-seeded, ranging from 45 to 62 g per 100 seeds, compared with the groups formed from mainly Middle American accessions which were small-seeded, ranging from 27 to 36 g per 100 seeds. The groups of mainly Andean accessions flowered (data not provided) and matured later than those of mainly Middle American accessions. The groups of mainly Andean accessions contained lower protein concentration, ranging from 19.8 to 21.1%, and higher phaseolin concentration, ranging from 39.3 to 42.5% of total protein, in contrast to groups of mainly Middle American accessions with 20.9–23.3% protein concentration and 37.6–39.9% phaseolin concentration.

Discussion

The correlation analysis showed that the accessions had opposite reaction patterns to Andean and Middle American pathogen isolates for ANT and ALS, which implies that the accessions that showed lower scores to Andean pathogen isolates, showed higher scores to Middle American pathogen isolates and *vice versa*. The results for ANT and ALS broadly support expectations for co-evolution of host and pathogen (Pastor-Corrales and Jara 1995).

Referring to groups of largely Andean origin, most accessions were resistant to ANT isolates of the Middle American origin. Only groups G1, G2, G3 and G7 gave the expected reaction of susceptibility to Andean pathogen isolates and that of resistance to Middle American ANT isolates. They also displayed some deviation from this pattern for ALS. For example, groups G2, G3, and G6 were not resistant to the Andean or the Middle American ALS isolates. Some groups (G1, G4 and G7) from the Andean region had intermediate to higher resistant patterns to Andean ALS isolates. The accessions in groups G4, G5, G6, G8 and G9 were highly resistant to ANT, irrespective of the origin of pathogen isolates. Thus, there were many exceptions to the expectations of co-evolution.

Table 3. The mean (± s.e.) for each of two morphological attributes and two biochemical attributes for each of the 15 groups found in the cluster analysis of 1082 common bean accessions from the primary centres of origin in CIAT's core collection

SWt, seed weight; M, days to maturity; PC, protein concentration; PhC, phaseolin concentration

Groups[A]	No. of acc.	SWt ± s.e. (g/100 seeds)	M ± s.e. (days)	PhC ± s.e. (%)	PC ± s.e. (%)
G1	75	57.9 ± 1.65	126 ± 1.3	20.8 ± 0.22	41.4 ± 0.80
G2	53	62.1 ± 2.17	139 ± 1.7	19.9 ± 0.27	42.4 ± 0.85
G3	129	59.9 ± 1.44	134 ± 1.4	20.9 ± 0.18	42.5 ± 0.55
G4	56	56.6 ± 2.78	129 ± 1.7	21.1 ± 0.29	39.3 ± 0.75
G5	23	51.3 ± 3.00	136 ± 3.9	19.8 ± 0.42	39.6 ± 1.20
G6	58	56.2 ± 2.25	128 ± 2.2	20.3 ± 0.26	41.7 ± 0.79
G7	44	44.9 ± 2.28	131 ± 2.4	21.0 ± 0.32	41.6 ± 1.04
G8	44	41.4 ± 2.80	127 ± 2.0	22.0 ± 0.36	38.6 ± 1.30
G9	112	35.4 ± 1.36	121 ± 1.3	21.6 ± 0.20	40.0 ± 0.66
G10	103	34.7 ± 1.26	123 ± 1.1	21.6 ± 0.23	38.6 ± 0.66
G11	25	27.0 ± 1.84	123 ± 2.3	23.3 ± 0.50	38.8 ± 1.06
G12	105	27.8 ± 1.05	120 ± 0.9	22.4 ± 0.19	38.7 ± 0.56
G13	33	35.5 ± 1.62	118 ± 1.4	20.9 ± 0.26	40.0 ± 1.24
G14	76	30.8 ± 1.11	118 ± 1.2	22.1 ± 0.25	37.6 ± 0.65
G15	146	28.3 ± 0.79	117 ± 0.8	22.3 ± 0.17	37.9 ± 0.50

[A]Groups G1–G7 are mainly Andean in origin and groups G9–G15 are mainly Middle American in origin, while group G8 is of mixed origin.

Only the accessions in groups G13 to G15 generally fulfilled the reactions to Andean and Middle American isolates of ANT and ALS predicted by co-evolution. These mainly Middle American groups were consistently resistant to Andean isolates of ANT and ALS and susceptible to the corresponding Middle American isolates.

Other results for the mainly Middle American groups include group G12 with only 11% of accessions resistant to ANT, even when the pathogen isolates were from the Andes, while the accessions of G11 could be of interest due to their higher resistance to the Andean and Middle American ALS isolates (100 and 80%, respectively) and to their higher protein concentration. Groups 9 and 10 displayed the co-evolutionary expectations for resistance to Andean isolates on ANT and ALS and those for susceptibility to ALS-m, but they also tended to be resistant to ANT-m contrary to the same expectations.

Overall, resistance to CBB occurs about equally in the Andean and Middle American germplasm (Table 2). In this regard, the accessions in groups G5 (44% resistant) and G13 (45% resistant) are promising for breeders, although the accessions in G5 were mostly susceptible to ALS irrespective of the origin of pathogen isolates. Since resistance to CBB is so rare in common bean germplasm, a list of accessions with multiple resistance, including that for CBB, has been extracted (Table 4). Curiously, resistance to CBB is almost always found in combination with resistance to ANT-a, often with resistance to ALS-a and to ANT-m, but infrequently with resistance to ALS-m. For the latter combination, only G23568B, an Andean accession, is available (Table 4). An examination of these accessions showed that they come principally from 2 genetic groups or races of common bean. Those of Middle American origin from Mexico are largely from race Durango, and principally from subrace D2 as determined by RAPD analysis (Beebe *et al.* 2000). There is even 1 resistant accession from Ecuador, G17162A, classed as a subrace D2. This subrace appears to have particular diversity for multiple-resistance genes, including CBB resistance. Another 5 accessions from Colombia and Peru were Andean climbers that would be classified as race P according to the classification system proposed by Singh *et al.* (1991*c*). However, no accessions showed resistance to CBB and all of the ANT and ALS isolates tested. Resistance to ALS-m is a particular lack in the sources identified with resistance to CBB. To achieve this, complementary crosses are required with corresponding multiple disease-resistance screening of segregants in order to transfer comprehensive multiple resistance to elite breeding lines. Mahuku *et al.* (unpublished data) provide lists of cultivated and wild accessions with resistance to ALS, which will contribute to this end.

As there is little evidence for pathogen races in CBB, the core collection was inoculated with the 1 common isolate that is highly virulent to both common bean major gene pools. The evidence for co-evolution of host and pathogen does not include CBB, but indicates a different selection pressure for evolution of resistance to CBB, which appears to be common in both gene pools.

The present work has implicitly used the reaction with different races of pathogens to classify accessions into gene pools of cultivated common bean. Gene pools have usually been defined using other criteria, such as plant-categorical

Table 4. Accessions with resistance to common bacterial blight (CBB) and at least two more resistances, their origin, disease scores and group to which they were assigned

Disease scores 1–3 are considered to be resistant, scores 4–6 intermediate, and scores 7–9 susceptible. ALS, angular leaf spot; ANT, anthracnose; a, isolate of Andean origin; m, isolate of Middle American origin

Acc. no.	Origin	ALS-a	NT-a	S-m	NT-m	BB	Group[A]
G23568B	Peru	4	1	2	1	3	G4
G4278	Mexico	1	1	8	1	3	G5
G8925	Mexico	1	1	6	1	3	G5
G21132	Mexico	2	1	8	3	3	G5
G22024	Mexico	2	1	7	1	3	G5
G12120	Peru	7	1	7	1	3	G5
G23766A	Peru	7	2	8	1	3	G5
G23874	Peru	6	3	6	1	3	G5
G7257	Colombia	2	6	7	1	3	G13
G17162A	Ecuador	1	2	8	7	2	G13
G2660	Mexico	3	1	7	8	3	G13
G3322	Mexico	1	1	8	7	3	G13
G10962	Mexico	2	1	8	7	3	G13
G19048	Mexico	2	1	6	6	3	G13
G19326	Mexico	3	1	7	5	3	G13

[A]Groups G4 and G5 (mainly Andean accessions) and G13 (mainly Middle American accessions) from Table 2.

Table 5. Correspondence between classification of accessions by plant, pod and seed attributes and grouping of accessions by disease reaction

Groups found in present analysis	Gene-pool classification[A]				
	Andean	North Andean	Mixed	Middle American	Total
Mainly Andean (G1–G7)	324	24	59	25	432
Mixed (G8)	21	1	13	9	44
Mainly Middle American (G9–G15)	94	13	143	346	596
Total	439	38	215	380	1072

[A]Classification based on plant categorical and pod and seed quantitative attributes as per Islam *et al.* (2001*a*), where 1072 accessions from the primary centres were used.

and pod and seed quantitative attributes (Islam *et al.* 2001*a*, 2001*b*). Islam *et al.* (2001*a*) classified the same collection, less 10 accessions, into 4 groups: the major Andean and Middle American gene pools, a mixed intermediate group and an incipient gene pool from the Northern Andes. The correspondence between that classification and the present one (for the accessions in common) is presented in Table 5. Accessions that were classified as either from the Andean gene pool or from mainly Andean groups by both criteria (30%) or from the Middle American gene pool or from mainly Middle American groups by both criteria (32%) accounted for 62% of total. Thus, while relatively few accessions (255 or 24%) fell into groups G13–G15, and generally fulfilled the profile expected by co-evolution, a far greater number could be classified approximately by pattern analysis.

Although there was broad agreement in the classification of accessions to Andean and Middle American gene pools by the 2 criteria of disease reaction and seed proteins, differences were found too. Disease reaction and seed proteins resulted in a contrary classification between the 2 major gene pools (Middle American for Andean or *vice versa*) for 11% (25 + 94 of 1072) of accessions. Most of these (94 of 119) possessed an Andean protein profile but presented a Middle American disease reaction (Table 5). This suggests germplasm exchanges between the 2 gene pools, possibly through introductions and introgressions.

Forty-two accessions of the 94 with Andean protein types had molecular information that was used to study introgression of Middle American genes into the Andean gene pool by Islam (2001). The results indicated that 33% (14 of 42) of the accessions showed introgressions between these 2 gene pools. The 38 accessions that pertained to the North Andean gene pool tended to cluster with the Andean gene pool, as noted previously (Islam 2001).

There is also evidence for the addition of Andean germplasm to the Middle American gene pool. On the basis of seed and protein characteristics, at least 13 Middle American accessions that classified with mainly Andean groups on disease patterns were Andean introductions. Other Middle American accessions with Andean disease patterns in groups G4, G5 and G6 were mainly of Durango subrace D2, with a high frequency of the uncommon phaseolin type M (Beebe *et al.* 2000). Also, in the mainly Middle American groups G9, G10 and G12, 8, 6 and 20 accessions, respectively, appear to be Middle American introductions (introgressions) into the Andean gene pool. Even though the 2 major gene pools show evidence of different evolution under the combination of natural and manual selection (Islam *et al.* 2001*b*), they were not completely isolated. However, the influence of introduction and introgression between gene pools appears to have had only a minor influence on the broad evolutionary separation of these gene pools.

In general, the findings support the theory of co-evolution of common bean and the pathogens causing ANT and ALS as was found with smaller collections of host genotypes by other researchers. However, they also indicate that the co-evolution of host and pathogen isolates was not straight-forward. Accessions from the Andean gene pool were not necessarily always resistant to Middle American pathogen isolates and *vice versa*. A relatively small number of accessions fulfilled the expectations of the relationship of co-evolution (groups G13–G15) completely with regards to reaction to isolates causing ANT and ALS. Differentiation of gene pools by disease reactions was reasonably similar to that by plant and pod and seed attributes, although the Andean gene pool presented considerable introgression. Groups formed by disease data presented characteristics previously associated with the 2 major gene pools. The accessions of the mainly Andean groups were large-seeded with lower protein and higher phaseolin than those groups of mainly Middle American accessions.

Pattern analysis proved to be useful for this type of investigation, by which breeders can select a specific group of accessions based on their need for multiple disease resistance.

Acknowledgment

This research was financially supported by a grant from the Australian Centre for International Agricultural Research (ACIAR), Canberra, ACT. Constructive comments from the referees and the Managing Editor were most helpful.

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Received 10 February 2001, accepted 7 September 2001