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Genetic and Physiological Variation Among Bean Lines Resistant and Susceptible to Bean Anthracnose

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Summary. Electrophoresis was used to determine genetic and or biochemical variation, if any, among bean lines resistant and susceptible to anthracnose. This was based on two enzyme systems: peroxisase and esterase. It was revealed that resistant and suceptible plants differed in their band patterns and intensities. Band intensity differences occurred mainly among monomorphic bands with higher intensities expressed by susceptible plants, while band pattern differences were expressed both by resistant and susceptible plants. These differences appeared only at certain stages of development. These stages were identified as 3 and 40 days after emergence and were considered as critical stages for screening purposes. The peroxidase isozyme A_5 and the esterase isozyme C_1 at 3 days, and the peroxidase band C_1 and esterase bands A_1 and A_2 at 40 days were important because these differences could be used as 'genetic/biochemical' markers for screening the population for resistance. Thus, electrophoretic differences could be used as a screening aid and this could save time and effort in breeding programmes. Comparisons between inoculated and non-inoculated leaves of resistant and susceptible lines indicated that infection induced changes in both the amount and kind of peroxidases even before symptoms of the disease appeared. However, there were no specific differences between resistant and susceptible lines, indicating that resistant and susceptible lines responded to infection in the same manner.

Key words: Disease resistance – Bean anthracnose – Genetic, physiological variation

Introduction

Varying results have been reported in electrophoretic comparisons of disease resistant and susceptible plants. These variations have been reported not only for different crops but also for various enzyme systems. Some workers (Stavely and Hanson 1967; Veech 1969) reported a similarity of patterns in both resistant and susceptible plants while Orlob and Arny (1961) and Seevers et al. (1971) obtained different patterns for resistant and susceptible plants. Barrett and McLaughlin (1954) compared protein fractions of healthy susceptible and resistant wheat seedlings and found no differences in their protein type and mobility. Similar results were reported for wheat (Macko et al. 1968), soybean (Hilty and Schmitthenner 1966) and tobacco (Veech 1969). On the other hand, Orlob and Arny (1961) found that healthy barley plants resistant to the yellow dwarf virus differed significantly form those that were susceptible. The activity of the latter was usually higher. Similarly, it was shown by Seevers et al. (1971) that the activity of isozyme 9 of a near isogenic wheat line was consistently associated with the Sr6 allele conferring resistance to stem rust. Jennings et al. (1969) detected no marked differences in peroxidase activity between resistant and susceptible plants. However, upon inoculation with *Helminthosporium* sp. the increase in peroxidase activity was greater in susceptible than in resistant plants. They concluded that peroxidases were more closely associated with susceptibility than with resistance. Similarly, peroxidase activities in the leaves of potato clones were tested against the level of field resistance to *Phytophthora infestans* (Kedar 1959; Umaerus 1959). In both these studies it was found that peroxidase activity was correlated positively with field resistance. The present study was undertaken to study possible electrophoretic differences between bean lines susceptible and resistant to anthracnose and to determine whether these differences could be used for initial screening of large populations for resistance.

Materials and Methods

Twelve bean lines (Table 1) were used for this study, six resistant and six susceptible. These lines had been evaluated

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and rated as being resistant or susceptible to anthracnose on the basis of repeated field tests. Five seeds per line were planted in an unsterilized soil mixture in 250 mm plastic pots and on emergence they were thinned to three plants per pot. The soil mixture comprised of top forest soil, sand and horse manure in a ratio of 2 : 1 : 1 with an addendum of diammonium phosphate fertilizer (1 g: 1 litre of soil). The pots were lightly watered every other day to minimise seed rot common in cold oversoaked soils. The experiments were carried out in a greenhouse.

Sampling of the plant material started with seeds and 3-day-old seedlings and was continued at weekly intervals up to 38 days. The final leaf sampling was conducted on the 40th day after emergence. Except for 3- and 10-day-old seedlings, sampling was done on the first trifoliate leaves. One gram of leaf tissue (without petioles) was ground in prechilled mortars and homogenised with 5 ml of 0.9% sodium chloride solution. The homogenate was centrifuged twice for 30 min at 9,000× *g* and the supernatant obtained was used for electrophoresis. Electrophoresis was conducted according to Gupta and Stebbins (1969). After the electrophoretic run, the gel was sliced horizontally. One half was stained for peroxidase and the other for esterase. Peroxidases were localized with a mixture of equal amounts of benzedine solution and 3% hydrogen peroxide. The zymogram pattern appeared immediately and was photographed. Esterases were localized using alpha-naphthyl acetate as the substrate and Fast Blue-RR as a dye coupler. The gel was put in the incubator at 37 °C for 1 h and then washed in running water.

Comparison of inoculated and non-inoculated leaves of resistant and susceptible lines was done after artificial inoculation. The inoculum used was a mixture of the alpha, beta, gamma and delta races and a new race (not yet named), identified at I.P.O., Wageningen. Plant inoculations were done when the first trifoliate leaves had opened – about 3 weeks after planting. The spore concentration was determined with a haemocytometer and adjusted to 10^6 per ml by dilution. The inoculum was applied to the plants with an atomiser. Both lower and upper surfaces of primary and trifoliate leaves were dampened with the suspension. After inoculation, the plants were covered with polythene bags that had been moistened on the inside and left overnight in the greenhouse. The control plants received a similar treatment except that they were sprayed with water instead of a spore suspension. The harvesting of leaves from both the inoculated and control plants was done six times for primary leaves and four times for trifoliate leaves. Lesions were cut with little healthy tissue. The extract from these tissues was used for biochemical assay.

Table 1. Nairobi Bean (NB) collection numbers, common names and reaction to anthracnose of twelve bean lines used in this study. Numbers as used in text

Number	NB number	Common name	Disease reaction
1	86	Red Haricot	R
2	1401	Large Rose Coco	S
3	863	Rose Coco	R
4	518	Mwezi Moja	S
5	248	Mwezi Moja	R
6	1122	Small Rose Coco	S
7	16	Black Haricot	R
8	26	Canadian Wonder	S
9	259	Rose Coco	R
10	510	Large Rose Coco	S
11	84	Red Haricot	R
12	1897	Zebra Bean	S

R = resistant to bean anthracnose; S = susceptible to bean anthracnose

Results

Electrophoretic Variations

Starch gel stained for peroxidase and esterase revealed differences in the intensity of staining both among tissues at the same stage and among different stages of development of the same tissue. Several stages were studied but only two of them, 3- and 40-day-old, were critical for screening purposes because these showed specific differences between the resistant and susceptible plants. Other stages, such as 17-, 24- and 31-day-old plants, also showed differences in peroxidase zymograms. However, these differences were not as specific as in the 3-and 40-day-old plants, although they were helpful in the interpretation of our results.

In the 3-day-old stage (Fig. 1, Table 2) peroxidase band A_5 showed marked differences between susceptible and resistant plants. It was a monomorphic band that stained moderately to darkly in susceptible lines

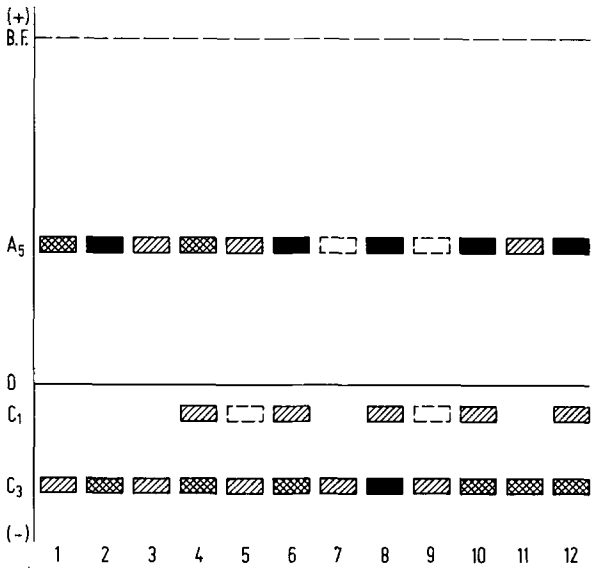

Fig. 1. Schematic diagram showing peroxidase isozyme patterns produced in bean cultivars resistant and susceptible to bean anthracnose in 3-day old plants. The degree of stippling is proportional to the staining intensity. Key: 0 = Point of sample application; + = Anodic side; — = Cathodic side; - - - = Borate buffer front; variation in bands: ■ dark; ▩ moderate; ▨ light; ⬚ faint

Table 2. Staining intensity of peroxidase isozymes A_5, A_4 and C_1 at different developmental stages

Days after emergence	Band	Lines											
		1	2	3	4	5	6	7	8	9	10	11	12
3	A5	3	4	2	3	2	4	1	4	1	4	2	4
	A4												
	C1				2	1	2		2	1	2		2
17	A5	3		2				2				3	
	A4		3		4	3	3		3	3	4		3
	C1												
24	A5	4		3				3				3	
	A4		4		4	4	4		3	4	3		4
	C1												
31	A5	2		2		1		3		3		3	3
	A4		3		4	3	4		4	4	4		4
	C1												
40	A5												
	A4	3	3	4	3	4	4		4	4	3	2	4
	C1		2		2	2	2		4		3		3

and quite lightly in resistant lines. For the same stage of development the esterase zymogram (Fig. 2) showed differences in its band C_1. This band appeared in the susceptible lines but was absent in resistant lines except in line 11, where it was present but quite faint.

At 17, 24 and 31 days after emergence, both bands A_5 and A_4 were present in all the susceptible lines as well as in the resistant lines 5 and 9, while band A_5 stained only in 4 lines, all of them resistant (Table 2). None of the lines had both bands A_4 and A_5. There were some changes in these two bands at 31 days. Band A_5 stained in all the resistant lines and the susceptible line 12. Furthermore, lines 5, 9 and 12 had both bands, A_4 being darker than A_5.

In the 40-day-old plants, peroxidase band C_1 expressed specific differences between resistant and susceptible plants (Table 2). This band was present in all the susceptible plants except line 5. The esterase zymogram for the same stage (Fig. 3) expressed differences in three bands. These bands were A_1, A_2 and C_1. Bands A_1 and A_2 showed greater activity (stained darker) in susceptible lines than the resistant lines. Band C_1 showed specific qualitative differences between susceptible and resistant lines, being present in all the susceptible lines and absent in the resistant lines except line 9. Other bands were not important since they did not show any consistent differences between the resistant and susceptible lines.

Inoculation Studies

In artificial inoculation studies, symptoms of disease did not appear until about a week after inoculation. The disease was first detected in the susceptible lines. The first test on inoculated primary leaves was done 7 days and, on trifoliate leaves, 8 days after inoculation. Both primary and trifoliate leaves responded to inoculation, but in different ways.

Primary leaves showed differences between inoculated and non-inoculated leaves. Infection at this stage induced higher peroxidase activity (darker bands). Two bands, A_5 and A_4, also showed qualitative difference

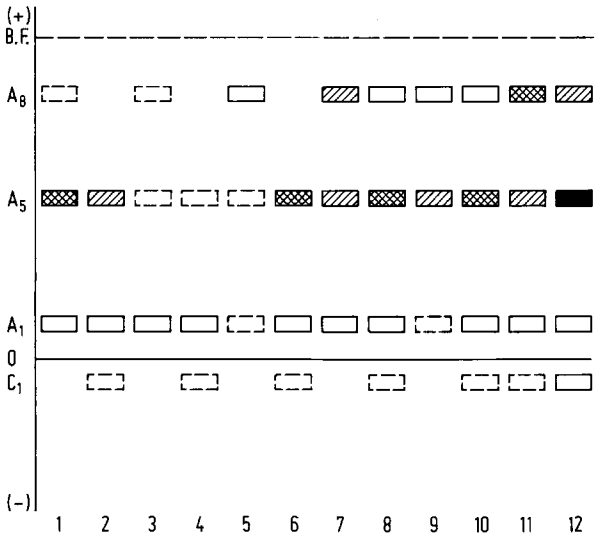

Fig. 2. Schematic diagram of esterase isozymes at 3 days in bean cultivars resistant and susceptible to bean anthracnose. The degree of stippling is proportional to the staining intensity. Refer to Fig. 1. Key as for Fig. 1

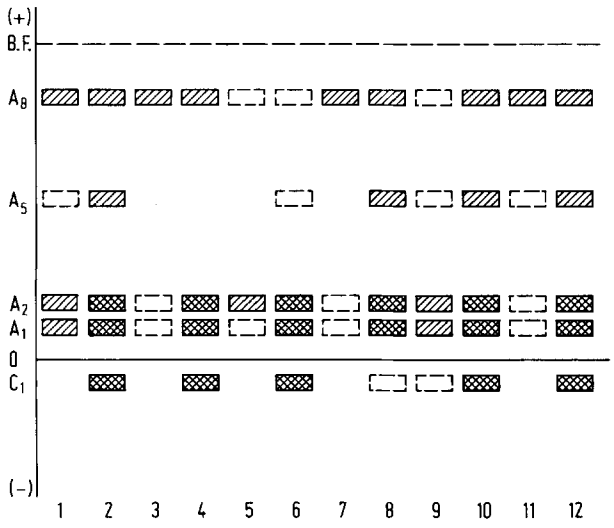

Fig. 3. Schematic diagram of esterase isozymes in bean cultivars at 40 days after emergence. The degree of stippling is proportional to the staining intensity. Key as for Fig. 1

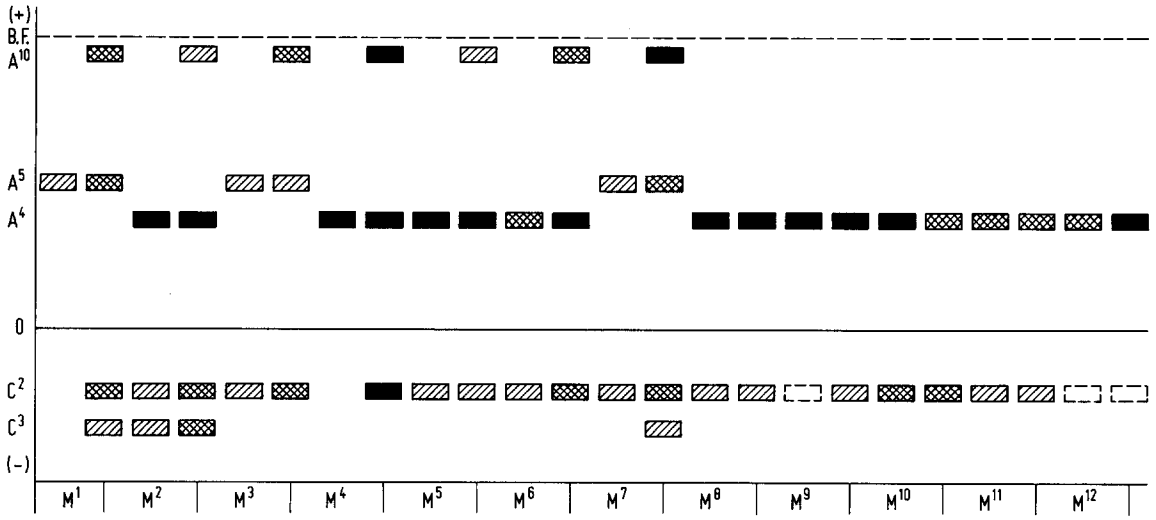

Fig. 4. Schematic diagram showing peroxidase isozymes pattern produced in trifoliate infected and non-infected leaves 8 days after infection. Non-infected plants (H) and infected plants (I). Key as for Fig. 1

between susceptible and resistant lines. A_5 was present in four of the resistant lines only, while A_4 was present in all the susceptible lines and two of the resistant ones: lines 5 and 9. Band A_5 and A_{10} showed variations between inoculated and non-inoculated leaves; while band A_5 exhibited quantitative changes, band A_{10} showed qualitative changes. Similarly, trifoliate leaves also gave differences between inoculated and non-inoculated leaves (Fig. 4). There were quantitative as well as qualitative changes. Band A_{10} showed only qualitative changes in some lines. There were no specific differences between resistant and susceptible lines in their biochemical response to artificial inoculation.

Discussion

Our study showed differences between resistant and susceptible lines both in the peroxidase and esterase enzyme system, specially in 3- and 40-day-old plants. Important in peroxidases were bands A_5 in the 3-day-old plants and C_1 in 40-day-old plants; and in esterases, bands C_1 in 3-day-old plants and A_1 and A_2 together with C_1 in 40-day-old plants. However, at each of these stages both enzyme systems were essential for complete screening since none of these bands discriminated perfectly between resistant and susceptible lines at any stage. While no single band was a perfect marker, any combination of two or more of the above mentioned bands allowed discrimination between the resistant and susceptible lines in the 12 lines used in our study.

If one wants to save time and greenhouse space and choses to screen at 3 days, peroxidase band A_5 will wrongly indicate line 1 as susceptible and esterase band C_1 will do the same for line 11. However, using both enzyme systems together both line 1 and 11 will be classified as resistant. Similarly, at 40 days, isoperoxidase C_1 was present in all the susceptible lines and also in line 5 (resistant). Line 5, however, can be identified from the susceptible lines with the help of esterase bands A_1 and A_2. Where data from two bands conflict, the conclusion must be that the particular line is resistant. Although interesting differences were also found between resistant and susceptible lines at 17, 24 and 31 days (peroxidase bands A_4 and A_5), the clearest differences were found at 3 and 40 days, both for peroxidases and esterases.

The patterns described above show that electrophoretic screening can be used for screening populations for resistance as early as 3 days after emergence. Our results are in agreement with those from various other workers in the field (Staples and Stahman 1964; Stavely and Hanson 1967; Wheeler et al. 1971). The peroxidases have been shown to be involved in disease resistance by several workers. Johnson and Cunningham (1972) showed in wheat that inoculated resistant line LR10 (TC) had 109% higher peroxidase than the healthy controls 9 days after infection. Similarly, Kedar (1959) and Umaerus (1959) showed that peroxidase activity was positively correlated with field resistance to *P. infestans* in potato clones. Fehrmann and Dimond (1967) also found a positive and highly significant correlation between peroxidase activity in the different organs of potato and resistance to *P. infestans.* Bassiri and Adams (1978) have used electrophoretic patterns for the identification of bean cultivars. They emphasized the importance of a multi-enzyme system for complete identification of the cultivars. These citations and many others are evidence of the involvement of peroxidase in the resistance reactions of several plants to diseases. The work of Seevers et al. (1971) and Yamamoto et al. (1978) show that this relationship is not always present.

Electrophoretic comparisons between inoculated and non-inoculated leaves showed that anthracnose

induced changes in peroxidase isozymes. These changes were both qualitative and quantitative. Since these changes did not affect all isozymes it suggests that not all isozymes are involved in the disease reaction of the plant. From our results it appears that band A_{10} is somehow associated with disease response. The observation is similar to that made by Seevers et al. (1971). Similarly the induction of new isozymes by infection, as A_{10} in our case, was reported by Yu and Hampton (1964). Our results do not show specific changes for resistant and susceptible plants. These observations are in agreement with those of Novacky and Wheeler (1970). They observed that victorin, a phytotoxic compound of *Helmintosporium victoriae,* induced similar changes in resistant and susceptible oat plants.

From this and other similar studies it is clear that the isozyme technique is a useful tool for screening populations for resistance. The specificity of the electrophoretic method is very important. It can show genetic differences where problems exist in the identification of genotype, especially where phenotypes do not differ.

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