

## INHERITANCE OF HALO BLIGHT RESISTANCE IN COMMON BEAN

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### ABSTRACT

Halo blight caused by (*Pseudomonas syringae* pv. *phaseolicola* (Burkh) (*Psp*)) is an important disease of common bean (*Phaseolus vulgaris* L.) world-wide. Several races of the *Psp* exist and likewise some sources of resistance in common bean have been identified. CAL 143, is a CIAT-bred common bean line, which was released as a variety in Malawi and several other countries in southern Africa. It possesses good resistance to *Psp*. However, the mode of inheritance for resistance to *Psp* in CAL 143, which is essential for devising a proper breeding strategy for developing bean varieties with durable resistance, has not been established. A study was conducted on the mode of inheritance for resistance to *Psp* using populations generated from crosses between three susceptible female parents (Chimbamba, Nasaka, and RC 15) and CAL 143 as a resistant male parent. The progenies were advanced to F<sub>2</sub> and F<sub>3</sub> generations in greenhouses at Bunda College of Agriculture and Bvumbwe Research Station. The F<sub>2</sub> and F<sub>3</sub> populations were evaluated for resistance to *Psp* in the field at Dedza and Ntchenachena. The results from both F<sub>2</sub> and F<sub>3</sub> populations indicated that there was a single dominant gene mode of inheritance for resistance to *Psp* in CAL 143. This monogenic resistance can be utilised easily and quickly in breeding for resistance to *Psp*. CAL 143 is, therefore, an ideal source of resistance and a valuable parent in breeding for resistance to *Psp* in Malawi.

*Key Words:* Halo blight, common bean, *Phaseolus vulgaris*, *Pseudomonas syringae*, resistance genes

### RÉSUMÉ

Le flétrissement du Halo causé par le *Pseudomonas syringae* pv. *phaseolicola* (Burkh) (*Psp*) est une maladie importante du haricot commun (*Phaseolus vulgaris* L.) au monde. Plusieurs races de *Psp* existent ainsi que quelques sources de résistance dans le haricot commun ont été identifiées. CAL 143 est une lignée de CIAT améliorée du haricot commun, qui était disseminée comme une variété au Malawi et d'autres de l'Afrique australe. Elle possède une bonne résistance au *Psp*; cependant, le mode d'acquisition pour résistance au *Psp* dans CAL 143, essentiel pour dresser une stratégie appropriée au développement des variétés de haricot à résistance durable, n'a pas été établi. Une étude était conduite sur le mode d'acquisition pour résistance au *Psp* par l'utilisation des populations issues des croisements entre trois parents femelles susceptibles (Chimbamba, Nasaka, et RC 15) et le parent mâle résistant CAL 143. Les progénies étaient portées aux générations F<sub>2</sub> et F<sub>3</sub> en serre au Bunda College of Agriculture et Bvumbwe Research Station. Les populations F<sub>2</sub> et F<sub>3</sub> étaient évaluées pour résistance au *Psp* sur terrain au Dedza et Ntchenachena. Les résultats des populations F<sub>2</sub> et F<sub>3</sub> ont indiqué que CAL 143 possédait un mode de gène dominant de résistance au *Psp*. Cette résistance monogénique peut être facilement et rapidement utilisée en amélioration pour résistance au *Psp*. CAL 143 est, par conséquent, une source idéale de résistance et un parent de valeur en amélioration pour résistance au *Psp* au Malawi.

*Mots Clés:* Flétrissement du Halo, haricot commun, *Phaseolus vulgaris*, *Pseudomonas syringae*, gène de résistance

## INTRODUCTION

Halo blight caused by *Pseudomonas syringae* *pv phaseolicola* (*Psp*) is a serious seed-borne disease of common bean worldwide (CIAT, 1981; Schwarts and Pastor-Corrales, 1989). It is a disease of economic importance in areas of moderate to cool temperatures in the highlands of Africa (Beebe and Pastor-Corrales, 1991). The pathogen is widely distributed in Malawi (Msuku *et al.*, 2000) and causes serious damage in Africa (Worthmann *et al.*, 1998). The typical symptoms are small brown spots that are surrounded by a light-green or yellow halo. The halo is caused by a toxin produced by the *Psp* bacterium when temperatures are less than 21°C for at least part of the day. The disease also causes water soaked greasy looking spots on pods and may appear as small, brown, scabby spots.

Nine races of the pathogen have been reported based on their reactions on differential cultivars and lines (Taylor *et al.*, 1996a). The cultivar Red Mexican U.I.3, GN Nebraska # Sel. 27 and U.I.35 have hypersensitive resistance to race 1 isolates, controlled by a single gene (Taylor *et al.*, 1996b; Beebe and Pastor-Corrales, 1991). A recessive gene controlling tolerance to races 1 and 2 was reported in genotype P.I. 150414 from USA, while a two complementally gene model was found in a USA variety, Montcalm. A series of three recessive alleles complementally to either of two other genes were identified in a Malawian bean line 1212D (Msuku, 1984; Kelly *et al.*, 1985).

An effective breeding strategy for resistance to any disease requires a thorough understanding of the nature of inheritance of the genes controlling resistance to the pathogen causing the disease. CAL 143 has shown good resistance to *Psp* in Malawi, but the mode of inheritance has not been determined; hence the need for this study. The identification of additional sources of resistance to *Psp* and knowledge of its mode of inheritance will facilitate the incorporation of durable resistance in new improved bean varieties. The objective of this study was to determine the mode of inheritance of halo blight resistance in the common bean.

## MATERIALS AND METHODS

Crosses were made between three recipient (susceptible) parents to *Psp*: Chimbamba, Nasaka, and RC 15 and one donor (resistant) parent to *Psp*: CAL 143. Chimbamba is a local land race, climbing bean cultivar of Type IV, which is adapted in Malawi. It is normally grown with stakes or in association with maize for support. Nasaka is a local land race, bush bean cultivar of Type I, which is adapted in Malawi, and RC 15 is also a bush bean line of Type I, which originated from the bean breeding programme at Bunda College of Agriculture in Malawi. CAL 143 is a CIAT bred line that has been released as a variety called Napilira in Malawi, where it is resistant to *Psp*, in addition to good adaptation as well as high yields (mean yield over 2300 kg ha<sup>-1</sup>). The variety is also grown in other countries in southern Africa region (Angola, southern Democratic Republic of Congo, Mozambique, Swaziland and Zambia). The F<sub>1</sub>s were generated and advanced to F<sub>2</sub> and F<sub>3</sub> in the greenhouses at Bunda College of Agriculture and Bvumbwe Agricultural Research Station.

The parents, F<sub>2</sub> and F<sub>3</sub> populations were evaluated in the fields at Dedza and Ntchenachena sites, which represent the normal bean growing conditions during the rainy season, where halo blight is a common disease. The site in Dedza was located at 1500 meters above sea level (masl), on a geographical position: 14° 20' S; 34° 18' E, with annual average temperature of 20.9 °C and soils which were classified as clay loam. The site for Ntchenachena was located at 1600 masl, on a geographical position: 10° 40' S; 34° 5' E, with annual mean temperature of 22.4 °C and soils which were classified as clay loam. Both sites have favourable environments for *Psp* because of the cool temperatures and adequate wet conditions during the bean crop growing period (Msuku, 1984). The field trials were planted during the third and last week of December, respectively at Dedza and Ntchenachena. The segregating F<sub>2</sub> and F<sub>3</sub> progenies resulting from the crosses between the recipient parents (Chimbamba, Nasaka, and RC 15) and a common

donor parent (CAL 143) were planted alongside the parental lines. Each plot had a single row of 6 meters long, and the rows were spaced at 0.75 m apart. Seeds were planted at a spacing of 10 cm for bush beans (Nasaka and RC 15) and their resulting progenies, while the climbing bean (Chimbamba) and the resulting progenies were planted at and 15 cm apart, because climbing bean plants are more vigorous and require more space. Nitrogen fertiliser was applied ( $40 \text{ kg N ha}^{-1}$ ) at the first trifoliolate leaf stage and weeding was done on regular basis. Insect pests were controlled by occasional spray with Dimethoate pesticide. Rainfall data were collected during the crop growing period at both sites using rain gauges mounted at about 50 m away from the experimental fields.

The individual plants were assessed based on their reaction to *Psp* using a CIAT scale of 1 to 9 (CIAT, 1987), where 1=immune and 9=very susceptible. The scores were grouped into 2 categories; 1-4 for resistant plants and 5-9 for susceptible plants. The disease evaluation was based on natural occurrence of the pathogen in the trial plots. The plants were scored twice during the entire growing period: at flowering (R6) and pod filling (R8) stages. The observed disease score ratios were tested against the expected ratios through a Chi-square test using the statistical package for the social sciences (SPSS

Version 9.0. The observed phenotypic classes were tested for goodness of fit to postulated ratios based on the possible number of genes involved, and this was used to determine the mode of inheritance for resistance to *Psp*. The frequency distributions of plants based on disease scores were plotted to determine the overall reaction of the parental lines and segregating populations to *Psp* in the field.

## RESULTS

Among the parental lines, CAL 143 was resistant to *Psp*, while Chimbamba showed differential reaction, and both Nasaka and RC 15 were susceptible (Figs. 1, 3 and 5). In the segregating populations, a total of 17  $F_2$  and 20  $F_3$  plants from Chimbamba and CAL 143 cross were evaluated for resistance to *Psp*. The  $F_2$  population showed a bimodal distribution, while the  $F_3$  population showed a trimodal distribution pattern (Fig. 2). In the Nasaka and CAL 143 cross, a total of 21  $F_2$  and 20  $F_3$  plants were evaluated for resistance to *Psp* and both  $F_2$  and  $F_3$  populations showed a bimodal distribution pattern (Fig. 4). In the RC 15 and CAL 143 cross, a total of 25  $F_2$  and 22  $F_3$  plants were evaluated for resistance to *Psp* and both  $F_2$  and  $F_3$  populations showed a bimodal distribution pattern (Fig. 6). Overall, the results from the segregating populations indicated that

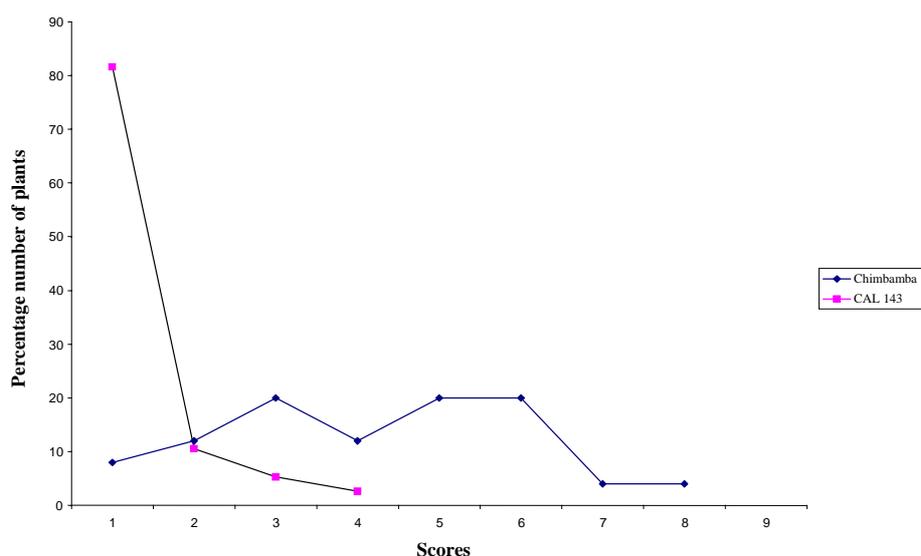


Figure 1. Percentage distribution of Chimbamba and CAL 143 plants resistant to *Psp*.

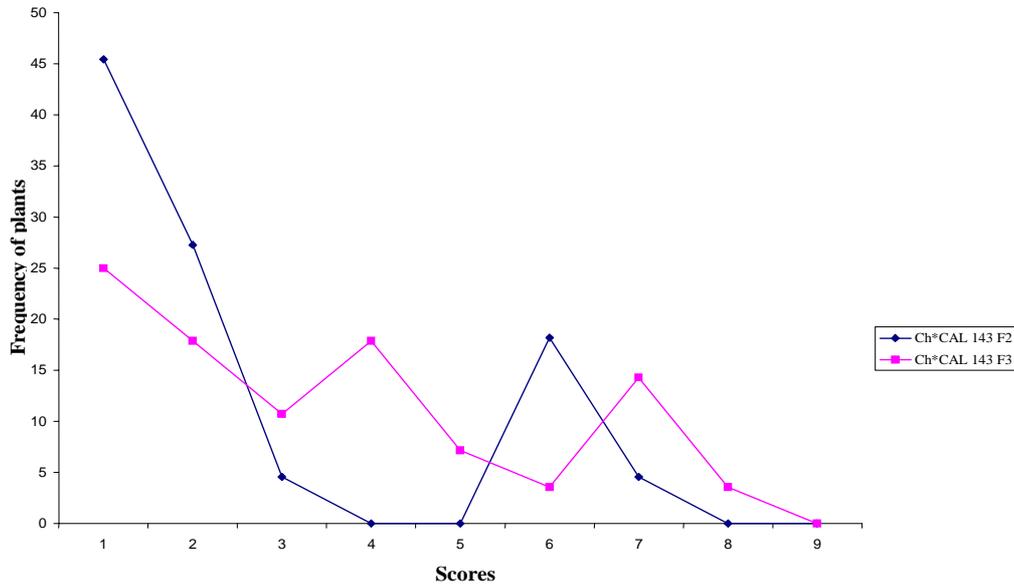


Figure 2. Percentage distribution of F<sub>2</sub> and F<sub>3</sub> plants resistant to Psp from Chimbamba and CAL 143 population.

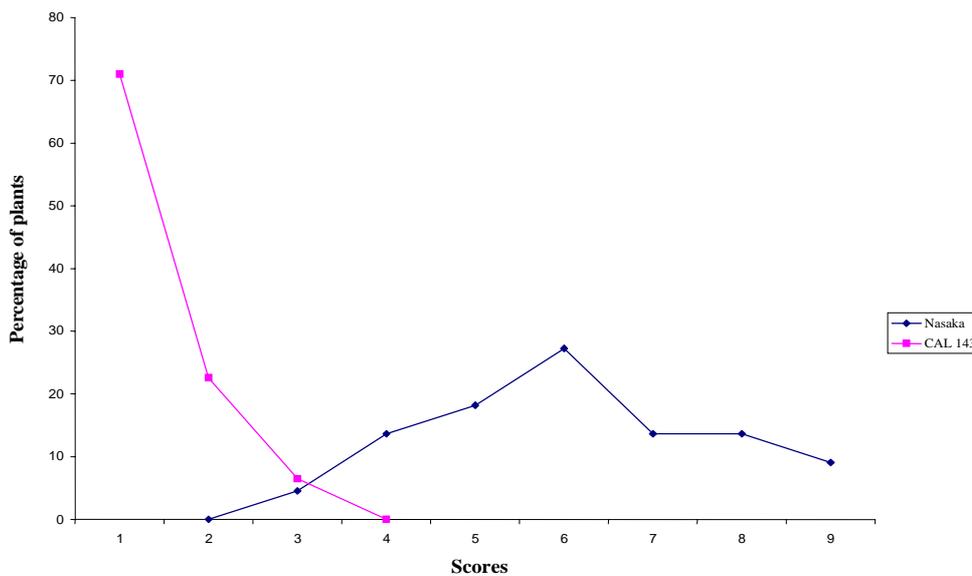


Figure 3. Percentage distribution of Nasaka and CAL 143 plants resistant to Psp.

all F<sub>2</sub> populations from the three recipient parents showed a bimodal distribution pattern in their reaction to *Psp*. Among the F<sub>3</sub> populations only plants from Chimbamba as a female parent segregated differently from the rest by showing a trimodal distribution pattern while those from Nasaka and RC 15 showed a bimodal distribution pattern in reaction to *Psp*.

**Inheritance of resistance to *Psp*.** The data collected from the evaluation of the F<sub>2</sub> and F<sub>3</sub> populations, were arranged in two categories: resistant (disease scores 1-4) and susceptible (disease scores 5-9) based on their reaction to *Psp*. These data were analysed using a Chi-square test. The Chi-square additivity test on both F<sub>2</sub> and F<sub>3</sub> progeny plants suggested that the data

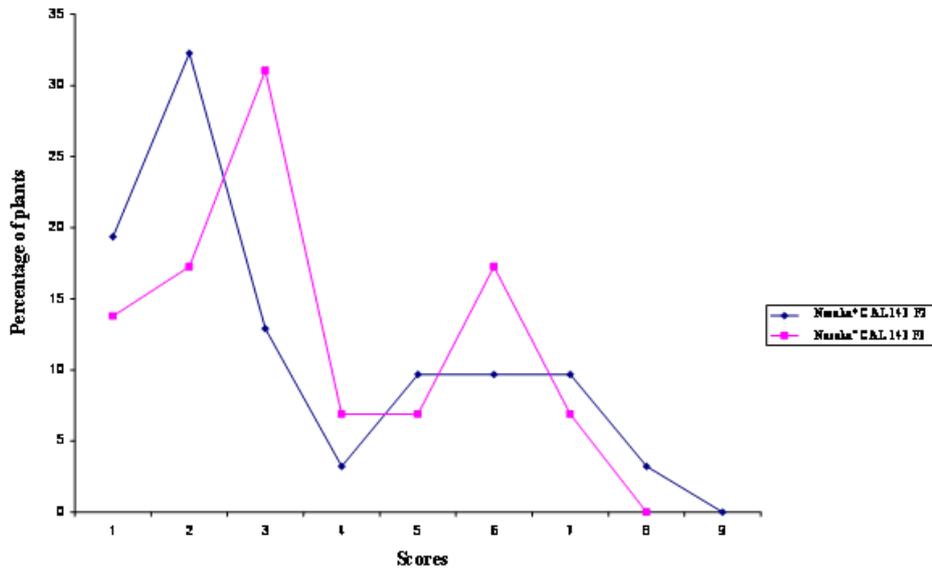


Figure 4. Percentage distribution of F<sub>2</sub> and F<sub>3</sub> plants resistant to Psp from Nasaka and CAL 143 population.

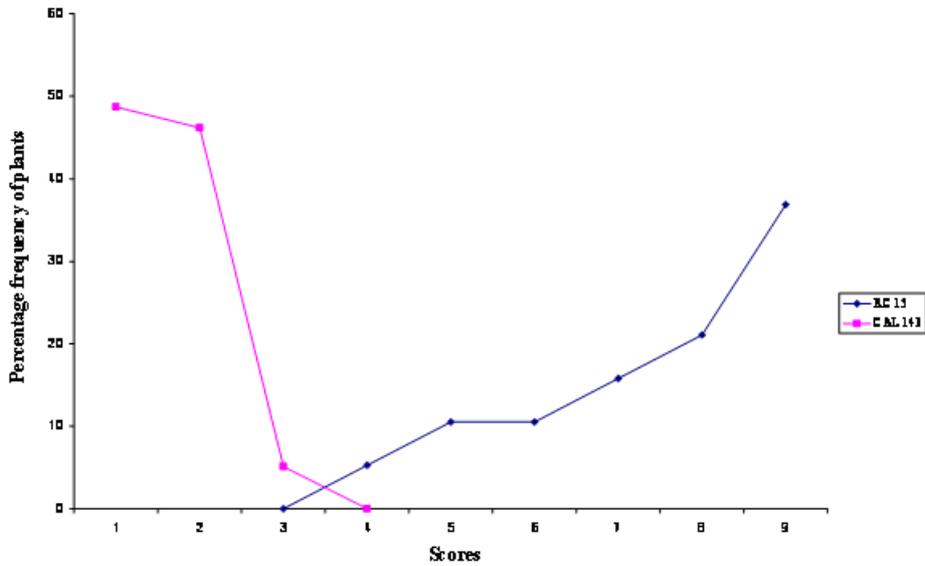


Figure 5. Percentage distribution of RC 15 and CAL 143 plants resistant to Psp.

were homogeneous,  $X^2$  additivity = 0.465,  $P=0.793$  at F<sub>2</sub> and  $X^2$  additivity = 0.181,  $P=0.913$  at F<sub>3</sub> (Table 1). The segregation ratios were, therefore, tested based on pooled Chi-square and the results indicated that the data were in compliance with

the resistant: susceptible ratio of 3:1 at F<sub>2</sub> and 5:3 at F<sub>3</sub>, indicating that CAL 143 had one dominant gene conferring resistance to *Psp* ( $X^2$  pooled= 1.01,  $P=0.799$  for F<sub>2</sub> and  $X^2$  pooled= 1.722,  $P=0.632$  for F<sub>3</sub>).

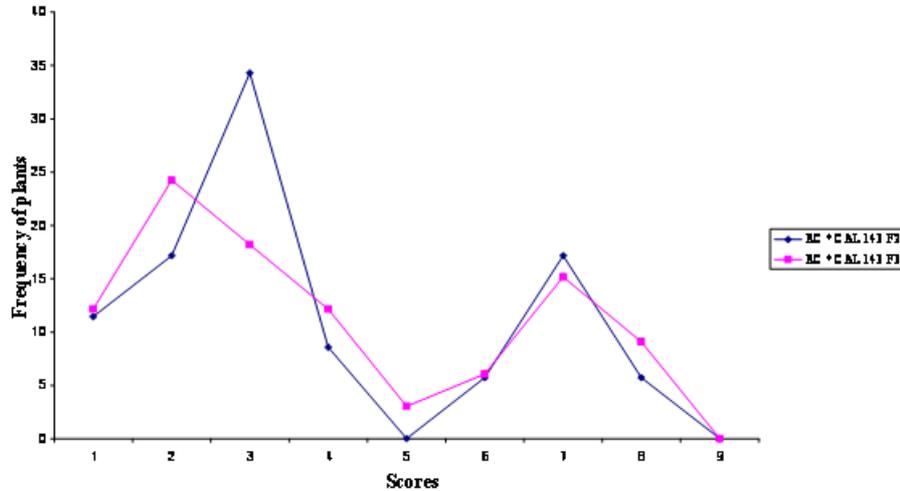


Figure 6. Percentage distribution of F<sub>2</sub> and F<sub>3</sub> plants resistant to Psp from RC 15 and CAL 143 population.

## DISCUSSION

The findings from this study suggest that it is practically possible to phenotype individual plants for resistance to *Psp* under natural conditions as long as the pathogen is present and the environmental conditions are favourable. Asensio *et al.* (2010) conducted field evaluations under inoculated conditions and identified two accessions (BGE 002189 and BGE 029592) out of 199 from a Spanish core collection that had immune reaction to two races of *Psp* (race 6 and 7). Msuku (1984) suggested that field tests would be useful in detecting field resistance more accurately than greenhouse or laboratory tests. The results from this study also suggest that there is one dominant gene conferring resistance to *Psp* in CAL 143 (Table 1). This mode of inheritance of resistance to *Psp*, which is controlled by a single dominant gene, was also reported by Miklas *et al.* (2009). They conducted allelism tests involving UI-3 (formerly 'Red Mexican UI-3') and host differential cultivars: Canadian Wonder, ZAA 54 (A52), Tendergreen, ZAA 55 (A53), ZAA 12 (A43) and Guatemala 196-B, where a single dominant gene, *Pse-1*, was reported to condition resistance to races 1, 7, and 9 of *Psp*. Thus the single dominant gene was found to be a valuable source of broad spectrum resistance to *Psp*. This gene has been mapped to linkage group B 10 and found to be near quantitative trait loci (QTL) for resistance to

fusarium wilt, halo blight, and angular leaf spot (Fall *et al.*, 2001; Blair *et al.*, 2003; Lopez *et al.*, 2003; Miklas *et al.*, 2006). In a study conducted by Msuku (1984), two dominant complementary genes were found to determine resistance to race 2 of *Psp* in Montcalm. In the same study, a Malawian land race, 1212D had an allelomorphic series of three alleles, which were assumed to control resistance to the same race in complement with either of two genes.

**Source of broad resistance.** Although in this study, the actual number of prevalent halo blight races in the field were not determined, Msuku (1984) identified four races (1, 2, 3 and 4) after screening 32 isolates, which were obtained from various bean growing areas of Malawi, including Dedza and Nchenachena. These races were identified based their reaction to five differential cultivars (Red Mexican UI-3, Red Mexican UI-34, Great Northern 123, Jubila and Namajengo (a landrace in Malawi). Nasaka and Montcalm were used as susceptible and resistant controls. Many of the isolates belonged to race 2 (16 isolates) and race 1 (13 isolates), indicating that these races might be common in most bean production environments where *Psp* is predominant. Since Msuku's (1984) results suggested more than one race of *Psp* in the bean production environments, it would appear that CAL 143 may potentially offer a good source of resistance to a broader range of *Psp* races, which are prevalent in Dedza

TABLE 1. Frequency distributions of the number of plants, from parental lines and F<sub>2</sub> and F<sub>3</sub> progenies reacting to Psp

Parent/crosses	Frequency distribution of plants on a 1-9 scale									Observed# of plants		Expected Ratio of R:S	X <sup>2</sup>	P
	1	2	3	4	5	6	7	8	9	R	S			
Chimbamba (P <sub>1</sub> )	2	3	5	3	5	5	1	1	0					
CAL 143 (P <sub>2</sub> )	31	4	2	1										
F <sub>2</sub> (P <sub>1</sub> /P <sub>2</sub> )	10	6	1	0	0	4	1	0	0	17	5	3:1	0.061	0.806
F <sub>3</sub> (P <sub>1</sub> /P <sub>2</sub> )	7	5	3	5	2	1	4	1	0	20	8	5:3	0.952	0.329
Nasaka (P <sub>2</sub> )	0	0	1	3	4	6	3	3	2					
CAL 143 (P <sub>2</sub> )	22	7	2	0	0	0	0	0	0					
F <sub>2</sub> (P <sub>2</sub> /P <sub>2</sub> )	6	10	4	1	3	3	3	1	0	21	10	3:1	0.829	0.363
F <sub>3</sub> (P <sub>2</sub> /P <sub>2</sub> )	4	5	9	2	2	5	2	0	0	20	9	5:3	0.517	0.472
RC 15 (P <sub>3</sub> )	0	0	0	1	2	2	3	4	7					
CAL 143 (P <sub>2</sub> )	19	18	2	0	0	0	0	0	0					
F <sub>2</sub> (P <sub>2</sub> /P <sub>2</sub> )	4	6	12	3	0	2	6	2	0	25	10	3:1	0.120	0.699
F <sub>3</sub> (P <sub>2</sub> /P <sub>2</sub> )	4	8	6	4	1	2	5	3	0	22	11	5:3	0.253	0.615
Σ(P <sub>1</sub> , P <sub>2</sub> and P <sub>3</sub> )	2	3	6	7	11	13	7	8	9					
Σ(P <sub>2</sub> )	72	29	6	1	0	0	0	0	0					
ΣF <sub>2</sub> across crosses	20	22	17	4	3	9	10	3	0	63	25	3:1	0.545	0.460
ΣF <sub>3</sub> across crosses	15	18	18	11	5	8	11	4	0	62	28	5:3	1.541	0.214
Chi-square value for additivity in F <sub>2</sub>													0.465	0.793
Chi-square value for additivity in F <sub>3</sub>													0.181	0.913
Pooled Chi-square value (total) in F <sub>2</sub>													1.01	0.799
Pooled Chi-square value (total) in F <sub>3</sub>													1.722	0.632

and Ntchenachena, the selected two major bean growing areas in this study where *Psp* is predominant. That a single gene can offer broad resistance to *Psp* was demonstrated by Miklas *et al.* (2009), and this was found in the differential cultivar UI-3. Teverson (1991) and Taylor *et al.* (1996b), have reported five putative R genes (R1, R2, R3, R4, and R5) that have been identified to confer resistance to *Psp*, and the gene action, dominant or recessive are symbolised *Pse* or *pse* respectively (Basset, 2004). Fourie *et al.* (2004) reported that genes conferring resistance to *Psp* occur in a tight cluster. CAL 143 has shown to potentially have *Pse* gene which confers broad resistance to *Psp*, at least against the races that might occur across the two bean production environments (Dedza and Ntchenachena) in Malawi. Asensio *et al.* (2010) also identified two accessions from a Spanish core collection that had resistance to *Psp* races 6 and 7, which have not been reported in Malawi.

**Implications for breeding.** The pooled Chi-square value for  $F_2$  populations strongly supported a single gene model of inheritance in CAL 143 to *Psp*. The  $F_3$  data were equally supportive and confirmed the same model. CAL 143, with broad resistance to *Psp*, is already released as a variety in Malawi, because of high yield and its attractive grain type for the market (medium to large seed size and red-mottled grain colour). The variety can be widely promoted for production in the areas where *Psp* is a constraint to bean production. In addition, CAL 143 can be utilised in the breeding programmes when making future crosses for developing new varieties with high yield and resistance to *Psp*. The use of a broad genetic base has been advocated by Singh (2001) as a good strategy for varietal improvement. The fact that its resistance is monogenic and dominant makes the breeding process easier and quicker to make progress and achieve the intended breeding objectives..

### CONCLUSION

The inheritance of resistance to *Psp* in CAL 143 is determined by a single dominant gene (*Pse*). This genotype is an ideal source of resistance to *Psp* in Malawi and a good parent in breeding for

resistance to *Psp* in common beans. It would be easier to breed desirable bean varieties with resistance to *Psp* using CAL 143 for Malawi and other countries in southern Africa, where the preference is for large seeded (Andean) bean types, because the resistance gene is already in the large seeded background and it is determined by a single dominant gene.

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