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Evaluation of yellow common bean (*Phaseolus vulgsaris* L.) genotypes for resistance to anthracnose

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Anthracnose, caused by the fungus *Colletotrichum lindemuthianum*, is a significant disease affecting common bean (*Phaseolus vulgaris L.).* The identification of sources of anthracnose resistance within the yellow market class is crucial for developing yellow bean varieties resistant to anthracnose. The objective of this study was to assess a set of yellow common bean genotypes for resistance to eight races of *C. lindemuthianum*. A total of 30 yellow-seeded bean genotypes were evaluated for their resistance to races 5, 19, 38, 39, 51, 183, 1050 and 1105. Among these genotypes, Y1612-04 demonstrated exceptional performance as it exhibited resistance to seven of the eight races used in this study. This genotype holds potential as a source of resistance to enhance the Manteca yellow bean market class, which is popular in East and Southern Africa.

Key words: Anthracnose, *Colletotrichum lindemuthianum*, Common bean, races, yellow beans.

INTRODUCTION

Common bean (*Phaseolus vulgaris L.)* is a significant source of protein, carbohydrates, fiber, and essential micronutrients (iron and zinc) in numerous African and Latin American countries (Uebersax et al., 2022). The common bean is categorized into several market classes primarily based on seed characteristics such as seed shape, size, and color. One of these market classes includes yellow beans, which consist of genotypes with varying shades of yellow seed color. Examples of these shades of yellow seed color comprise bright yellow (known as the Manteca) and dull yellow (known as the Njano) (Sadohara et al., 2022). Yellow beans are popular in Africa (Katungi et al., 2009), and in countries such as Zambia, the landrace "Lusaka," which has a Manteca seed color, fetches a higher price than other market classes (Sichilima et al., 2016). Some of the contributing factors to the popularity of yellow beans include their nutritional superiority and faster cooking time compared to other market classes (Cichy et al., 2015; Wiesinger et al., 2018). Low soil fertility, drought (Hamabwe et al., 2023), aluminum toxicity (Njobvu et al., 2020), and diseases are major contributors to low yields of common bean in Africa. Common bean is susceptible to several diseases, which cause significant yield losses on the continent (Binagwa et al., 2021). Anthracnose (ANTH), caused by the fungus *Colletotrichum lindemuthianum*, is a major seed-borne

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disease of common bean globally and can lead to yield losses of up to 100% (Ferreira et al., 2013). Sharing farm-saved seed, a popular informal seed system in Africa, facilitates the spread of ANTH between farms and production years. While ANTH can be effectively controlled using fungicides, smallholder resource-poor farmers, who are the primary producers of common beans in Africa, often cannot afford them (Nalupya et al., 2021). Furthermore, there are human safety concerns associated with chemical usage. Therefore, developing and utilizing ANTH-resistant varieties is the most cost-effective, human, and environmentally friendly management strategy for addressing ANTH (Dillard and Cobb, 1993). The highly variable nature of *C. lindemuthianum*, with over 298 races reported globally (Nunes et al., 2021) and 58 highly diverse races reported in Zambia alone (Sansala et al., 2023), poses challenges for developing varieties with durable resistance, as new races can overcome the resistance of newly developed varieties. There is no single genotype resistant to all races of *C. lindemuthianum*.

Given this extensive variability and evolution, there is a continuous need to identify new sources of resistance to support the development of ANTH-resistant varieties. Preferably, these sources of resistance should belong to the same market class as the one targeted for genetic improvement, thereby avoiding breeding challenges associated with utilizing resistance sources from outside the target market class. For instance, there is a low recovery rate or frequency of yellow-seed progenies when yellow beans are crossed with other market classes. Therefore, identifying yellow-seeded genotypes as sources of ANTH resistance represents an ideal strategy for enhancing ANTH resistance within the yellow market class of beans. The objective of this study was to assess a set of genotypes consisting of yellow-seeded varieties for their resistance to eleven races of *C. lindemuthianum*.

MATERIALS AND METHODS

Plant materials

A set of 30 Andean variable yellow-seeded genotypes, including both the Manteca and Njano types, was utilized in this study (Table 1). All 30 genotypes were advanced pure breeding lines obtained from Dr. Karen Cichy's Breeding program at Michigan State University. The landrace "Kabulangeti" served as susceptible checks, while the well-known Middle American genotype G2333, which exhibits high resistance to diverse races of C. lindemuthanum, was employed as a resistant check.

C. lindemuthianum races

A total of eight races of *C. lindemuthianum* (5, 19, 38, 39, 51, 183, 1050, and 1105) were employed in this study. These races were previously characterized using 12 race differential cultivars from isolates collected from various parts of Zambia (Sansala et al., 2023). The eight races were preserved on filter paper and maintained in the Bean Breeding and Genetics Lab at the University of Zambia. Selection of these eight races for the current study was based on their virulence and frequency of isolation from various geographic regions reported in the previous study. For example, race 19 is highly prevalent in Zambia and was reported in several bean-growing regions in previous studies (Sansala et al., 2023). Races 1050 and 1105 are highly virulent and widespread in many bean-growing regions of Zambia (Sansala et al., 2023).

Screening for resistance to eight races of *C. lindemuthianum*

The 30 genotypes and checks were assessed for resistance to eight races of *C. lindemuthianum* in both the growth chamber and greenhouse at the University of Zambia, following a protocol previously described (Mungalu et al., 2020; Kuwabo et al., 2023). Seeds of the 30 genotypes and checks were planted on Styrofoam trays using a completely randomized block design with three replications. Each tray contained 60 wells, and two seeds of each genotype were planted per well. Therefore, a well-served as an experimental unit, resulting in six seeds (seedlings) per genotype being evaluated with three replications. Inoculation of the seedlings occurred when the cotyledon leaves were fully expanded. Evaluations were performed for one race at a time, resulting in a total of eight separate evaluations for the eight races, with a two-week interval between each evaluation.

Each race was transferred from the filter paper onto PDA media in petri dishes and stored in a dark room for 14 days to allow for sporulation and mycelium growth. After this period, the petri dishes were flooded with distilled water, and the mycelium was carefully scraped and mixed to create inoculum. The inoculum was then standardized to a spore concentration of 1.2×10^6 spores per ml using a haemocytometer. Tween 20 was added to the inoculum, which was then sprayed onto the seedlings using a small handheld sprayer until the leaves were sufficiently wet and inoculum started to drip from the leaves. The inoculated seedlings were kept at room temperature for two hours to allow the inoculum to dry off before being transferred to a high humidity chamber maintained at 98% humidity and ambient temperature. The seedlings were kept in the chamber for 72 h to allow for infection to take place, after which they were transferred to the greenhouse and placed on benches for five days to allow for ANTH development. ANTH severity for each genotype was then assessed on a severity scale of 1 to 9 (Nalupya et al., 2021), where a score of 1 to 3 indicated seedlings with no visible symptoms or only a few very small lesions mostly on primary leaf veins, 4 to 6 indicated moderate resistance with small lesions on leaves and the seedling stem, and 7 to 9 indicated susceptibility with numerous small or enlarged lesions, sunken cankers on leaves and the seedling stem, or dead plants.

Data analysis

ANTH severity score data were analyzed in SAS 9.3 (SAS Institute, 2011). To determine the significance of severity scores differences among genotypes, PROC Mixed was used to conduct Analysis of variance (ANOVA) using the following statistical model: $Y_{ik}=\mu+\alpha_i+E_{ik}$, where: Y_{ik} was anthracnose severity score, for accession *i*, in replication *k*; αi was the fixed variable effect of the accession *i*; E was the residual associated with replication *k* for accession *i*. The severity scores data of accessions for all eight races were not normally distributed, therefore, were transformed (logarithmic transformation) before use in ANOVA.

RESULTS

Significant ($p<0.05$) differences in anthracnose severity scores were observed among the 30 yellow genotypes

Table 1. Anthracnose severity scores for 30 yellow genotypes and checks inoculated with *C. lindemuthianum* races 5, 19, 38, 39, 51, 183, 1050 and 1105 at University of Zambia

Genotype	Race number and severity scores (1 – 9)							
	5	**19**	**38**	**39**	**51**	**183**	**1050**	**1105**
Yellow genotypes								
Y1612-04	1	1	9	9	1	-	1	1
PIC 86	-	9	1	1	-	9	9	9
Y1608-02	9	9	9	9	9	9	3	9
Y1608-03	-	9	9	9	7	1	9	9
Y1608-04	9	2	9	9	9	2	9	9
Y1608-06	9	1	9	9	9	1	7	9
Y1608-09	9	1	-	9	9	1	7	9
Y1608-14	9	1	-	9	9	1	8	1
Y1608-15	9	9	9	9	9	9	8	9
Y1609-01	9	9	9	9	-	9	8	8
Y1609-02	9	1	9	9	-	1	8	9
Y1609-09	9	9	9	9	-	9	7	1
Y1609-14	9	9	9	9	1	9	7	9
Y1609-16	9	9	9	9	9	9	8	9
Y1610-01	9	1	9	9	9	1	9	9
Y1612-01	9	9	9	9	9	9	1	1
Y1612-02	9	9	9	9	8	9	-	1
Y1612-03	-	9	9	9	8	9	1	1
Y1612-05	9	1	9	9	-	9	1	1
Y1612-06	9	9	9	9	-	9	1	1
Y1701-03	9	9	9	9	9	9	9	9
Y1701-09	-	9	9	9	-	9	9	9
Y1702-10	-	9	9	8	9	1	9	9
Y1702-15	9	9	9	-	9	9	9	9
Y1702-18	9	9	9	9	-	9	9	9
Y1702-22	9	9	9	9	9	1	9	9
Y1703-01	9	-	9	9	9	9	8	9
Y1703-20	9	9	9	9	-	9	7	9
Y1703-21	9	9	9	9	9	9	9	9
Y1703-22	9	9	9	9	9	9	8	9
Checks								
G2333	1	1	1	1	1	1	-	1
Kabulangeti	3	9	1	9	1	9	9	9

G2333 is resistant check; Kabulangeti is susceptible check; Severity scores are on a scale of 1- 9, where 1-3 is resistant, 4-6 is moderately resistant and 7-9 is susceptible; - is missing severity score.

inoculated with nine diverse races of *C. lindemuthianum*. In general, the reaction was skewed towards susceptibility. As expected, the resistant check G2333 was highly resistant while the susceptible check Kabulangeti was highly susceptible to all eight races (Table 1). Race 5 was the least virulent race used in this study, and all genotypes except Y1612-04 were highly susceptible (Table 1 and Figure 1).

For Race 19, eight genotypes (Y1608-04, Y1608-06, Y1608-09, Y1608-14, Y1609-02, Y1610-01, Y1612-04, Y1612-05) were highly resistant to race 19 while the others were susceptible (Figure 2). All genotypes except PIC 86 were highly susceptible to races 38 and 39 (Figures 3 and 4). For race 51, only two genotypes (Y1609-14 and Y1612-04) were resistant while the other genotypes were highly susceptible (Figure 5). Nine genotypes (Y1608-03, Y1608-04, Y1608-06, Y1608-09, Y1608-14, Y1609-02, Y1610-01, Y1702-10, and Y1702-22) were resistant to race 183 while the other 21 genotypes were highly susceptible (Figure 6). Six genotypes (Y1608-02, Y1612-01, Y1612-03, Y1612-

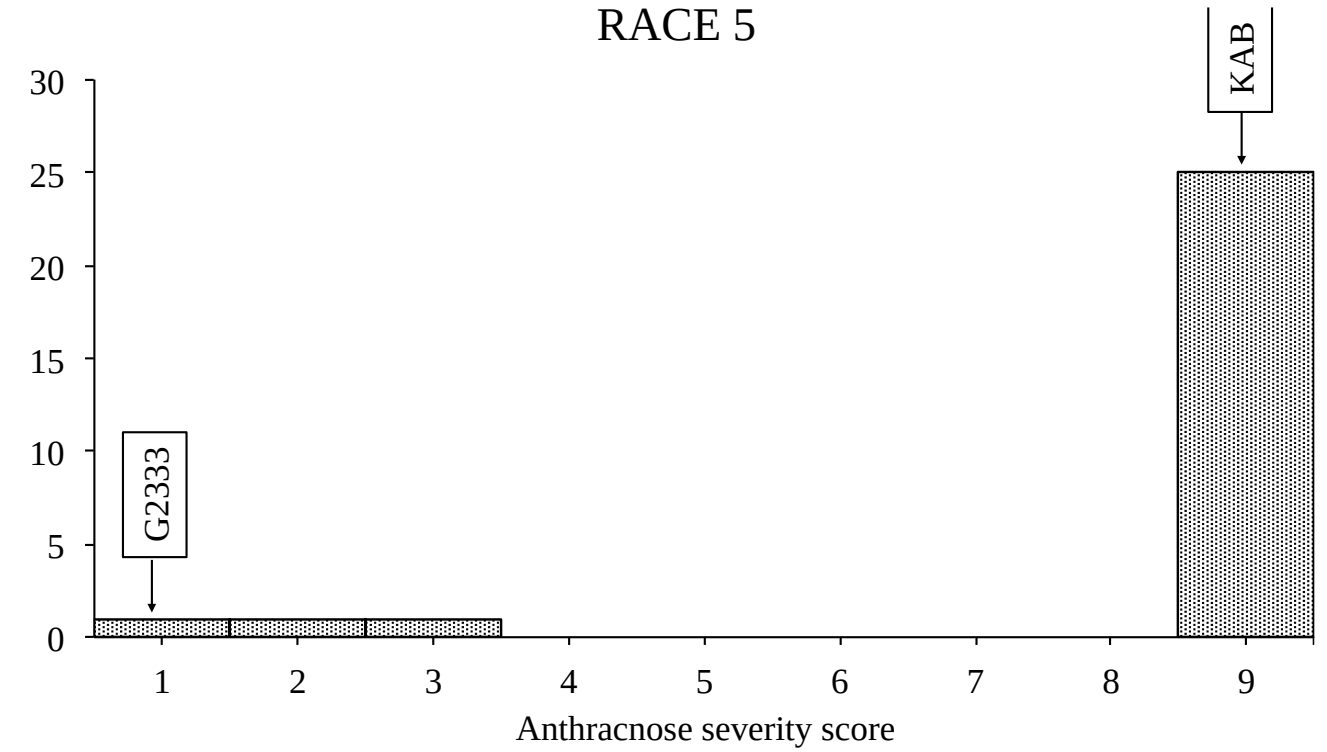

Figure 1. Frequency distribution of anthracnose severity scores for the 30 yellow genotypes and checks inoculated with race 5 of *C. lindemuthianum* at University of Zambia.

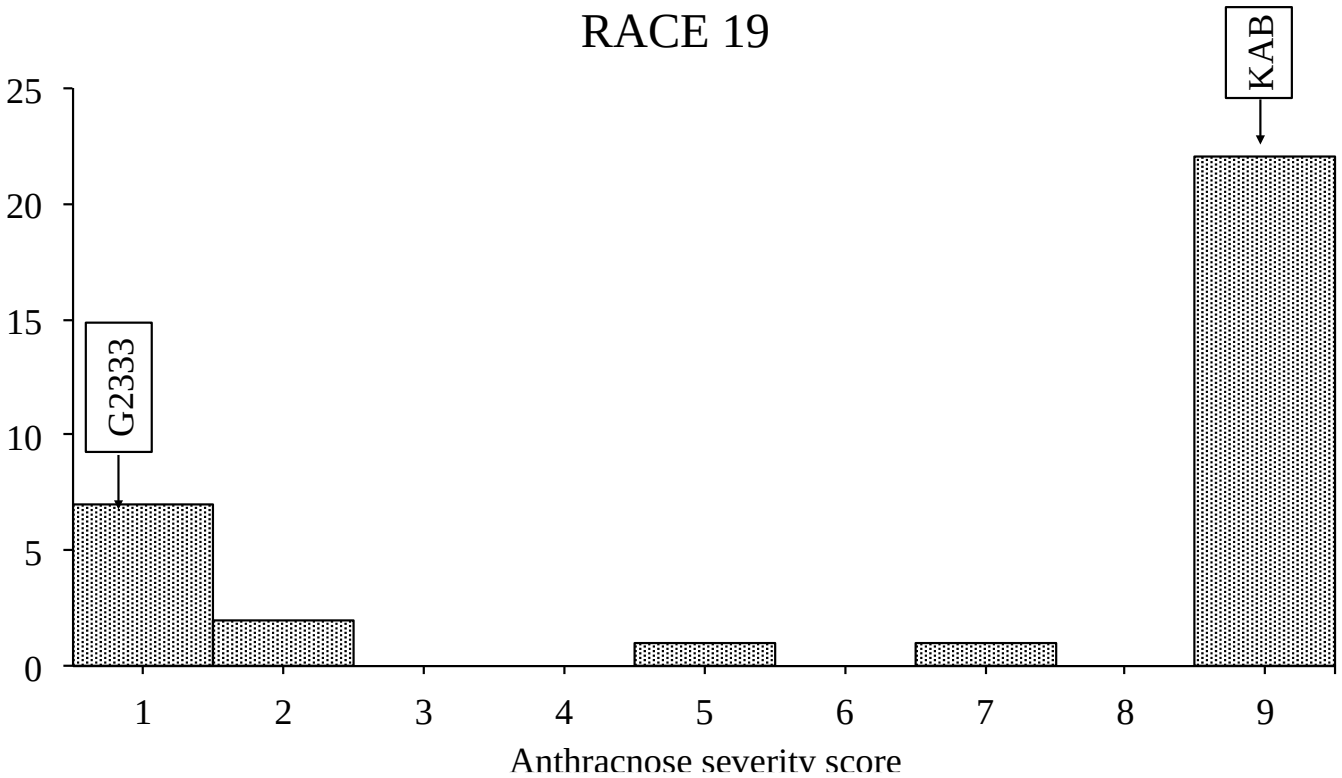

Figure 2. Frequency distribution of anthracnose severity scores for the 30 yellow genotypes and checks inoculated with race 19 of *C. lindemuthianum* at University of Zambia.

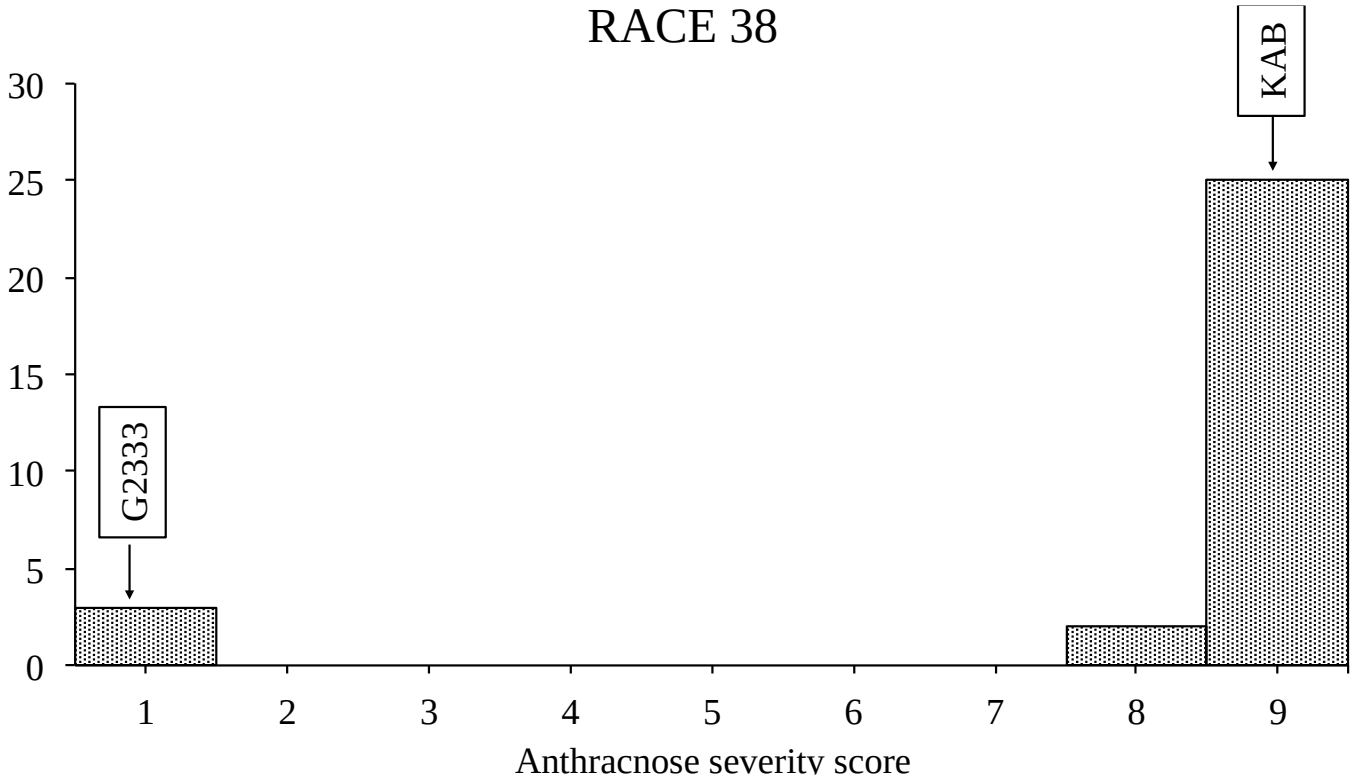

Figure 3. Frequency distribution of anthracnose severity scores for the 30 yellow genotypes and checks inoculated with race 38 of *C. lindemuthianum* at University of Zambia.

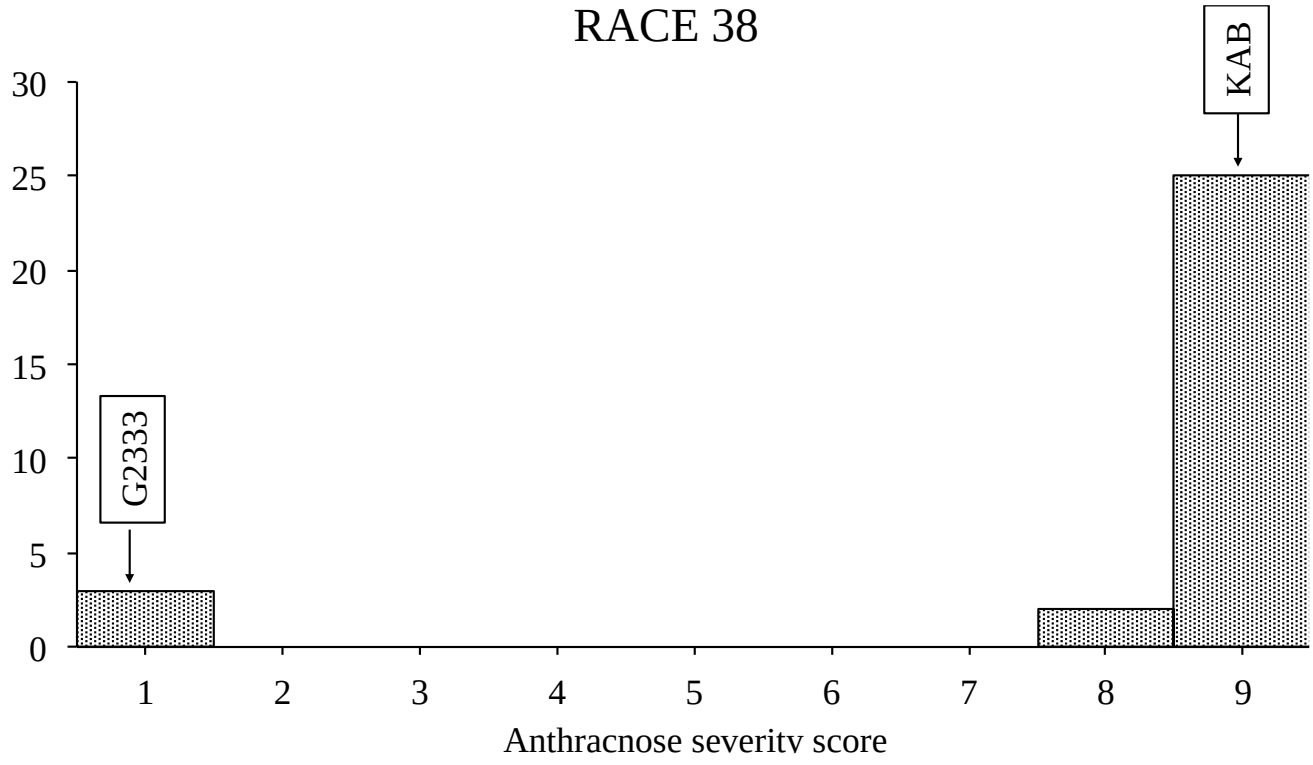

Figure 4. Frequency distribution of anthracnose severity scores for the 30 yellow genotypes and checks inoculated with race 39 of *C. lindemuthianum* at University of Zambia.

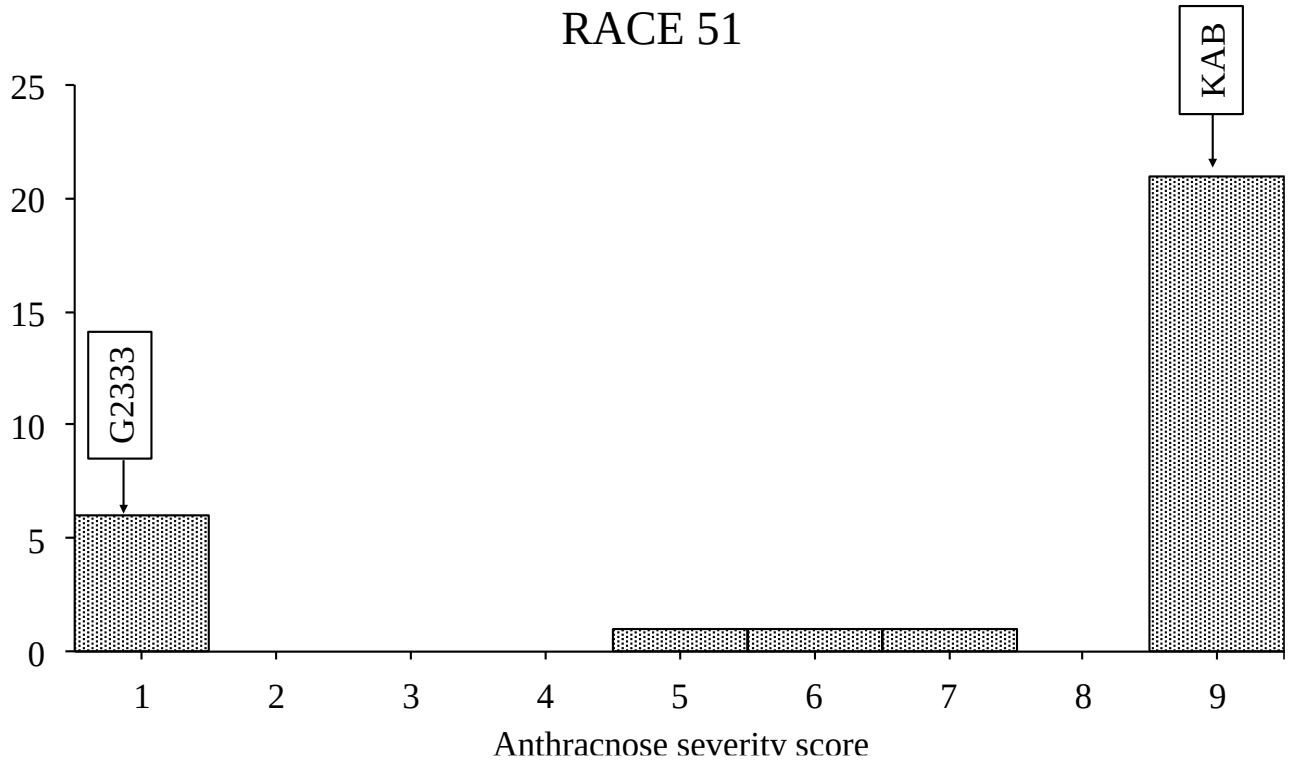

Figure 5. Frequency distribution of anthracnose severity scores for the 30 yellow genotypes and checks inoculated with race 51 of *C. lindemuthianum* at University of Zambia.

04, Y1612-05, and Y1612-06) were resistant to race 1050 while the other genotypes were susceptible (Figure 7). For race 1105, eight genotypes (Y1608-14, Y1609-09, Y1612-01, Y1612-02, Y1612-03, Y1612-04, Y1612-05, and Y1612-06) were resistant to race 1105 while the other genotypes were susceptible (Figure 8).

DISCUSSION

Manteca yellow beans are a major market class of beans that is popular in East and Southern Africa (Farrow and Muthoni-Andriatsitohaina, 2020). Anthracnose is a major constraint of yellow bean productivity in Africa. This study was aimed at evaluating 30 Manteca yellow bean genotypes for resistance to eight diverse races of *C. lindemuthianum*. Of the 30 yellow-seeded genotypes evaluated, 11 were susceptible to all eight races. The other 19 were resistant or moderately resistant to at least one race. Race 19 is particularly important in Zambia because of its wide geographic spread across bean producing regions in Zambia (Mungalu et al., 2020; Sansala et al., 2023). It is, therefore, important to identify yellow bean genotypes that can be used as sources of resistance to race 19. Among the 30 genotypes evaluated, five genotypes were highly resistant to race 19. In fact the resistance levels of these five genotypes were similar to that of the resistant check G2333, which carries resistance genes *Co-4*^{2} and *Co-5*. This result is encouraging as the five genotypes can be used as sources of resistance to race 19.

Among the 30 genotypes, Y1612-04 exhibited broad-spectrum resistance to *C. lindemuthianum*. Notably, it displayed high resistance to the most prevalent race in Zambia (race 19), as well as to the two highly virulent races (1050 and 1105) utilized in this study. Additionally, Y1612-04 demonstrated high resistance to the less virulent race 5 and moderate resistance to race 51. This genotype should be prioritized as a source of ANTH resistance by breeding programs aiming to enhance ANTH resistance within the yellow market class. Introgressing ANTH resistance from other market classes has proven challenging due to the low recovery of yellow seed color in progeny. Therefore, the identification of Y1612-04 as resistant is significant because it possesses a yellow seed color, thus overcoming seed color challenges in progeny crosses with other yellow genotypes. Additionally, conducting genetic studies to elucidate the genetic basis of Y1612-04's broad-spectrum resistance to *C. lindemuthianum* is crucial. However, it's important to note that Y1612-04 was susceptible to races 38 and 39. PIC 86, which was susceptible to all seven races to which Y1612-04 was resistant, displayed resistance to races 38 and 39. This underscores the challenges of breeding for

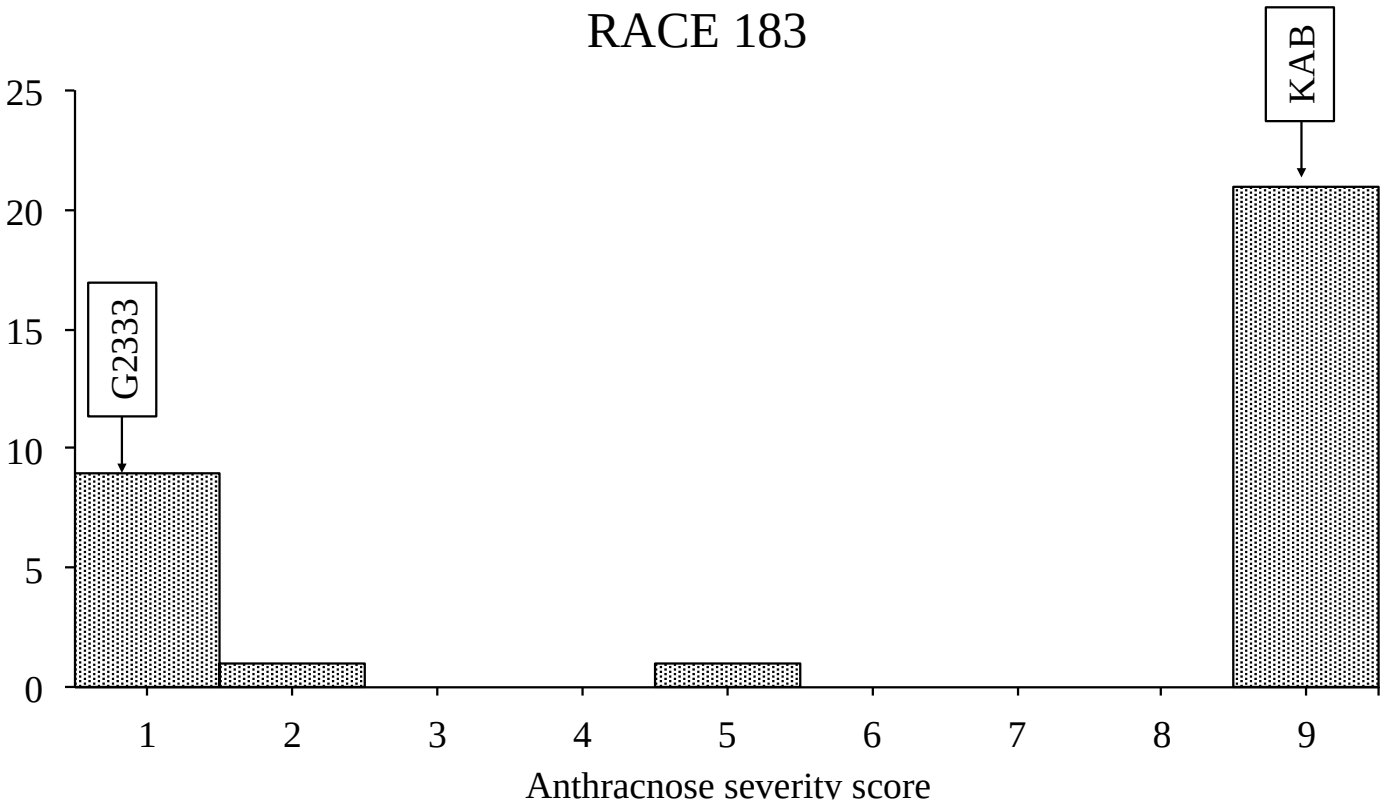

Figure 6. Frequency distribution of anthracnose severity scores for the 30 yellow genotypes and checks inoculated with race 183 of *C. lindemuthianum* at University of Zambia

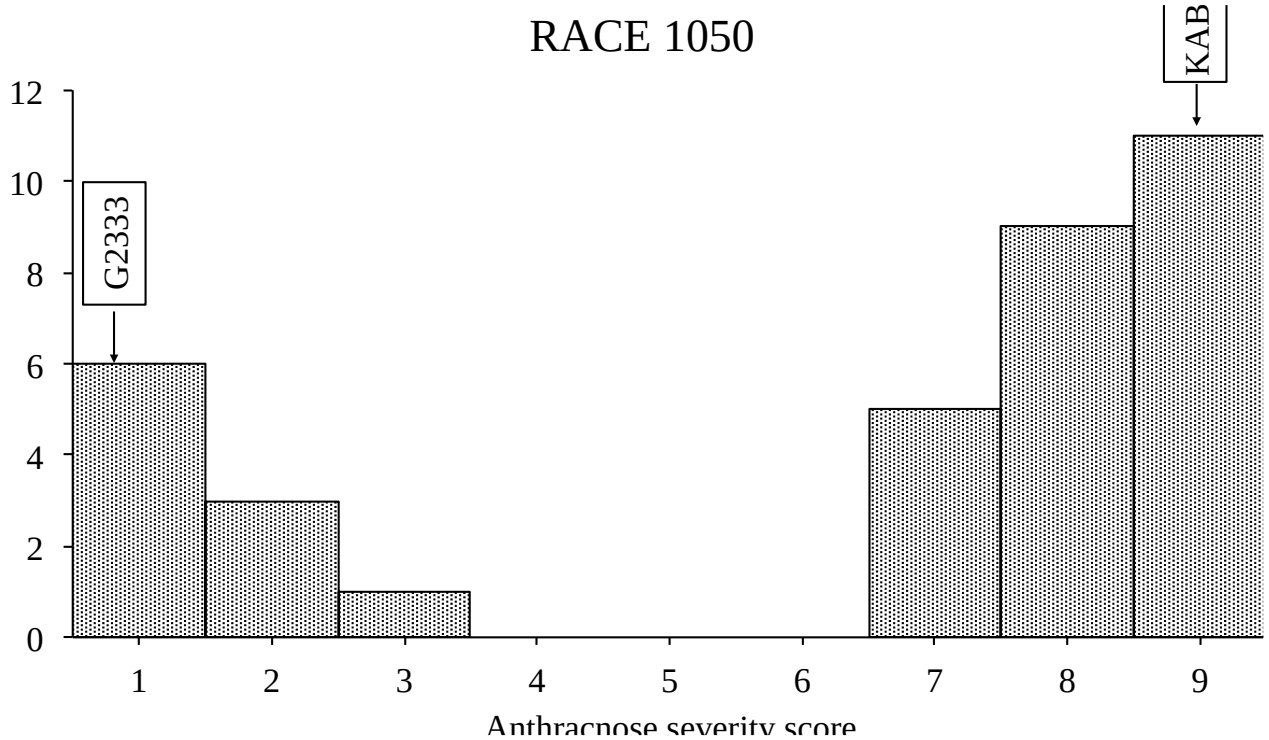

Figure 7. Frequency distribution of anthracnose severity scores for the 30 yellow genotypes and checks inoculated with race 1050 of *C. lindemuthianum* at University of Zambia

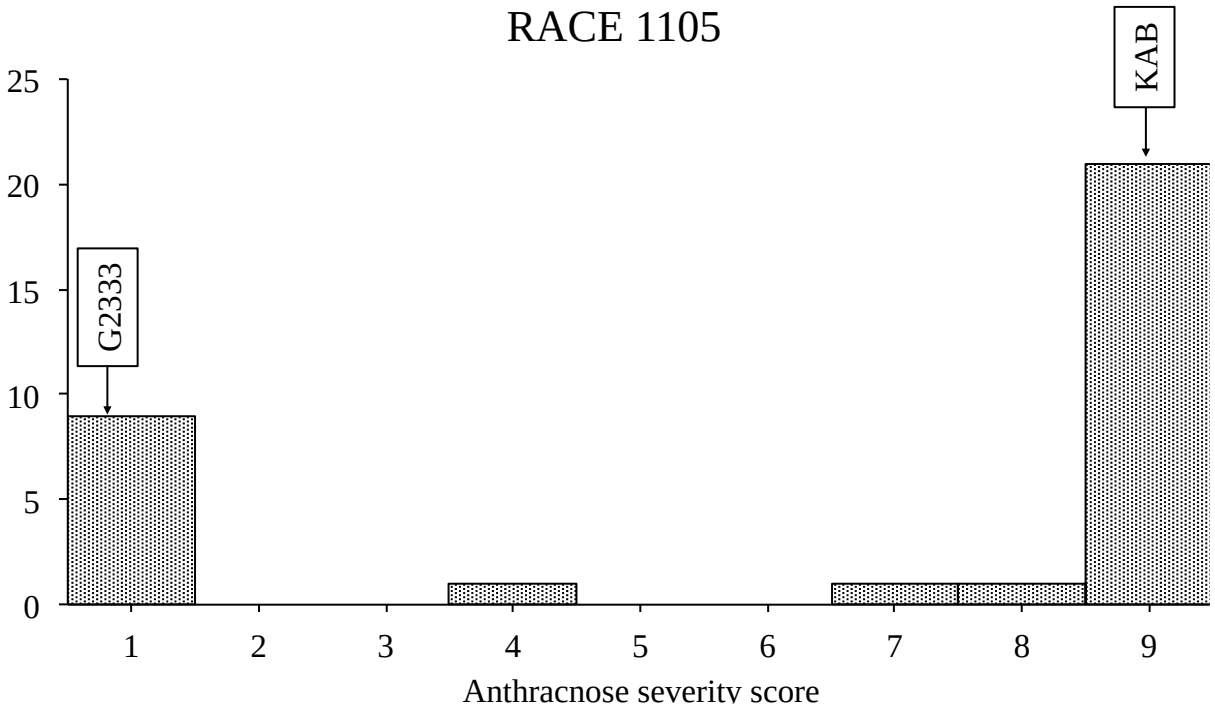

Figure 8. Frequency distribution of anthracnose severity scores for the 30 yellow genotypes and checks inoculated with race 1105 of *C. lindemuthianum* at University of Zambia.

anthracnose resistance, as no single genotype is resistant to all races of *C. lindemuthianum*, given that resistance follows a gene-for-gene resistance model (Flor, 1955), where host plant resistance genes confer resistance to specific races of *C. lindemuthianum* (Mungalu et al., 2020). The frequency distribution of anthracnose severity scores for the 30 yellow genotypes to all eight races used in this study followed a bimodal distribution. This pattern suggests that a single or a few major-effect genes could have been the basis of the observed resistance for some of the 30 genotypes to the eight races of *C. lindemuthianum*. This finding aligns with previous studies that have reported major-effect loci as the basis of resistance to these eight races, albeit in different genetic backgrounds and market classes from those used in this study (Mungalu et al., 2020; Kuwabo et al., 2023).

Conclusion

A total of 30 yellow bean genotypes were assessed for resistance to eight races of *C. lindemuthianum*. The frequency distribution of anthracnose severity scores for these genotypes across all eight races exhibited a bimodal distribution, suggesting that the observed resistance to ANTH among the 30 genotypes may be controlled by a single or few major-effect genes. This study has identified yellow bean genotypes with Manteca seed color that are resistant to eight races of *C. lindemuthianum* found in Zambia, including race 19, which is widely distributed in Zambia, and races 1050 and 1105, known for their high virulence. Among these genotypes, Y1612-04 stood out as it exhibited resistance to seven of the nine races used in this study. This genotype holds promise as a source of resistance to enhance the Manteca yellow bean market class, which is popular in East and Southern Africa.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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