Evaluation of 20 enset (Ensete ventricosum) landraces for response to Xanthomonas vasicola pv. Musacearum infection

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Abstract Xanthomonas wilt, caused by Xanthomonas vasicola pv. musacearum (Xvm), formerly X. campestris pv. musacearum, is the most threatening and economically important disease of enset (Ensete ventricosum), the multipurpose food security crop orphan in south and southwestern Ethiopia. Xvm has also had a major impact on banana and plantain production in east Africa following its detection in Uganda in 2001 and subsequent spread. The only current effective control of this disease relies on integrated disease management strategies including minimizing field pathogen inoculum and deployment of wilt-resistant enset landraces. Identifying landraces with stable and durable Xvm resistance will greatly accelerate breeding programmes. While previous reports have identified landraces with varying degrees of tolerance to Xvm, no systematic study has collectively assessed their relative resistance. Here we undertook a detailed “common garden” analysis of 20 enset landraces previously reported to exhibit lower susceptibility to Xvm using an aggressive Xvm inoculum isolated from a disease hotspot area. Detailed longitudinal and survival analyses were applied to each landrace, using a combination of area-under-disease progress stairs, disease index and apparent infection rate to capture disease metrics as well as disease progression symptoms. Considerable variation was observed among the 20 landraces; however, none exhibited full immunity to Xvm infection. Landraces Haella, Mazia and Lemat showed the lowest susceptibility to Xvm as evidenced by reduced disease units and higher survival rates compared to the susceptible control landrace Arkiya, which exhibited the highest infection level and lowest survival rate, consistent with a high degree of susceptibility to Xvm. Thus, we have in this controlled experiment identified new material suitable for incorporation into future breeding programmes to develop Xvm-resistant enset varieties.

Keywords Enset landrace · Xanthomonas wilt · Resistance · Susceptibility

Introduction

Enset (Ensete ventricosum (Welw.) Cheesman) is a diploid (2n = 18), herbaceous, perennial monocarpic crop belonging to the family Musaceae. Enset is often
Enset is tolerant to prolonged drought and provides a year-round source of staple nutritious food. Enset’s long history of cultivation across diverse ethnic groups is reflected in its significant cultural and socio-economic value in Ethiopia (Shigeta, 1990; Olango et al., 2014) and today it is widely cultivated across south and southwestern Ethiopia enhancing food security for more than 20 million Ethiopians (Yesuf & Hunduma, 2012; Yemataw et al., 2017a; Borrell et al., 2019). For farmers, enset is more than a year-round staple food. It provides multiple additional daily benefits yet requires little management input. Multipurpose benefits are derived from different enset landraces such as those suited for animal feed and bedding, fibre or packaging, construction material or as a medicinal source (Brandt et al., 1997; Nurfeta et al., 2008; Yemataw et al., 2017b).

Xanthomonas wilt disease caused by Xanthomonas vasicola pv. musacearum (Xvm) (Ashagari, 1981, 1985; Archido & Tezera, 1993; Tessera et al., 2008; Yesuf & Hunduma, 2012) poses a significant threat to enset production. Enset Xanthomonas wilt was first reported in Kaffa district of Ethiopia in the 1960’s (Yirgou & Bradbury, 1968) and was subsequently identified on banana (Yirgou & Bradbury, 1974), although the first observations likely date back to 1930s (Castellani 1939). The causative agent was previously known as Xanthomonas musacearum and X. campestris pv. musacearum, but following recent taxonomic re-evaluation was renamed Xanthomonas vasicola (Studholme et al., 2020). Currently, the disease is distributed in all enset growing areas of southern and southwestern Ethiopia, where it has a continuing devastating impact on enset production (Blomme et al., 2017; and Nakato et al., 2018).

Enset has been cultivated for millennia in the different agro-ecologies of south and southwestern Ethiopia by multiple ethno-linguistic communities that have selected and adapted diverse landraces with agronomically important characteristics resulting in a complex landrace structure (Yemataw et al., 2014a; Yemataw et al., 2016). Such selection is a key factor in enset cultivation and a typical enset farm comprises multiple landraces, including those exhibiting resistance/tolerance to Xvm.

Enset landraces in Ethiopia might offer enhanced resistance to Xvm, thus potentially offering a huge genetic resource to farmers and ultimately banana growers. Yet to date, no systematic patho-testing for responses to Xvm has yet been undertaken. This is partly due to the challenges associated with enset’s longevity and requirement for clonal propagation. Farmers often attest that certain enset landraces show relatively low levels of infection to Xvm and often these are incorporated into their backyard landrace mixture as one option for disease management (Ashagari, 1985; Yemataw et al., 2016). Despite this, identification of resistant landraces has been challenging. Enset landraces with lower but varying susceptibility to Xvm have been reported. However, inconsistency in response to Xvm has been observed (Ashagari, 1985; Handoro & Michael, 2007; Welde-Michael et al., 2008; Tessera et al., 2008; Haile et al., 2014, 2020; Hunduma et al., 2015; Wolde et al., 2016; Handoro & Said, 2016) have been reported. While such approaches in Ethiopia would necessitate adoption and acceptance of genetic modification technologies, the availability of enset germplasm resistant to Xvm could both facilitate enset breeding and simultaneously provide new genetic tools for improvement of banana, including application of gene editing (Tripathi et al., 2021), which is more acceptable than traditional GMO technologies.

To date, no systematic, collective screening of the core enset landraces reported to support low levels of Xvm infection has been undertaken. This is largely due to the lengthy timeframes involved in propagating and testing these landraces and access to necessary field sites and resources. Such a study would greatly assist breeding/selection efforts to identify elite landraces exhibiting enhanced Xvm resistance. To address this, we have independently rescreened and comprehensively evaluated selected enset landraces previously reported to have reduced susceptibility to Xvm, identifying specific enset landraces with potential to contribute towards future sustainable management of the disease. We used a common garden experiment at a single site to minimize environmental variability inherent when
comparing the previous studies where we undertook a detailed study of infection progression and ranked enset landraces with significantly enhanced tolerance to Xvm, thus prioritizing key germplasm to be deployed in future breeding programmes.

Materials and methods

Description of the study site

Evaluation of enset landraces for resistance to Xanthomonas vasicola pv. musacearum (Xvm) was undertaken in an open-field pot experiment at Southern Agricultural Research Institute (SARI), Hawassa, Ethiopia. The site is situated 7°4′ N and 38°3′ E with an elevation of 1700 m above sea level. It has an annual rainfall of 1100 mm, with annual average, minimum, and maximum temperatures 20.6 °C, 13.5 °C and 27.6 °C, respectively.

Plant material acquisition and multiplication

We sourced 18 enset landraces previously reported to exhibit low level infection or having contrasting infection phenotypes following Xvm inoculation. In addition, landrace ‘Arkiya’, was included as it was previously reported to support a high level of Xvm growth (Handoro & Michael, 2007), and as a precaution landrace ‘Bota Arkiya’ was included as it has a similar name to landrace ‘Arkiya’ though of different origin. The total 20 landraces are detailed in Table 1.

Nineteen enset landraces were sourced from enset farmers in six enset growing zones of southern Ethiopia (Fig. 1). Prior to collection, farmers in the regions where the landraces reported to have originated were interviewed. Following this a rapid on-site appraisal was conducted to avoid possible misidentification before clonal material for replicated pathotesting was collected. Underground corms of two-to-three-year-old mother plants from each of the 20 landraces (the 19 collected plus landrace Mazia) were first macro-propagated to produce true-to-type suckers. Mother corms of Mazia were kindly provided by Areka Agricultural Research Center (AARC), Areka, Ethiopia.

Following collection, enset landraces were propagated conventionally at AARC in June 2016 using an enset macro-propagation method as previously described (Yeshitila et al. 2009). Briefly, the mother corm was dissected into two equal halves and the apical tissue removed from the centre of each half corm to allow secondary meristems to develop into suckers for planting. These dissected corms are dried in the shade for 3 h before planting. Each half corm was then planted in a slanted orientation in holes of 1 m × 1 m and covered with topsoil (Fig. 1b and c).

After 12 months, multiplied enset suckers were uprooted and transplanted into 5 L capacity plastic pots filled with a sun-dried mixture of soil: sand: manure at a ratio of 3:1:1 and placed in an open field experimental site at SARI. Suckers were allowed to establish for two months to get sufficient leaves (>3) to allow inoculation with Xvm. During this establishment period, suckers in plastic pots were watered daily, both prior to inoculation with Xvm and for the subsequent two months after Xvm inoculation. Watering thereafter was reduced to twice per week for the remaining experimental period.

Preparation of bacterial suspension for inoculation

Virulent Xvm was collected from a disease hot spot in the Hagereselam district, Sidama region, southern Ethiopia, ensuring it was geographically distinct from any landrace sampling sites. Xvm bacterial ooze from young leaves and/or pseudostem of diseased enset plants was harvested into sterile distilled water and preserved at 4 °C until use. Two sets of bacterial suspensions were tested for hypersensitivity and pathogenicity responses. One inoculum comprised the uncultured bacterial suspension. The other was derived from day-old field harvested Xvm isolates streaked on YPSA (5 g yeast extract, 10 g peptone, 20 g sucrose and 15 g agar per litre) a commonly used growth and isolation medium for selecting pure Xvm colonies (Haile et al., 2014). These plates were incubated at 28 °C for 24 h (Schaad & Stall, 1998). Single colonies with a yellow, convex, mucoid morphology typical of Xvm were harvested and preserved on YPSA slants at 4 °C.

Hypersensitivity and pathogenicity tests

Both uncultured and cultured suspensions were tested for hypersensitivity and pathogenicity on two-month-old tobacco (Nicotiana tabacum) or the highly susceptible control enset landrace ‘Arkiya’. Hypersensitivity
tests were initially conducted on *N. tabacum* using 2 mL of a bacterial suspension adjusted to OD$_{600}$ = 0.5 (~$1.0 \times 10^8$ colony forming units (cfu) per mL). A positive hypersensitive response was scored if tissue exhibited clearing chlorosis zone around the point of injection.

For initial assessment of pathogenicity tests, 14-month-old (i.e., 2 months after transplanting) disease-free enset suckers of the susceptible landrace ‘Arkiya’ (Handoro & Michael, 2007) were infected with 4 mL of bacterial suspension (~$10^8$ cfu/mL at OD$_{600}$ = 0.5) from uncultured and cultured inocula, infiltrating into adaxial or abaxial petiole of the youngest open leaf using a 5 mL syringe fitted with 28-gauge needle.

Experimental design and inoculation of *Xvm* to test landraces

Preliminary testing showed that the uncultured bacterial suspension gave both faster and more severe symptoms on both *N. tabacum* (hypersensitive response) and enset landrace Arkiya (susceptible) during the pathogenicity
tests. Thus field harvested, uncultured Xvm suspensions preserved at 4 °C were used as inoculum for landrace testing. As above, suckers of 14-month-old enset landraces (two months after transplanting to pots) were inoculated with a 4 mL aliquot of the bacterial suspension (~10^8 cfu/mL) using a sterile hypodermic syringe fitted with 28-gauge needle, infiltrating the youngest innermost leaf petiole (Fig. 2). Control plants were infiltrated with 4 mL of sterile distilled water.

This pot experiment was organized in four replicates of 20 landraces. Each replicate consisting of 10 plants and landraces within each replication was laid out in randomized complete block design (RCBD). The entire experiment comprised 800 plants in total, including 30 individuals of the 20 enset landraces inoculated with uncultured Xvm suspension and a further 10 individuals of the 20 landraces inoculated with sterile distilled water (negative controls).

Data collection and analysis

Data recording and quantification of disease progression

Data were collected a week after inoculation then subsequently at two-week intervals for 155 days. To evaluate key phenotypes induced by Xvm infection we captured all visible symptoms in each of the 800 landraces both descriptively and digitally. Interestingly, during the first two weeks post-inoculation, some enset landraces showed unusual phenotypes and symptoms, include twisting of the leaf blade in some landraces, rolling or curling of the leaf tip and leaf edges in others. From the third week onward following Xvm innoculation, typical wilting symptoms were observed. This included severe drooping of the top 25% of the challenged leaf blade, collapsing of the challenged leaf blade, wilting of both inoculated and adjacent uninoculated leaves, chlorosis across the majority of leaves and, in certain landraces, complete death. At each evaluation point, the number of leaves per plant that showed these consistent wilting symptoms and total number of
asymptomatic leaves per plant were counted. Symptoms from the first two-week period were excluded from analysis due to inconsistent initial phenotypes described above.

Four response variables were used to quantify disease phenotypes: disease index (DI), area under disease progress stairs (AUDPS), apparent infection rate (AIR) and survival. DI is the percentage of symptomatic leaves per individual at each evaluation period averaged across the 4 replicates (S Chandry, 2017). DI quantifies disease symptoms over the evaluation period using a scale of 0–4, with 1 and 4 corresponding to 25% and 100% of total wilted leaves per plant respectively.

\[
\text{Disease Index (DI)} = \frac{w}{t} \times 4
\]

where, \(w\) is the number of symptomatic leaves and \(t\) is the number of total leaves of a single plant. AUDPS (Simko & Piepho, 2012) estimates disease accumulation and progress over time and is considered to provide better estimates of disease by giving a weight closer to the optimal than that derived from AUDPC assessments (Madden et al., 2007). AIR, corresponding to the speed at which an epidemic develops (Meena et al., 2011), was calculated as the slope of disease index development.

Finally, survival analysis was applied to study the fraction of survivors per time-point among enset landraces challenged with Xvm. Survival data was generated according to Schandry (2017) with a customized DI cut-off point of 2.23. This cut-off DI was used for comparative purposes and is the maximum DI value in the current study for Mazia, a landrace frequently cited (Table 1) for its resistance/tolerance to Xvm. For survival analysis, we assumed infection scores beyond this cut-off level which are equivalent to 55.75% symptomatic leaves which would have lead to death or non-recovery of the landrace.

\[
\text{Area Under Disease Progress Curve (AUDPC)} = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)
\]

\[
\text{Area Under Disease Progress Stairs (AUDPS)} = \text{AUDPC} + \left[ \frac{y_1 + y_n}{2} \times \frac{D}{n-1} \right]
\]

where, \(y_i\) is disease index at the \(i^{th}\) observation, \(t_i\) is time in days at the \(i^{th}\) observation, \(n\) is the number of total observations, and \(D\) is duration from the first to the last observation (\(D = t_n - t_1\)).

**Data analysis and visualization**

The following R packages were used for data manipulation and formatting: *broom* (Robinson et al., 2015), *dplyr* (Wickham et al., 2021), *magrittr* (Bache and Wickham, 2020), *stringr* (Wickham, 2016), *stargazer* (Marek, 2018), *tidyverse* (Wickham et al., 2019) and *lme4* (Bates et al., 2015) to appropriately account for...
differential disease development in enset landraces over time. The linear model (lm) function from the base stats package was employed. For model comparison in the disease units and survival analysis, Akaike information criterion (AIC) and Schwarz’s Bayesian information criterion (BIC) were used to identify the best model among the alternatives. Models with lower AIC and BIC values were used for analysis. Functions from lsmeans (Lenth, 2016) and lmerTest (Kuznetsova et al., 2016) were used to extract means from data fitted to linear and linear mixed models respectively, applying the process “data manipulation for result visualization”. Graphic visualizations used ggplot2 (Wickham, 2016).

Functions from the following packages were used for analysis: MESS (Ekstrøm, 2020), survival (Therneau & Lumley, 2011), (Schröder et al., 2011), (Harrell Jr, 2016), coxme (Therneau, 2020), (Bates et al., 2015), lmerTest (Kuznetsova et al., 2016), multcomp (Hothorn et al., 2008). Packages rmarkdown (Allaire et al., 2021) and knitr (Xie, 2021) were used to format reports from R Markdown (.rmd) files. Recorded disease data and R scripts used in data manipulation, analysis and visualization are available in Rmarkdown and HTML format (supplementary file 1–3), which contains a full description of data management and analysis.

Generalized linear hypothesis testing, adjusted for multiple comparisons using Tukey’s method (Hothorn et al., 2008), was used to assess statistically significant differences at a \( p \) value cut-off of 0.05. Statistical significance among treatments was determined using the “compact letter displays (cld)” method in multcomp (Hothorn et al., 2008) package for graphic visualization. Treatments within the same “letter group” are not significantly different whereas treatments with different letters display a significant difference.

Results

Screening of 20 enset landraces against Xanthomonas vasicola pv. musacearum (Xvm)

Hypersensitivity and pathogenicity tests

A typical hypersensitive response (HR) was observed on \( N. \) tabacum leaves from both of uncultured and cultured Xvm suspensions. However, the uncultured Xvm suspension caused a severe HR on \( N. \) tabacum within three days after inoculation (DAI) whereas symptoms were delayed for up to eight days with the cultured Xvm inoculum (Data not shown). Similarly, the susceptible “control” landrace Arkia also developed stronger symptoms with uncultured inoculum. It took just 21 DAI with uncultured Xvm suspension to cause wilting and complete collapse of susceptible Arkia, whereas it took 30 to 45 DAI for the cultured suspension to cause the same disease symptoms, indicating culturing induced a loss of virulence.

Symptom description

A range of symptoms were observed during the course of infection and subsequent disease development on Xvm-challenged enset landraces. Necrosis around the point of inoculation and surrounding tissues was observed 3 DAI in most landraces (Fig. 3). At early stages of infection, up to 15 DAI, landraces showed varying and inconsistent symptoms, including twisting and slight leaf curling, and drooping of the blade and tip of the inoculated leaf. The leaf laminar surrounding the Xvm inoculated area became deformed, twisted, or curved inwards. By 15 DAI these symptoms severe curling of the leaf edge, drooping and folding back of leaf blade was consistently observed in all landraces. Gradually, drooping from the leaf apex and folding back or collapsing of leaves became the most prominent symptoms as the disease developed. All tested enset landraces showed one or more these symptoms.

On severely infected enset landraces such as Dirbo and the susceptible control Arkia, symptoms extended to leaf chlorosis, initiating from the apex and, then leading to gradual collapsing with clear wilting of the inoculated leaf and symptoms spreading to other leaves. Eventually all leaves wilted, with subsequent death and rotting of the whole plant (Fig. 4a). However, on landraces that showed mild infection symptoms, for example Lemat or the resistant/tolerant control, Mazia, the inoculated leaf collapsed then dried or at worse the symptoms extended to just a few adjacent leaves and the entire plant remained healthy (Fig. 4b). At no stage were classical HR or HR-like symptoms observed in any tested enset landraces.
Area-under-disease progress stairs (AUDPS)

Plotting the area-under-disease progress stairs revealed more-or-less distinct curves and disease progression for the tested 20 enset landraces (Fig. 5a). Analysis of AUDPS also showed a spectrum of significant differences ($p < 0.05$) among landraces with landrace Arkiya having the highest and Haella the lowest AUDPS value (Fig. 5b). In addition, the analysis revealed that AUDPS score seems to group landraces into clusters that accumulate lower, intermediate, or higher disease load as the disease progress, with Haela and Arkiya showing the lowest and highest value, respectively.

Disease index and apparent infection rate

Disease severity expressed as unit of DI illustrated the progress of disease development of Xvm infection in the 20 enset landraces. AIR calculates the rate of disease index development as a function of slope at each disease evaluation period (See Material and Methods). Analysis of variance of the DI and apparent infection rate (AIR) showed marked statistical differences ($P < 0.05$) among landraces (Supplementary file 4).

Mean disease index value ranges from 0.65, 0.80, 0.81 in Haella, Mazia, Lemat, corresponding to 16.25%, 20.00%, 20.25% severity up to 1.98 (49.50%), 2.05 (51.25%), and 2.59 (64.75%) in Yesha, Godere and the susceptible control Arkiya, respectively (Table 2). As evidenced from the lowest AIR, disease development rate was slowest in landrace Lemat, and fastest in Genticha, Abatemerza, Nechuwe, Gefetano and Godere. Generally, AIR did not show a direct linear relationship with disease index suggesting that AIR varies among enset landraces irrespective of disease severity of landraces as measured by disease index.

![Fig. 3 Xvm infection symptoms during the early stage of disease development on enset landraces. Labels are placed near to Xvm infected leaves indicated by asterisk. a Curling of inoculated leaf, b Twisting of inoculated leaf, c Drooping of leaf apex of inoculated and other leaves and d Folding around point of inoculation](image-url)
Survival analysis

To ascertain whether strong symptoms were associated with reduced survival, a DI of ≥2.23 was used as a time-to-event cut-off point for survival analysis. This cut-off DI represents the peak of infection level of the resistant/tolerant control enset landrace “Mazia”. The survival data generated with the survfit function of the survival package against landraces best fitted to a Gaussian distribution that showed the lower AIC (19,176.7) and BIC (19,317.4) values (Supplementary file 5) among Weibull, Logistic and Lognormal distributions. The Gaussian distribution fitted survival data was then used to generate Kaplan-Meier estimates of survival (Fig. 6). The linear fit of survival analysis showed non-proportionality of hazard ratio, but as an alternative option, data fitted to a mixed effects Cox model for subsequent comparison of estimates (Table 3 and Supplementary file 1–3 for details).

Only those enset landraces that exhibited mild or severe infection to Xvm showed significant estimates of hazard ratio (HR) and Gaussian distribution (Table 3). Lemat, Haella and Mazia respectively had estimated hazard ratios of 0.230, 0.272 and 0.294, indicative of a mild infection to Xvm. This result indicates that for landraces Lemat, Haella and Mazia there is, respectively, a 77.0%, 72.8%, 70.6% decreased risk of getting severely infected with Xvm up to greater than the cut-off DI value of ≥2.23. The susceptible control, Arkiya, showed an estimated hazard ratio of 1.866 indicative of an 86.6% higher risk of showing a DI value ≥2.23 after infected with Xvm.

Fig. 4 Comparison of Xvm disease symptoms on enset landraces. Development of Xvm disease symptoms on the susceptible enset landrace Dirbo (a) contrasted to the resistant/tolerant landrace Mazia (b). *DAI = Days after inoculation
Survival estimates of the 12 enset landraces that fitted a Gaussian distribution reinforce that a higher proportion of plants survive from landraces that showed mild infection than landraces with severe infection (see peak disease index) during the course of disease development (Fig. 6). For example, 95 DAI 50% of landrace Arkiya was estimated to be severely infected whereas for the highest ranked tolerant landraces, Lemat, Haella and Mazia, this was less than 7%.

**Discussion**

Xanthomonas wilt disease caused by Xvm is a major bottleneck to enset (*Ensete ventricosum*) cultivation in south and southwestern Ethiopia, where diverse enset landraces and wild types of the crop are found. Today, it also represents a major threat to banana cultivation in east Africa (Ocimati et al., 2019). Farmers traditionally adopted host plant resistance in enset as a management option for this Xanthomonas wilt disease by incorporating certain enset landraces they perceived to be more resistant/tolerant to Xvm (Ashagari, 1985; Archido & Tezera, 1993; Handoro & Michael, 2007; Welde-Michael et al., 2008; Haile et al., 2014; Hunduma et al., 2015; Wolde et al., 2016; Handoro & Said, 2016), to date there has been no systematic comparison of enset landraces’ responses to Xvm infection. Here we have undertaken a common garden experiment using an aggressive Xvm inoculum to conduct a detailed assessment of disease development on enset landraces previously reported for their low susceptibility to Xvm. This has provided the most definitive insight today into Xvm disease phenotypes on enset landraces, and thus informs both future objectives of identifying the underlying component(s) of Xvm resistance and germplasm to incorporate into enset breeding programmes. Early infection symptoms calculated on the percentage of symptomatic leaves at each evaluation stage were determined and combined with various parameters such as area under disease progress stairs, disease index, apparent infection rate and survival rate into a detailed statistical analysis.

Comparison of Xvm infection results between different studies has been previously confounded by the inevitable variability between batches of freshly collected inoculum. Conversely, a stock of “standard” Xvm inoculum may be
perceived as a solution (though this has never been implemented in practise), long-term storage and reculturing of isolates often leads to loss of virulence (Ansari & Butt, 2011). This would have a major impact on such long-term experiments as these, where parental material must be first clonally propagated and replicated plants established. Given the constraints of available infrastructure in the geographic region of this study, the only practical approach was to use freshly harvested inoculum from already infected plants (derived directly from bacterial ooze of cut leaves). Indeed, preliminary experiments showed that culturing Xvm from fresh inoculum resulted in some attenuation of virulence, based on both disease symptoms on landrace Arkiya and HR in tobacco. Many previous enset screens have used Xvm isolates from disease hot-spot areas in the southern Ethiopia districts of Hagereselam where the tolerant/resistant landrace Mazia showed greatest disease symptoms to Xvm inoculum compared to isolates from four other districts (Handoro & Michael, 2007). Clearly, further comprehensive studies on classical and molecular epidemiology on Xvm strains sourced geographically distinct enset-growing areas in Ethiopia are required and a major study to address this is currently underway. However, even if regional variation in virulence was observed, it would still not be possible to definitively associate subtle differences in tolerance to local adaptation. Consequently, based on preliminary studies (as well as the initial in field studies underpinning the selection of the 19 landraces) the choice to use Xvm inoculum derived from a single uniform batch of uncultured Xvm suspensions to simultaneously inoculate all plants was the most logical approach to eliminate such batch effects.

Given the nature of the Xvm infection, it is important to understand the temporal and spatial development of symptoms on enset from initial visible phenotypes to the actual wilting and subsequent death, rather than simply conduct an endpoint analysis and simultaneously determine how different closely related genotypes respond to the same Xvm challenge. In this study we showed that during early stages of Xvm infection on enset landraces (up to 15 DAI), varying transient symptoms were observed, including twisting and leaf curling, and drooping of the leaf blade and apex. Symptoms associated with wilting, folding or collapsing of the leaf blade, severe drooping from leaf apex and wilting, tended to appear from week 3 following Xvm infection. Thus, for consistent, robust scoring, analyses were restricted to disease data gathered from the third week onwards.

This study revealed HR-like symptoms in a limited number of Xvm-challenged enset landraces, reminiscent of that seen in classical resistance-gene mediated HR (Balint-Kurti, 2019). This raises the possibility that traditional disease resistance genes may contribute to the enset-Xvm interaction, warranting further investigation. Involvement of HR cannot be excluded even in those landraces that do not show HR-like symptoms as local resistance-gene-mediated reactions can also induce wilting like phenotypes (Jakobek & Lindgren, 1993; Chasan, 1994; Pajerowska-Mukhtar & Dong, 2009). All the 20 tested landraces showed at least partial symptoms of Xvm infection at different infection stages whereas mock infected plants were asymptomatic. Bacterial multiplication was not assessed at each disease

Table 2  Mean of disease index and mean apparent infection rates (AIR) and standard errors of Xvm on the 20 enset landraces

| Landraces | Disease index | Apparent infection rate (AIR) |
|-----------|---------------|------------------------------|
| Haella    | 0.65±0.06 a*  | 1.3×10^{-2}±0.002 ac**       |
| Mazia     | 0.80±0.06 ab  | 1.4×10^{-2}±0.002 acd        |
| Lemat     | 0.81±0.05 ab  | 0.7×10^{-2}±0.002 a          |
| Kuro      | 1.00±0.06 ac  | 1.7×10^{-2}±0.002 bce        |
| Gezewet   | 1.04±0.07 acd | 1.6×10^{-2}±0.002 bce        |
| Bedediet  | 1.05±0.06 acd | 1.5×10^{-2}±0.002 ae         |
| Alagena   | 1.14±0.06 bed | 1.5×10^{-2}±0.002 ae         |
| Ado       | 1.32±0.08 ce  | 1.9×10^{-2}±0.002 bce        |
| Bota Arkia| 1.44±0.06 def | 1.3×10^{-2}±0.002 ab          |
| Gefetano  | 1.56±0.08 ef  | 2.2×10^{-2}±0.002 def        |
| Dirbo     | 1.64±0.08 efg | 1.4×10^{-2}±0.002 ae         |
| Nechuwe   | 1.69±0.09 eh  | 2.2×10^{-2}±0.002 ef         |
| Unjame    | 1.73±0.07 fh  | 1.6×10^{-2}±0.002 bce        |
| Ginbuwe   | 1.76±0.06 fh  | 1.6×10^{-2}±0.002 bce        |
| Abatemerza| 1.77±0.08 fh  | 2.1×10^{-2}±0.002 cef         |
| Wachiso   | 1.80±0.08 fh  | 1.6×10^{-2}±0.002 bce        |
| Genticha  | 1.85±0.07 fh  | 2.0×10^{-2}±0.002 bee         |
| Yesha     | 1.98±0.07 gh  | 1.8×10^{-2}±0.002 bee         |
| Godere    | 2.05±0.09 h   | 2.8×10^{-2}±0.002 f           |
| Arkiya    | 2.59±0.06 i   | 1.5×10^{-2}±0.002 ae          |

* and ** are compact letter display of the result of multiple comparison tests using of means of disease index and apparent infection rate using Tukey’s method p < 0.05. Enset landraces with similar letters are statistically not different from each other and vice versa.
evaluation; rather the focus was on distinguishing symptoms associated with disease progression and potential phenotypes associated with an immune response. However, it is important to note that resistant plants still display a low level of infection symptoms, as has been reported in several crops (Iglesias-Bernabé et al., 2019; Lima et al., 2017). This is consistent with most classical gene-for-gene interactions during which bacterial enumeration reveals a low level of accumulation of the pathogen until effective resistance sets in, thus likely accounting for the weak phenotypes recorded in these landraces. Previous reports that claim a lack of complete immunity to Xvm infection in enset due to the presence of some level of infection in tested landraces (Archido & Tezera, 1993; Handoro & Michael, 2007; Welde-Michael et al., 2008; Haile et al., 2014; Hunduma et al., 2015; Wolde et al., 2016; Handoro & Said, 2016) probably highlight the host challenges faced in deploying effective resistance against a mobile vascular pathogen, compared with those deploying a more classical foliar infection strategy where multiplication occurs apoplastically.

Complementing these disease metrics, survival analysis provided insight into the inherent infection risk. Inoculations leading to a cut-off disease index value of ≥2.23 (55.75% symptomatic leaves). This cut-off value was chosen as a reference for comparison purposes and was the maximum infection level recorded for landrace Mazia, which in this study showed average lower infection among other landraces. Notably, landrace Mazia has also previously reported in several studies for its consistently lower susceptibility to Xvm (Table 1).
infection. Importantly we have, based on our comprehensive analysis of symptomologies and disease data rank landraces, compiled robust evidence for deploying selected landraces into labour intensive enset breeding programmes. It is important to mention that there is a great deal of evidence of sharing the vernacular of enset landraces within and in neighbouring enset growing districts in Ethiopia (Olango et al., 2015; Yemataw et al., 2014a; Yemataw et al., 2016) that might not be pheno-
typical or genetically identical. For example, simple sequence repeat (SSR) analysis revealed that two enset landraces with identical vernaculars, ‘Gena’, from Wolaita zone and Sidama region in Southern Ethiopia were genetically distinct (Olango et al., 2015). Futhermore, genome-wide comparison of single nucleotide polymorphism (SNP) data from the landrace Mazia sourced from Dawro and Wolaita zones of Southern Ethiopia revealed different SNP profiles (Yemataw et al., 2018). Thus, caution is needed when identifying enset landraces for study as variation in the reaction of landraces with identical or similar vernaculars might not be only due to the variation in virulence of Xvm isolates or environmental factor but also due to of the presence distinct genotypes sharing the identical vernacular.

Notably, most of the landraces that showed higher and moderate tolerance/resistance to Xvm infection viz. Mazia, Haella, Lemat, Kuro, Gezewet and Bedediet originated from areas with the richest on-farm enset landrace diversity (Yemataw et al., 2014b; Yemataw et al., 2016). These areas are the Dawro (Landrace Mazia and Kurro), Kembata-Tembaro (Landrace Heturna), and Gurage (Landrace Lemat, Gezewet and Bedediet) districts of southern Ethiopia. The recent disease pressure in these districts is comparatively higher than other enset growing areas in southern Ethiopia with the exception of the Kembata Tembaro district (Sadik Muzemil per. Com.). Furthermore, these areas also reside along the belts where Xvm was initially discovered (Castellani 1939) in southern Ethiopia. Hence, we hypothesize that the co-existence of Xvm with enset might have driven combined evolution/selection of landraces with lower susceptibility to Xvm infection.

In summary, the landraces with lower susceptibility to Xvm identified in this study, Mazia, Haella and

| Enset landraces | Mean disease index | Hazard ratio (coxme) | Hazard ratio (coxme) | Gaussian distribution |
|-----------------|--------------------|----------------------|----------------------|----------------------|
|                 |                    | p value              | p value              |                      |
| Haella          | 0.65               | 0.272                | <0.0001              | <0.0001              |
| Mazia           | 0.80               | 0.294                | <0.0001              | <0.0001              |
| Lemat           | 0.81               | 0.230                | <0.0001              | <0.0001              |
| Kuro            | 1.00               | 0.441                | <0.0001              | <0.0001              |
| Bedediet        | 1.04               | 0.403                | <0.0001              | <0.0001              |
| Gezewet         | 1.04               | 0.410                | <0.0001              | <0.0001              |
| Alagena         | 1.14               | 0.435                | <0.0001              | <0.0001              |
| Ado             | 1.32               | 0.626                | 0.0024               | 0.0143               |
| Bota Arkiya     | 1.44               | 0.564                | 0.0003               | 0.002                |
| Gefetano        | 1.56               | 0.871                | 0.330                | 0.692                |
| Dirbo           | 1.64               | 1.013                | 0.930                | 0.060                |
| Nechuwe         | 1.69               | 1.102                | 0.470                | 0.249                |
| Unjame          | 1.73               | 0.966                | 0.800                | 0.331                |
| Ginbuwe         | 1.75               | 1.001                | 1.000                | 0.687                |
| Abatemenza      | 1.77               | NA                   | NA                   | NA                   |
| Wachiso         | 1.80               | 1.070                | 0.620                | 0.107                |
| Genticha        | 1.85               | 1.164                | 0.250                | 0.108                |
| Yesha           | 1.98               | 1.331                | 0.027                | 0.002                |
| Godere          | 2.05               | 1.505                | 0.0013               | 0.002                |
| Arkiya          | 2.59               | 1.866                | <0.0001              | <0.0001              |

1 mixed effects Cox model
Lemat, along with highly susceptible landraces provide the foundation for further testing in advanced experimental settings and wider environment conditions to better understand the underpinning resistance/tolerance components. In this respect high quality genome assemblies are in progress. Critically, it is important that not only the source of landraces, but also the pathogen inoculum need to be considered for future studies and resources to enable this are also being established.

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