

Screening of Chickpea Germplasm for Resistance to Fusarium Wilt Under Sick-Plot Conditions

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ABSTRACT

Fusarium wilt, caused by Fusarium oxysporum f. sp. ciceris, is among the most damaging soil-borne diseases of chickpea, and the sick plot has long been the workhorse of resistance screening: a field in which inoculum has been built up so that susceptible plants reliably die. This narrative review asks how far a resistance call made in such a plot can be trusted. It draws on published work on the races of the pathogen, the genetics of resistance, the sources of resistant germplasm, the available molecular markers and the statistics of field trials, and it reports no new data. The main difficulty is instability. Races differ in aggressiveness and symptom type, soil temperature and inoculum density alter the speed and extent of wilting, and sick plots are rarely uniform, so a line that looks resistant in one season or field may wilt in another. Genotype-by-race interaction adds a further layer, because a line resistant to one race can be fully susceptible to another. We argue that a single sick-plot score is best treated as a provisional observation, to be confirmed by repetition, spatial adjustment, race-specific testing and, where available, marker information, and we set out what a transparent screening report should contain.

Keywords: Chickpea; Fusarium oxysporum f. sp. ciceris; Fusarium wilt; Sick plot; Host resistance; Pathogenic races; Germplasm screening; Narrative review

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1. Introduction

Chickpea is grown for its protein-rich seed across the semi-arid tropics and the Mediterranean basin, and often on stored soil moisture with few inputs. In that setting, a disease that kills plants from below the soil line is hard to manage by chemical means, and the usual advice to growers is to rely on resistant cultivars, a suitable sowing date and clean seed [1, 2]. Fusarium wilt, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *ciceris*, is the disease around which much of that advice was written. The fungus survives in the soil

for long periods, it is carried on seed and plant debris, and once a field is infested it tends to stay that way [1].

Breeders therefore need to know which lines resist the disease, and the standard way of finding out is the sick plot. A sick plot is a piece of land in which the pathogen has been deliberately built up, usually by repeated chickpea cultivation and by returning infected residue, until susceptible plants die in predictable numbers. Lines are sown in it beside susceptible checks, and the survivors are called resistant [3]. The method is cheap, it

exposes plants to the pathogen in something close to the conditions farmers face, and it can handle large collections. Those are real advantages, which explains why the method has lasted.

The trouble is that the call it produces is less stable than its tidy output implies. The pathogen is not one thing but a group of races that differ in symptom type and in the cultivars they attack [4, 5]. Disease development depends on soil temperature, inoculum density and the timing of infection [6, 7]. And no field is uniform: inoculum, moisture and soil texture change from one corner of a plot to another. A line can therefore look resistant because it is resistant, because the race in that plot cannot attack it, because the soil was cool that season, or because it happened to occupy a clean patch of ground. The aim is a fair account of what has been shown and what has merely been repeated.

This article is a narrative review. It asks how far a resistance call from a sick plot can be trusted, summarises what is known about the pathogen, the genetics of resistance, germplasm sources and markers, and sets out the sources of instability in turn. It reports no new screening data. Where the literature is silent or ambiguous we say so, and we avoid quoting percentages or counts of resistant accessions that we could not check against the primary papers.

2. Literature Review

2.1 The Pathogen and Its Races

Fusarium oxysporum is a species complex rather than a single uniform fungus. Its members include harmless soil saprophytes and a large number of plant pathogens, each of which is restricted to a narrow range of hosts and is named as a forma specialis [8, 9]. The group is of such importance that plant pathologists have ranked it among the most significant fungal pathogens of crops [10]. The relationship between a forma specialis and its evolutionary history is not simple: some formae speciales arose more than once, and the same name can cover genetically distinct lineages [11, 12].

Within *F. oxysporum* f. sp. *ciceris*, races are defined by the reaction of a set of differential chickpea cultivars. Races 1 to 4 were first separated on such a set in the early 1980s [4], and further races were later recognised in the Mediterranean region, giving the series that is now written as 0, 1A, 1B/C, 2, 3, 4, 5 and 6 [1, 2]. The races also fall into two groups by the syndrome they cause. Some produce a yellowing syndrome, with slow decline of the foliage, while

others produce a wilting syndrome in which plants collapse quickly [1, 13]. This is not a trivial distinction for a screener, since yellowing is easily missed or mistaken for stress.

Races are not evenly distributed. Some are reported mainly from the Indian subcontinent, others from the Mediterranean basin, and some from both regions, and a given field may carry more than one [1, 14]. Fingerprinting studies suggest that races have arisen stepwise from within a limited number of lineages instead of independently, which means the races are related and their behaviour on a host can overlap [14]. Comparative genomics of another *F. oxysporum* pathogen has shown that lineage-specific chromosomes carrying pathogenicity genes can move between strains, so the capacity to change host range is not purely theoretical [15, 16]. This is one reason why the same line can look excellent in one report and unremarkable in the next.

For several races a DNA-based assay now exists that distinguishes them without a differential set [17], and diversity within the pathogen has been explored with array-based markers [18]. These tools matter because race identification by differentials is slow and relies on the same kind of biological assay it is trying to calibrate. They have not, however, been applied to every plot or every screening programme, and the race composition of many sick plots remains an unrecorded variable.

2.2 Disease Development and Its Environment

The fungus enters through the roots and colonises the xylem, and the vascular blockage produces the characteristic wilting [1]. Symptoms may appear at the seedling stage, in which case the crop is thinned early, or later around flowering and podding, when the loss of plants shortens grain filling. The timing of onset is not a detail: the relation between the pattern of an epidemic and the yield finally lost is a tight one, and epidemics that start earlier and progress faster do more harm [7].

Environment shapes all of this. Work by a Spanish research group showed that temperature and inoculum density influence how disease develops in the soil and how well control measures perform [6], and that changing the sowing date altered the severity of wilt, which is consistent with the pathogen favouring warmer soil [19]. The practical meaning for a screener is plain. Two sowings of the same line in the same plot, a month apart, may meet the pathogen at different soil temperatures and give different results. A small difference in sowing date can therefore matter more than a

large difference in genotype.

Other organisms share the same fields. Wilts and root rots of chickpea have more than one cause, and symptoms from different pathogens can look alike in the field [13]. A plant scored as wilted may therefore have died of something other than *Fusarium* wilt unless its stem is checked. Conversely, a pathogen that is present but not causing symptoms inside an apparently healthy plant is not visible to a field scorer at all. Table 1 compares the main environments in which resistance can be assessed with these difficulties in mind.

2.3 Genetics of Resistance

Classical genetic studies found that resistance to the wilting races is often controlled by a small number of genes, with resistance to a given race frequently inherited as a simple trait [5, 20]. That simplicity is part of why the trait attracted marker work early. Several race-specific genes have been placed close together on one linkage group, which suggests a cluster instead of scattered, independent loci [21, 22]. Resistance to the yellowing races, race 0 in particular, has been described as more complex and less clear-cut [22].

Table 1. Screening environments for *Fusarium* wilt resistance compared.

Environment	Main strength	Main weakness
Sick plot (field)	Natural conditions; many lines at once [1, 3]	Patchy inoculum; unknown race mix [17, 41]
Pot or glasshouse, soil inoculum	Defined isolate and dose [6]	Small scale; root growth unlike the field
Controlled seedling assay	Rapid; race-specific [5, 32]	Differs from field expression of resistance
Marker-assisted check	Independent of weather and season [21, 37]	Only for loci already mapped [22]
Multi-site field testing	Exposes genotype-by-environment effects [43, 45]	Costly; sites differ in race

Note: Qualitative comparison drawn from the cited methodological and epidemiological literature; no performance figures are implied.

Race-specific genes fit the older gene-for-gene picture of plant resistance, in which a host gene matches an avirulence factor in the pathogen [23]. If a line carries a gene effective against one race only, its resistance disappears the moment a different race dominates the plot. Quantitative resistance, built from several genes of smaller effect, behaves differently, tends to show up as slower wilting rather than none, and is generally

thought to be less easily overcome [24, 25]. Modern accounts of breeding for disease resistance stress that the two kinds of resistance coexist, and that the type present in a line determines how it should be tested [26].

A line with slow-wilting partial resistance poses a problem for sick-plot scoring. If plants are scored only once, a slow-wilter may be classed as resistant because it was still standing on the day of scoring, and the same line may look susceptible when scored later or in warmer soil [7, 24]. The scoring scale and its timing are thus part of the genetic definition of the trait, not a technical afterthought.

2.4 Sources of Resistant Germplasm

Cultivated chickpea passed through several population bottlenecks during domestication and breeding, which narrowed its genetic base and limits the variation available for traits such as disease resistance [27]. Genebank collections are the obvious place to look, and large reference collections have been assembled to capture the diversity of the cultivated species with a manageable number of accessions [28]. Sequencing of the genome and resequencing of large sets of accessions have since provided a picture of how diversity is arranged and which regions of the genome have been shaped by selection [29, 30, 31].

The wild relatives of chickpea offer a second source. Wild *Cicer* species were screened against several races of the pathogen, and reactions varied among species and among races, which showed that useful resistance exists outside the cultivated gene pool but also that it cannot be assumed to be effective against every race [32]. Using such material is slow, since it needs crossing and backcrossing before the resistance can be assessed in an agronomically useful background [33].

Flowering time deserves a mention here. The genetics of phenology determine when a crop is in the field and which stage it has reached when soil conditions favour the pathogen, so an early or late line can escape or meet disease for reasons unrelated to resistance genes [34]. This is escape, not resistance, and the two must be separated if a screening result is to be useful to a breeder.

2.5 Screening Methods and the Sick Plot

The literature on screening cool-season food legumes for root diseases describes a range of techniques, from field plots to pot tests, to seedling assays in controlled conditions [3]. Each trades realism for control. A sick plot preserves

natural conditions but gives little control over inoculum and race. A pot test with defined inoculum controls the dose but removes the soil structure and the wider environment in which the plant would grow. Seedling assays are quick and race-specific but may not reflect field behaviour at later stages [5, 32].

In practice a screening programme usually uses the sick plot first, to sort a large number of lines, and a more controlled method later, to confirm a small number of candidates. That sequence is sensible, but it only works if the first step is treated as a filter, not a verdict, and if the confirmation step is actually carried out. The risk is that a line which passed the plot once is carried forward in the pedigree as resistant without further question.

2.6 Markers, Genomics and Phenotyping

DNA markers linked to resistance were reported from the 1990s onward [20, 35], and the development of larger marker sets and genetic maps widened the toolkit [33, 36]. A study that introgressed race 1 resistance into an elite cultivar using markers showed that the approach works when the race and the locus are known [37]. Reviews of the impact of genomics on chickpea breeding argue that markers can reduce the amount of field testing needed, but not eliminate it [38, 39].

Markers do not escape the problem of phenotype. A marker is useful because it tracks a phenotype recorded somewhere, and if that original phenotype was an unstable sick-plot score, the marker association inherits the instability. The more reliable the phenotyping, the more useful the marker. This is a recurring theme in genomics-assisted breeding: the genome can be read cheaply, but the trait still has to be measured well [39]. It is a habit worth keeping even when the answer looks obvious.

3. Materials and Methods

3.1 Scope and Question

The review asks a single question in two parts: how reliable is a resistance call made in a sick plot, and what would make it more reliable? The question was broken into sub-questions that a screening report should answer: which race or races were present, how the inoculum and soil were characterised, what the sowing date and temperature were, how the plot was laid out and analysed, how and when plants were scored, and whether the call was confirmed elsewhere. None of these questions is hard to ask, but each is easy

to leave unasked.

3.2 Literature Assembly

The review draws on peer-reviewed work in five areas: the biology and races of the pathogen; the epidemiology of the disease; the genetics and markers of resistance; the diversity and sources of chickpea germplasm; and the statistical analysis of field and multi-environment trials. Sources were chosen for relevance, standing in the field and clarity of method. Studies on other crops or other *formae speciales* are used for what they teach about evolution of pathogens, resistance types and statistics, and not as evidence of results in chickpea.

We did not run a formal systematic search and do not claim one. This is a narrative synthesis, and the choice of sources reflects the authors' judgement. The consequence is that the review is a better guide to the structure of the problem than to the proportion of lines found resistant in any programme, and it avoids quoting counts or percentages from individual screening studies that could not be checked against the primary papers for this article.

3.3 Appraisal Framework

To judge how far a screening report can be trusted, we used four questions. Is the race composition of the plot known or at least acknowledged [4, 17]? Are environment and timing recorded, including soil temperature and sowing date [6, 19]? Does the design separate genotype from position in the plot, and does the analysis match [40, 41]? And has the result been repeated across seasons or sites, so that the genotype-by-environment component can be seen [42, 43]? These questions structure the next section.

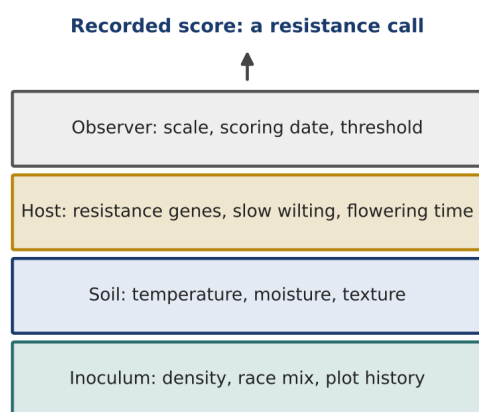
4. Results

4.1 Why a Sick-Plot Score Is Not a Fixed Property of a Line

A sick-plot score is the outcome of several layers (Figure 1). At the base is the inoculum, whose density and race composition are set by the history of the plot. On top of it sits the soil, with its temperature, moisture and texture, which governs how quickly the fungus infects and spreads [6, 7]. The host contributes its genes and its phenology [5, 34]. Only at the top does the observer enter, choosing a scoring scale, a date and a threshold. Every layer can change between seasons, and the score is a product of all of them.

This is why we prefer to speak of a resistance call, not a resistance value. A value suggests something

measured once and fixed. A call is a judgement, made under particular conditions, that might be reversed under others. In plant breeding more broadly, the way genotypes rank can change with the environment, and the breeder must decide whether the change is predictable or not [42]. For wilt screening the predictable part is, to a degree, controlled by sowing date and race; the unpredictable part is the season. The point is not that a plot is badly run, only that its verdict travels less far than the table of scores suggests.



Schematic of layers, not data; each layer can change between seasons

Figure 1. Layers between inoculum and the score recorded in a sick plot (schematic, not data).

4.2 Race Variability and Genotype-by-Race Interaction

Because resistance is often race-specific [4, 5], the central interaction in wilt screening is between genotype and race. Figure 2 sketches how it arises. A line that is resistant to one race and susceptible to another will be ranked high in a plot dominated by the first race and low in a plot dominated by the second. A different line may show the reverse. When the race composition of the plot is unknown, the ranking cannot be interpreted, and combining results from plots with different race mixes can bury a real effect.

The interaction has a practical consequence for how germplasm is used. A line recorded as resistant in one country may be of little help in another where a different race prevails, and the resistance may fail when a cultivar is moved [1, 32]. Under the gene-for-gene view, selection by the crop on the pathogen, and the reverse, may also change the race mix over time [23, 25]. Durable protection is more plausible from lines with several resistance sources than from one gene deployed alone [25, 26].

Part of the confusion in the literature arises from variable naming. Race numbers were assigned in different places and decades, on differential sets that did not always overlap, and the

correspondence between series is not always explicit. A concise differential set was proposed to make results comparable [5], but not every programme uses it, and a report that says only that a line was resistant to *Fusarium* wilt without naming the race should be read as incomplete. Read this way, a single healthy row in a susceptible field is an invitation to retest, not a conclusion.

	Race A	Race B	Race C	Race D
Line 1	R	S	S	R
Line 2	S	R	S	S
Line 3	R	R	R	S
Line 4	S	S	S	S

■ R: resistant reaction ■ S: susceptible

Hypothetical pattern to show interaction; not data

Figure 2. Hypothetical genotype-by-race reaction matrix showing how interaction arises (schematic, not data).

4.3 Environment, Inoculum and Timing

Soil temperature and inoculum density are the two environmental factors with the best documentation. Warmer soil favours the pathogen, and an increase in inoculum density generally raises disease pressure, so a line with partial resistance may hold at a lower density or temperature and fail at a higher one [6, 19]. The dynamics of the epidemic are important too, because the time at which disease first appears and its rate of increase shape both the final proportion of plants affected and the losses [7]. Table 2 summarises the sources of instability and what can be done about each.

A practical response is to fix the sowing date within each year, and to record soil temperature during the critical period, so that a later reader can judge whether conditions were favourable to the pathogen. Where late sowing is used to produce a milder epidemic, it must be understood that such milder conditions test fewer lines to destruction and so separate fewer of them [19]. A screening that is too harsh culls partially resistant lines that would be valuable; one that is too mild lets susceptible lines through. A resistance that disappears when the temperature rises is not a resistance a grower would value.

Scoring time interacts with all of this. Because slow-wilting lines may stay green until late, a single early score can class them as resistant, and a single late score can class them as susceptible. Repeated observations through to maturity capture the shape of the epidemic and allow areas under the progress curve to be compared, which is more informative than a snapshot [7, 24]. The extra effort is modest compared with the cost of carrying a wrongly classified line into a breeding programme.

Table 2. Sources of instability in a sick-plot resistance call.

Source	How it alters the score	Countermeasure
Race composition	Resistance may be race-specific [4, 5]	Race-specific confirmation
Soil temperature	Changes speed and extent of wilting [6, 7]	Record temperature; fix sowing date
Inoculum density	Higher dose can overcome partial resistance [6, 19]	Estimate and report inoculum
Plot heterogeneity	Escapes mistaken for resistance [41, 44]	Replicate; adjust for space
Plant maturity and timing	Late wilting scored as healthy [7]	Score repeatedly to maturity
Mixed pathogens	Other root diseases confuse symptoms [1, 13]	Confirm by isolation

Note: Effects are described qualitatively; their size varies with site, season and pathogen population.

4.4 Heterogeneity of the Sick Plot

The word sick suggests uniformity that the field rarely delivers. Inoculum builds up unevenly because residue is spread unevenly, because irrigation or runoff moves spores, and because soil texture and moisture differ across the plot [1, 3]. Where a susceptible check is sown only at intervals, it can show that the plot is working on average while hiding the clean patches in which a susceptible test line would survive. A survivor in a clean patch is an escape, and it looks exactly like resistance.

Statistical design can help. Treating the unit that was actually randomised as the experimental unit protects against false precision [40], and models that account for spatial trends and neighbouring plots reduce the influence of fertility or disease gradients on the comparison between lines [41, 44]. These techniques are well known in field trials generally but are used inconsistently in resistance screening, where the response is binary at the plant level and the patchiness of disease is the main source of noise. Interleaving susceptible checks through the plot, not only at its edge, gives a direct map of disease pressure that a spatial model can use.

Replication across sites and seasons tells a different story. Multi-environment analyses, such as additive main effects and multiplicative interaction models and genotype plus genotype-by-environment biplots, separate the stable part of a line's performance from the part that depends on the site [43, 45]. Applied to wilt screening, they would show which lines keep their rank across plots, which is exactly what the breeder wants to know. Such analyses require data from more than one environment, which not every screening report provides.

4.5 Confirming a Provisional Call

A provisional call becomes reliable when it survives independent tests. Figure 3 sets out a simple order of confirmation: repeat the plot in a second season and, if possible, a second site; confirm the line against a known race in a controlled assay; check the symptom is wilt by isolation from the stem [3, 5]; and, where the resistance locus is known, test for the marker [21, 37]. Not every step is available to every programme, but the sequence indicates where confidence is gained.

What a report should contain follows from the same logic (Table 3). The race or races present, the history of the plot, the susceptible checks, the layout and replication, the sowing date and temperature, the scale and the timing of scoring, and whether results were repeated across seasons or sites are the minimum a reader needs in order to interpret the result [3, 42, 43]. Reports that omit them are not necessarily wrong, but they cannot be compared or reused with any confidence.



Schematic order of confirmation, not data

Figure 3. Decision path for confirming a provisional sick-plot resistance call (schematic).

Table 3. What a transparent screening report should state.

Item	Why it matters	Often missing?
Pathogen race(s)	Resistance differs by race [4, 22]	Often
Plot history	Explains inoculum build-up [1]	Often
Susceptible checks	Shows the plot was working [3]	Sometimes
Design and replication	Allows separation of line and position [40, 41]	Sometimes
Soil temperature, sowing date	Controls disease expression [7, 19]	Often
Scoring scale and timing	Defines resistance class [7]	Sometimes
Repeat seasons or sites	Tests stability [42, 43]	Often

Note: Items reflect good practice discussed in the cited work and are not drawn from any single study.

5. Discussion

5.1 Interpretation and Practical Reading

The main conclusion of this review is a cautionary one. A resistance call from a single sick-plot season is a useful filter and a poor verdict. Lines that fail in a well-run plot can be discarded with reasonable confidence, since a susceptible line in a high-pressure plot will usually wilt. Lines that pass are more problematic, because passing has several explanations and only one of them is resistance [1, 3]. Nothing here argues against the sick plot; it argues for reading its results with the care they deserve.

The second point concerns race. Because resistance to the wilting races is frequently race-specific [4, 5], a programme that does not know which races are present in its plot does not know what it has selected for. This does not make the screening useless, since a line resistant to the local race is exactly what local growers need, but it does limit the range over which the result can be exported. A name such as *Fusarium* wilt resistant on a seed packet is thus a regional statement rather than a general one.

The third point concerns environment, which is the least controllable of the factors. Variation in soil temperature between seasons can move the same line across the boundary between resistant and susceptible [6, 7]. This is a reason to prefer lines that hold across several seasons, and to value partial resistance only when it has been shown to persist when conditions favour the pathogen. Durability is easier to claim than to demonstrate [25]. These points apply with equal force to screening for other soil-borne diseases, not only

chickpea wilt.

Three findings would change this reading. Evidence that scores from a single season predict performance across several seasons and sites, for a large and diverse set of lines, would justify more reliance on the single-plot call. A demonstration that resistance in the commonly used donors is effective against all races present in a region would reduce the weight placed on race identity. And proof that spatial variation within sick plots is small in properly managed plots would reduce the need for spatial adjustment [41, 44]. We are not aware of syntheses showing any of the three, though absence of such work is not proof that it does not exist.

5.2 Limitations

This is a narrative review and shares the limitations of that form. The selection of sources reflects judgement, no formal search or quality scoring was done, and the review does not quote numerical results from individual screening studies, since these could not be checked against the primary reports for this article. A reader who needs the proportion of accessions resistant in a given collection should consult the original studies and apply the appraisal questions above, or wait for a formal systematic review with meta-analysis.

The review also leans on work from a small number of research groups and regions, notably Mediterranean and South Asian programmes, and on a literature that is uneven in how fully it reports methods. Evidence from other crops and other formae speciales has been used to frame hypotheses about resistance types and pathogen evolution [11, 15], and these hypotheses should not be treated as established for chickpea. Reviews of this kind are more useful when they say where the evidence stops than when they smooth it over.

5.3 Implications for Screening Programmes

Programmes can improve the reliability of their calls without large new investment. They can identify the races present in each plot, using the DNA-based assay where one exists [17] or a differential set otherwise [5]. They can fix and report sowing date, record soil temperature, and interleave susceptible checks through the plot. They can score repeatedly instead of once. And they can lay out lines in a design that allows spatial analysis [41, 44].

A staged pipeline follows naturally. A broad first pass in the sick plot removes the clearly susceptible; a second season, ideally at a second site, removes the lines that benefited from a clean

patch or a cool year; a race-specific controlled assay identifies what each survivor is resistant to; and markers, where the locus is known, add independent support [21, 33, 37]. It is slower than a single season, but it produces calls that other programmes can use.

5.4 Directions for Future Work

Three needs stand out. First, a formal systematic review of published chickpea wilt screenings, with transparent search and quality appraisal and explicit extraction of race, plot and environment information, would show how much of the literature can be compared at all. Second, shared reference panels of lines with known race reactions, grown across many sick plots, would give a direct measure of how stable calls are, and genotype-by-environment methods would make the result quantitative [43, 45]. Third, work that links resistance loci to race-specific reactions and, in turn, to stable field performance would help markers fulfil the promise outlined in genomics reviews [38, 39].

6. Conclusion

The sick plot is a valuable instrument, but it measures a joint outcome of host, pathogen race, inoculum, soil temperature, timing and chance, and only one of those is the resistance of the line. The evidence summarised here shows how each of the others can change the score, and how a line can change its apparent standing without changing its genes. It does not, however, give a figure for how often that happens, and we have been careful not to supply one.

For the practitioner the message is practical rather than discouraging. Screen in the sick plot, but record the race, the plot history, the sowing date and the temperature; sow checks through the plot; score repeatedly; and confirm before declaring a line resistant. For the researcher, the opportunity is a set of coordinated, well-reported trials, across plots and seasons, that turn a provisional call into a measured and transferable result.

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9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

A.E. conceived the review and wrote the sections on genetics of resistance and germplasm. J.S. wrote the sections on the pathogen, races and disease epidemiology. J.E. wrote the sections on screening methods, field design and appraisal. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No human participants or animals were involved and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

13. References

- [1] Jiménez-Díaz, R. M., Castillo, P., Jiménez-Gasco, M. M., Landa, B. B., & Navas-Cortés, J. A. (2015). Fusarium wilt of chickpeas: Biology, ecology and management. *Crop Protection*, 73, 16–27. DOI: [10.1016/j.cropro.2015.02.023](https://doi.org/10.1016/j.cropro.2015.02.023)
- [2] Jendoubi, W., Bouhadida, M., Boukteb, A., Béji, M., & Kharrat, M. (2017). Fusarium wilt affecting chickpea crop. *Agriculture*, 7(3), 23. DOI: [10.3390/agriculture7030023](https://doi.org/10.3390/agriculture7030023)
- [3] Infantino, A., Kharrat, M., Riccioni, L., Coyne, C. J., McPhee, K. E., & Grünwald, N. J. (2006). Screening techniques and sources of resistance to root diseases in cool season food legumes. *Euphytica*, 147(1–2), 201–221. DOI: [10.1007/s10681-006-6963-z](https://doi.org/10.1007/s10681-006-6963-z)
- [4] Haware, M. P., & Nene, Y. L. (1982). Races of *Fusarium oxysporum* f. sp. *ciceris*. *Plant Disease*, 66(9), 809–810. DOI: [10.1094/PD-66-809](https://doi.org/10.1094/PD-66-809)
- [5] Sharma, K. D., Chen, W., & Muehlbauer, F. J. (2005). Genetics of chickpea resistance to five races of Fusarium wilt and a concise set of race differentials for *Fusarium oxysporum* f. sp. *ciceris*. *Plant Disease*, 89(4), 385–390. DOI: [10.1094/PD-89-0385](https://doi.org/10.1094/PD-89-0385)
- [6] Landa, B. B., Navas-Cortés, J. A., Hervás, A., & Jiménez-Díaz, R. M. (2001). Influence of temperature and inoculum density of *Fusarium oxysporum* f. sp. *ciceris* on suppression of Fusarium wilt of chickpea by rhizosphere bacteria. *Phytopathology*, 91(8), 807–816. DOI: [10.1094/PHYTO.2001.91.8.807](https://doi.org/10.1094/PHYTO.2001.91.8.807)
- [7] Navas-Cortés, J. A., Hau, B., & Jiménez-Díaz, R. M. (2000). Yield loss in chickpeas in relation to development of Fusarium wilt epidemics. *Phytopathology*, 90(11), 1269–1278. DOI: [10.1094/PHYTO.2000.90.11.1269](https://doi.org/10.1094/PHYTO.2000.90.11.1269)

- [8] Gordon, T. R., & Martyn, R. D. (1997). The evolutionary biology of *Fusarium oxysporum*. *Annual Review of Phytopathology*, 35, 111-128. DOI: [10.1146/annurev.phyto.35.1.111](https://doi.org/10.1146/annurev.phyto.35.1.111)
- [9] Michielse, C. B., & Rep, M. (2009). Pathogen profile update: *Fusarium oxysporum*. *Molecular Plant Pathology*, 10(3), 311-324. DOI: [10.1111/j.1364-3703.2009.00538.x](https://doi.org/10.1111/j.1364-3703.2009.00538.x)
- [10] Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., ... Foster, G. D. (2012). The top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology*, 13(4), 414-430. DOI: [10.1111/j.1364-3703.2011.00783.x](https://doi.org/10.1111/j.1364-3703.2011.00783.x)
- [11] O'Donnell, K., Kistler, H. C., Cigelnik, E., & Ploetz, R. C. (1998). Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences*, 95(5), 2044-2049. DOI: [10.1073/pnas.95.5.2044](https://doi.org/10.1073/pnas.95.5.2044)
- [12] Correll, J. C. (1991). The relationship between formae speciales, races, and vegetative compatibility groups in *Fusarium oxysporum*. *Phytopathology*, 81(9), 1061-1064. DOI: [10.1094/Phyto-81-1061](https://doi.org/10.1094/Phyto-81-1061)
- [13] Trapero-Casas, A., & Jiménez-Díaz, R. M. (1985). Fungal wilt and root rot diseases of chickpea in southern Spain. *Phytopathology*, 75(10), 1146-1151. DOI: [10.1094/Phyto-75-1146](https://doi.org/10.1094/Phyto-75-1146)
- [14] Jiménez-Gasco, M. M., Milgroom, M. G., & Jiménez-Díaz, R. M. (2004). Stepwise evolution of races in *Fusarium oxysporum* f. sp. *ciceris* inferred from fingerprinting with repetitive DNA sequences. *Phytopathology*, 94(3), 228-235. DOI: [10.1094/PHYTO.2004.94.3.228](https://doi.org/10.1094/PHYTO.2004.94.3.228)
- [15] Ma, L.-J., van der Does, H. C., Borkovich, K. A., Coleman, J. J., Daboussi, M.-J., Di Pietro, A., ... Rep, M. (2010). Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature*, 464(7287), 367-373. DOI: [10.1038/nature08850](https://doi.org/10.1038/nature08850)
- [16] Rep, M., & Kistler, H. C. (2010). The genomic organization of plant pathogenicity in *Fusarium* species. *Current Opinion in Plant Biology*, 13(4), 420-426. DOI: [10.1016/j.pbi.2010.04.004](https://doi.org/10.1016/j.pbi.2010.04.004)
- [17] Jiménez-Gasco, M. M., & Jiménez-Díaz, R. M. (2003). Development of a specific polymerase chain reaction-based assay for the identification of *Fusarium oxysporum* f. sp. *ciceris* and its pathogenic races 0, 1A, 5, and 6. *Phytopathology*, 93(2), 200-209. DOI: [10.1094/PHYTO.2003.93.2.200](https://doi.org/10.1094/PHYTO.2003.93.2.200)
- [18] Sharma, M., Nagavardhini, A., Thudi, M., Ghosh, R., Pande, S., & Varshney, R. K. (2014). Development of DArT markers and assessment of diversity in *Fusarium oxysporum* f. sp. *ciceris*, wilt pathogen of chickpea (*Cicer arietinum* L.). *BMC Genomics*, 15, 454. DOI: [10.1186/1471-2164-15-454](https://doi.org/10.1186/1471-2164-15-454)
- [19] Landa, B. B., Navas-Cortés, J. A., & Jiménez-Díaz, R. M. (2004). Integrated management of *Fusarium* wilt of chickpea with sowing date, host resistance, and biological control. *Phytopathology*, 94(9), 946-960. DOI: [10.1094/PHYTO.2004.94.9.946](https://doi.org/10.1094/PHYTO.2004.94.9.946)
- [20] Tekeoglu, M., Tullu, A., Kaiser, W. J., & Muehlbauer, F. J. (2000). Inheritance and linkage of two genes that confer resistance to *Fusarium* wilt in chickpea. *Crop Science*, 40(5), 1247-1251. DOI: [10.2135/cropsci2000.4051247x](https://doi.org/10.2135/cropsci2000.4051247x)
- [21] Winter, P., Benko-Iseppon, A. M., Hüttel, B., Ratnaparkhe, M., Tullu, A., Sonnante, G., ... Muehlbauer, F. J. (2000). A linkage map of the chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* × *C. reticulatum* cross: Localization of resistance genes for *Fusarium* wilt races 4 and 5. *Theoretical and Applied Genetics*, 101(7), 1155-1163. DOI: [10.1007/s001220051593](https://doi.org/10.1007/s001220051593)
- [22] Sharma, K. D., & Muehlbauer, F. J. (2007). *Fusarium* wilt of chickpea: Physiological specialization, genetics of resistance and resistance gene tagging. *Euphytica*, 157(1-2), 1-14. DOI: [10.1007/s10681-007-9401-y](https://doi.org/10.1007/s10681-007-9401-y)
- [23] Flor, H. H. (1971). Current status of the gene-for-gene concept. *Annual Review of Phytopathology*, 9, 275-296. DOI: [10.1146/annurev.py.09.090171.001423](https://doi.org/10.1146/annurev.py.09.090171.001423)
- [24] Poland, J. A., Balint-Kurti, P. J., Wisser, R. J., Pratt, R. C., & Nelson, R. J. (2009). Shades of gray: The world of quantitative disease resistance. *Trends in Plant Science*, 14(1), 21-29. DOI: [10.1016/j.tplants.2008.10.006](https://doi.org/10.1016/j.tplants.2008.10.006)
- [25] Mundt, C. C. (2014). Durable resistance: A key to sustainable management of pathogens and pests. *Infection, Genetics and Evolution*, 27, 446-455. DOI: [10.1016/j.meegid.2014.01.011](https://doi.org/10.1016/j.meegid.2014.01.011)
- [26] Nelson, R., Wiesner-Hanks, T., Wisser, R., & Balint-Kurti, P. (2018). Navigating complexity to breed disease-resistant crops. *Nature Reviews Genetics*, 19(1), 21-33. DOI: [10.1038/nrg.2017.82](https://doi.org/10.1038/nrg.2017.82)
- [27] Abbo, S., Berger, J., & Turner, N. C. (2003). Evolution of cultivated chickpea: Four bottlenecks limit diversity and constrain adaptation. *Functional Plant Biology*, 30(10), 1081-1087. DOI: [10.1071/FP03084](https://doi.org/10.1071/FP03084)
- [28] Upadhyaya, H. D., Dwivedi, S. L., Baum, M., Varshney, R. K., Udupa, S. M., Gowda, C. L. L., ... Singh, S. (2008). Genetic structure, diversity, and allelic richness in composite collection and reference set in chickpea (*Cicer arietinum* L.). *BMC Plant Biology*, 8, 106. DOI: [10.1186/1471-2229-8-106](https://doi.org/10.1186/1471-2229-8-106)
- [29] Varshney, R. K., Song, C., Saxena, R. K., Azam, S., Yu, S., Sharpe, A. G., ... Cook, D. R. (2013). Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. *Nature Biotechnology*, 31(3), 240-246. DOI: [10.1038/nbt.2491](https://doi.org/10.1038/nbt.2491)
- [30] Jain, M., Misra, G., Patel, R. K., Priya, P., Jhanwar, S., Khan, A. W., ... Chattopadhyay, D. (2013). A draft genome sequence of the pulse crop chickpea (*Cicer arietinum* L.). *The Plant*

- Journal*, 74(5), 715–729. DOI: [10.1111/tjp.12173](https://doi.org/10.1111/tjp.12173)
- [31] Varshney, R. K., Thudi, M., Roorkiwal, M., He, W., Upadhyaya, H. D., Yang, W., ... Liu, X. (2019). Resequencing of 429 chickpea accessions from 45 countries provides insights into genome diversity, domestication and agronomic traits. *Nature Genetics*, 51(5), 857–864. DOI: [10.1038/s41588-019-0401-3](https://doi.org/10.1038/s41588-019-0401-3)
- [32] Kaiser, W. J., Alcalá-Jiménez, A. R., Hervás-Vargas, A., Trapero-Casas, J. L., & Jiménez-Díaz, R. M. (1994). Screening of wild *Cicer* species for resistance to races 0 to 5 of *Fusarium oxysporum* f. sp. *ciceris*. *Plant Disease*, 78(10), 962–967. DOI: [10.1094/PD-78-0962](https://doi.org/10.1094/PD-78-0962)
- [33] Millán, T., Clarke, H. J., Siddique, K. H. M., Buhariwalla, H. K., Gaur, P. M., Kumar, J., ... Winter, P. (2006). Chickpea molecular breeding: New tools and concepts. *Euphytica*, 147(1–2), 81–103. DOI: [10.1007/s10681-006-4261-4](https://doi.org/10.1007/s10681-006-4261-4)
- [34] Kumar, J., & Abbo, S. (2001). Genetics of flowering time in chickpea and its bearing on productivity in semiarid environments. *Advances in Agronomy*, 72, 107–138. DOI: [10.1016/S0065-2113\(01\)72012-3](https://doi.org/10.1016/S0065-2113(01)72012-3)
- [35] Mayer, M. S., Tullu, A., Simon, C. J., Kumar, J., Kaiser, W. J., Kraft, J. M., & Muehlbauer, F. J. (1997). Development of a DNA marker for *Fusarium* wilt resistance in chickpea. *Crop Science*, 37(6), 1625–1629. DOI: [10.2135/cropsci1997.0011183X003700050042x](https://doi.org/10.2135/cropsci1997.0011183X003700050042x)
- [36] Thudi, M., Bohra, A., Nayak, S. N., Varghese, N., Shah, T. M., Penmetsa, R. V., ... Varshney, R. K. (2011). Novel SSR markers from BAC-end sequences, DArT arrays and a comprehensive genetic map with 1,291 marker loci for chickpea (*Cicer arietinum* L.). *PLoS ONE*, 6(11), e27275. DOI: [10.1371/journal.pone.0027275](https://doi.org/10.1371/journal.pone.0027275)
- [37] Varshney, R. K., Mohan, S. M., Gaur, P. M., Gangarao, N. V. P. R., Pandey, M. K., Bohra, A., ... Gowda, C. L. L. (2014). Marker-assisted backcrossing to introgress resistance to *Fusarium* wilt race 1 and *Ascochyta* blight in C 214, an elite cultivar of chickpea. *The Plant Genome*, 7(1). DOI: [10.3835/plantgenome2013.10.0035](https://doi.org/10.3835/plantgenome2013.10.0035)
- [38] Gaur, P. M., Jukanti, A. K., & Varshney, R. K. (2012). Impact of genomic technologies on chickpea breeding strategies. *Agronomy*, 2(3), 199–221. DOI: [10.3390/agronomy2030199](https://doi.org/10.3390/agronomy2030199)
- [39] Varshney, R. K., Thudi, M., Pandey, M. K., Tardieu, F., Ojiewo, C., Vadez, V., ... Siddique, K. H. M. (2018). Accelerating genetic gains in legumes for the development of prosperous smallholder agriculture: Integrating genomics, phenotyping, systems modelling and agronomy. *Journal of Experimental Botany*, 69(13), 3293–3312. DOI: [10.1093/jxb/ery088](https://doi.org/10.1093/jxb/ery088)
- [40] Hurlbert, S. H. (1984). Pseudoreplication and the design of ecological field experiments. *Ecological Monographs*, 54(2), 187–211. DOI: [10.2307/1942661](https://doi.org/10.2307/1942661)
- [41] Gilmour, A. R., Cullis, B. R., & Verbyla, A. P. (1997). Accounting for natural and extraneous variation in the analysis of field experiments. *Journal of Agricultural, Biological, and Environmental Statistics*, 2(3), 269–293. DOI: [10.2307/1400446](https://doi.org/10.2307/1400446)
- [42] Allard, R. W., & Bradshaw, A. D. (1964). Implications of genotype-environmental interactions in applied plant breeding. *Crop Science*, 4(5), 503–508. DOI: [10.2135/cropsci1964.0011183X000400050021x](https://doi.org/10.2135/cropsci1964.0011183X000400050021x)
- [43] Yan, W., Hunt, L. A., Sheng, Q., & Szlavnics, Z. (2000). Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Science*, 40(3), 597–605. DOI: [10.2135/cropsci2000.403597x](https://doi.org/10.2135/cropsci2000.403597x)
- [44] Besag, J., & Kempton, R. (1986). Statistical analysis of field experiments using neighbouring plots. *Biometrics*, 42(2), 231–251. DOI: [10.2307/2531047](https://doi.org/10.2307/2531047)
- [45] Gauch, H. G. (2006). Statistical analysis of yield trials by AMMI and GGE. *Crop Science*, 46(4), 1488–1500. DOI: [10.2135/cropsci2005.07-0193](https://doi.org/10.2135/cropsci2005.07-0193)