

Agro-climatic suitability of Viroflay spinach in Punjab, Pakistan and the risk of downy mildew (*Peronospora effusa*) - A Review

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Abstract

Spinach (*Spinacia oleracea* L.) is a short-duration winter vegetable of increasing importance in Punjab, Pakistan. Viroflay, an open-pollinated semi-savoy cultivar of spinach, has shown vigorous growth in preliminary, non-replicated plantings in Dera Ghazi Khan, but its wider introduction into Punjab raises question about plant-health. Viroflay is the universally susceptible host in the international differential set for spinach downy mildew, caused by the obligate oomycete *Peronospora effusa* (Grev.) Rabenh., and is susceptible to all the 20 officially recognized races of the pathogen. This review summarizes current knowledge of *P. effusa* biology, seed-borne oospore transmission, race evolution and host-pathogen interaction, and evaluates these against the agro-climatic and production conditions of province Punjab. There is no race survey or confirmed record of spinach downy mildew from Pakistan in the literature. The cool Rabi growing season, rising winter rainfall reported for Dera Ghazi Khan, dense sowing, and reliance on imported seed are identified as the main factors that could favor introduction and establishment of the pathogen. A phased surveillance approach is proposed. Viroflay seed lots will be tested for oospores, unsprayed Viroflay strips will be used as sentinel plots for early detection, and any recovered isolates will be race-typed using the international differential set. Although Viroflay's universal susceptibility limits its suitability for commercial production, the same characteristic makes it useful as a sentinel cultivar for detecting *P. effusa* and documenting its occurrence in Pakistan.

Keywords: Downy mildew, Pakistan, *Peronospora effusa*, *Spinacia oleracea*, Viroflay.

Introduction

Spinach is a nutritious, fast-growing leafy vegetable cultivated widely in Pakistan, where its short cycle and multiple cuttings make it attractive to smallholders. Pakistan was ranked fifteenth in the world spinach production in 2020, with about 71,008 t harvested from 6,430 ha (Aslam *et al.*, 2026). Viroflay is an open-pollinated, semi-savoy cultivar of French origin spinach that has been grown internationally for many decades. It occupies a special place in plant pathology. It does not carry any known downy mildew resistance loci and is therefore used worldwide as the universally susceptible reference in the differential set that defines races of *Peronospora effusa* (Correll *et al.*, 2011; Clark *et al.*, 2024). It is also the cultivar from which the chromosome-scale reference genome of spinach was assembled (Hulse-Kemp *et al.*, 2021).

Preliminary, non-replicated plantings of Viroflay by the author in Dera Ghazi Khan, an arid district of southern Punjab, have shown vigorous growth. Before the cultivar is promoted widely, it is essential to assess the risk that a cultivar without downy mildew resistance may suffer from or contribute to the establishment of this disease, which is regarded as the most important constraint on spinach production worldwide (Kandel *et al.*, 2019a). The objective of this review is to assess the

suitability of Viroflay spinach for Punjab, with emphasis on Dera Ghazi Khan, by evaluating the risk posed by downy mildew caused by *P. effusa*, and outlining a surveillance approach for evaluating the cultivar safely.

The pathogen *Peronospora effusa*

Taxonomy and host range: Spinach downy mildew is caused by *P. effusa*, an obligate biotrophic oomycete of the family Peronosporaceae, previously known as *P. farinosa* f. sp. *spinaciae* (Klein *et al.*, 2020). As an oomycete, it is not a true fungus as it has cellulose-containing cell walls and is controlled by a distinct set of fungicide chemistries. Because it cannot be cultured on artificial media, isolates must be maintained on living spinach plants, which complicates diagnosis and race identification (Klein *et al.*, 2020). A telomere-to-telomere genome assembly has recently been produced, revealing 9,745 coding genes including 307 predicted effectors (Fletcher *et al.*, 2022).

Symptoms and disease cycle: Infected leaves develop chlorotic lesions on the upper surface, with grey to violet masses of sporangia on the lower surface; affected leaves become unmarketable (Clark *et al.*, 2024). The asexual cycle is polycyclic. Sporangia produced in large numbers are dispersed by wind and initiate new infections both locally and

over long distances, allowing rapid epidemics within a single season (Kandel *et al.*, 2019a). Under controlled inoculation at 19 °C with a 24 h dew period, chlorosis and light sporulation appear on susceptible plants about five days after inoculation (Clark *et al.*, 2024).

Sexual oospores are formed in infected leaves and seeds when opposite mating types meet (Kandel *et al.*, 2019a). Oospores can remain viable for at least six years, and seed transmission has been demonstrated experimentally: in isolated glass chambers, plants grown from oospore-infested seed lots, or from clean seed coated with infested leaf debris, developed downy mildew 34 to 37 days after planting, whereas plants from oospore-free seed remained healthy (Klosterman *et al.*, 2024). Sexual recombination also contributes to the emergence of resistance-breaking isolates (Skiadas *et al.*, 2022). The risk-relevant traits are summarized in Table 1.

Race structure and the position of Viroflay

Race evolution: Races of *P. effusa* are defined by their pattern of compatibility on a standard set of differential cultivars and near-isogenic lines. The number of officially denominated races has increased rapidly over the past three decades. Race 4 was described from the United States in 1991 (Brandenberger *et al.*, 1991), and three further races were reported from the United States and Europe in 2003 (Irish *et al.*, 2003). New races and deviating strains continued to appear (Feng *et al.*, 2014; Feng *et al.*, 2018), with races Pe:18 and Pe:19 denominated in 2021 and Pe:20 in 2024 by the International Working Group on *Peronospora* (Correll *et al.*, 2021; Correll *et al.*, 2024a). Pe:19 in particular has limited commercial resistance available (Clark *et al.*, 2024). Races have also been characterized from Asia, for example in Japan (Kubota *et al.*, 2017), showing that the race problem is not confined to Western production systems.

Viroflay as a universal susceptible variety: Six resistance loci (*RPF1–RPF6*) have been identified in spinach, and near-isogenic lines carrying each locus, were developed by backcrossing resistant sources into Viroflay (Clark *et al.*, 2024). These lines, together with Viroflay and selected commercial cultivars, form the differential set used to name races (Correll *et al.*, 2024b). *RPF1*, *RPF2* and *RPF3* map to chromosome 3, and candidate genes encoding nucleotide-binding leucine-rich-repeat proteins have been fine-mapped (Irish *et al.*, 2008; Bhattarai *et al.*, 2021). Field-resistance QTLs have also been reported from worldwide germplasm (Bhattarai *et al.*, 2022). By design, Viroflay carries none of these loci. Table 2 summarizes the main race milestones and the corresponding Viroflay reaction.

What molecular studies reveal about Viroflay's susceptibility: Transcriptome comparisons explain part of Viroflay's behaviour. When Viroflay and the resistant cultivar Solomon were inoculated, Solomon

rapidly induced defence-associated genes such as pathogenesis-related gene *PR-1* and glutathione-S-transferases, whereas Viroflay supported full pathogen development and sporulation (Kandel *et al.*, 2020). In a dual transcriptome study using races R13 and R19, homologues of known resistance genes were strongly up-regulated in resistant near-isogenic lines, but in Viroflay they were either not expressed, expressed at very low levels or down-regulated (Clark *et al.*, 2024). The same study identified RxLR and Crinkler effector genes expressed by the pathogen on Viroflay, confirming that the cultivar allows the pathogen to deploy its full virulence repertoire.

Spinach diseases in Pakistan

Published plant-pathology work on spinach in Pakistan has concentrated on *Alternaria* leaf spot. *Alternaria alternata* was first reported on spinach in Pakistan in 2019 (Aslam *et al.*, 2019). A later survey of 25 fields in five districts of Punjab during 2021–22 found mean district incidence ranging from 43.96% in Gujranwala to 67.86% in Hafizabad, with multiloci sequencing revealing a genetically distinct regional population (Aslam *et al.*, 2026). The same authors list downy mildew among the most destructive spinach diseases globally, but their survey did not report it from Punjab.

No survey, first report or race characterization of *P. effusa* on spinach in Pakistan has been reported in the literature reviewed. This absence should be interpreted with care. It may reflect genuine absence, low disease pressure under local conditions, or simply the lack of targeted surveillance and of laboratories equipped to maintain an obligate pathogen. Distinguishing among these explanations is the first research priority identified in this review (Table 3).

Agro-climatic compatibility and downy mildew risk in Punjab

Downy mildew epidemics are favored by cool temperatures, high humidity and prolonged leaf wetness, conditions that in intensive production are intensified by very dense sowing and overhead irrigation (Kandel *et al.*, 2019a; Clark *et al.*, 2024). In California, baby-leaf spinach sown at about three to four million seeds per acre under sprinklers provides near-optimal conditions for the disease (Koike *et al.*, 2011), and a shift to drip irrigation has been studied partly for this reason (Montazar *et al.*, 2019).

Spinach season in Punjab overlaps the pathogen's favorable window. Sowing runs from about mid-September into the winter months, so the crop grows through the coolest part of the year when night temperatures fall and dew forms. In DG Khan, a Mann-Kendall analysis of 2003–2022 records found a significant decrease in average minimum temperature and a significant increase in rainfall

(Shah *et al.*, 2024). Both trends would, if they continue, lengthen periods of leaf wetness and move winter conditions closer to those that favour downy mildew.

Other features of local production cut the other way. Spinach in Punjab is typically grown in small plots under flood or furrow irrigation rather than sprinklers, at lower densities than mechanized baby-leaf systems, and DG Khan's arid climate gives long dry spells between rain events. These features probably lower disease pressure relative to the systems in which most races were described. The balance of these factors is set out in Table 4. Its overall conclusion is that the risk cannot be dismissed on climatic grounds and has to be measured.

Management options

Seed health: Because seed-borne oospores are the most plausible route by which *P. effusa* could enter Punjab, seed health is the first line of defence. Viroflay seed should be obtained from suppliers able to certify origin and, where possible, tested for oospores using seed-wash and molecular assays before sowing (Klosterman *et al.*, 2024). Seed should not be saved from crops that showed downy mildew symptoms.

Cultural practices: Cultural measures reduce leaf wetness and inoculum carry-over: moderate plant density, avoidance of overhead irrigation, irrigation timed so that foliage dries before night, removal and destruction of infected crop residue, and rotation away from spinach and related Amaranthaceae on infested land, since oospores persist in debris (Kandel *et al.*, 2019a; Klosterman *et al.*, 2024).

Host resistance: Resistant cultivars are the principal means of control worldwide, particularly in organic production (Kandel *et al.*, 2020; Bhattarai and Shi, 2021). Viroflay offers no such protection, so its use should be limited to areas where the pathogen has not been detected. If *P. effusa* is found in Punjab, cultivar choice should be matched to the races identified locally rather than to international race lists.

Chemical and biological control: Fungicides specific to oomycetes are used against spinach downy mildew in intensive systems, and field evaluations continue (Matheron *et al.*, 2019). Biopesticides have been evaluated for organic production with variable results (Kandel *et al.*, 2019b). Products labelled for spinach downy mildew in extension guidance include mefenoxam, mandipropamid, oxathiapiprolin, fosetyl-aluminium and ametoctradin with dimethomorph. Most are single-site chemistries at risk of resistance, so products from different FRAC groups should be rotated. Registration status in Pakistan must be confirmed before any recommendation is made, and

pre-harvest intervals are critical for a leafy crop that is harvested repeatedly.

Viroflay as a sentinel, a surveillance framework for Punjab

The feature that makes Viroflay risky for growers also makes it useful to plant pathologists. Because it is susceptible to every known race, a Viroflay plant will show disease if any *P. effusa* is present. Unsprayed strips of Viroflay sown alongside farmer fields can therefore act as sentinel plots, detecting the pathogen earlier and more reliably than scouting of cultivars with partial or race-specific resistance. This mirrors Viroflay's established role as the universal susceptible in the international differential set (Correll *et al.*, 2024b).

A four-step framework is proposed. First, seed lots are tested for oospores before sowing. Second, sentinel strips of Viroflay are sown each Rabi season at representative sites, including DG Khan and the main peri-urban production belts. Third, sentinel and commercial plots are scouted weekly for chlorotic lesions with lower-surface sporulation, and suspect samples are confirmed by microscopy and PCR. Fourth, any isolates recovered are maintained on live plants and race-typed on the international differential set. If no pathogen is detected over several seasons, phased use of Viroflay can continue with seed testing and cultural precautions. If the pathogen is confirmed, the finding is published as a first report with its race profile, and commercial sowings shift to cultivars carrying matching resistance while Viroflay is retained only as a sentinel.

Research gaps and priorities

- A structured survey of spinach-growing districts, supported by sentinel Viroflay plots, is needed to establish whether the pathogen is present in Punjab.
- Commercial and imported spinach seed lots, including Viroflay, should also be screened for oospores to quantify the risk of pathogen introduction.
- Any recovered isolates should be characterized using the international differential set and, ideally, sequenced to determine their relationship with global pathogen populations.
- Microclimate monitoring of leaf wetness, dew, and minimum temperature in DG Khan and the main spinach production belts would further support disease-risk modelling during Punjab's Rabi season.
- Randomized trials comparing Viroflay with local cultivars and resistant hybrids across multiple locations and seasons are also required to quantify yield and quality alongside disease reaction. If the disease is detected, locally conducted trials should evaluate appropriate

cultural practices, seed treatments, and fungicides registered for use in Pakistan under local production conditions.

Conclusion

Viroflay's vigour in preliminary DG Khan plantings is encouraging, but the cultivar carries a well-documented and absolute vulnerability as it is susceptible to all 20 denominated races of *P. effusa*. The pathogen is seed-transmissible, survives for years as oospores, evolves new races through recombination, and is favored by the cool, dewy conditions of Punjab's Rabi season, conditions that climatic trends in DG Khan may be reinforcing. At the same time, no confirmed record of spinach downy mildew from Pakistan was found, and local irrigation and farm structure may lower disease pressure. The evidence therefore supports neither unrestricted promotion nor rejection of Viroflay. It supports a phased, monitored introduction in which

seed is tested, Viroflay itself is used as a sentinel, and any isolates are race-typed. Such a programme would answer the suitability question for Viroflay and, at the same time, give Pakistan its first evidence-based baseline for one of the world's most important spinach diseases.

Contribution of authors

SA conceived and designed the review, conducted the literature search and synthesis, critically evaluated and interpreted the available evidence, formulated the conclusions, reviewed and verified all AI-assisted outputs, and wrote and revised the manuscript, assuming full responsibility for its accuracy, integrity, and content.

Conflict of interests

The author declares no conflict of interest.

Table 1: Biological characteristics of *Peronospora effusa* and their relevance to Viroflay introduction in Punjab.

Attribute	Evidence from the literature	Relevance to Punjab
Obligate biotroph	Cannot be cultured; maintained on live plants (Klein <i>et al.</i> , 2020)	Diagnosis and race typing need live differential hosts and trained staff
Airborne sporangia	Wind dispersal drives local and distant epidemics (Kandel <i>et al.</i> , 2019a)	Once established, spread between neighboring fields is rapid
Oospores in seed and debris	Viable at least six years; seed transmission shown (Klosterman <i>et al.</i> , 2024)	Imported seed lots are the most likely entry route
Sexual recombination	Generates resistance-breaking isolates (Skiadas <i>et al.</i> , 2022)	Local populations could diversify once introduced
Race-specific resistance	20 races denominated; new races overcome deployed genes (Clark <i>et al.</i> , 2024)	Resistant cultivars must be matched to races actually present

Table 2: Selected race-denomination milestones for *Peronospora effusa* and the reaction of Viroflay.

Period	Race(s)	Source	Viroflay
1991	Pe:4	Brandenberger <i>et al.</i> (1991)	Susceptible
2003	Three new races (US and Europe)	Irish <i>et al.</i> (2003)	Susceptible
2014	New races and deviating strains	Feng <i>et al.</i> (2014)	Susceptible
2018	New races and novel strains	Feng <i>et al.</i> (2018)	Susceptible
2021	Pe:18, Pe:19	Correll <i>et al.</i> (2021)	Susceptible
2024	Pe:20	Correll <i>et al.</i> (2024a)	Susceptible

Table 3: Status of selected spinach diseases in Pakistan based on the literature reviewed.

Disease	Pathogen	Status in Pakistan	Source
Alternaria leaf spot	<i>Alternaria alternata</i>	First reported 2019; 44–68% mean incidence in five Punjab districts (2021–22)	Aslam <i>et al.</i> (2019, 2026)
Downy mildew	<i>Peronospora effusa</i>	No confirmed report or race data found	This review
White rust	<i>Albugo occidentalis</i>	Listed among major diseases internationally; no Pakistani survey found	Aslam <i>et al.</i> (2026)
Anthracnose	<i>Colletotrichum spinaciae</i>	Listed among major diseases internationally; no Pakistani survey found	Aslam <i>et al.</i> (2026)

Table 4: Factors that may raise or lower downy mildew risk for Viroflay in Punjab, with emphasis on DG Khan.

Factor	Direction	Basis
Cool Rabi season with dew	Raises risk	Pathogen favoured by cool, humid conditions (Kandel <i>et al.</i> , 2019a)
Falling minimum temperature and rising rainfall in DG Khan, 2003–2022	Raises risk	Trend analysis by Shah <i>et al.</i> (2024)
Imported seed of an open-pollinated cultivar	Raises risk	Oospores carried on seed (Klosterman <i>et al.</i> , 2024)
Farmer-saved seed from infected crops	Raises risk if disease establishes	Oospores form in leaves and seed (Kandel <i>et al.</i> , 2019a)
No resistance in Viroflay	Raises risk	Universal susceptible (Clark <i>et al.</i> , 2024)
Flood or furrow irrigation	Lowers risk	Less leaf wetting than sprinklers (Koike <i>et al.</i> , 2011)
Arid climate, long dry spells	Lowers risk	Fewer uninterrupted wetness periods
Small, scattered plots	Lowers spread	Less continuous host area for sporangial spread

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