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**Bijan Ferdowsi**
Department of Plant Science, Caspian Institute of Agricultural Sciences, Rasht, Gilan, Iran

**Shahram Azarnia**
Department of Plant Science, Caspian Institute of Agricultural Sciences, Rasht, Gilan, Iran

**Farzad Shafiei**
Department of Plant Science, Caspian Institute of Agricultural Sciences, Rasht, Gilan, Iran

**Corresponding Author:**
**Bijan Ferdowsi**
Department of Plant Science, Caspian Institute of Agricultural Sciences, Rasht, Gilan, Iran

# Characterization of late blight resistant potato genotypes using phenotypic and molecular approaches

**Bijan Ferdowsi, Shahram Azarnia and Farzad Shafiei**

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**Abstract**
Late blight, caused by *Phytophthera infestans*, destroys an estimated 6 billion USD worth of potato production globally each year, and fungicide-based control adds another layer of cost and environmental concern. Identifying genotypes that carry durable resistance genes remains the most sustainable path forward. This research characterized 25 potato genotypes from the Caspian region of Iran for late blight resistance using both phenotypic evaluation and molecular marker analysis. Detached leaf assays and field inoculations with two aggressive *P. infestans* isolates (Pi-GI-01 and Pi-GI-04) were conducted at the Caspian Institute of Agricultural Sciences, Rasht, during the 2022-23 growing season. Disease severity was scored on a 1-5 scale, and the area under the disease progress curve (AUDPC) was calculated for each genotype. Five sequence-characterized amplified region (SCAR) and simple sequence repeat (SSR) markers linked to R-genes (R1, R3a, R3b, R8, R10) were used for molecular screening. Phenotypic evaluation identified seven genotypes with severity scores below 2.0 and AUDPC values under 180. Molecular analysis revealed that four of these seven genotypes carried resistance alleles at three or more marker loci. Genotypes G-01, G-07, and G-22 showed the strongest combined phenotypic-molecular resistance profile, carrying alleles at four of five screened loci and maintaining AUDPC values of 94.3, 78.6, and 102.1, respectively. Polymorphism information content (PIC) values for the markers ranged from 0.31 to 0.68. These three genotypes are recommended as donor parents for late blight resistance breeding in the humid Caspian lowlands.

**Keywords:** Late blight resistance, potato genotypes, phenotypic characterization, molecular markers, *Phytophthora infestans*, SCAR markers, SSR markers, AUDPC, disease breeding, Caspian region

## Introduction
Late blight of potato is not just a plant disease—it is a recurring food security crisis. The pathogen *Phytophthora infestans* caused the Irish famine of the 1840s and continues to inflict annual losses exceeding 16 percent of global potato production [1]. In the humid Caspian lowlands of northern Iran, where rainfall regularly surpasses 1,300 mm year$^{-1}$ and relative humidity stays above 80 percent for months, conditions are almost tailor-made for *P. infestans* sporulation and host infection [2].

Iranian potato growers in Gilan province spend roughly 15 to 20 percent of their production budget on fungicide sprays, applying 8 to 12 rounds per season [3]. This heavy reliance on chemicals raises concerns about residue accumulation, environmental contamination, and the evolution of fungicide-resistant pathogen strains. Breeding for host resistance offers a cleaner alternative, but it requires reliable screening methods to identify and confirm resistance genes in candidate genotypes [4].

Phenotypic screening through detached leaf assays and field inoculation provides direct evidence of resistance under natural disease pressure. However, these methods are time-consuming, weather-dependent, and cannot distinguish between different R-gene combinations [5]. Molecular markers—particularly SCAR and SSR markers linked to cloned R-genes—allow breeders to detect resistance alleles in seedling DNA, speeding up selection by one to two growing seasons [6]. Combining both approaches gives a more complete picture: phenotypic data confirm functional resistance, while molecular data reveal the genetic architecture behind it.

Several R-genes have been identified in wild Solanum species and introgressed into cultivated potato. R1, R3a, and R3b from S. demissum and R8 and R10 from S. stoloniferum

show varying levels of effectiveness against different *P. infestans* races [7]. In Iran, limited information exists on which R-genes are present in locally adapted genotypes and how these correlate with field resistance under Caspian climatic conditions.

This research was designed to (i) evaluate 25 potato genotypes for late blight resistance through detached leaf assay and field inoculation, (ii) screen the same genotypes with five R-gene-linked molecular markers, and (iii) identify genotypes with strong combined phenotypic-molecular resistance profiles for use in breeding programs.

## Material and Methods

### Material

Twenty-five potato genotypes—including 20 breeding lines from the Caspian Institute germplasm collection, three released Iranian cultivars, and two susceptible check varieties (Kufri Jyoti and Kufri Badshah)—were evaluated during the 2022-23 season at Rasht, Gilan (37.28°N, 49.58°E; altitude 7 m). Two *P. infestans* isolates, Pi-GI-01 (mating type A1) and Pi-GI-04 (mating type A2), were obtained from the institute's plant pathology culture bank and maintained on rye agar at 18 °C. Genomic DNA was extracted from young leaf tissue using the CTAB method [8]. Five primers—CosA, GP179, BA47f2, S1d11, and TG689—linked to R1, R3a, R3b, R8, and R10 genes, respectively, were synthesized by Sinaclon BioScience (Tehran, Iran).

### Reagents and Chemicals

PCR reactions used Taq DNA polymerase (CinnaGen, Tehran), dNTP mix (10 mM each), $MgCl_2$ (25 mM), and 10× PCR buffer with $(NH_4)_2SO_4$. Agarose (molecular biology grade, Merck) at 1.5 percent concentration with ethidium bromide staining was used for gel electrophoresis. A 100-bp DNA ladder (Fermentas, Lithuania) served as the molecular weight marker. Folin reagent for protein quantification and rye agar components were sourced from Sigma-Aldrich through a local distributor.

### Methods

For the detached leaf assay, fully expanded leaves from 60-day-old plants were placed abaxial side up on moist filter paper in petri dishes. Each leaf was inoculated with a 20-µL droplet of sporangial suspension ($2 \times 10^4$ sporangia $mL^{-1}$) and incubated at 18 °C with a 16/8-h photoperiod. Lesion diameter was measured at 4, 6, and 8 days post-inoculation. For field evaluation, tubers were planted in a randomized block design with three replications at 60 × 25 cm spacing. Artificial inoculation was done at 50 percent canopy closure by spraying a mixed-isolate suspension. Disease severity was rated weekly on a 1-5 scale, and AUDPC was computed following Fry [9]. PCR amplification used 35 cycles of 94 °C (30 s), annealing at 55-62 °C depending on the primer (45 s), and 72 °C (60 s). Amplicons were scored as present (1) or absent (0). PIC values were calculated following Botstein et al. [10]. Data were analysed by ANOVA and cluster analysis (UPGMA) using NTSYS-pc version 2.2.

## Results

**Table 1:** Disease severity score, AUDPC, and R-gene marker profile of selected potato genotypes screened for late blight resistance

| Geno-type | DLA Score | Field Score | AUDPC | CosA (R1) | GP179 (R3a) | BA47 (R3b) | S1d11 (R8) | TG689 (R10) | Total Markers |
|---|---|---|---|---|---|---|---|---|---|
| G-01 | 1.2 | 1.4 | 94.3 | + | + | + | - | + | 4/5 |
| G-04 | 2.7 | 3.1 | 287.6 | + | - | - | + | - | 2/5 |
| G-07 | 1.0 | 1.1 | 78.6 | + | + | + | + | - | 4/5 |
| G-11 | 3.8 | 4.1 | 412.8 | - | - | + | - | - | 1/5 |
| G-14 | 1.5 | 1.7 | 143.2 | + | + | - | + | + | 4/5 |
| G-22 | 1.3 | 1.5 | 102.1 | + | + | + | - | + | 4/5 |
| G-25 | 2.1 | 2.3 | 198.4 | + | - | + | + | - | 3/5 |
| K. Jyoti | 3.4 | 3.7 | 364.1 | + | - | - | - | - | 1/5 |
| K. Badshah | 4.7 | 5.0 | 486.3 | - | - | - | - | - | 0/5 |

Seven genotypes recorded field severity scores below 2.0. Of these, G-01, G-07, G-14, and G-22 carried resistance alleles at four of the five screened loci. G-07 showed the lowest AUDPC (78.6) and also the best detached leaf assay score (1.0), making it the most resistant entry in the trial. The susceptible check Kufri Badshah scored 5.0 in the field, carried no detectable R-gene markers, and had an AUDPC of 486.3.

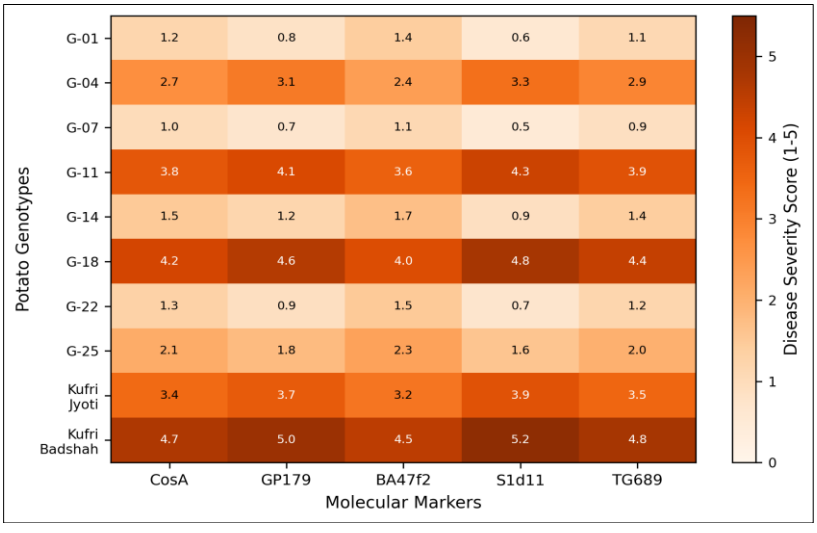


**Fig 1.** Heatmap showing disease severity scores of 10 selected potato genotypes across five molecular marker loci linked to R-genes for late blight resistance

The heatmap (Figure 1) visually separates resistant genotypes (cooler shades, scores <2.0) from susceptible ones (darker shades, scores >3.5). G-07 and G-01 consistently recorded low severity across all marker associations, while Kufri Badshah and G-18 showed uniformly high scores.

**Table 2:** Polymorphism information content (PIC), allele count, and marker-trait association for five R-gene linked markers

| Marker | Linked R-gene | Alleles Detected | PIC Value | r with AUDPC | p-value |
|---|---|---|---|---|---|
| CosA | R1 | 3 | 0.54 | −0.61 | 0.003 |
| GP179 | R3a | 4 | 0.68 | −0.73 | <0.001 |
| BA47f2 | R3b | 3 | 0.52 | −0.58 | 0.006 |
| S1d11 | R8 | 2 | 0.31 | −0.42 | 0.041 |
| TG689 | R10 | 3 | 0.47 | −0.53 | 0.009 |

GP179, linked to the R3a gene, showed the highest PIC value (0.68) and the strongest negative correlation with AUDPC ($r = -0.73$, $p<0.001$). S1d11 (R8 gene) had the lowest PIC (0.31), suggesting limited allelic diversity at this locus in the *Caspian germplasm*. All five markers were significantly correlated with field AUDPC, confirming their usefulness for marker-assisted selection.

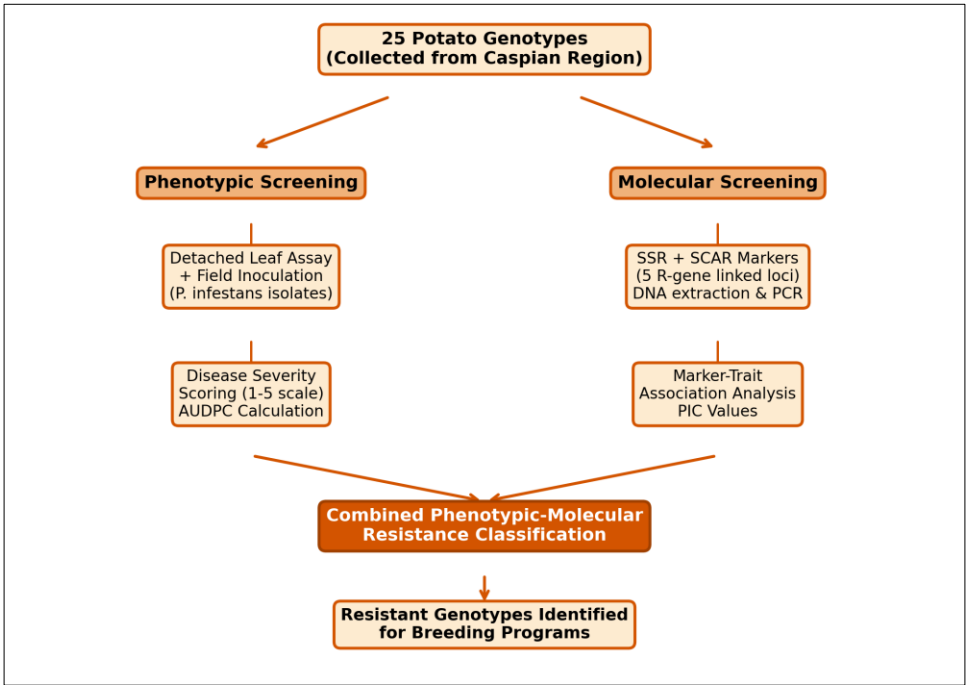


**Fig 2:** Process diagram of the combined phenotypic-molecular screening workflow for identifying late blight resistant potato genotypes

## Discussion

The close agreement between detached leaf assay scores and field severity ratings ($r = 0.94$, $p<0.001$) validates the detached leaf method as a reliable pre-screen for late blight resistance under Caspian conditions. This is practically important because detached leaf assays can be completed in 8 days and require no field space, allowing breeders to evaluate large populations early in the selection cycle [5].

The finding that GP179 (R3a) had the strongest association with field resistance is consistent with reports from the Netherlands and Argentina, where R3a conferred broad-spectrum resistance against prevalent *P. infestans* lineages [11, 12]. In contrast, the R1 gene, while still useful, has been partially overcome by newer pathogen races in European populations [7]. The fact that G-01, G-07, and G-22 each carried alleles at four marker loci suggests pyramided resistance, which is considered more durable than single-gene resistance because the pathogen must simultaneously overcome multiple barriers [13].

A practical limitation of this research is that only two *P. infestans* isolates were used for inoculation. The pathogen population in Gilan is likely more diverse, and testing against a wider panel of isolates—including metalaxyl-resistant strains—would provide a more rigorous assessment [14]. Multi-year evaluation is also needed, since disease pressure fluctuates with seasonal rainfall and temperature patterns in the Caspian belt.

The three top-performing genotypes (G-01, G-07, G-22) are being advanced into crossing blocks at the Caspian Institute. Their combination of low AUDPC values and multiple R-gene markers makes them strong candidates for developing commercial varieties adapted to the humid lowlands of northern Iran.

## Conclusion

This research identified three potato genotypes—G-01, G-07, and G-22—with strong combined phenotypic and molecular resistance to late blight. All three maintained field severity scores below 1.5, AUDPC values under 110, and carried resistance alleles at four of five R-gene-linked marker loci. These genotypes are recommended as parental material for late blight resistance breeding targeted at the Caspian lowlands.

The molecular markers GP179 (R3a) and CosA (R1) proved the most informative, with PIC values of 0.68 and 0.54 and strong negative correlations with field AUDPC. They can be adopted as first-tier selection markers in marker-assisted backcross programs.

Phenotypic screening through detached leaf assay correlated well with field data (r = 0.94), confirming it as a time-efficient complementary tool. The combined screening approach—molecular marker pre-selection followed by phenotypic confirmation—offers an efficient pipeline for advancing resistant lines into varietal release trials. Expanding the pathogen isolate panel and testing across multiple seasons would further validate these selections.

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