

Assessment of apple germplasm for resistance against *Alternaria* blotch triggered by *Alternaria alternata* f. sp. *mali* under natural epiphytotic conditions

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Received: 18 September 2024 / Accepted: 19 March 2025

Published online: 31 March 2025

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Abstract

Alternaria blotch, caused by *Alternaria alternata* f. sp. *mali*, poses a substantial economic threat to apples (*Malus domestica* Borkh.) cultivation. Despite its significance, a comprehensive understanding of the resistance responses of recently introduced commercial apple cultivars remains underexplored in the scientific literature. This study explored the resistance potential within the apple germplasm collection, focusing on identifying cultivars with inherent resistance to the pathogen. Evaluation of the apple gene pool for resistance to *Alternaria alternata* f. sp. *mali* identified five cultivars, Skyline Supreme, Red Fuji, Red Chief, Oregon Spur, and Bright N Early, as resistant to *Alternaria* blotch in the Mashobra region. Conversely, under Nauni conditions, 12 cultivars (Early Red One, Oregon Spur, Oregon Spur II, Gale Gala, Dorset Golden, Anna, Co Fuji, Golden Spur, Granny Smith, Honey Crisp, Washington Delicious, and Sansa) demonstrated disease resistance. Notably, this study highlights the potential of the resistance sources identified in this investigation as valuable genetic resources for inclusion in breeding programs aimed at enhancing apple cultivars with improved resistance against *Alternaria* blotch. This study underscores the importance of further exploring genetic variability within the germplasm of apples to bolster disease management strategies in apple production.

1 Introduction

The apple (*Malus domestica* Borkh.) is a globally cultivated tree fruit of historical significance in Europe and Asia, and plays a crucial role in agricultural production. East Asia is a key production region contributing to over 50% of the world's apple yield [1, 8, 11]. However, widespread cultivation faces a significant challenge due to *Alternaria* blotch disease, which affects the majority of apple varieties in these pivotal regions. *Alternaria* blotch manifests from late spring to early summer and is characterized by circular patches on leaves that expand to diameters of 2–5 mm. Severe cases lead to uneven necrosis, premature leaf fall, compromised fruit quality, and decreased marketability, owing to the early removal of infected leaves. In addition to producer livelihoods, these losses are highly significant in terms of the food supply chains associated with healthy apple production.

Notably, *Alternaria* blotch poses a significant economic threat in Japan and other Asian countries, particularly affecting vulnerable groups [1, 3, 4, 10]. This concern extends to the United States, where the pathotype is crucial in the southeastern region [5, 9, 18]. To address this challenge, cultivating resistant apple cultivars has emerged as a strategic approach

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for disease management, aligning with the global push for safe high-quality fruit production with minimal pesticide reliance [13]. Although certain fungicides have limited efficacy, they can reduce disease incidence.

Despite persistent control efforts, significant gaps exist in thorough research pertaining to the levels of resistance and susceptibility of different varieties of apples. Isolation and characterization of resistance genes will provide essential inputs for upgrading breeding programs. In addition, precise information on yield losses caused by *Alternaria* blotch, which have economic consequences in different regions of cultivation, may help in decision-making policies as well as market strategies. In this regard, filling this research gap is of prime importance in providing effective disease management practices and ensuring future resilience in apple production systems against *Alternaria* blotch.

2 Materials and methods

The susceptibility of various apple cultivars to *Alternaria* blotch under natural epiphytic conditions transpired during the cropping seasons 2015–2016 and 2016–2017. The study was conducted at the research farm of the Department of Fruit Science, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, and RHR & TS, Mashobra. Disease severity assessments were performed using a 0–4 scale as recommended in previous studies [19]. Random recordings of the severity of each cultivar and disease selection were conducted during specified cropping seasons. Following the methodology outlined in [14], the percentage of disease severity was computed using the following formula:

$$\text{Disease Severity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

Based on the disease severity, the responses of various cultivars and selections were categorized into four classes: resistant (0–5%), moderately resistant (6–20%), susceptible (41–60%), and highly susceptible (> 60%).

2.1 Statistical analysis

The data were statistically analyzed using SPSS 27.0. The mean values of the cultivars were compared using the post-hoc Tukey's test at a significance level of 5% ($p < 0.05$).

3 Results and discussion

In the present study, we examined the manifestation of *Alternaria alternata* f. sp. *mali* in the field by evaluating leaf blotch symptoms within the apple germplasm collection at the Regional Horticultural Research Station in Mashobra and the Department of Fruit Science at Dr. Yashwant Singh Parmar University of Horticulture and Forestry in Nauni, Solan, during the cropping seasons—2015–2016 and 2016–2017. The collections at both locations comprised 18 and 21 apple cultivars, respectively, with assessed tree ages ranging from 5 to 8 years. Evaluation of leaf blotch symptoms and categorization of genotypes based on the percent disease index (PDI) offers crucial insights into the resistance patterns of various apple cultivars. Screening of 18 varieties of Mashobra revealed that the Skyline Supreme, Red Fuji, Red Chief, Oregon Spur, and Bright N Early varieties were resistant to *Alternaria* blotch, as shown in Table 1. Previous studies have established the importance of identifying resistant varieties for effective planning and sustainable disease management in apple cultivation [9, 12, 22]. The cultivars Red Spur, Red Baron, Lalla Delicious, and Golden Hornet were rather resistant, with PDI values of 16.67, 12.50, 6.17, and 17.00 percent, respectively, and therefore offer other possibilities for the choice of cultivars in regions affected by *Alternaria* blotch infection [2, 7]. Others fell into the category of moderate susceptibility, with PDIs between 30.67 and 63.50 percent, and the latter showed different reactions, including those of highly susceptible ones. This agrees with the observed variability and genetic bases in apple cultivars, which are noted to be complex, as indicated by research conducted by [15, 16]. Understanding such genetic diversity is crucial for breeding programs aimed at developing cultivars with improved resistance characteristics [16]. Several studies have investigated resistance to *Alternaria* blotch disease in various apple cultivars. Reference [20] described a mutant strain (ID-120-S40) of apples resistant to *Alternaria* blotch selected from gamma-ray-irradiated apical meristem cultures of the highly susceptible cv. Indo. Interestingly, [21] suggested that resistance to *Alternaria* blotch is controlled by a single recessive gene, with the

Table 1 Screening of apple germplasm growing under natural condition against *Alternaria* blotch of apple at RHR&TS-Mashobra

Cultivar	Percent disease index		Pooled mean	Reaction category
	1st Season	2nd Season		
Red Spur	15.33 ± 0.07 ^j	18.00 ± 0.10 ⁱ	16.67	MR
Red Baron	11.33 ± 0.10 ^k	13.67 ± 0.02 ^j	12.50	MR
Lalla Delicious	5.67 ± 0.02 ^l	6.67 ± 0.03 ^k	6.17	MR
Skyline Supreme	5.33 ± 0.02 ^m	6.33 ± 0.03 ^l	5.83	R
Manchurian Crab	36.00 ± 0.10 ^e	37.00 ± 0.10 ^e	36.50	MS
Red Fuji	0.00 ± 0.00 ^p	1.00 ± 0.10 ^o	0.50	R
Starking Delicious	42.00 ± 0.10 ^c	43.00 ± 0.10 ^c	42.50	S
Golden Hornet	16.00 ± 0.05 ⁱ	18.00 ± 0.05 ⁱ	17.00	MR
Red Chief	2.33 ± 0.02 ^o	4.00 ± 0.10 ^m	3.17	R
Oregon Spur	3.00 ± 0.10 ⁿ	3.00 ± 0.05 ⁿ	3.00	R
Tydemans Early Worcestor	38.00 ± 0.10 ^d	41.00 ± 0.10 ^d	39.50	MS
Hardeman	30.00 ± 0.10 ^g	33.33 ± 0.08 ^f	31.67	MS
Vance Delicious	44.00 ± 0.10 ^b	46.00 ± 0.10 ^b	45.00	S
Stark Spur Golden	30.00 ± 0.50 ^g	33.00 ± 0.10 ^g	31.50	MS
Gold Spur	29.00 ± 0.10 ^h	32.33 ± 0.10 ^h	30.67	MS
Scarlet Gala	32.00 ± 0.05 ^f	33.00 ± 0.05 ^g	32.50	MS
Top Red Delicious	63.00 ± 0.10 ^a	64.00 ± 0.10 ^a	63.50	HS
Bright N Early	3.00 ± 0.05 ⁿ	4.00 ± 0.10 ^m	3.50	R

Data are presented as mean ± standard deviation. Mean values followed by the same letter are not significantly different according to post hoc Tukey's test ($p < 0.05$)

proposed gene symbol Alt alt for susceptible vs. resistant conditions. This genetic information could be valuable for breeding programs aimed at the development of resistant varieties.

Table 2 shows the PDI data of *Alternaria* blotch of different apple cultivars under the experimental conditions of the Department of Fruit Science, Dr Y S Parmar University of Horticulture, Nauni. Accordingly, these were considered *Alternaria* blotch-resistant cultivars: Early Red One, Oregon Spur, Oregon Spur II, Gale Gala, Dorset Golden, Anna, Co Fuji, Golden Spur, Granny Smith, Honey Crisp, Washington Delicious, and Sansa. Cultivars with lower PDI might be further developed as important genetic resources for further breeding programs in an attempt to develop varieties possessing optimum adaptability under local conditions, with added resistance potential. The localized specificity relative to resistance traits is in agreement with research on environmental variables influencing disease resistance among apple cultivars [6, 13, 17]. However, Super Chief, Gipson Golden, Golden Delicious, Ace Spur, Schelet Spur, and Red Chief showed only intermediate resistance, varying from 5.84–10.00 PDI. The rating that Scarlet Spur is only moderately susceptible stresses the need for special monitoring of this cultivar under any disease management program. Differences in susceptibility detected in the remaining cultivars were able to elaborate on the magnitude and complexity of host–pathogen interactions. Additionally, specific attention to disease management cannot rely on a single approach. Finally, approaches to *Alternaria* blotch management in apple orchards should consider both genetic and environmental factors [2, 6].

4 Conclusion

A wide number of apple genotypes have been tested against *Alternaria* blotch under natural epiphytic conditions. Prominent resistance against *Alternaria* blotch on apples was evident in Skyline Supreme, Red Fuji, Red Chief, Oregon Spur, and Bright N Early. In contrast, Top Red Delicious showed a highly susceptible reaction to denote it as susceptible. Manchurian Crab, Tydemans Early Worcestor, Hardeman, Stark Spur Golden, Gold Spur, and Scarlet Gala at RHR & TS Mashobra showed a moderate degree of susceptibility, whereas only Royal Delicious and Vance Delicious were rated as susceptible. This will greatly reduce the levels of *Alternaria* blotch loss when cultivating resistant cultivars, such as Skyline Supreme and Red Fuji, which can ultimately be used to produce market-quality apples. Therefore, the current findings provide vital insights into the farming approach, thus enlightening farmers on the hardness of their orchards and producing economically viable apples. Further molecular studies on the molecular basis of

Table 2 Screening of apple germplasm growing under natural condition against *Alternaria* blotch of apple at Nauni

Cultivar	Percent disease index		Pooled mean	Reaction category
	1st Season	2nd Season		
Early Red One	2.67 ± 0.05 ^l	3.00 ± 0.10 ^l	2.84	R
Oregon Spur	4.33 ± 0.05 ⁱ	4.67 ± 0.05 ⁱ	4.50	R
Super Chief	7.67 ± 0.11 ^e	10.33 ± 0.15 ^d	9.00	MR
Oregon Spur II	4.00 ± 0.10 ^j	6.00 ± 0.10 ^f	5.00	R
Gale Gala	3.67 ± 0.05 ^k	4.67 ± 0.05 ⁱ	4.17	R
Dorset Golden	4.00 ± 0.10 ^j	5.67 ± 0.15 ^g	4.84	R
Anna	0.00 ± 0.00 ⁿ	0.00 ± 0.00 ^m	0.00	R
Co Fuji	0.00 ± 0.00 ⁿ	0.00 ± 0.00 ^m	0.00	R
Scarlet Spur II	22.67 ± 0.10 ^c	23.67 ± 0.10 ^c	23.17	MS
Gipson Golden	9.33 ± 0.11 ^d	10.67 ± 0.10 ^d	10.00	MR
Golden Delicious	5.67 ± 0.05 ^g	6.00 ± 0.10 ^f	5.84	MR
Golden Spur	2.67 ± 0.10 ^l	3.33 ± 0.10 ^{kl}	3.00	R
Granny Smith	4.33 ± 0.05 ⁱ	5.33 ± 0.11 ^h	4.83	R
Ace Spur	5.33 ± 0.10 ^h	7.00 ± 0.10 ^e	6.17	MR
Honey Crisp	2.33 ± 0.05 ^m	3.67 ± 0.10 ^k	3.00	R
Schelet Spur	5.33 ± 0.10 ^h	7.33 ± 0.10 ^e	6.33	MR
Washington Delicious	3.67 ± 0.10 ^k	4.00 ± 0.10 ^j	3.84	R
Sansa	0.00 ± 0.00 ⁿ	0.00 ± 0.00 ^m	0.00	R
Royal Delicious	58.00 ± 0.10 ^a	59.67 ± 0.15 ^a	58.84	S
Red Chief	6.00 ± 0.10 ^f	7.00 ± 0.10 ^e	6.50	MR
Vance Delicious	51.67 ± 0.10 ^b	53.00 ± 0.15 ^b	52.34	S

Data are presented as mean ± standard deviation. Mean values followed by the same letter are not significantly different according to post hoc Tukey's test ($p < 0.05$)

resistance in the identified cultivars might provide more tools to perform targeted breeding programs with the aim of increasing the resistance of apples to *Alternaria* blotch.

Acknowledgements The authors express their appreciation for the continued support provided by the Department of Plant Pathology, Dr. Y.S. Parmar, University of Horticulture and Forestry, Solan.

Author Contributions All authors have uniformly contributed in manuscript preparation. P. C. and A.G wrote the main manuscript and J.S. and M. S. prepared tables. All authors have collectively reviewed, revised, and approved the manuscript for publication.

Data Availability All the data are available in the manuscript.

Declarations

Ethics approval and consent to participate The apple (*Malus domestica* Borkh.) cultivars used in this study were obtained from the germplasm collections of the Department of Fruit Science, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, and the Regional Horticultural Research and Training Station, Mashobra, Himachal Pradesh. The cultivars collected during experimentation were duly permitted and approved by the university, and the samples were properly documented.

Competing interests The authors declare no competing interests.

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