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Alterations in ash and mineral nutrition of leaves and shoots of *Rumex maritimus* under *Ustilago parletoreii* infection

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Abstract

The present investigation evaluates changes in ash content and mineral composition in leaves and shoots of *Rumex maritimus* during infection by *Ustilago parletoreii* at different developmental stages (pre-flowering, flowering, pre-sporulation, young sporulation, mature sporulation, and spore stage). Ash content in leaf tissue increased from 10.19% in the pre-flowering control to a maximum of 14.95% during mature sporulation, while shoot ash content rose from 10.77% to 12.81% over the same stages, indicating enhanced accumulation of inorganic constituents during fungal development. Potassium content in leaves declined from 16.46 mg/50 mg ash at the pre-flowering stage to 10.27 mg/50 mg ash during young sporulation, followed by a marked increase to 20.93 mg/50 mg ash at mature sporulation and 22.80 mg/50 mg ash in spores. A similar trend was observed in shoots, with potassium reaching 22.80 mg/50 mg ash in the spore stage. In contrast, calcium content showed a continuous decline with disease progression, decreasing in leaves from 1.46 mg/50 mg ash in the pre-flowering control to 0.53 mg/50 mg ash at mature sporulation, and in shoots from 1.45 to 0.62 mg/50 mg ash. Ferric iron content remained relatively stable during vegetative and early sporulation stages (≈1.01–1.15 mg/50 mg ash) but declined sharply in the spore stage to 0.19 mg/50 mg ash in both tissues. Phosphorus content decreased steadily with infection, falling in leaves from 3.45 mg/50 mg ash at pre-flowering to 1.03 mg/50 mg ash at mature sporulation, and in shoots from 2.59 to 0.91 mg/50 mg ash. The results demonstrate that *U. parletoreii* infection causes pronounced, stage-dependent disruption of mineral homeostasis in *R. maritimus*, with more pronounced alterations in leaf tissues than shoots, particularly during sporulation.

Keywords: Rumex maritimus, Ustilago parletoreii, ash, mineral, nutrition

Introduction

Mineral nutrition plays a crucial role in plant growth, development, and defense against biotic stresses, including fungal pathogens. Plant disease development is governed by complex interactions among the host, pathogen, and environment, in which mineral nutrients significantly influence host susceptibility or resistance (Datnoff *et al.*, 2007; Tripathi & Tewari, 2022) [1, 2]. Balanced mineral nutrition enhances plant vigor, strengthens structural barriers, and supports biochemical defense mechanisms, whereas nutrient deficiencies or imbalances often predispose plants to pathogen attack (Huber & Graham, 1999; Marschner, 2023) [3, 4].

Macronutrients such as potassium (K) and phosphorus (P) play key roles in regulating host metabolism and disease resistance. Potassium contributes to enzyme activation, osmotic regulation, and synthesis of phenolic compounds, thereby reinforcing cell walls and reducing disease severity (Tripathi & Tewari, 2022; Wang *et al.*, 2024) [2, 6]. Potassium deficiency has frequently been associated with increased incidence of fungal diseases due to weakened epidermal tissues and altered carbohydrate metabolism (Palti, 2005) [5]. Phosphorus, an essential component of nucleic acids and energy-transfer molecules, influences plant defense responses, and its altered availability can significantly affect pathogen development and host resistance (Datnoff *et al.*, 2007) [1].

Calcium (Ca) plays a dual role in plants by providing structural support to cell walls and acting as a secondary messenger in defense signaling pathways. Calcium-mediated signaling is essential for the activation of pathogen-associated molecular pattern (PAMP)-triggered immunity and maintenance of membrane stability during pathogen attack (Tripathi & Tewari, 2022) [2]. Reduced calcium availability weakens cell wall integrity and facilitates fungal penetration, leading to increased disease susceptibility (Huber & Graham, 1999) [3].

Micronutrients such as iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) also influence plant defense mechanisms. Iron is particularly important due to its involvement in redox reactions and oxidative burst responses during pathogen attack (Greenshields *et al.*, 2007) [7]. However, iron is also actively targeted by pathogens through specialized acquisition systems, leading to competition between host and pathogen for available iron (Cesco *et al.*, 2020; Herlihy *et al.*, 2020) [8, 9]. Zinc acts as a cofactor for numerous defense-related enzymes, and Zn-deficient plants often show impaired immune responses (Cabot *et al.*, 2019) [10]. Similarly, manganese contributes to lignin biosynthesis and antioxidative defense systems, further strengthening resistance to pathogens (Huber & Graham, 1999) [3].

Fungal pathogens are known to manipulate host nutrient pathways to support their growth and reproduction. Biotrophic fungi, in particular, depend on living host tissues and actively reprogram host metabolism to redirect nutrients toward infection sites (Brefort *et al.*, 2012) [11]. Members of the genus *Ustilago* are well-known biotrophic pathogens that induce profound physiological and biochemical changes in host plants during their infection cycle, including nutrient redistribution during sporulation (Schirawski *et al.*, 2010) [12].

Previous studies have demonstrated that fungal infection can significantly alter mineral allocation within host tissues, affecting both macro- and micronutrient distribution between vegetative and reproductive organs (Cesco *et al.*,

2020) [8]. Such changes are often stage-dependent, reflecting varying metabolic demands of the pathogen during colonization and spore formation (Chung *et al.*, 2017) [13]. Despite growing evidence on the role of mineral nutrition in plant disease resistance, information on tissue-specific mineral dynamics during *Ustilago* infection remains limited.

Rumex maritimus is a wild plant species that serves as a suitable host for studying mineral changes under fungal infection due to its distinct vegetative organs and susceptibility to smut fungi. The present study investigates changes in ash content and mineral composition in leaves and shoots of *R. maritimus* during infection by *Ustilago parletoreii*. Understanding these nutrient dynamics provides insights into host–pathogen interactions and the role of mineral nutrition in disease development.

Materials and Methods

Study Site and Plant Material

Healthy and *Ustilago parletoreii*–infected plants of *Rumex maritimus* L. were collected from natural populations during different developmental stages, namely pre-flowering, flowering, pre-sporulation, young sporulation, mature sporulation, and spore stage. Plants were identified based on morphological characteristics, and disease stages were distinguished by visible symptoms of fungal infection and sporulation on aerial parts.

Freshly collected plant samples were immediately transported to the laboratory for further processing. Leaves and shoots were separated manually, washed thoroughly with tap water followed by distilled water to remove adhering soil and debris, and blotted dry using filter paper.

Sample Preparation

The cleaned plant materials were shade-dried at room temperature (25–30 °C) until constant weight was achieved. Dried samples were then ground into a fine powder using a clean mortar and pestle and stored in airtight containers to prevent moisture absorption prior to analysis.

Determination of Ash Content

Ash content was determined following standard procedures by Peach and Tracy (1956). Accurately weighed powdered samples (approximately 1 g) of leaves and shoots were placed in pre-weighed silica crucibles. The samples were incinerated in a muffle furnace at 550 ± 25 °C for 5–6 h until a uniform white or light grey ash was obtained. After cooling in a desiccator, the crucibles were reweighed, and ash content was calculated as a percentage of dry weight.

$$\text{Ash content } (\%) = \frac{\text{Weight of ash}}{\text{Weight of dry sample}} \times 100$$

Mineral Analysis

Mineral analysis was carried out using the ash obtained from the incinerated samples. A known quantity of ash (50 mg) was dissolved in a suitable volume of dilute nitric acid (HNO_3) and filtered to obtain a clear solution. The final volume was made up using distilled water.

Sodium (Na), potassium (K), calcium (Ca), and ferric iron (Fe) concentrations were determined using a flame photometer under standard operating conditions. Appropriate hollow cathode lamps were used for each element, and calibration curves were prepared using analytical-grade standard solutions. Phosphorus (P) content was estimated using a colorimetric (Mishra, 1968) method after suitable reagent treatment, and absorbance was measured using a Kelt Summerson colorimeter. Mineral concentrations were expressed as milligrams per 50 mg ash (mg/50 mg ash).

Results and Discussion

Leaf Tissue of *Rumex maritimus*

The leaf tissue of *R. maritimus* exhibited pronounced changes in ash and mineral composition with the progression of *Ustilago parletoreii* infection. Ash content increased from the pre-flowering and flowering control stages to a maximum during mature sporulation, indicating enhanced accumulation or redistribution of inorganic constituents in infected leaves. Such increases in ash content are commonly associated with pathogen-induced metabolic stress and altered ion homeostasis in host tissues.

Potassium content declined during early infection stages but increased markedly during mature sporulation and spore formation. Potassium plays a central role in osmotic regulation, enzyme activation, and stress tolerance, and its enrichment during later stages likely reflects both host defense responses and fungal nutrient demands. Similar potassium accumulation in infected tissues has been reported as a consequence of pathogen-mediated nutrient reallocation (Tripathi & Tewari, 2022; Haro & Benito, 2019) [2, 15].

In contrast, calcium content in leaves showed a continuous decline with disease progression. Calcium is critical for cell wall stability and membrane integrity, and its depletion is known to increase host susceptibility by facilitating cell wall degradation and pathogen penetration. Reduced calcium levels in infected tissues have been widely linked with enhanced fungal colonization (Tripathi & Tewari, 2022) [2].

Ferric iron remained relatively stable during most stages but dropped sharply at the spore stage. This decline may be attributed to sequestration of iron by the pathogen or its utilization during sporogenesis. Iron competition between host and pathogen is a well-documented phenomenon, as many fungi actively acquire iron through siderophore-mediated mechanisms (Cesco *et al*., 2020) [8].

Phosphorus content decreased steadily from control to mature sporulation stages, indicating intensive utilization during fungal growth and reproduction. Phosphorus is essential for ATP production and nucleic acid synthesis, and its depletion in infected leaves suggests strong metabolic demand during sporulation (Tripathi & Tewari, 2022) [2].

Shoot Tissue of *Rumex maritimus*

Shoot tissues also showed notable alterations in ash and mineral content following infection. Ash content increased moderately during flowering and sporulation stages, suggesting systemic changes in mineral allocation under disease stress. Compared with leaves, the magnitude of ash increase in shoots was lower, indicating tissue-specific responses to infection.

Potassium levels in shoots remained high during flowering and pre-sporulation but declined at mature sporulation, followed by a sharp increase in the spore stage. This pattern suggests translocation of potassium towards fungal reproductive structures, consistent with the high potassium requirement for fungal metabolism and osmotic balance (Haro & Benito, 2019) [15].

Calcium content in shoots declined progressively with disease advancement, mirroring the trend observed in leaves. This systemic reduction in calcium may contribute to weakened structural integrity and increased vulnerability of shoot tissues to pathogen spread.

Iron content in shoots remained relatively constant during vegetative and early sporulation stages but decreased markedly in the spore stage, supporting the hypothesis of iron mobilization or sequestration during fungal reproduction. Similar redistribution of iron during fungal infection has been reported in other host–pathogen systems (Cesco *et al*., 2020) [8].

Phosphorus concentration declined steadily with infection progression, reaching minimum values during mature sporulation. This decrease again highlights the strong phosphorus demand associated with fungal growth and sporogenesis, reflecting host–pathogen competition for essential nutrients.

Collectively, the combined results demonstrate that *U. parletoreii* infection significantly alters mineral nutrition in both leaves and shoots of *R. maritimus*, with more pronounced changes observed in leaf tissues. Increased ash content and potassium enrichment during sporulation, coupled with depletion of calcium, iron, and phosphorus, reflect complex host–pathogen interactions involving nutrient redistribution, structural weakening of host tissues, and high metabolic demands of the fungus during spore formation. These findings are consistent with established concepts that mineral nutrition plays a pivotal role in determining disease progression and pathogen success.

Table 1: Changes in the ash and mineral content in the leaf tissue of *Rumex maritimus* infected with *Ustilago parletoreii*.

Leaves	Ash (%)	Sodium (mg/50 mg ash)	Potassium (mg/50 mg ash)	Calcium (mg/50 mg ash)	Ferric iron (mg/50 mg ash)	Phosphorus (mg/50 mg ash)
Pre-flowering (Control)	10.19	4.58	16.46	1.46	1.098	3.45
Flowering (Control)	9.83	3.99	10.89	1.40	1.06	2.96
Pre-sporulation	11.11	3.07	11.45	1.02	1.15	2.52
Young sporulation	13.835	2.835	10.27	0.875	1.24	1.7
Mature sporulation	14.95	5.31	20.93	0.53	1.15	1.03
Spore	10.99	3.56	22.80	1.30	0.19	1.26

*The data represent the means of three replications

Table 2: Changes in the ash and mineral content in the shoot tissue of *Rumex maritimus* infected with *Ustilago parletoreii*.

Shoot	Ash (%)	Sodium (mg/50 mg ash)	Potassium (mg/50 mg ash)	Calcium (mg/50 mg ash)	Ferric iron (mg/50 mg ash)	Phosphorus (mg/50 mg ash)
Pre-flowering (Control)	10.77	4.41	17.20	1.45	1.01	2.59
Flowering (Control)	12.10	5.52	20.07	1.36	1.04	2.46
Pre-sporulation	11.36	5.08	19.56	1.11	1.09	2.12
Young-sporulation	11.98	4.625	18.175	0.89	1.05	1.625
Mature-sporulation	12.81	3.34	12.30	0.62	1.05	0.91
Spore	10.99	3.56	22.80	1.30	0.19	1.26

*The data represent the means of three replications

Conclusion

Ustilago parletoreii infection causes marked, stage-dependent changes in ash content and mineral composition of *Rumex maritimus*. Increased ash and potassium levels during sporulation indicate significant nutrient redistribution to support fungal development, whereas continuous declines in calcium, iron, and phosphorus reflect structural weakening of host tissues and increased metabolic demand during infection. Mineral alterations were more pronounced in leaves than shoots, highlighting tissue-specific responses to pathogen stress. Overall, the study underscores the importance of mineral nutrient dynamics in host–pathogen interactions and provides insight into nutrient-mediated mechanisms underlying disease progression.

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Competing Interests

The authors have declared that no competing interests exist.

Authors' Contributions

All the authors have given equal contributions. All the authors read and approved the final manuscript.

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