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Mineral composition modulates *Erwinia amylovora* resistance in pear based on path analysis

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Abstract The effects of mineral composition (N, P, K, Mg, Fe, Cu, and Zn) against fire blight caused by *Erwinia amylovora*, the most devastating disease of pome fruits, was investigated in pear. Due to the transport physiology of minerals, the leaf contained higher concentrations of every mineral analyzed, especially macro-minerals (N, P, K, and Mg) compared to the fruit. Minerals obtained from the leaves were not statistically correlated with resistance to fire blight, however all the minerals examined in the fruit, except for K, were found to be significant. Increased P and Mg concentrations were associated with disease resistance, while N, Zn, Fe, and Cu were associated with susceptibility. Nitrogen-causing susceptibility exerted 61% of this impact through itself directly and was sharply distinguished from other mineral compounds. Furthermore, the indirect effect of nitrogen on disease susceptibility through Cu (39%), Zn (33%), and Fe (30%) was even higher than the direct effect of these minerals (21%, 24%, and 29%, respectively). The direct effects of P (13%) and Mg (10%), which are associated with an increase in resistance, were lower than the indirect effects (19% and 29%, respectively) due to their negative correlation with nitrogen, showing that the main effect of these minerals was in suppressing the negative effects of nitrogen on susceptibility by maintaining mineral balance.

Keywords Direct and indirect effects · Disease management · Fire blight · *Pyrus communis* · Mineral nutrition

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Pears are the second most popular pome fruit after apples in terms of both cultivation area (1 417 980 hectares) and production quantity (26 324 873 tonnes). 10% of the pears produced are traded between countries as fresh fruit. In addition, pear is also processed into multiple products, such as juices, marmalades, jams, formula, muesli, and formula, etc. At the same time, technological developments in fruit storage have made pears an increasingly viable crop for farmers, and this is clearly seen in the rising production quantity year by year (FAO, 2022).

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To maintain optimal pear production and trade, control of fire blight, the most devastating disease of all pome fruits especially pears, should be given high attention. *Erwinia amylovora* is able to infect both the plant's above and below-ground organs, and can kill the whole plant and cause significant crop and financial losses (Gaaliche et al., 2018). Controlling the disease primarily requires the use of resistant rootstocks and cultivars (Kellerhals et al., 2017) because the use of antibiotics is restricted due to the emergence of resistant strains, chemical control is not a permanent solution and, along with residue issues, has detrimental effects on the health of people, animals and the environment (Gusberti et al., 2015; Jimenez Madrid & Ivey, 2023).

Since fire blight resistance is polygenic (Evrenosoğlu et al., 2019) and the resistance mechanisms are complex (Mertoğlu et al., 2020), controlled hybridizations are commonly utilized in breeding programs. Selection of parents with superior fruit quality and disease resistance is extremely important for breeding new desirable genotypes. Thus, research has been carried out to reveal the level of susceptibility of currently available genetic resources (Mitrev et al., 2020; Şahin et al., 2020; Simionca Marcașan et al., 2023).

Determination of appropriate early selection criteria is crucial in minimizing the time, labor, and costs associated with long-term studies such as breeding. In the pre-selection of genotypes for resistance to fire blight, morphological and biochemical markers are frequently used (Sahin, 2022; Tadayon & Sadeghi, 2023). One such marker may be the mineral level of the pome fruits or leaves, which regulate different physiological events (Günen et al., 2003; Gupta et al., 2022; Neumann et al., 2004). In this study, the direct and indirect effects of minerals on the resistance of pears to *Erwinia amylovora* were explored and discussed via path analysis for the first time.

A total of 36 different hybridization combinations were made using 13 parental cultivars (Santa Maria, Williams, Magness, Conference, Kaiser Alexandre, Kieffer, Moonglow, Akca, Ankara, Bursa, Guz, Limon, and Tas). The seeds derived from fruits, were folded at 4 °C for 45 days before being sowed in plastic bags with a 1:1:1 mixture of sand, peat, and perlite, in which they were allowed to germinate and sprout. Supplementary Table 1 provides comprehensive details on the hybridization combinations. One F_1 hybrid from each hybridization combination that was resistant or susceptible to fire blight (36 resistant, 36 susceptible) was used in the study.

Artificial inoculation was used to determine the susceptibility levels of the hybrids. Seven *E. amylovora* isolates with very high virulence – according to pathogenicity tests performed on pear shoots among 75 *E. amylovora* isolates, isolated within the scope of the studies conducted by Saygılı et al. (2004) and Aysan et al. (2004) – were used for inoculations. When the plants' shoots had grown to a height of about 15–30 cm, they were inoculated twice using these isolates (each isolate cultured on its own and mixed before inoculation), which were grown in King B for 48 h. One ml of bacteria culture with 10^8 cell ml^{-1} density was injected into each branch. After inoculation, plants were routinely fertilized and irrigated in the greenhouse at 80–90% humidity and 27 ºC. The length of the infected part of the shoots was measured at the end of 8 weeks according to the formula below (1), and then the average of two values was taken and the genotype susceptibility (GS) value was calculated for each hybrid. Hybrids with susceptibility between 0 and 10% were classified as resistant, and those with susceptibility between 60 and 100% as susceptible (Thibault et al., 1987). Before disease assessments, the pathogen was re-isolated by growing in 5% SNA and testing for fluorescence pigment formation on King B medium (Gür & Baştaş, 2023).

$$\text{Genotype Susceptibility (GS)} = \frac{\text{Length of the Infected Part (cm)}}{\text{Total Length of Shoot (cm)}} \times 100 \quad (1)$$

The genotypes chosen as a result of the artificial inoculations were transplanted into the field and cultivated until fruiting. The formation of the abscission layer, coloration, and taste were taken into consideration as criteria for harvesting the fruits (Akkurt et al., 2024). Leaf samples were taken from the newest leaves, which had finished growing in July. All samples were immediately transferred to the laboratory and prepared for analysis. Ground leaf and fruit samples (0.2 g) were digested using a wet digestion method with HNO_3 and H_2O_2 (2:3) in a microwave oven (MarsXpres CEM, Matthews, USA) for elemental analysis. Total essential element (potassium (K), phosphorus (P), zinc (Zn), iron (Fe), copper (Cu), and magnesium (Mg)) concentrations of the extracted samples were determined using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7500ce, Agilent Technologies, Santa Clara, USA) (Dağhan et al., 2020). Nitrogen determination was measured using the Kjeldahl method proposed by Kacar and Inal (2008) with some modifications. The mineral analyses were verified by determining the elemental contents of a reference sample (tomato leaf; 1573a-NIST, Gaithersburg, MD, USA) using the same analytical methods.

The study was established according to a randomized plot experimental design with 36 replications. A t-test was used to determine whether minerals were associated with differences between resistant and susceptible genotypes. The direct effects of minerals on disease resistance and indirect effects through other minerals were determined using path analysis (Zar, 2013). Minitab-17 (Minitab Inc., State College, Pennsylvania, USA) was used in the analysis.

The results of the mineral analysis performed on fruit and leaf samples are given in Table 1. The quantities of all minerals were found to be higher in the leaf than in the fruit. While the levels of macronutrients (N, P, K, and Mg) in leaf and fruit were comparable, the difference in micronutrients (Fe, Cu, and Zn) was seen to increase. Similar results have been found in many pome fruit species such as apple, quince, and pear (Wojdylo et al., 2021).

The mineral quantities in the leaf had no statistically significant effect on the plant's resistance to fire blight. However, in the fruit, P and Mg were correlated with disease resistance, whereas rising levels of N, Fe, Cu, and Zn were correlated with increased susceptibility. Even though K was higher in the fruit of genotypes that were resistant to disease, it had no statistically significant impact on resistance. Assimilation products are mostly produced by leaves, while fruits are the primary transportation points. It has therefore been suggested that assessing the mineral nutrition and disease resistance from fruit samples would be more accurate (Abdel-Sattar et al., 2022). Our study results are in accordance with that.

The average concentrations of N (0.31%), Fe (7.82 ppm), Zn (5.94 ppm), and Cu (3.99 ppm) in fruits of susceptible genotypes were higher than those of resistant genotypes (0.21%, 3.22 ppm, 3.29 ppm, and 2.1 ppm, respectively). Conversely, P (0.12%) and Mg (0.14%) levels were lower in susceptible genotypes than in the resistant group (P: 0.17%, Mg: 0.23%). The results obtained are comparable with previous research conducted on the effects of minerals on resistance to *Erwinia amylovora* (Aguila-Clares et al., 2018; Bastas et al., 2006; Günen et al., 2003).

It is inconsistent and contradictory to say that minerals improve resistance or susceptibility to pathogens (Dordas, 2008; Nadeem et al., 2018; Opara & Umoh, 2016). Even the effect of minerals on resistance to the same disease agents in the same species differ from study to study. The causes of this contradiction are still not completely understood. However, generally, N-supported development increased succulent tissues which would favor the growth of pathogens (Neumann et al., 2004). Fe is the most important mineral in the life cycle of bacteria due to the physiological events in which it is involved such as increasing virulence (Müller et al., 2022), and in case of its scarcity, bacteria form siderophores to increase Fe uptake (Ferreira et al., 2019). Therefore, in plants, Fe usually causes susceptibility to bacterial diseases such as fire blight (Bastas et al., 2006; Günen et al., 2003). Zn

Table 1 change intervals and T-test results of minerals investigated according to different plant parts

Minerals	Range	Susceptible	Resistant	*P*-Value
Fruit				
N (%)	0.13–0.43	0.31 ± 0.04	0.21 ± 0.05	0.000
P (%)	0.05–0.27	0.12 ± 0.06	0.17 ± 0.06	0.009
K (%)	0.26–1.05	0.70 ± 0.04	0.69 ± 0.11	0.840
Mg (%)	0.06–0.31	0.14 ± 0.08	0.23 ± 0.08	0.001
Fe (ppm)	0.1–16.26	7.82 ± 4.29	3.22 ± 3.79	0.001
Cu (ppm)	0.03–6.07	3.99 ± 1.51	2.16 ± 1.29	0.000
Zn (ppm)	0.01–13.07	5.94 ± 3.54	3.29 ± 1.87	0.004
Leaf				
N (%)	1.08–2.05	1.68 ± 0.15	1.56 ± 0.22	0.112
P (%)	0.18–0.47	0.30 ± 0.06	0.31 ± 0.56	0.684
K (%)	0.46–1.67	1.14 ± 0.19	1.22 ± 0.34	0.481
Mg (%)	0.28–0.55	0.42 ± 0.07	0.43 ± 0.08	0.767
Fe (ppm)	41.98–134.70	75.9 ± 25.6	69.10 ± 22.2	0.478
Cu (ppm)	8.02–23.26	13.37 ± 3.42	14.82 ± 4.89	0.387
Zn (ppm)	12.94–41.47	26.58 ± 7.0	26.78 ± 6.02	0.937

increases the amount of amino acids in the apoplast, reducing the sugar content in plant tissues favored by disease agents (Mogazy & Hanafy, 2022). Membrane stability is maintained by Zn which decreases oxidative damage through the detoxification of superoxide radicals (Faizan et al., 2021; Iranbakhsh et al., 2021). It is also involved in the production of secondary compounds that increase resistance in the phenylpropanid pathway (Siddiqui et al., 2019). For these reasons, Zn generally promotes plant resistance (Bastakoti, 2023; Cabot et al., 2019; Wadhwa & Joshi, 2016). In the present study, Zn was found to increase disease susceptibility. This is thought to be because Zn content was below the optimum level. In actuality, the measured Zn level was relatively low in comparison to other research done on pears (Arzani et al., 2008; Michailidis et al., 2021). Although copper is needed for the production of disease-related enzymes, secondary metabolites, and receptors for ethylene, an important hormone that regulates plant development and disease resistance, it has been found to increase disease susceptibility. It is thought that this is possibly caused by the synergistic relations of Cu with Ni, Fe, and Zn. P, the main constituent of genetic material (DNA, RNA), the energy cycle (ATP), and fundamental structures (phospholipids), is involved in numerous metabolic cycles including systemic induced resistance (SIR) and systemic acquired resistance (SAR) at transcriptome level (Mitra et al., 2020) and is crucial for the formation of healthy roots (Liu, 2021). Mg, one of the most effective minerals in the treatment of bacterial infections, promotes the expression of genes related to resistance through salicylates, jasmonates, and ethylene (Ishihara et al., 2012). In the xylem, magnesium produces disease-related enzymes including chitinase and -1,3-glucanase, and aids in the production of tylosis layers, which serve as a barrier against disease agents (Imada et al., 2016). The obtained results are mostly in parallel with the literature.

Significant positive correlations were found between the susceptibility to fire blight caused by *Erwinia amylovora* and N (0.63), Cu (0.58), Fe (0.49), and Zn (0.44), while negative correlations were found with Mg (-0.47) and P (-0.37) (Table 2). The direct and indirect effects of minerals on susceptibility are given in Table 2. Path analysis has been used in various species to reveal the effects of chemical composition on quality, biotic and abiotic stresses (Bawa et al.,

Table 2 Correlation and path coefficients (with effects) of minerals

Minerals		Correlation coefficient	Path coefficient	Effects (%)
N		0.63***		60.85 (Direct)
	P		−0.01	1.43 (Indirect)
	K		0.11	12.43 (Indirect)
	Fe		0.05	6.11 (Indirect)
	Cu		0.08	9.61 (Indirect)
	Zn		0.07	7.47 (Indirect)
	Mg		−0.02	2.13 (Indirect)
P		−0.37*		13.21 (Direct)
	N		−0.10	19.23 (Indirect)
	K		−0.07	12.97 (Indirect)
	Fe		−0.08	16.10 (Indirect)
	Cu		−0.09	18.23 (Indirect)
	Zn		−0.05	10.22 (Indirect)
	Mg		−0.05	9.99 (Indirect)
Fe		0.49**		29.14 (Direct)
	N		0.17	29.86 (Indirect)
	P		−0.03	5.72 (Indirect)
	K		−0.01	0.87 (Indirect)
	Cu		0.09	16.41 (Indirect)
	Zn		0.07	12.70 (Indirect)
	Mg		0.03	5.31 (Indirect)
Cu		0.58***		20.99 (Direct)
	N		0.29	39.33 (Indirect)
	P		−0.04	5.55 (Indirect)
	K		−0.04	5.78 (Indirect)
	Fe		0.10	14.07 (Indirect)
	Zn		0.08	11.31 (Indirect)
	Mg		0.02	2.97 (Indirect)
Zn		0.44**		23.54 (Direct)
	N		0.22	32.55 (Indirect)
	P		−0.02	3.31 (Indirect)
	K		−0.09	13.76 (Indirect)
	Fe		0.08	11.60 (Indirect)
	Cu		0.08	12.04 (Indirect)
	Mg		0.02	3.25 (Indirect)
Mg		−0.47**		9.74 (Direct)
	N		−0.17	29.10 (Indirect)
	P		0.06	10.18 (Indirect)
	K		−0.05	9.16 (Indirect)
	Fe		−0.09	15.22 (Indirect)
	Cu		−0.10	16.42 (Indirect)
	Zn		−0.06	10.20 (Indirect)

2020; Bhimappa et al., 2017; Dar et al., 2014; Song et al., 2016). Nitrogen-causing susceptibility exerted 61% of this impact through itself directly and was sharply distinguished from other mineral compounds. Furthermore, the indirect effect of nitrogen on disease susceptibility through Cu (21%), Zn (24%), and Fe (29%) was even higher than the direct effect of these minerals (39%, 33%, and 30%, respectively). Numerous investigations have indicated that nitrogen has a significant impact on other minerals (Guo et al., 2019; Kumar et al., 2021). The direct effects of P (13%) and Mg (10%), which increase resistance, were lower than the indirect effects (19% and 29%, respectively) due to their negative correlation with nitrogen shows that the main effect of these minerals suppressing the negative effects of nitrogen by maintaining mineral balance. The results obtained show how mineral balance is effective in disease resistance.

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Author contributions YE and KM conceived and designed the study. YE, KM, EA, and MFY performed the experiments. NG made the mineral analysis. KM made the statistical analysis and wrote the original draft. All authors in this manuscript have read and approved the final version of the manuscript before submission.

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Data availability The data will be available upon a reasonable request from the corresponding author.

Declarations

Ethical approval This article does not contain any studies with human participants or animals by any of the authors.

Conflict of interest The authors have not a conflict of interest to declare.

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