Anatolian Journal of Botany
7(2): 122-127 (2023)
Research article *doi:10.30616/ajb.1287442*

Nitrate content in roots of pepper seedlings exposed to *Phytophthora capsici*

Esra KOÇ[1]*, Belgizar KARAYİĞİT[2]
[1,2]*Ankara University, Faculty of Science, Department of Biology, Ankara, Türkiye*
**ekoc@science.ankara.edu.tr,[2]belgizar.06@gmail.com*

Received : 25.04.2023
Accepted : 24.05.2023
Online : 04.07.2023

Phytophthora capsici'ye maruz bırakılan biber fidelerinin köklerinde nitrat içeriği

Abstract: *Phytophthora capsici* causes root rot, a deadly plant disease. Resistance to diseases is produced by the activation of many defense substances, so knowledge of this natural defense mechanism allows the development of new strategies for disease control. In this study, the response of nitrate (NO_3^-), which is effective in plant growth and development, in different pepper genotypes exposed to pathogen infection was investigated. For this, resistant and sensitive pepper genotypes were exposed to 10^2, 10^3, and 10^4 zoospore/mL of *P. capsici-22* strain and changes in NO_3^- content were determined from root samples taken on the 2nd, 4th and 6th days after infection. All zoospore concentrations resulted in an overall increase in NO_3^- content in roots of CM-334 on all days. In KM-181 and SD-8 genotypes, the highest NO_3^- content was determined on the 6th day of 10^3 zoospore/mL application. In SD-8 and KM-181 genotypes, a significant decrease in the amount of NO_3^- was determined on the 4th and 6th days of treatment of 10^4 zoospore/mL. In these genotypes, a decrease in the amount of NO_3^- was found with the increase in infection time at high zoospore concentration. When the three pepper genotypes were compared, the highest NO_3^- content was determined in the resistant CM-334 genotype, which was exposed to 10^4 zoospore/mL on the 6th day following the infection. In this study, changes in the amount of NO_3^- in resistant and susceptible pepper genotypes indicated that NO_3^- may be effective in plant defense against *P. capsici-22*.

Key words: Nitrate, pepper, *Phytophthora capsici*-22

Özet: *Phytophthora capsici* ölümcül bir bitki hastalığı olan kök çürüklüğüne neden olur. Hastalıklara karşı direnç, birçok savunma maddesinin aktivasyonu ile üretilir, dolayısıyla bu doğal savunma mekanizmasının bilinmesi, hastalık kontrolü için yeni stratejilerin geliştirilmesine olanak tanımaktadır. Bu çalışmada, bitki büyüme ve gelişmesinde etkili olan nitratın (NO_3^-) patojen enfeksiyonuna maruz kalan farklı biber genotiplerindeki tepkisi araştırılmıştır. Bunun için dirençli ve duyarlı biber genotipleri 10^2, 10^3 ve 10^4 zoospore/mL *P. capsici*-22 izolatına maruz bırakılmış ve enfeksiyondan sonraki 2., 4. ve 6. günlerde alınan kök örneklerinden NO_3^- içeriğindeki değişimler belirlenmiştir. Tüm zoospor konsantrasyonları, tüm günlerde CM-334'ün köklerindeki NO_3^- içeriğinde genel olarak bir artışa neden olmuştur. KM-181 ve SD-8 genotiplerinde en yüksek NO_3^- içeriği 10^3 zoospor/mL uygulamasının 6. gününde belirlenmiştir. SD-8 ve KM-181 genotiplerinde, 10^4 zoospor/mL uygulamasının 4. ve 6. günlerinde NO_3^- miktarında önemli bir azalma saptanmıştır. Bu genotiplerde, yüksek zoospor konsantrasyonunda enfeksiyon süresinin artışı ile birlikte NO_3^- miktarında azalma bulunmuştur. Üç biber genotipi karşılaştırıldığında, en yüksek NO_3^- içeriği, enfeksiyondan sonraki 6. günde 10^4 zoospor/mL'ye maruz bırakılan dirençli CM-334 genotipinde belirlenmiştir. Bu çalışmada, dirençli ve duyarlı biber genotiplerinde NO_3^- miktarındaki değişimler, NO_3^- 'ün *P. capsici-22* 'ye karşı bitki savunmasında etkili olabileceğini işaret etmektedir.

Anahtar Kelimeler: Biber, nitrat, *Phytophthora capsici*-22

Citation: Koç E, Karayiğit B (2023). Nitrate content in roots of pepper seedlings exposed to *Phytophthora capsici.* Anatolian Journal of Botany 7(2): 122-127.

1. Introduction

The soil-borne pathogen *Phytophthora capsici* Leonian, which causes root rot, causes great economic damage in pepper growing areas in many countries of the world. Root and crown rot agent *P. capsici* is effective on plant species in the *Solanaceae*, *Cucurbitaceae*, and *Leguminaceae* families (Krasnow and Hausbeck, 2015). This fungus, which was first seen on peppers in New Mexico in the world, had a wide host range in later years (Leonian, 1922). In Turkey, for the first time in 1974, the disease caused by *P. capsici* was seen in pepper cultivation areas in the Central Anatolia region and it caused sudden drying of the plants (Karahan and Maden, 1974). The disease is seen in almost all areas where pepper is grown and causes significant crop losses in years when it occurs (Leonian, 1922).The spots on the root and root collar are typical brown-colored spots and these lesions dry out after a while, causing the plant to die completely (Van Steekelenburg, 1980).

In suitable ecological conditions, zoospores released from the sporangia of the agent, which are formed abundantly in the soil irrigation water, infect the pepper seedlings from their roots, while drying from the root part of the seedling and causing its death (Leonian, 1922). These infections are more common in poorly drained areas where irrigation water or rainwater accumulates during the rainy season. The disease can be observed in different periods of the plant, depending on environmental conditions and the occurrence of infection. It causes a collapse in seedlings in the early period. It starts to appear close to fruit formation in the field and spreads in a very short time, infecting the plants and causing death (Naegele and Hausebeck, 2014). It is sometimes impossible to get products in the fields where this disease is intense. When it infects ripe fruits, it causes a large number of quality and quantity losses in the product. To date, many researchers have focused on the effects of nitrogen (N) on growth, development, and yield in plants.

Nitrogen (N), an essential element for plant metabolism, affects a series of events involved in disease development such as inoculation, colonization, and infection as a result of plant- pathogen interaction (Zhou et al., 2017). Different N forms are effective in physiological events such as enzyme, photosynthesis rate, water balance, respiration rate, and signalling pathway. On the other hand, studies on the importance of N in plant defense against pathogens have been more limited. In this study, the changes in nitrate (NO_3^-) content in the roots of pepper seedlings resistant and susceptible to *P. capsici* were investigated. In the literature survey, there is no record on NO_3^- content in peppers infected with *P. capsici*.

2. Materials and Method

2.1. Plant materials

In the current study, resistant Criollo de Morelos-334 (CM-334), susceptible Kahramanmaras-181 (KM-181) and Sera demre-8 (SD-8) pepper genotypes were used as plant material. Sterilized pepper seeds were placed in pots containing the same amount of sterilized soil, fertilizer and sand and left to germinate in the growing chamber. The seedlings were grown under the conditions of a photoperiod (24 ± 2 °C) of 16 hours light and 8 hours dark at two months of old.

2.2. *Phytophthora capsici*-22 strain and plant inoculation

Phytophthora capsici-22 strain (Ankara University Faculty of Agriculture) to be used in the inoculation process was grown on V_8 agar plates (Jones et al., 1975) (Fig. 1), and zoospore were obtained from growing mycelia (Blaker and Macdonald, 1981; Ward and Stoessl, 1974; Satour and Butler, 1967; Hachler and Hohl, 1984).

After the sterilization process of the roots of the two-month-old seedlings, the seedlings were taken into the Hoagland solution and placed in the growth chamber. After 3 days, the roots of the seedlings were inoculated with 10^2, 10^3, and 10^4 zoospore/mL for 1 hour. After the applications, the seedlings were placed in glass bottles containing Hoagland solution and transferred back to the growing chamber, and then random samples were taken from the roots on the 2nd, 4th, and 6th days (Koç et al., 2011; Koç and Üstün, 2012; Koç, 2022). After the samples were passed through liquid nitrogen, they were stored at -70°C until analysis.

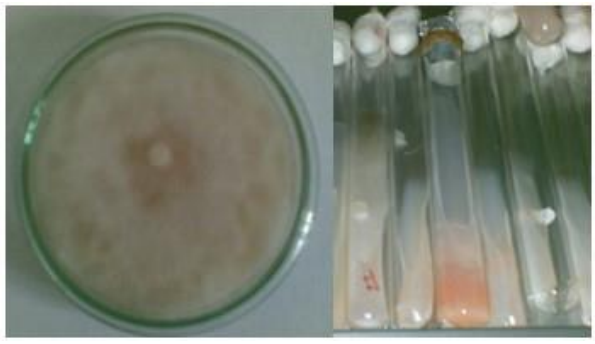

Figure 1. *Phytophthora capsici*- 22 strain.

2.3. NO_3 analysis

The NO_3^- extraction was carried out according to Ruiz et al. (1999). The NO_3 amount was determined according to the salicylic acid method (Cataldo et al., 1975) and the absorbance was measured at 410 nm and calculated as µmol L^{-1} g^{-1} FW against the NO_3^- standard.

2.4. Statistical analysis

The data obtained in terms of NO_3^- were analyzed with repeated measures analysis of variance (ANOVA: genotype×day×treatment). Significance differences were determined by the Tukey test (5% significance level).

Capital letters indicate the difference in genotypes for the same day and treatment. Lowercase letters indicate the difference in treatments of the same genotype and the same day. Superscript lowercase letters indicate the difference in days for the same genotype and treatment.

3. Results

In of root all three pepper genotypes, the most disease was observed in the application of 10^4 zoospore/mL of *P. capsici*-22 isolate on the 6th day following infection. Infection progressed more rapidly in KM-181 and SD-8 and SD-8 genotype was severely damaged, especially on the 6th day. On the 6th day of infection, severe disease symptoms such as leaf wilting, detachment of leaves, and brown lesions on stem length and roots were detected (Fig. 2).

Figure 2. Disease development on the 6th day in three pepper genotypes infected with 10^4 zoospore/mL.

When the NO_3^- contents of CM-334 genoype with 10^2 and 10^4 zoospore/mL were compared on the 2nd and 6th days, a significant increase in NO_3^- content was detected in the roots of the seedlings applied with 10^4 zoospore/mL ($p<0.05$) (Fig. 3). The difference between NO_3^- contents on the 2nd and 6th days was significant in the CM-334 seedlings inoculated with 10^3 zoospore/mL ($p<0.05$). Compared to the control, the difference between NO_3^- contents measured on all days in the CM-334 genotype inoculated with 10^2 zoospore/mL was not significant. Compared to both control and other treatments, the highest NO_3^- increase was determined on the 6th day treatment of 10^4 zoospore/mL ($p<0.05$) (Fig. 3).

An increase in the amount of NO_3^- was detected on the 2nd day of infection in the roots of the KM-181 genotype exposed to 10^4 zoospores/mL treatments. However, with the increase in the duration of infection, a significant decrease in the amount of NO_3^- was determined ($p<0.05$) (Fig. 4). On the other hand, in lower zoospore/mL treatments, the increase in NO_3^- content was determined on the 4th and 6th days after the infection. Increases in NO_3^- contents were determined on the 4th day in the roots of the seedlings inoculated with 10^2 zoospore/mL compared to the control, and on the 6th day in the application of 10^3 zoospore/mL ($p<0.05$). The highest NO_3^- content among all treatments was determined on the 6th day following the infection in the roots of the seedlings inoculated with 10^3 zoospore/mL ($p<0.05$) (Fig. 4).

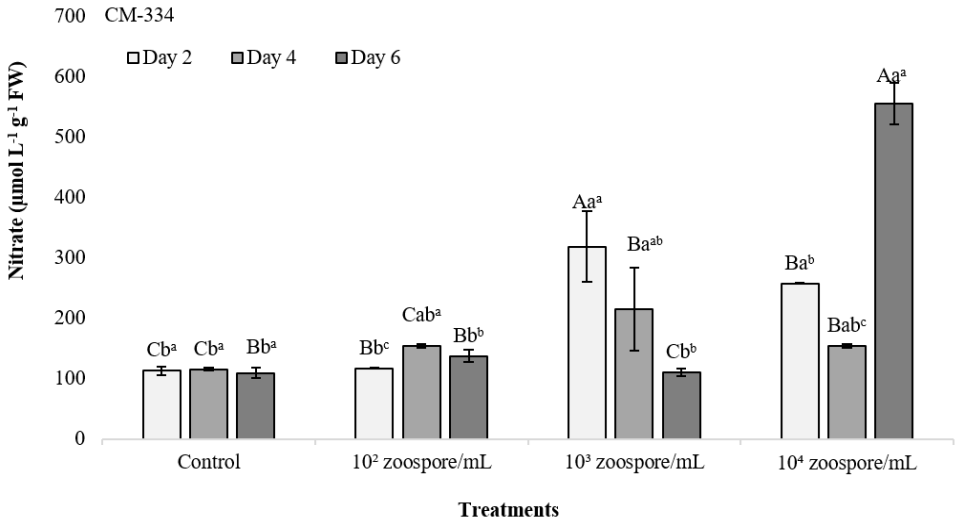

Figure 3. NO_3^- content in roots of CM-334 genotype exposed to different zoospore concentrations (n=3)

In the roots of SD-8 seedlings, with compared to the control, a decrease in NO_3^- content was determined on the 2nd and 6th days following the infection in 10^2 zoospore/mL ($p<0.05$) (Fig. 5). Compared with the control, a decrease in NO_3^- content on the 2nd and 4th days after the infection in the roots of SD-8 genotype inoculated with 10^3 zoospore/mL and an increase in the 6th day were found, the difference was significant ($p<0.05$) (Fig. 5).

According to the control , a decrease in NO_3^- content was determined on the 2nd and 6th days in 10^2 zoospore/mL applications. The highest NO_3^- increases were determined on the 4th day in the 10^2 zoospore/mL application and on the 6th day in the 10^3 zoospore/mL treatment, respectively ($p<0.05$). In the treatment of 10^4 zoospores/mL, NO_3^- contents decreased on all days compared to the control, and the lowest NO_3^- content was determined on the 6th day ($p<0.05$) (Fig. 5).

When the control group of all three pepper genotypes were compared, the highest NO_3^- amount was determined in KM-181 genotype (Fig. 4). This was followed by SD-8 and CM-334 genotypes, respectively (Fig. 3, 5). The difference between NO_3^- contents in the roots of three pepper genotypes was found to be significant in 10^2 zoospore/mL application on the 2nd day, and the highest NO_3^- content was found in SD-8 genotype ($p<0.05$). When the NO_3^- amounts in the roots of three pepper genotypes were compared in 10^2 zoospore/mL application on the 4th and 6th days following the infection, the highest NO_3^- was determined in KM-181 genotype ($p<0.05$) (Fig. 4). In all three pepper genotypes, the highest NO_3^- contents were detected in CM-334 genotype in 10^4 zoospore/mL treatment, and KM-181 genotype at 6th day following infection in 10^3 zoospore/mL treatment ($p<0.05$) (Fig. 3, 4).

4. Discussions

Nitrogen may limit pathogen growth and affect the activation of the plant defense system. Compared with its effects on plant growth and development, the role of NO_3^- in the defense of plants against pathogens is less well known (Fagard et al., 2014; Soulie et al., 2020). It has been reported that different N forms such as NO_3^- are effective on plant disease resistance by using different assimilation and metabolism pathways (Mur et al., 2017). Gupta et al. (2013) found that NO_3^- increased the disease resistance to *Pseudomonas syringae* pv in tobacco by stimulating the synthesis of salicylic acid and NO. Fagard et al. (2014) found that low N decreased the resistance of *Arabidopsis* to *Erwinia amylovora*. In tomato plants exposed to *Botrytis cinerea*, it was determined that low NO_3^- content increased the susceptibility to the pathogen, whereas higher concentrations of NO_3^- decreased the disease symptoms and

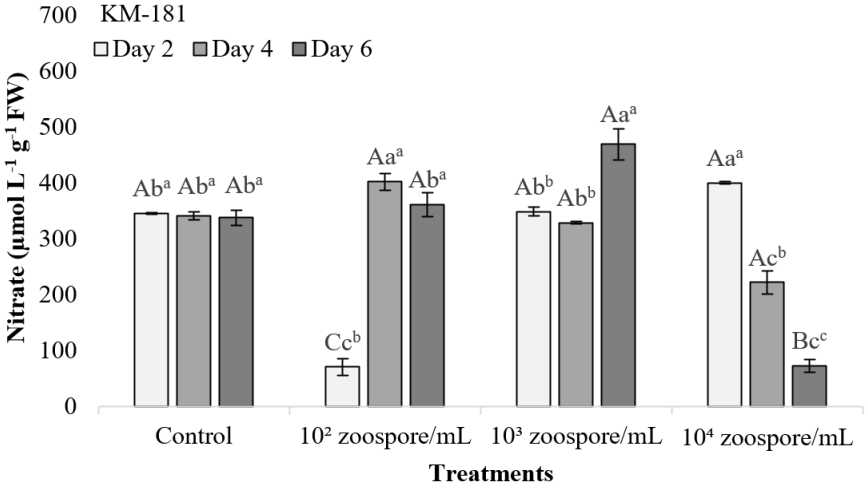

Figure 4. NO_3^- content in roots of KM-181 genotype exposed to different zoospore concentrations (n=3)

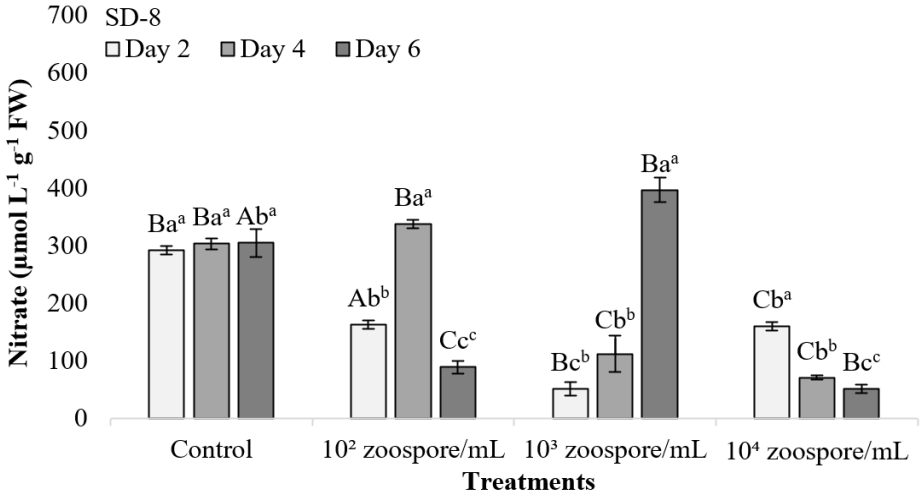

Figure 5. NO_3^- content in roots of SD-8 genotype exposed to different zoospore concentrations (n=3)

lesion, and an increase in defense substances. Similarly, Zhou et al. (2017) and Wang et al. (2016) found that higher NO_3^- in cucumber seedlings exposed to *Fusarium oxysporum* increased the tolerance to *Fusarium* wilt by inhibiting pathogen colonization due to the decrease in membrane damage and pathogen toxin production in plants. It has been reported that there is an opposite relationship between the NO_3^- content in the roots and the disease index, and it has been stated that the efficacy of the disease varies depending on the NO_3^- content in the root (Zhou et al., 2017). In this study, all *P. capsici* zoospore concentrations caused a general increase in NO_3^- amount in the roots of CM-334 genotype on all days. A significant decrease in NO_3^- content was detected on the 4th and 6th days of 10^4 zoospore/mL application in SD-8 and KM-181 genotypes. In other words, a decrease in the amount of NO_3^- was determined due to the increase in the duration of infection at high zoospore concentrations. The CM-334 genotype continued to respond to this severe stress with its high NO_3^- content. This different defense response can be attributed to having different genotypes. It has been reported that the toxin produced by fungi in vitro provides N-regulation and, in general, the effects of N on disease vary depending on the host type, a pathogen type, and the duration of the infection (Bolton and Thomma, 2008). Changes in the amount of NO_3^- depending on the day after the application show that it acts as a defense mechanism against the disease, and the results seem to be compatible with the data obtained from the above mentioned studies. Increases and decreases in the amount of NO_3^- also indicate that there may be a connection between the occurrence of symptoms of disease (hence the severity of disease). These data also support the conclusion that CM-334 genotype have a higher resistance to *P. capsici* isolate than KM-Hot and SD-8 genotypes in our previous studies (Koç and Üstün, 2012), and have shown that NO_3^- has an effect on the formation of this tolerance. The severity of the disease and the length of necrosis were determined at least in CM-334 genotype and maximum in SD-8 genotype (Koç and Üstün, 2012). It has been reported that NO_3^- increases the hypersensitive response (HR)-mediated resistance to pathogens and increases the production of polyamines (Mur et al., 2017). Polyamines are also involved in systemic resistance (Mur et al., 2017). Sagor et al. (2009) and Tiburcio et al. (2014) reported that oxidative deamination of polyamines with amine oxidases reduces pathogen entry by strengthening the cell wall, while polyamine oxidase inhibitors cause weakening of the host's defense system by blocking signal transduction pathways. In a previous study, it was determined that *P. capsici* stress increased the activities of enzymes such as polyaminoxidase (PAO), diaminoxidase (DAO), which are involved in the catabolism of polyamines in CM-334 and KM-Hot pepper genotypes (Koç, 2015). In this study, in parallel with the increase in infection duration, increases in H_2O_2 amount and high DAO and PAO activities were detected. H_2O_2, which is formed as a result of catabolism of polyamines with aminoxidases such as PAO and DAO, contributes to stimulating host cell death (Yoda et al., 2003; Moschou et al., 2009). It has been stated that this DAO and PAO activities occur during hypersensitive response induction after inoculation (Cowley and Walters, 2002). Recent studies indicate that NO_3^- stimulates the production of ethylene and jasmonic acid and other signaling molecules as a signal molecule, and the production of pathogenesis-related proteins (PR) and plant defensin proteins with antifungal properties (Mur et al., 2017; Soulie et al., 2020; Farjad et al., 2021). Changes such as NO, phytoalexin synthesis, SA accumulation and stimulation of defense genes encoding proteins increase resistance against pathogens (Wendehenne et al., 2014). In a previous study, PR-R and PR-S proteins in the PR-5 group and PR-2, PR-3 were found in KM-Hot pepper genotype exposed to 10^4 zoospore/mL concentrations of *P. capsici* (Koç and Üstün, 2009). In the CM-334 genotype, it was determined that a protein with a weight of 28 kDA corresponding to Chi28 (chitinase property) of the PR-3 group was accumulated (Koç and Üstün, 2009). Therefore, changes in NO_3^- content and synthesis of PR proteins after infection with *P. capsici* support studies indicating that NO_3^- functions as a signaling molecule.

Developing disease-resistant genotypes and using them in cultivation is the method struggle with the highest added value in the long term in terms of health and environment. Additionally, the prevention of product loss due to disease will also increase economic gain. The development of pepper genotypes resistant to *P. capsici* is seen as the best control method against this disease. Therefore, it is important to know and use the natural defenses in the fight against pathogens and to develop new strategies for disease control.

In this study, the changes in NO_3^- amounts in resistant and sensitive pepper genotypes to *P. capsici* were investigated. However, further studies are needed to better understand the molecular, biochemical and physiological changes (association with hormones such as JA, SA, ethylene that act as signal molecules in the defense system, amino acid metabolism such as glutamate and aspartate, defense-related secondary metabolites, nitrate transporters, defense-related key genes etc.) underlying the differential effect of NO_3^- availability in pepper on plant susceptibility to *P. capsici.*

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

EK: designing the study, conducting and supervising analyzes, manuscript writing, BK: editing of references section and nitrate analysis.

References

Blaker NS, Macdonald JD (1981). Predisposing effects oil moisture extremes on the susceptibility of *Rhododendron* to *Phytophthora* root and crown rot. Phytopathology 71: 831-834.

Bolton MD,Thomma BP (2008). The complexity of nitrogen metabolism and nitrogen-regulated gene expression in plant pathogenicfungi. Physiological and Molecular Plant Pathology 72: 104-110.

Cataldo DA, Haroon M, Schrader LE, Youngs V (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Communications in Soil Science and Plant Analysis 6: 71-80.

Cowley T, Walters DR (2002). Polyamine metabolism in barley reacting hypersensitively to the powdery mildew fungus *Blumeria graminis* f.sp. *hordei*. Plant Cell Environment 25: 461-468.

Fagard M, Launay A, Clément G, Courtial J, Dellagi A, Farjad M, Krapp A, Soulié MC, Masclaux-Daubresse C (2014). Nitrogen metabolism meets phytopathology. Journal of Experimental Botany 65: 5643-5656.

Farjad M, Clément G, Launay A, Jeridi R, Jolivet S, Citerne S, Rigault M, Soulie MC, Dinant S, Fagard M (2021). Plant nitrate supply regulates *Erwinia amylovora* virulence gene expression in Arabidopsis. Molecular Plant Pathology 22: 1332-1346.

Gupta KJ, Brotman Y, Segu S, Zeier T, Zeier J, Persijn ST (2013). The form of nitrogen nutrition affects resistance against *Pseudomonas syringae pv. phaseolicola* in tobacco. Journal of Experimental Botany 64: 553-568.

Hachler H, Hohl HR (1984). Temporal and spartial distrubition patterns of collar and papillae wall appositions in resistant and susceptible tuber tissue of *Solanum tuberrosum* infected by *Phytophthora infestans*. Physiological Plant Pathology 24: 107-114.

Jones DR, Unwin CH, Ward EWB (1975).Capsidiol induction in pepper fruit during interactions with *Phytophthora capsici* and *Monilinia fructicola*. Phytopathology 65: 1417-1419.

Karahan O, Maden S (1974). Orta Anadolu Bölgesinde Karaağaç (*Ulmus* spp.) ve kavak (*Populus* spp.) ' larda görülen kurumalar ve buna sebep olan etmenler. Bitki Koruma Bülteni 19(4): 175-180.

Koç E, Üstün AS (2009). Pathogenesis related proteins in stem and leaf tissues of peppers *Capsicum annuuum* L. infected by the root fungus *Phytophthora capsici* Leon. Advances in Food Sciences 31(3): 146-150.

Koç E, Üstün AS, İşlek C, Arıcı YK (2011). Defence responses in leaves of resistant and susceptible pepper (*Capsicum annuum* L.) cultivars infected with different inoculum concentrations of *Phytophthora capsici* Leon. Scientia Horticulturae 128(4): 434-442.

Koç E (2015). Exogenous application of spermidine enhanced tolerance of pepper against *Phytophthora capsici* stress. Plant Protection Science 51(3): 127-135.

Koç E (2022). Physiological responses of resistant and susceptible pepper plants to exogenous proline application under *Phytophthora capsici* stress. Acta Botanica Croatica 81(1): 89-100.

Koç E, Üstün AS (2012). Influence of *Phytophthora capsici* L. inoculation on disease severity, necrosis length, peroxidase and catalase activity, and phenolic content of resistant and susceptible pepper (*Capsicum annuum* L.) plants. Turkish Journal of Biology 36(3): 357-371

Krasnow CS, Hausbeck MK (2015). Pathogenicity of *Phytophthora capsici* to *Brassica* vegetable crops and biofumigation cover crops (*Brassica* spp.). Plant disease 99(12): 1721-1726.

Leonian LH (1922). Stem and fruit blight of peppers caused by *Phytophthora capsici sp. nov.* Phytopathology 12: 401-408.

Moschou PN, Sarrıs PF, Skandalıs N, Andrıopoulou AH, Paschalıdıs KA, Panopoulos NJ (2009). Engineered polyamine catabolism preinduces tolerance of tobacco to bacteria and oomycetes. Plant Physiology 149: 1970-1981.

Mur LA, Simpson C, Kumari A, Gupta AK, Gupta KJ (2017). Moving nitrogen to the centre of plant defence against pathogens. Annals of Botany 119(5): 703-709.

Naegele RP, Hausbeck MK (2014). Evaluation of pepper fruit for resistance to *Phytophthora cap*sici in a recombinant inbred line population, and the correlation with fruit shape. Plant Disease 98(7): 885-890.

Ruiz JM, Rivero RM, Garcia PC, Baghour M, Romerao L (1999). Role of $CaCl_2$ in nitrate assimilation in leaves and roots of tobacco plants (*Nicotiana tabacum* L.). Plant Science 141: 107-115.

Sagor GHM, Cong RZ, Berberich T, Takahashi H, Takahashi Y, Kusano T (2009). Spermine signaling in defense reaction against avirulent viral pathogen in *Arabidopsis thaliana*. Plant Signaling and Behavior 4(4): 316-318.

Satour MM, Butler EE (1967). A root and crown rot of tomato casused *Phytophthora capsici* and *Phytophthora parasitica*. Phytopathology 57: 510-515.

Soulie MC, Koka SM, Floch K, Vancostenoble B, Barbe D, Daviere A, Soubigou-Taconnat L, Brunaud V, Poussereau N, Loise E, Devallee A, Expert D, Fagard M (2020). Plant nitrogen supply affects the *Botrytis cinerea* infection process and modulates known and novel virulence factors. Molecular Plant Pathology 21: 1436-1450.

Tiburcio AF, Altabella T, Bitrian M, Alcazar R (2014). The roles of polyamines during the lifespan of plants: from development to stress. Planta 240: 1-18.

Wang M, Sun Y, Gu Z, Wang R, Sun G, Zhu C, Guo S, Shen Q (2016). Nitrate protects cucumber plants against *Fusarium oxysporum* by regulating citrate exudation. Plant and Cell Physiology 57(9): 2001-2012.

Wendehenne D, Gao QM, Kachroo A, Kachroo P (2014). Free radical-mediated systemic immunity in plants. Current Opinion in Plant Biology 20: 127-134.

Ward EWB, Stoessl A (1974). Isolataion of the phyoalexin capsidiol from pepper leaves and stems. Proceedings of 66th Annual Meeting of the American Phytopathological Society, Vancouver. Pp. 11-15.

Van Steekelenburg NAM (1980). Phytophthora root rot of sweet pepper. Netherlands Journal of Plant Pathology 86: 259-264.

Yoda H, Yamaguchı Y, Sano H (2003). Induction of hypersensitive cell death by hydrogen peroxide through polyamine degradation in tobacco plants. Plant Physiology 132: 1973-1981.

Zhou J,Wang M, SunY, Gu Z, Wang R, Saydin A, Shen Q, Guo S (2017). Nitrate increased cucumber tolerance to *Fusarium* wilt by regulating fungal toxin production and distribution. Toxins 9(3): 100.