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RESEARCH PAPER

# Effect of N fertilizing on gas exchange, leaf photosynthetic performance and nutrient concentrations of Sweet Cherry cv. 0900 ziraat

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## Abstract

In this study, the effect of nitrogen (N) fertilization on gas exchange and the photosynthetic performance of cherry leaves were investigated. In the study, 4 different doses of N were applied from the soil, and N, P, K, Ca, Mg, Fe, Mn, Zn, and B concentrations were determined in leaf samples taken from the middle part of the shoots 65-70 days after full flowering. Assimilation rate (A), the concentration of intercellular $CO_2$ (Ci), transpiration rate (Tr), stomatal conductance to water vapor (Gsw), total conductance to $CO_2$ (Gtc), and total conductance to water vapor (Gtw) were measured simultaneously with leaf collection for mineral analysis. Leaf water use efficiency (WUE) and instantaneous carboxylation efficiency (ICE) were calculated. N fertilizing affected the leaf accumulation of some macro (N, P, K, Ca, and Mg) and micro (B) nutrients. As N doses increased, N content of leaf increased, while decreasing leaf P, K, and B contents. N fertilizing negatively affected Tr, A, Gsw, Gtw, Gtc, and ICE. While there were negative correlations between leaf N concentration and gas exchange and leaf photosynthetic performance, they were positive for P and K. It means that changes in gas exchange and leaf photosynthetic performance were not related to increasing leaf N concentration, but decreasing leaf K and/or P concentrations depending on N fertilizing.

## Introduction

Photosynthesis is a very important processes to acquisition carbon supply for plant growth and development (Cheng & Fuchigami, 2000). The epidermis of leaf is covered with a waxy cuticle to prevent water loss. But it lets diffusion of atmospheric $CO_2$ toward the inner photosynthetic tissues. Gas exchanges are primarily realized through stomata (Lebaudy et al., 2008). Stomata regulates leaf gas exchange (the uptake of $CO_2$) and the loss of water vapor in response to changing environmental conditions (Hetherington, 2001). The stomata occupy a central position in the pathways for both the loss of water from plants and the exchange of $CO_2$. It is commonly assumed that they therefore provide the main short-term control of both transpiration and photosynthesis (Jones, 1998).

Sweet cherry trees, like others fruit trees, need various nutrients to grow and produce high yields. Nitrogen (N), phosphorus (P) and potassium (K) are the most important nutrients and mineral nutrition can markedly affect photosynthesis (Longstreth & Nobel, 1980). Bottrill et al. (1970) stated that all nutrient disorders, excluding iron (Fe) and molybdenum (Mo), inhibit photosynthesis when chlorophyll was the basis of their calculation; manganese (Mn)-, copper (Cu)-, P-, and K-deficient plants had the greatest depression. Use of mineral fertilizer is the quickest way of increasing crop production. It is clear that the level of fertilizer applied influenced many processes like fruit quality in orchards (Bybordi, 2013). Hasanuzzaman et al. (2018) pointed that among all nutrients, K is one of the most vital elements used for plant growth and physiology. Physiological processes such as stomatal regulation and photosynthesis depend on K. Wang et al. (2012) reported that K deficiency increased the root abscisic acid (ABA) concentration of cotton. Hetherington (2001) showed that ABA regulates the aperture of stomatal pores. Basile et al. (2003) proved that K deficiency affected the leaf photosynthetic capacity through biochemical limitations.

Cultivars and rootstocks show different responses to nutrient deficiencies. Hu et al. (2016) realized that effect of K fertilization was more important on photosynthesis, chlorophyll fluorescence, and carbohydrates contents in sensitive-K cultivar of cotton. Fallahi et al. (2001) investigated effect of rootstocks on net photosynthesis, leaf nutrition of apple trees and determined rootstock was important on leaf photosynthesis and leaf mineral concentrations.

Boussadia et al. (2015) stated that most limiting factor for tree growth is N deficiency, which induced stunted growth and reduced yield and poor product quality. According to Cheng & Fuchigami (2002), N and carbohydrate metabolism are interrelated, and carbon assimilation depends on N metabolism to meet the needs of the photosynthetic machinery. There is a negative linear relationship between tree N concentration and total nonstructural carbohydrates concentration. Cheng & Fuchigami (2000) express that calculated intercellular $CO_2$ concentration, tended to decrease with increases in leaf N, indicating that stomatal conductance did not limit photosynthesis in leaves with low N concentration. Like K and N, P also affects some physiological process. Lauer et al. (1989) determined that low phosphate nutrition results in increased chlorophyll fluorescence, reduced photosynthetic rate, accumulation of starch and sucrose in leaves, and low crop yields. Bernardi et al. (2015) studied the effect of different doses of N, P, and K on photosynthesis. The results showed that the high levels of N photosynthesis negatively. When K was applied at intermediate fertilization levels, it had positive effects, but P had little effect.

The aim of this study was to investigate the effect of increasing N doses applied to soil on gas exchange and leaf photosynthetic performance of sweet cherry trees.

## Material and Method

We carried out this study with '0900 Ziraat' sweet cherry cultivar grafted on Gisela 5 rootstocks. The experiment was carried out according to "Randomized Complete Block" design as 6 replicates and one tree in each replicate. We used 24 trees with 4 different N doses and 6 replications in each N dose. We planted the orchard used for the experiment in 2008 at 5x2 m planting distances. The orchard was full yield in 2014 and treatments were applied in 2015, 2016, 2017 and 2018, but took measurements only in 2018.

Ammonium nitrate (33.0.0), monopotassium phosphate (0.52.34) and potassium sulphate (0.0.50) used as fertilizer sources. Fertilizers were applied between April and June at approximately 15-day intervals ($19^{th}$ of April, $3^{rd}$ of May, $24^{th}$ of May and $7^{th}$ of June). The required amounts were weighed for each tree at the recommended dose (0, 50, 125 and 250 g N) dissolved in water and applied beneath the tree canopy in 4 different periods. K (125 g $K_2O$/tree) and P (50 g $P_2O_5$/tree) were stable in all treatments.

Plant analysis: Leaf samples were collected 65 to 70 days after full bloom from the middle part of the shoots. Firstly, leaf samples were washed through tap water, then washed through HCl (0.1 normality) and finally washed with deionized water. We placed them in paper bags and dried at 65-70 °C in a drying chamber until a constant weight (for about 48 hours). Dried leaves were ground and weighed to determine N, P, K, Ca, Mg, Fe, Mn, Zn and B concentrations. Kjeldahl wet digestion (for N) and dry ashing methods (for P, K, Ca, Mg, Fe, Mn, Zn and B) were carried out the extraction of nutrients (Ryan et al., 2001) and determined by ICP-OES.

In the middle of the vegetative period, we carried out gas exchange and leaf photosynthetic measurements simultaneously and collected leaves for mineral analysis. For gas exchange and leaf photosynthetic, assimilation rate (A), concentration of intercellular $CO_2$ (Ci), transpiration rate (Tr), stomatal conductance to water vapor (Gsw), total conductance to $CO_2$ (Gtc), total conductance to water vapor (Gtw) were taken from the third fully expanded upper leaves between 10:00-11:00 am using Li-Cor 6800 Photosynthesis System (Li-Cor, Lincoln, NE, USA). Measurement conditions: photosynthetic photon flux density, 1000 $\mu mol_{photon}\ m^{-2}\ s^{-1}$, operational or chamber ambient $CO_2$ concentration, 400 $\mu molCO_2\ mol_{air}^{-1}$. Leaf temperature and leaf to air vapor pressure deficit ranged from 25.6 to 28.8 °C and from 1.22 to 3.06 kPa, 27 °C, respectively. Leaf water use efficiency (WUE) and instantaneous carboxylation efficiency (ICE) were calculated respectively using the formula of WUE=A/Tr and ICE= (A/Ci).

**Table 1.** Effects of nitrogen treatments on leaf nutrient concentration of sweet cherry cv. 0900 Ziraat grafted on Gisela 5 rootstock and reaching full yield. All results were based on dry weight.

| N doses (g/tree) | N (%) | P (%) | K (%) | Ca (%) | Mg (%) |
|---|---|---|---|---|---|
| 0 | 1.84 ± 0.054 c | 0.35 ± 0.035 a | 2.10 ± 0.12 a | 2.37 ± 0.085 a | 0.48 ± 0.031 a |
| 50 | 1.91 ± 0.048 c | 0.26 ±0.012 b | 2.16 ± 0.11 a | 2.02 ± 0.075 b | 0.44 ± 0.021 b |
| 125 | 2.15 ± 0.081 b | 0.18 ± 0.004 c | 1.67 ± 0.078 b | 2.13 ± 0.077 ab | 0.47 ± 0.016 ab |
| 250 | 2.43 ± 0.047 a | 0.18 ± 0.004 c | 1.48 ± 0.060 b | 2.33 ± 0.097 a | 0.50 ± 0.018 a |
| P value | P<0.01 | P<0.01 | P<0.01 | P<0.05 | P<0.05 |
| **N doses (g/tree)** | **Fe (mg kg$^{-1}$)** | **Cu (mg kg$^{-1}$)** | **Mn (mg kg$^{-1}$)** | **Zn (mg kg$^{-1}$)** | **B (mg kg$^{-1}$)** |
| 0 | 106 ± 6.27 | 10.2 ± 0.48 | 20.5 ± 1.87 | 11.0 ± 0.49 | 75 ± 4.22 a |
| 50 | 107 ± 8.19 | 11.5 ± 0.79 | 23.8 ± 2.97 | 11.3 ± 0.73 | 73 ± 5.05 a |
| 125 | 103 ± 8.45 | 11.0 ± 0.78 | 27.9 ± 4.34 | 11.5 ± 1.34 | 67 ± 5.56 b |
| 250 | 101 ± 4.99 | 10.3 ± 0.26 | 29.3 ± 4.20 | 10.9 ± 0.49 | 66 ± 4.36 b |
| P value | NS | NS | NS | NS | P<0.01 |

NS: non-significant, ±: standard error mean

**Table 2.** Effects of nitrogen treatments on leaf photosynthetic performance of sweet cherry cv. 0900 Ziraat grafted on Gisela 5 rootstock and reaching full yield

| N doses (g/tree) | Tr mmol m$^{-2}$ s$^{-1}$ | A (µmol m$^{-2}$ s$^{-1}$) | Ci µmol mol$^{-1}$ | Gsw mol m$^{-2}$ s$^{-1}$ |
|---|---|---|---|---|
| 0 | 2.03 ± 0.31 a | 8.67 ± 0.80 a | 230 ±14.94 | 0.099 ± 0.016 a |
| 50 | 1.62 ± 0.12 b | 6.85 ± 0.38 b | 236 ± 8.70 | 0.074 ± 0.005 b |
| 125 | 1.19 ± 0.19 c | 5.96 ± 0.93 b | 223 ± 9.17 | 0.055 ± 0.008 bc |
| 250 | 0.91 ± 0.12 c | 4.34 ± 0.55 c | 216 ± 6.41 | 0.041 ± 0.006 c |
| P value | P<0.01 | P<0.01 | NS | P<0.01 |
| **N doses (g/tree)** | **Gtw mol m$^{-2}$ s$^{-1}$** | **Gtc mol m$^{-2}$ s$^{-1}$** | **WUE** | **ICE** |
| 0 | 0.096 ± 0.015 a | 0.060 ± 0.009 a | 4.61 ±0.42 | 0.038 ±0.002 a |
| 50 | 0.073 ± 0.005 b | 0.046 ±0.003 b | 4.35 ± 0.21 | 0.029 ± 0.002 b |
| 125 | 0.054 ± 0.008 bc | 0.034 ±0.005 bc | 5.38 ± 0.85 | 0.028 ± 0.005 b |
| 250 | 0.041 ± 0.006 c | 0.026 ±0.003 c | 4.76 ± 0.11 | 0.020 ±0.002 c |
| P value | P<0.01 | P<0.01 | NS | P<0.01 |

NS: non-significant, ±: standard error mean, A: assimilation rate, Ci: concentration of intercellular $CO_2$, Tr: transpiration rate, Gsw: stomatal conductance to water vapor, Gtc: total conductance to $CO_2$, Gtw: total conductance to water vapor, WUE: water use efficiency, ICE: instantaneous carboxylation efficiency

Statistical analysis: Data means were separated using one-way ANOVA with "JMP© 8.0" (SAS Institute, Inc.) according to LSD (Least Square Difference). Statistical differences based on $P<0.05$ and $P<0.01$. In addition, with pairwise correlations between the nutrients and physiological parameters were examined.

## Results and Discussion

Nitrogen fertilization applied to the soil at different doses affected N, P, K, Ca, Mg and B leaf accumulations. As N doses increased, N concentration of leaves increased, but decreased nutrients such as P, K and B. While the highest N values (2.43%) were obtained from the highest N dose (250 g N/tree), the lowest values (1.84%) resulted from the lowest N dose (0 g N/tree). The situation contrasted with P, K and B concentrations of leaves and the highest values of P, K and B were obtained at the lowest N dose. In other words, while regression between leaf N and increasing N doses was linear and positive, it was linear and negative for P, K, B. Ca, and Mg had the same trend and we obtained the highest values of Ca and Mg at 0 g N/tree and 250 g N/tree treatments (Table 1). Assimilation rate (A), transpiration rate (Tr), stomatal conductance to water vapor (Gsw), total conductance to $CO_2$ (Gtc), and instantaneous carboxylation efficiency (ICE) were also affected from increasing N doses and while the highest values were determined at the lowest N dose, and the lowest ones were in the highest N dose (Table 2). According to correlation analysis, of all nutrients, N, P, K and Mg had effect on gas exchange and leaf photosynthetic performance. While correlations were negative for leaf N and Mg concentrations, they were positive for P and K (Table 3).

Fallahi et al. (2001a), Klein (2002), Prsa et al. (2007) and Souza et al. (2013) reported that as N supply increase, it results in high N concentration in the leaves. Fallahi et al. (1984) reported that they fertilized apple trees with N applied to the soil and determined increasing Mg concentration and reducing K and P concentration in apple leaves. Neilsen et al. (1984) realized N, which is applied from soil at different doses, increased the N and Mn concentration of the apple leaves. Klein et al. (1989) informed that fertilizer N applied to the soil reduced significantly the K amount of soil solution in top soil layer (0-30 cm) and the according to increasing N dose, N concentration of leaf increased. Neilsen et al. (1999) found that the N concentration of the leaves and fruits increased with increasing of applied N in the apple orchard, but the P concentration of the fruits and the K concentration of the leaves and fruits decreased. Yang et al. (2015) determined a significant negative correlation between the N and B concentrations in the leaves of litchi trees. Uçgun & Altindal (2021) determined that as N fertilization applied from soil increased, the nutrient levels of sweet cherry leaves changed with increasing N and Mn and decreasing P, K and B concentrations.

Cheng & Fuchigami (2000) determined that the

**Table 3.** Correlations between nutrient concentrations of leaf and photosynthetic performance of leaf of sweet cherry cv. 0900 Ziraat grafted on Gisela 5 rootstock and reaching full yield

| | Tr | A | Ci | Gsw | Gtw | Gtc | WUE | ICE |
|---|---|---|---|---|---|---|---|---|
| **N** | -0.62** | -0.67** | -0.15 | -0.61** | -0.61** | -0.61** | 0.16 | -0.66** |
| **P** | 0.48* | 0.60** | -0.16 | 0.50* | 0.50* | 0.50* | 0.05 | 0.70** |
| **K** | 0.48* | 0.55** | 0.11 | 0.50* | 0.50* | 0.50* | 0.10 | 0.49* |
| **Ca** | -0.20 | -0.08 | -0.30 | -0.18 | -0.18 | -0.18 | 0.14 | 0.09 |
| **Mg** | -0.53** | -0.40* | -0.49* | -0.53** | -0.53** | -0.53** | 0.18 | -0.13 |
| **Fe** | -0.24 | -0.15 | -0.01 | -0.18 | -0.19 | -0.19 | 0.38 | -0.15 |
| **Cu** | -0.16 | -0.19 | -0.05 | -0.19 | -0.19 | -0.19 | 0.05 | -0.18 |
| **Mn** | -0.14 | -0.32 | 0.02 | -0.18 | -0.18 | -0.18 | -0.31 | -0.36 |
| **Zn** | -0.17 | -0.32 | 0.07 | -0.18 | -0.18 | -0.18 | -0.22 | -0.30 |
| **B** | 0.23 | 0.23 | 0.39 | 0.31 | 0.31 | 0.31 | 0.09 | 0.09 |

A: assimilation rate, Ci: concentration of intercellular $CO_2$, Tr: transpiration rate, Gsw: stomatal conductance to water vapor, Gtc: total conductance to $CO_2$, Gtw: total conductance to water vapor, WUE: water use efficiency, ICE: instantaneous carboxylation efficiency calculated intercellular $CO_2$ decreased with increasing leaf N and found curvilinear relationship between leaf N concentration and photosynthetic capacity in apple leaves. Tóth et al. (2002) performed a study to determined effect of the different N doses (30, 60, 90, 120 and 150 N kg/ha) on photosynthesis of in maize plants and found no significant differences. Cechin & De Fátima Fumis (2004) obtained that the $CO_2$ assimilation of the sunflower leaves for photosynthesis was remarkably increased by high nitrogen supply. N did not affect statically stomatal conductance, but high-N grown plants had lower intercellular $CO_2$ concentration. Reddy et al. (1996) characterized net photosynthetic rate, stomatal conductance and transpiration of cotton were positively correlated with leaf N concentration. Prsa et al. (2007) stated that the treatment with 80 kg N/ha (recommended dose in integrated apple production) had no or little effect on physiological parameters according to control (no fertilizer).

Hu et al. (2016) proved non-stomatal factors such as chlorophyll and decreased carboxylation efficiency supervised photosynthesis level when K was deficiency. Basile et al. (2003) determined that leaf potassium concentration didn't affect stomatal conductance significantly and leaves having low potassium had the highest calculated internal $CO_2$ concentrations. Zhao et al. (2001) stated that photosynthetic rate of cotton (*Gossypium hirsutum* L.) grown in K deficiency-environment was only 23% of the control plants receiving a full K supply. It was mainly associated with mainly low chlorophyll concentration, poor chloroplast ultrastructure, and restricted saccharide translocation. There was no relationship between stomata conductance and photosynthetic rate. Fallahi et al. (2001b) revealed the scion leaf net photosynthesis and leaf mineral concentrations were affected by rootstock. Bud.9 rootstock had lower net photosynthesis, higher Ca and Mn but lower K concentrations than those on the other rootstocks. Bednarz & Oosterhuis (1999) indicated that reductions in leaf physiological processes and growth of cotton plants occur after the petiole K concentration fell below 0.88% on a dry weight basis. According to Terry & Ulrich (1974)'s results, low K apparently decreased photosynthesis through an increase in mesophyll resistance to $CO_2$ (rm). Kanai et al. (2007) stated that K had a positive effect on biomass of tomato plants and K deficiency decreased severely biomass of all organs and depressed leaf photosynthesis and transport of $^{13}C$ assimilates. Behboudian & Anderson (1990) also showed that K deficiency caused lower rate of photosynthesis in tomato plants. This decreasing effect in -K leaves were due to impairment of photosynthetic capacity and not to stomatal closure. Peaslee & Moss (1966) stated that photosynthetic capacity in maize leaves were primarily associated with leaf K concentration and the critical level was about 2 mg/g for K in fresh weight. Normal-appearing leaves living K deficiency showed a sharply decreasing in photosynthesis rates. Reddy & Zhao (2005) determined photosynthesis rate decreased in cotton with decreasing K levels.

In the study of Fujita et al. (2003), P-deficiency treatment affected negatively leaf photosynthesis, stomatal conductance of tomato plants. Kondracka & Rychter (1997) stated that phosphate deficiency affects plant growth and the rate of photosynthesis. Bernardi et al. (2015) evaluated the effect of N, P and K fertilizing on gas exchange and leaf photosynthetic performance in sweet orange. The results indicated that photosynthesis rate was depressed by the high levels of N, improved by K at intermediate fertilization levels and affected a little by P. Li et al. (2021) studied the nutrient uptake and distribution in mycorrhizal cuttings of Populus × canadensis 'Neva' under drought stress and determined that gas exchange parameters positively correlated with the concentrations of leaf P, K, Ca, Fe, Mn, Cu, and Zn while negatively with N.

In many studies as mentioned above, it was revealed nutrients have positive or negative effects on gas exchange and gas exchange and leaf photosynthetic performance. These effects may occur directly or indirectly. The excessive or deficiency of a mineral element affects some enzyme activities and hormone syntheses and these enzymes and hormones regulate some physiological process affecting gas exchange and leaf photosynthetic performance.

## Conclusion

While N fertilization affected positively the leaf accumulation of N, its effect was negative on the leaf accumulation of P and K. As for Tr, A, Gsw, Gtw, Gtc, and ICE, they decreased with increasing N fertilizing. There were negative correlations between decreasing gas exchange and leaf photosynthetic performance and increasing leaf N level, but contrarily for P and K. It is known that there is antagonistic and synergic interaction between mineral nutrients. We determined an antagonistic effect of N fertilization applied to the soil on the accumulation of P and K. It shouldn't be forgotten that overfertilizing with any nutrient causes environmental pollution, soil salinization (which also precludes the absorption of mineral nutrients), decreased yield, and decreases gas exchange and leaf photosynthetic performance. Increased fertilization also has a high cost decreasing profit margins for fruit producers.

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## Author Contributions

**KU:** Conceptualization, data curation, investigation, methodology, visualization, initial drafts, writing, review and editing; **BT:** data curation, investigation; **MC:** investigation; **MA:** investigation, writing, initial drafts, review and editing.

## Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper

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