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STABILIZATION OF NITROGEN METABOLISM IN CHICKPEA THROUGH FOLIAR FERTILIZATION

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ABSTRACT

It is well-known that nitrogen is essential for plant growth and development. Although chemical fertilization remains the main strategy to provide nitrogen requirements for majority of plants, legume crops including chickpea often have a natural symbiosis with bacteria for nitrogen fixation. Meanwhile, a source of molybdenum is still needed for activation of molybdoenzymes for nitrogen metabolism. However, this becomes an issue in some areas with molybdenum-deficient soil. Therefore, molybdenum supplementation is crucial for nitric oxide production in plants. Herein, chickpea plant samples were supplemented with nitrate and molybdenum through foliar fertilization to test nitrate and nitrite reductase activities. As a result, the enzymatic activities were shown to be very high after five days post-supplementation. This study demonstrates the need for molybdenum fertilizers in successful plant growth management.

Keywords: chickpea; nitrogen metabolism; molybdoenzymes; molybdenum; foliar fertilization

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most widely grown legume crop with 15 million tons of global production after dry beans and dry peas [1]. Chickpeas are large source of proteins, carbohydrates, fats, fibers as well as few bioactive compounds such as phenolic acid and isoflavones [2]. Among these, some nutrients provide the crop with high biopharmacological traits such as lipid-lowering, hypocholesterolemic, antioxidant, hypoglycemic and hypotensive properties [3]. Another interesting fact about chickpea is that it can obtain up to 85% of required nitrogen (N) from symbiotic N_2 fixation provided by a bacteria called *Rhizobium* [4]. The remaining sources are soil-based inorganic N, organic minerals, and fertilizers [5, 6]. Indeed, biofertilization by *Rhizobium* has been proven to be environmental-friendly solution to replace nitrate fertilizers [7]. In general, nitrogen metabolism in plants is regulated by nitrate reductase (NR) and nitrite reductase (NiR). However, these are inducible molybdoenzymes meaning they require not only their substrate but also molybdenum (Mo) in their active sites for activation [8, 9].

Mo is a rare transition element that has an essential significance for almost all biological systems due to its requirement for activation of enzymes, which catalyse several key reactions in carbon, sulfur and nitrogen metabolism [10]. Accordingly, deficiency of this particular element can cause severe consequences in growth of all kinds of plants including chickpea. Moreover, apart from nitrogen assimilation, there are other consequences of Mo deficiency in chickpea. To date, Mo stress decreases the seed germination, seedlings growth and even nutrient content [11]. Particularly, it can result in low amount of lysine and methionine content within chickpea proteins and therefore such product is not recommended for human consumption [12]. As it was mentioned in the previous paragraph, molybdoenzymes required for nitrogen assimilation in plants need a source of Mo for their activation. However, the Mo content in plants depends on the bioavailability of Mo in soils [13]. Therefore, there is a need for studies on potential applications of Mo fertilizers in regions with naturally Mo-deficient soil.

Herein, chickpea plant samples were treated with a source of nitrate and molybdate through foliar fertilization to test NR and NiR activities. For that reason, final products of their activities – nitrite and nitric oxide were detected after the supplementation. As a result, high level of enzymatic activities was absorbed demonstrating the importance of Mo-supplementation through foliar fertilization. This in turn would improve nitrogen metabolism in plants grown on molybdenum-deficient soils.

MATERIALS AND METHODS

Plant materials and foliar fertilization

The chickpea plants (Kyabra cultivar) were grown in growth room at 25 + 2°C and 16 h/8 h photoperiod under controlled environment using N-and-Mo-free soil. The leaves of 14-day-old chickpea seedlings were sprayed with 50 mM KNO_3 (Sigma-Aldrich, USA) and 5 µM Na_2MoO_4 (Sigma-Aldrich, USA). In addition, deionized water was sprayed on another group of plants, grown in different room with the same parameters, as non-supplemented controls. Crude extracts were collected from the leaves daily for next ten days post-supplementation for detection of nitrite and nitric oxide.

Nitrite and nitric oxide detection

For nitrite content determination, the crude extracts from plant leaves were centrifuged at 10,000 rpm. Then, 500 µL of 1% sulfanilamide (Glentham Life Sciences, UK) dissolved in 20% HCl (Sigma-Aldrich, USA) and 500 µl of 0.12% N-(1-Naphthyl) ethylenediamine dihydrochloride (Tokyo Chemical Industry, Japan) were added into the supernatant. The detection was performed using the method described by [14]. Colour intensity was determined spectrophotometrically at 548 nm. The quantitative value was determined using the construction of a calibration curve with approximations (R^2>0.95). Nitric oxide content was determined using a method described by [15] and was measured with nmol per gram of leave biomass. Standard deviation of three indepen-

dent experimental repeats were calculated for both NO_2^- and NO detection.

RESULTS AND DISCUSSION

NR is the first enzyme in nitrate nitrogen assimilation pathway in plants as it is responsible for nitrite production. The enzyme molecules start being highly synthesized 12-15 hours nitrate supplementation, and hours may vary depending on the plant species [16]. Meanwhile, optimal concentration of the supplementation ranges from 20 to 70 mM [17]. In addition, potassium nitrate has demonstrated a higher inductive effect on plant leaves rather than sodium nitrate most likely due to a potential role of K^+ ions in controlling the nitrate channel or influence of those ions on nitrate penetration into plant tissues [18]. As for the Mo supplementation, it has been claimed that the risk of disease development starts increasing in animal organisms consuming plants that grown in soil where molybdenum content is over 10 mg/kg [19]. Because of mentioned parameters above, 50 mM KNO_3 and 5 µM molybdate were used in this study to spray the plant leaves. Then, nitrite detection in the leaves was performed daily in 10-days period after the foliar fertilization (Figure 1).

The results illustrated in Figure 1 showed that NR activity started after 3 days post-supplementation (dps) forming around 164 ng nitrite quantity per gram of leave mass. Then, the nitrite production kept growing in plant tissues until it reached its pick at 673 ng after 5 dps. Notably, this value kept gradually decreasing after 6 dps and there was no NO_2^- content detected at 9 dps. The use of water-supplemented control proved that the detected high nitrite content was result of activated NR in plants due to enzyme substrate and Mo supplementation. Moreover, NiR activity test was conducted to explain the nitrite content disappearance within 9 dps as well as to check the complete nitrogen assimilation pathway in chickpea plants by determining NO content in the same samples (Figure 2).

The results of the NiR activity test showed that the NO production by the enzyme started a day after small amount of nitrite was formed. As expected, the synthesis of NO kept increasing day by day as NO_2^- content was also high between 4 and 6 dps, according to the data presented in Figure 1 and 2. However, at tenth day there was no more formed NO_2^- and NO detected at all. This could occur due to limited amount of enzymatic substrate and Mo supplementation at the starting

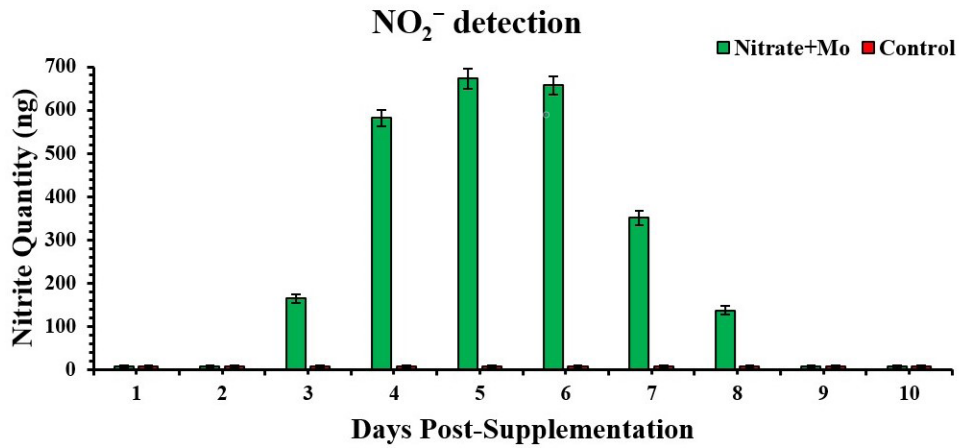

Figure 1. NR activity test by detection of its final product – NO_2^-. The plant leaves were supplemented with KNO_3 and Na_2MoO_4 by spraying. In addition, deionized water was sprayed instead of molybdate on the other plant leaves as a control. Crude extracts were collected daily for next ten days post-supplementation. The nitrite quantity was determined by correlating absorbance values to ng (per gram of leave mass) using a standard curve (R^2>0.95). Error bars represent standard deviation of technical triplicates.

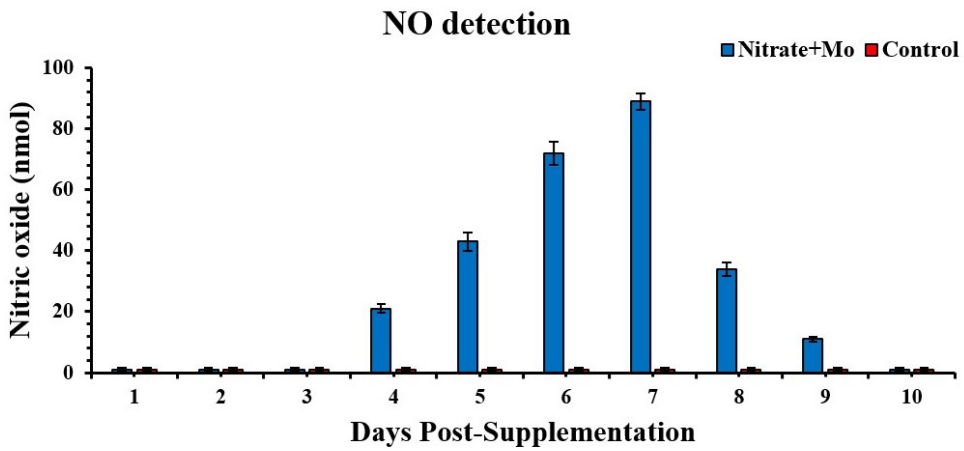

Figure 2. NiR activity test by detection of its final product – NO. The plant leaves were supplemented with KNO_3 and Na_2MoO_4 by spraying. In addition, deionized water was sprayed instead of molybdate on the other plant leaves as a control. Crude extracts were collected daily for next ten days post-supplementation. NO was measured with nmol per gram of leave mass using a method described by [15]. Error bars represent standard deviation of technical triplicates.

day of experiments.

According to earlier studies, the potential application of *Rhizobium* bacterial species could supply constant source of nitrogen to crops [4]. This sort of natural biofertilizers needs to be studied further to create reliable commercial products for large-scale agricultural industries. This study suggests Mo supplementation is also a key factor for nitrogen assimilation especially in plants that grown in areas with naturally less molybdenum content. As such, this must be taken into consideration in sustainable agriculture practices.

CONCLUSION

This study demonstrates that the chickpea plants can be supplemented with enough source of molybdenum through foliar fertilization for efficient level of nitrogen metabolism in chickpea. Such fertilizers are needed I n all agriculturally important areas with naturally molybdenum-deficient soil as the demand for global food security is increasing due to demographic growth on our planet. Taking into account that chickpea is one of the legume crops that can grow in a symbiosis with bacteria that provides a significant source of nitrogen, this biofertilization needs to be studied further in future for a potential nitrogen fixation solution for other crops. Thus, environmental-friendly fertilizers are in demand considering the chemical pollution consequences leading to global climate change.

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ЖАПЫРАҚТЫҚ ТЫҢАЙТУ АРҚЫЛЫ НОҚАТТАҒЫ АЗОТ АЛМАСУЫН ТҰРАҚТАНДЫРУ

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ТҮЙІН

Азот өсімдіктердің өсуі мен дамуы үшін өте қажет екені белгілі. Химиялық тыңайту өсімдіктердің көпшілігінің азотқа деген қажеттілігін қамтамасыз етудің негізгі стратегиясы болып қала берсе де, бұршақ дақылдары, соның ішінде ноқат азотты бекіту үшін жиі бактериялармен табиғи симбиозға ие. Бұл ретте молибден көзі азот алмасуына жауапты молибдоферменттерді белсендіру үшін әлі де қажет. Дегенмен, бұл молибден жетіспейтін топырағы бар кейбір аймақтарда мәселеге айналады. Сондықтан молибден қоспасы өсімдіктерде азот оксидін алу үшін өте маңызды. Мұнда ноқат өсімдігінің үлгілері нитрат пен нитритредуктаза белсенділігін тексеру үшін жапырақтық тыңайту арқылы нитрат пен молибденмен толықтырылды. Нәтижесінде, ферменттік белсенділік қосымша қабылдағаннан кейін бес күннен кейін өте жоғары екендігі көрсетілді. Бұл зерттеу өсімдіктің табысты өсуін басқару үшін молибден тыңайтқыштарының қажеттілігін көрсетеді.

Негізгі сөздер: ноқат; азот алмасуы; молибдоферменттер; молибден; жапырақтық тыңайту

УДК 581.19

СТАБИЛИЗАЦИЯ АЗОТНОГО ОБМЕНА У НУТА ЧЕРЕЗ ВНЕКОРНЕВУЮ ПОДКОРМКУ

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АННОТАЦИЯ

Хорошо известно, что азот необходим для роста и развития растений. Хотя химическое удобрение остается основной стратегией обеспечения потребности большинства растений в азоте, бобовые культуры, такие как нут, часто вступают в естественный симбиоз с бактериями для фиксации азота. В тоже время, для активации молибдоферментов азотистого обмена необходим источник молибдена. Однако это становится проблемой в некоторых районах с почвами с дефицитом молибдена. Таким образом, добавки молибдена имеют решающее значение для производства оксида азота растениями. В данном случае образцы растений нута были дополнены нитратом и молибденом посредством внекорневой подкормки для проверки активности нитрат- и нитритредуктазы. В результате было показано, что ферментативная активность очень высока через пять дней после приема добавки. Это исследование демонстрирует необходимость молибденовых удобрений для успешного управления ростом растений.

Ключевые слова: нут; азотистый обмен; молибдоферменты; молибден; внекорневая подкормка