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Effect of Atmospheric Nitrogen Dioxide on Mulukhiya (*Corchorus olitorius*) Growth and Flowering*

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Abstract: This study focuses on evaluation of the effect of NO_2 at ambient concentrations on vegetative growth and reproduction of mulukhiya (*Corchorus olitorius*) plants. Treatment of mulukhiya plants with 50 ppb NO_2, in a controlled chamber, promoted vegetative growth and flowering as well. Treated plants produced more biomass and flowered early, whereas the control plants produced less biomass and flowered late. This result indicates that ambient NO_2 boost plants' growth parameters and enhance development. NO_2 may act directly as an external signaling molecule to stimulate flowering and/or indirectly to promote leaf growth and hence affect the flowering signals that moves from the leaf through the phloem to the shoot apical meristem (SAM) leading to the overall performance of plant development and reproduction.

Key words: Mulukhiya, nitrogen dioxide, growth, flowering, fumigation

INTRODUCTION

Flowering in plants is controlled internally and externally by developmental and environmental signals. Genetic studies of the timing of flowering in *Arabidopsis thaliana* have revealed four major controlling pathways. The photoperiod and vernalization pathways integrate environmental signals into the floral decision, whereas the autonomous and gibberellin (GA) pathways act independently of environmental cues (Simpson and Dean, 2002). Recently, Tamaki *et al*. (2007) and Corbesier *et al*. (2007) proved that florigen, the signal that tells a plant to flower, is a protein that moves from leaves through the phloem to induce flowering in the shoot apical meristem (SAM). Exogenously supplied NO_2 plays important roles in plant growth and metabolism (Morikawa *et al*., 2004; Takahashi *et al*., 2005, 2006; Adam *et al*., 2008).

In contrast to the general view that NO_2 acts as a destructive gas, at ambient levels it has been found to stimulate vegetative growth of plants such as *Nicotiana plumbaginfolia* and horticultural plants including lettuce, sunflower, cucumber and pumpkin by acting as a signaling molecule rather than as a significant nutrient source (Takahashi *et al*., 2005; Adam *et al*., 2008). The effect of atmospheric NO_2 on decontamination of cadmium by kenaf (*Hibiscus cannabinus*) has been investigated (Takahashi *et al*., 2008). Growing of kenaf seedlings in air supplemented with NO_2 increased the cadmium content per stem by more than 30% than that in plants grown in NO_2 free air (Takahashi *et al*., 2008).

Studies on the effects of NO_2 on plants have mainly concentrated on measuring changes in vegetative growth parameters and much less are known about alteration and phenology of reproductive organs. Regulation of flowering by NO_2 has not previously been reported.

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In this study, the relationship between the pollutant NO_2 and the floral initiation in mulukhiya (*Corchorus olitorius*) has been investigated. Mulukhiya, [also called moroheiya in Japanese], is a nutritious vegetable known throughout the world, especially in the Middle East and Africa. While investigating the effects of NO_2 on this vegetable species, we found that fumigation with 50 ppb NO_2 stimulates its vegetative growth as well as flowering initiation. Present findings suggest that NO_2 may act on mulukhiya by modulating the flowering regulator(s) directly as an external signal or indirectly by modulating the endogenous flowering stimulus.

MATERIALS AND METHODS

This study was carried out at the Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Japan from March 2005 to March 2007.

Mulukhiya seeds were sown in plastic pots containing perlite and vermiculite (v/v) and then divided into two groups. Each group was placed in a growth chamber, one supplied with 50 ppb NO_2 and the other NO_2-free as described by Adam *et al*. (2008) and Takahashi *et al*. (2005). Double chambers were used (Fig. 1). Briefly, two glass-walled NO_2-fumigation chambers (1.5 · 1 · 0.7 m in width, height and depth; model HM1500; Nippon Medical and Chemical Instruments Co., Osaka, Japan) were placed in a confined glasshouse (6.9 · 2.4 · 3.0 m in width, height and depth; model BTH-P1-TH; Nippon Medical and Chemical Instruments Co.). The air entering the glasshouse (at a rate of 4 m^3 min^{-1}) from outside was scrubbed of NO, NO_2 and O_3 by using a scrubber with an activated charcoal and $KMnO_4$ system (Purelite, Nippon Puretec Co., Tokyo, Japan) as described by Wildt *et al*. (1997) so that the NO, NO_2 and O_3 concentrations in the glasshouse were kept at <5 nL L^{-1}. Temperature and relative humidity in the glasshouse were controlled at 22±0.3°C and 70±4%, respectively, while CO_2 concentration was kept at 340±80 μl L^{-1} by a climate control system (CTH-G 100; Nippon Medical and Chemical Instruments Co.). Air entering the +NO_2 chamber (at a rate of 1 L min^{-1}) was supplemented with NO_2. The final concentration of NO_2 in the chamber was kept at 50±10 nl L^{-1}. Air entering the -NO_2 chamber at the same rate as that for +NO_2 chamber

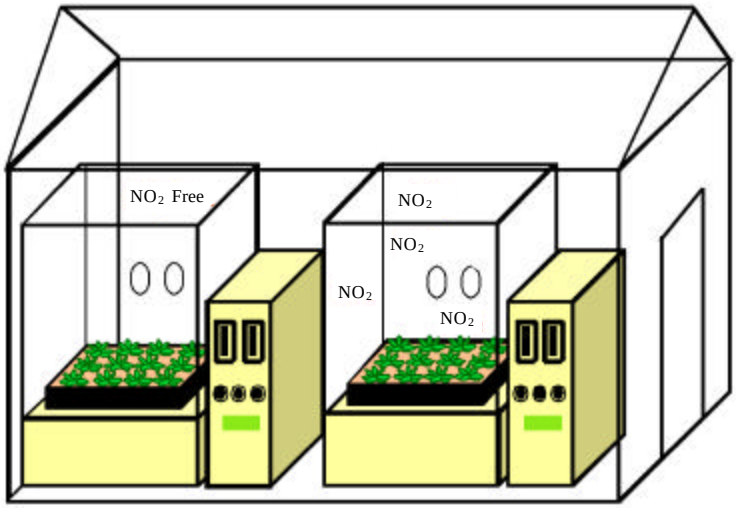

Fig. 1: Sketch describes the fumigation chambers used in this research

was not supplemented with NO_2 but was kept at <5 nL L^{-1} NO_2. Both chambers were equipped with independent climate control systems (CTH-C 70; Nippon Medical and Chemical Instruments Co.) to maintain temperature, CO_2 concentration and relative humidity at the same values as those for the glasshouse.

Plants were grown for 6 weeks, irrigated with half-strength MS medium (Murashige and Skoog, 1962). The numbers of flowers and flower buds were counted at the time of harvest. Shoot biomass and root biomass were measured after the samples were freeze-dried. Total leaf area and stem length of each sample were measured.

For further analysis of flowering time and seed formation, experiments under the same conditions were performed for a longer time (about 9 weeks) to allow complete flowering and seed setting. Statistical significance of the data was evaluated using student's t-test.

RESULTS AND DISCUSSION

Treatment with NO_2 for a prolonged period (6 weeks) at a concentration of 50 ppb activated flowering and seed formation in mulukhiya (Fig. 2). Moreover, the treatment stimulated all measured growth parameters (Fig. 3). Shoot and root biomass were increased in treated plants compared to the controls and similar results were obtained with total leaf area and stem length. However, there was no difference in the number of leaves.

Fig. 2: NO_2 enhances mulukhiya growth and development. (A) A photograph of plants fumigated treated with 50 ppb NO_2 (right) for 6 weeks and the controls (NO_x <5 ppb) (left). (B) 9 weeks-old pods of the fumigated treated (right) and the control plants (left). (C) The relative maturity of the seeds of the treated plants (top) and the controls (bottom). Bar, 1 cm

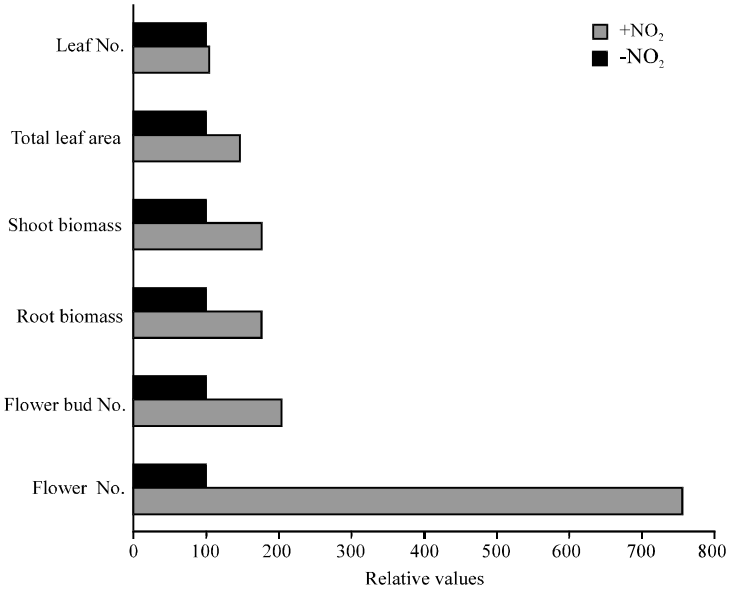

Fig. 3: Effects of NO_2 treatment on the growth and flowering of mulukhiya plants. Shown are leaf number, total leaf area, shoot biomass, root biomass, flower bud number and flower number from plants treated with 50 ppb NO_2 for 6 weeks under natural lighting and irrigation with a medium containing 10 mM KNO_3. Values for treated plants (grey columns) are normalized relative to a value of 100 for the control plants (black columns). Control plants had leaf number 9.40±1.64, total leaf area 30.58±7.80 (cm^2), shoot biomass 0.05±0.02 (g), root biomass 0.007±0.003 (g), flower bud number 2.10±0.74 and flower number 0.01±0.32 (n = 8, except for the total leaf area n = 3)

During the 6 weeks, treated plants started to flower earlier than the control plants. At the time of harvest, 7 out of 8 plants had at least one open flower. The mean value of the flowers number was 0.8 for the treated plants and 0.1 for the control plants and all plants had a larger number of flower buds (4.25) compared to the controls (2.1) with no open flowers (Fig. 2, 3).

Shoot and root biomass showed a significant increase upon treatment: 0.1 and 0.01 g for shoot and root, respectively, while the controls showed 0.05 and 0.007 g for shoot and root, respectively (Fig. 3), which is in a general agreement with the previous reports (Morikawa *et al*., 2004; Takahashi *et al*., 2005; Adam *et al*., 2008) that showed NO_2 stimulates plant growth.

The number of leaves was not affected by NO_2. The number of leaves produced on the primary shoot before the first flower is initiated is a standard indicator for flowering time. Plants with more leaves flower later than those with fewer leaves (He *et al*., 2004). However, the total leaf area increased upon treatment with NO_2. The growth conditions of the plants generally and leaves in particular might have a direct effect on the flowering decision, in addition to the other factors that control flowering time. Hence, while the direct flowering signal moves from leaves to SAM, the effect of NO_2 on flowering time might be by indirectly affecting leaf growth and the floral stimulus that is produced in the leaves, although Corbesier and Coupland (2006) stated that early flowering is associated with small leaves. Another possibility is that NO_2 might directly affect the floral decision center to speed up this process, or it may interacts with or be integrated with flowering pathways to regulate the floral stimulus. In contrast, Nitric Oxide (NO) suppresses flowering of Arabidopsis plants by affecting the expression of regulatory genes in flowering pathways (He *et al*., 2004).

In conclusion, our results indicated that 50 ppb NO_2 promotes not only the vegetative growth but also accelerates flowering and reproduction in mulukhiya. The importance of this observation lies on the fact that keeping all generally considered harmful gases at their naturally balanced states remediates the environment devastation threats. However, the mechanism by which NO_2 modulates flowering time remains to be investigated. Further study will uncover the mechanism behind these results and support the hypothesis that NO_2 is capable of significant regulation of the plant life cycle.

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