Nitrate concentration and accumulation on vegetables related to altitude and sunlight intensity

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Abstract. Nitrate absorption and concentration in consumed vegetables should be also interrelated with its quality, where it is now less noticed in standard vegetable quality. The higher nitrate content is associated with the human health impact such as the baby blue syndrome and stimulating the tumor growth. The environmental status on especially sunlight intensity and quality has a special role to control nitrate concentration in the leaves. The purpose of this study was to characterize the nitrate concentration and accumulation in \textit{Brassica rapa} \textit{L.} grown in an open field and screen shading at lower and medium altitudes. Plant sampling was arranged at lower altitudes under 500 m asl (meters above sea level) and medium altitudes 500-700 m asl. Plant growth under shading and unshaded condition were observed for nitrate status as absorbed and its concentration in the sap plants. The study site at the lower altitude exposed sunlight intensity by 27.2% higher, where it decreased nitrate accumulation by 39.3%. Nitrate accumulation under shaded condition rate of 54.9% exhibited more 17.7% nitrate concentration in the vegetable.

1. Introduction
Nitrate is one of the forms of nitrogen that are available more in well-drained soils. Therefore, in terrestrial aerobic soil, \textsubscript{NO}3\textsuperscript{-} is the primary form of nitrogen absorbed by vegetation. Nitrogen is a significant nutrient in plants is required in large quantities for the entire growth process, especially in vegetative growth. However, it can cause adverse environmental impacts due to leaching, volatilization, denitrification, and surface flow. Environmental considerations on human health, including the consumption of vegetable crops, especially leafy vegetables, can accumulate nitrates over the safe limits for human beings but not for the plants themselves. Excessive nitrogen fertilization, light intensity, temperature, cultivation methods, fertilizers used, and post-harvest storage play a significant role in nitrate accumulation in vegetable crops.

The presence of nitrates correlates with the risk of stomach cancer caused by the formation of endogenous compounds N-nitroso [1]. Nitrate is relatively non-toxic, but 5% of its absorption is transformed into nitrates in the digestion metabolism. Nitrites can react with hemoglobin and become methemoglobin. The impact of methemoglobin formation is the disruption of oxygen distribution to the body’s tissues. Methaemoglobin cannot react with oxygen and causes hypoxemia. Blue skin discoloration due to the existence of deoxygenated blood. This condition is commonly called methemoglobinemia or blue baby syndrome [2,3,4]. Nitrates are naturally found with higher concentrations in certain vegetables rather than in fruit products [5]. The evidence of nitrate with a
variable concentration in various vegetable plants related to the agroecological ecosystem and culture [6,7,8,9,10,11] has a consequence of managing the product output due to the human health consideration. About 30 – 90% of the overall nitrate intake is sourced from vegetables, especially leafy vegetables. Regarding potential mechanisms of toxicity, international organizations for food safety issues had established a cut-off point for the effects of nitrates. Hence the EFSA and the WHO as well recommended an acceptable daily intake in the value of 3.7 mg kg$^{-1}$ body weight/day [9,12, 13].

*Brassica rapa* L. with local names could be caisim, cainsin, or choy sum, belongs to the vegetable plant of the family Brassicaceae. The plant can be cultivated from lowland to highland, but it is usually cultivated in areas of 100 - 500 m above sea level. Some of them are cultivated at 500-800 m above sea level. A lower intensity of sunlight reduces the capacity of the plant to reduce the absorbed nitrate to ammonium. In this case, ammonium is furtherly available for physiological metabolism in the plant. An additional artificial green-light as a supplement under blue and red light promoted more efficiency in plant nutritional metabolism to improve net photosynthetic rates and maximal photochemical efficiency [14]. In the open field without an ancillary appropriate continuous lightning source combined with minimum sunlight flux, absorbed nitrate should be more accumulated in the plant.

For vegetables’ nitrate absorption reduction purposes, split application during the plant growth stages was recommended [15]. Another technique to minimize nitrate absorption and accumulation in vegetables was incorporating an osmolyte alleviating plant stress such as glycine betaine into the plant growth media [16]. In the growing stage, the nitrate accumulation development tended to increase daily for lettuce, but fastly to decrease by kale [17]. Additional organic manure application on vegetables reduced the nitrate loss due to emission and reduced the fertilizer application regarding nitrate absorption optimization [18]. Additional continuous LED light to inhibit nitrate concentration in vegetables should consist of 90% red light and 10% blue light [19]. The quantification of nitrate status in *Brassica rapa* in a lower sunlight intensity will be discussed furtherly.

The purpose of this study covered the identification of the growth, yield, and nitrate concentration and accumulation in leafy vegetable *Brassica rapa* L. grown in shade conditions with less sunlight intensity and in open field culture. On both conditions were differentiated between the vegetable growth at lower altitudes and medium altitudes. Moreover, it was also to confirm the development of nitrate concentration and accumulation at the post-harvest time.

2. Methodology

The field experiment was arranged in lowland or lower altitude levels 200 – 500 m above sea level (asl). This lower altitude was compared with medium altitude at the level of 500–700 m asl. Three locations at each level category were taken as replication. All of the six study sites were situated in Bogor. At each site location, *Brassica rapa* was planted and observed at open condition without any shading and compared with under shading of plastic greenhouse installation. This multisite experiment was conducted from June to August 2013. The plant observation included field growth observation and post-harvest observation. Post-harvest plant samples were stored at the Post-Harvest Laboratory of the Department of Agronomy and Horticulture, Bogor Agricultural University.

After land clearing and preparation, chicken manure in the dosage of 20 kg was applied for the strip plots area of 18 m$^2$ (12 m x 1.5 m). The shading level of planting in the plastic greenhouse was approximately 50%.

Tosakan varieties aged 18 - 20 days were set with a space distance planting of 20 cm x 20 cm. Weeding and watering were carried out appropriately. Harvesting was succeeded in the 3rd week after planting at each location, wherein the second week, ten plant samples were taken to observe growth, yield, and nitrate status. GPS Garmin-Map76CSX was applied to determine altitude, Horiba Cardy ion meter C-141 was used for nitrate analyses and lightmeter (T-TOOL-1634) HS1010 in adjustment with Licor Li-250, Licor Li-200, and Li-210, to measure the sunlight intensity.

The experimental parameters included vegetative growth of plants, moisture content, and nitrate concentrations in fresh plants sap. Observation at the post-harvest time was conducted at 0-1-2-3 days
after harvest, where the vegetables were stored in the laboratory at room temperature. Moisture content was obtained from wet weight measurements and dry weights at a temperature of 105°C for 24 hours.

3. Result and discussion

After two weeks, the growth performance of *Brassica rapa* was supported better in lower altitude, where the plant height, leaf length, leaf width, and even in biomass yield above the ground. The vegetative parameters showed ranging 1.3-1.7 times higher in the measurements. Nitrate concentration in the sap plant leaf at the lower altitude of 200-500 m asl exposed ranging in a value of 2000–3044 ppm with an average value of 2522 ppm. The nitrate concentration in the medium altitude was found higher, with an average value of 3507 ppm. The nitrate accumulation in the plant was also found higher in the higher altitude with ranging values of 2969 – 3541 mg kg⁻¹ and a median value of 3255 mg kg⁻¹ instead of in the less elevation level, which reached the average value only 2336 mg kg⁻¹. There is a standard quality for vegetables in European Union, which regulates that the maximum concentration in iceberg lettuce vegetables was set at 2000 ppm grown in the open field and 2500 ppm under shading or cover [13]. The relation between altitude level and plant vegetative performance, yield, and nitrate status of *Brassica Rapa* L in 2 and 3 weeks after planting is presented in Table 1.

Compared to the growth in the second week, the growth acceleration of the *Brassica rapa* plant in the third week in the higher altitude showed a much better measurement value, where its value showed 166% on plant height, 152% on leaf length, even 236% on biomass yield. On the other side, the plant's vegetative growth in the lower altitude showed a relatively stagnant value. Plant in the lower altitude showed a faster maturity process than plants in the higher altitude.

In the third week, the average nitrate concentration in all altitude levels showed a lower value than in the second week. However, in this harvest time, the effect of altitude group exposed no significant difference on final vegetative growth parameters, yield and edible part of the vegetable, nitrate concentration, and nitrate accumulation. The nitrate concentration and accumulation difference at both altitudes in the third week showed a convergent result value.

| Vegetative performance/ yield/nitrate status | 2 weeks after planting** | 3 weeks after planting** |
|---------------------------------------------|--------------------------|--------------------------|
|                                             | Lower altitude | Medium altitude | Lower altitude | Medium altitude |
| Plant height (cm)                           | 27.2a           | 17.4b            | 27.2a           | 28.9a           |
| Leaves number /plant                        | 4.8a           | 4.3a             | 5.7a           | 5.3a           |
| Leaf length (cm)                            | 25.5a           | 18.0b            | 25.0a           | 27.4a           |
| Leaf width (cm)                             | 9.0 a           | 6.9b             | 7.9a           | 10.1a           |
| Biomass yield (g)                           | 17.5a           | 10.5b            | 19.8a           | 24.8a           |
| Water content (%)                           | 92.4a           | 92.7a            | 97.9a           | 92.0a           |
| Edible part/plant (g)                       | -***           | -***             | -***           | -***           |
| Sap nitrate concentration (ppm)             | 2 522a          | 3 507b           | 2 438a          | 2 245a          |
| Nitrate content/plant (mg kg⁻¹)             | 2 336a          | 3 255a           | 2 388a          | 2 066a          |

*Lower altitude 200-500 m asl, medium-altitude 500-700 m asl
**Unequal indexed letters (a,b) at the same row and plant age represent a significant difference level at p=0.05
***Still in vegetative growth, not harvested

The response of the vegetable plant related to shading treatment under plastic greenhouse shading in approximately 50% shading level was similar to the effect of altitude levels in the second week after two weeks in the field.

The shading significantly impacted the vegetative growth components plant height, leaf length, and biomass yield above the ground. The vegetative parameters showed approximately 1.2-1.3 times higher
in the measurements. Nitrate concentration in the sap plant leaf at the shading treatment exposed an average value of 3303 ppm. This value is higher than the nitrate concentration at open field culture, where without shading, it reached an average value of only 2727 ppm. The nitrate accumulation in the plant was also higher under the greenhouse culture, with a median value of 3077 mg kg$^{-1}$.

The plant vegetative performance, yield, and nitrate status of *Brassica rapa* L in 2 and 3 weeks after planting related to the growth at open field culture or greenhouse shading is presented in Table 2.

The growth of *Brassica rapa* related to the shading and unshaded treatment in the third week showed almost similar with the effect of in the altitude levels, wherein the third week there was no significant difference on the vegetative growth and yield components including edible part of the plants, except the effect on plant height. Under shading conditions, the plants grew under the lower intensity of sunlight, where the plant underwent an etiolation process, in which the plant height under shaded conditions increased by 20%. The intensity of light captured by plants affects vegetative growth and the concentration of nitrates accumulated by plants. Plants in open land get more intensity of sunlight than shaded plants. The nitrate reductase enzyme requires the appropriate light intensity to reduce nitrates in the plants. The greater the intensity that plants capture, the higher the nitrate reduction. Additional red-blue-green light in short-term continuous lightning in 24 or 48 hours significantly diminished nitrate content in vegetable leaves [20]. Lower sunlight intensity with lower PAR-value increased nitrate accumulation in the plant and its productivity [21], even though it was still capable of growing at a PAR value of 160 μmol m$^{-2}$ s$^{-1}$[22]. The reduction of sunlight intensity at PAR exposure of 100 μmol m$^{-2}$ s$^{-1}$ increased nitrate content in the leaf up to 3.4 times higher and NO$^2$ contents 34 times higher than under 200 μmol m$^{-2}$ s$^{-1}$[23]. Additional ultraviolet and far-infrared light were capable of inhibiting the nitrate building in the vegetables [24]. As a result, nitrate concentrations in shaded plants are higher than those grown in open land.

In the third week, the average nitrate concentration and nitrate accumulation in the plant under shading conditions showed accordingly with the measurement values in the second week. Under shading conditions, nitrate concentration was 2759 ppm, and the accumulation reached 2452 mg kg$^{-1}$. These values were approximately 25% higher than the measurement value at open field culture. Both nitrate concentration and accumulation in the third week showed lower values compared to the second week. During the growing process from the second week to the third week, the plant was capable of reducing nitrate to ammonium for the further growth metabolism of the plant. However, the total nitrogen content in the harvest time was not analyzed to further identification of nitrogen status in the plant.

**Table 2.** Vegetative performance, yield, and nitrate status of *Brassica rapa* L at 2 and 3 weeks after planting (WAP) under shading of plastic greenhouse* and unshaded condition.

| Vegetative performance/ yield/nitrate status | 2 Weeks After Planting** | 3 Weeks After Planting** |
|---------------------------------------------|--------------------------|-------------------------|
| **Field open culture** | **Shading** | **Field open culture** | **Shading** |
| Plant height (cm) | 20.4a | 24.2b | 25.3a | 30.9b |
| Leaves number /plant | 4.7a | 4.5a | 5.5a | 5.5a |
| Leaf length (cm) | 19.0a | 24.5b | 29.2a | 23.3a |
| Leaf width (cm) | 7.3a | 8.6a | 9.5a | 8.5a |
| Biomass yield (g) | 12.1a | 15.9a | 23.7a | 20.9a |
| Water content (%) | 91.9a | 93.2a | 95.0a | 94.9a |
| Edible part/plant (g) | **.....** | **.....** | 20.8a | 19.0a |
| Sap nitrate concentration (mg kg$^{-1}$) | 2.727a | 3.303b | 2.104a | 2.579a |
| Nitrate content/plant (mg kg$^{-1}$) | 2.531a | 3.077b | 2.001a | 2.452a |

*Approximately 50% shading level under plastic greenhouse construction

**Unequal indexed letters(a,b) at the same row and plant age represent a significant difference level at p=0.05
In the post-harvest time, the nitrate concentration in the vegetable sap increased significantly day by day. Vegetable from the open culture containing only 2210 ppm at the harvest time, the concentration in room temperature increased to 2347 ppm the first day, 2350 ppm on the second day, and 2853 ppm on the third day. The harvested vegetable from the shading in the greenhouse showed nitrate concentration in the value of 2667 ppm. After that, it increased to 2753, 2789, and 3360 ppm in the first until third days, respectively. For the consumption purpose, the nitrate accumulation was about 7-9% lower than the concentration in the sap plants since the water content in the vegetable ranging 90-95% of the fresh weight. The daily development nitrate concentration and accumulation of harvested *Brassica rapa* L from shading of Plastic Greenhouse and unshaded condition are presented in Table 3.

Since the nitrate source from this vegetable is about 70% of other consumed food and the recommendation of acceptable daily intake of 3.7 mg kg\(^{-1}\) personal weight day\(^{-1}\) [12], the consumption of a 70kg personal weight should not be over 259 mg nitrate per day. This result represents the highest daily consumption of 89 g of *Brassica rapa* growth at an open field, but only 73 g grown in shading condition, even only 61 g three days later, if the vegetable is stored at room temperature. At the post-harvest time, the nitrate concentration and accumulation increased day by day.

**Table 3.** The daily nitrate development of harvested *Brassica rapa* L from shading under plastic greenhouse* und unshaded condition.

| Vegetative performance/ yield/nitrate status | Nitrate accumulation in the post-harvest day | 0 | 1 | 2 | 3 |
|---------------------------------------------|---------------------------------------------|---|---|---|---|
|                                             | Nitrate concentration in the vegetable sap (mg kg\(^{-1}\)) | 2210a | 2347a | 2350a | 2853a |
| Open field culture                          |                                             | 2667b | 2753b | 2789b | 3360b |
| Plastic Greenhouse                          | **Unequal indexed letters(a,b) at the same column for concentration and at the same column for accumulation represent a significant difference level at p=0.05** | 2028a | 2125a | 2103a | 2519a |
| Open field culture                          |                                             | 2462b | 2502b | 2518b | 2942b |
| Plastic Greenhouse                          |                                             | **4. Conclusion**

Plant vegetative parameters of *Brassica rapa* comprising plant height, leaf width, leaf length, and even biomass yield above ground had a better performance at the lower altitude just only until two weeks. In the third week, its vegetative performance was not differentiable. Higher values of nitrate concentration and accumulation in the plants in the second week were also remarked, at the lower altitude of 200-500 m asl nitrate accumulation in the plants reached averagely 2336 mg kg\(^{-1}\) and it increased to 3255 mg kg\(^{-1}\) at a medium altitude of 500-700 m asl. In the third week, there was no difference observed because of the altitude category.

Under shading of the plastic greenhouse at shading level ±50%, the plant underwent an etiolation process, so that the vegetable grew longer in the second week until the third week compared to growing plant at the open field. An increase of nitrate accumulation in the plant at shading conditions was noticed only in the second growth week, where its value reached 3077 mg kg\(^{-1}\) or about 20% higher than in open field culture. At the postharvest time, the nitrate concentration and accumulation in room temperature storage increased day by day.

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