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Relationship between growth and N:P of cabbage (*Brassica oleracea* L., var. *capitata*) plug seedlings according to moisture content and nitrogen and phosphorus application after transplanting

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ABSTRACT

The aim of this study was to evaluate the effect of moisture on N and P uptake of cabbage (*Brassica oleracea* L., var. *capitata*) and to determine whether relative growth rate (μ) is correlated to either the concentration of N (N_P), P (P_P), or their ratio (N_P:P_P) in shoots. Seedlings were transplanted and exposed to different levels of moisture, N and P under controlled conditions. μ, leaf area, N_P, and P_P were determined and the relationship between μ and N_P, P_P, and N_P:P_P were examined using generalized linear models. Increased moisture levels enhanced the uptake of both N and P. A larger N supply increased μ and N_P but tended to decrease P_P, whereas a larger P supply increased P_P but had little effect on μ and N_P. N_P:P_P could be predicted from individual N and P supply or from their ratio. The regulatory coefficient (*H* value) of cabbage seedlings, expressing the extent of homeostasis, was estimated as 4.10, which is relatively high among herbaceous plants. μ and N_P were highly correlated, whereas P_P and N_P:P_P did not show a significant relationship to μ. We concluded that nutrient uptake can be enhanced by moisture management, and that μ is highly correlated to N_P while there was a weak relationship between μ and N_P:P_P in cabbage seedlings because of the relatively strong homeostasis.

1. Introduction

N and P are major elements of fertilizers, but they can also be a cause of pollution of rivers or groundwater if they are applied in surplus, beyond the requirement of plants and end up flowing out of fields (Almasri and Kaluarachchi 2007; McLay et al., 2001; Sims et al., 1998; Sturm et al., 2010). In addition to environmental impacts, with the price of fertilizers rising worldwide, excessive application of fertilizers should be avoided to reduce costs. Thus, an appropriate fertilization management system to meet the requirement of plants and to increase the nutrient use efficiency is required (Mueller et al., 2011; Sanchez and Doerge 1999).

In general, the nutrient use efficiency decreases with increase in the nutrient supply and increases with the increase in the amount of soil moisture that results from frequent irrigation or due to higher level of ground water. However, the excessive supply of water can also lead to leaching of fertilizer leading to a decrease in nutrient use efficiency (Erdem et al., 2010; Gauer et al., 1992; Home et al., 2002; Rahman et al., 2000; Sørensen 1995; Zhang et al., 2015). Hence, the soil moisture level in the field is an important factor for fertilizer management and the necessity to control the level of soil moisture by supplying or draining water becomes essential. In Japan, new subsurface irrigation systems, called ‘FOEAS’ and ‘OPSIS’, have been developed in recent years, which enable the control of subsurface water level (Matsuo et al., 2013; Nakano et al., 2014; Sasaki et al., 2014; Shimada et al., 2012; Wakasugi and Fujimori 2009). Therefore, revealing the plants’ responses, such as, the changes in the relative growth rate (μ) and the concentration of nutrients, to different soil moisture levels has become important.

In Japan, as cabbage (*Brassica oleracea* L., var. *capitata*) is cultivated in open fields in general, its growth can be disturbed easily by unusual and abnormal weather. However, for cabbage harvesters, uniformity of growth at harvest is of high priority as it allows savings on labor (Yamamoto et al., 2016). Transplanting cabbage seedlings nursed in cell trays is widespread in cabbage cultivation, though, transplants are sensitive to environmental stress such as drying or high temperature just after transplanting due to their limited root zone (Yoshioka et al., 1998). Delays in growth at this stage negatively affects cabbage head

Abbreviations: μ, relative growth rate of the shoot; N_P, N concentration in plant shoots; P_P, P concentration in plant shoots; N:P, ratio of N to P concentrations; N_P:P_P, ratio of N to P concentrations in plant shoots; N_S, N amount that was supplied; P_S, P amount that was supplied; N_S:P_S, ratio of N to P weight that was supplied; LA, Leaf area; EC, electrical conductivity

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uniformity and reduces yield. Hence, for leafy vegetables, transplanting is a key step that can influence the management practices employed to ensure good crop yield (Fujiwara et al., 2000). In fact, the highest yield of cabbage was obtained when irrigated the day before and the day after transplanting (Sturm et al., 2010). It has also been demonstrated that the increase in dry matter of cabbage transplants was largely dependent on the soil moisture condition (Takahashi et al., 2017), whereas the application of nitrogen fertilizer for transplanted cauliflowers significantly influenced the N uptake efficiency (Sørensen 1995). Nitrogen deficiency at transplanting can also cause the production of small unmarketable curds called buttons in broccoli production (Masarirambi et al., 2011). As the process of transplanting can affect the yield and quality of the crop, it is important to focus on and improve the process, to enable transplants to establish roots and grow fast.

The growth rate hypothesis proposes that organisms growing fast are associated with a low ratio of N and P concentration (N:P) (Elser et al., 2003). Although it has been studied and supported in zooplankton, arthropods, and microbes (Acharya et al., 2004; Elser et al., 2000; Hessen et al., 2007; Main et al., 1997; Makino et al., 2003; Watts et al., 2006), the relationship between N:P in plant shoots (N_P:P_P) and μ for vascular plants is still controversial (Yan et al., 2015; Yu et al., 2012) because some studies report a relationship (Lovelock et al., 2007; Niklas and Cobb 2005; Peng et al., 2016; Yan et al., 2015), whereas others do not (Agren 2004; Cernusak et al., 2010; Matzek and Vitousek 2009; Yu et al., 2012). Yan et al. (2015) and Peng et al. (2016) showed that the relationship between μ and N_P:P_P was a hump-shaped curve, i.e., the μ increased to a maximum, then decreased with increasing N_P:P_P. They also showed that the optimal N_P:P_P at which μ was maximized was approximately 16 for *Arabidopsis thaliana* and 2.38 for *Amaranthus mangostanus* at the flowering stage. Garrish et al. (2010) also described that the optimal whole-plant N:P was approximately 6, at which the largest dry mass of the plant was achieved for *Ficus insipida*. Another study has reported that more than 40% of cereals and oilseeds attaining maximum yield had N_P:P_P in a range between 4 and 6 and legumes had approximately 9 (Sadras 2006). If an optimal N_P:P_P exists for cabbage transplants, this could be a valuable indicator for their growth. Furthermore, if N_P:P_P can be regulated by N:P supply (N_S:P_S), fertilization at transplanting can control N_P:P_P as transplanting is the process exposing seedlings to the different N_S:P_S. However, 10-fold variation in N_S:P_S usually causes only two- to three-fold variation in N_P:P_P because of homeostatic regulation by plants (Gusewell 2004; Gusewell and Koerselman 2002). As the extent of homeostasis can be expressed by the value of the regulatory coefficient (H), revealing the H value of cabbage enables to understand how influential N_S:P_S is on N_P:P_P. Thus, for effective cabbage cultivation, it is meaningful to clarify the relationship between plant growth and N_P:P_P, to discover the H value and the optimal N_P:P_P for cabbage transplants. At the same time, the N and P concentration in plant shoots (N_P and P_P), individually, can influence plant growth and the concentration of one element can complicate the influence of the other (Boon et al., 1990; Burns 1992; Mimura et al., 1996; Rouached et al., 2011; Yan et al., 2015; Yu et al., 2012). Therefore, careful characterization is necessary when associating the apparently non-linear relationships between plant growth and nutrient concentrations or their ratio.

The Objectives of this study is 1) to understand the effect of moisture on the growth and nutrient uptake, 2) to clarify the interaction effect of N_S and P_S on N_P and P_P, 3) to estimate the H value and the relationship between N_P:P_P and N_S:P_S and 4) to evaluate the relationship between μ and N_P, P_P, and N_P:P_P of cabbage transplants. Therefore, we performed two experiments in this study. First, cabbage seedlings were transplanted into pots with different moisture conditions in a greenhouse, and the optimal moisture condition was determined for growth and nutrient uptake. Second, transplants were grown in pots with different N and P supply in a growth chamber at the optimal moisture condition. We observed the effect of N and P supply and its interaction on plant characteristics such as μ, leaf area (LA), N_P, and P_P. Then, we estimated the H value and the relationship between N_P:P_P and N_S:P_S using a generalized linear model (GLM) approach as a flexible framework for considering the interaction term and distribution pattern of the response variable (Crawley 2005). Finally, we examined and evaluated the relationship between μ and N_P, P_P, and N_P:P_P using the GLM approach for finding the most reliable indicator of the growth of cabbage transplants.

2. Materials and methods

2.1. Growth under different moisture conditions (Experiment 1)

Cabbage seeds (*Brassica oleracea* L. 'Satsukio') were sown in cell trays (25 mL × 128 cells) filled with a culture medium (N:P:K = 50:219:83 mg L^{-1}) (NAPLA type S, YANMAR Co., Ltd., Japan) in a greenhouse at Tsukuba Vegetable Research Station, Kannondai, Tsukuba. Three weeks after sowing, the seedlings were fertilized with 1 L of liquid fertilizer, OK-F-1 (N:P:K = 150:35:141 mg L^{-1}) (OAT Agrio Co., Ltd., Japan) per cell tray. Four weeks after sowing, one seedling was transplanted to a 360-mL plastic pot 9 cm in diameter and filled with a culture medium (N:P:K = 12:10:481 mg L^{-1}) (Bear mix, Hokkaido Peatmoss Co., Ltd., Japan) and fertilized with 200 mg of ammonium nitrate (supplying N) and 437 mg of superphosphate of lime (supplying P) to one liter of culture media. Moisture levels of the culture medium were adjusted as follows: 30% (W_1), 50% (W_2), and 66% (W_3) water content by weight. These culture mediums showed approximately −49, −21, and −5.0 kPa of the matric potential measured by tensiometers, respectively. To keep the moisture content constant, pots were watered once per day according to fixed weights, which were unique to each water volume. Shoots were sampled at 15 days after transplanting, and dried at 80 °C for 2 days. Each condition had nine replicates randomly placed in the green house. Relative growth rate of the shoot (μ) was calculated by the following equation (Yan et al., 2015; Yu et al., 2012):

$$\mu = Ln(M_T/M_0)/T$$

Where T is the elapsed time after transplanting (day), M_0 is the shoot dry weight at the day of transplanting (mg) and M_T is the shoot dry weight at T days after transplanting (mg).

2.2. Growth under different N and P supply conditions (Experiment 2)

The processes followed before transplanting were the same as those mentioned above. One seedling was transplanted to a 660-mL plastic pot, 12 cm in diameter, and filled with a culture medium made of Bear mix and vermiculite (N:P:K = 4:13:42 mg L^{-1}) mixed at a volume ratio of 1:1. Therefore, about N:P:K = 8:12:262 mg L^{-1} is initially contained in the medium used in Experiment 2. After transplanting, pots were put in a growth chamber (with 220 µM m^{-2} s^{-1} of photosynthetic photon flux density (PPFD) and exposed to 12 h of light at 20 °C and 12 h of darkness at 15 °C). According to the standards of previous studies (Yan et al., 2015; Yu et al., 2012), sixteen solutions were arranged in a two-way full-factorial design which included four N levels (0, 2, 8, 32 mM N L^{-1}, added as NH_4NO_3) and four P levels (0, 0.3, 1.2, 4.8 mM P L^{-1}, added as KH_2PO_4 and NaH_2PO_4). Other elements were supplied at the same concentration, and were based on the treatment known as the Hoagland's formula (Hoagland and Arnon 1950) and modified for our experiment as follows: 4 mM K, was added as KH_2PO_4 and KCl, 0.8 mM $MgSO_4$, 2 mM $CaCl_2$, and microelements (Mn, B, Fe, Cu, Zn, Mo) as 50 mg L^{-1} of OAT house #5 (OAT Agrio Co., Ltd., Japan) were added. The pH of the solutions was maintained at 6.2 using NaOH. The electrical conductivity (EC) of the solutions is shown in Table 1. Pots divided to sixteen experimental blocks were watered by each solution to the W_3 level of water content once per day. The places of pots in a block and the places of blocks in the chamber were randomly replaced