JARQ 51 (3), 271 – 277 (2017) http://www.jircas.affrc.go.jp

Effect of Moisture, Nitrogen and Phosphate on Initial Growth and Shoot:Root Ratio of Cabbages Following Transplantation

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Abstract

Cabbage transplants (*Brassica oleracea var. capitata*) nursed in cell trays are often unable to absorb sufficient soil moisture after field transplantation. To determine the optimal soil conditions necessary to enable transplants to adjust smoothly, we transplanted cabbage seedlings to pots containing soil of varying moisture and nutrient content. Plants in the moist experimental group (less than 1.7 pF-value) with fertilizer (200 mg $N{\cdot}L^{-1}$) showed an exponential increase in shoot dry weight (SDW). Observations revealed that plants in the dry group (2.8 pF-value), plants in the moist group (1.7 pF-value) without fertilizer, and plants in the relatively dry (2.4 pF-value) group with fertilizer showed significantly ($P < 0.01$) lower SDW at 6, 9, and 12 days, respectively, after transplanting as compared with the highest growth achieved. The nutrient levels of 80, 200 and 500 mg $N{\cdot}L^{-1}$, and 300, 1000 and 3000 mg $P_2O_5{\cdot}L^{-1}$ were also examined. Moisture and nitrogen levels had major impacts on total dry weight (TDW). It was also revealed that deficiency in either moisture or nitrogen restricted plant growth. For the less than 1.5 pF-value experimental group, the low nitrogen content tended to increase the root portion of TDW. To promote the growth of cabbage transplants in moist soil, it is important to keep the shoot portion of TDW high with sufficient nitrogen fertilizer.

Discipline: Horticulture
Additional key words: phosphate, seedling, soil water content, transplant

Introduction

Transplant cultivation is the major method of cabbage production in Japan, as it simplifies the management of cabbage seedlings and uses fields efficiently. Cell trays are widely used in plant nurseries to produce highly suitable seedlings for the transplanting machine. Seedlings grown in a cell tray, however, form root balls, which result in root systems shallower than those of conventional transplants (Yoshioka et al. 1998). Therefore, transplants are sensitive to drying stress just after transplanting, and delays in growth at this stage reduce yield and negatively impact cabbage head uniformity (Fujiwara et al. 1998a). According to Fujiwara et al. (2000), the uniformity of growth at harvest depends on the condition of the root system just after transplanting; therefore, successful rooting after transplantation is one of the most important steps in cabbage production. Hence, it is important to prepare optimally formulated soil for transplants. Although the irrigation and fertility aspects of cabbage farming (Sanchez et al. 1994, Thomas et al. 1970), physical properties or agricultural method such as tilling (Moriyama et al. 2000), transplanting depth (Fujiwara et al. 1998b), and planting density (Fujiwara et al. 2000) for cabbage transplants have been widely examined, few studies have considered the soil conditions at transplanting. Furthermore, soil conditions can have a large impact on the allocation of photoassimilate to tissues (Aerts et al. 1991, Hilbert 1990). And because the edible part of cabbage is aboveground vegetative tissue, increasing the shoot ratio may directly result in a higher yield or shorter cultivation period. It is worth noting that cabbage is a shallow-rooted plant; therefore, it is susceptible to dryness and easily affected by a lack of certain elements such as calcium and boron (Chandler 1940, Palzkill et al. 1976). Excess shoot growth can increase the risk of

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Received 31 May 2016; accepted 11 October 2016.

element deficiency as demand also increases. In addition to these morphological and physiological characteristics, cabbage tends to be grown in open fields, where crops are typically exposed to weather conditions. Therefore, root development is important for cabbage plants to acquire resources and physically support the shoot. The relationship between the shoot:root ratio and crop yield is not well understood, but it would be worthwhile to study changes in photoassimilate distribution in response to soil conditions.

Cabbage is expected to be introduced to crop rotations in paddy fields because of its relatively high adaptability to different soil conditions compared to other vegetables. However, water retention or drainage in paddy fields is usually unsuitable for vegetables to grow. To solve this problem, the Farm-Oriented Enhancing Aquatic System (FOEAS) was developed for draining and supplying water to paddy fields through culvert pipes, and thus controlling the subsurface water level (Wakasugi & Fujimori 2009). Nakano et al. (2014) reported that early stage cabbage growth was promoted in a plot where a high ground-water level had been maintained by FOEAS, but growth in the same plot was later suppressed due to excess water stress. In addition to FOEAS, the Optimum Subsurface Irrigation System (OPSIS) was also developed in recent years to supply water to fields below the ground level, thereby keeping soil moisture tension low, and is expected to stabilize or increase vegetable production (Sasaki et al. 2014).

As such new technologies as FOEAS and OPSIS that allow for precise water control are expected to become more widespread, basic knowledges about the effect of soil moisture and fertility on plant growth and photoassimilate distribution should be quantitatively organized. The purpose of this study was to elucidate the following three subjects, while focusing on the early growth stage of cabbage, just after transplanting.

1) The stage at which differences in soil conditions would reflect plant growth
2) The optimal moisture and fertility conditions for plant growth
3) The effect of soil conditions on the root ratio of total dry weight

First, we observed the continuous changes in shoot dry weight (SDW) up to 15 days after transplanting (DAT) for various treatments in Experiment 1. Next, we compared the total dry weight (TDW), defined as the sum of SDW and root dry weight (RDW), for different moisture, nitrogen, and phosphate conditions in Experiment 2. Finally, we analyzed the effect of soil conditions on the ratio of RDW to TDW.

Materials and Methods

1. Growth conditions and experimental design

(1) Experiment 1

Cabbage seeds (*Brassica oleracea* L. cv. Satsukio) were sown in cell trays (25 ml × 128 cell) filled with a culture soil ($N:P_2O_5:K_2O$ = 50:500:100 mg·L^{-1}) (NAPLA type S, YANMAR Co., Ltd., Japan) on September 17, 2014 in a greenhouse at Tsukuba Vegetable Research Station, Kannondai, Tsukuba. Three weeks after sowing, the seedlings were fertilized with 1 L of liquid fertilizer ($N:P_2O_5:K_2O$ = 150:80:170 mg·L^{-1}) (OK-F-1, Otsuka Chemical 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 soil containing fertilizer ($N:P_2O_5:K_2O$ = 200:1000:200 mg·L^{-1}) (Yokabaido, Hokkaido Peatmoss Co., Ltd., Japan, designated (+)) or a culture soil without fertilizer (Bear mix, Hokkaido Peatmoss Co., Ltd., Japan, designated (-)). Except for fertility, both soils are expected to have the same physical properties as both Yokabaido and Bear mixes are composed of the same materials, mainly peat moss (that contains $N:P_2O_5:K_2O$ = 12:24:580 mg·L^{-1} innately). Since the moisture levels of culture soils must be accurately adjusted, peat moss having high moisture retentivity was suitable for this experiment. In this study, 200 mg N·L^{-1} is equivalent to 200 kg N·ha^{-1} in the field, provided that the plow layer is set as 10 cm. The moisture levels of the culture soils were adjusted as follows: 10, 30, 50, and 66% water content by weight (designated W_0, W_1, W_2, and W_3), representing approximately 2.8, 2.4, 1.7, and less than 1.5 pF-value, respectively. To keep the soil moisture constant, pots were watered according to fixed weights, which were unique to each water volume, once per day. Shoots were sampled on 3, 6, 9, 12, and 15 DAT, and dried at 80ºC for two days. For each test, six plants were sampled from each treatment.

(2) Experiment 2

Seeds of the same cultivar were sown on April 9, May 7, and May 14 of 2015, and grown under the same conditions as Experiment 1. Four weeks after sowing, one seedling was transplanted to a 360-ml plastic pot filled with the Bear Mix culture soil, adjusted to W_1, W_2, and W_3 water weight content, with 80, 200 or 500 mg N·L^{-1} of ammonium nitrate (designated N_1, N_2 and N_3), and 300, 1000 or 3000 mg P_2O_5·L^{-1} of superphosphate of lime (designated P_1, P_2, and P_3) and 200 mg K_2O·L^{-1} by potassium chloride. The ranges of nitrogen and phosphate content were set from approximately 0.3 to 3 times to the (+) condition in Experiment 1. Soil moisture was kept constant using the same method described in Experiment

1. The soil was carefully washed out by water to preserve the lateral root and root hair. Shoots and roots were sampled at 15 DAT, and dried at 80ºC for two days. Three plants were sampled from each treatment three times, and nine plants were used in total.

2. Statistical analysis

The data were subjected to an analysis of variance (ANOVA) and regression analysis using the statistical software, R-Console (Rx64 3.0.2) (Team 2015).

Results

1. Changes in growth for different soil conditions

Growth of W_0(+)/(-) was significantly (P < 0.01) delayed compared to W_2(+) and W_3(+) at 6 DAT (Fig. 1). Plants in W_1(-), W_2(-), and W_3(-) grew significantly slowly compared to W_1(+), W_2(+), and W_3(+) at 9 DAT. The growth of W_1(+) was significantly delayed compared to W_2(+) and W_3(+) at 12 DAT. Plants in both W_2(+) and W_3(+) grew exponentially, and the highest SDW was found to be W_3(+) in the end, but no significant difference appeared between these two treatments by 15 DAT. These results clarified the specific timing of the soil moisture and fertility levels, and their effect on SDW of cabbage transplants after transplanting.

2. Investigation of optimal moisture and fertility levels

There were no significant differences in TDW for W_1 and W_2, regardless of different fertility conditions (Fig. 2, Table. 1). However, all TDWs in W_1 were significantly (P < 0.01) lower than those in W_2. The effect of nitrogen was clear for W_3; in other words, TDWs in W_3 increased according to the amount of nitrogen. TDWs were slightly lower in W_3N_1 and slightly higher in W_3N_2 than most of those in W_2. TDWs in W_3N_3 were the highest among the treatments, and the increase of phosphate from P1 to P3 resulted in a significant (P < 0.05) increase of TDW for this group.

3. Changes in root ratio in response to soil conditions

The ratio of RDW to TDW was much smaller than that of SDW, but RDW generally tended to increase with moisture level, and positively correlated with TDW for all fertility treatments (Fig. 3). The regression line for N_3 clearly shows this tendency and was highly reliable (R^2 = 0.889) (Fig. 3 (C)). However, as the nitrogen content decreased from N_3 to N_1, inclinations became larger and regression lines less reliable (R^2 = 0.681 or 0.578) (Fig. 3 (A) and (B)). Regression lines derived from each phosphate condition were almost the same and fairly

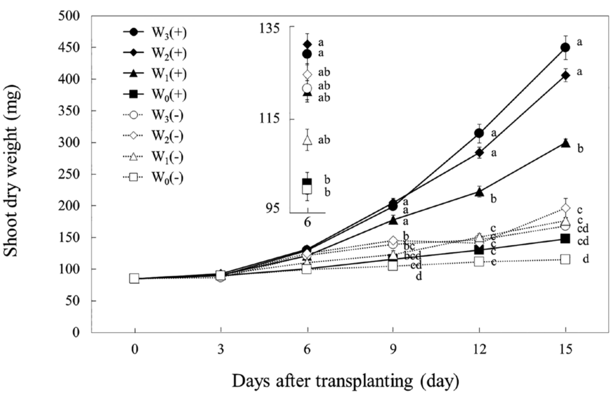

Fig. 1. Changes in shoot dry weight (SDW) of cabbage after transplanting.

W_0, W_1, W_2, and W_3 indicate the moisture treatments: approximately 2.8, 2.4, 1.7, and less than 1.5 pF-values, respectively. (+) and (-) indicate treatments with and without fertilizer ($N:P_2O_5:K_2O$ = 200:1000:200 mg•L^{-1}). The enlarged figure is displayed for six days after transplanting. Different letters indicate significant differences among the treatments within the same day at P < 0.01 by the Tukey-HSD test. Vertical bars indicate the SE.

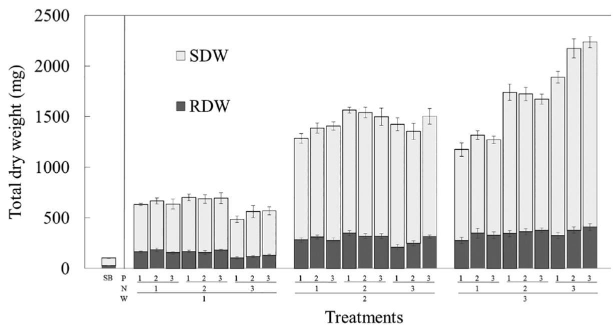

Fig. 2. Total dry weight (TDW) at 15 days after transplanting for different moisture, nitrogen, and phosphate treatments.

Total dry weight (TDW) is defined as the sum of shoot dry weight (SDW) and root dry weight (RDW). SB indicates the TDW of seedlings before transplanting. The numbers 1, 2, and 3 in the bottom line beginning with W indicate moisture treatments W_1 (2.4 pF-value), W_2 (1.7 pF-value), and W_3 (less than 1.5 pF-value), respectively. The numbers 1, 2, and 3 in the middle line beginning with N indicate nitrogen treatments N_1 (80 mg N•L^{-1}), N_2 (200 mg N•L^{-1}), and N_3 (500 mg N•L^{-1}), and in the upper line beginning with P indicate phosphate treatments P_1 (300 mg P_2O_5•L^{-1}), P_2 (1000 mg P_2O_5•L^{-1}), and P_3 (3000 mg P_2O_5•L^{-1}), respectively. Vertical bars indicate the SE.

Table 1. Significant differences between total dry weights of treatments in Experiment 2.

W[z]			1									2									3								
	N[y]		1			2			3			1			2			3			1			2			3		
		P[x]	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
		1		NSw	NS	NS	NS	NS	NS	NS	NS	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
	1	2			NS	NS	NS	NS	NS	NS	NS	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
		3				NS	NS	NS	NS	NS	NS	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
		1					NS	NS	NS	NS	NS	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
1	2	2						NS	NS	NS	NS	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
		3							NS	NS	NS	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
		1								NS	NS	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
	3	2									NS	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
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[z] The numbers 1, 2, and 3 in the first line and row beginning with W indicate moisture treatments W_1(2.4 pF-value), W_2 (1.7 pF-value), and W_3 (less than 1.5 pF-value), respectively.
[y] The numbers 1, 2, and 3 in the second line and row beginning with N indicate nitrogen treatments N_1 (80 mg N·L^{-1}), N_2 (200 mg N·L^{-1}), and N_3 (500 mg N·L^{-1}), respectively.
[x] The numbers 1, 2, and 3 in the third line and row beginning with P indicate phosphate treatments P_1 (300 mg P_2O_5·L^{-1}), P_2 (1000 mg P_2O_5·L^{-1}), and P_3 (3000 mg P_2O_5·L^{-1}), respectively.
[w] NS, * and ** indicate non-significant or significant differences of $P < 0.05$ and 0.01 by the Tukey-HSD test, respectively.

reliable (R^2 = 0.673 ~ 0.755) (Fig. 3 (D), (E) and (F)). Our results showed that root growth was strongly affected by moisture and nitrogen levels, but that phosphate had little influence.

Discussion

There are other indexes to demonstrate the growth of cabbages such as plant height, the number of leaves, and specific leaf area. However, the allometric relationship between leaf or stem and TDW indicates a similar allocation irrespective of water stress treatments (Cella Pizarro & Bisigato 2010). And SDW, in which totalized plant growth can be reflected, is usually adopted to evaluate the cabbage growth itself (Fujiwara et al. 1998a).

In Experiment 1, it was shown that cabbage transplants required moist (less than 1.7 pF-value) conditions and fertilizer for exponential growth, and that an insufficient level of either moisture or fertilizer mutually restricted the growth of cabbage transplants (Fig. 1). The lowest SDW in group W_0(-) may have been caused by insufficient moisture and/or nutrient levels. The compensation for this delay by adding fertilizer in W_0(+) was limited, although observed in W_1, W_2, and W_3 regardless of fertilizer. Finding that fertilizer did not contribute much to the delayed plant growth in W_0 indicates that the limiting growth factor at 2.8 pF-value (W_0) was insufficient moisture not fertilizer. As plants in the W_1(+) group grew significantly (P < 0.01) better than W_1(-), W_2(-), and W_3(-), all of which grew similarly, the moisture level at 2.4 pF-value (W_1) was sufficient for plants under the (-) condition, and then extra moisture did not promote further growth. The difference in SDW between W_1(+) and W_2(+) became significant (P < 0.01) at 12 DAT, thus demonstrating that the amount of moisture at 2.4 pF-value (W_1) was restricting plant growth under the (+) condition. However, as there was no significant difference between W_2(+) and W_3(+), the moisture demand by plants under the (+) condition was satisfied at 1.7 pF-value (W_2), and the limiting growth factor here may shift toward fertilizer. This implies that the addition of fertilizer could promote growth at less than 1.7 pF-value (W_2 and W_3). The amount of nitrogen and phosphate for the N_2P_2 treatment in Experiment 2 is equivalent to the (+) condition in Experiment 1, and the addition of fertilizer, such as in the N_3 treatment, was

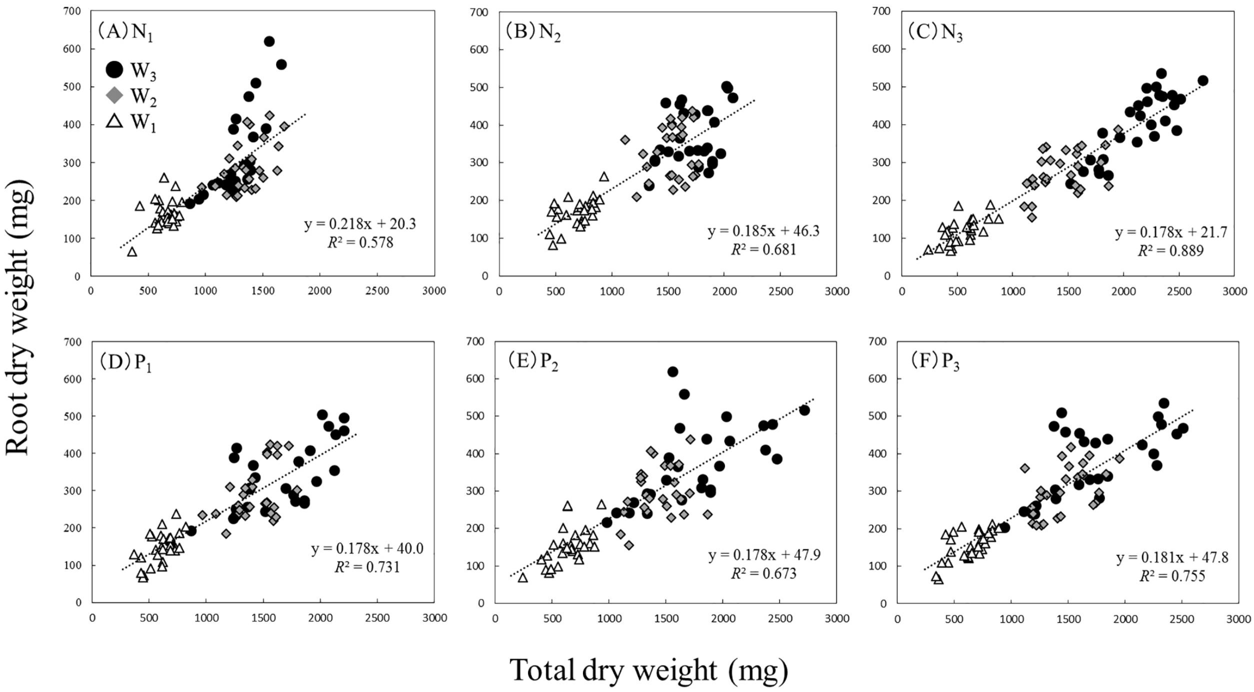

Fig. 3. Correlation between total dry weight (TDW) and root dry weight (RDW) for different soil conditions.
One point denotes one plant. A dotted line in each graph is a regression line. W_1, W_2, and W_3 indicate the moisture treatments: approximately 2.4, 1.7, and less than 1.5 pF-values, respectively. (A), (B), and (C) indicate nitrogen treatments N_1 (80 mg N•L^{-1}), N_2 (200 mg N•L^{-1}), and N_3 (500 mg N•L^{-1}, respectively. (D), (E), and (F) indicate phosphate treatments P_1 (300 mg P_2O_5•L^{-1}), P_2 (1000 mg P_2O_5•L^{-1}), and P_3 (3000 mg P_2O_5•L^{-1}), respectively.

actually effective on TDW only for the W_3 group (Fig. 2). Hence, it was insufficient moisture, at 1.7 pF-value (W_2), in the (+)/N_2P_2 group, and insufficient nitrogen, at less than 1.5 pF-value (W_3), which restricted the growth of cabbage transplants in Experiment 1. Once growth was delayed, the underdeveloped plants would rarely achieve the same level of growth as that of the plants that were not delayed (Fujiwara et al. 1998b, Gedroc et al. 1996). Our results showed the same tendency in cabbage transplants for at least 15 days (Fig. 1), and the initial differences just after transplanting are likely to influence not only SDW at 15 DAT but also later growth and even yield, emphasizing that proper soil conditions during transplant are crucial for cabbage cultivation.

Our results showed the importance of both the level and timing of moisture and nitrogen for cabbage seedlings during transplant. It is known that cabbages are intermediately susceptible to water stress, and insufficient irrigation reduces both marketable and total yield (Smittle et al. 1994, Xu & Leskovar 2014). As these reports showed the negative impacts of deficient irrigation over the whole cultivation period, the results of our experiment may be the first report revealing the extent this deficiency can have on cabbage growth just after transplanting. It is also known that increased nitrogen application results in higher cabbage yields (Everaarts & De Moel 1998, Freyman et al. 1991, Turan & Sevimli, 2005). Freyman et al. (1991) demonstrated this fact in the field using up to 500 $kg{\cdot}ha^{-1}$ of urea. This nitrogen ratio is considered equivalent to the N_3 treatment in this experiment. Although we used ammonium nitrate as the nitrogen source, Turan & Sevimli (2005) showed that both ammonium nitrate and urea have positive effects on cabbage yield. Collectively, as long as the moisture is sufficient for cabbages, the TDW of the plants just after transplanting increases with the amount of nitrogen applied, at least up to the N_3 level, and this amount of nitrogen is unlikely to have a negative effect on later growth. For the effect of phosphate on cabbage, Dechassa et al. (2003) reported that cabbage attained 80% of its maximum yield with no additional phosphate supply in soil originally containing 16 $mg{\cdot}kg^{-1}$ of soluble phosphate, whereas carrot (*Daucus carota* L.) and potato (*Solanum tuberosum* L.) reached only 4% and 16% of their highest yields, respectively. Additional data indicating that cabbage is tolerant to low phosphate (Temple-Smith & Menary 1977) or weakly responsive to phosphate addition have also been reported (Alt et al. 1998). Consistent with these reports, the impact of phosphate was generally small in our experiment. Though the amount of phosphate in P1 seemed insufficient in W_3N_3, the standards of phosphate could generally be higher than the demands of plants in other treatments. Therefore, the effect of phosphate might be obscure especially between P_2 and P_3 treatments. We can only conclude that adding phosphate to soil already saturated with phosphate has little effect.

It was a general tendency that RDW increased linearly with TDW, but there were some departures of RDW from this linearity under low nitrogen levels (Fig. 3). The number or architecture of roots could also change according to soil conditions, though we focused on changes in photoassimilate distribution to the roots. Then, consistent with the results in Cella Pizarro & Bisigato (2010), the ratio of RDW to TDW was hardly influenced by moisture conditions, as added water increased both RDW and TDW. However, the amount of nitrogen can change the allocation of dry matter (Fig. 3 (A), (B) and (C)). For the N_1 treatment, the RDW of some samples in W_3 was much higher than expected from the regression line, which made the inclination larger and the regression coefficient less reliable. The increase in the root fraction compared to shoots (caused by nitrogen restriction) seems to be the general nature of many plant species (Bonifas et al. 2009, Davidson 1969, Gedroc et al. 1996). Some reports stated that the increase in the root portion compared to shoots was caused by the decrease of SDW in corn (*Zea mays*.) (Anderson 1988) and barley (*Hordeum vulgare* L.) (Welbank & Williams 1968). In our experiment, the increase in the RDW ratio to TDW for the low nitrogen group was also due to reduced SDW, since RDWs in the W_3 treatments were almost the same, but SDW decreased as nitrogen decreased (Fig. 2). As this seems clear only for the W_3 group, sufficient moisture is a prerequisite for this plasticity in dry matter allocation. Although Leskovar et al. (1990) indicates that pepper (*Capsicum annuum* L.) transplants with lower RDW showed greater fruit production than plants seeded directly, when considering that the period and cultivation environment, edible parts, and individual resource demands vary between plant species, it is difficult to determine a general relationship between the shoot:root ratio and yield. Further research is needed to reveal how the shoot:root ratio influences yield. However, it is noteworthy that cabbage which maintained a high shoot:root ratio by sufficient nitrogen application also gained the highest TDW during the early growth stage. The response to soil conditions and the plasticity of the cabbage shoot:root ratio may be seasonally influenced, but this study demonstrated that moisture and nitrogen levels can also be adjusted to control plant growth. From this aspect, our data can be instrumental in managing accurate watering and fertilization for cabbage transplants.

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