Journal of Plant Biology, February 2007, 50(1) : 8-11

Estimation of Leaf Number and Leaf Area of Hydroponic Pak-Choi Plants (*Brassica campestris* ssp. *chinensis*) Using Growing Degree-Days

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Temperature is a principal environmental factor that directly affects the growth and timing of appearance for crop leaves. To estimate the leaf number and leaf area of 'Seoul' pak-choi plants (*Brassica campestris* ssp. *chinensis*), we applied the concept of growing degree-days {GDD=(T_{avg}-T_{base})×days}, where T_{avg}, T_{base}, and days were the daily average air temperature, base temperature, and days after transplanting, respectively. Leaves that were beginning to unfold with a leaf area ≥1 cm^2 were counted every 2 to 3 d. Linear relationships were found between leaf number and days after transplanting as well as between leaf number and GDD. The rate of appearance and the number of leaves per GDD were 0.542 leaves d^{-1} and 0.051 leaves °C^{-1} d^{-1}, respectively. In contrast, the relationship was non-linear between leaf number and leaf area, with the latter being calculated as $[(128.9+11.6\times GDD-0.03\times GDD^2)/\{1+(0.051\times GDD+3.5)/13.7)^{(-3.9)}\}]$ (cm^2 °C^{-1} d^{-1}). Using model validation, we found that the estimated leaf number and leaf area showed strong agreement with measured values. Our results demonstrate the usefulness of modeling to estimate total leaf area and growth from hydroponically grown pak-choi plants.

Keywords: base temperature, growing degree units, leaf area, leaf number, model, pak-choi

Pak-choi (*Brassica campestris* ssp. *chinensis*) is the most important leafy vegetable in Asia, especially in China (Liu et al., 2003), and is considered an alternative to lettuce in Summer. However, little information is available regarding the physiological characteristics and cropping system of this plant. For systematic, large-scale production, growers require an understanding of the cultivation environment and must analyze its developmental traits, particularly those of the leaves.

Leaf area is one of the most important parameters when estimating photosynthesis rates, light interception, water and nutrient use, crop growth, and yield potential (Smart, 1985; Williams, 1987; Williams and Martinson, 2003). This parameter depends on rates of leaf appearance and expansion, as well as the duration of leaf expansion and the leaf senescence rate (Warrington and Kanemasu, 1983). The expansion of leaf area can be successfully predicted from leaf number (Dwyer and Stewart, 1986; Hammer et al., 1987; Muchow and Carberry, 1989, 1990).

Nevertheless, leaf area, number, and appearance rates all are affected by environmental factors such as temperature, photoperiod, radiation, water stress, and composition of the nutrient solution (Warrington and Kanemasu, 1983; Ritchie and NeSmith, 1991; Faust and Heins, 1993; Yin and Kropff, 1996; NeSmith, 1997; Karlsson and Werner, 2001). Of these, temperature is generally the primary consideration when developing models for predicting leaf area (Ritchie and NeSmith, 1991; NeSmith, 1997), development (Faust and Heins, 1993), and appearance (Yin and Kropff, 1996).

Reaumur introduced the concept of heat units (or thermal time) in 1730; since then, many methods for its calculation have been devised (McMaster and Wilhelm, 1997). Heat units can be expressed as growing degree-days (GDD) or growing degree units (GDU), and are frequently used to describe the timing of biological processes (McMaster and Wilhelm, 1997). Leaf number can be either linearly related to the accumulation of thermal units (°C d) from the onset of seedling emergence (Warrington and Kanemasu, 1983; Dwyer and Stewart, 1986; Muchow and Carberry, 1990; Slafer et al., 1994) or nonlinearly related (Faust and Heins, 1993; Yin and Kropff, 1996; NeSmith, 1997). Whereas the effect of temperature on leaf number has been extensively researched in grain crops, very little information is available for most vegetable species (NeSmith, 1997).

Our study objectives were to delineate the rate of leaf appearance and determine leaf number, and to analyze the relationship between leaf number and leaf area. From this data we could then develop models for predicting leaf number and leaf area based on GDD in hydroponically-grown pak-choi plants.

MATERIALS AND METHODS

The experiments were conducted in venlo-type glasshouses at the experimental farm of Seoul National University (37.3°N, 127.0°E). Seeds of 'Seoul' pak-choi plants (Syngenta Seed, Korea) were germinated on polyurethane cubes (2.5×2.5×2.5 cm) moistened with tap water. The sowing dates were July 12, 19, and 26; August 12, 19, and 26; and September 2 and 14 of 2003. Plants obtained from seed sown on 25 March 2003 were used to validate our models.

When 3-5 true leaves appeared, seedlings were transplanted to a nutrient film technique (NFT) system (3.5×0.7×0.8 m), with a 200 L tank. Plant spacings were 0.15×0.1 m, between- and within- rows, at a density of 67 plants per square meter. The nutrient solution, applied at 2 L min^{-1} per

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trough, consisted of 224.2 mg L^{-1} NO_3-N, 18.6 mg L^{-1} NH_4-N, 41.3 mg L^{-1} P, 312.8 mg L^{-1} K, 160.3 mg L^{-1} Ca, 48.6 mg L^{-1} Mg, 64.1 mg L^{-1} SO_4-S, 3.0 mg L^{-1} Fe, 0.5 mg L^{-1} B, 0.5 mg L^{-1} Mn, 0.05 mg L^{-1} Zn, 0.02 mg L^{-1} Cu, and 0.01 mg L^{-1} Mo (as specified by the Horticultural Experiment Station in Japan). These nutrients already began to be supplied when the first true leaf appeared. The pH and electrical conductivity (EC) of this solution were measured every 2 to 3 d with a pH meter (HM-14P; TOA Electronics, Japan) and an EC meter (CM-14P; TOA Electronics). The pH was adjusted to 5.5 to 6.5 with KOH or H_2PO_4, while EC was maintained at 2.0 d S m^{-1}, using stock solution and tap water. This nutrient solution was circulated at 10 min ON/ 10 min OFF from the time of transplant to harvest, and was not renewed during the experimental period.

Leaves that were beginning to unfold, and which had a leaf area of ≥1 cm^2, were counted every 2 to 3 d. Abnormal plants were eliminated from the data analysis. Total leaf area for each plant was measured with an LI-3100 leaf area meter (LI-COR; Lincoln, USA). Photosynthetically active radiation (PAR, MJ m^{-2} d^{-1}) inside the greenhouse was calculated according to external global radiation and transmissivity of the greenhouse (0.56). Outdoor global radiation data were obtained from the Suwon Weather Center, 200 m from the experimental site. The set points for indoor air temperature were 15 and 28°C, and were controlled using hot-water heating and pads and a fan cooling system. These temperatures were measured with T-type thermocouples every 10 min, and were recorded with a data logger (CR10X; Campbell Scientific, USA).

Thermal time (growing degree-days, GDD, °C d) was calculated according to the following formula:

$$GDD = (T_{avg} - T_{base}) \times days \quad (1)$$

where, T_{avg}, T_{base}, and days were daily average air temperature, base temperature, and number of days after transplanting, respectively. Germination tests were performed at five constant temperatures (15, 20, 25, 30, and 35°C). The base temperature, set at 13.5°C, was estimated by regressing the inverse of the time to 50% germination rate ($1/GR_{50}$) against the temperature gradient.

Leaf area was modeled using the following logistic function:

$$Y = M\times[1 + (N/a)^b]^{-1} \quad (2)$$

where Y, M, N, a, and b were leaf area, maximum potential leaf area, leaf number, and constants, respectively. Parameters were estimated according to the Gauss-Newton algorithm, a nonlinear least squares technique. The parameter M was fitted to a parabolic function (Eq. 3) because M and temperature were shown to be positively correlated:

$$M=a+b\times GDD+c\times GDD^2 \quad (3)$$

where a, b, and c were the intercept, and first- and second-order regression coefficients, respectively. A completely randomized block design included four beds in the NFT system and eight plant replications. All data were analyzed using the SAS (Statistical Analysis Software) program, and the experimental results were subjected to an analysis of variance (ANOVA). Parameters and regression coefficients of the models were estimated using SAS REG and NLIN procedures.

RESULTS AND DISCUSSION

Data from the eight sowing dates provided various thermal and radiation regimes on which to base our initial model development (Fig. 1). Daily average temperatures ranged from 17.2°C to 44.5°C during the experiment. To calculate GDD, maximum, and minimum temperatures, one generally must know the base temperature and number of days after transplanting. However, in this study, the base (13.5°C) and maximum (47.6°C) temperatures were determined by germination rates over a range of temperatures, such that we could instead use the average temperature because it was between the base and the maximum temperature.

No variability was apparent in leaf number per plant with respect to days after transplanting over the eight sowing dates. A linear relationship was obtained between leaf number and days after transplanting (Fig. 2). The leaf appearance rate was 0.544 leaves·d^{-1} (R^2=0.980***, F=3135.7, RMSE =1.53), with a new leaf emerging every 2 d over this experimental period.

Leaf number and GDD were closely and linearly related (Fig. 3), with a value of 0.05 leaves $°C^{-1}$ d^{-1} (R^2=0.920***, F=725.7, RMSE=1.68). Similar results have been reported

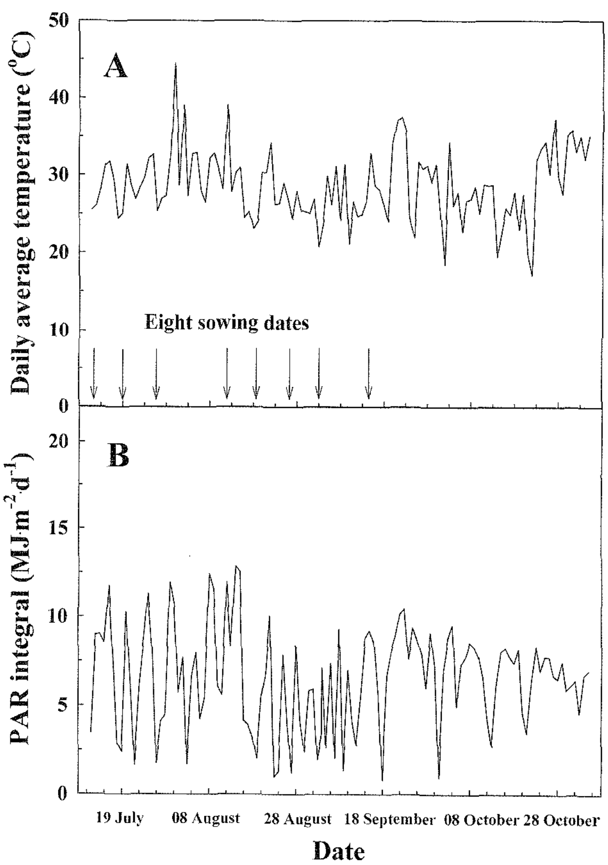

Figure 1. Changes in indoor daily average temperature (**A**) and outdoor daily PAR integral (**B**) from July to October 2003. Arrows indicate 8 sowing dates.

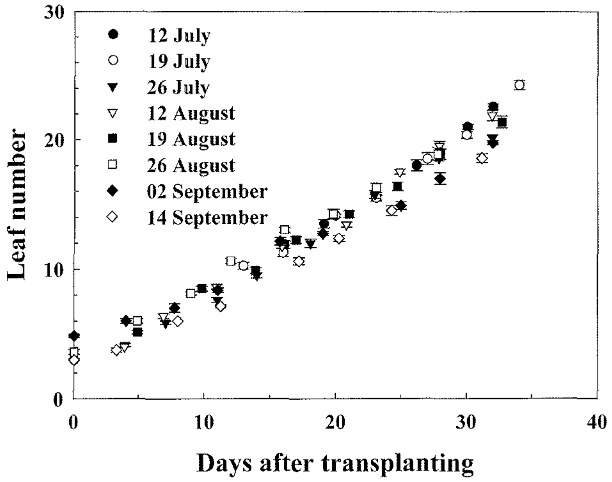

Figure 2. Leaf number per plant as function of days after transplanting. Vertical bars indicate standard error (SE) of 8 replications.

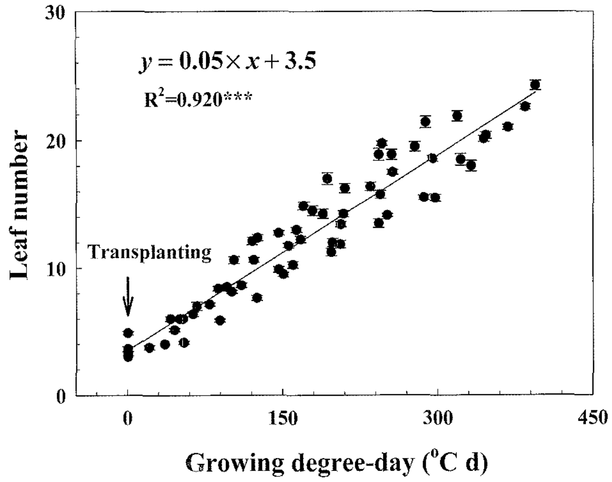

Figure 3. Relationship between leaf number per plant and growing degree-days (GDD) after transplanting: y=ax+b; where y is leaf number, x is GDD after transplanting, a is constant, and b is initial leaf number at transplanting. Leaf number: y=0.051x+3.5 (R^2= 0.920, RMSE=1.68 and P <0.0001). Vertical bars indicate standard error (SE) of 8 replications.

by Gallagher (1979), Kirby et al. (1985), Baker et al. (1986), Dwyer and Stewart (1986), Muchow and Carberry (1990), and Slafer et al. (1994). In contrast, NeSmith (1997) has found that the relationship between GDD and leaf number is nonlinear. Therefore, leaf area may be estimated as a function of GDD, with either a linear or nonlinear relationship. GDD models have also been used to predict growth stages, such as leaf emergence, transplanting, flowering, fruiting, and harvesting. With this function, appropriate transplant and harvest periods can also be estimated. Therefore, based on the results from our current model, we can predict that seedlings transplanted at 50 to 70°C d after the appearance of the first true leaf will have three or four leaves, not including the cotyledon. This means that a pakchoi plant with a shoot fresh weight of 60 g will be ready for harvest at 160 to 190°C d after transplanting (data not shown).

Interestingly, a nonlinear relationship was obtained between

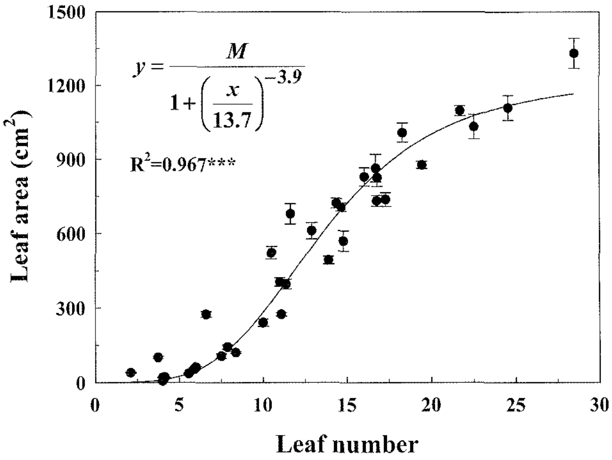

Figure 4. Relationship between leaf number and leaf area. Vertical bars indicate standard error (SE) of 7 replications. Leaf area: y=M/ $[1+(x/13.7)^{-3.9}]$ (R^2=0.967, and P <0.0001), where x is leaf number and M is maximum potential leaf area.

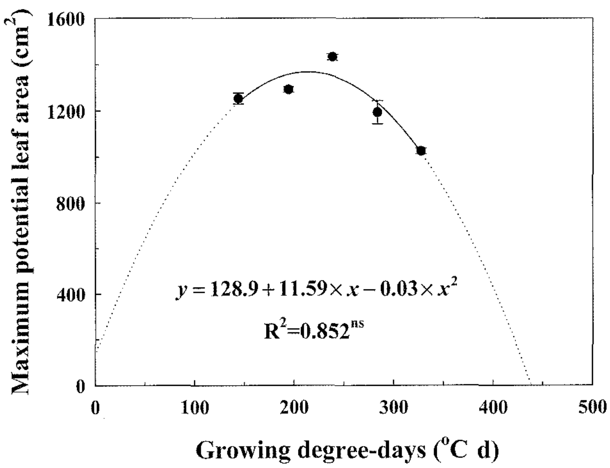

Figure 5. Relationship between growing degree-days (GDD) and leaf area. Maximum potential leaf area: $y=128.9+11.59x-0.03x^2$ (R^2= 0.852, RMSE=80.95, and P=0.148), where x is GDD after transplanting. Vertical bars indicate standard error (SE) of 5 replications.

leaf number and leaf area (Fig. 4). Any increase in the former is significant in terms of the expansion and senescence of leaves. The overall curve for leaf area was sigmoid, as was the curve for leaf number. The maximum potential leaf area, M, showed a curvilinear relationship with GDD (Fig. 5) and could be expressed as a quadratic model ($y= 128.9+11.59x-0.03x^2$, $R^2=0.852^{ns}$, F=5.75, RMSE=80.95). Therefore, we propose that leaf area can be estimated using the function of GDD, so that leaf area = $(128.9+11.6\times GDD-0.03\times GDD^2)/\{1+((0.05\times GDD+3.5)/13.7)^{(-3.9)}\}$ (cm^2 $°C^{-1}$ d^{-1}) (R^2=0.967***, F=4649.1). Because many previous studies have reported successful predictions of leaf area development based on leaf number (Warrington and Kanemasu, 1983; Yin and Kropff, 1996; NeSmith, 1997), we also can confirm that leaf number and leaf area can be estimated with GDD.

The number of leaves from leafy vegetables is a very important aspect of marketplace economics. Leaf area is associated with many agronomic and ecological processes,

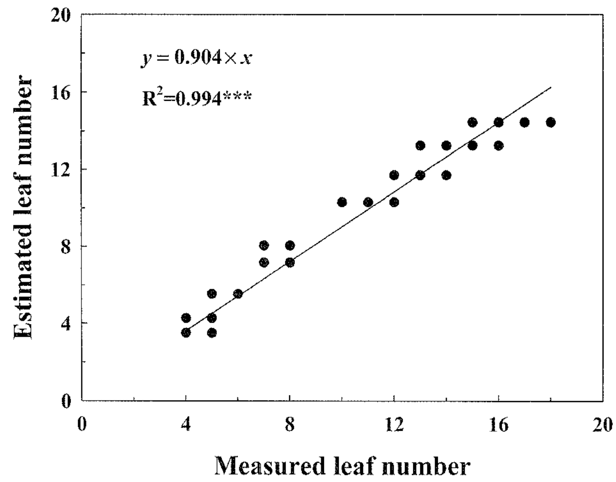

Figure 6. Measured and estimated numbers of leaves (n=76) (R^2=0.994, RMSE=0.695, and P <0.0001).

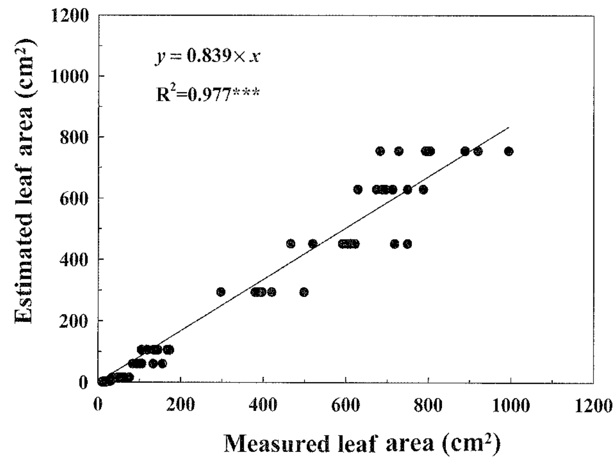

Figure 7. Measured and estimated leaf areas (n=76) (R^2=0.977, RMSE=55.38, and P<0.0001).

including photosynthesis, transpiration, and the energy balance (Gardner et al., 1990). Plant physiologists and agronomists have demonstrated the importance of this parameter in estimating growth rates and yield potential (Gallagher and Biscoe, 1978; Williams and Martinson, 2003). Therefore, our results would be especially useful for developing models that predict leaf area as well as those that can describe the overall growth models of hydroponically grown pak-choi plants.

Our comparison of estimated leaf numbers versus measured values (Fig. 6) showed a reasonably good fit at 0.904 (R^2=0.994***). Measured and estimated leaf areas also were compared here (Fig. 7), and presented another good fit at 0.839 (R^2=0.977***).

In conclusion, we have demonstrated here that GDD is a very effective tool for establishing models to predict leaf number and leaf area as a function of temperature in hydroponically grown pak-choi plants.

Received September 20, 2006; accepted January 15, 2007.

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