BRIEF COMMUNICATION

Leaf area prediction model for sugar beet (*Beta vulgaris* L.) cultivars

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Abstract

In two successive years (2003 and 2004), a set of 16 commercial sugar beet cultivars was established in Randomized Complete Block experiments at two sites in central Greece. Cultivar combination was different between years, but not between sites. Leaf sampling took place once during the growing season and leaf area, LA [cm^2], leaf midvein length, L [cm] and maximum leaf width, W [cm] were determined using an image analysis system. Leaf parameters were mainly affected by cultivars. Leaf dimensions and their squares (L^2, W^2) did not provide an accurate model for LA predictions. Using L×W as an independent variable, a quadratic model ($y = 0.003 x^2 - 1.3027 x + 296.84$, $r^2 = 0.970$, $p<0.001$, $n = 32$) provided the most accurate estimation of LA. With compromises in accuracy, the linear relationship between L×W and LA ($y = 0.5083 x + 31.928$, $r^2 = 0.948$, $p<0.001$, $n = 32$) could be used as a prediction model thanks to its simplicity.

Additional key words: leaf length; leaf width; morphology; non-destructive methods.

Plant morphology is determined by a combination of gene action and environmental effects (Iwata *et al.* 2002a,b, Kessler and Sinha 2004). Quantification of organ shapes is often useful for many field studies in agronomy, zoology, genetics, ecology, and taxonomy (Iwata and Ukai 2002). For example, leaf shape could be used for plant species identification (Camargo Neto *et al.* 2006, Du *et al.* 2007) or cultivar classification (Iwata *et al.* 2002a). Special interest is focused on leaf area (LA) estimation because of its relationship with plant and crop physiology (Goudriaan and van Laar 1994). However, LA measurements, especially in the field, are time-consuming, laborious, or demand expensive and sophisticated equipment. Thus, for many crop species, non-destructive, easily applied models were developed for LA estimation based on simple measurements of leaf dimensions (length and width). Such models have already been reported for maize (Stewart and Dwyer 1999), bean (Bhatt and Chanda 2003), taro (Lu *et al.* 2004), white clover (Gamper 2005), sugar beet (Květ and Marshall 1971, Tsialtas and Maslaris 2005), sunflower (Rouphael *et al.* 2007), radish (Salerno *et al.* 2005), and zucchini (Rouphael *et al.* 2006). An ideal model should be based on its simplicity, hence it should be derived from the measurement of only one leaf dimension and a linear function should relate this dimension with LA. Often, this is not feasible.

A linear model based on leaf width (W) measurements has already been established for sugar beet cv. Rizor (Tsialtas and Maslaris 2005). In sugar beet, LA is a trait related with photosynthesis and LAI (Scott and Jaggard 1993) but also relative leaf expansion rate (the algebraic sum of LA between two successive measurements) could be used as a screening tool for drought tolerance of sugar beet genotypes (Ober and Luterbacher 2002, Ober *et al.* 2005). However, the above-mentioned model is of restricted usefulness since sugar beet cultivars are diploid and triploid hybrids differing in their leaf shape and morphology (Bosemark 1993). Thus, another model based on measurements of sugar beet cultivars of

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Abbreviations: ANOVA – analysis of variance; L – leaf midvein length; LA – leaf area; LAI – leaf area index; W – maximum leaf width.
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divergent morphology should be launched in order to be widely used in sugar beet LA estimations. The aim of this work was to study the relationships between leaf dimensions and LA in a wide range of cultivars and to establish a prediction model of LA in sugar beets.

Experimentation took place in two successive years (2003 and 2004) at two sites in central Greece. Site 1 was located in the farm of *Hellenic Sugar Industry SA*, Larissa factory (39º43'N, 22º28'E, 76 m a.s.l.) on a soil formed by naturally drained alluvion with solonetz and solontchak in the depressions and with a high level of water table. Site 2 was laid beneath the foothills of eastern Mount Olympos (39º55'N, 22º39'E, 22 m a.s.l.) on a gray and dark coloured marshy soil with scattered solonetz and solontchak and a high level of water table, too. With a growing season from March to November, Site 1 has a typical Mediterranean climate with mild, rainy winter and hot, dry summer (mean temperature: 19.4 ºC, rainfall: 280 mm) while the microclimate of Site 2 is positively affected by the sea and is more rainy and cooler during summer (mean temperature: 17.8 ºC, rainfall: 380 mm). In each year, a different set of 16 commercial cultivars obtained from four breeders (*SESVANDERHAVE*, NV/SA, Tienen, Belgium, *Hellenic Sugar Industry SA*, Thessaloniki, Greece, *Danisco Seed*, Holefy, Denmark, *Florimond Desprez Co*., Cappelle en Pévèle, France) was arranged in a Randomized Complete Block design with six replications. The seeds were mechanically drilled (*Hege 80*, *Wintersteiger AG*, Ried, Austria) in four rows (8 m long) per plot, at 45 cm apart and at 9.1 cm spacing in each row. Sowing was conducted on 17–18 April 2003 and 18–19 March 2004. In 2003, sowing was delayed due to high rainfall during winter and early spring. Adequate fertilization [150 kg(N) ha^{-1}, 90 kg(P) ha^{-1}, 265 kg(K) ha^{-1}] was applied as basal and top-dressing. Supplemental irrigation was provided according to the needs of the crop and full protection was taken against cercospora, powdery mildew, weeds, and insects by sprayings.

In 2003, leaf sampling was conducted on 23rd August at both sites. In 2004, the leaves were collected on 24th August at Site 2 and on 5th September at Site 1. Three upper, healthy, and full-expanded sunlit leaves were collected from each plot, sealed in a plastic bag, and put in a portable refrigerator. The samples were transferred to the Physiology Laboratory of Larissa factory, *Hellenic Sugar Industry SA*, for determinations. LA, maximum W, and midvein length (L) were measured using *WinDias* image analysis system (*Delta-T Devices*, Cambridge, UK). Each year, a total of 576 leaves were measured. The average of the three measurements comprised the value of each replication. The mean value of each cultivar was derived from six replications (a total of 18 leaves) per site.

Data of each year were analyzed as a Randomized Complete Block design combined over sites with cultivars as main factor. The *M-STAT* statistical package (version *1.41*, Crop and Soil Sciences Department, Michigan State University) was used for the analysis. Figures were displayed using *Excel 98* software *(MSOffice, Microsoft)* and the significance level of the best-fitted curves was estimated by *SPSS 14* (*SPSS*).

ANOVA revealed that cultivars had the most significant effect on the leaf parameters, especially in 2004 (Table 1). The site×cultivar interaction was significant in no case. In 2003, no site effect on W and no cultivar effect on LA were evident. Based on the significant effect of cultivars, cultivar means were used to establish relationships between LA and leaf dimensions. According to

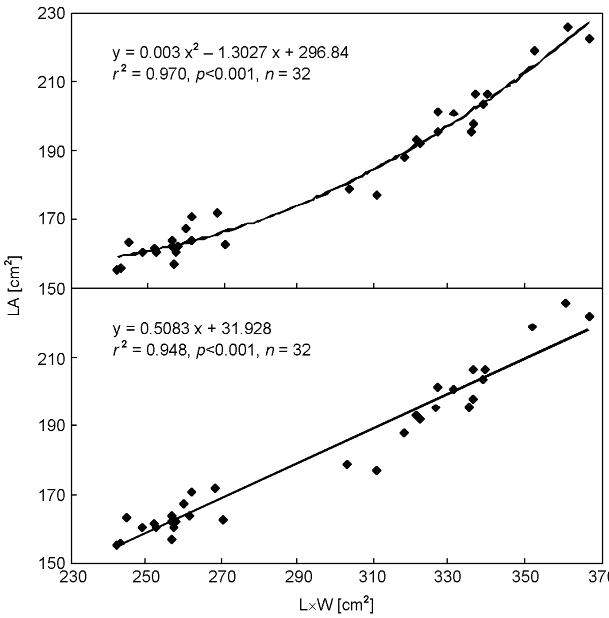

Fig. 1. Quadratic and linear curves fitted to the (L×W)–LA relationship.

Table 1. ANOVA of the leaf traits determined. df = degree of freedom, ns = not-significant ($p>0.05$), $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$.

Source of variation	df	2003 LA	2003 L	2003 W	2004 LA	2004 L	2004 W
Site (S)	1	*	*	ns	**	*	**
Cultivar (C)	15	ns	***	***	***	***	***
S×C	15	ns	ns	ns	ns	ns	ns
CV [%]		10.41	7.04	7.90	12.17	6.94	7.60

Lu *et al.* (2004), simple, linear relationships between leaf dimensions and LA would be preferable. In our work, the best-fitted curves between LA and leaf dimensions (L and W) were those of quadratic functions. Although significant, the L–LA relationship ($y = 0.4771\,x^2 - 9.9889\,x + 159.47$, $r^2 = 0.641$, $p<0.001$, $n = 32$) had a low r^2, which is a main criterion for selecting a curve as a prediction model (Rouphael *et al.* 2007). In accordance with previous reports (Tsialtas and Maslaris 2005, Rouphael *et al.* 2007), a higher r^2 was found for the W–LA relationship ($y = 2.587\,x^2 - 51.226\,x + 409.03$, $r^2 = 0.792$, $p<0.001$, $n = 32$) but it was still too low for providing an accurate, non-destructive prediction of LA. In order to establish a more accurate LA prediction model, L^2 and W^2 were used as suggested by other researchers (Salerno *et al.* 2005, Serdar and Demirsoy 2006). As regards the L^2–LA relationship, the best-fitted curve was a linear function ($y = 0.2607\,x + 44.607$, $r^2 = 0.641$, $p<0.001$, $n = 32$) with r^2 not higher than that of the L–LA relationship. Analogous results were found for the W^2–LA relationship ($y = 0.0027\,x^2 - 0.3061\,x + 155.54$, $r^2 = 0.794$, $p<0.001$, $n = 32$). A quadratic model having L×W as an independent variable showed to have a rather high r^2 and thus it could serve as an accurate LA prediction model for sugar beet cultivars (Fig. 1). Models based on L×W have already been established for other species such as zucchini (Rouphael *et al.* 2006), kiwi (Mendoza-de Gyves *et al.* 2007), and hazelnut (Cristofori *et al.* 2007). Also, L×W was linearly related with LA ($y = 0.5083\,x + 31.928$, $r^2 = 0.948$, $p<0.001$, $n = 32$) and thus this simple equation could also be used for LA predictions thanks to its simplicity (Lu *et al.* 2004) although its accuracy is lower compared with the quadratic function (Fig. 1).

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