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Research Note

Porometer for estimating stomatal conductance in maize: Determination of trueness and precision according to ISO 5725

Chiara Rusconi [a], Roberto Confalonieri [a,*], Ermes Movedi [a], Angela Gazzoli [b], Gregorio Arrigoni [b], Gloria Brocca [b], Anna Diva Cosentino [b], Tommaso Foglia [b], Federico Lombardo [b], Brando Mandelli [b], Marika Pavasini [b], Giacomo Pigni [b], Livia Paleari [a]

[a] Università Degli Studi di Milano, ESP, Cassandra Lab, Via Celoria 2, 20133, Milan, Italy
[b] Università Degli Studi di Milano, Cropping Systems MS Course, Via Celoria 2, 20133, Milan, Italy

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ABSTRACT

Stomatal conductance (g_s) is a key variable for quantifying crop water status and different technologies have been developed for its determination. While infrared gas analysers (IRGA) are widely recognised as a reference for g_s measurement, their limited usability and portability, and their cost, are making porometers an increasingly seductive alternative. However, few studies have compared porometers with other methods, and key information on performance metrics along the g_s biophysical range is missing. The accuracy (precision and trueness) of the LI-600 porometer for g_s measurement in maize was evaluated using the ISO 5725 protocol. A ring trial was carried out by growing plants in pots under three irrigation regimes to identify different g_s levels. Measurements of g_s were performed by three independent groups of operators at two growth stages using the porometer and the IRGA (reference). For intermediate g_s values, precision was satisfactory (mean relative standard deviation of repeatability and reproducibility <19%), whereas marked underestimations were observed. In cases of severely stressed and well-watered plants, the trueness was good (overall R^2 was 0.62), whereas the poor precision could be compensated by the possibility of taking a high number of replicates (very short time is needed for acquiring data). This, together with the high usability, make porometers an alternative in the case of intensive or time-constrained field campaigns.

1. Introduction

Water is considered as the major driver to meet the food demand of a growing global population (De Fraiture & Wichelns, 2010), and optimising its use through improved irrigation strategies is fundamental to increase crop productivity (Chartzoulakis & Bertaki, 2015). Besides the detrimental consequences on overall productivity, water stress episodes may have a variety of impacts on plant physiology (Yousaf et al., 2023). For this reason, many variables have been used to quantify crop water status (e.g., Devi et al., 2022), and different technologies have been developed to estimate water stress, mostly based on remote sensing (e.g., Costa-Filho et al., 2020), simulation models (e.g., Zhou et al., 2024), and proximal sensors (e.g., Mertens et at., 2021). However, the extremely rapid response of stomatal conductance (g_s, mmol m^{-2} s^{-1}) to water stress (Medrano, Escalona, Bota, Gulías, & Flexas, 2002) makes this variable one of the most reliable indicators of crop water status (e.g., Jones, 2004), and potentially able to support the optimisation of irrigation strategies (Liao et al., 2022).

Portable infrared gas analysers (IRGA) and leaf porometers are among the most popular instruments to estimate g_s. The former (e.g., CIRAS-3; PP Systems, Amesbury, MA, USA) quantifies g_s in real time by measuring the exchange of gases between the atmosphere and the interior airspace of the leaf, whereas the latter (e.g., LI-600; LI-COR, Lincoln, NE, USA) estimates g_s by measuring the water vapour produced by plants during transpiration. Currently, IRGA is regarded as the most reliable method for g_s determination (Toro et al., 2019). However, some limitations of this technology (price, time for acquiring measurements, usability) are increasingly making porometers a seductive alternative for a variety of users. As well as being user-friendly and fairly inexpensive compared to other instruments, modern porometers are highly portable

* Corresponding author.
E-mail address: roberto.confalonieri@unimi.it (R. Confalonieri).

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and extremely fast in acquiring measurements.

Although porometers are used in a variety of research, very few studies have compared their performances with those from other approaches (e.g., Batke et al., 2020; Toro et al., 2019), and, even in these cases, no standard protocols have been adopted for their evaluation. Indeed, no information is available on key performance metrics for porometers, such as precision, accuracy, repeatability and reproducibility, this being a frequent gap in the environmental and agronomic literature, regardless of the variable investigated (Confalonieri et al., 2014). The absence of this information leads to uncertainty in the interpretation of measurement results, and it hinders (i) a reliable comparison of results obtained using different technologies and (ii) a straightforward selection of the most suitable method for the specific experimental context (González & Herrador, 2007).

In various disciplines (e.g., chemistry), protocols for methods validation (e.g., ISO, 1994) are considered indispensable to provide users with standardised information about the methods themselves, and to ensure objectivity to the whole evaluation process (Slezak & Waczulikova, 2011). The difficulties in measuring living entities with non-destructive procedures and in creating homogeneous reference values (Rambla-Alegre et al., 2012) have discouraged scientists from evaluating *in vivo* field methods according to standard validation protocols. However, a few exceptions have demonstrated how standard protocols are crucial for certifying the quality of methods and for certifying their suitability for a specific purpose or context before routine adoption (Acutis et al., 2007).

A full validation would require the determination of many metrics – selectivity/specificity, limit of detection, limit of quantification, recovery, working range and linearity, accuracy (composed by trueness and precision, the latter including repeatability and reproducibility), robustness (ISO, 1994) – with some of them impossible to be determined on living entities. However, the exclusion of certain metrics does not compromise the validation procedure (e.g., Scaglia et al., 2007), and determining the different components of the accuracy is sufficient to ensure the quality of field data obtained using *in vivo* methods (Confalonieri et al., 2014).

The objective of this study was the determination of the accuracy of the LI-600 porometer – for estimating g_s in maize – according to the ISO 5725 validation protocol (ISO, 1994) adapted to *in vivo* field methods.

2. Materials and methods

2.1. Experimental data

The ring trial was carried out on the campus of the Faculty of Agriculture of the University of Milan, (latitude 45°28′N, longitude 9°13′E) in 2023. Three different maize hybrids (Pioneer P1096, Pioneer P2088, Pioneer P0937) were sown on May 4 in 10 l pots (280 mm diameter, 275 mm height). Plant management prevented any nutrient stress and damages caused by pests and diseases. For each hybrid, a total of 12 plants was grown. To explore a wide range of water availability, three treatments were applied on July 9 (V7–V8 stage) and July 15 (V9–V10) using a drip irrigation system: fully irrigated, half of the water amount used in the first treatment, and no irrigation. For the remaining parts of the crop cycle, plants were fully irrigated. Plastic screens were positioned 50 mm above the top border of the pots (around the stems) to prevent rainfall from altering the irrigation treatments while allowing air circulation at the soil-atmosphere interface.

For each pot, g_s was measured on abaxial leaf surfaces by using a LI-600 steady-state porometer on the two youngest undamaged, completely unfolded sunlit leaves. In addition, the total g_s was measured on the same leaves with a CIRAS-3 portable gas exchange analyser. To allow the comparison between the measurements collected with the two instruments, values of total g_s from porometer were derived according to the percentages of stomata in the adaxial and abaxial maize leaf surfaces (Driscoll et al., 2006; Revilla et al., 2016; Zheng et al., 2013).

Measurements were collected around midday at two dates, three days after the day when the irrigation treatments were applied. For both dates, average air temperature, relative humidity and solar radiation during the measurement sessions are shown in Fig. S1.

2.2. Accuracy determination (ISO 5725)

The different components of the porometer accuracy were quantified by following the ISO 5725 protocol for analytical methods (ISO, 1994) as adapted to *in vivo* field methods by Confalonieri et al. (2014). According to ISO (1994), accuracy is given by both precision and trueness. Precision is in turn defined as composed by repeatability (agreement between measurements performed by the same operator under the same conditions) and reproducibility (agreement between measurements performed by different operators under different conditions). Trueness represents the agreement between the mean value of all the measurement replicates and the reference value. Given the impossibility of producing standard reference materials (*true values*) when working on living entities, measurements obtained from a method considered as the reference for g_s (i.e., IRGA; Toro et al., 2019) were used as *true values* (Confalonieri et al., 2014). The inter-laboratory effect required by the ISO protocol was reproduced by dividing operators into three independent groups (laboratories hereafter) and providing them with the porometer and related user manual. Each laboratory performed measurements in different moments of the day between 11.00 a.m. and 2.00 p.m., under different temperature, humidity, and irradiance levels (Fig. S1), and without any communication between laboratories. This mimicked the effects (reproducibility) of different conditions during the measurements and of possible discrepancies in the interpretation of the method from different laboratories (Confalonieri et al., 2014).

The ISO 5725 protocol evaluates a method at different levels of the analyte (i.e., of the target variable) by using, for example, reference materials with different pre-defined analyte concentrations. Given (i) the already mentioned impossibility to get reference materials with living entities and (ii) the indirect relationship between irrigation treatments and g_s values (other factors can be involved besides irrigation volumes), the different levels were defined *a posteriori* according to the values obtained using the reference method.

According to ISO (1994), after checking the normality of data using the Shapiro and Wilk (1965) test, outlier detection was carried out on the variances (Cochran (1941) test) and on the means (Grubbs (1969) test) of the replicates of each laboratory. Data from the remaining laboratories were then used to calculate, within each level, the standard deviations of repeatability (S_r) and reproducibility (S_R) (Eqs. (1)–(5)):

$$S_r = \sqrt{\frac{\sum_{i=1}^{p}(n_i - 1)s_i^2}{\sum_{i=1}^{p}(n_i - 1)}} \tag{1}$$

where p is the number of laboratories, n_i is the number of measurement replicates for the i th laboratory, and s_i^2 is their variance. The between-laboratory standard deviation (S_L) is calculated as:

$$S_L = \sqrt{\frac{S_d^2 - S_r^2}{\bar{\bar{n}}}} \tag{2}$$

with

$$S_d^2 = \frac{\sum_{i=1}^{p} n_i(\bar{y}_i - \bar{\bar{y}})^2}{p - 1} \tag{3}$$

and

$$\bar{\bar{n}} = \frac{1}{p-1}\left(\sum_{i=1}^{p} n_i - \frac{\sum_{i=1}^{p} n_i^2}{\sum_{i=1}^{p} n_i}\right) \tag{4}$$

where $\bar{y}_i$ and $\bar{\bar{y}}$ are the mean of the i th laboratory and the overall mean,

respectively.

$$S_R = \sqrt{S_r^2 + S_L^2} \quad (5)$$

In cases when S_r was larger than S_R, S_L was set to zero and S_R equal to S_r (Horwitz, 1995). Finally, the limits of repeatability (r) and reproducibility (R), representing the maximum expected values of the absolute difference between two results under repeatability and reproducibility conditions, were calculated by multiplying S_r and S_R by $\sqrt{2}t_\infty$, with t_∞ being the Student's t (two tails) for ∞ freedom degrees and $\alpha = 0.05$ (ISO, 1994).

The trueness of the method was calculated by comparing the reference values and the mean of all the replicates using the following metrics: relative root mean square error (RRMSE; 0 to $+\infty$, optimum 0; Jørgensen et al., 1986), modelling efficiency (EF; $-\infty$ to +1; optimum +1; Nash & Sutcliffe, 1970), and the coefficient of residual mass (CRM; $-\infty$ to $+\infty$; optimum 0; Loague & Green, 1991).

3. Results

Table 1 shows the precision metrics of the porometer for six levels of g_s. To facilitate the comparability of results with those from other methods, S_r and S_R are expressed on a relative basis, by simply calculating their coefficient of variation. Resulting values are the relative standard deviation of repeatability (RSD_r) and reproducibility (RSD_R).

The precision metrics covered a wide range of values, with both r and R ranging from 64.43 to 325.93 mmol m^{-2} s^{-1}. The best performance in terms of precision resulted for the levels 2, 3, and 4, i.e., reference g_s ranging from 100 to ~200 mmol m^{-2} s^{-1}, where the lowest repeatability and reproducibility limits were achieved. On the contrary, poor precision metrics were estimated for the levels corresponding to reference g_s values < 56 mmol m^{-2} s^{-1} and >268 mmol m^{-2} s^{-1}. In particular, the worst precision was obtained for level 1 (i.e., the lowest value of reference g_s), with RSD_r and RSD_R exceeding three times those calculated for the other levels.

R and RSD_R were always very similar to the corresponding r and RSD_r, and no clear relationships (e.g., positive or negative trends) were found between their values and reference ones (Fig. 1).

Fig. 2 and Table 2 show the agreement between reference g_s values and those measured with the porometer. With the exception of what observed for the lowest (<~120 mmol m^{-2} s^{-1}) and highest (>~300 mmol m^{-2} s^{-1}) reference g_s values, the porometer presented marked underestimations (positive value of CRM), which led to an R^2 equal to 0.62 ($p = 0.06$) and to a positive EF.

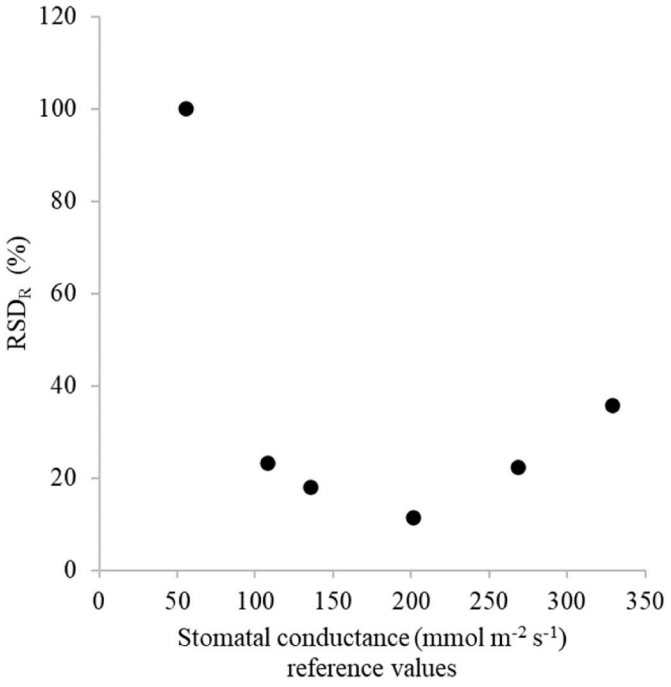

Fig. 1. Relationship between relative standard deviation of reproducibility (RSD_R) and reference values of stomatal conductance (corresponding values of repeatability are not shown since always very close to reproducibility ones; see Table 1).

4. Discussion

The porometer showed, in general, a satisfactory level of precision, except for g_s values close to the extremes of the explored range. For intermediate values (from about 100 to ~270 mmol m^{-2} s^{-1}), precision metrics agreed with those reported in the literature for methods aimed at estimating variables describing canopy architecture (Confalonieri et al., 2017). On the contrary, Confalonieri et al. (2015) reported considerably higher precision for methods estimating plant nitrogen content implemented in commercial instruments, with average values of RSD_r and RSD_R almost ten times lower than those achieved in this study. Similarly, Bocchi et al. (2008) obtained lower precision metrics for a method used to quantify the stability of soil aggregates, with RSD_r never exceeding 8%, and RSD_R lower than 9% for all levels but one, where the metric was 23.83%. Scaglia et al. (2011) also reported lower precision metrics (RSD_r and RSD_R never exceeded 15.5%) for a method aimed at estimating the biological stability of municipal solid waste.

The unexpected agreement between the values of RSD_r and RSD_R

Table 1
Precision metrics (repeatability and reproducibility) of the porometer according to the ISO 5725 protocol (ISO, 1994).

Level	Date	Mean stomatal conductance (mmol m^{-2} s^{-1})		Repeatability		Reproducibility	
		Reference	Porometer	r[a]	RSD_r[b]	R[c]	RSD_R[d]
1	2	55.56	82.22	139.17	90.38	154.37	100.25
2	1	107.50	80.83	69.53[e]	23.33[e]	69.53	23.33
3	1	135.56	79.17[e]	68.46[f]	18.22[e]	68.46	18.22
4	1	200.83	73.75	64.43[e]	11.57[e]	64.43	11.57
5	1	268.33	126.39	167.31[e]	22.49[e]	167.31	22.49
6	2	328.89	280.56	325.93[e]	35.75[e]	325.93	35.75

[a] Repeatability limit (mmol m^{-2} s^{-1}).
[b] Relative standard deviation of repeatability (%).
[c] Reproducibility limit (mmol m^{-2} s^{-1}).
[d] Relative standard deviation of reproducibility (%).
[e] One laboratory detected as outlier according to the Cochran test.
[f] Corrected values (if $S_r > S_R$, S_r set equal to S_R; Horwitz, 1995).

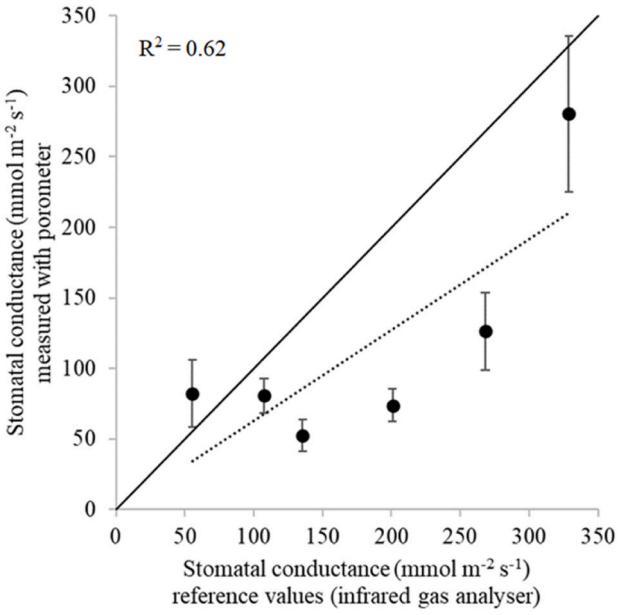

Fig. 2. Comparison between stomatal conductance values form the reference method and from the porometer. The dotted line represents the regression line, whereas the solid line shows the perfect (theoretical) agreement between the two methods.

Table 2
Trueness metrics of the porometer.

	Relative root mean square error (RRMSE; %)	Modelling efficiency (EF)	Coefficient of residual mass (CRM)
LI-600 porometer	48.4	0.12	0.37

seems to indicate that the method was more affected by an intrinsic uncertainty during repeated measurements rather than by effects depending on the operator or on environmental conditions. This could be partly explained by considering the time needed to take measures with the porometer in light of the temporal variability of g_s (Matthews et al., 2017). In the case of IRGA, high-frequency variations in g_s are captured, as the leaf chamber requires ~ 120 s to equilibrate before providing the measurement result (Paleari et al., 2024). In contrast, the porometer provided nearly instantaneous measurements, preventing the instrument from capturing high-frequency fluctuations in g_s. However, the short time needed to acquire measurements with the porometer could represent, together with its high usability and portability, a positive feature in contexts where the alternative would be not measuring g_s at all, given the poor suitability of IRGA for intensive field campaigns. For these kind of campaigns, the lower precision of the porometer could be compensated by increasing the number of replicates.

Concerning the trueness, results highlighted an overall underestimating behaviour of the porometer, contrary to that observed by other authors (i.e., Lavoie-Lamoureux et al., 2017; Batke et al., 2020), who reported overestimations ranging from about 25% to 50% compared to IRGA. However, the R^2 achieved in this study was slightly more satisfactory than those achieved by other authors. An exception was reported by Toro et al. (2019), where stronger correlations (R^2: 0.78 to 0.92) were observed for four species, although only under water stress conditions, whereas correlations were poorer for well-watered treatments (e.g., R^2 for well-watered maize plants was 0.095). Besides the under- or over-estimating aspect, the results reported in literature were consistent with those obtained in this study in highlighting how the worst agreement was achieved for intermediate g_s values. A possible explanation is that the porometer could be insufficiently sensitive to the partial closure of stomata.

Despite the satisfactory trueness at low g_s values, the method exhibited the lowest precision under severe water stress conditions. This means that, with a low number of replicates, the reliability of the method for low g_s could be penalised to the extent that some authors (e.g., Pietragalla & Pask, 2012) have discouraged its use in cases of severe stress. As already mentioned, according to the trueness values at low g_s levels achieved in this study, a high number of replicates could partly compensate this low precision.

5. Conclusions

This study provided valuable insights into the performance of the porometer for estimating g_s in maize crops. The poor precision observed under well-watered and, especially, severe water stress conditions could be partly compensated for by performing a high number of replicates. This is made possible, even in intensive or time-constrained field campaigns (and contrarily to IRGA-based instruments), by the very short time needed to acquire measurements and by its high usability and portability. The results also highlighted that the porometer was best suited for low and high g_s values (corresponding to severely stressed and well-watered plants), whereas it seems to not be sensitive enough under conditions characterised by the partial closure of stomata.

This study also underlined the need for rigorous and quantitative validation protocols (e.g., ISO, 1994) for *in vivo* methods, in order to provide users with clear information on quantitative metrics describing the different components of accuracy and with indications on possible differences in the methods reliability along the biophysical range of the variables of interest. Moreover, the use of standard validation protocols allowed the comparability of alternative methods and the possibility to identify the most suitable for a specific research context.

CRediT authorship contribution statement

Chiara Rusconi: Data curation, Formal analysis, Investigation, Writing – original draft. **Roberto Confalonieri:** Conceptualization, Formal analysis, Supervision, Writing – review & editing. **Ermes Movedi:** Data curation, Investigation. **Angela Gazzoli:** Data curation, Investigation, Supervision. **Gregorio Arrigoni:** Data curation, Investigation. **Gloria Brocca:** Data curation, Investigation. **Anna Diva Cosentino:** Data curation, Investigation. **Tommaso Foglia:** Data curation, Investigation, Methodology. **Federico Lombardo:** Data curation, Investigation, Methodology. **Brando Mandelli:** Data curation, Investigation. **Marika Pavasini:** Data curation, Formal analysis. **Giacomo Pigni:** Data curation, Investigation. **Livia Paleari:** Conceptualization, Formal analysis, Methodology, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biosystemseng.2024.12.013.

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