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3D laser triangulation, a simple and robust method for automated growth determination of crop plants in challenging environments

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Abstract: To increase the understanding of plant genotype x environment interactions using high-throughput plant phenotyping, simple and robust methods should be developed. This is necessary in order to broaden the application range of phenotyping across the plant research community, but it is also important that future research on plant phenotypic responses to abiotic stresses includes studying the plants in their natural environments. This will increase our understanding of how multiple stresses interact in defining the plant phenotype, and will give us a realistic understanding of the interaction between plant responses and epigenetic regulation. In the present paper we validated a commercial 3D NIR-laser scanner (PlantEye, Phenospex B.V., Herleen, The Netherlands) to track daily changes in plant growth with high precision in challenging environments. Firstly, we demonstrated that the NIR laser beam of the scanner does not affect plant photosynthetic performance. Secondly, we demonstrated that it was possible to estimate phenotypic variation amongst 10 genotypes of *Brassica napus* L. (rapeseed), using a linear correlation between scanned parameters and destructive growth measurements. Our results demonstrate the high potential of 3D laser triangulation for simple measurement of phenotypic variation in challenging environments and in a high temporal resolution.

1. Introduction

Plant screening and phenotyping technologies with an appropriate resolution and at high frequencies in fluctuating climate environments are essential to improve the efficiency of high-throughput plant phenotyping (HTPP) towards understanding how phenotypic variation is linked to environmental conditions. Most sensors enabling non-destructive measurements of plant growth are based on optical principles, and these measurements are therefore greatly affected by changes in illumination. This requires complicated normalisation and calibration software, which makes their application impracticable, if not impossible, under conditions with fluctuating illumination. This issue is easily solved by using closed cabinets to which plants are transported for imaging, or by measuring under dark conditions [1, 2]. However, though transport was shown not to affect plant growth [3], it comes along with lower throughput. Furthermore, in many phenotyping platforms, plants are often placed in rows with large distances between the pots [1, 4], or screened individually following a plant-to-sensor concept, where the plant is transported on a conveyor belt to the screening unit [2, 5]. These applications do not reflect the situation in the field or in production units where plants are grown at high density and where the structure of the individual plant is affected by competition for light and resources. Moreover, diurnal measurements are usually not possible because of insufficient throughput, even though high temporal resolution is a fundamental requirement to understand how external and internal factors determine differences in canopy structure, and how different genotypes adapt to the dynamic and changing environment. For instance, leaves within natural canopies are constantly changing orientation due to endogenous mechanisms and external factors such as light and water availability [6]. Direct methods to quantify these structural changes are important for the understanding of how plants optimise the canopy structure to maximise light utilisation and minimise water loss under heat and drought stress.

The development of 3D imaging techniques for estimating canopy structure, shoot growth and biomass has expanded during the last couple of years. Stereo camera systems using two RGB (red, green, blue) cameras give the ability to capture three-dimensional images [7, 8], whereas sensors more suitable for greenhouse and field conditions are light detection and ranging (LIDAR) and laser triangulation [9. 10]. In contrast to camera systems which are passive, LIDAR and laser triangulation are active devices, in which a light beam (laser line or dot) is projected onto plants and the energy scattered from the plant is used for the computation of depth maps and 3D point clouds.

Light sources in the visible spectrum have direct effects on plant photosynthesis and primary carbohydrate metabolism, and affect the circadian clock if applied in dark periods. Furthermore, near-infrared light that is not reflected can induce transitions in the manganese cluster of photosystem II (PSII) above 800 nm [11], and recent findings illustrated that far-red light up to 800 nm can drive PSII electron transport [12], suggesting that photochemistry may also be affected by even longer wavelengths. This can have implications for processes related to the photosynthetic apparatus in plants, and can affect plant yield. However, to our knowledge, no effort has been put into studying these effects when validating the use of light sources in plant growth sensors.

The purpose of the present paper was to evaluate a commercial 3D laser triangulation scanner and to test its suitability for HTPP in a greenhouse environment.

2. Material and Methods

2.1 The 3D laser triangulation scanner

The automated plant growth measurements were performed using the commercial 3D laser triangulation scanner PlantEye F300 developed by Phenospex B.V. (Heerlen, the Netherlands, http://phenospex.com/products/plant-phenotyping/science-planteye-3d-laser-scanner/) (Fig.1A). The sensor projects a laser line in the near infrared (NIR) region of the light spectrum vertically downwards and captures the scattered light with an integrated CMOS-camera. NIR is used to increase data guality

Figure 1. A) Drawing of the 3D triangulation scanner, the red lines shows the width and projection of the laser line, the blue line is the projection and width of the camera and the green line/area displays the crop stand. B) The set up showing the positioning system in its starting position with the 3D triangulation scanner mounted.
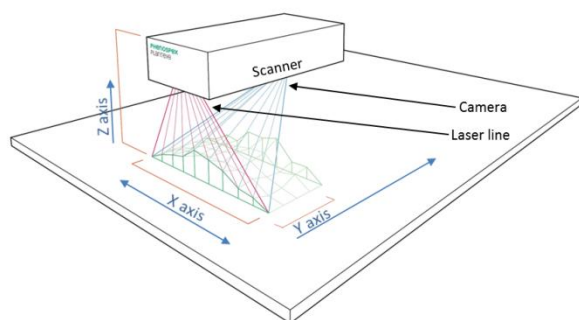
A   B
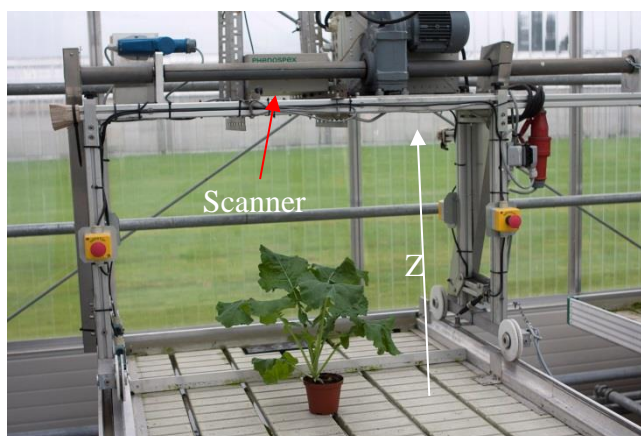
since most of the light is reflected from plants, compared to other commercial laser scanners, which typically use light in the visible spectrum. A sunlight filter reduces all artefacts like reflections or

background noise from sunlight or other light sources, allowing reproducible measurements under direct sunlight or high irradiances. Moreover, all internal parts like the laser diode and camera is temperature-controlled by a thermoelectric cooler, allowing the operation of the sensor at ambient temperatures of up to 45°C without any cutback or loss of data quality.

The 3D laser triangulation scanner was mounted on a boom (Technical University of Southern Denmark, Odense, Denmark) (Fig. 1B). The boom was placed on a greenhouse table (1.2 x 8 m), with the distance of the scanner to the table of 850 mm, resulting in a scan width (x-direction) of 640 mm. The automated boom can be programmed to run at six different velocities (denoted 1 − 6), and at specific time points during the day and on specific days, controlled by a digital timer system. To check on the evenness of the velocity of the boom, the time was recorded after every 20 cm at velocity 6. The velocity was measured to be 50.4 mm s^{-1} with a standard error of 1.5 mm s^{-1} over a distance of 6 m.

During the scanning process the scanner moves linearly over the plants in the y-direction (Fig. 1), and collects images of the projected laser light on the plants. The scanning field (greenhouse table) can be divided into a number of subfields and, from each image of a subfield, depth profiles in each x-z plane are computed. From these a 3D point cloud is calculated for the subfield canopy. From each 3D point cloud, the meshing of neighbouring points (segmentation) is automatically carried out, and plant height, projected leaf area, 3D leaf area and leaf angle distribution of the subfield canopy are computed. The resolution in the y-direction depends on the scanning speed of the scanner, which generates 50 xz depth profiles per second, with a resolution of 0.8 mm in the x-direction (width) and a resolution of 0.2 mm in the z-direction (height information). For instance, if the scanning speed is 50 mm s^{-1}, the resolution in the y-direction is 1 mm. To start and stop the scanning process, metal barcodes with a size of 20 x 40 mm are positioned at the end of the scanning area on the table. The scanning area is 6 m x 0.64 m. If the number of subfields is defined as 10 in the scanner software, the software separates the depth profiles (3D point clouds) in 10 data files, one for each subfield being 600 x 640 mm in size.

The height of plants placed in each subfield is calculated from the histogram of the height data. The scanner software arranges the 3D point cloud as a histogram displaying the number of point measurements in relation to the distance from the sensor (z-direction). The height information is then arranged in percentiles, from which only part is used for the computation of the plant height. This process is called cropping and is defined in the sensor settings. In most plant species, 80% of the lower points and 10% of the higher points of the histogram are discarded. The average of the remaining points (between the 80 to 90% percentiles) has been found to give a robust estimate of plant height. The projected leaf area is then calculated, as a proportion of the subfield area. The 3D leaf area is computed by considering the distances in 3D taking the steepness of the leaf angle into account. This is a far better estimation of leaf area compared to the projected leaf area which is delivered by 2D

imaging. A comparison between the estimate of 3D leaf area and projected leaf area (2D) was made by scanning a flat object with an area of 116.2 cm^2 with the angle of the object towards the scanner increased incrementally in the y-direction (Fig. 2A-B) or x-direction (Fig. 2C-D).

The results showed that the surface of the object was estimated to 116.4 ± 2 cm^2 at an angle of 0º for 3D area and to 118.9 ± 1 cm^2 for 2D area. At an angle of 80° in the y-direction, the 3D area was 107.6 ± 3 cm^2 and the projected area was 221.8 ± 1 cm^2; a similar result was obtained when tilting the object in the x-direction. Above the angle of 80º, the 3D leaf area was incorrectly estimated, especially when the object was tilted in the x-direction. This was caused by insufficient scattering of light back into the camera. These results demonstrate the advantages of 3D measurements, being sufficiently precise in predicting the area, even at increasing leaf-angles in both the x- and y-direction, compared to 2D which only allows the measurement of the projected leaf area.

Figure 2. Validation of the parameters "projected leaf area" (projected LA) and "3D leaf area" (3D LA) automatically computed in the 3D triangulation scanner software. An object with defined area of 116.2 cm^2 was tilted stepwise to the z-plane in the y- and x-direction and measured by the 3D triangulation scanner. Projected LA and 3D LA were then computed of this object for different angles. Area of object (white dots), computed area of object (black dots). Values are average of three ± SE, with SE´s below 1 cm^2 for most values.
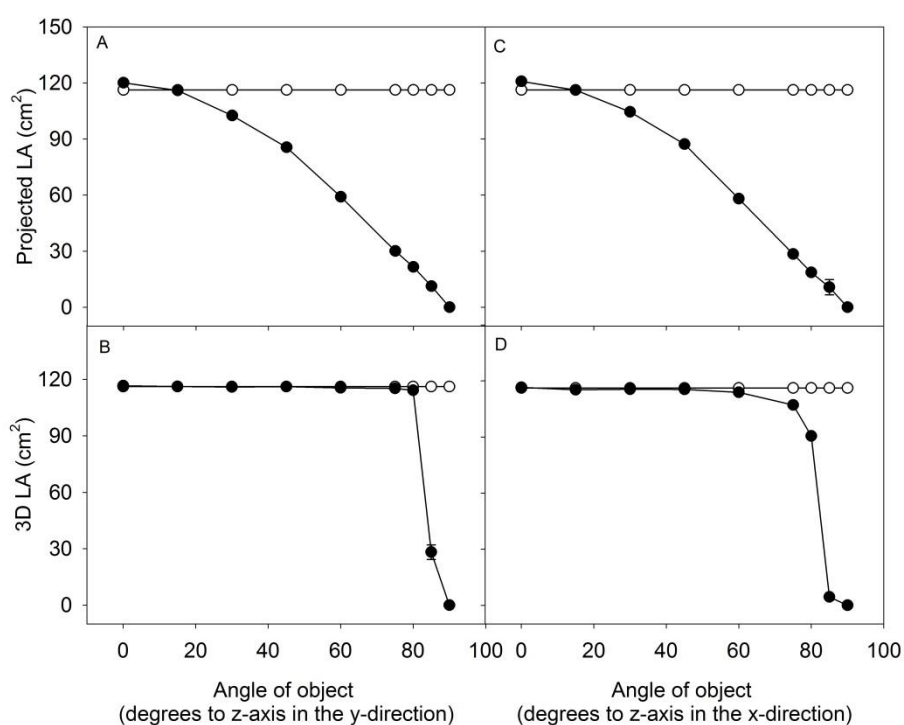
2.2   *Experiment   1:*

Validating a potential influence of the projected laser line on photosynthetic activity

The 3D laser triangulation scanner is equipped with a NIR laser belonging to the laser class 1M (940 nm), meaning that it is eye-safe to use in all conditions except when passed through magnifying optics. In order to test whether a laser line of this power had any effects on the plant physiological performance, the PSII photochemistry of rapeseed plants was monitored continuously with a PAM fluorimeter (MONI-PAM, Walz, Effeltrich, Germany) for plants placed underneath the scanner. Half of the laser line was covered using black tape (TESA 4613, tesa A/S, Amsterdam, The Netherlands) and six plants were placed on the table so that three plants were exposed to the laser and three plants were not exposed to the laser. The system was set to measure every 30 min (00:00 h and 00:30 h) at a scanning speed of 20 mm s^{-1}. The measurements took 5 min from start to end, and the MONI-PAM was set to measure every 20 min (00:05, 00:25 and 00:45 h). The chlorophyll fluorescence measuring system consisted of six emitter-detector units (MONI-heads), each representing independent fluorimeters. Three MONI-heads were placed directly underneath the path of the laser line, and the three other MONI-heads were placed outside that path. Rapeseed leaves of similar age were fixed in the MONI-heads leaf clips, and measurements of photosynthetic active irradiation in the range of 400-700 nm (PAR, µmol m^{-2} s^{-1}) using the integrated quantum sensor, maximum photochemical efficiency of PSII during the dark period; $F_v/F_m = (F_m-F_0)/F_m$, Quantum yield of PSII (Φ_{PSII}); $F'_q/F'_m = (F'_m-F')/F'_m$ and the electron transport rate (ETR) during the light period [13], were recorded continuously as described above. The intensity of the light saturating pulse was 1800 µmol photons m^{-2} s^{-1} and the duration of the pulse was 0.8 s. The measurements were done on eight consecutive days. Every day at 10:00 h the MONI-heads were randomised, and a new part of the leaf was used, and after four days the black tape was moved to cover the other 50% of the laser line in order to obtain a completely randomised design.

2.3 Experiment 2: Predicting growth parameters of rapeseed by 3D laser triangulation

The experiment was conducted to develop a model from which it would be possible to predict destructive growth parameters of rapeseed by using the parameters obtained by 3D laser triangulation. The experiment on rapeseed was carried out from 24 September to 6 October 2013 (plant batch 1), and repeated from 8 – 29 November 2013 (plant batch 2) in a greenhouse located at the Department of Food Science, University of Aarhus (Aarslev, Denmark, Lat. 55°N). The light period was 20 h per day, supplemented by artificial light provided by high-pressure sodium lamps (SON-T agro, 600 W, Phillips, Eindhoven, The Netherlands) at 130 µmol m^{-2} s^{-1} photosynthetic flux density. The temperature set point was 20°C with opening of the vents at 25°C. The plants were watered by flooding for a short period every second day.

Seed of the rapeseed genotype 'DH5' was sown in 11 cm pots containing peat and placed immediately on the table where the 3D triangulation scanner was mounted. The field of the 3D triangulation scanner was divided into ten longitudinal subfields (640 x 600 mm) with six pots in each subfield, distributed in two rows with three pots in each. The distance between the two rows was 80 mm and the distance between pots in the rows was 60 mm. Plants from each of 10 subfields were harvested at growth stages defined by the number of leaves, to allow comparison of destructive and non-destructive measurements at different stages of plant development. The scanning measurements of each subfield were conducted with a scan velocity of the boom of 20 mm s^{-1}, giving a resolution of less than 1 mm in the scanning direction (y). Scans were carried out every hour over a period of three weeks.

The destructive harvests were carried out at 12:00 h, and all six plants from a subfield were harvested. For each plant, leaves were separated from the petioles, the number of leaves was counted (LN) and the total leaf area (LA) was determined using a leaf area meter (LI-3000, LI-COR, Lincoln, NE). Plant fresh weight (FW) was determined, plant material was dried at 70°C for 24 h and plant dry weight (DW) was determined. The leaf area index (LAI) was calculated as total leaf area of the six plants per ground area of the subfield (1350 cm^2). The average values per plant of the destructive growth measurements (FW, DW, LN, LA and LAI) were related to the estimated values of projected leaf area (cm^2 plant^{-1}), 3D leaf area (cm^2 plant^{-1}) and height (cm). The estimated values were calculated from the scanning measurements conducted in the middle of the dark period (00:00 h) the night before the destructive harvests. This time point was chosen based on earlier observations that variation in environmental conditions have least effect on the plant structure at this time of day, making the day-to-day measurements of growth most reliable. The calculated parameters were based on four out of the six measured plants to avoid two plants at the edge, which were expanding their leaves outside the plot. These two plants were manually removed from the 3D point cloud. The correlation analysis was carried out on plants with leaf numbers ranging from two to seven (including cotyledons) from six subfields out of 10 from each batch (12 in total). In the last four subfields of both batches, the plants had too many overlapping leaves, or leaves were extending out of the plot.

2.4 Experiment 3: Validating the model on 10 rapeseed genotypes

A screening experiment with 10 rapeseed genotypes was carried out in order to validate the linear models on the rapeseed genotype 'DH5' from experiment 2. The experiment was carried out from 26 July to 19 August 2013 with greenhouse climate set points similar to those for Experiment 2. The seeds of 10 rapeseed genotypes, 0. Chuosenshu, 1. Cobra, 2. Expert OSR, 3. Palu, 4.Olympiade, 5.Major, 6.S13, 7. Resyn HO48, 8. Markus and 9. DH5, were sown in 11 cm pots containing peat and

placed directly in 10 subfields on the table where the scanner was mounted. The distance between the pots was similar to that in experiment 2. Scanning measurements of each subfield were conducted every hour at a scan velocity of 20 mm s^{-2}, as in experiment 2. At the final harvest 24 days after sowing (DAS), all plants from each subfield were harvested. For each plant, the leaves were counted (LN) and the total leaf area (LA) was determined using a leaf area meter (LI-3000, LI-COR, Lincoln, NE). Plant fresh weight (FW) was determined, plant material was dried at 70°C for 24 h and plant dry weight (DW) was determined. The linear models obtained in experiment 2 were used to calculate daily values for plant FW, DW and total leaf area, to generate growth curves for the 23 DAS until the 18 August, the day before harvest.

2.5 Data analysis

Data analyses were carried out using the R-language stat package [14]. For experiment 2, the effects of independent variables of plant batch and measured scanning parameters on the dependent variables of the destructive harvest measurements were tested. There was no effect of plant batch number and therefore Pearson's correlation coefficients were calculated for all plants and between all variables using the rcorr() function and corrected for multiple interferences using the Holm's method. Linear correlations were calculated using the lm() function and the regression lines were fitted to the curves showing the relation between the scanning parameters and the destructive harvest measurements in Sigmaplot (ver. 11, Systat software, 2008).

In the screening experiment (Expt 3) the measurements of the destructive harvest after 24 DAS were analysed using a one-way ANOVA with genotypes as main effect, and the average values were separated across the different genotypes using the glht() function to analyse for multiple comparisons using the Tukey correction (95% confidence intervals).

3. Results and discussion

2.1 Photosystem II efficiency of rapeseed was not affected by the near-infrared (NIR) laser line

The diurnal measurements of PSII operating efficiency, (Φ_{PSII}) and the electron transport rate (ETR) varied greatly in relation to the changing light conditions during the eight consecutive measurement days, and in relation to the actual light level at which the individual MONI-heads were positioned (results not shown). Furthermore, flashing with high light every 20 min during the night decreased the maximum photochemical yield of PSII (F_v/F_m), possibly because the leaves became less and less dark-adapted. However, F_v/F_m was always above 0.8, which is in the range of F_v/F_m values (0.79-0.84) shown not to affect plant yield [13]. As seen in figure 3 there was no significant effect of

the NIR laser line after 0, 20 and 40 min of exposure on the F_v/F_m or Φ_{PSII} (F'_q/F'_m) values. These measurements were made continuously every hour throughout the eight days, but the figure only shows mean values from the two time points 00:00 h (beginning of dark period) and 04:00 h (beginning of light period) for the eight consecutive days in order to avoid effects of differences in light irradiance between consecutive days. The electron transport rate (ETR) was linear related to PAR under the low light conditions in the two treatments showing that photochemistry and photosynthesis of the plants was unaffected by the NIR laser line (Fig. 4).

Figure 3. Values of maximum photochemical efficiency of PSII at the beginning of the dark period (F_v/F_m) and Quantum yield of PSII at the beginning of the light period (F'_q/F'_m) in rapeseed plants exposed to the near-infrared laser line from the 3D triangulation scanner or placed in a control treatments. The mean values are shown for the time point of 00:00 h and 04:00 h. Control plants (grey bars) and scanned plants (white bars), n=3.
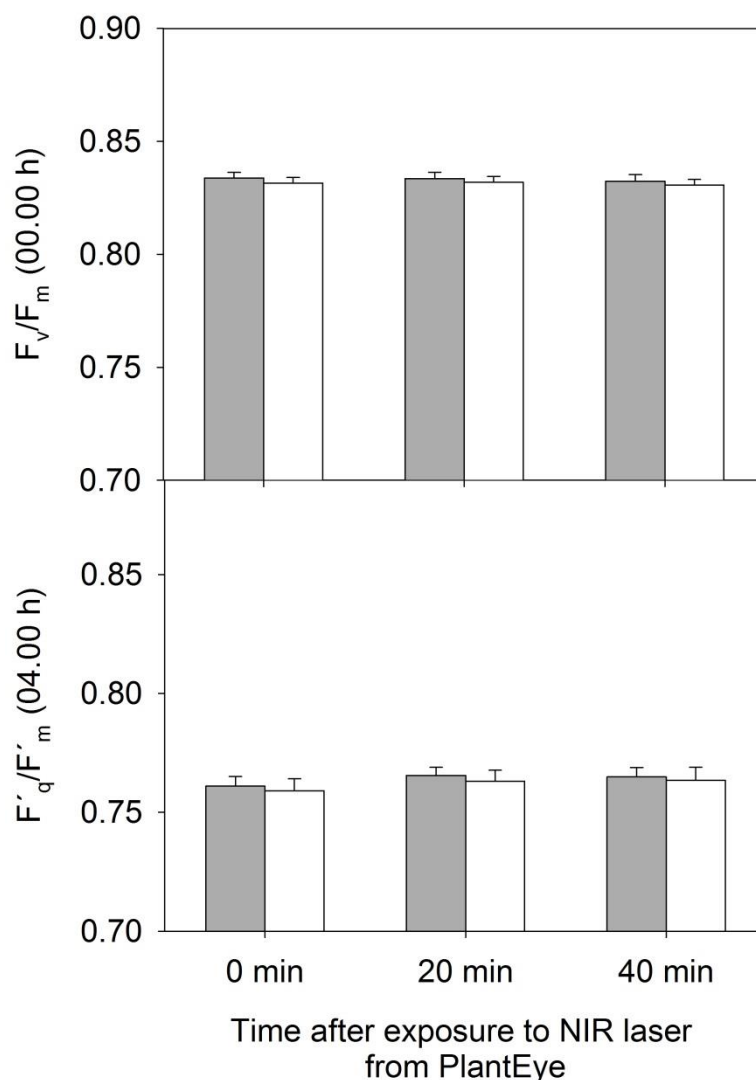
Figure 4. Correlation between the electron transport rate (ETR) and the photosynthetic active radiation (PAR) for continuous chlorophyll fluorescence measurements carried out in rapeseed plants exposed to the near-infrared laser line from the 3D triangulation scanner or placed in a control treatment. The values shown are from day 5 and 6. Control plants (black dots) and scanned plants (white dots), n =3.
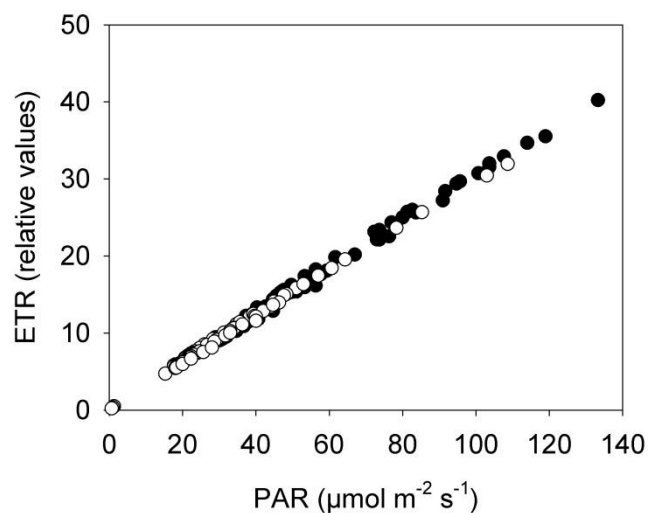
2.2 The scanning parameters are highly correlated with growth measurements of rapeseed, but underestimate total leaf area

Table 1 shows the correlation coefficients between the scanning parameters and the destructive growth measurements based on the dataset from 47 rapeseed plants of the cultivar 'DH5'. It is clear that the correlation coefficients were generally very high, ranging from r^2 = 0.93-0.97 and with corresponding low *p*-value (<0.001) for all variables except when correlated with the height parameter and leaf number. The reason for this is that young rapeseed plants establish a rosette with older leaves at the base, increasing in size, and smaller, younger leaves developing in the centre on a stem of increasing height up to a leaf stage of nine to 30 leaves, depending on the growth conditions. This means that the plants without an erect stem make the height parameter from the scanner less informative about the 3D structure. Also, the older leaves that expand continuously cause a large variation in the leaf area of plants with the same number of leaves, height and weight. The 3D leaf area had the highest correlation coefficient (Holm's method) in predicting the biomass production of young rapeseed plants in terms of DW or FW (r^2 = 0.97, *p* = 0.001), compared to the projected leaf area and the defined parameters of shoot area (Table 1). Therefore, it was decided to base the prediction of plant weight and leaf area on the parameter of 3D leaf area (Fig 5). As seen in this figure, the fitted linear correlation fits well to the

data up to a 3D leaf area of 200 cm^2 plant^{-1}, but the last two points are not well fitted, showing that the method is not reliable for larger size plants. It is further seen that the scanner underestimated the total leaf area of a plant; the 3D leaf area was 50-90% of the measured leaf area depending on the size of the plant (Fig 5C, stippled line). The main reason for this underestimation is the overlapping of leaves, which increases in time and with developmental stage, but also because any leaf surfaces with angles larger than 85° to the z-axis would not be detected (see Fig. 2). Overlapping leaves and

leaves expanding out of the plot are common limitations faced by the usual non-invasive methods used within the phenotyping community [4]. In the present set-up, the maximum scan width, the distance from the scanner and the distance between the pots limited the number of plants that could be measured in each subfield. This happened when the leaves from different individual plants started to overlap and extend out of the field. Despite this, we found that there were good correlations using the 3D point cloud monitored from one angle on the rapeseed plants with leaf areas ranging from 2.5-400 cm^2 and shoot dry weight ranging from 0.01 g – 1.5 g per plant. This was in the same range as seen for individual rapeseed plants monitored by light curtain arrays (LCs) from one to 16 different angles [4], and in cereals monitored individually from three different angles using a conveyor based imaging station [2]. It has been shown that 3D laser scanning with the use of at least three different angles can estimate above-ground biomass of juvenile trees in the range from 3 to 12 g [15], and a set-up with several 3D triangulation scanners placed at different angles would improve the estimation of larger plants. A similar approach was shown to improve the accuracy of estimation by light curtain arrays [4].

Table 1. Correlation coefficients; r^2 and adjusted *p*-values (Holm´s method) for correlation between the scanningparameters and dectructive growth parameters in one genotype (DH5) of rapeseed. The destructive growth parameters are LAI (leaf area index), fresh weight (FW), dry weight (DW), leaf number (LN), leaf area (LA) and the scanning parameters are height, 3D leaf area (3D LA) and projected leaf area (Proj LA).

		LAI	FW	DW	LN	LA
Height	r^2	0.86	0.93	0.93	0.89	0.94
	p	< 0.01	< 0.001	< 0.001	< 0.01	< 0.001
3D LA	r^2	0.93	0.97	0.97	0.88	0.97
	p	< 0.001	< 0.001	< 0.001	< 0.01	< 0.001
Proj LA	r^2	0.93	0.97	0.97	0.88	0.97
	p	< 0.001	< 0.001	< 0.001	< 0.01	< 0.001

Thee leaf area index (LAI) is an important parameter for defining canopy structure, light use efficiency and in predicting primary production. It follows a near-linear relationship with crop growth rate and net photosynthesis up to a species-specific optimum LAI value, which often approximates three [16]. Our results show that LAI can be well estimated up to a value of two. At this value, crop growth rate is directly related to light interception over the whole leaf area and is not limited by overlapping leaves.

2.3 3D laser triangulation identified phenotypic variation in rapeseed

Scanning measurements taken every hour for 23 days after sowing (DAS) on 10 different rapeseed genotypes with similar architecture were transformed to plant FW, plant DW and total leaf area based on the linear correlation between the destructive plant growth measurements and the estimated 3D leaf area from the scanner in experiment 2 on one of the cultivars ('DH5'). Growth curves for plant FW, plant DW and total leaf area of four selected genotypes of rapeseed are shown in figure 6A-C.

Figure 5. A-D) Destructive measurements of plant FW, plant DW, total leaf area and Leaf area index (LAI) are shown in relation to the 3D leaf area parameter (3D LA) from the 3D triangulation scanner measurements, Experiment 1 (white dots), experiment 2 (black dots). N = 12, each data point display the mean of 6 plants ± SE. The stippled line shows the hypothetical 1:1 relationship between total leaf area and 3D leaf area.
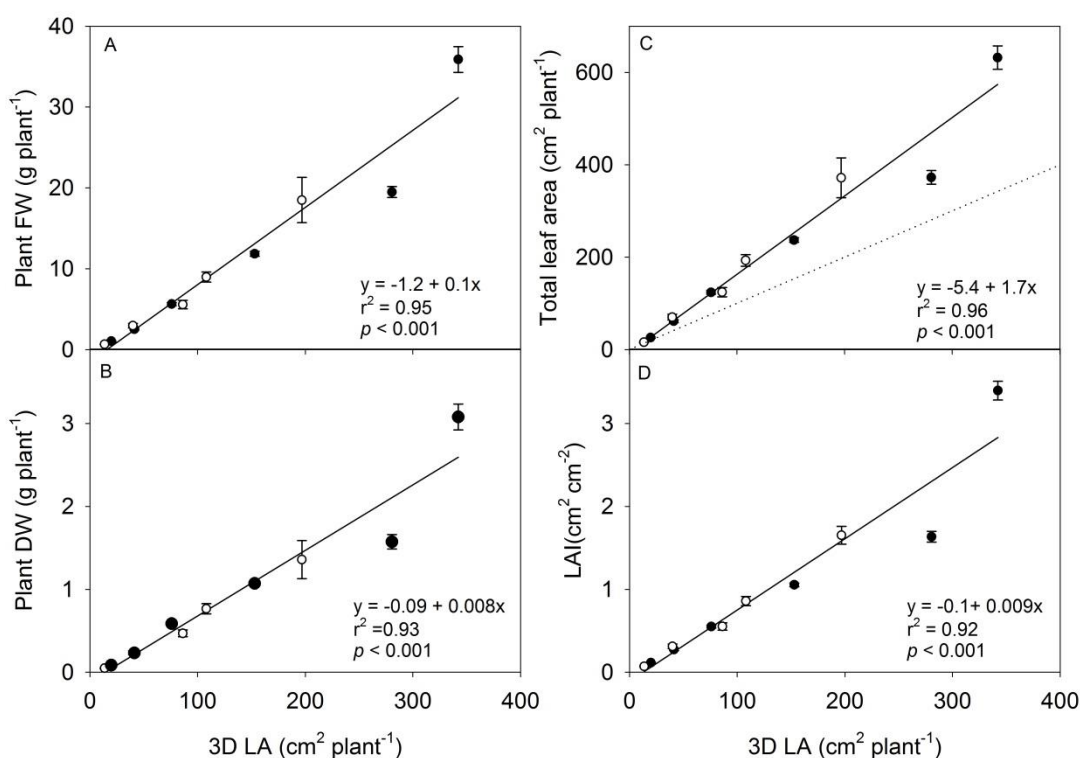
For the destructive harvest values, the statistical analysis revealed significant differences between the cultivars for all three growth parameters, and the result of the multiple comparisons of means tests is shown as letters above the bars (Fig. 6D-F). The results show that despite large variances in between the cultivars, only some of them were significantly different. According to the test, cultivars 7.Resyn and 2.Expert were not significantly different, but they were both significantly different from 4.Olympiade, and 7.Resyn also differed significantly from 9.DH5 in terms of FW and DW. For total leaf area, 7.Resyn only differed significantly from 4. Olympiade. It is clear from the predicted values of weight and leaf area after 23 DAS that the 3D laser scanning successfully distinguished the two cultivars 7.Resyn and 4.Olympiade, which were also significantly different in terms of destructive growth parameters after 24 DAS. Furthermore, the 3D scanning also placed the two genotypes 2.Expert and 9.DH5 in between the significantly different genotypes, and in the same sequence, using the mean values from the destructive harvest. The predicted values and the harvested values from the four genotypes formed linear correlations with r^2-values ranging from 0.94-0.99 (results not shown). However, when including all 10 genotypes, the r^2-values dropped due to the insignificance of the harvest results. This underlines, not surprisingly, the point that non-invasive methods like the 3D laser triangulation do not predict genotypic differences in plant growth *better* than destructive methods. However, the temporal resolution is improved, as was also shown for another image-based approach [17]. Further, it demonstrates that 3D laser triangulation from just one perspective is a valid alternative to destructive harvest measurements.

2.4 Diurnal patterns in scanning parameters contain information on diurnal changes in 3D plant structure

The scanning parameters of height and 3D leaf area oscillate in a diurnal pattern (Fig 7). The magnitude of these oscillations depends on the climate conditions and is caused by a combination of nyctinastic leaf movements and diurnal variation in leaf and stem expansion, and these may differ a lot between plant species [18]. In figure 7, the values of plant height and 3D leaf area (cm^2 $plant^{-1}$) are shown for the four genotypes of rapeseed on an hourly basis from 7 – 16 DAS, 2-12 August (Fig. 7A-B), with the corresponding climate parameters (Fig. 7C-D). The amplitude of the diurnal measurements varied between the different days, but these differences were not directly an indicator of differences in the actual increase in plant size during the 24 h day from 00:00 h to 00:00 h the following night. It was seen that a clear warm day (e.g. 7 August) with high light intensity and low air RH resulted in a large drop of the 3D leaf area by midday in all four genotypes and that the genotype with the highest growth rate experienced the largest drop (4.Olympiade). However, the corresponding diurnal pattern in plant height was not directly related to this drop in 3D leaf area, as similar

oscillations in the plant height measurements were seen on 8 August, where the diurnal oscillation in 3D leaf area was less pronounced. The temporal variation in the non-invasive measurements may illustrate that circadian movements of leaves from a horizontal to a more vertical position at night [19] alter the 3D structure of the plant, causing differences in the plant height measurements throughout the day, and that low light intensities reduce the circadian leaf movements [20]. These circadian leaf movements may also have increased the area of leaf surface with an angle larger than 85° to the z-axis, beyond the detection limit of the scanner.

Figure 6. A-C) Growth curves from 6-23 DAS for estimated values of plant FW, plant DW and total leaf area in four selected genotypes of rapeseed. D-F. Destructive harvest values of plant FW, plant DW and total leaf area in 10 genotypes of rapeseed at 24 DAS. The estimated values were based on a linear relationship between the 3D triangulation scanner measurements and destructive measurements in one rapeseed genotype (DH5) for the mean of 4 plants. For the destructive parameters the values are mean of 6 plants ± SE, letters represent significant differences between means ($p < 0.05$). The coloured bars represent the same genotypes as in the curves displayed in figures A-C.
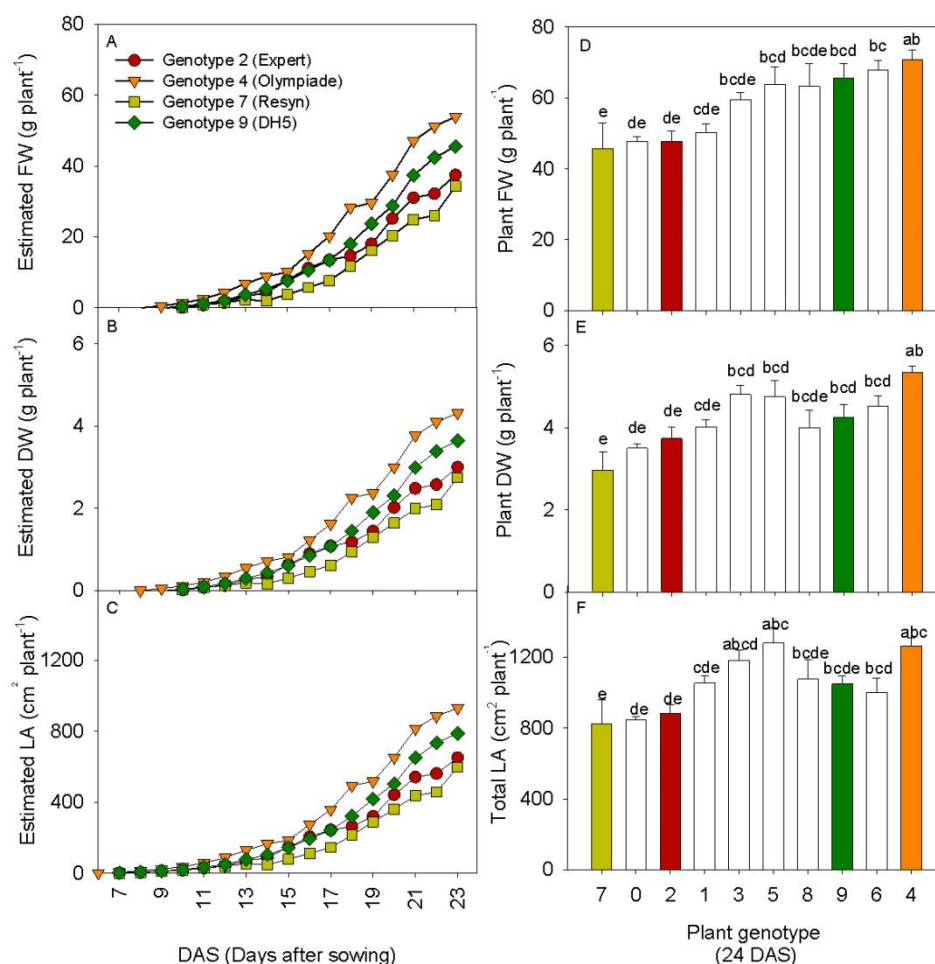
Nyctinastic leaf movements are a combination of circadian leaf movements and heliotropism [21-22]. Heliotropism is where the plants optimise their light interception (diahelitropism), or avoid high light intensities (paraheliotropism), by adjusting the angle of their leaves in relation to the incident light. We suggest that the drop in 3D leaf area on the 7 August and the other days with high light intensities was an effect of paraheliotropism, induced by the low humidity and a corresponding high vapour pressure deficit (results not shown), closing leaf stomata, decreasing leaf turgor, and thus increased leaf angle, so that a larger surface area was beyond the detector's detection limit. It is tempting to suggest that these genotypic-specific heliotropic movements, related to water status, explain some of the variation in growth, but it is beyond the present paper to validate this.

Figure 7. A-B) Growth curves from 7-16 DAS (02.08.2013 – 12.08.2013) for the 3D triangulation scanner measurements of height (mm) and 3D LA (cm^2 $plant^{-1}$) in four selected genotypes of rapeseed. C) Light intensity (grey area) and air temperature (black line). D) relative air humidity (% RH) in the same period, shown as mean value per hour.
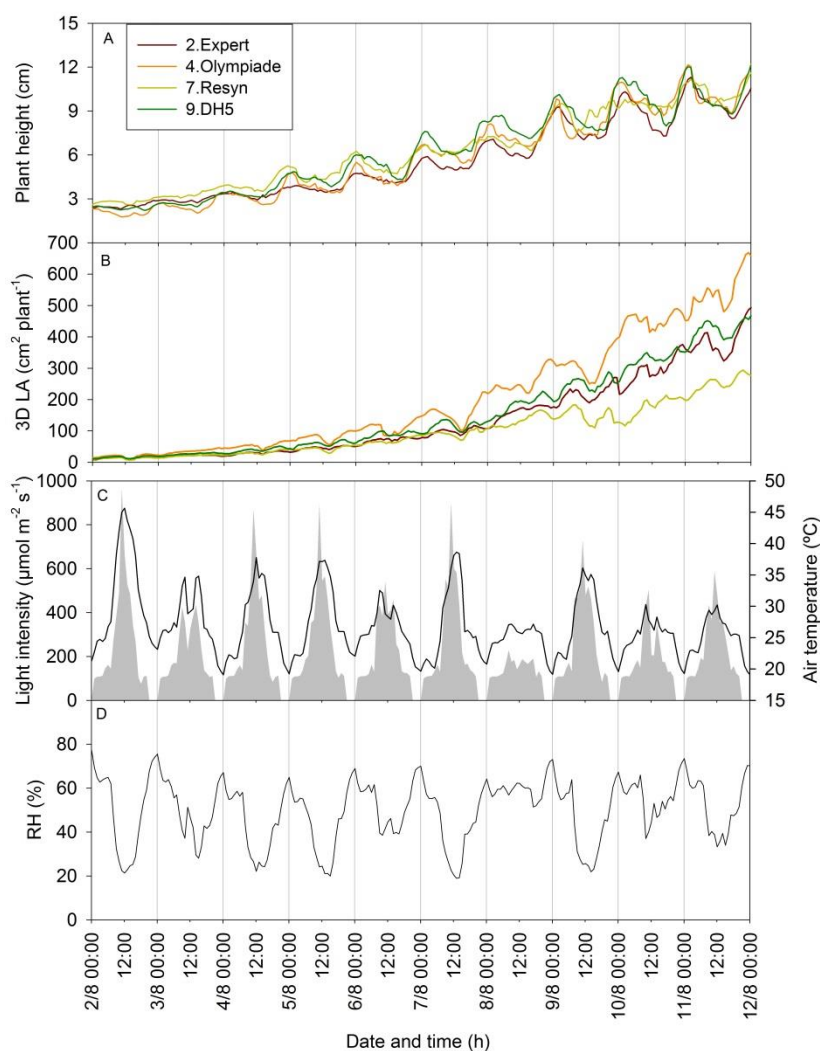
2.5 Conclusion

The development of methods to phenotype plants with a high frequency and revealing short and long term growth patterns is of importance for research. 3D laser triangulation is a valid tool to estimate growth and structural information of plants, and can be used for active "sensor-to-plant" approaches. This enables frequent measurement and the possibility to study fast responses and adaptations of plant structure to fluctuating environmental conditions. The laser line which is projected on plants, in order to compute height profiles, does not affect the plant photosynthetic performance at the frequency of measurements used. It can give a rapid estimate of plant height, weight and leaf area in contrasting genotypes of rapeseed with similar architecture based on linear correlations from one genotype. The estimation of plant growth parameters was sufficiently accurate for rapeseed plants up to the 7th leaf stage, but these results may be improved by increased spacing of the plants to avoid leaf-overlap, and by increasing the height, and hence scan width, of the scanner to allow for even taller plants to be measured. Scanning from more perspectives could improve the accuracy, but with a need for more algorithms. In addition, the analysis of measurements in high temporal resolution could reveal information on crop responses to abiotic stresses, showing how plants alter their 3D canopy structure to optimise light interception and avoid water loss under natural light, drought and temperature stresses.

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Author contributions

KHK installed the 3D laser triangulation phenotyping platform, performed the experimental work, carried out the data analysis and interpretation and wrote the manuscript. COO contributed to conceiving this work and assisted in designing the experiments. Both authors contributed in reading, editing and approving the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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