Protocol/Materials and methods for 2023 Halm til det hele

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Varieties and plants m^{-2}

- Spring barley: 300 pl m^{-2} and 200 pl m^{-2}
- Spring wheat: 450 pl m^{-2} and 300 pl m^{-2}

Table 1: Five varieties of spring barley: Skyway, Halfdan, Wish, Feedway and Flair. Five varieties of spring wheat: KWS-Fixum, Bravens, Selina, Kapitol and Nimrod. Tkw = Thousand kernel weight. Plot code represents the plot codes used on the illustration of field design.

Crop	Sort	Akronym	Tkw (gr)	Germination%	200 pl/m2	300 pl/m2	450 pl/m2	Plot code	Seeding kg/ha (200 pl/m2)	Plot code	Seeding kg/ha (300 pl/m2)	Plot code	Seeding kg/ha (450 pl/m2)
Barley	Skyway	A	56	94	x	x		AL	119	AH	179		
Barley	Halfdan	B	59	95	x	x		BL	124	BH	186		
Barley	Wish	C	56	95	x	x		CL	118	CH	177		
Barley	Feedway	D	50	94	x	x		DL	106	DH	160		
Barley	Flair	E	53	95	x	x		EL	112	EH	167		
Wheat	KWS_Fixu	F	53	94		x	x			FL	169	FH	254
Wheat	Bravens	G	50	97		x	x			GL	155	GH	232
Wheat	Selina	H	50	94		x	x			HL	160	HH	239
Wheat	Kapitol	I	42	94		x	x			IL	134	IH	201
Wheat	Nimrod	J	53	96		x	x			JL	166	JH	248
	Seeding (kg/ha) = (Plants pr m2* Tkvw)/germination %												

Experimental design

Plot sizes: 3*6 m. Design: RCBD, with block size 10 plots and four repetitions (=blocks). Due to a mistake while sowing AL and AH (see table 1) have two replicates within each block and not just one. Plots are sown by a 21 row Pöttinger mounted on the Robotti autonomous robot (called Robotti area in figure 1), where 10 rows are used for combine harvesting, thus not touched during season. Two rows are not sown (seed tubes blocked) and then eight rows are used for biomass cutting and PAR measurements 3 times during season. In the eastern end of the experiment, all varieties are sown in one replicate of both normal and low plant densities using a 1.5 m Wintersteiger seeder to illustrate how standard plots will look like. Overall design and plot design is shown in figure 1. 12 Ground Control Points (GCP) are placed to assure that the entire experiment has known fix-points measured on ground using an RTK GPS, and thus can be used to align pictures taken according to a known reference point on ground. This procedure is described in Holman et al., 2016.

Seeds m^{-2}

Calculations of how many kg of seeds to sow m^{-2} is calculated as $\frac{\text{Desired plants m2} * \text{tkw}}{\%\ germination}$. Calculations are shown in table 1.

Plant protection and fertilization

Standard plant protection (herbicides, fungicides and insecticides) and standard fertilization according to norms.

Drone flying

Drone equipment

Four different drones are used for the acquisition of images. Their specs. Can be found using below links, and their use according to purpose of the images is shown in table 2.

Phantom 4 pro RTK (P4 RTK)

Specs: https://www.dji.com/dk/phantom-4-rtk/info

Mavic 2 pro (M2 pro)

Specs: https://www.dji.com/dk/mavic-2/info

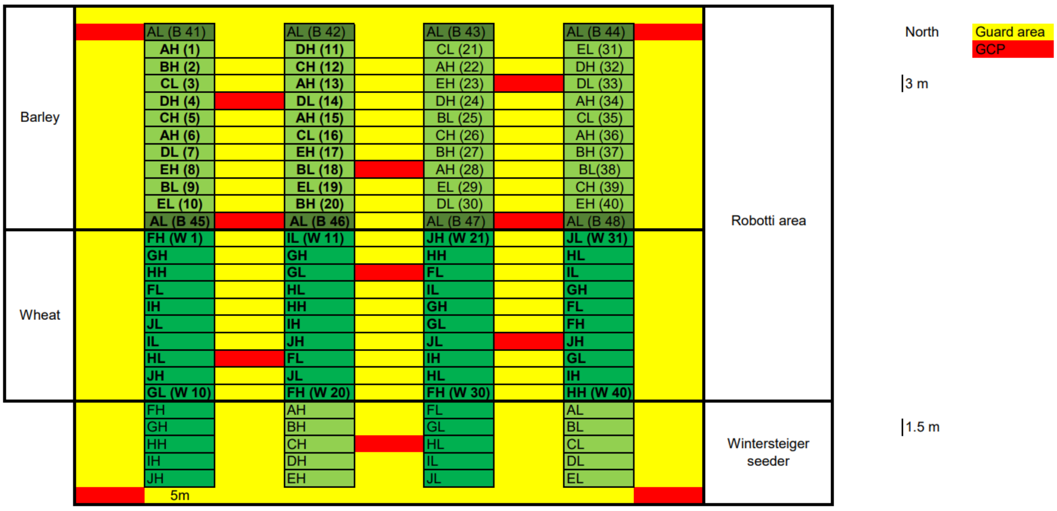
Phantom 4 Multispectral (P4 M)

Specs: https://www.dji.com/dk/p4-multispectral/specs

Mavic 3 multispectral (M3 M)

https://ag.dji.com/mavic-3-m/specs

A



B

C

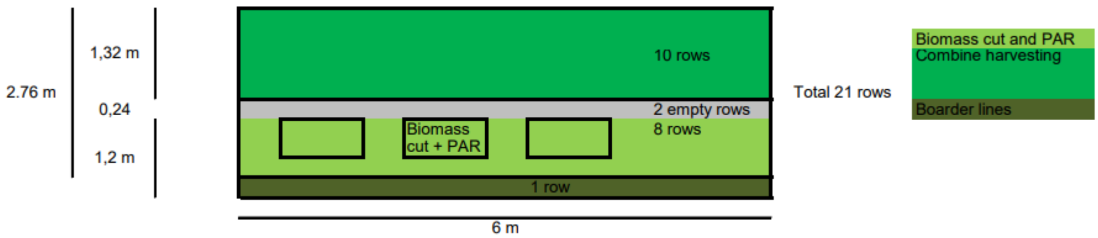


Figure 1: A: Overall plot design and GCPs (in red). Robotti area means plots sown with Pöttinger seeder. Wintersteiger area means plots sown with standard 1.5 m Wintersteiger seeder. B: Drone image of plot experiment in May – left is an orthomosaic, right is a single image. Yellow marking is a plot seeded by the Pöttinger. Black arrow point to a GCP. White markings are GCPs. C: Individual plot design when sown with Pöttinger seeder. 10 rows are for combine harvesting and 8 row are for biomass cut and PAR measurements. Empty rows are made by blocking these two tubes on the seeder.

Software

Drone flights are carried out mainly as a flying following a predefined route plan to acquire overlapping images that will be stitched together to become a so-called orthomosaic. Image overlaps will normally be set to around 80%. Stitching will take place either in the software called Pix4D mapper (vs 4.8.4) or in Agisoft Metashape Professional (vs 2.0.3) – both are professional photogrammetry software.

Route plans for drone flying and the actual automatic drone flight according to route plan is carried out using the app DJI GS-Pro. The plan is made to assure that the route goes well beyond borders of the experiment to avoid edge effect due to light artifact in the outer images of a flight, just as recommended by Rasmussen et al. (2016)

Plot average values of either the R, G and B channels of the RGB cameras or R, G, B, RE and NIR of the multispectral cameras is extracted plot-wise using ArcGIS and QGIS tools for zonal statistics. Afterwards extracted average values from these bands is used to calculate different vegetation indices (VI) – see further down.

Altitudes vs use-case

A range of different altitudes are used when flying (driving in case of the Robotti) in order to get an extensive dataset to estimate plot-wise vegetation indices, crop height, counting of specific crop features like emerging plants, heads and stubble. Table 2 illustrates setups used to estimate the different measures to be used for the final estimation of straw yield.

Table 2: Combination of 1) Purpose of drone/Robotti images (vegetation indices (VI), crop height estimation etc.), 2) vehicle used, 3) distance from ground to camera, 4) single and multiple images for different types of analyses and 5) camera angle to the surface of the plots.

Purpose	Drone/Robotti	Altitudes (m)	Single images	Ortomosaics	Camera angle (degre
VI Multispectral (NDVI, NDRE etc)	P4 Multispectral	30		x	90
VI RGB (nExG, VARI etc)	P4 RTK	25		x	90
Crop height	P4 RTK	15, 25		x	90
Crop height sensitivity	P4 RTK, M2 Pro	15, 25, 50		x	90/60
Plant counting	P4 RTK	2, 4, 8	x		90/75
	Robotti	1,5			
Head counting	M2 Pro	Varying heights	x		90
	M2 Pro	8 , 15	x	x	90
Stubble counting	M3 M	2	x		90

Ground Control Points and RTK GPS measurements

IKEA cutting boards are placed flat on the ground just after sowing, and their center x, y and z coordinate is measured using an Emlid Reach RS2 RTK gps (figure 1 B)

Vegetation indices (VI)

NDVI $=\frac{NIR-R}{NIR+R}$

NDRE $=\frac{NIR-RE}{NIR+RE}$

nExG $=\frac{2*G-R-B}{G+R+B}$

VARI $=\frac{G-R}{G+R-B}$

Height measurements

Height measurements from drone images is estimated using a Digital Elevation Model estimated from drone flight just after the experiment was established and a Digital Surface Model estimated continuously while the crop is growing. Crop height is then estimated as nDSM (m) = DSM (m) – DEM (m). Procedure is described in Holman et al (2016) and illustrated in figure 2. DSM and DTM are estimated in Agisoft Metashap and Pix4D respectively to test different photogrammetry software. GCPs can be used to align photos and optimize DSM and DTM.

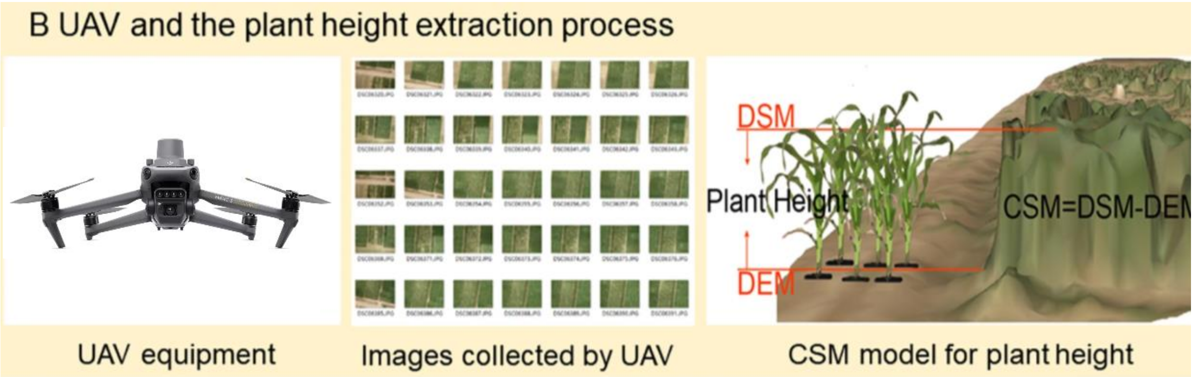


Figure 2 modified from Wang et al. (2019) showing procedure from acquiring multiple overlapping photos using UAV to the estimation of a digital elevation model (DEM) and digital surface model (DSM) using photogrammetry, and final estimation of crop height via crop surface model (CSM).

Counting using drone imaging

Germinated plants: Two flights at low altitude and two overdriving using Cropeye on Robotti are made after germination (one to two leaves) to estimate germinated plants. At GS 21 and 22 (BBCH)

Heads: Flights in different altitudes are made when crops is at anthesis (table 2).

Stubble: After harvest, flights in different altitudes are made to count stubbles (table 2)

Standard images on ground using Robotti

Camera setup on Robotti is based on two industrial RGB cameras called Cropeye with 5.1 mpix (2448*2048 pixels) and field of view of approx. 90*75 cm, equaling an area of approx. 0,675 m2. Rows follow the vertical part of the image, thus rows observed are 90 cm long. Plants m^{-2} is calculated as defined in VEJLEDNING I BEDØMMELSER I LANDSFORSØGENE® (2023)

Visual evaluation

Plant counting

Plants are counted in five random rows of one meter length each in different locations within each harvest plot: one row in each end, two rows in the middle and one outer row. Plants m^{-2} is evaluated and calculated as described in VEJLEDNING I BEDØMMELSER I LANDSFORSØGENE® (2023)

Crop height

Crop height is measured using a specially developed measuring stick with a transparent plate. When transparent plate touch crop surface, plant height is observed and noted down as the distance from ground of crop to the transparent plate. Measurements are done in three locations within harvest plots – one in both end and one in the middle.

Manuel head counts

Heads are counted in a few plots to make a ground truth. Using a ring with a defined area heads m^{-2} is estimated. At the same time images in different altitudes using the M2 pro drone is acquired over the rings, thus manually counted heads can be compared to digital estimations.

Stubble count and stubble height measurements in period after harvest

After harvest low-altitude images are acquired using the M3 M drone to count stubble and estimate stubble height. Images for counting is acquired in approx. two meter altitude. A flight in 15 meter altitude is used to make a point cloud of the plots, and can be used to estimate stubble height.

Biomass cutting and PAR interception

3 times biomass harvest (tillering, elongation, grain filling) + Intercepted PAR measurements (LICOR setup)

Measurements of intercepted PAR is measured as described by Wang et al. (2020) using a line quantum sensor from Skye instrument https://www.skyeinstruments.com/line-quantum-sensor Measurements are carried out between 10.00-11.30 and 14.00-15.30 in order to avoid to many reflections of light inside canopy when sun is at zenith around 13.00. Furthermore, measurements are made on dry canopy to avoid reflections from water droplets. The aim should be to measure a day with diffused light, as full sun can cause problematic shadows. PAR measurements are made in three locations of the plot – at both ends and in the middle. Sensor is placed between two rows of the crop, and three measurements across the plot width is made – thus nine measurements represent a plot. Biomass is then harvested, where four rows of one meters each is cut and pooled into two samples. From this biomass can be compared to intercepted PAR.

We only measure in the plots that are laid out in normal plant density.

Harvest and post-harvest treatments

A full bag of straws is collected from each harvest plot. A weight is used in the field to calculate the full fresh matter weight of the harvested material. A subsample from each plot is extracted, weighed and dried for 48 hours to estimate % drymatter (DM). This number is used to adjust the entire harvested biomass into kg DM.

10 bags containing full biomass from the different barley and wheat varieties is sorted into straw material and other materials (chaff, heads) in order to estimates the different fractions of the harvested material leaving the combine harvester, and to get a precise estimate of what is actual straw.

Desired results

- Grain yield (kg DM/ha)
- Straw yield (kg DM/ha)
- Plant number
- Height (m) and VI indices continuously during growth
- Heads
- Stubble height (m)
- Stubble number and size
- PAR interception
- Data on both Robotti plots where there is no leftover straw, and on standard 1.5 m plots (Wintersteiger) where we leave the string of straw – figure 1.

References

Holman, F. H., Riche, A. B., Michalski, A., Castle, M., Wooster, M. J., & Hawkesford, M. J. (2016). High throughput field phenotyping of wheat plant height and growth rate in field plot trials using UAV based remote sensing. *Remote Sensing*, *8*(12), 1031.

VEJLEDNING I BEDØMMELSER I LANDSFORSØGENE® (2023): https://www.landbrugsinfo.dk/-/media/landbrugsinfo/public/a/8/e/planter_kval_landsfors_regi_bedoemmelser-i-landsforsoegene.pdf

Rasmussen, J., Ntakos, G., Nielsen, J., Svensgaard, J., Poulsen, R. N., & Christensen, S. (2016). Are vegetation indices derived from consumer-grade cameras mounted on UAVs sufficiently reliable for assessing experimental plots?. *European Journal of Agronomy*, *74*, 75-92.

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