Additional information

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1. Experimental field setup
Table A1: Overview of the selected maize genotypes used in the experiment

| Genotype number | Genotype  | Line type | Breeder/ Company                                      |
|-----------------|-----------|-----------|-------------------------------------------------------|
| 0               | Lapriora  | hybrid    | KWS SAAT AG, Einbeck, Germany                         |
| 1               | DKC2960   | hybrid    | DeKalb Genetics Corp., Dekalb, IL, USA                |
| 2               | Tiago     | hybrid    | Delley seeds and plants, Delley, Switzerland (DSP)    |
| 3               | Pralinia  | hybrid    | DSP                                                   |
| 4               | Bonfire   | hybrid    | DSP                                                   |
| 5               | Swiss301  | hybrid    | DSP                                                   |
| 6               | DSP1771E  | dent line | DSP                                                   |
| 7               | DSP5009S3 | dent line | DSP                                                   |
| 8               | DSP5049A31| dent line | DSP                                                   |
| 9               | DSP5145X1 | dent line | DSP                                                   |
| 10              | DSP5164A3 | dent line | DSP                                                   |
| 11              | DSP2563E3 | flint line| DSP                                                   |
| 12              | DSP2637A  | flint line| DSP                                                   |
| 13              | UH003     | flint line| University of Hohenheim, Germany                      |
| 14              | UH008     | flint line| University of Hohenheim, Germany                      |
| 15              | SMxxx     | flint line| Freiherr von Moreau Saatzucht GmbH, Altdorf, Germany  |
2. Field management:

Preceding crop: Grain maize
Tillage operations: Plough / rotary harrow

Fertilization:

- **20/ April**  Diammonium phosphate (DAP): 25 kg N ha\(^{-1}\), 64 kg P ha\(^{-1}\)
- **04/ May**   Korn-Kali®: 96 kg K ha\(^{-1}\), 14 kg MgO ha\(^{-1}\)
- **10/ May**   KAS 138 kg N ha\(^{-1}\), 69 kg CaO ha\(^{-1}\)

Sum: N: 163 kg ha\(^{-1}\), P: 64 kg ha\(^{-1}\), K: 96 kg ha\(^{-1}\), MgO: 14 kg ha\(^{-1}\)

Plant protection:

- **27/ April**  1.1 l ha\(^{-1}\) Spectrum + 2.2 l ha\(^{-1}\) Stomp Aqua
- **14/ July**   0.125 l ha\(^{-1}\) Steward (European corn borer)
3. Weather information

Figure A1: Cumulated precipitation, relative humidity and Wind speed during the time of the maize experiment
4. Camera set up

Table A2: Weight of the parts of the imaging tool

| Part                      | Weight (g) | Comment                                                                 |
|---------------------------|------------|-------------------------------------------------------------------------|
| Handle frame              | 675 + 110+8| Aluminium + two brass screws+ 2 metal screws                             |
| Canon XSI                 | 717        | 85 mm lens attached                                                     |
| Eos 400                   | 770        | 85 mm lens attached                                                     |
| Connection cable          | 20 + 155   | Cable connects the two canon cameras using the remote cable port, security belt is attached to the handling frame |
| and security belt         |            |                                                                         |
| VarioCam                  | 1445       | Industrial grade IR camera, needs a computer to be controlled, 75 mm lens attached |
| Battery+ cable            | 1380 + 95  | Two 6 V batteries combined and attached with, IR compatible connector (self-made) |
| Laptop+ Accessories       | 1590+195+44| Laptop + Fire wire cable+ fire wire card                                |
| Sum                       | 7204       |                                                                         |

Table A3: Threshold settings for image segmentation for plant and soil differentiation

| Flight | Date       | NDVI | Grey value |
|--------|------------|------|------------|
| 1      | 16.06.2011 | 0.1  | 20000      |
| 2      | 05.07.2011 | 0.1  | 13500      |
| 3      | 11.07.2011 | 0.1  | 16000      |
| 4      | 26.07.2011 | 0.1  | 16000      |
| 5      | 02.08.2011 | 0.1  | 11000      |
| 6      | 12.08.2011 | 0.1  | 11000      |
| 7      | 16.08.2011 | 0.1  | 11000      |
| 8      | 15.09.2011 | 0.1  | 18000      |
| 9      | 29.09.2011 | 0.1  | 7000       |
Figure A2: A sample area shown for raw NDVI, RGB and IR images (left to right) taken on 11.07.2011 (das 81). Shown is a corner position in the experimental field in rectangle 2, with a field marker in the upper right corner, tracks left and right of plots (right side downwards), and a row from top down: genotype 0, 0, 12, 12, 12, 15, 15.

5. Details on ground measurements

![Graphs showing relationships between leaf weight index, leaf area, and fresh weight](image)

Figure A3: Tested relationship for calculation of LAI based on leaf fresh weight.

\[
\text{LWI} = \frac{\text{FM}_{\text{leaf}}}{(\text{SL} \times \text{RD})} \times 1000000
\]

LWI= leaf fresh weight index (mg cm\(^{-2}\)), FM\(_{\text{leaf}}\) = fresh matter of leaves (kg), SL = sampling length (cm), RD row distance (cm)

LAI= leaf area/(SL*RD), LAI leaf area = index

Sampled genotypes were: 3 Pralinia, 1 DKC2960, 8 DSP5049A31, 11 DSP2563E3, 13 UH003, 6 DSP1771E
6. Examples for the observed skewness of three genotypes during the season

The skewness of the NDVI images indicates additional parameters?

The applicability of skewness as an additional parameter, quantifying the distribution of the greenness parameter NDVI (Figure A5) within the image or within the segmented area, was investigated as well. The skewness of NDVI (Figure A6) showed a different seasonal pattern than NDVI. The skewness of the NDVI\textsubscript{Plot} was relatively constant with a small reduction at the beginning of flowering and a stronger increase at the end of the season. Average values were usually close to zero. For NDVI\textsubscript{Plant} different phases were significantly more pronounced due to significant differences from normal distribution (> 0) in the early growth phase (371 °Cd) and particularly during the phase of senescence (> 940 °Cd). Repeatability of the skewness of NDVI was generally higher in larger plots (h\textsuperscript{2} = one < two < three < four row plots) but did not always reflect the plot size differences. It strongly increased by the segmentation procedure. The repeatability of the skewness was generally lower as the one for the CC and NDVI and plot size effects were more pronounced making it less suited to differentiate among genotypes.

The NDVI skewness parameter may indicate whether an observed lower NDVI\textsubscript{Plant} is caused by lower overall leaf greenness or by ongoing senescence reducing the green leaf area (example in additional file 2 section 6). However, mixed pixels at the border between plant and soil may influence this signal, linking the skewness of NDVI\textsubscript{Plant} also to differences in canopy cover. Indeed, the skewness of NDVI was linearly related to CC (Figure A7) where the relationship was stronger for NDVI\textsubscript{Plant}. It can be assumed that the skewness of NDVI\textsubscript{Plant} mostly reflects the onset of senescence, but the mixed pixel and the senescence effect cannot be clearly disentangled here. Thus, we conclude that a pixel size of 2.5 cm on the ground (see Table 1) as in this study, is not sufficient to utilize the skewness as additional parameter.
We also tested, whether the distribution parameter the skewness of the NDVI\textsubscript{Plant} would be valuable as additional parameter. The skewness of NDVI\textsubscript{Plant} was a valuable indicator for nitrogen nutrition status in pearl millet [1]. It may be used as indicator, whether a low NDVI\textsubscript{Plant} is caused by a general low leaf greenness or by senescence, which increases the patchiness of green, yellow and brown leaf parts [2]. In our study, the explanatory power of skewness was limited: The increase in skewness with advanced senescence was likely related to a combination of senescent plant tissue (increased patchiness) and an increased portion of pixels containing a mixture of soil and plant signal. Thus, a high sensor resolution is a precondition to derive pure canopy pixels to measure senescence “patchiness” based on the skewness of NDVI\textsubscript{Plant}. Because the skewness of NDVI\textsubscript{Plant} is linked to the amount of mixed soil and plant pixels it might serve as a parameter for the quality of the segmentation.

However, if the NDVI distribution (skewness) is of interest it turned out to be important to have three or more maize rows for a high repeatability. For the reliable detection of CC at least two or better three rows are needed.

In conclusion the ‘skewness’, a parameter quantifying the histogram asymmetry of NDVI signals within images, proved very useful for evaluation of segmentation quality and development of senescence.

**Figure A5 The skewness value (s) is a distribution parameter.** A skewness value higher than zero indicates a shift towards low average NDVI values caused by a higher number of soil or senescent plant pixels. In contrast, skewness values below zero reflect a shift towards green pixels indicating a well-covered plot or plants in a fully green stage.
Figure A6: Skewness of NDVI and its repeatability as affected by plot size. Skewness of NDVI is shown in four row plots (A, B) and repeatability of the skewness of NDVI in one, two, three and four row plots (C, D) of plot (A, C) and plant NDVI (B, D).

Figure A7: Relationship between canopy cover and skewness of NDVI_{plot} (A) and NDVI_{plant} (B). The indicated linear regression lines reflect the functions $y = 0.5 - 0.82x$, $r^2 = 0.18^{**}$ (A) and $y = 1.57 - 1.65x$, $r^2 = 0.56^{***}$ (B).
Figure A8: NDVI_{Plant} and its skewness at six measurement points during the season 2011. The skewness identifies differences between genotype 7 and 13 in the late growth stages, where at the same time NDVI_{Plant} doesn’t.
Figure A9: Distribution of $\text{NDVI}_{\text{Plant}}$ of three different genotypes shown for 6 points during seasonal development illustrating the change of the skewness.
7. Thermal measurements related to repeatability

Figure A10 Canopy temperature of the three row plots (n= 64) as related to air temperature. Measurement time (°Cd) and the corresponding repeatability (h²) is indicated.

References

1. Gerard B, Buerkert A, Hiernaux P, Marschner H: Non-destructive measurement of plant growth and nitrogen status of pearl millet with low-altitude aerial photography (reprinted from plant nutrition for sustainable food production and environment, 1997). Soil Science and Plant Nutrition 1997, 43:993-998.
2. Thomas H: Senescence, ageing and death of the whole plant. New Phytologist 2013, 197:696-711.