GEVES
Expertise & Performance

Multispectral camera and imaging for assessment of resistance to *Fusarium graminearum*

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A Multispectral Imaging Algorithm has been developed by GEVES to assess the phenotypic resistance of wheat to *Fusarium* (*F. graminearum* and *F. culmorum*). The results obtained were compared with visual disease assessment (VDA) and qPCR analysis.

The aim is to find a new and faster method of phenotyping to replace VDA currently used for the resistance assessment in the frame of VCUS studies (Value for Cultivation, Use and Sustainability) for registration in the French National List.

PRINCIPLE OF VIDEOMETERLAB®

Based on multispectral imaging

- 20 different wavelengths from UV to IR (360 to 1050 nm)
- more discriminative than RGB imaging

UV — VISIBLE RANGE — I.R.
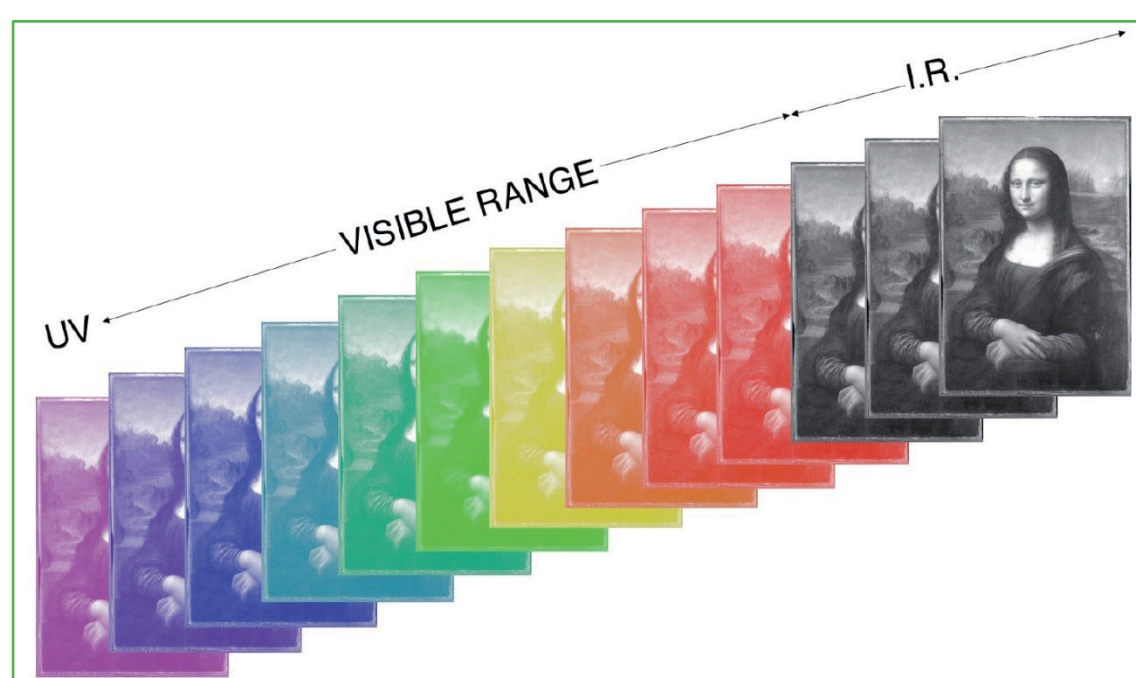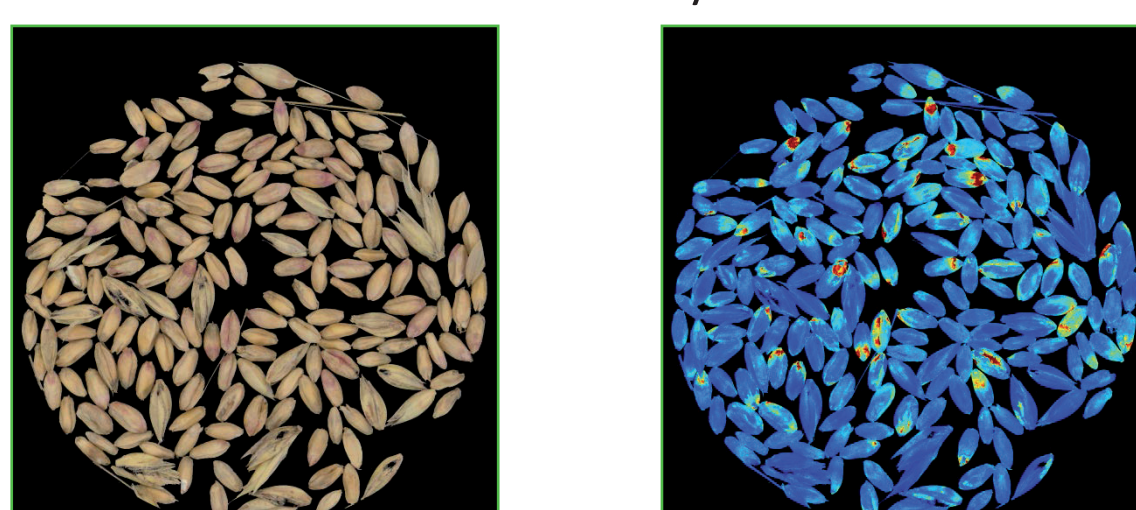
Image acquisition on conveyor belt

- 2056x2056 pixels per band
- 5 to 10 mins. for 1000 seeds

VideometerLab® and conveyor belt

Classification of areas infected by *Fusarium*

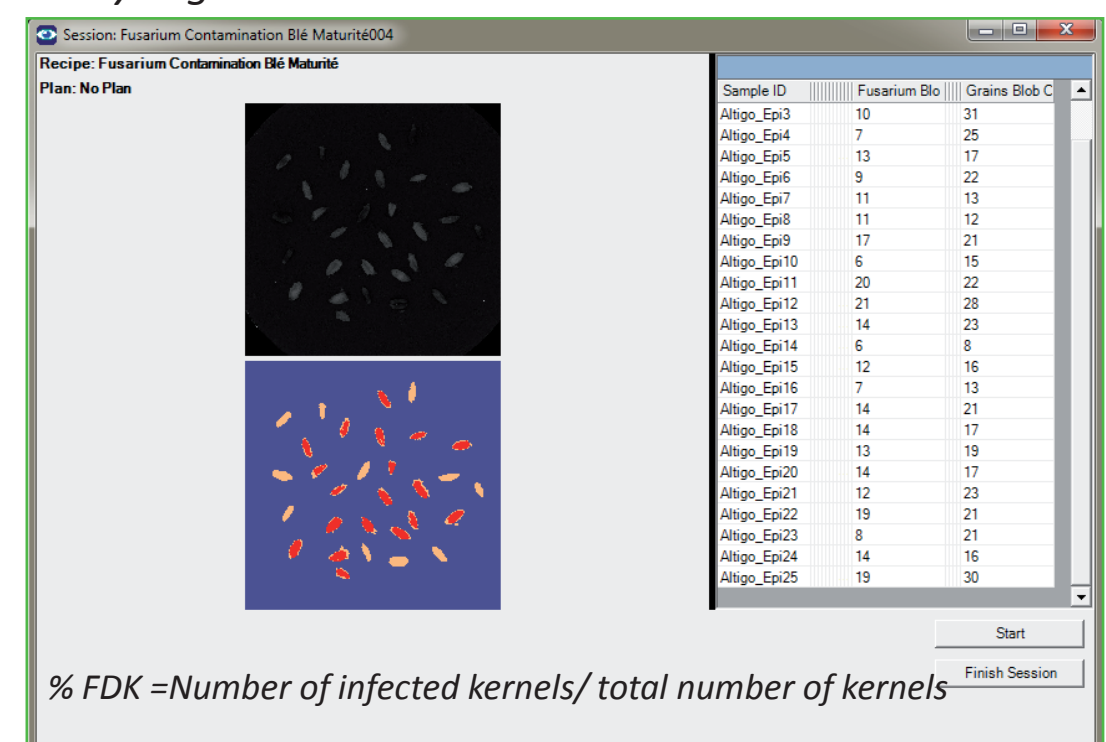Based on Canonical Discriminant Analysis (CDA) between infected kernels (yellow/red) and not infected (blue)

Wheat kernels infected by *F. graminearum* — CDA image

Quantification of *Fusarium* Damaged Kernels (FDK)

- Thresholding of the classified pixels
- Results exploited on Excel

% FDK =Number of infected kernels/ total number of kernels

MATERIAL AND METHODS

✓ **Experimental design:**
- Spray inoculation : *F.graminearum* & *F. culmorum*
- Winter wheat: 5 cultivars (resistance controls)
- 2 replicates of 25 spikes (used for both VDA & Videometer)

✓ **in field visual scoring :**
  - **At 360°C dpi :** % scabbed spikelets ; % FDK
  - **At maturity :** % FDK

✓ **Videometer :** % FDK
  - **in Petri Dish**
    - Individual spike : 25 spikes/cv from the 1st replicate
    - In bulk: ≈1000 kernels/cv collected from 25 spikes of the 2nd replicate
  - **With the conveyor belt**
    - In bulk≈1000 kernels/cv collected from 25 spikes from the 2nd replicate

✓ **qPCR**
  - 5 cultivars, with ≈1000 kernels/cv from the 2nd replicate
  - Grinding of all kernels for each cultivar and DNA extraction from 50 mg of flour
  - qPCR with specific TaqMan® probe of *F. graminearum*

Fig1: Scale of visual assessment of % scabbed spikelets
0 5 10 20 40 50 60 90 100

RESULTS

1. Correlations between Visual Disease Assessment and Videometer

✓ on individual spikes & in bulk, at different physiological stages

Fig 2: Analysis of correlation between %FDK by VideometerLab® and % scabbed spikelets by VDA at 360° dpi on 125 individual individual spikes/cultivar
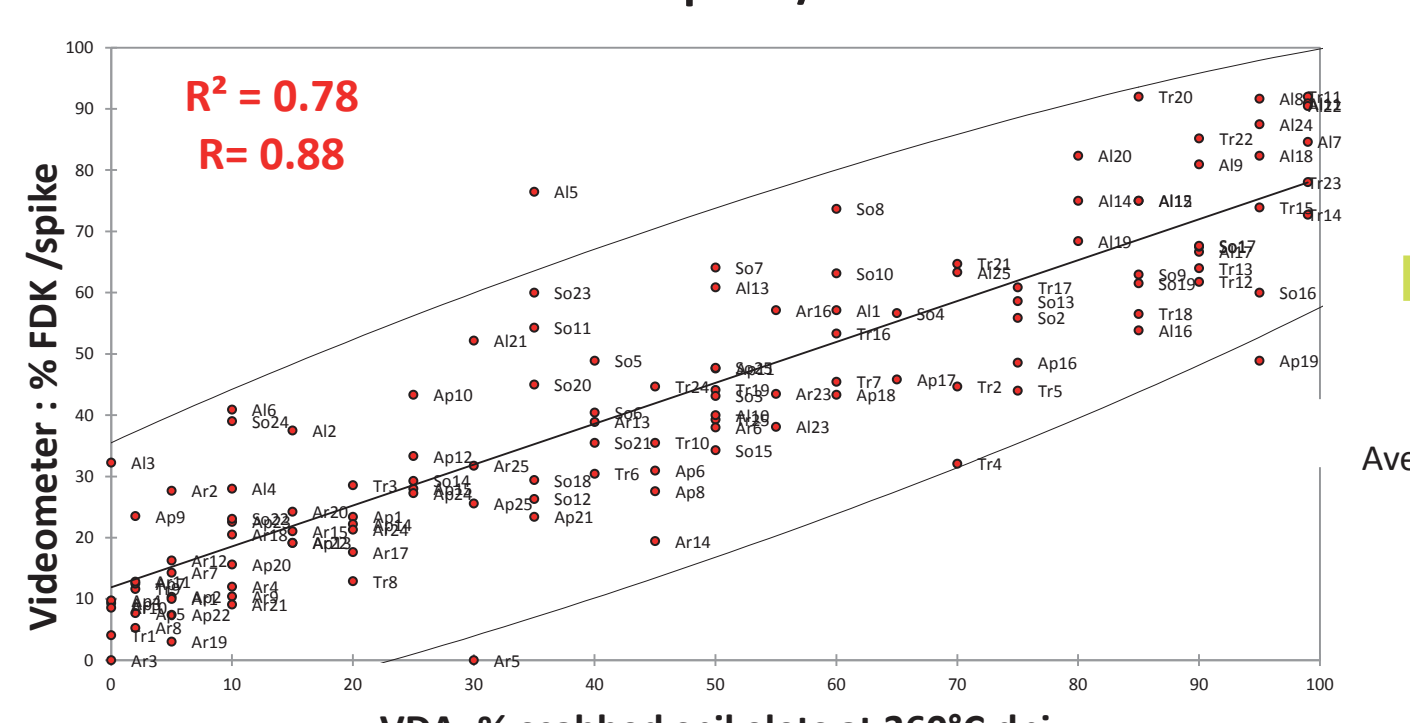R² = 0.78 R= 0.88

Fig 3: Analysis of correlation between % FDK by VideometerLab® and % scabbed spikelets by VDA at 360° dpi on 25 individual spikes/cultivar
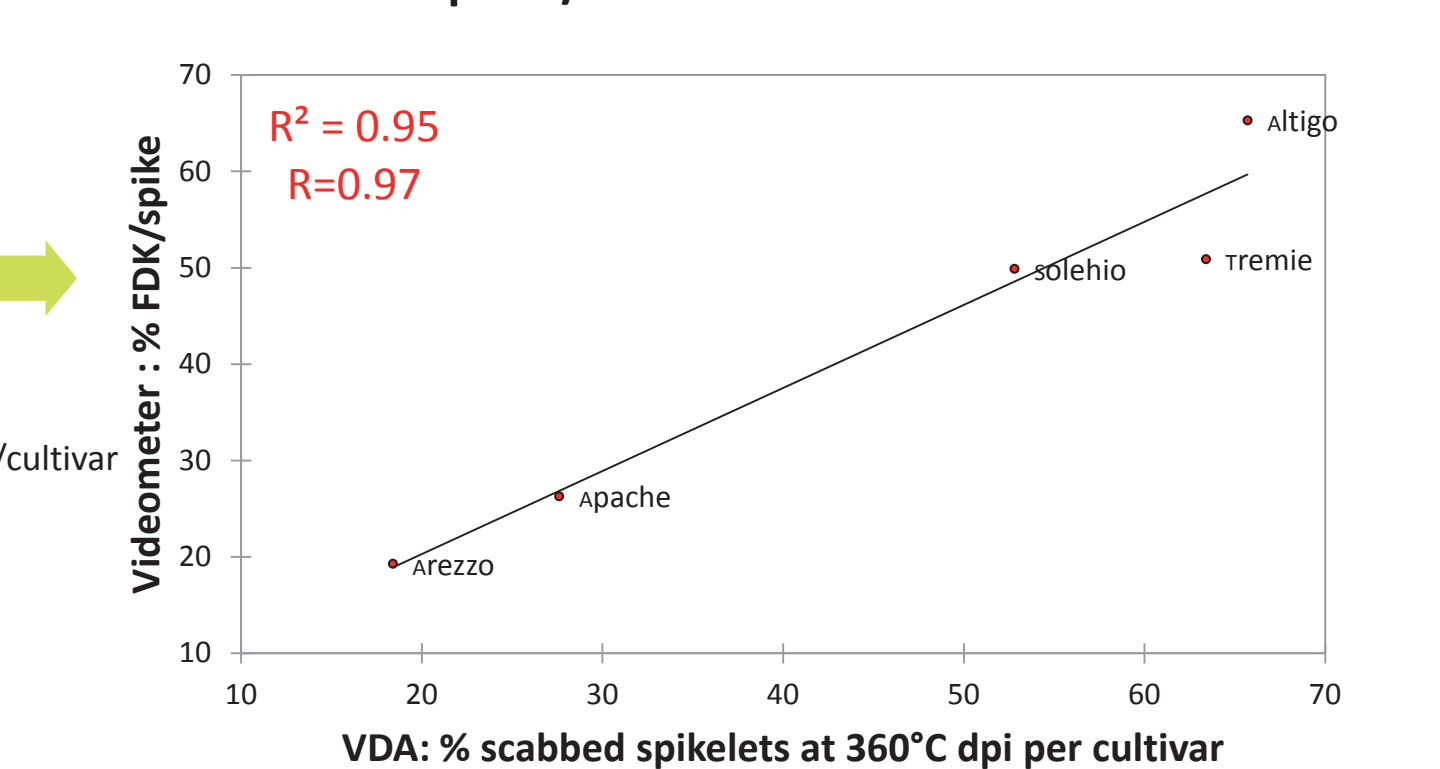R² = 0.95 R=0.97
Average/cultivar

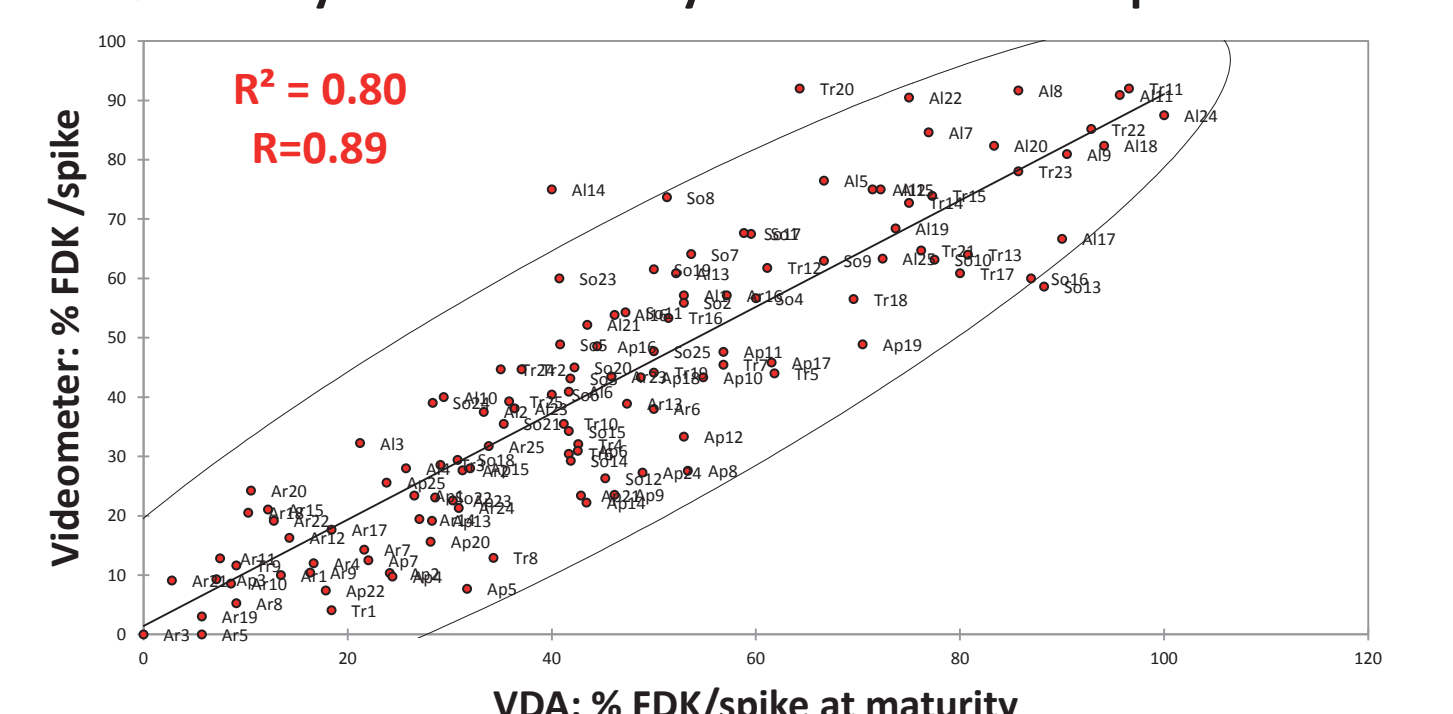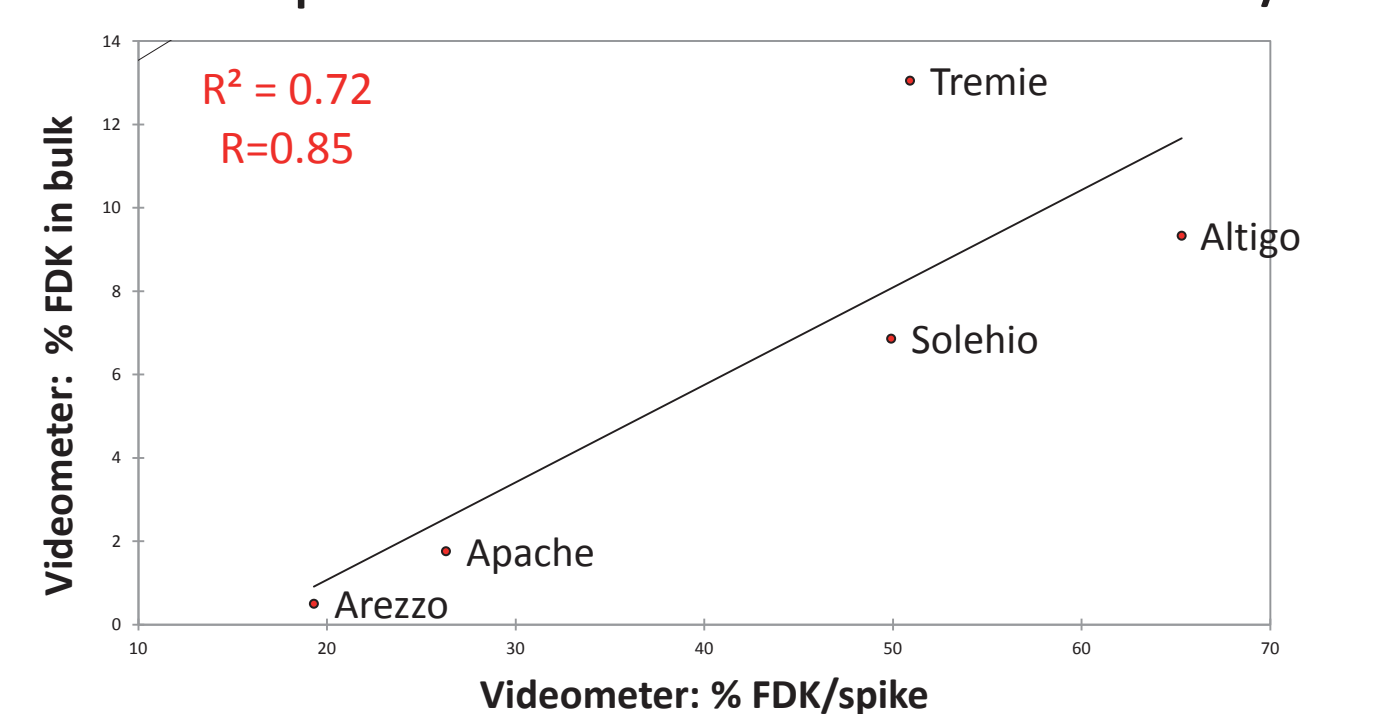Fig 4: Analysis of correlation between % FDK by VideometerLab® and % FDK by VDA at maturity on 125 individual spikes
R² = 0.80 R=0.89

Fig 5: Analysis of correlation between % FDK in bulk and on individual spikes with VideometerLab® on 1000 kernels/cv
R² = 0.72 R=0.85

✓ Summary of correlations obtained
- % scabbed spikelets & % FDK
- Individual spikes & in bulk
- Videometer: in Petri dish & with conveyor belt
- Scoring at 360°C dpi & at maturity

Correlation Matrix (Pearson) :	VDA, at 360°C dpi	VDA, at maturity	VideometerLab®+Petri dish, at maturity		VideometerLab® + conveyor belt, at maturity
Variables	% scabbed spikelets in bulk	% FDK/spike	% FDK/spike	% FDK in bulk	% FDK in bulk
VDA at 360°C: % scabbed spikelets in bulk	1	0.851	0.908	0.918	0.920
VDA at maturity : % FDK/spike	0.851	1	0.893	0.871	0.924
VideometerLab®+ Petri dish: % FDK/spike	0.908	0.893	1	0.850	0.984
VideometerLab®+ Petri dish: % FDK in bulk	0.918	0.871	0.850	1	0.798
VideometerLab®+ conveyor belt: % FDK in bulk	0.920	0.924	0.984	0.798	1

Bold values have a level of significance *alpha=0.05*
Table 1: Summary of correlations obtained, using VDA and VideometerLab® to assess Fusarium quantification

Strong correlations were found between VDA (at 360°C on spikelets, and at maturity on kernels) and Videometer at maturity on kernels, for the following modalities:
  - on individual spikes & in bulk,
  - for Videometer with Petri dish & conveyor belt.

In other trials inoculated with *F. graminearum*, a weaker correlation was observed in case of natural contamination with *Microdochium* spp (data not shown).

2. Correlations between qPCR, VideometerLab® and Visual Disease Assessment

Fig 6 : Comparisons of *Fusarium graminearum* infection level for winter wheat between qPCR (DNA content of *F. graminearum*), VideometerLab® with conveyor (% FDK) and VDA (% scabbed spikelets at 360°C), In brackets: official resistance cotation: 1= Susceptible to 9= Resistant
R² = 0.82 R=0.91
R² = 0.91 R=0.95
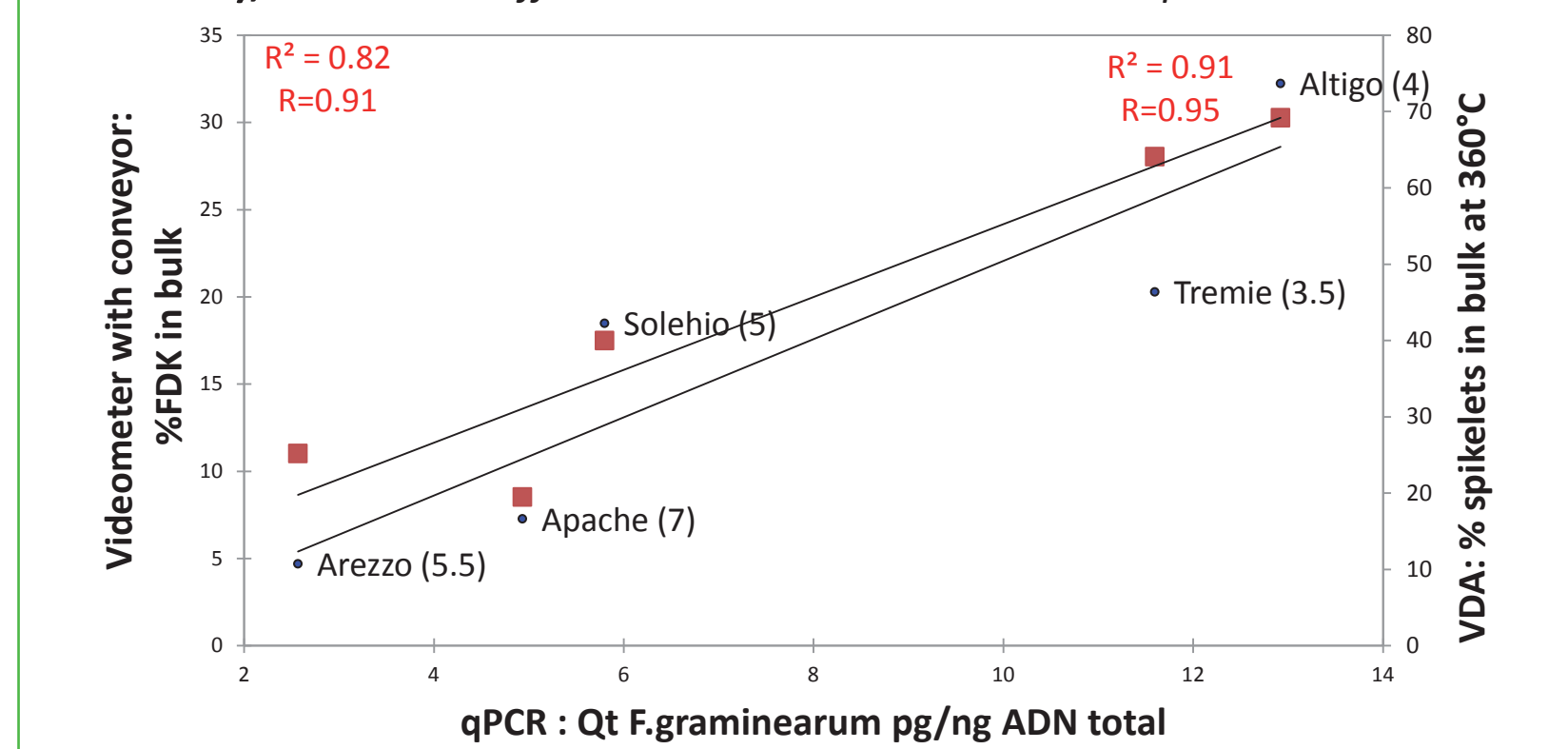
High correlations were obtained between qPCR and Videometer used with the conveyor (R=0.91) for kernels collected in bulk, and also between qPCR and VDA at 360°C dpi (R=0.95), confirming the VideometerLab® ability to assess accurately and rapidly the quantification of *Fusarium* in kernels at maturity, in agreement with the resistance classification.

CONCLUSION AND OUTLOOKS

Good results were obtained using the VideometerLab® to assess the resistance of wheat cultivars to *Fusarium graminearum and culmorum,* with the algorithm developed by GEVES.
More tests will be carried out in 2014 to confirm these results on a higher number of wheat varieties, and on other species of cereals (triticale, durum wheat,...). New research program will be planned to develop another algorithm able to distinguish *Microdochium* spp from *Fusarium graminearum* on wheat kernels.

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