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PHENOquad: A new multi sensor platform for field phenotyping and screening of yield relevant characteristics within grapevine breeding research

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Summary

Balanced and stable yield is a major trait in grapevine breeding and breeding research. Grapevine yield hereby is a complex quantitative trait, as it is influenced by multiple plant parameters, like berry size, number of berries per bunch, number of bunches per shoot, management, and environmental factors. In the current breeding process, the complexity of this trait has shown that a classification according to descriptive factors for marker development is only possible to a limited extent. Precise field phenotyping of yield-related traits is the basic prerequisite to be able to measure such quantitative traits. This, however, is the major bottleneck due to labor, time and constrains of plant material in the breeding process. For this reason, one of our main goals with the newly developed phenotyping platform PHENOquad with its multisensor system PHENOboxx is to improve phenotyping efficiency of grapevine yield to overcome the phenotyping bottleneck.

The newly developed embedded vision system PHENOboxx is mounted on an "all-terrain vehicle (ATV)". This allows a fast data acquisition on a large number of individual vines. In order to evaluate the yield potential of breeding material in comparison to established grapevine cultivars, various yield-related parameters of the vines are quantified directly in the field with high spatial and temporal resolution. As key parameters for yield-related phenotyping, the number of shoots, bunches, berries and the weight of dormant pruning wood was identified. The image data acquired are annotated to train the artificial intelligence (AI). Within the process, the image analysis results are compared to annotated ground truth data and correlated with the field reference data.

We expect to increase the precision, target specificity and throughput of screening grapevine material without reducing its accuracy over time by using the PHENOquad. In addition, a weighting of yield-relevant parameters would be possible. This opens up new possibilities for efficient plant evaluation in the scope of grapevine breeding. Also new application possibilities for precision viticulture are conceivable.

Key words

Phenoliner, PHENOboxx, High-throughput, phenotyping, precision viticulture, sensor, data management, image analysis, yield components

Introduction

In viticulture, wine quality is closely linked to yield per vine. If the vine is overcropped, wine quality decreases (Clingeleffer *et al.*, 2005; Poni *et al.*, 2018). Yield and wine quality are regarded as the two most important economic indicators for winegrowers (Santiago-Brown *et al.*, 2015). Therefore, a stable and balanced yield is one of the major breeding traits besides resistance/tolerance against biotic factors like powdery- and downy mildew and abiotic factors like frost or drought (Töpfer *et al.*, 2011; Töpfer and Trapp, 2022). In the current breeding process, breeders have to evaluate phenotypic traits manually by visual scorings. In an early breeding stage, this is especially labor-intensive for seedlings because the traits of interest occur in the same period for all seedlings, leading to an assessment of more than 10,000 plants in a short time span. Due to the high number of individuals, this process is prone to errors and accuracy decreases over time. With grapevine being a perennial crop, new varieties need to be monitored over a long period (~25 years) under field conditions. During this time yield depressions in new cultivars can occur even after several years in the field. Due to new major challenges in term of climate change and the increase

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in frequency of various extreme weather events in recent years, robust and climate change adapted vines become an additional main breeding goal. The new challenges cause a need for new strategies to be developed to make grapevine breeding more efficient (Töpfer and Trapp, 2022).

To overcome the phenotyping bottleneck that exists with the traditional evaluation, different high performance digital solutions have been published in recent years. For image acquisition different platforms are described. Kicherer *et al.*, (2015) introduced an automated platform based on a chain vehicle, Victorino *et al.*, (2020) used an autonomous robot system. Other studies used "all terrain vehicles" (ATV) (Aquino *et al.*, 2018; Millan *et al.*, 2018; Nuske *et al.*, 2014b), a converted grapevine harvester (Kicherer *et al.*, 2017a), other agricultural vehicles (Arnó *et al.*, 2013; Milella *et al.*, 2019) or "unmanned aerial vehicles" (UAVs) (Ballesteros *et al.*, 2020; Di Gennaro *et al.*, 2019; Torres-Sánchez *et al.*, 2021). Image processing is respected as one of the most utilized techniques for attempting an early yield estimation (Barriguinha *et al.*, 2021).

The different imaging systems have to deal with various challenges during data acquisition in the field. The biggest technical challenge in phenotyping of grapevine breeding material in the field are the rapidly changing light exposure conditions. One way to solve this problem is to perform the data acquisition under constant illumination conditions, such as in the tunnel of a grapevine harvester (Kicherer *et al.*, 2017a). Another possibility is to take data acquisition out at night with the help of artificial lighting (Millan *et al.*, 2018; Nuske *et al.*, 2014b). Furthermore the detection, segmentation, and counting of individual bunches is complex due to overlap with other bunches, leaves or shoots as well as contrast with other objects in the background (Font *et al.*, 2015; Nuske *et al.*, 2014a; Pérez-Zavala *et al.*, 2018). In addition, environmental dynamics such as leaf movements due to wind can negatively influence the image quality (Nellithimaru and Kantor, 2019). In order to obtain a proper correlation between image evaluation and true yield, it is important to consider management practices, such as the trellis system, defoliation of the bunch area, shoot positioning and shoot/bunch thinning (Nuske *et al.*, 2014b). Similar results were obtained by Zabawa *et al.*, (2020), who showed that the accuracy of berry detection differs between vertical shoot positioning and semi minimal pruned hedges training systems. According to Victorino *et al.* (2022) the percentage of occluded bunch area can be estimated using the visible bunch area and the canopy porosity as variables in a multiple regression model. It is also possible to estimate the number of berries hidden by leaves (Kierdorf *et al.*, 2022). Another challenge is the overlapping of recorded images. Here, the counted berries must be corrected to obtain a reliable yield estimate (Zabawa *et al.*, 2020).

Yield prediction models are mostly variety dependent. Therefore, a solution applicable to grapevine breeding research needs to cope with all the different varieties. However, for newly crossed grapevine varieties, there are no historic data to train new yield prediction models. Identifying the flower number per inflorescence has the theoretical potential to be variety-independent, albeit being insufficient for reliable yield estimations. A combination with the fruit set rate and the average berry weight would be necessary to solve this issue (Millan *et al.*, 2017). The same problem occurs for the early yield estimation based on shoot counting. In this case, historical data about the number of bunches per shoot and the average bunch weight for a variety are needed to obtain a reliable estimation (Liu *et al.*, 2017).

According to Clingeleffer *et al.* (2001), the yield of grapevines is composed of the following components:

1. 60-70% of the yield variance is determined by the number of bunches per vine
2. 30% of the yield variance is determined by the number of berries per bunch
3. 10% of the yield variance is determined by berry size

This classification is based on medium- to long-term yield data from diverse climatic zones for a wide range of varieties in commercial vineyards. The three yield components in their sum describe 100% of variance in grapevine yield. Therefore, most studies are focused on the detection of these components. For yield estimation under field conditions, berry number and bunch area could be used together. In particular the bunch-projected area appears to be a crucial variable for grapevine yield estimation (Victorino *et al.*, 2020) and the most promising period for monitoring vines with an on the go sensor setup seems to be between the phenological stages berry-set BBCH71 and bunch-closure BBCH79 (Aquino *et al.*, 2018).

In addition to the yield components described above, the balance between generative and vegetative growth, also called vine balance plays an important role in classifying seedlings for their breeding purposes. Vine balance can be examined through vine indices such as the ratio of total yield to mass of dormant pruning wood (Ravaz, 1903). An accurate image-based evaluation for pruning mass has been shown by (Kicherer *et al.*, 2017b). This method could be used in grapevine breeding research as well as by the industry to monitor vine balance.

The goal of this study is to increase grapevine breeding efficiency and the selection of suitable breeding lines by developing a new phenotyping tool; sensor-based, fast, with high precision and non-invasive for high throughput in grapevine breeding research.

Material and Methods

Plant material

Field tests within the study were conducted in the years 2021 and 2022 in two experimental vineyard plots at the JKI Geilweilerhof located in Siebeldingen, Germany (49◦21.747´N, 8◦04.678´E). Rows were planted in north-south direction and vines were cultivated in a vertical shoot positioned trellis system with one cane and around 10 buds per vine for both plots.

Four economically important grapevine varieties as well as seven elite breeding lines out of the intermediate testing phase (Trapp & Töpfer, 2022) were used in the presented study (Table 1). Plants are grafted on SO4 root stocks with an inter-row distance of 2 m and grapevine spacing with 1.1 m for both plots.

Table 1: List of genotypes evaluated in the field trials

Variety	VIVC	Accession number	Rows	Number of vines	Year of planting
Dornfelder	3659	DEU098-2008-057	1	21	2008
Pinot noir	9279	DEU098-2008-075	1	22	2008
Pinot blanc	9272	DEU098-2008-072	1	22	2008
Riesling	10077	DEU098-2008-080	1	24	2008
Gf.2010-011-0048	-	-	2	50	2015
Gf.2001-041-0004	-	-	2	46	2016
Gf.2001-041-0003	-	-	2	46	2016
Gf.2004-043-0010	-	-	2	46	2016
Gf.2004-043-0021	-	-	2	45	2016
Gf.2004-043-0034	-	-	2	40	2018
Gf.2000-305-0081	-	-	2	38	2019
				Σ 400	

Platform

For the PHENOquad a KYMCO MXU550 was used as sensor carrier vehicle. On the ATV, both the area in front of the handlebars and the area behind the seat can be used as mounting space. The PHENOboxx was mounted in the front area and a power generator was mounted in the back area. The power generator was used for the external power supply of the PHENOboxx (Fig. 1).

PHENOboxx

The multi sensor system PHENOboxx combines a total of four different sensors. The main sensor is a five channel multi spectral camera from JAI (JAI Fusion FS-3200T-10GE, JAI A/S, Valby Copenhagen, Denmark). The camera is equipped with three 1/1.8” CMOS sensors with 3MP each. One covers the visible wavelengths with a typical Bayer pattern RGB sensor, the other two are monochromatic sensors with bandpass filters. Inside the camera is a prism that divides the optical path into three, so that despite there being three sensors, each sensor is illuminated with the same scene. The five channels are sensitive at a wavelength of 400 – 1.000 nm. The position of the camera is about 1.7 m from the vine and with the 8 mm lens from VST Europe (VS-0818H/3CMOS, VST Europe B.V., CN Amsterdam, The Netherlands) the whole leaf wall can be imaged.

The system has two VNIR LED bars (EFFI-FLEX-20-000-850-WW-PP, EFFILUX Deutschland GmbH, HÜRTH, Germany) to enable data acquisition in low light conditions or even at night. The light bars have the function to flash after triggering. The power increases to 300% when operated in this mode. The JAI camera is able to output trigger signals. We use this configuration to maximize light and minimize power consumption. With this equipment, the ATV is able to drive at 5 km h^{-1} during the day without the images becoming blurred.

Right next to the multi spectral camera is a depth camera from Lucid Vision (Helios2 + HTP003S-001, LUCID Vision Labs, Inc., Richmond, BC, Canada). With this additional sensor, the

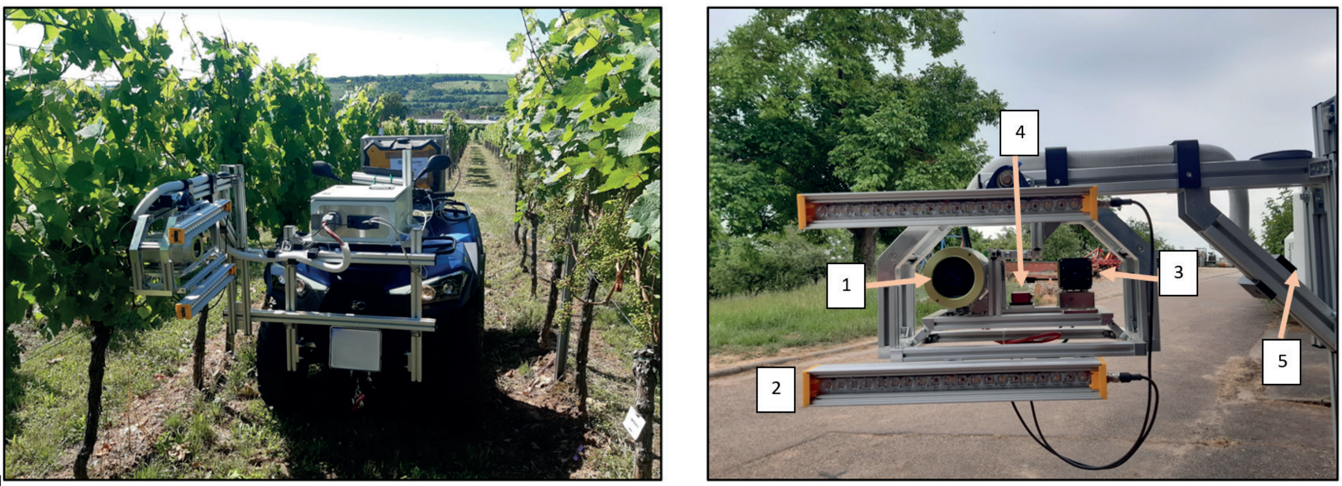

Fig. 1: The new phenotyping platform PHENOquad (left) with the PHENOboxx (right), a multi sensor setup used for data acquisition in grapevine breeding research. 1) Multi spectral camera (400-1.000 nm) 2) VNIR LED light Bars 3) TOF camera 4) GPS system 5) Ambient Light Sensor (ALS).

differentiation of foreground and background is easier and therefore it is possible to georeference every vine plant. The Time-of-Flight camera (ToF camera) provides an image with a resolution of VGA with 16 bits. A simultaneous operation with the multispectral camera is not possible because of the NIR laser that the ToF camera is working with and the VNIR LED bar. The light bar overlays the signal from the laser and the ToF camera becomes blind. Therefore, the ToF camera image is acquired directly after the multispectral image. This is accomplished by an inverted trigger signal from the JAI camera.

Each captured image is assigned its global coordinates by the onboard GNSS system (Ellipse-N, SBG SYSTEMS, Carrieres-sur-Seine, France). It is spatially located between the cameras and communicates via serial interface. Besides the coordinates, the SBG system also provides information about its relative orientation. In combination with the ToF camera each grapevine plant can be assigned to its coordinates as mentioned above.

To reduce motion blur due to ATVs vibration, all of the sensors are mounted on a gimbal-like structure (Fig. 1). Our previous studies have indicated which frequencies dominate, and these are now mechanically damped by the gimbal.

Independent of the gimbal structure is the use of a 6-channel spectrometer as an ambient light sensor (ALS) (Spectral 3 Click, MikroElektronika, Belgrade, Serbia). It is mounted opposite to the cameras viewing direction. With this sensor, the integration time can be adjusted during the ongoing measurement depending on the ambient light and thus improve the image quality even under difficult and changing conditions. The Spectral 3 Click is equipped with an IC named AS7263 (ams-OSRAM AG, Premstaetten, Austria) which is sensitive to wavelengths 610 nm, 680 nm, 730 nm, 760 nm, 810 nm and 860 nm with 20 nm full width at half maximum (FWHM). Parallel to each captured image, the spectrometer is read out and its values are stored.

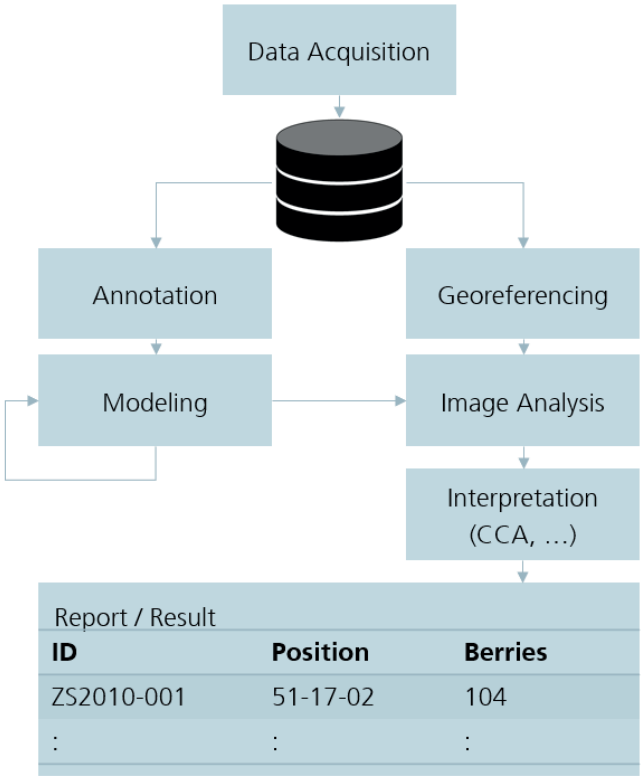

Fig. 2: Workflow of the image evaluation. After the image acquisition, a small subset is separated for modeling. After each image is georeferenced, the model is applied to the remaining not annotated images. The result is interpreted with a non-AI approach such as the number of berries via connected component analysis (CCA) depending on the use case. With this information, the desired parameters of each vine can be recorded and thus reported.

Data Workflow

The data flow in general is structured as in Fig. 2. After the image acquisition, the image that best shows the individual plant is selected for georeferencing. The new data are integrated into the meta data of the particular image. A small subset of the images (ca.150) is then separated for annotation and model training, test and validation. The annotation part can be adapted to the individual use case like the detection of berries or buds. The resulting model is then applied to the remaining images. The interpretation of the model´s output depends on the use case and can be done by non-AI approaches such as the number of berries via connected component analysis (CCA) method. The results are also integrated into the meta data of the particular image. With this information, the desired parameters of each vine can be recorded and thus reported.

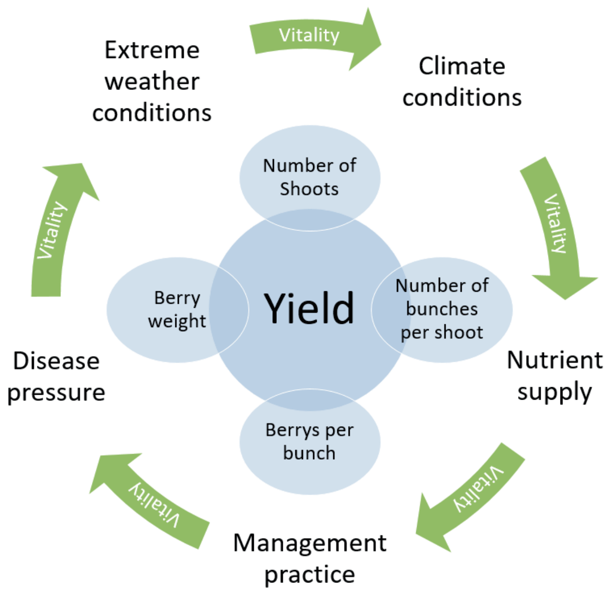

Fig. 3: Yield is made up of several components. These are the number of shoots, number of bunches per shoot, berries per bunch, and the berry weight. Yield is also influenced by a number of biotic and abiotic factors, that can in general be described as vitality of the vine.

Image data and reference data acquisition annotation

The multi sensor system PHENOboxx was used to collect data at four different points in the growing season in 2022 to identify the components that are significant for the yield composition (Fig. 3). At the growth stage pea size (BBCH75), we have recorded all the plants listed in Table 1. The ATV drives with approximately 4 km·h^{-1} and the camera runs with 10 frames per second. Therefore, the 400 vines had an image overlap of 80 – 90% and were captured in about 4400 frames.

In parallel to the image data acquisition with the PHENObox, corresponding reference data were collected at all of the four phenological stages for all vines on the same day (Fig. 4). At bud burst (BBCH10 in scale of **B**iologische Bundesanstalt, **B**undessortenamt und **Ch**emische Industrie (Lorenz *et al.*, 1995)), the number of shoots was recorded. Between fruit set (BBCH71) and pea size (BBCH75) as well as at harvest (BBCH89), the number of bunches was manually recorded. After flowering (BBCH69), the bunch zones of vines were defoliated on the east side in order to avoid any bunch coverage by canopy during image acquisition. At harvest, bunch weight was determined after image capture for each plant. In winter, images of the dormant pruning wood were taken. After vine pruning, the mass of dormant pruning wood was determined which was used in order to calculate the Ravaz-Index as described by Ravaz (1903) (Table 2).

Between BBCH71 and BBCH75, total chlorophyll content (Chl) and nitrogen balance index (NBI) was measured for three leaves on five vines per row using the Dualex® 4 Scientific sensor (Force-A, Orsay, France). On the same selected vines, three bunches were phenotyped using a 3D scanner at harvest as described by Rist *et al.* (2018). Hereby, different bunch parameters can be determined automatically: berry number per bunch (BN), mean berry diameter (MBD), mean berry volume (MBV), total berry volume (TBV), bunch width (BW), and bunch length (BL).

Annotation

From the 4,400 images of the 400 vines, 116 random images were annotated by labeling only the center of the berry. These labeled images were used to train a model with u-net architecture (Ronneberger *et al.*, 2015). A detailed description of the training process is not part of this paper.

The model was applied to the remaining 4,284 images of the measurement series. One exemplary result image is depicted in Fig. 5. Using the center detection approach, the number of visible berries can now be determined in the next step via the CCA method. In general, the evaluation is done in two steps: the actual detection of the desired plant parameters and then the quantitative evaluation of these like number of berries.

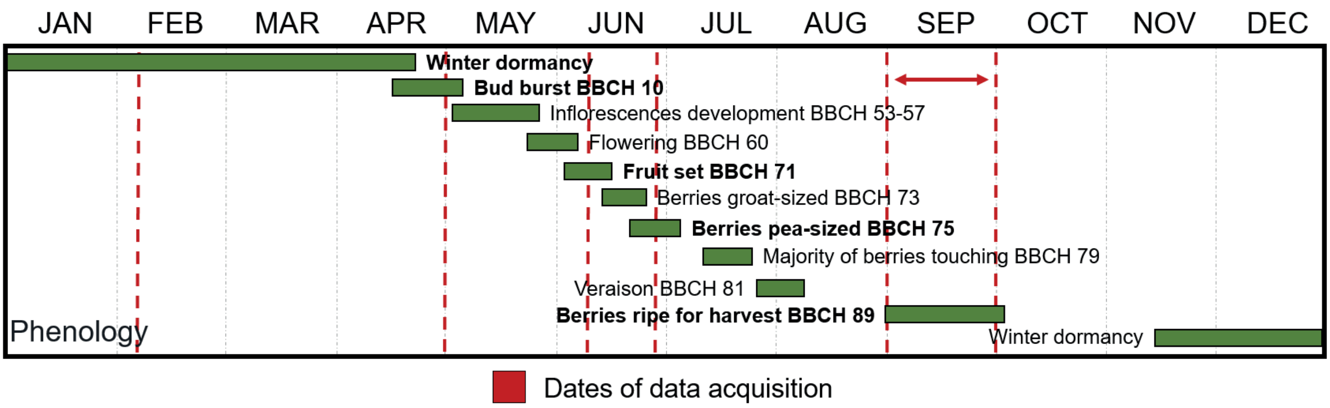

Fig. 4: Dates of data acquisition in the growing season to determine the important yield components (number of shoots, – bunches, yield, berry number in the images and the dormant pruning wood) at the respective phenological stages.

Table 2: List of field reference data acquired with the corresponding phenological stage, BBCH stage and the annotated ground truth data in the image annotation

Phenological stage	Stage	Field reference data	Ground truth data (image)
Bud burst	BBCH10	Number of shoots	Number of shoots
Fruit set – Berries pea sized	BBCH71-75	Number of bunches Chl & NBI[1]	Number of berries Total bunch area
Harvest	BBCH89	Number of bunches Yield 3D-Scan[2]	Number of berries Total bunch area
Winter dormancy	BBCH99	Dormant pruning wood	Pruning wood area

[1] chlorophyll content (Chl) and nitrogen balance index (NBI) for 3 leaves at 5 vines per row
[2] for 3 bunches at 5 vines per row

Fig. 5: Exemplary image of berry counting at BBCH75. The center of each berry is marked with a dot on a new mask and are reliably detected. The dots on the mask are counted with the following connected component analysis (CCA).

For validation purposes, the berries in 271 images from Dornfelder at the stage of pea-sized (BBCH75) were counted manually to obtain ground truth data.

Results and Discussion

Stable and balanced yield is an important trait for grapevine breeders. In order to be able to select new crossings for the further breeding process yield estimation and forecasting are of particular interest for the grapevine breeders. The usual phenotyping process in the field is very labor intensive, error-prone and subjective as it is done manual by skilled experts, who assess several hundreds of seedlings showing the same traits at the same time. The application of the multisensory system PHENOboxx could be used for preselection of seedlings in the breeding process and thereby reduce costs and workload.

The new phenotyping platform PHENOquad with the multisensory system PHENOboxx has been successfully tested in the 2022 season. The idea of a platform based on an ATV is not new and has already been successfully tested in several studies (Aquino *et al.*, 2018; Millan *et al.*, 2018; Nuske *et al.*, 2014b). Compared to previously used phenotyping platforms for grapevine breeding research at the Institute for Grapevine Breeding Geilweilerhof, PHENObot and Phenoliner, a high throughput data acquisition using PHENOquad seems to be possible. The driving speed of 4 km h^{-1}, which roughly approaches the speed of a tractor at work in vineyards, and an image acquisition of 10 Hz is clearly above the 0.6-1 km·h^{-1} and 5Hz that were possible with the Phenoliner (Kicherer *et al.*, 2017a). With the PHENObot, in comparison, no image acquisition was possible on-the-go, so it had to stop for the image acquisition of each vine so that the image acquisition with the PHENObot per grapevine took about 15 s (Kicherer *et al.*, 2015). In studies that also used an ATV for on-the-go image acquisition, speed ranges between 5.4 and 7 km h^{-1}. However, in these studies, data acquisition was conducted at night under constant environmental light conditions and not under constantly changing daytime lighting conditions (Aquino *et al.*, 2018; Millan *et al.*, 2018; Nuske *et al.*, 2014b).

Another advantage of the PHENOquad over the Phenoliner is the lower weight of the platform, which minimizes undesirable soil compaction and thus a negative influence on soil biota and root development of the vines. This is of particular importance, as data acquisition is intended to take place several times a year at different points in the growing season. The integration of further sensors and the expansion of the research questions may additionally add more crossings of the vineyard. Due to the better adaptation to viticulture requirements, data acquisition in vineyards of winegrowers outside of the JKI research fields is also possible, which enables a transfer of the platform into viticulture practice. As a validation of image analysis for the number of berries, the results from image analysis were correlated with the manually annotated ground truth data for the dataset of 271 images from Dornfelder at pea-size stage (BBCH75). The result is plotted in Fig. 6. The coefficient of r=0.963 shows a high positive correlation between the results of image analysis and the ground truth data. Hence, the image based berry detection produces a representative result.

The first results for detecting the number of berries in (variable) daylight conditions confirm the possibility of gaining valid results without standardized conditions such as in the moving tunnel of the Phenoliner (Kicherer *et al.*, 2017a) or image acquisition at night with the PHENObot or other platforms (Aquino *et al.*, 2018; Kicherer *et al.*, 2015; Millan *et al.*, 2018). This is a significant advance towards usability in practical viticulture. The flashing LED bars also allow data acquisition at night. The remaining limitation in the data acquisition is rain or wet parts of the vine, as they reflect the light and are not visible in the images. The results of berry counting at the pea-sized stage correspond with previous results for image analyses from on-the-go data acquisition in the field (Aquino *et al.*, 2018).

The next step is to determine the accuracy of detection of the berry count for the varieties 'Riesling', 'Pinot Noir' and 'Pinot

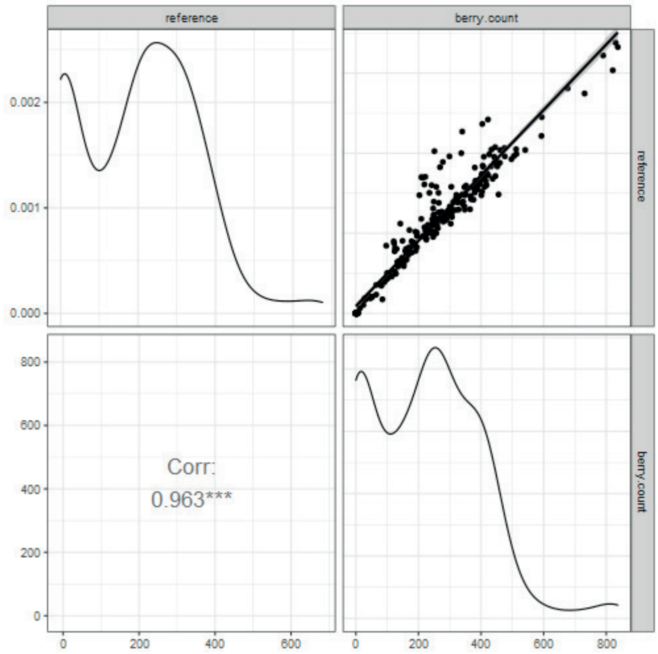

Fig. 6: Validation of the berry count from the image analysis with the annotated ground truth reference data.

Blanc' as well as for the seven breeding lines in the interterm testing. Besides berry count, the total bunch area in the images should also be evaluated for all genotypes. Thus, it was assessed whether there are differences in the analysis between different genotypes, in particular between loose and dense clustered varieties. Such differences between various genotypes in the detection of the number of berries has already been observed in previous studies (Millan *et al.*, 2018). The same analysis should be carried out for data acquisition at harvest. In addition to the immediate yield components, the number of shoots and the dormant pruning wood will be analyzed.

A future objective is to supplement the results of image analysis with other data such as weather, soil and vitality data to be able to model yield predictions. The image data will be available to grapevine breeding research in a database developed specifically for this purpose, so that the images can be analyzed retrospectively.

This would make it possible to compare the performance of seedlings from different vintages and check why individual genotypes do not meet the breeding objectives in some years but certainly do in other years.

Conclusion

With the PHENOquad, a new robust phenotyping platform for grapevine breeding research was successfully tested. By using a quad bike as platform and the multi sensor system PHENOboxx, grapevines can be screened directly in the field without negative side effects, especially soil compaction due to a high dead weight. Compared to previous phenotyping platforms, a higher driving speed for data acquisition increases the potential for a high-throughput field phenotyping of yield components. In this sense, routine data collection for phenotyping studies within grapevine breeding research can be considered solved by using the PHENOquad. Data analysis, however, needs to be further elaborated. A transfer of the principle to other phenotypic traits is conceivable as well as to practical viticulture.

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

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