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High-throughput phenotyping of yield parameters for modern grapevine breeding

Dissertationen aus dem Julius Kühn-Institut

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

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Anna Kicherer

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Abbreviations

a1, a2, a3	Three semi-axis of an ellipsoid
ANOVA	Analysis of variance, a collection of statistical models
AJGWR	Australian Journal of Grape and Wine Research
ARC	Australian Research Council, a Commonwealth entity within the Australian Government
B	Total number of berries per region
BAT	Berry Analysis Tool, MATLAB script for image analysis
BBCH	Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie, phenological development stages of a plant
BIV	Berries in Vineyards, MATLAB script for image analysis
BIVcolor	Berries in Vineyards-color, MATLAB script for image analysis
BMBF	Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung)
c	ratio between mm and pixel
C4	Carbon fixation within photosynthesis, one of three biochemical mechanisms, along with C3 and CAM
CAT	Cluster Analysis Tool, MATLAB script for image analysis
cm	Centimetre, SI unit of length
CT	Computed Tomography
DPPN	Deutsches Pflanzen Phänotypisierungsnetzwerk
DNA	Desoxyribonucleic acid
E	East, UTM coordinates
EPPN	European Plant Phenotyping Network
FTIR	Fourier transform infrared spectroscopy
FKZ	Förderkennzeichen
g	Gram, SI unit of mass
GB	Giga byte, unit of digital information
GHz	Giga hertz, SI unit of frequency
GigE	Gigabit Ethernet, transmitting Ethernet frames at a rate of a gigabit per second
GPS	Global Positioning System, a space-based satellite navigation system
GRA.LE.D	GRApevine LEaf Digitalization, image analysis tool
GUI	Graphical user interface
^{1}H	Hydrogen, chemical symbol
ha	Hectare, SI unit of area
ha h^{-1}	Hectare per hour
HSV	Hue-saturation-value
HT	High-throughput
I	Image
IAP	Integrated Analysis Platform
ID	Identifier
INRA	Institut National de la Recherche Agronomique
IPPN	International Plant Phenotyping Network
IPK	Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung
IR	Infrared

JKI	Julius Kühn-Institut
kg	Kilogram, SI base unit of mass
kg m^{-2}	Kilogram per square meter
km h^{-1}	Kilometre per hour, unit of speed
l	Length
LAI	Leaf area index
LDA	Linear discriminate analysis, statistical analyse
m	Meter, SI unit of length
m²	Square meter, SI unit of area
mm	Millimetre, SI unit of length
mm²	Square millimetre, SI unit of area
min.	Minutes, unit of time
mL	Millilitre, SI unit of volume
MABC	Marker assisted back crossing
MAS	Marker assisted selection
MB	Mega byte, unit of digital information
MC	Monochrome
MCS	Multi-camera-system
MDPI	Multidisciplinary Digital Publishing Institute
MRI	Magnetic Resonance Imagers
n	Number of samples/observations, statistical parameter
N	North, UTM coordinates
NDVI	Normalized Difference Vegetation Index
NIR	Near infrared
nm	Nanometre, SI unit of length
OIV	International Organization of Vine and Wine
p	Number of parameters, statistical parameter
pm	Picometre, SI unit of length
p-value	significance level, statistical parameter
PA	pruning area
PC	Personal computer
PET	Positron Emission Tomography
PHENObot	Phenotyping robot
PIAS	Prototype Image Acquisition System
PLA	Plant location administration, name of a database
PW	pruning weight
QTL	Quantitative trait loci
R^2, r^2	Determination coefficient, statistical parameter
R’	Region consisting of one sub region
r	Correlation coefficient
$R_{S, 1,...s,...,S}$	Regions of pixels grouped together; S = number of regions; s = index of considered region
RAPD	Randomly amplified polymorphic DNA
RGB	red-green-blue
RMSE	Root-Mean-Squared-Error, statistical parameter
RTK	Real-time-kinematic
SAS	Statistical Analysing System; statistic software
SLR	Single-lens reflex camera

SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
SQL	Structured query language, a special-purpose programming language
TST	Trait size tool, MATLAB script for image analysis
U-Go	Unmanned ground outdoor
UAP	Unmanned aerial platform
UAV	Unmanned aerial vehicle
UTM	Universal Transverse Mercator, coordinate system
VIS	Visible Imaging Spectrometer
*V*IVC	*Vitis* International Variety Catalogue, database
w	width
YiPa	Yield per pruning area, vine balance index
y	each pixel
μ_d	Mean diameter of fitted ellipse through R’
µm	Micrometre, SI unit of length
σ_d	Standard deviation
2D	Two-dimensional space, geometric bi-parameter model
3D	Three-dimensional space, geometric three-parameter model
$^{11}CO_2$	Radioactive isotope Carbon-11
64 bit	Operating system
°C	Degree Celsius, unit of temperature
°S	Degree South
°N	Degree North

Summary

Grapevine is grown on about 1% of the German agricultural area requiring one third of all fungicides sprayed due to pathogens being introduced within the 19th century. In spite of this requirement for viticulture a reduction is necessary to improve sustainability. This objective can be achieved by growing fungus resistant grapevine cultivars. The development of new cultivars, however, is very time-consuming, taking 20 to 25 years. In recent years the breeding process could be increased considerably by using marker assisted selection (MAS). Further improvements of MAS applications in grapevine breeding will come along with developing of faster and more cost efficient high-throughput (HT) genotyping methods.

Complementary to genotyping techniques the quality, objectivity and precision of current phenotyping methods is limited and HT phenotyping methods need to be developed to further increase the efficiency of grapevine breeding through sensor assisted selection. Many different types of sensors technologies are available ranging from visible light sensors (Red Green Blue (RGB) cameras), multispectral, hyperspectral, thermal, and fluorescence cameras to three dimensional (3D) camera and laser scan approaches. Phenotyping can either be done under controlled environments (growth chamber, greenhouse) or can take place in the field, with a decreasing level of standardization. Except for young seedlings, grapevine as a perennial plant needs ultimately to be screened in the field. From a methodological point of view a variety of challenges need to be considered like the variable light conditions, the similarity of fore- and background, and in the canopy hidden traits.

The assessment of phenotypic data in grapevine breeding is traditionally done directly in the field by visual estimations. In general the BBCH scale is used to acquire and classify the stages of annual plant development or OIV descriptors are applied to assess the phenotypes into classes. Phenotyping is strongly limited by time, costs and the subjectivity of records. Therefore, only a comparably small set of genotypes is evaluated for certain traits within the breeding process. Due to that limitation, automation, precision and objectivity of phenotypic data evaluation is crucial in order to (1) reduce the existing

phenotyping bottleneck, (2) increase the efficiency of grapevine breeding, (3) assist further genetic studies and (4) ensure improved vineyard management. In this theses emphasis was put on the following aspects: Balanced and stable yields are important to ensure a high quality wine production playing a key role in grapevine breeding. Therefore, the main focus of this study is on phenotyping different parameters of yield such as berry size, number of berries per cluster, and number of clusters per vine. Additionally, related traits like cluster architecture and vine balance (relation between vegetative and generative growth) were considered. Quantifying yield parameters on a single vine level is challenging. Complex shapes and slight variations between genotypes make it difficult and very time-consuming.

As a first step towards HT phenotyping of yield parameters two fully automatic image interpretation tools have been developed for an application under controlled laboratory conditions to assess individual yield parameters. Using the Cluster Analysis Tool (CAT) four important phenotypic traits can be detected in one image: Cluster length, cluster width, berry size and cluster compactness. The utilization of the Berry Analysis Tool (BAT) provides information on number, size (length and width), and volume of grapevine berries. Both tools offer a fast, user-friendly and cheap procedure to provide several precise phenotypic features of berries and clusters at once with dimensional units in a shorter period of time compared to manual measurements.

The similarity of fore- and background in an image captured under field conditions is especially difficult and crucial for image analysis at an early grapevine developmental stage due to the missing canopy. To detect the dormant pruning wood weight, partly determining vine balance, a fast and non-invasive tool for objective data acquisition in the field was developed. In an innovative approach it combines depth map calculation and image segmentation to subtract the background of the vine obtaining the pruning area visible in the image.

For the implementation of HT field phenotyping in grapevine breeding a phenotyping pipeline has been set up. It ranges from the automated image acquisition directly in the field using the PHENObot, to data management, data analysis and the interpretation of obtained phenotypic data for grapevine breeding aims. The PHENObot consists of an automated guided tracked vehicle system, a calibrated multi camera system, a Real-Time-Kinematic GPS system and a computer for image data handling. Particularly developed software was applied in order to acquire geo referenced images directly in the vineyard.

The geo-reference is afterwards used for the post-processing data management in a database. As phenotypic traits to be analysed within the phenotyping pipeline the detection of berries and the determination of the berry size and colour were considered. The high-throughput phenotyping pipeline was tested in the grapevine repository at Geilweilerhof to extract the characteristics of berry size and berry colour using the Berries In Vineyards (BIVcolor) tool. Image data acquisition took about 20 seconds per vine, which afterwards was followed by the automatic image analysis to extract objective and precise phenotypic data. In was possible to capture images of 2700 vines within 12 hours using the PHENObot and subsequently automatic analysis of the images and extracting berry size and berry colour. With this analysis proof of principle was demonstrated. The pilot pipeline provides the basis for further development of additional evaluation modules as well as the integration of other sensors.

Zusammenfassung

Weinbau wird auf 1% der deutschen Agrarfläche betrieben. Auf dieser vergleichsweise kleinen Anbaufläche wird jedoch ein Drittel aller in der deutschen Landwirtschaft verwendeten Fungizide appliziert, was auf die Einführung von Schaderregern im 19. Jahrhundert zurück zu führen ist. Für einen nachhaltigen Anbau ist eine Reduktion des Pflanzenschutzmittelaufwands dringend notwendig. Dieses Ziel kann durch die Züchtung und den Anbau neuer, pilzwiderstandsfähiger Rebsorten erreicht werden. Die Rebenzüchtung als solche ist sehr zeitaufwendig, da die Entwicklung neuer Rebsorten 20 bis 25 Jahre dauert. Der Einsatz der markergestützten Selektion (MAS) erhöht die Effizienz der Selektion in der Rebenzüchtung fortwährend. Eine weitere Effizienzsteigerung ist mit der andauernden Verbesserung der Hochdurchsatz Genotypisierung zu erwarten.

Im Vergleich zu den Methoden der Genotypisierung ist die Qualität, Objektivität und Präzision der traditionellen Phänotypisierungsmethoden begrenzt. Die Effizienz in der Rebenzüchtung soll mit der Entwicklung von Hochdurchsatz Methoden zur Phänotypisierung durch sensorgestützte Selektion weiter gesteigert werden. Hierfür sind bisher vielfältige Sensortechniken auf dem Markt verfügbar. Das Spektrum erstreckt sich von RGB-Kameras über Multispektral-, Hyperspektral-, Wärmebild- und Fluoreszenz-Kameras bis hin zu 3D-Techniken und Laserscananwendungen. Die Phänotypisierung von Pflanzen kann unter kontrollierten Bedingungen in Klimakammern oder Gewächshäusern beziehungsweise im Freiland stattfinden. Die Möglichkeit einer standardisierten Datenaufnahme nimmt jedoch kontinuierlich ab. Bei der Rebe als Dauerkultur erfolgt die Aufnahme äußerer Merkmale, mit Ausnahme junger Sämlinge, deshalb auch überwiegend im Freiland. Variierende Lichtverhältnisse, Ähnlichkeit von Vorder- und Hintergrund sowie Verdeckung des Merkmals stellen aus methodischer Sicht die wichtigsten Herausforderungen in der sensorgestützen Merkmalserfassung dar. Bis heute erfolgt die Aufnahme phänotypischer Merkmale im Feld durch visuelle Abschätzung. Hierbei werden die BBCH Skala oder die OIV Deskriptoren verwendet. Limitierende Faktoren dieser Methoden sind Zeit, Kosten und die Subjektivität bei der Datenerhebung. Innerhalb des

Züchtungsprogramms kann daher nur ein reduziertes Set an Genotypen für ausgewählte Merkmale evaluiert werden. Die Automatisierung, Präzisierung und Objektivierung phänotypischer Daten soll dazu führen, dass (1) der bestehende Engpass an phänotypischen Methoden verringert, (2) die Effizienz der Rebenzüchtung gesteigert, und (3) die Grundlage zukünftiger genetischer Studien verbessert wird, sowie (4) eine Optimierung des weinbaulichen Managements stattfindet.

Stabile und über die Jahre gleichbleibende Erträge sind für eine Produktion qualitativ hochwertiger Weine notwendig und spielen daher eine Schlüsselrolle in der Rebenzüchtung. Der Fokus dieser Studie liegt daher auf Ertragsmerkmalen wie der Beerengröße, Anzahl der Beeren pro Traube und Menge der Trauben pro Weinstock. Die verwandten Merkmale Traubenarchitektur und das Verhältnis von generativem und vegetativem Wachstum wurden zusätzlich bearbeitet. Die Beurteilung von Ertragsmerkmalen auf Einzelstockniveau ist aufgrund der genotypischen Varianz und der Vielfältigkeit des betrachteten Merkmals komplex und zeitintensiv.

Als erster Schritt in Richtung Hochdurchsatz (HT) Phänotypisierung von Ertragsmerkmalen wurden zwei voll automatische Bildinterpretationsverfahren für die Anwendung im Labor entwickelt. Das Cluster Analysis Tool (CAT) ermöglicht die bildgestützte Erfassung der Traubenlänge, -breite und -kompaktheit, sowie der Beerengröße. Informationen über Anzahl, Größe (Länge, Breite) und das Volumen der einzelnen Beeren liefert das Berry Analysis Tool (BAT). Beide Programme ermöglichen eine gleichzeitige Erhebung mehrerer, präziser phänotypischer Merkmale und sind dabei schnell, benutzerfreundlich und kostengünstig.

Die Möglichkeit, den Vorder- und Hintergrund in einem Freilandbild zu unterscheiden, ist besonders in einem frühen Entwicklungsstadium der Rebe aufgrund der fehlenden Laubwand schwierig. Eine Möglichkeit, die beiden Ebenen in der Bildanalyse zu trennen, ist daher unerlässlich. Es wurde eine berührungsfreie, schnelle sowie objektive Methode zur Bestimmung des Winterschnittholzgewichts, welches das vegetative Wachstum der Rebe beschreibt, entwickelt. In einem innovativen Ansatz wurde unter Kombination von Tiefenkarten und Bildsegmentierung die sichtbare Winterholzfläche im Bild bestimmt.

Im Zuge dieser Arbeit wurde die erste HT Phänotypisierungspipeline für die Rebenzüchtung aufgebaut. Sie umfasst die automatisierte Bildaufnahme im Freiland unter Einsatz des PHENObots, das Datenmanagement mit Datenanalyse sowie die Interpretation

des erhaltenen phänotypischen Datensatzes. Die Basis des PHENObots ist ein automatisiert gesteuertes Raupenfahrzeug. Des Weiteren umfasst er ein Multi-Kamera-System, ein RTK-GPS-System und einen Computer zur Datenspeicherung. Eine eigens entwickelte Software verbindet die Bilddaten mit der Standortreferenz. Diese Referenz wird anschließend für das Datenmanagement in einer Datenbank verwendet. Um die Funktionalität der Phänotypisierungspipeline zu demonstrieren, wurden die Merkmale Beerengröße und -farbe im Rebsortiment des Geilweilerhofes unter Verwendung des Berries In Vineyard (BIVcolor) Programms erfasst. Im Durschnitt werden 20 Sekunden pro Weinstock für die Bildaufnahme im Feld benötigt, gefolgt von der Extraktion der Merkmale mittels automatischer, objektiver und präziser Bildauswertung. Im Zuge dieses Versuches konnten mit dem PHENObot 2700 Weinstöcke in 12 Stunden erfasst werden, gefolgt von einer automatischen Bestimmung der Merkmale Beerengröße und -farbe aus den Bildern. Damit konnte die grundsätzliche Machbarkeit bewiesen werden. Diese Pilotpipeline bietet nun die Möglichkeit zur Entwicklung weiterer innovativer Programme zur Erhebung neuer Merkmale sowie die Integration zusätzlicher Sensoren auf dem PHENObot.

1. General introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest domesticated and most worldwide-grown perennial fruit crops. Its evolution is closely linked to the cultural development of humankind and has an important economic and social value. The primary centre of domestication is most likely the Transcaucasia region (Töpfer et al., 2011). Nowadays it is cultivated at latitudes from 50°N to 30°N and 40°S to 30°S that approximate to the 10°C and 20°C isotherms (Mullins et al., 1992). It is supposed, that worldwide 8,000 to 12,000 grapevine cultivars exist, mainly used for wine production (56.8%) but also for table grapes (27.0%), a mixed usage for both wine and table grape production (7.3%), dried fruits (0.7%), and finally other genotypes are used as rootstocks (Töpfer et al., 2011). Besides wine production and fresh or dried food consumption grapes are used for juice, jam, syrups, ethanol, vinegar and seed oil production.

1.1 Grapevine breeding

History of grapevine breeding

One of the oldest known genotypes, first mentioned by Philippe de Beaumanoir in 1283, is 'Weißer Heunisch'. Together with the old 'Pinot' cultivar family it forms the parentage of many cultivars of present importance (Boursiquot et al., 2004; Bowers et al., 1999). It remains unclear how these cultivars emerged. It might be reasonably assumed that they originated from random selections rather than from organized breeding activities. The first clear cut evidence for controlled grapevine breeding is found in America during the late 18th century (Töpfer et al., 2011). First known cultivars like 'Sage', 'Cunningham' and 'Catawba' are well known as American hybrids. In European countries, above all in France, breeding activities turned up as a consequence of the introduction of different pathogens in the 19th century. Powdery mildew (*Erysiphe* (syn. *Uncinula*) *necator*,

Schwein.1834) was introduced to Europe in 1845 causing 80% harvest failures (Creasy and Creasy, 2009). Around 1863 phylloxera (*Daktulosphaira vitifoliae*, Fitch) arrived in Europe. Only the grafting of vines using scions of traditional cultivars (with leaf resistance to phylloxera) and root tolerant rootstocks saved the viticulture production (Campbell, 2004). Tragically another pathogen, downy mildew (*Plasmopara viticola*, Berk. & Curt *ex*. De Bary) came along with such rootstocks in 1878. Millardet suggested in 1878 to combine the *V. vinifera* L. subsp. *vinifera* fruit quality and the resistance against powdery and downy mildew found in American wild species. The outcome of these breeding activities was recognised as the so-called French hybrids. Due to the poor wine quality neither of the American and French hybrids succeeded in the market. Whereas the breeding activities stopped in France, countries like Germany used the French material for their own breeding efforts. While generating F1-populations by interspecific crosses was quite successful for rootstock breeding the quality of the achieved wine grapes was insufficient. Making it necessary to have more than two generations from the wild species to select reasonable genotypes and even more crosses to obtain really elite lines and new quality cultivars (Töpfer et al., 2011). Husfeld was the first proving that resistance and quality can be combined (Alleweldt, 1977). His cultivars (‘Aris’, ‘Siegfriedrebe’) convinced with good wine quality and high mildew resistance but were insufficient in terms of yield and virus susceptibility (Alleweldt, 1977). Except for the step of marker assisted selection (MAS) the illustration of Figure 1 shows a breeding scheme and gives an idea about the time frame of breeding programs already used by Husfeld and Alleweldt. Classical breeding programs obtain several successive steps decreasing the number of individuals in each step. Assuming a breeding program of wine grapes starts with 50,000 seedlings a year, greenhouse testing will lead to 5,000 vines planted in a seedlings plot.

Apart from the seedling stage all further steps require three to five years of growth. The first three years are needed to get the vine established and the following years to achieve a full crop. Vines from breeding lines showing good viticultural performance and high resistance levels will then be used for quality assessments. This so-called micro-vinification is crucial in wine grape breeding. Starting from a single vine level with no more than one litre it is by far one of the most time consuming evaluations in classical grapevine breeding. Reducing the required time for this step could accelerate the duration of grapevine breeding. This could only be realized through the development of early marker based genotyping methods. Not only wine quality traits like sugars, acids, flavours, off-flavours, etc. could be interesting for this application but also the yield traits correlated

to important quality traits like berry size, berry number, cluster architecture and phenology traits like time of ripening and ripening duration.

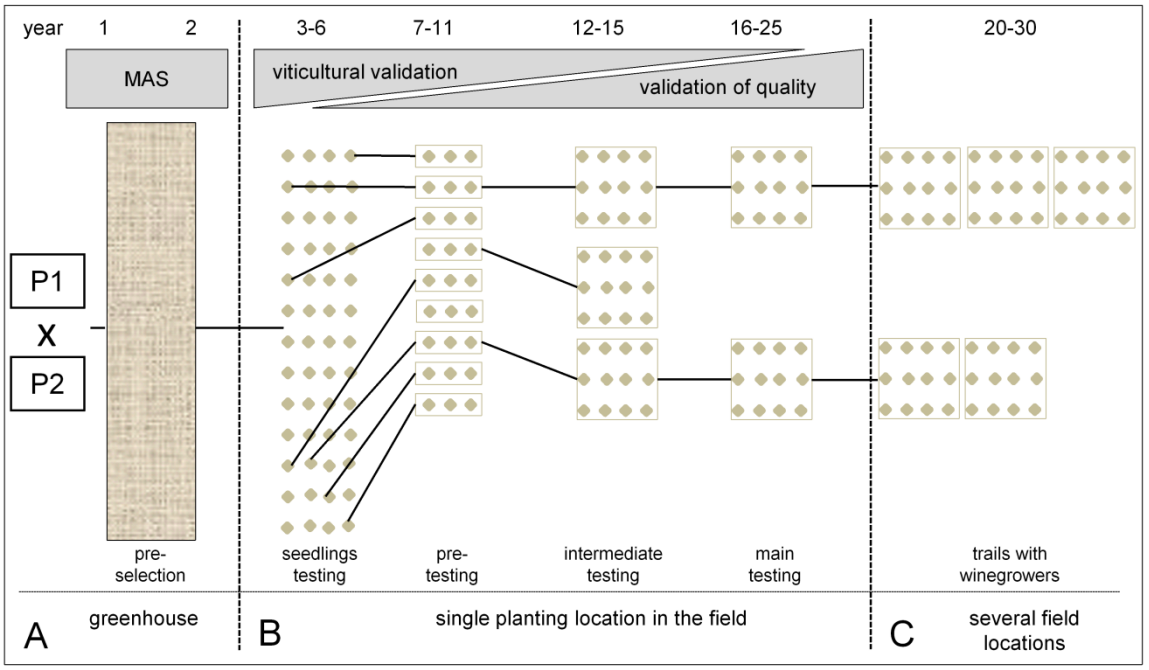

Figure 1 Timescale and steps of grape wine breeding. A: pre-selection in the greenhouse to eliminate e.g. genotypes with high susceptibility to fungal diseases (*Plasmopara viticola*, *Erysiphe necator*). For the early evaluation of traits like yield and quality the importance of MAS is increasing since these traits are difficult to evaluate prior to planting and growing in the field. B: Increasing number of vines per genotype in various steps of testing, seedlings- (1 vine), pre- (10 vines), intermediate- (50 vines) and main testing (500 vines). C: Followed by test trails with viticultural practice. Usually developing a new cultivar through classical wine grape breeding requires 25-30 years. With the utilization of MAS the expected savings in time are up to 10 years.

Grapevine was the fourth one of the first flowering plants and the first fruiting perennial crop whose genome was completely sequenced (Jaillon et al., 2007; Velasco et al., 2007) and therefore progress was made easier by the relatively small size of the genome (Bouquet, 2011). The rapid development of molecular techniques and genome sequencing, most important the development of molecular markers, accelerates grapevine breeding. First genetic mapping studies used RAPD (randomly amplified polymorphic DNA) markers (Weeden et al., 1994), followed by SSR (simple sequence repeats) markers (Bowers et al., 1996; Di Gaspero et al., 2007; Di Gaspero et al., 2005; Merdinoglu et al., 2005; Welter et al., 2007) which proved to be reliable, comparable and robust while permitting a more detailed analysis of genetically determined grapevine traits (Töpfer et al., 2011). As a next generation of markers in grapevine breeding, single nucleotide polymorphism (SNP) based markers have been used for genetic analysis (Myles et al.,

2010; Salmaso et al., 2004; Salmaso et al., 2008). Using SSR or other marker types, as well as a combination of them to develop genetic maps, provides the genetic framework required for QTL (quantitative trait loci) mapping and therefore the combination of genotypic and phenotypic information. This analysis permits the dissection of complex polygenetic traits and provides a rough localization of possible underlying genes.
With emerging new and effective genotyping methods in the last decade, the major missing tools are efficient and objective high-throughput phenotyping methods to accomplish modern grapevine breeding.

Objectives of grapevine breeding

A long generation cycle, limited plant material, slow propagation rates due to hard wood cuttings and the requirement of several repetitions to break down the environmental influence of a trait make grapevine breeding very time consuming (compare Figure 1). Two methods of grapevine breeding can be distinguished: (1) clonal selection of variants within the cultivar (asexual) for keeping cultivars healthy and stable in yield and (2) cross breeding (sexual reproduction) divided into breeding of rootstocks, table and wine grapes. To achieve the specific breeding goals of all three categories totally independent breeding programs based on different kinds of genetic resources are needed. Mainly non-*vinifera* vines of North American origin have been used to improve rootstocks through interspecific crosses. Agronomical performance and the resistance to phylloxera are the major breeding issues for rootstocks. In table grape breeding mainly crosses within *V. vinifera* L. subsp. *vinifera* are performed and the main breeding goals are quality (seedlessness, taste, sweetness, colour, uniformity of colour an cluster architecture, crispness, berry size, Botrytis stability) and post-harvest traits (time of ripening, transport stability) (Truel, 1983). The major objectives in wine grape breeding are high wine quality combined with high disease resistances and good climatic adaption. The most important traits for wine grape breeding are summarized in Table1.

Table 1 Objectives in wine grape breeding.

Breeding traits		**range of traits**	
wine quality			
red	dark colour	moderate colour	
white	fruity	neutral	muscat/aromic
rich in various components	tannins, flavonols	amino acids	potassium
sugar	medium	high	
acidity	high	medium	
off-flavours	none		
other wine characters	well balanced taste	wine with rich body	
aging potential	medium aging potential	high	long lasting wine
viticulture performance			
resistances- fungi	*Erysiphe necator* (syn. *Uncinula necator*)	*Plasmopara viticola*	*Botryotinia fuckeliana* (syn. *Bortytiy cineria*)
	Black rot	*Anthracnose*	
resistance- bacteria	*Pierce`s disease*	*Agrobacterium*	*Phomopsis viticola*
resistances-insects	*Daktulospharia vitifoliae*	*Xiphinema index* (vector for viruses)	
resistances- abiotic stress	frost	drought	sunburn
growth	upright		
wood maturation	early	middle	
yield			
yield	< 1 kg m^{-2}	1.5 kg m^{-2}	> 1.5 kg m^{-2}
fruit characters	loose cluster	thickness of berry skin	
berry ripening	early	middle	late
berry size	small (13 mm)	medium (18 mm)	wide (23 mm)
berries per cluster	< 200	200-300	> 300
cluster per cane	2	3	4

modified after Töpfer et al. 2011

1.2 Phenotyping bottleneck

With the rapid development of plant genomic technologies the ability to dissect the genetics of quantitative traits is limited due to a lack of access to plant phenotyping instruments. Although molecular breeding strategies have laid greater effort on genetic selection, phenotypic data are still needed for selection of breeding material, to identify genetic markers and for genetic studies. Current assessments of phenotyping characteristics in grapevine breeding are manly done by visual estimations using the BBCH scale (Lorenz et al., 1995) or OIV (Anonymous, 2009) descriptors. These methods are subjective, very time consuming and therefore also expensive. Modern plant phenotyping intended to measure complex traits non-invasive at a certain accuracy and precision at different scales of organization, from whole plant level to organs, to increase the efficiency in grapevine breeding. To achieve this goal modern phenotyping involves expertise from biological and computer science, mathematics and engineering to develop so-called machine vision

systems. These kinds of systems are already widely used in industrial production, medicine, radar guidance and document analysis for examination, monitoring or controlling. Within the agricultural sector modern phenotyping methods are also used in food industry for post-harvest fruit recognition and in precision agriculture. Phenotyping with such systems can take place in different environments (controlled or field) and depending on the experimental design different sensors can be used. To analyse the gene-environment interaction and to display the phenotypic response it is crucial to capture quantitative reference measurements and interpret the gained sensor results.

Sensor type

The sensors used to detected and quantify the phenotype of plants express the interaction between light and plants such as the reflectance, absorbance or transmission of photons. Different plant components show various wavelength-specific characteristics. For example, chlorophyll absorbs primary in the blue (420-480nm) and red (630-790nm) spectral region whereas liquid water has its absorption characteristics in the infrared. Therefore imaging at different wavelength is used for different plant phenotyping aspects. Imaging techniques manly include visible light, fluorescence, thermal infrared and spectroscopy imaging among others (MRI, PET, CT). Table 2 gives an overview of sensors currently used in plant research.

Controlled environment phenotyping systems

In the recent years, many efforts have been made to build up platforms, which allow the assessment of large quantities of phenotypic data under controlled environments. These platforms can be divided into two principal approaches depending on the movement of either the sensor or the plant: sensor-to-plant system and plant-to-sensor system.
PHENOPSIS (Granier et al., 2006) was developed to assess the plant growth in *Arabidopsis thaliana* and follows the sensor-to-plant principle. Another representative of this group is the pepper plant imaging facility in Wageningen developed within the SPICY project (van der Heijden et al., 2012). Phenotyping systems representing the plant-to-sensor principle have been set up at Jülich Plant Phenotyping Centre (GROWSCREEN, Nagel et

al., 2012, Jansen et al,. 2009, Walter et al., 2007), at INRA Montpellier (Phenoscope, Tisné et al., 2013), and at the University of Ghent (WIWAM, Skirycz et al., 2011).

Only a few companies offer individual solutions of HT plant phenotyping systems, such as the LemnaTec Scanalyzer (LemnaTec AG, Aachen, www.lemnatec.de) or PlantScreen Conveyor systems (Qubit Phenomics, Kingston, Ontario, Canada, www.qubitphenomics.com). LemnaTec systems have for instance been installed at public research institutions in Adelaide (The Plant Accelerator as part of the Australian Plant Phenomics Facility, http://www.plantphenomics.org.au/), at INRA Dijon and Montpellier (PPHD and Phenoarch, http://bioweb.supagro.inra.fr/phenoarch/index.php/en/), and at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK; http://www.ipk-gatersleben.de/en).

Qubit Phenomics Trayscan systems are for instance operating at the High Resolution Plant Phenomics Centre Canberra (http://www.csiro.au/Outcomes/Food-and-Agriculture/HRPPC/PlantScan.aspx), the ARC Centre of Excellence in Plant Energy Biology, Acton, Australia, (http://www.plantenergy.uwa.edu.au/research/tech_platforms_main.shtml), and the C4 Rice Centre at the International Rice Research Institute in Los Baños, Laguna, Philippines (Junker et al., 2015).

All of these systems require several components:

- one or more sensors for raw data acquisition.
- a physical system for the integration of different sensors if needed and power supply.
- devises for plant or sensor positioning (depending on the type of platform).
- analytical capabilities for reference measurements.
- software systems to log sensor data and for managing and analysing potentially large and complex datasets.

Novel techniques are appearing in the course of phenotyping research within the frame of networks like DPPN (Deutsches Pflanzen Phänotypisierungsnetzwerk; http://www.dppn.de/dppn/DE/Home/home_node.html), EPPN (European Plant Phenotyping Network; http://www.plant-phenotyping-network.eu/) or IPPN (International Plant Phenotyping Network; http://www.plant-phenotyping.org/).

Field environment phenotyping systems

Field–based systems always rely on the sensor-to-plant principle. The required components of field-based phenotyping systems are manly the same as the ones used in controlled environments. Compared to controlled environments like greenhouses and growing chambers field-based systems need to be robust enough to cope with harsh environmental influences (dust, vibration and weather conditions). This affects the sensor, the physical equipment and the construction framework. Three kinds of device approaches to position the sensor in the field can be distinguished: (1) ground-based, (2) aerial-based, and (3) satellite-based systems.

Ground-based platforms include vehicles equipped with navigation GPS system device and sensors, they are often referred to as "phenomobiles" (Araus and Cairns, 2014). The vehicle the sensor system is attached to can either be a tractor (Andrade-Sanchez et al., 2013; Braun et al., 2010; Llorens et al., 2011), an agricultural harvester (Montes et al., 2007) or an independent vehicle (Berenstein et al., 2010; Calcante et al., 2012; Nuske et al., 2011).

Aerial-based platforms include small airplanes or helicopters, blimps (helium-filled balloons) (Losos et al., 2013), and unmanned aerial platforms (UAP) such as polycopter.

Further field-based systems include "phenotowers" (Rascher et al., 2011) or systems inspired by greenhouse applications like the LemnaTey Scanalyzer system (LemnaTec AG, Aachen, www.lemnatec.de) for field application.

An automated field phenotyping platform has been introduced for the application in cotton (*Gossypium barbadense* L.). The system carried four sets of sensors to measure canopy height, reflectance and temperature simultaneously on four adjacent rows, enabling the collection of phenotypic data at a rate of 0.84 ha h^{-1} (Andrade-Sanchez et al., 2013). A high-throughput phenotyping platform employing light curtains and spectral reflectance sensors mounted on a tractor and evaluating the performance of different maize (*Zea mays*) genotypes under field conditions was developed (Montes et al., 2011). Furthermore a semi-automatic system was developed to monitor micro-plots of wheat cultivars under field conditions. The system is based on a hyperspectral radiometer and two RGB cameras observing the canopy from ~1.5 m distance to the top of the canopy (Comar et al., 2012). Other applications have been introduced for the field phenotyping of maize

(Ruckelshausen et al., 2009) and small grain cereals (Busemeyer et al., 2013). A robot application for viticulture was suggested by Longo et al. 2011.

Table 2 Comparison of different sensors used in plant phenotyping.

Technique	sensor	data	parameters phenotyped	environment
visible light	cameras (visible spectrum: 380-780nm)	pixel- based grey or color value images (RGB channels)	- growth (dynamics, area, biomass, yield, height) - phenology (development stages: e.g. germination, flowering,) - whole plant, plant organs,: e.g. roots, leaves.	controlled, field
fluorescence	fluorescence cameras (and setup; 400-800nm)	pixel-based emitted fluorescence (blue, red, far-red)	- health status, fertilisation (N-supply, chlorophyll) - photosynthetic status, drought stress - secondary products (e.g. stilbens, anthocyanins)	controlled, field
thermal	infrared cameras (3.5-15μm)	pixel-based map of surface temperature (infrared)	- health status - water status	controlled, field
near infrared	near-infrared cameras, multi-spectral line scanning cameras, active thermography (0.78-3.0μm)	pixel-based continuous or discrete spectra (near-infrared)	- health status - water status - composition of secondary products	controlled, field
multi-/hyper-spectral	near-infrared instruments, spectrometers, multi / hyper spectral cameras, thermal cameras (different spectral channels)	pixel-based continuous or discrete spectra (various ranges)	- health status - canopy size - water status	controlled, field
3D	stereo camera system, time-of-flight cameras	depth maps	-plant architecture (e.g. shoot, leaf angle, canopy, root, height)	controlled, field
laser	laser scanning instruments (widely different ranges)	depth map, 3D point clouds	- canopy size - plant architecture	controlled, field
MRI	magnetic resonance imagers (gamma radiation < 5pm)	altering magnetic field(radio frequency range) water (^{1}H) mapping	- morphometric parameters (3D; 200-500μm) - water content	controlled
PET	Positron emission detectors for short-lived isotopes (e.g. $^{11}CO_2$)	positron emission signal	- transport portioning- biochemical and physiological functions (1-2 mm)	controlled
CT	X-ray computed tomography, (0.25nm-1 pm)	voxel based spectra	- morphometric parameters (3D; 100μm) - e.g. grain quality	controlled

Applications of sensor technology in viticulture

Due to the fact, that grapevine is a perennial plant and traits need to be screened under natural field conditions, most of the sensor-based methods developed and used in viticulture research are mainly field-based methods. Nevertheless it is much easier to develop sensor systems under controlled environmental conditions wherefore a set of such methods exists. Table 3 gives an overview over the sensor technology used in viticulture.

On the basis of RGB images, programs with user-interaction like the GRA.LE.D (Bodor et al., 2012) or SuperAmpelo (Soldavini et al., 2009) offer the opportunity to analyse simply leave characters (Bodor et al., 2012; Michels et al., 2013) or as well further ampelographic traits like cluster, berry and seed characteristics (Soldavini et al., 2009). Further image analysis tools are available to detect cluster characteristic like cluster compactness (Cubero et al., 2015), berry size (Tardaguila et al., 2012; Tardaguila et al., 2013; Wycislo et al., 2008) based on RGB images. To quantify the vitality and morphology of berries, fluorescence microscopy imaging and semi-automatical image analysis has been used to detect the shrivel index (berry area per berry perimeter) and tissue vitality of berries (Fuentes et al., 2010). A combination of VIS and NIR sensors (NIR spectrometer, GreenSeeker RT100 and Crop Circle) has been used to compute *Plasmopara viticola* infection on leaves collected in the field (Calcante et al., 2012). For the monitoring of grapevine ripening characteristics (berry colour, volume, uniformity, sugar and acidities) of single berries, a commercially available device (Dyostem, Seferis, Villeneuve les Maguelone Cedex, France) can be used in the laboratory.

An intermediate step between the very controlled environments indoors and the sensor applications under field conditions are hand-held devices. They work with artificial backgrounds fixed to the sensor to guarantee a consistent distance object-to-sensor and to eliminate the natural background making image analysis easier. These setups are used to detect grapevine inflorescences (Diago et al., 2014) or grape clusters (Rabatel and Guizard, 2007). The Multiplex (Force A, Paris, France) uses fluorescence to assess the grapevine health status (Lejealle et al., 2012), maturity (Agati et al., 2013) and physiological status (photosynthesis, photochemical reactions, secondary metabolites) (Cerovic et al., 1999).

The two main traits assessed by sensor-based field applications, predominantly developed and used for precision viticulture so far, are yield and canopy characteristics.

First step to predict yield is to detect grape clusters in the image and to be able to distinguish between leave and grape clusters either automatically (Font et al., 2014; Liu et al., 2013; Reis et al., 2012) or by user interaction (Dey et al., 2012; Diago et al., 2012; Dunn and Martin, 2004). Counting of grape berries using laser scanners also showed good results of 84% accuracy (Djuricic et al., 2014). To get better yield prediction not only the detection of grape pixels is important, but also the measuring of berry size (Roscher et al., 2014) or counting of detected berries is taking it a step further (Nuske et al., 2011). Recently they updated their method by using calibration data either from previous harvests or a small set of destructive handpicked samples (Nuske et al., 2014). Other studies used a combination of images with laser technologies (Grocholsky et al., 2011), terahertz time-domain spectroscopy (Federici et al., 2009) as well as remote sensing approaches (Cunha et al., 2010) to detect and predict yield.

Canopy performance, including canopy characterisation like vigour, size, density and shape are a key indicator of value in viticultural production and therefore one of the most sensor-based assed traits. In most approaches remote sensing is used to assess vine vigour, either based only on the multispectral satellite imagery (Johnson et al., 2003; Lamb, 2000; Llorens et al., 2011; Mazzetto et al., 2010; Strever, 2007) or in combination with VIS sensors (Fuentes et al., 2014) to detect the leafe area index (LAI). In some other studies a laser scanner approach is used to detect the canopy size and density (Grocholsky et al., 2011; Llorens et al., 2011).

Remote sensing is further used for the site-specific assessment of health status (Calcante et al., 2012; Mazzetto et al., 2010) whereas the gained information can be used for targeted spraying applications (Berenstein et al., 2010; Braun et al., 2010; Strever et al., 2012). Furthermore chlorophyll fluorescence imaging has been used to detect downy mildew infections (Cséfalvay et al., 2009).

As another important trait of vineyard management, water stress status has been determined by using thermal IR images (Fuentes et al., 2012a; Jones et al., 2009; Möller et al., 2007). Nevertheless one of the most expensive tasks in vineyard management is vine pruning, therefore different studies intend to develop methods of image processing (McFarlane et al., 1997; Ming and Tien-Fu, 2006) and artificial intelligence (Corbett-Davies et al., 2012) to automate this step. The detection of winter pruning wood as another important indicator of vine vigour has been carried out using a remote sensor approach (multi-spectral-radiometric) (Dobrowski et al., 2003) and a 2D laser scanner sensor (Tagarakis et al., 2013).

Table 3 Comparison of different sensors used in viticulture.

Technique	sensor	parameter	reference	environment
visible light	RGB camera	number		
		- inflorescences	(Diago et al., 2014)	controlled*
			(Grossetete et al., 2012)	controlled*
		- berries	(Font et al., 2014)	controlled
			(Rabatel and Guizard, 2007)	controlled*
			(Nuske et al., 2011 and 2014)	field
			(Grocholsky et al., 2011)	field
		size		
		- leaves	(Bodor et al., 2012)	controlled
			(Soldavini et al., 2009)	controlled
		- cluster, seed	(Soldavini et al., 2009)	controlled
		- berries	(Tardaguila et al., 2013)	controlled
			(Tardaguila et al., 2012)	controlled
			(Soldavini et al., 2009)	controlled
			(Wycislo et al., 2008)	controlled
			(Roscher et al., 2014)	field
		colour of berries, seeds (maturity, browning index)	(Rodríguez-Pulido et al., 2012)	controlled
		cluster compactness	(Cubero et al., 2015)	controlled
		amount of pixel (fruit/leaves)	(Diago et al., 2012)	field
			(Dunn and Martin, 2004)	field
		LAI	(Fuentes et al., 2014)	field
		health status		
		- leaves	(Li et al., 2012)	controlled
			(Meunkaewjinda et al., 2008)	controlled
			(Boso et al., 2004)	controlled
		- leaf disc	(Peressotti et al., 2011)	controlled
fluorescence	imaging	berry size	(Fuentes et al., 2010)	controlled
		tissue vitality	(Fuentes et al., 2010)	controlled
	non-imaging	health status	(Lejealle et al., 2012)	controlled*
			(Cséfalvay et al., 2009)	controlled*
		maturity	(Agati et al., 2013)	controlled*
		physiological status	(Cerovic et al., 1999)	controlled*
thermal	IR cameras	water stress	(Fuentes et al., 2012a)	field
			(Jones et al., 2009)	field
			(Möller et al., 2007)	field
spectral	spectrometer	NDVI	(Mazzetto et al., 2011)	field
	non-imaging		(Mazzetto et al., 2010)	field
		vine vigour	(Llorens et al., 2011)	field
			(Mazzetto et al., 2010)	field
			(Lamb, 2000)	field
			(Strever, 2007)	field
			(Johnson et al., 2003)	field
		pruning weight	(Dobrowski et al., 2003)	field
	terahertz spectroscopy	differentiate between cluster parameter	(Federici et al., 2009)	controlled
laser		number of berries	(Djuricic et al., 2014)	field
			(Grocholsky et al., 2011)	field
		pruning weight	(Tagarakis et al., 2013)	field
		canopy size	(Grocholsky et al., 2011)	field
3D	stereo camera system	canopy dimensions	(Klodt et al., 2015)	field

* hand-held devices

The technologies mentioned above represent either manually recording from a constant distance to the canopy (Diago et al., 2012; Diago et al., 2014; Fuentes et al., 2012a; Fuentes et al., 2014), mounted to a tractor (Braun et al., 2010; Llorens et al., 2011; Mazzetto et al., 2010), truck-crane (Möller et al., 2007) or include modified vehicles (Berenstein et al., 2010; Calcante et al., 2012; Nuske et al., 2011) equipped with global positioning systems (GPS) devices (Grocholsky et al., 2011; Mazzetto et al., 2011; Nuske et al., 2014). A robot application for viticulture was suggested by Longo et al. 2011. The U-Go robot was developed as a multipurpose vehicle with the aim to facilitate work during the season (harvesting, pruning, transportation of bins) (Longo et al., 2011). Its technical specification allows a remote control or autonomous motion using GPS waypoints (Longo et al., 2011). Automated analysis of the foliage distribution pattern in the canopy (Berenstein et al., 2010; Braun et al., 2010) are the foundation for selective spraying and spraying robots (Longo et al., 2012; Ogawa et al., 2006).

Nevertheless, the existing platforms with corresponding sensor technology operate mainly on a whole field level but miss out on the opportunity to assess phenotypes on a single vine level, but this is urgently needed for modern phenotyping applications in grapevine breeding.

The vineyard of the future (https://vineyardofthefuture.wordpress.com) on Waite campus in Adelaide, Australia, follows another approach. A one hectare vineyard which contains an advanced integrated vineyard monitoring and logging system to do online real-time measurements aims providing information about all vine responses at all times. The assessment of growth, plant health, water status and berry quality through a web-based system is using in-soil, in-vine and remote sensing technologies (Fuentes et al., 2012b).

Nonetheless, all of these studies focus mainly on vineyard management, site-specific information to improve crop load, water or health status of the considered plot. Adequate methods for single vine evaluation to be used within the breeding programmes are still demanding.

1.3 Yield parameters

Yield is a commonly measured but poorly understood trait. As an example of a perennial plant, that has to adapt to yearly variations the underlying genetics of yield traits

is complex. Yield has an extremely quantitative character, both because of the number of segregating loci controlling all of the traits involved in yield and of the influence of non-genetic factors like physiological and environmental factors (Conner et al., 1998; Fanizza et al., 2005; Garcia et al., 2000; King et al., 2000; Wang et al., 2000). A number of investigations have been conducted on the inheritance of yield and yield components in fruit tree species using classical biometrical approaches, and while these studies have been useful for making predictions on the genetic progress occurring in plant breeding programs, they have not provided information on individual genes influencing QTLs (Fanizza et al., 2005). Table 4 gives a summary of grapevine yield parameters and their underlying influencing factors.

Fruit size and shape are two major factors determining yield and quality. The fruit shape is more important in table grape breeding (Wycislo et al., 2008) as the shape of the berries and the uniformity of the whole cluster are important quality traits and have great influence on consumer acceptability. Therefore berry size is the most frequently assessed yield parameter in genetic studies. The underlying variation of used progenies mostly segregated for the seedlessness trait. However, seedlessness is negatively correlated with berry size in grapevine (Fanizza et al., 2005) since seed tissues provide important hormones for fruit development (Coombe, 1960; Pérez et al., 2000). Due to the fact that berry size and seedlessness have strong interactions it is difficult to get stable QTLs for berry size (Cabezas et al., 2006; Doligez et al., 2002; Fanizza et al., 2005; Mejia et al., 2007; Mejia et al., 2011). As in most other fleshy fruits the developmental stages of grape berries follow a double sigmoid curve, corresponding to the three development stages (stage I: berry growth due to cell division; stage II: slow berry growth; stage III: berry growth due to cell enlargement) (Coombe, 1960). Fernandez et al. 2006a showed that cell enlargements might explain different berry sizes between three cultivars. In some clones the number of cells was also affected (Fernandez et al., 2006a; Fernandez et al., 2006b). Furthermore the berry size is also influenced by factors such as the berry location within the cluster, the number of berries per cluster and the plants source and sink ratio (Dai et al., 2009; Ollat et al., 2002). Berry size has also been considered to influence wine composition and quality but it has also been concluded that the viticultural practices used to control yield in a vineyard may be more important than the yield or berry size values per se in determining the quality of the resulting grapes and wines (Matthews and Nuzzo, 2005). It has been shown that vineyard management can influence yield potential (Smart et al., 1990) by applying different methods like dormant pruning, shoot thinning before flowering and cluster

thinning at veraison for instance (Dunn et al., 2001). Higher shoot numbers per vine can decrease the number of clusters per shoot and the number of berries per cluster (Junquera et al., 2011).

Table 4 Yield parameters in grapevine and main influence factors.

Yield parameters	**influences**		
	genetic	**viticultural**	**environmental**
vines per unit area		- inter-row, - planting distance	
shoots per vine		- pruning level, - number of fruiting branches, - desuckering	
clusters per shoot	- variety		weather conditions during: - flower formation
berries per cluster	- variety		weather conditions during: - flower formation, - flowering
berry size/ berry weight	- variety		weather conditions during: - flowering, - period flowering-beginning of ripening, - period beginning of ripening-harvest
vine balance (generative/vegetative growth)	- variety - rootstock	- pruning during growth period - nutrition supply - health status	- soil

Another important parameter influenced by several of the yield parameters listed in Table 4, like number of berries per cluster and berry size, is cluster architecture. *Botrytis*, bunch rot of grapes, is an important disease of grape and grape cluster architecture may be an important variable in expressing the severity of bunch rot in the field (Vail and Marois, 1991).

Besides the aim of breeding to break down the genetics behind yield parameters, the seasonal variation in yield enhances the industries emphasis on forecasting and controlling their yield to achieve optimal yield and outcomes. Yield components measured for this purpose are:

- bunches/bud during dormancy (Jones et al., 2013; Wisdom et al., 2004)
- shoots and bunches per unit length of row approx. six weeks after budburst (Dunn and Martin, 2007)
- berries per bunch, bunches per unit length of row and bunch weight at the onset of veraison (Tardaguila and Martinez de Toda, 2007)

- weight/vine, bunches/vine, weight/bunch, berries/bunch, weight/berry at harvest (Dunn and Martin, 2004)

Modelling the yield forecast can also be done using the airborne pollen concentration (Besselat and Cour, 1990; Cunha et al., 2003).

Within the breeding program yield parameter and the following quality assessments of berries, must and wine can first be recorded four to five years after the cross. In addition the amount of yield available for experimental assessment is limited.

1.4 Objectives

The general goal of this thesis was to set up a phenotyping pipeline for high-throughput field phenotyping in modern grapevine breeding based on yield parameters. In particular the objectives were the:

1) usage of non-destructive visible light sensors (RGB camera, MC camera) as cost-efficient and fast sensors in different environments (laboratory and on a phenotyping platform).

2) determination of yield parameters that could be detected using RGB images and image analysis.

3) collection and validation of ground truth data (reference data) to assess the gained sensor data. Investigation of the possibility to record objective and precise phenotypic data of yield parameters by using RGB images and automated image analysis.

4) set up of an automated data acquisition in the field and the guarantee of an automated data handling of the sensor data in cooperation with an interdisciplinary team. Establishment of an opportunity to record phenotypic data on a single vine level for grapevine breeding purposes.

5) interpretation and evaluation of the gained phenotypic sensor data for grapevine breeding.

The aims of these developments are a precise and objective detection of parameter to increase the sample size and furthermore reduce the errors of assessment. These HT-phenotyping methods aim at providing reliable data that can moreover be analysed in retrospective to increase grapevine breeding efficiency.

1.5 Publications

The present cumulative doctoral thesis consists of four scientific articles[5]. Three of these articles have been published (publication II and IV) or submitted (publication III) in peer reviewed academic journals. Additionally publication I was published as reviewed conference proceeding. Publication II is reproduced with the corresponding permission of Vitis, Julius Kühn-Institut, Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany. The author's pre-print version of publication I and III is reproduced with the corresponding permission of the International Society for Horticultural Science, Leuven Belgium (I) and John Wiley & Sons Ltd.West, Sussex United Kingdom (III).

Publication I

Kicherer, A., Roscher, R., Herzog, K., Förstner, W. and Töpfer, R. 2015. Image based Evaluation for the Detection of Cluster Parameters in Grapevine. 11th International Conference on Grapevine Breeding and Genetics. Acta Horticulturae. 1082:335-340. DOI: 10.17660/ActaHortic.2015.1082.46 http://dx.doi.org/10.17660/ActaHortic.2015.1082.46

Publication II

Kicherer, A., Roscher, R., Herzog, K., Šimon, S., Förstner, W. and Töpfer, R. 2013. BAT (Berry Analysis Tool): A high-throughput image interpretation tool to acquire the number, diameter, and volume of grapevine berries. Vitis 52 (3):129-135.

[5] Each one of the following four chapters represents one article. The reference system and spelling of each journal, to which the article was submitted, is maintained.

Publication III

Kicherer, A., Klodt, M., Sharifzadeh, S., Cremers, D., Töpfer, R. and Herzog, K. 2015. Automatic image based determination of pruning weight as a determinant for yield potential in grapevine management and breeding. Australian Journal of Grape and Wine Research. In revision.

Publication IV

Kicherer, A., Herzog, K., Pflanz, M., Wieland, M., Rüger, P., Kecke, S., Kuhlmann, H. and Töpfer, R. 2015. An automated phenotyping pipeline for application in grapevine research. Sensors 15 (3):4823-4836.

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Image based Evaluation for the Detection of Cluster Parameters in Grapevine

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Keywords: automation, cluster morphology, cluster analysis tool, high-throughput phenotyping, image interpretation, Matlab

Abstract

Automated image interpretation is a powerful instrument for the acquisition of objective and precise phenotypic data with high throughput. Cluster length, cluster width, berry size and cluster compactness are four important phenotypic traits with impact on cluster morphology, health status and yield. For the image-based evaluation of this grapevine cluster morphology traits, the automated *Cluster Analysis Tool (CAT)* was developed in Matlab®. The comparison of precise reference measurements with *CAT* ratings on 100 cluster of 'Riesling' and 'Pinot Noir' showed a significant correlation of r=0.94 (0.97) for cluster width, r=0.90 (0.95) for cluster length and r=0.61 (0.23) for berry size. Variation of compactness could be detected in a crossing population calculating a compactness factor. To assess grapevine cluster morphology traits under laboratory conditions the automated image interpretation tool *CAT* presents a fast and user-friendly tool. The present study provides an improved and relevant phenotyping method for grapevine breeding. It could also be applied in genetic and ampelographic studies.

INTRODUCTION

Cluster and berry morphology are two key parameters which have an impact on (1) cluster health status (cluster architecture and compactness), (2) size characteristics and (3) yield. Traditionally the manual measurement of yield components are evaluated by visual estimations using defined descriptors such as OIV standards (Anonymous, 2009). For example berry width (OIV 221), cluster length (OIV 202) and cluster width (OIV 203). These OIV descriptors imply the classification into five predefined notes (1 – very small; 3 – small; 4 – medium; 7 – large; 9 – very large). A classification according to OIV descriptors is labour-intensive, requires trained people and the amount of samples and repetitions is restricted. In contrast, image based methods provide an automatic analysis of large sample sets, saving time and providing more objective information with the same or even increased accuracy. For viticulture, image analysis under laboratory conditions has so far mainly been applied for assessing the berry morphology. RGB images are used for characterisation of the number, size and volume of berries (Kicherer, et al., 2013) as well as berry weight (Tardaguila, et al., 2012), shape factors and compactness of the clusters (Wycislo, et al., 2008). The present study aims at the development of an easy image acquisition setup and an automated image interpretation tool in order to assess precise cluster morphology traits of grapevine under laboratory conditions.

MATERIALS AND METHODS

Plant Material

Grape clusters of *Vitis vinifera* ssp. *vinifera* cultivars 'Riesling' and 'Pinot Noir' were sampled in the experimental vineyard of Geilweilerhof at Siebeldingen, Germany (N

49°21.747, E 8°04.678). One hundred clusters per cultivar were harvested at developmental stage BBCH 89 (berries ripe for harvest (Lorenz, et al., 1995)) and were used for image acquisition.

In contrast to the established cultivars a F1-population (Gf.Ga-47-42 x Villard Blanc; 150 genotypes) shows large variability in berry size (OIV 221; notes 1-9) and grape cluster architecture. Therefore the population was used to detect the variability of cluster compactness using the CAT calculated values. Six clusters per genotype were harvested at BBCH 89, captured and analysed using the Cluster Analysis Tool **(**CAT).

Image Acquisition

A black box with a metal rod (4.1 mm) and a hook was used to capture a photograph of each cluster. An orange label (39 mm x 51 mm) was fixed at the rod next to the hanging cluster as a scale reference. One RGB image was captured per harvested cluster from the front by using a single-lens reflex camera (Canon® EOS 40D) mounted on a camera stand. A white background was used to capture the 'Pinot noir' grapes.

Reference Data

As reference, the cluster length, cluster width and size of 30 berries were manually measured by analysing the images of the intact cluster with the semi-automated Trait Size Tool (*TST)* (Herzog, et al., 2014).

Cluster Analysis Tool (CAT) Workflow

The image interpretation tool *CAT* which features a graphical user interface (GUI) was developed in Matlab® 7.5 (MathWorks, Ismaning, Germany). The workflow comprises image processing tools and machine learning algorithms for classification. The classification of the image aims at the assignment of each pixel to either the class 'cluster' or 'background'. The image interpretation process includes three steps.

1. Step One In order to determine cluster dimensions and the size parameters of single berries in mm, an orange label is used as a scale reference. It enables an automated calculation of the conversion ratio between mm and pixel. The label is detected automatically by *template matching* utilizing normalized cross correlation (Lewis, 1995).

2. Step Two All pixels of an image are classified into 'background' or 'cluster' using Active Contours (Chan and Vese, 2001), software can be downloaded from http://www.mathworks.com/matlabcentral/fileexchange/19567-active-contour-segmentation. In order to define an initial input mask for the *Active Contours* algorithm, the *Circular Hough Transform* (Peng, et al., 2007) was used for detection of single berries which are the most distinctive, round objects. Using the colour of the detected berries the contour of the berry cluster is found so that the inner part of the contour is at most similar to the colour of the detected berries.

3. Step Three Objects like the hook are often assigned to the 'cluster'. Thus, a *Morphological Opening* is used which removes thin and or small objects with a diameter less than 5 mm (Haralick, et al., 1987). Moreover a morphological reconstruction-based *Opening* and *Closing* is used in order to estimate the number of visible berries in the image. Assuming the flash light causes bright spots on the berries, each area of light pixels (blob) surrounded by dark pixels can be detected and counted as one berry. The local maxima of the blobs are used as the centroids of the berries. If some of the centroids are missed, the centres of berries detected by Circular Hough Transform (see step two) are also used as centroids. Duplicate centroids are removed by introducing a minimum distance of 5 mm between the centroids. Finally, the number of detected centroids is used for

estimation of the number of visible berries in the image. Dimensions of the berry clusters are derived from the determination of a bounding box around the classified cluster, which is parallel to the image axis. In addition to the diameter of detected berries obtained from the circle detector (see step two), the area of the classified cluster in the image is also deduced from the image using the obtained classification from step two.

Finally, a summary of *CAT* results from all investigated images is given as a text file including the length and width of the bounding box (= length and width of the cluster in mm), the cluster area (mm^2) and the berry size (mm).

Cluster Compactness

The cluster compactness was evaluated using the CAT calculated values: (1) area of the bounding box (cluster length x cluster width) and (2) cluster area. Compactness is defined as 'bounding box area/cluster area'. The compactness ratio was afterwards classified into five notes: (1) very loose cluster, compactness factor ≥ 1.91; (2) loos, compactness factor 1.91> x ≥1.81, (3) medium, compactness factor 1.81> x ≥1.71, (4) dense, compactness factor 1.71> x ≥1.61 (5) very dense, compactness factor < 1.61. To validate the digital cluster compactness evaluation the clusters were also rated using the OIV 204 (bunch dense; notes 1-9) as a reference.

Statistical Analysis

Statistical analysis was performed using SAS 4.3 (SAS Institute, Cary, NC, USA). The Pearson correlation coefficient was used for data evaluation.

RESULTS AND DISCUSSION

The *Cluster Analysis Tool (CAT)* was developed for image-based phenotyping of cluster morphology. The RGB images from 100 'Riesling' and 'Pinot Noir' clusters were automatically analysed applying *CAT*. Thus, the cluster dimension and berry size could be extracted. The *CAT*-based data was compared with reference data which was acquired with the *Trait Size Tool* (TST) from the same pictures used for the CAT analyse. The comparison of the cluster length revealed a significant correlation of r=0.90 for 'Riesling' (Fig.1 A) and r=0.95 for 'Pinot Noir' (Fig.1 D). Cluster width showed a significant correlation of r=0.94 ('Riesling'; Fig.1 B) and r=0.97('Pinot Noir'; Fig.1 E). Due to practical reasons of the cluster attachment in an upright position, the secondary cluster was also considered in the *CAT* compared to the OIV descriptors for cluster width (OIV 202) and cluster length (OIV 203). It thus proved important to attach the cluster straight in the image because the bounding box is set parallel to the image axis to acquire the cluster dimensions with the *CAT*. The automatic determination of the conversion ratio makes the system very flexible since it is independent of image format, image resolution or the distance between camera and object. Moreover, the system can be easily handled by other users since single parts, e.g. the template, are interchangeable.

For validation of the *CAT*-calculated values of the berry size (diameter of detected berries), 30 berries per image where measured using the *TST* as reference data. Both, the *CAT* and the *TST* only consider visible berries in an image and miss out on the hidden ones. Comparison of *TST* measured berry sizes with the *CAT*-calculated values showed a significant correlation of r=0.61 ('Riesling'; Fig.1 C) and r=0.23('Pinot Noir'; Fig.1 F). A reason for the rather low correlation of 'Pinot noir' berry size might be the very inhomogeneous berry size of the clone used (see picture in Fig.1). It could not be guaranteed that the automatically selected and measured berries of the CAT are equal to the ones measured by a person during the reference measurements using the interactive tool *TST*. In contrast the 'Riesling' berry size was more homogenous and therefore shows a better correlation. Top priority of the CAT was the validation of the cluster size.

Nevertheless, the berry size is a useful additional reference parameter. As it is difficult to detect the berry sizes in inhomogeneous clusters we recommend to destem such clusters and use programs like the Berry Analysing Tool (*BAT)* (Kicherer, et al., 2013) to obtain more reliable data of the berry size.

Using the *CAT*-detected area of the bounding box and the visible area of the cluster, a ratio describing the compactness of the cluster was computed and used for the evaluation of a F1-population. The classification into five notes showed a significant correlation of r=0.55 compared to the OIV classification (OIV 204). Using the OIV notes the F1-population was only determined as notes 3, 5 and 7 (Frequency 22, 58 and 41 genotypes). Appling the CAT based classification a higher variability could be achieved (Fig.2). This is a benefit when thinking about using these data for QTL analysis.

CONCLUSION

Cluster morphology is one of the most important traits influencing the health status of the grapes and the yield itself. The present study shows that an automated image interpretation tool like *CAT* provides a fast and user-friendly tool to assess cluster morphology traits of grapevine under laboratory conditions.

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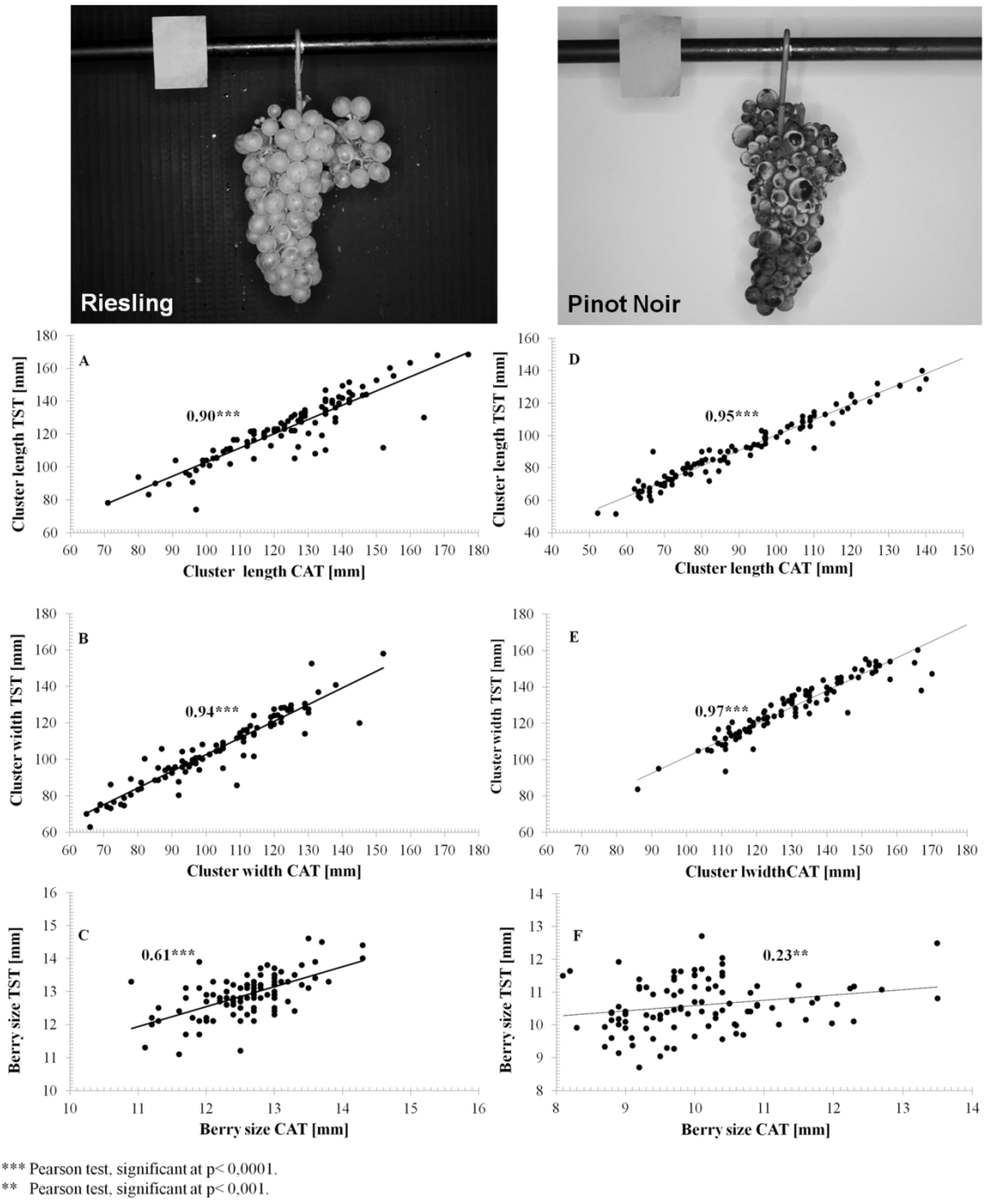
Figures



*** Pearson test, significant at p< 0,0001.
** Pearson test, significant at p< 0,001.

Fig. 1 Correlation plots of fast automatic (*Cluster Analysing Tool - CAT*) and laborious semi-automatic (*Trait Size Tool - TST*) determined cluster data from 100 'Riesling' (A, B, C) and 100 'Pinot Noir' (D, E, F) images. A, D: Significant correlation of cluster length (r=0.90; r=0.95). B, E: Significant correlation of cluster width (r=0.94; r=0.97). C, F: Significant correlation of berry size (r=0.61; r=0.23).

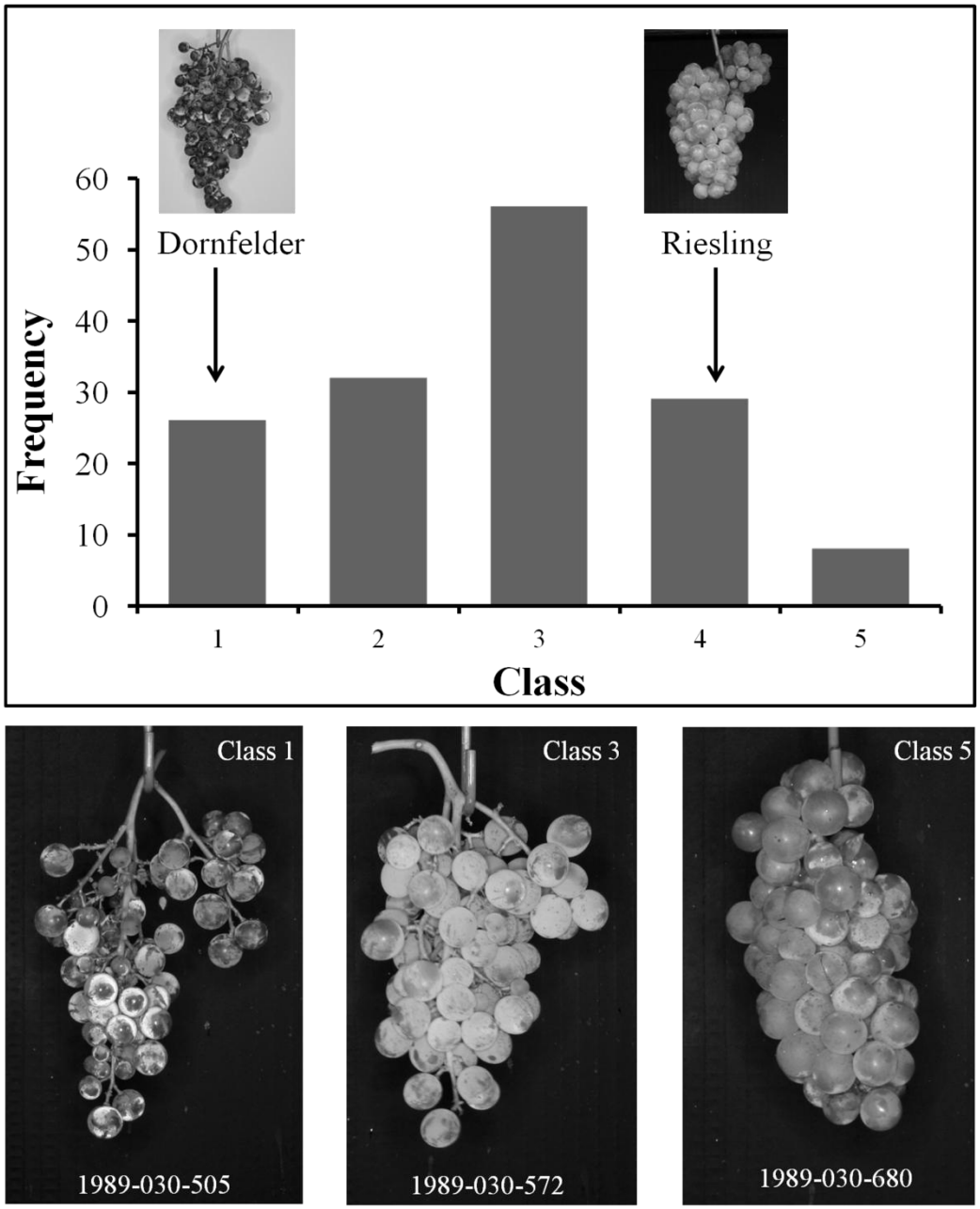


Fig. 2. Frequency distribution of the compactness classes (1: very loose cluster, compactness factor ≥ 1.91; 2: loose, compactness factor 1.91> x ≥1.81, 3: medium, compactness factor 1.81> x ≥1.71, 4: dense, compactness factor 1.71> x ≥1.61 5: very dense, compactness factor < 1.61) in a crossing population. The compactness factor is calculated as the ratio of the CAT calculated values area of the bounding box (cluster length x cluster width) to the cluster area. Data is based on the mean of 6 images per genotype of 150 individuals. Arrows indicate two representative cultivars (‘Riesling’, ‘Dornfelder’) for the respective class.

Vitis **52** (3), 129–135 (2013)

BAT (Berry Analysis Tool): A high-throughput image interpretation tool to acquire the number, diameter, and volume of grapevine berries

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Summary

QTL-analysis (quantitative trait loci) and marker development rely on efficient phenotyping techniques. Objectivity and precision of a phenotypic data evalua- tion is crucial but time consuming. In the present study a high-throughput image interpretation tool was devel- oped to acquire automatically number, size, and volume of grape berries from RGB (red-green-blue) images. Individual berries of one cluster were placed on a black construction (300 x 300 mm) to take a RGB image from the top. The image interpretation of one dataset with an arbitrary number of images runs automatically us- ing the BAT (Berry-Analysis-Tool) developed in MATLAB. For validation of results, the number of berries was counted and their size was measured using a digital calliper. A measuring cylinder was used to determine reliably the berry volume by displacement of water. All placed berries could be counted by BAT 100 % correctly. Manual ratings compared with BAT ratings showed strong correlation of r = 0.96 for mean berry diameter/ image and r = 0.98 for cluster volume.

K e y w o r d s : HT-phenotyping, image interpretation, grapevine berry size, berry morphology.

Introduction

The combination of high wine quality and longterm resistance against various fungal pathogens combined with good climatic adaptation reflects the major objectives in grapevine breeding (TÖPFER *et al.* 2011). Many traits of grapevine can only be evaluated in the vineyard being highly influenced by environmental factors and thus requiring several repetitions. Particularly for berry related traits it is cumbersome to separate genetic and environ- mental interactions due to the non-controlled environment. Yield is the most commonly measured trait in viticulture (FANIZZA *et al.* 2005). It belongs to the most complex traits in grapevine breeding besides berry and wine quality and is influenced by numerous genetic loci (FANIZZA *et al.* 2005) and non-genetic factors.

Marker-assisted selection (MAS) in grapevine breeding has become a very valuable tool for early monitoring genetic loci for resistance in breeding material and is nowadays used routinely to screen seedlings in order to pyramide resistances (SCHWANDER *et al.* 2012, EIBACH *et al.* 2007, FISCHER *et al.* 2004). Besides identifying the most appropriate genotype, phenotyping of plant material is widely known as the very labour-intensive and time consuming part of this process. The variation in yield per vine is explained by the number of clusters per vine (60 %), the number of berries per cluster (30 %) and the berry size (10 %) (NUSKE *et al.* 2011). Berry size is considered one of the most important characters concerning yield for both wine grape and table grape breeding. For quality reasons in wine grape breeding small to medium sized berries (width
13-18 mm) are desired. For table grape cultivars grape productivity plays an important role in the table grape market, as seedlessness is especially demanded but negatively correlated to fruit size (DOLIGEZ *et al.* 2002, FANIZZA *et al.*
2005). Currently, phenotyping of berry length and width is done according to OIV descriptors (OIV 220 and 221) at maturity on 30 berries. Other traits like the cluster form and cluster size (OIV 208 and 222) are rather vague and subjective for a proper scientific analysis and QTL detection. Using OIV descriptors, it is difficult to detect slight differences in fruit size, it is time-consuming, and expensive. Fine mapping of known QTL regions requires precise phenotypic data of berry features using a large number of fruits of a mapping population. The utilisation of manual measurements of fruit size is non-practical. Digital analysis promises a much faster, precise and less time-consuming technique to receive phenotypic data.

To achieve large quantities of phenotypical data high-throughput phenotyping has recently been introduced to plant research. Therefore, computer vision has been used for fruit recognition. Focus was laid on grading, defect detection, classification and state of ripeness detection based on the appearance in the post harvest process (KODAGALI and BALAJI 2012). Various sorting and grading tools for fruits and vegetables have been developed e.g. for apple (LEEMANS *et al.* 2002, THROOP *et al.* 2005, LI *et al.* 2002), date (AL OHALI 2011), peaches (ESEHAGHBEYGI *et al.* 2010), watermelon (SADRNIA *et al.* 2007), banana (WANG *et al.*
2009), sweet cherry (BEYER *et al.* 2002), tomato (BREWER *et al.* 2006, MORIMOTO *et al.* 2000) and oranges (FELLEGARI and NAVID 2011, BAMA *et al.* 2011). The methods used are based on colour, size and defect features which play an important role in the production of this fruits and vegetables. WYCISLO *et al.* (2008) analyzed digital images using Sig-

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maScan® to characterise fruit shapes of table grapes. The major:minor ration, shape factors and the compactness value was detected out of RGB images. The commercially available maturity analysis system by Vivelys (DYOSTE 2010) measures berry colour, volume and uniformity by a sensor and in addition it analysis e.g. sugar load and acidities.

In order to improve precision and efficiency of phenotyping methods in grapevine breeding, the present study aims at developing an automated image interpretation tool to acquire berry morphology traits, especially the number of berries per cluster and the mean berry diameter. Supplementary determined values of the berry diameter will be used to calculate single berry volume.

Material and Methods

Plant material: Grape clusters were sampled in the vineyard of Geilweilerhof located in Siebeldingen and used for image acquisition. 100 clusters from the *Vitis vinifera* subspec. *vinifera* cultivars 'Riesling' and 'Müller- Thurgau' at BBCH 79 (phenological development stage scale; Majority of berries touching) (MEIER 2001) were used to validate the method regarding to berry number and sizes determination using BAT. 1,500 clusters from 130 genotypes of a F1 mapping population ('Gf.Ga-47-42' x 'Villard Blanc') were used to verify the berry volume calculated using BAT. All genotypes were harvested at BBCH 89 (berries ripe for harvest) at 70 °Oechsle. In contrast to the established cultivars 'Riesling' and 'Müller-Thurgau' the genotypes of the F1-population showed large variability in berry shape (OIV 223; notes 1-4), berry sizes (OIV 221; notes 1-5) as well as in grape cluster architecture.

Image acquisition: A black perforated met- al plate with a size of 300 x 300 mm (14 x 14 evenly arranged holes, 10 mm diameter) was placed on a black tray of equal size with bolts positioned in all four edges giving the construction an entirely black colour. The perforation causes a considerable proportion of the berries to be separated without the need to exactly place each berry in one hole what would be too time-consuming. This construction was placed on a red background to permit an automatic identification of the construction boarders in order to de- rive the berry sizes in mm rather than in pixel. All berries of one cluster were removed from the rachis and placed on the black construction. RGB images were taken from the top using a single-lens reflex camera (Canon® EOS 60D) fixed to a camera stand (Fig. 1).

The number of berries per cluster (image) was counted and the diameter of berries (as described in OIV 221 (OIV 2009)) was measured using a digital calliper (Insize

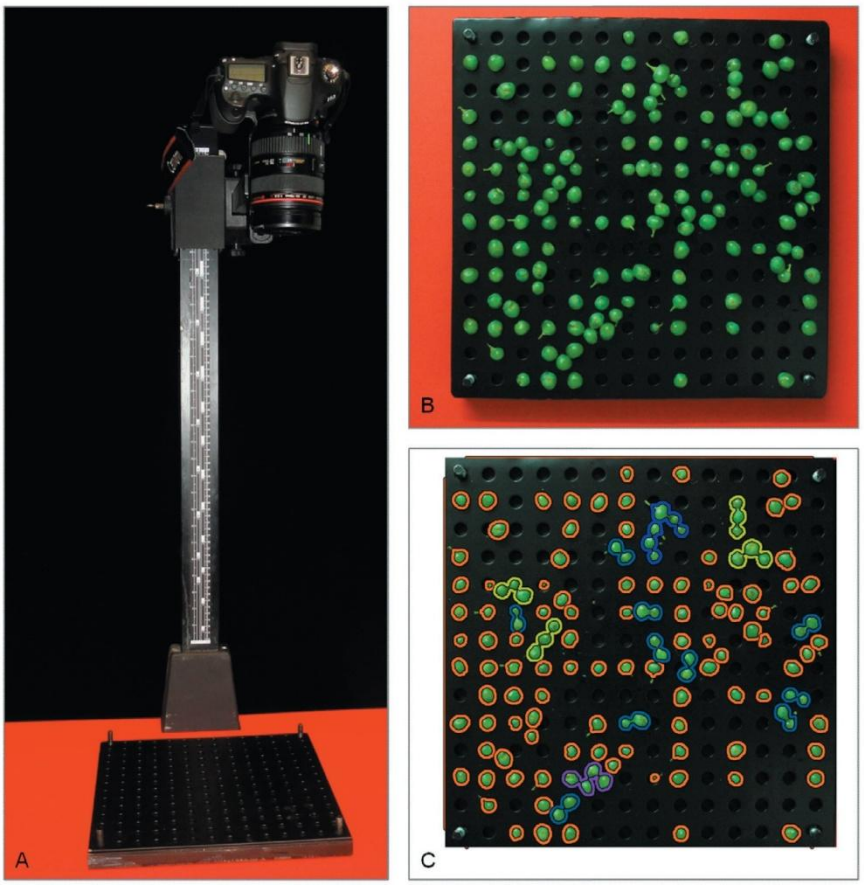

Fig. 1: Image acquisition, detection and quantification of grapevine berries using BAT algorithm. **A**: Image acquisition setup: Camera stand with a DSLR camera, black perforated metal plate (300 x 300 mm), black tray of equal size with bolts positioned in all four edges and the red background **B**: Original image of the perforated construction with berries **C**: Object Extraction: Image generated by MATLAB® with the detected berries (the number of contained berries in a region is colour-coded in order to distinguish the number of berries per region).

Co. Ltd.; DIGITAL CALIPER 300 mm; China). The measurements were taken as references to validate the BAT. Measurement accuracy was 1 mm. Due to measurement accuracy and practical reasons berry volume of one cluster (only berries) was raised instead of single berry volumes. Therefore a glass measuring cylinder, size of 1000 mL (10 mL scale steps) was used to record the water displacement.

Data analysis: Data sets of manually and software based values were analysed by Pearson correlation and ANOVA (Tukey Test). Statistical analyses were per- formed using SAS 4.3 (SAS Institute, Cary, NC, USA).

BAT (Berry analysis tool) workflow: The development of the image interpretation tool BAT was done using MATLAB® 7.5 (MathWorks, Ismaning, Germany). The image interpretation system comprised image processing tools and machine learning algorithms for classification. A RGB image I is given, in which each pixel has an unknown label y , which is either "berry", "background" (black construction) or "red background".

The image interpretation algorithm includes six steps starting with the detection of the construction boundary up to calculation of berry volume.

Step 1: Detection of the constructi onboundary and the elimination of th e redbackground: The images were converted to the HSV (hue-saturation-value) colour space and the hue and saturation channel are summed yielding a one-dimensional image with a bright background and a dark metal plate (Fig. 2A). This procedure is more robust towards varying illumination effects within one image and between different recordings of the images than e.g. thresholding the RGB image. Each image, represented as matrix, is summed over all rows and second over all columns getting two onedimensional curves with high peaks. Since the background appears bright in the image and the metal plate dark, as can be seen in Fig. 2A, all background pixels sum to a high value and the foreground pixel to a small value. In order to deter- mine the transition between the background and the metal plate, the gradients of the curves are computed, which are afterwards squared and smoothed. The obtained curve for image rows is shown in Fig. 2B and for image columns in Fig. 2C.

The red background of the image was removed by cropping the image. In order to obtain berry diameter in mm, the detected 'background' is used to get the Conversion Ratio c between mm and pixel. The construction has a defined length I and width w of 300 x 300 mm and thus the ratio c is given by

$$c = \frac{300}{\frac{1}{2}(w + l)}$$

The automatic determination of the Conversion Ratio makes the system flexible, since it is independent of image format, image resolution or the distance between camera and the construction.

Step 2: Classification of "berry" and"back ground": Active Learning (SETTLES 2010) was applied in order to classify the whole image into the classes "berry" and "background" (black construction). The used Active Learning strategy approaches an automatic collection of a few, representative feature vectors for both classes. Using such training data a classification model is learned using Logistic Regression (BISHOP 2006) which facilitate the assignment of each pixel to the class "berry" or "background". The training data for the class "background" was acquired from the four edges of the black construction, in which the bolts are positioned. The Circular Hough Trans- form (PENG *et al.* 2007) was used to acquire training data for the class "berry" by detecting distinctive, round objects.

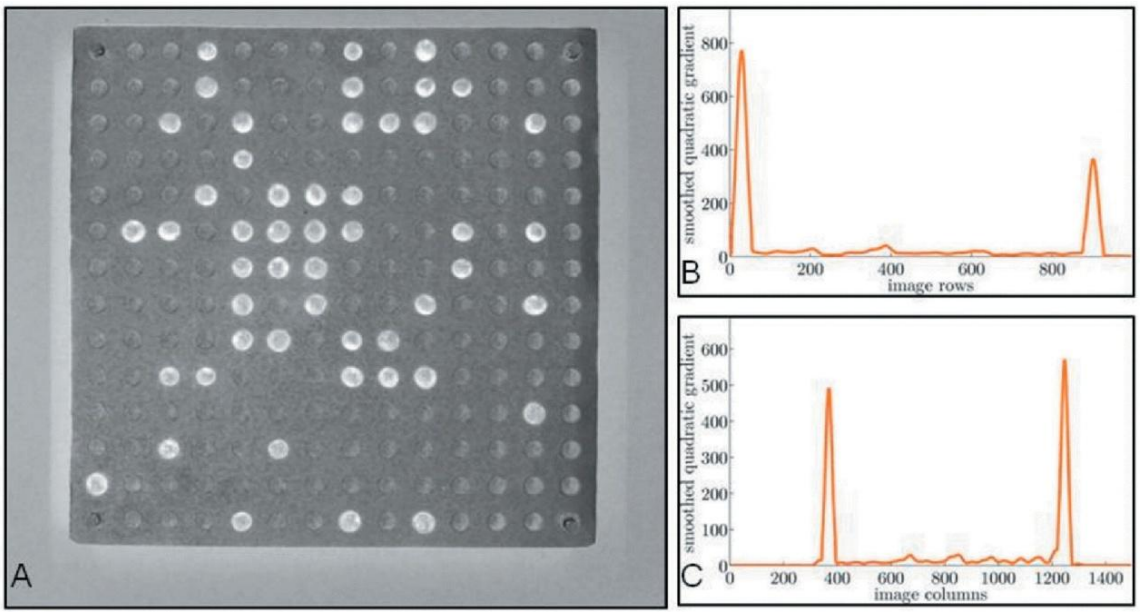

Fig. 2: Sum of the hue and saturation channel (**A**) and the obtained curves of the squared smoothed gradients-image rows (**B**) and image columns (**C**). The two largest values (Peaks) in each curve indicate the border of the metal plate. The difference between the largest values is the length l and the width w of the construction given in pixel.

The advantage of Active Learning is the adaption of the model to changing image conditions, like different position or colour of the construction, different sizes or positions or colours of berries.

Step 3: Morphological Operator tore move noise: The usage of opening, which is a morphological operator (HARALICK *et al*. 1987) allows for the removing of noise such as small parts of the rachis in the classification results. Using a disk-shaped structuring element of size $\frac{3}{c}$, regions with a radius less than three mm are removed. As we are looking at berries of BBCH 79 or higher, objects which are smaller than three mm are assumed to be foreign objects like parts of the rachis, in- sects or berry parts. This value can be reduced, however, with the need of a good image quality and the removal of all impurities on the metal plate. Neighboured pixel of the same class are grouped into one region $\mathcal{R}_s$, 1,...s,...,S where S is the total number of regions and s is the index of the considered region. One region that is classified as "berry" corresponds ideally one berry in the image. In this case the total number of berries equals the total number of regions *S*. However, if the berries touch each other, one region can comprise more berries which have to be separated (step 4, Fig. 3).

Step 4: Estimation of berry numbers: In the fourth step the total number of berries *B* is obtained by summing over the estimated number of berries in each region. For the determination of *B* in each region, Erosion is used as another Morphological Operator. Each region $\mathcal{R}_s$ is successively eroded step-by-step with a disk-shaped structuring element of increasing size in order to separate connected subregions (Fig. 3B-D). The size of a structuring element is increased as long as the number of subregions in $\mathcal{R}_s$ does not decrease. The maximum number of subregions equals the number of berries in $\mathcal{R}_s$ (Fig. 3D). All regions which number of subregions equals one are summarized in the set $\mathcal{R}$'.

Step 5: Estimation of single berry diameter: In the fifth step the diameters are estimated from each region in $\mathcal{R}$'. In this system the berry diameter is defined as the minor axis length of an ellipse fitted through all pixels in the region $\mathcal{R}$'. Furthermore, the mean diameter μ_d and the standard deviation σ_d is obtained.

Step 6: Calculation of single berry volume:For the determination of the volume of the berries they are supposed to be ellipsoids. Due to the two-dimensional data basis, two possibilities are considered for the experiments to calculate the volume of individual berries: either the berry shape is supposed to be (i) elliptical with the minor axis supposed to be equal. Therefore the following equation could be used for volume calculations where a1, a2, a3 are the three semi-axis of an ellipsoid with the two minor axis a2 = a3. Or (ii) the berries are round, meaning that the three semi-axis of the ellipsoid are equal (a1 = a2 = a3).

$$\frac{4}{3} \times \pi \times a1 \times a2 \times a3$$

Results and Discussion

The high-throughput image interpretation tool BAT was developed for automated image-based recording of the total number of berries per grape cluster, diameter of ber ries in mm and volume of individual berries in mL.

Validation of detected berry numbersand diameter using automated BAT: Development stage BBCH 79 was chosen because berries have their typical shape but compared to BBCH 89 are not too soft to get inaccurate manual measurements due to the softness.100 RGB images were captured (one image per cluster) from 'Riesling' and 'Müller-Thurgau'. All images were analysed using BAT to verify detected berry number and diameter with reference evaluations. The number of all berries was detected 100 percent correct in each image. On 2,500 berries the BAT-based measured berry diameter was compared to manually determined sizes (Fig. 4). The comparison revealed strong positive correlation at r = 0.96. Compared to the OIV method for the detection of berry width (OIV 221) not only 30 berries were measured, but the variation within the whole cluster was captured. Using OIV descriptors, it is difficult to detect slight differences in fruit size, e.g. OIV 221 (berry width) classifies 'Riesling' and 'Müller-Thurgau' as note 3. BAT recorded significant differences between 'Riesling' and 'Müller-Thurgau'. The BAT measured berry diameter showed an average 0.3 mm overestimation compared to the manual measurements. The minimal overestimation is caused either by the manual measurement accuracy of 1 mm or due to inaccuracies during the image interpretation process. The results show that the estimated diameter of individual berries depends on its posture on the black construction due to the fact that berries

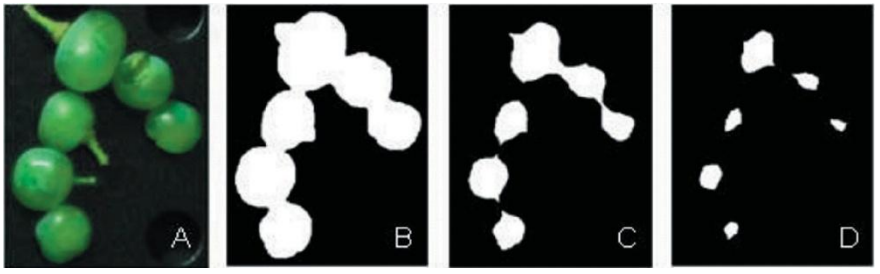

Fig. 3: Separation of single berries which are touched: Successive erosion with a disk-shaped structuring element of increasing size. **A**: Image detail of size 338 x 261 pixel of a detected region containing 6 berries. **B**: Classification result of the image detail after the opening. **C**: Erosion structuring element of size 15 pixel. **D**: Erosion structuring element of size 25 pixel, whereas all subregions $\mathcal{R}_s$ can be detected.

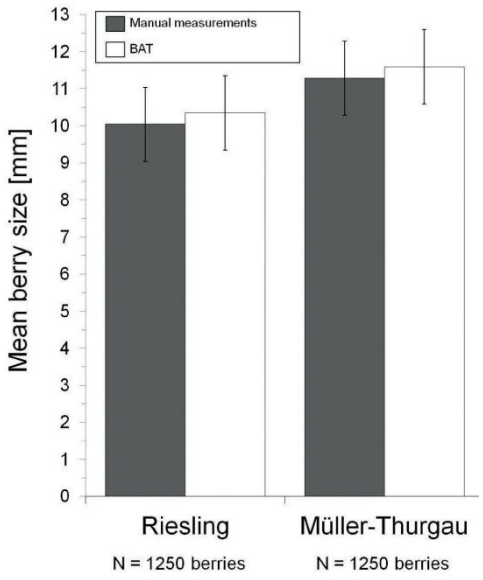

Fig. 4: Comparison of berry size (berry width) determined by manual measurements and by BAT. Error bars represent the standard deviation. An overestimation of 0.3 mm was observed. Difference of mean berry width between 'Riesling' and 'Müller- Thurgau' was 1.25 mm.

are not really symmetric like circles. In fact, a berry is defined by three axis (a1, a2, a3) like an ellipsoid. Therefore, it cannot be guaranteed that the minor axis length a1 can be determined accurately for all berries. Instead, a diameter in the range [a1, a2] is obtained, in which a1 < a2 < a3. Thus, it must be noted that in practice there will be always an overestimation which extent depends on the roundness and symmetry of the berries as long as the minor axis is the entity which is meant to be measured. Nevertheless the extent of the overestimation generally is very small.

Calculation of berry volume from image: 1,500 grape clusters were destemmed and photo- graphed. To validate the BAT-calculated values of berry volume the single berry volume of one image (one image represents one cluster) was summed up and compared to the cluster volume measured manually.

The berry shape can be assumed to be round or ellipsoid. Based on the statement that berry shape is ellipsoid the calculations of BAT showed an average 2 mL overestimation (1.7 %) of the cluster volume compared to the manual measurements. In comparison to that assuming the berry shape as round showed an average 7 mL underestimation (5.5 %) of the cluster volume. Both BAT calculations (round and ellipsoid shape volume) showed no significant difference compared with the manual measurements of the cluster volume. However, the BAT calculation of 'round shape' volume showed significant differences in comparison with the BAT calculation of 'ellipsoid shape' volume. Most of the analysed genotypes of the mapping population
'Gf.Ga.47-42' x 'Villard Blanc' possess a slightly ellipsoid berry shape which may cause a bigger calculation error assuming the berries are round. Furthermore as mentioned before the berry diameter is slightly overestimated and as we are using that value to calculate the volume it is not surprising that the ellipsoid volume is also slightly over- estimated. The 2 mL variance in calculation of ellipsoid volume could be explained due to manual measurement uncertainties which are maybe caused by using a measuring cylinder with an accuracy of only 10 mL. Comparison of manually measured volume data with BAT-calculated values (ellipsoid berry shape) showed strong positive correlation of 0.98 (Fig. 5). Assuming round berry shape the correlation between manually measured data and BAT- calculated values also showed strong positive correlation of 0.98 (data not shown).

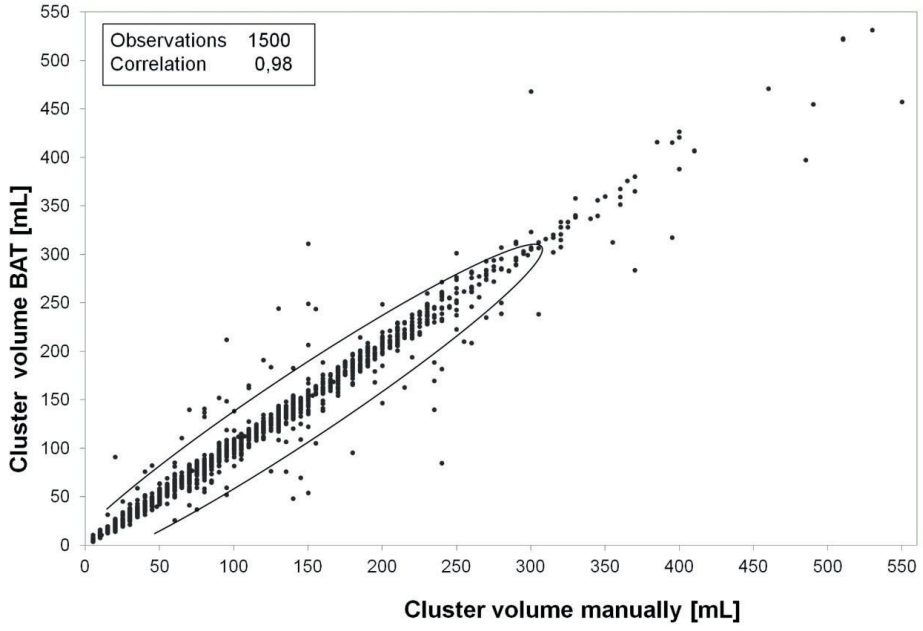

Fig. 5: Showing a correlation plot of the manually measured and BAT-calculated ellipsoid cluster volume (r = 0.98) from 130 F1 plants of the mapping population 'Gf.Ga 47-42' x 'Villard Blanc'. Each point represents the volume of one cluster/image. The density ellipse encompasses 95 % of the data points.

Altogether the obtained BAT-results showed strong correlations and only a minimal divergence to the manually received data. BAT is a fast image interpretation tool to acquire large and precise fruit trait data with high-through- put. BAT enables an automated and precise acquisition of three important phenotypic traits: the berry number, berry diameter and berry volume. In this study only green berries were used. To detect dark berries the construction needs to be painted white because the colour contrast of the berries and the metal plate as well as the metal plate and the background needs to be strong. Otherwise the circle detection step fails to proper identify representative berries or the boundary detection step fails resulting in an incorrect conversion ratio. In comparison to the study of WYCISLO *et al.* (2008) describing the detection of fruit shape based on 10 berries and giving results in pixel, BAT permits the investigation of an unlimited number of berries resulting in the detection of the whole variation. Another crucial ad- vantage of BAT is the export of berry diameter and berry volume as numbers with dimensional units (mm or ml in- stead of pixels). No machine like the Dyostem is needed. Since the automatic determination of the conversion ratio from pixel to mm the system is flexible regarding the used camera, image ratio and resolution and the distance between camera and construction. There is no need to cut the berry half before imaging as it is suggested in the current protocol of the "Tomato Analyzer" (BREWER *et al.* 2006).

In contrast to manual measurements, the image interpretation algorithm needs a much shorter period of time with equal accuracy. For example, the manual recording of 300 berries of one 'Riesling' cluster including counting of berries, measuring of one diameter per berry using digital calliper and determination of berry volume takes about 30 min. The application of BAT starts immediately after importing an image folder, in which all images of the folder were analysed automatically. The computation time depends on the specification of the used computer. It re- quires about one minute for analysing one image tested on a 64 bit system on common PC hardware (2 x 2.66GHz Intel Core2 Dou). Cluster volume could be detected by dip- ping the whole cluster (berries still on rachis) into a measuring cylinder obtaining the volume as water displacement. Nevertheless, berry number per cluster and berry diameter could not be detected in that step. Therefore, BAT offers the major advantage. It makes it possible to analyse multiple objects in one image at the same time.

Conclusion

The breeding of new grapevine varieties with regard to the yield depends on acquisition of different yield parameter. The number and size of berries per cluster are one of the most important parameters influencing the yield of grapevine. The present study shows that the fully-automatic interpretation of images by the utilisation of BAT is a fast, user-friendly and cheap procedure to supply precise phenotypic features of berries with dimensional units. It requires the sampling of berries, destemming and position- ing on a coloured construction in the lab and the taking of only one image from the top. Those phenotypic data are the basis for further investigations, e.g. QTL analyses or yield estimation. However, field-based phenotyping methods will be necessary to acquire further yield parameter (e.g. grape cluster per grapevine) in a non-destructive manner.

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Automatic image based determination of pruning weight as a determinant for yield potential in grapevine management and breeding

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Abstract

Background and Aims: Monitoring vines for yield potential and vine balance is an important tool not only in vineyard management but also in grapevine breeding. Vine balance is defined as a relation between vegetative and generative growth. It can be expressed as relation between grape yield and the weight of dormant pruning wood. In contrast to vineyard management for grapevine breeding emphasis is usually laid on the evaluation of individual seedlings instead of screening whole vineyards with the same cultivar. In this study we calculated the weight of dormant pruning wood by the assistance of an automated image-based method for estimating the area of dormant pruning wood. The evaluation of digital images in combination with depth map calculation and image segmentation is a new and non-invasive tool for objective data acquisition.

Methods and Results: The proposed method was tested on a set of seedlings planted at JKI, Institute for Grapevine Breeding Geilweilerhof, Germany. All images taken in the field were geo-referenced and manual segmentation was used to validate the automated method. Classification of the seedlings was done using yield parameters, vine balance indices and ripening values. Finally, 13 out of 138 investigated genotypes could be identified which fulfil the requirements concerning yield features and balanced vines from a breeding point of view.

Conclusion: The pruning area obtained using image based methods is an accurate, inexpensive and easy method to estimate pruning weight compared with the manual time-consuming measurements. Together with the yield parameters it is a suitable method for seedling evaluation and can also be used in precision viticulture.

Significance of the Study: This study demonstrates an image based evaluation of the pruning weight to be a highly valuable tool for grapevine research and grapevine breeding. Moreover, the tool might be used by industry to monitor vine balance. The key findings

reported have the potential to increase grapevine breeding efficiency by using an accurate and objective phenotyping method.

Key words: automated image segmentation, dense depth maps, grapevine breeding, pruning area, vine balance, *Vitis vinifera*

Introduction

Major objectives in wine grape breeding are superior wine quality at an optimal yield combined with high disease resistance, and a good climatic adaption. A balanced and stable yield with a preferably medium to late maturity belongs to important prerequisites for producing high wine quality in the European northern temperate grape growing areas (Töpfer et al. 2011). In terms of wine making yield and quality are negatively correlated. To assess the traits mentioned within grapevine breeding programs, adequate concepts are demanding in particular for single vine evaluation. Concepts which grapevine breeders use to evaluate yield in a seedling selection are borrowed from experiments of vineyard management practice with existing varieties. Yield components evaluated in this kind of studies are (1) yield per vine (kg) or more detailed (2) the number of clusters per vine plus the berries per cluster and berry weight (g) (Clingeleffer et al. 2004,Clingeleffer et al. 2001,Dunn et al. 2001). The validation of long-term datasets of yield components on different varieties in Australia showed that the seasonal variation in yield accounted for 60 % of variation for clusters per vine, 30 % to berries per cluster, and respectively 10 % for berry weight (Clingeleffer et al. 2001). Due to the fact that plant material is limited at the seedling stage yield potential cannot be sufficiently evaluated by determining just the parameters listed. In early stages of breeding programs only one genotype of a potential new variety is available but nevertheless the yield potential of this single vine needs to be evaluated in relation to wine quality. Therefore it would be advantageous to evaluate in addition (1) maximum crop level (the amount of grapes a vine of a given size can bring to maturity) and (2) vine balance. Vine balance can be examined by the use of vine indices like the ratio of crop weight to pruning weight which is applied in viticultural practice (Kliewer and Dokoozlian 2005,Poni et al. 2007). The determination of dormant pruning wood weight is usually done by manual measurement of the weight of pruned wood which is rather time-consuming. Efforts have been made to use a four-band aerial sensor (ADAR 5500) post-veraison to determine pruning weights (Dobrowski et al. 2003). A 2D laser scanner sensor has been used to map winter canes on a 10 x 20 m grid basis prior to pruning (Tagarakis et al. 2013). For high-throughput and objective acquisition of pruning weight covering a large set of single vines (i.e. seedlings) a fast ground-based sensor method is required. The use of cameras as optical sensors facilitates fast, low cost, and robust data recording in the field (Herzog et al. 2014). To avoid the use of an artificial background 3D stereo reconstruction and calculation of depth maps is suitable (Klodt et al. 2015).

Here we describe a non-invasive image based method to predict the pruning weight out of images by using automated depth segmentation and minor user interactions. The focus is on pruning weight and crop level for seedling selection.

Materials and Methods

Plant material

Tests involved 138 ungrafted seedlings planted in 1996 located at the experimental vineyards of JKI, Institute for Grapevine Breeding Geilweilerhof in Siebeldingen, Germany (N 49°13.005 E 8°02.671). The use of a set of seedlings consisting of different genotypes, respectively phenotypes guarantees a large variation in the plant material for image interpretation. Inter-row distance of trellis trained grapevines was 2.0 m, grapevine spacing was 1.0 m and rows were planted in a north-south direction. All vines observed in the experiment were surveyed using a Real-Time-Kinematic (RTK)-GPS system (Trimble® SPS852, Geo System GmbH, Jena, Germany) with 2 cm accuracy in order to link the gained sensor data to single grapevines.

Sensor and sensor data

Images were taken semi-automatically directly in the field using the extended Prototype-Image-Acquisition-System (PIAS) (Herzog et al. 2014) following the 2013 growing season. Therefore the PIAS was equipped with a multi-camera-system (MCS) consisting of two monochrome cameras (AVT GT-2450; objective: CVO 8 mm; 2448 x 2050 pixels) and one Red-Green-Blue (RGB) camera (AVT GT-2450C; objective: Schneider KMP-IR CINEGON 8 mm; 2448 x 2050 pixels). For the acquisition of geo-referenced images the software IGG-GEOTAGGER and a RTK-GPS system was used. The camera positions had a 10 cm difference between all cameras. The MCS was fixed in the middle of the PIAS with a distance of at least 1.0 m from the grapevine. Images were captured under natural illumination conditions with manually controlled exposure.

Reference evaluation

Yield components (yield/vine, cluster/vine) have been evaluated in the growing season 2013 for each genotype. Pruning weights (PW) for each of the 138 vines were obtained in the dormant period following the 2013 growing season and were used as ground truth for regression analysis. A subset of the field images was manually segmented and the pruning area PA (%) was used as reference data to verify the sensor data. The Ravaz index (yield/pruning weight) (Ravaz 1903) and the YiPa index (yield/pruning area) have been calculated. To draw a conclusion on the crop level of single genotypes, Brix values were used as index indicating the grape ripeness and sugar content. Brix was recorded during the ripening period using Fourier transform infrared spectroscopy (FTIR).

Experimental design

Within the present study two experiments have been performed.

Experiment (a): Development of an image based non-invasive detection method for pruning area (PA).

(a1) Automated image segmentation. Two monochrome images (MCS) captured with real background in the field (non-invasive).

(a2) Manual image segmentation. One RGB image (MCS) captured with a white artificial background in the field (non-invasive).

Experiment (b): An application example of the new method in grapevine breeding. Using the detected PA (YiPa index) and reference values (yield components, PW, Ravaz index) for the validation of a seedling selection.

Image-based phenotyping of pruning area

(a1) For the development of the automated image segmentation method a set of 39 vine images was used. Two monochrome images and one RGB image per vine (MCS) were captured with real background. Dense depth maps were calculated for each image pair and a subsequent segmentation step resulting in a partitioning of the image domain into foreground and background. The foreground class corresponds to the plant in the foreground and the background corresponds to the rest of the image. The area of the foreground provides the surface area (PA) of the visible part of the plant. Surface areas were computed using the dense depth reconstruction for all points in the foreground class. The complete method is described in Klodt et al. (2015).

(a2) Manual image segmentation. The same set of 39 vines was captured with a white artificial background. The RGB image (MCS) was used for the manual segmentation into the classes `background` and `foreground`(PA). CorelDraw X3 (Corel Corporation, Ottawa, Ontario, Canada) was used for the pixel-wise annotation of the two classes. To evaluate the automated image segmentation (a1) the percentage of the two classes was determined.

Statistical analysis

The statistical analysis was performed using SAS Enterprise Guide 5.1 (SAS Institute, Cary, NC, USA). The Pearson correlation coefficient was calculated. Linear regression analysis was performed to compute the regression line with a corresponding coefficient of determination R^2 as well as the Root-Mean-Squared-Error (RMSE). The RMSE is a function of the sum of squared residuals and the number of observations n minus the number of parameters p (1 parameter= PA). yi is PW and $\acute{y}$ represents the predicted PW calculated with the regression line.

$$\text{RMSE} = \sqrt{\frac{\sum(yi-\acute{y})^2}{n-p}} \quad (1)$$

Results and Discussion

Pruning area PA *vs. pruning weight* PW

The relationship between PW and PA (Figure 2) was investigated for the automated depth segmentation of images (a1) applying linear regression analysis. The manual image segmentation (a2) served as reference dataset. For both the determination coefficient R^2 and the correlation with the PW was calculated. The PA gained through the non-invasive manual segmentation (a2) showed a correlation of r=0.91 (r^2=0.83) and the automated segmentation (a1) correlated with r=0.61 (r^2=0.44). Root-Mean-Squared-Error was respectively *RMSE*=0.23 (a1) and *RMSE*=0.12 (a2) (Figure 2). The *RMSE* indicates that on average the predicted PW differs from the real PW about 230 g for automated segmentation and 120 g for manual segmentation. Thus, both image-based methods to detect PA showed good correlations with the measured PW. Whereas correlation between the two methods (a1; a2) was r=0.77.

Figure 3 shows the comparison of two analysed vines. The PW of the two vines was different (vine 1=0.15 kg; vine 2=0.62 kg), whereas the automatically detected PA was comparable (vine 1=9.8 %; vine 2=9.7 %). The automated segmentation lags behind on the detection of vines with thin shoots and lots of tendrils, these small objects could be easier segmented with the manual segmentation. The automated segmentation of thin and small objects using the applied algorithms is difficult because of the very homogeneous background as described by Herzog et al. (2014). This might be improved by incorporating prior knowledge about the scene structure to the segmentation step. A reconstruction algorithm that is specialized to fine-scaled features might help to improve segmentation results because thin features represent a dominant part of the prevailing scenes. Other possible improvements include the use of additional information from the RGB images to help to better distinguish between the foreground plant and the background. Currently, the segmentation is based only on the depth information. Especially the wires in the foreground cannot be distinguished from the plant in the current version of the program. Here, RGB information might provide useful additional information for a reliable separation by considering the different colour distributions of wires and stem. Furthermore, a part of the dormant wood of vine 2 is not visible in the monochrome image (Figure 3) which can cause underestimated PA detection due to manually exposed images. This shall be improved by using an automated system like the phenotyping robot PHENObot as showed by Kicherer et al. (2015). In addition, trunk size and perennial parts of the vine may vary from vine to vine. To improve the PA estimation a second image acquisition after pruning might be helpful to remove this second measurement from the first one and therefore get more accurate PA values.

Validation of a seedling selection

Experiment (b) shows an example of how the new method to detect PA developed in experiment (a) could be used in grapevine breeding. The evaluation of seedlings aims at finding the genotype that could be a superior selection and potential new variety. Therefore, the steps of the validation followed parameters used in viticulture to assess established varieties. Four parameters have been used to evaluate yield potential in a set of 138 seedlings: (1) yield (kg) per vine, (2) cluster per vine, (3) vine balance (using PW, PA for the Ravaz and YiPa index) and (4) crop level.

Table 1 gives an overview of the range of selection criteria used to validate the seedlings. Within the set of 138 genotypes (1) yield per vine varied from 0.03 kg to 6.51kg, (2) clusters per vine ranged from one cluster per vine up to 38 clusters per vine and the Ravaz index (3) was between 0.08-25.33 (PW 0.007-1.45 kg). The YiPa index in the subset of 39 genotypes covered a range of 0.003-0.68 (PA 4.2-14.7 %).

The maximum crop level (4) is defined as the amount of grapes a vine of a given size can bring to maturity. To validate the crop level we looked at the relationship between the time of ripening and the ripening index (Brix values). There are four different classes one can distinguish: class 1 contains genotypes with a small crop level which leads to accelerate ripening and a logarithmic curve shape. Class 2 includes the desired genotype to be selected with a crop level just below the maximum crop level. For class 3 and class 4 the time of ripening is linearly dependent upon the crop level whereas class three has an increased crop level and is slow ripening and class 4 is above the maximal crop level and never ripens (Figure 4). The distribution of classes over the whole set (138 genotypes) was 8 % in class 4, 16 % in class 3, 19 % in class 1 and 57 % in class 2.

Breeding goals for wine grapes concerning yield traits vary from vine yield smaller than 1 kgm^{-2} to more than 1.5 kgm^{-2} (Töpfer et al. 2011). Berry sizes from small to large, berries per cluster from less than 200 to more than 300 and the number of clusters from 2 to 4 per cane depending on the desired goal (Töpfer et al. 2011). As the German vine law specifies maximum vine yield depending on the growing region and wine quality, we used the maximum yield of 14,000 $kgha^{-1}$ as breeding goal to validate our seedlings. According to the desired breeding goal this value can be adapted any time. The amount of 14,000 $kgha^{-1}$ grapes means an average vine yield of 2.8 kg per vine. For this first evaluation step we excluded all genotypes producing less than 1.7 kg and of more than 3.5 kg grapes per vine. It has been shown that clusters per vine accounts for 60 % of the yield variation between the years in viticulture (Clingeleffer et al. 2001). Therefore the number of clusters per vine has been chosen as second criterion for the validation. The desired number of clusters per vine genotype was set to 12 - 18 clusters. The third selection criterion was vine balance which can be directly measured through pruning weight and yields at harvest. The dormant pruning weight can indicate the level of vegetative growth and whether a vine is of high, moderate, or low vigour. The most common way used to detect the relationship between vegetative and generative growth is the Ravaz index (yield/pruning weight). Using the data gained with the new automated method we calculated an additional index: the YiPa index (yield/pruning area) which is inspired by the Ravaz index, using the pruning area detected with the image-based method (a1) described earlier instead of the actual pruning weight. Generally, vines with Ravaz index levels between 5 - 10 are considered in the optimal range (Bravdo et al. 1984,Bravdo et al. 1985). But these levels can also vary depending on the cluster size and growing conditions (Kliewer and Dokoozlian 2005). For this selection example we chose genotypes varying between 5 – 11 (Ravaz index) and 0.1 - 0.3 (YiPa index). The targeted range of YiPa index was set using a box plot to display the variation of the YiPa values. Therefore, the lower first quartile (0.1) and the upper third quartile (0.3) were chosen as target range for this trait. YiPa index

could only be calculated for the subset of 39 genotypes for which automated image segmentation was done. Applying the first three selection criteria on the full set of 138 genotypes 17 genotypes matched all three. Three of them were also included in the subset of 39 vines in which the PA (a1) was calculated automatically and all three endorsed the selection of genotypes using the three criteria (yield/vine, cluster/vine, Ravaz index). Applying the fourth step of selection criteria, (4) crop level to the set of 17 genotypes selected by the three selection criteria the distribution was as followed: classes 1 and 4 contained one genotype each, two genotypes have been assigned to class 3 and 13 genotypes met all four selection criteria for yield potential proposed in this study (Figure 4). With regard to the climate change slowly ripening genotypes assigned to class 3 can also be consider valuable for breeding purposes. The criteria used for validating the set of seedlings is one example set of parameters and can always be adapted to changing breeding goals or scientific questions. The desired range of traits can also be used to control the amount of selected genotypes. One would not like to narrow down the amount too much and therefore lose interesting genotypes. On the other hand the selection window should not be to wide that too many genotypes will be selected. Besides the criteria chosen other interesting yield criteria are the number of clusters per shoot, the number of berries per cluster or the berry size. It will be challenging to set up algorithms to automatically monitor these traits under non experimental conditions. Therefore, the method presented is a good first step to assist breeding based on sensor techniques. Image acquisition in this study was done using the PIAS in combination with manual exposure of geo-referenced images. Using an autonomous platform like the PHENObot (Kicherer et al. 2015) can open up new opportunities and an even higher level of automation making it possible to screen a larger set of vines in a more objective way.

Conclusions

Utilizing an image based method to detect pruning weight it was shown that the pruning area detected automatically using depth segmentation was linearly correlated with the field measurements of pruning weight in the set of 39 different genotypes. Additionally, it was principally shown in this study that this new inexpensive and time saving method together with other selection criteria is suitable to validate the yield potential of a seedling selections.

Acknowledgements

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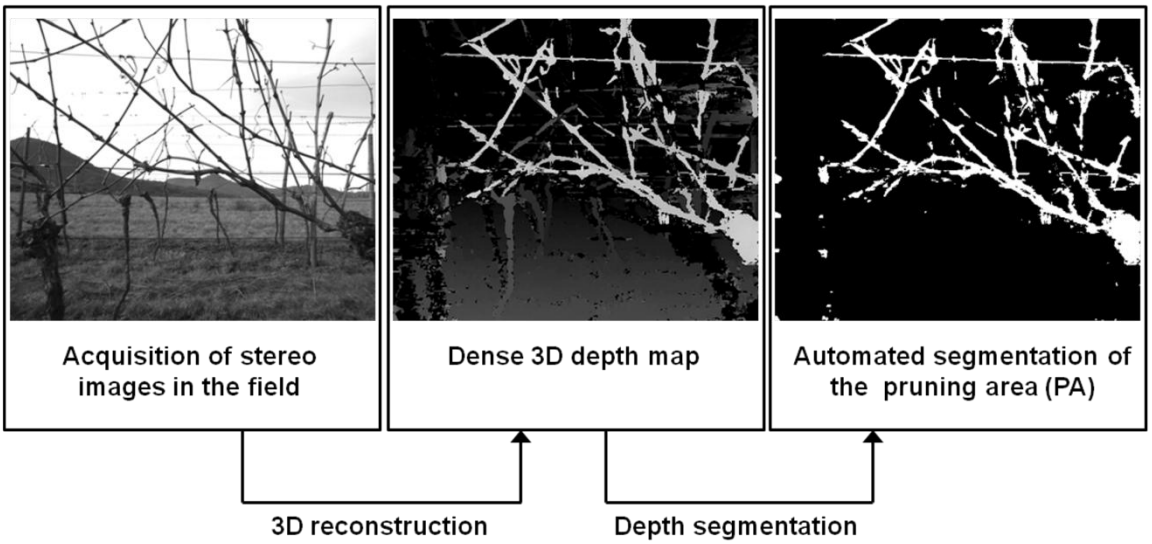

Figure 1 Workflow of automated image based depth segmentation and detection of pruning area (PA).

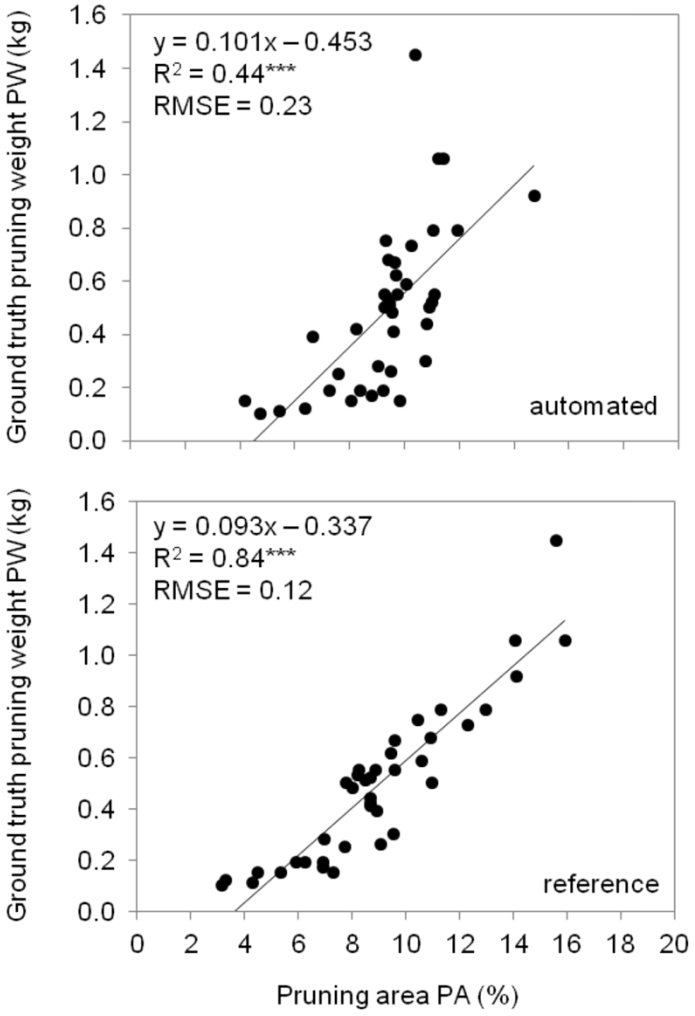

Figure 2 Pruning area (%) *vs.* ground truth pruning weight (kg). A subset of 39 grapevine images was used to fit a linear function. The results of the automated depth segmentation and of the reference (manual segmentation) are shown. y = linear regression line; R^2 = determination coefficient; RMSE = Root-Mean-Squared-Error; *** = *p-value* <0.001.

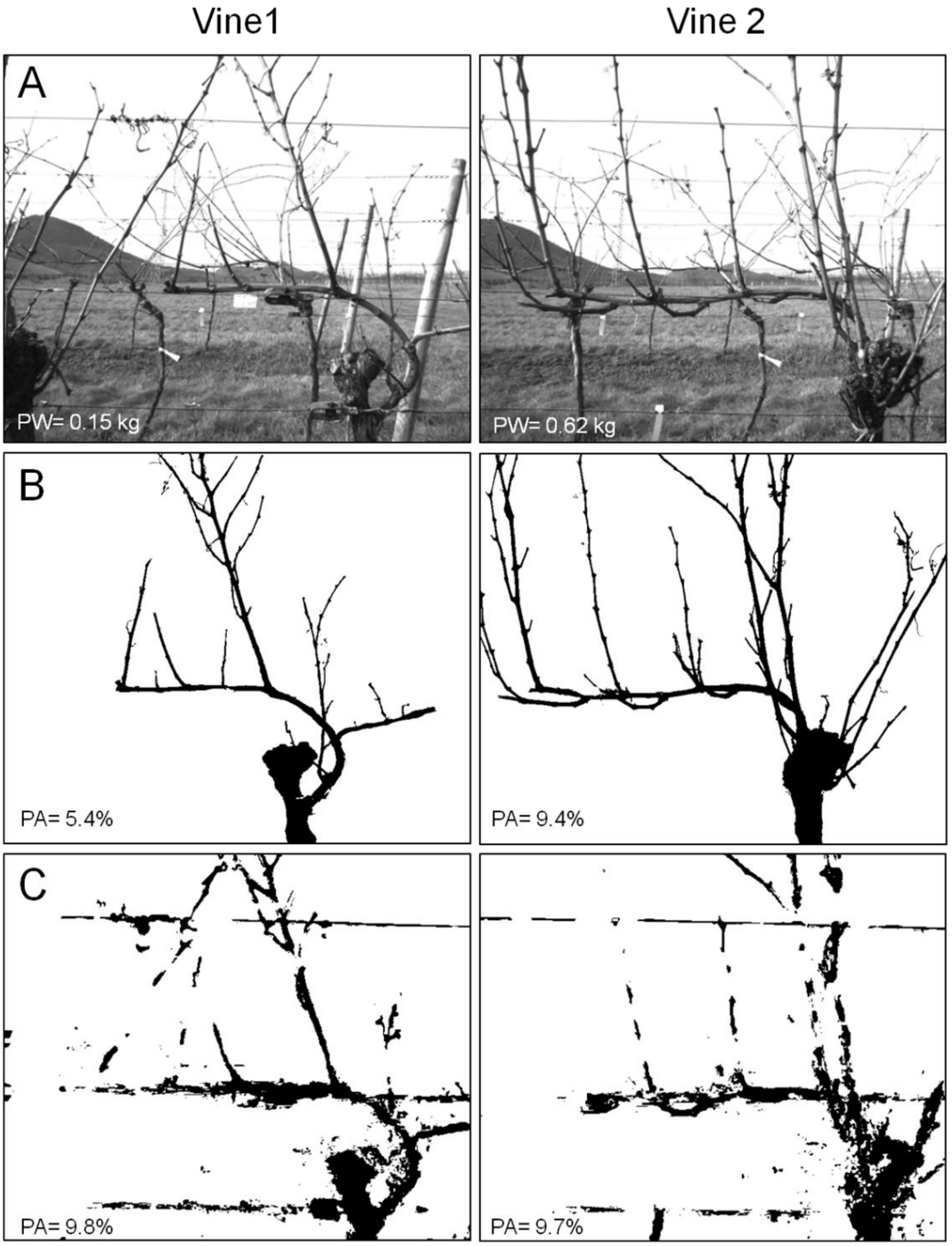

Figure 3 Comparison of the image based detection of pruning area (PA) of two different vines in the field. (A) Original monochrome image. (B) Manual image segmentation of the PA used as reference. (C) Automated depth segmentation and detection of PA. Both vines showed considerable differences for their pruning weight (PW) of 0.15 kg (vine 1) and 0.62 kg (vine 2) but comparable PAs.

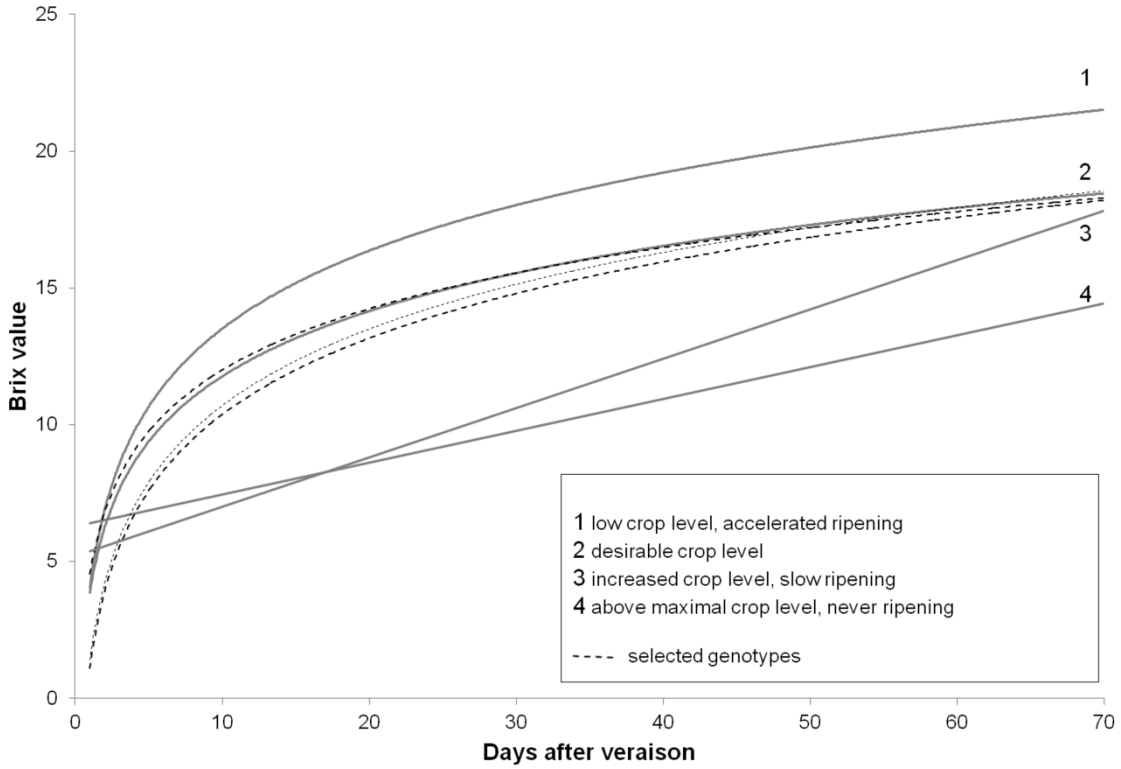

Figure 4 Time of ripening *vs.* ripening index relationship of exemplary genotypes. There are four different classes describing the various crop levels: 1= low crop level, accelerated ripening; 2= the desired crop level; 3= increased crop level, slow ripening, 4= above maximal crop level, never ripening). Three selected genotypes that meet all selection criteria (yield/vine 1.7-3.5 kg; Cluster/vine 12-18; Ravaz index 5-11; YiPa index 0.15-0.30) are displayed in the graph.

Table 1 Overview of the selection criteria for the evaluation of the seedling selection: (1) yield (kg) per vine, (2) cluster per vine, (3) vine balance (using PW, PA for the Ravaz/YiPa index). The range or the criteria detected in the screening, the targeted range for this study, and the number of evaluated and selected genotypes. 17 genotypes of the population met all criteria (yield/vine, cluster/vine, Ravaz index). A set of 39 images was used to detect the PA automatically and out of these results the YiPa index was calculated. 3 of the 18 genotypes also met this criterion.

	range observed	**range desired**	**number of evaluated genotypes**	**number of selected genotypes**	
(1) yield/vine	**0.03-6.51 kg**	**1.7-3.5 kg**	**138**	**49**	
(2) cluster/vine	**1-38**	**12-18**	**138**	**56**	**17**
(3) Ravaz index	**0.08-25.33**	**5-11**	**138**	**42**	
(3) YiPa index	**0.003-0.68**	**0.1-0.3**	**39**	**19**	
PW	**0.007-1.45 kg**	**-**	**138**	**-**	
PA	**4.2-14.7 %**	**-**	**39**	**-**	

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An Automated Field Phenotyping Pipeline for Application in Grapevine Research

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Abstract: Due to its perennial nature and size, the acquisition of phenotypic data in grapevine research is almost exclusively restricted to the field and done by visual estimation. This kind of evaluation procedure is limited by time, cost and the subjectivity of records. As a consequence, objectivity, automation and more precision of phenotypic data evaluation are needed to increase the number of samples, manage grapevine repositories, enable genetic research of new phenotypic traits and, therefore, increase the efficiency in plant research. In the present study, an automated field phenotyping pipeline was setup and applied in a plot

of genetic resources. The application of the *PHENObot* allows image acquisition from at least 250 individual grapevines per hour directly in the field without user interaction. Data management is handled by a database (*IMAGEdata*). The automatic image analysis tool *BIVcolor* (Berries in Vineyards-color) permitted the collection of precise phenotypic data of two important fruit traits, berry size and color, within a large set of plants. The application of the *PHENObot* represents an automated tool for high-throughput sampling of image data in the field. The automated analysis of these images facilitates the generation of objective and precise phenotypic data on a larger scale.

Keywords: robot; geoinformation; high-throughput analysis; image acquisition; plant phenotyping; grapevine breeding; *Vitis vinifera*

1. Introduction

With the fast development of genotyping methods to support grapevine breeding based on SSR (Simple Sequence Repeats) [1,2] or SNP (Single Nucleotide Polymorphism) analyses, including next generation DNA sequencing [3], genotyping efficiency has been greatly improved and costs have been reduced contemporaneously. However, plant phenotyping methods have only slowly improved during the last few decades, becoming now a major bottleneck. Therefore, the lack of sufficient phenotypic data and phenotyping methods constrains the possibility to reveal the genetics of quantitative traits, such as yield, growth and adaption to abiotic or biotic stresses. The development and implementation of high-throughput phenotyping platforms is therefore a key tool to improve the efficiency of grapevine (*Vitis vinifera* L. subsp. *vinifera*) or, more generally, plant breeding. In recent years, much effort has been made to build up such platforms, which allow the assessment of large quantities of phenotypic data under controlled environments [4–9]. Although these systems enable a detailed non-invasive plant assessment throughout the plant life cycle under controlled conditions, they neglect information about the genotype-environment interactions and do not take horticultural or viticultural plants into account. However, grapevine, for example, as a rather large perennial plant, needs to be evaluated directly in the field. Several studies of the implementation of new techniques for an improved management of vineyards in practical viticulture [10–14] have been conducted in recent years. Yield estimation is one of the most important traits in precision viticulture due to annual and spatial variations. The published studies aimed to improve yield estimation and forecasting by detecting bunches of grapes, berries [15–18] or the number of inflorescences [19] in images. Ground-based sensor data used in precision viticulture are than either recoded from a constant distance to the canopy [16,19–21], mounted to a tractor [10–12], truck crane [22] or include modified vehicles [13,15,23] equipped with global positioning systems (GPS) devices [18,24,25]. Another approach is the application of a field phenotyping robot. Such systems have already been introduced for application in maize [26] and small grain cereals [27]. A robot application for viticulture was suggested by Longo *et al.* [28]. The U-Go (Unmanned Ground Outdoor) robot was developed as a multipurpose vehicle with the aim of facilitating work during the season (harvesting, pruning, transportation of bins) [28]. Furthermore, the opportunity to be equipped with a modular remote sprayer [29] is given. Its technical specification allows remote control or autonomous motion using GPS

waypoints [28]. Nonetheless, all of these studies focus mainly on vineyard management, site-specific information to improve crop load, water or the health status of the considered plot. In contrast, grapevine breeding aims at the phenotyping of single grapevines, whereby genetic resources and large sets of breeding material need to be screened. That implies that in one experimental field plot, each plant can be a different genotype, showing its distinct phenotype, which needs to be assessed individually with high precision. Not only the resolution of phenotypic data towards one single grapevine may differ, also the variation of traits within breeding material is considerably higher than in commercial vineyards. Important phenotypic traits in grapevine breeding are the detection of fruit parameters, e.g., the berry size and color of berries. Current assessment of phenotypes in breeding programs relies largely on visual estimations, using the BBCH (phenological development stages of a plant; stands for Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) scale [30] or OIV (International Organization of Vine and Wine) descriptors [31]. These systems are laborious, time-consuming and, therefore, expensive. The data obtained are subjective and can vary significantly when evaluated by different persons. The biggest limitation, however, is the needed simultaneous screening of vines from several hectares of experimental vineyards, which limits a detailed evaluation of traits to a rather small number of breeding strains. The application of non-invasive, high-throughput sensor technologies is required to increase the efficiency of grapevine breeding by increasing the phenotyping efficiency (number of plants per time), improving the quality of phenotypic data recording and reducing the error variation. Such new methods progressively increase the amount of data that needs to be handled.

First steps towards a high-throughput phenotyping pipeline in grapevine breeding have been introduced by Herzog et al. [32]. The study implemented a Prototype Image Acquisition System (PIAS) for semi-automated capturing of geo-referenced images and a semi-automated image analysis tool to phenotype berry size. An automated phenotyping platform in grapevine breeding is needed to screen for phenotypic traits on a single-plant-level in a reasonable time, unlike the application in precision farming, whereas the overall appearance of a plot or at least single areas of a plot are of greatest interest.

Here, we describe the setup of an updated and expanded phenotyping pipeline involving automated data acquisition in the field, automated data management and data analysis. The challenges of this pipeline are the combination of: (1) automated simultaneous triggering of all cameras at a predefined position in the field; (2) automated acquisition of geo-referenced images; (3) data management via a database; and (4) automated image analysis for objective and precise phenotyping of the berry size and color. Moreover, we demonstrate the application of the pipeline in the grapevine repository at Geilweilerhof.

2. Material and Methods

2.1. Plant Material

The application of the phenotyping pipeline involved 2700 grapevines representing 970 accessions from the grapevine repository at the experimental vineyards of Geilweilerhof located in Siebeldingen, Germany (N 49°21.747, E 8°04.678). Interrow distance was 2.0 m, and grapevine spacing was 1.0 m. Rows were planted in a north-south direction. Colored size reference labels were fixed to the wires and used to scale the images.

2.2. Automated Image Acquisition

For the automated image acquisition directly in the field, the PHENObot (Phenotyping robot) was developed [33]. This phenotyping platform consists of a chain vehicle containing a control unit and a camera-light unit in combination with an industrial computer. In order to operate in a harsh outdoor environment and to enable the transportation and navigation of the camera-light unit for the non-destructive inspection of phenotypic grapevine traits, the chain vehicle had to meet certain requirements: a lifting capability up to 250 kg, low vibration drive at a speed between 4 to 6 $km \cdot h^{-1}$, an easily adjustable mounting system for the sensors, a navigation system based on GPS coordinates, the ability for path planning, as well as fulfilling safety standards [33]. For targeted image acquisition, path planning is needed for the PHENObot. Therefore, precise GPS positions of individual vines are necessary and, so, all grapevines have been surveyed. The camera-light unit used on the PHENObot consists of three monochrome cameras (AVT GT-2450; objective: CVO 8 mm; 2448 × 2050 pixels), one RGB camera (AVT GT-2450C; objective: Schneider KMP-IR CINEGON 8 mm; 2448 × 2050 pixels) and one NIR camera (AVT MANTA; objective: Schneider KMP-IR CINEGON 8 mm; 1388 × 1038). To enable an adequate illumination for standardized image acquisition, a lightning unit containing eight LED bars (12 LEDs; ODLW300 series; Smart vision lights, Muskegon, MI, USA) was combined with the camera unit (for the setup, see Figure 1A). The components are connected with the image acquisition computer by a fast Ethernet network (GigE). All cameras are synchronously triggered using this network, and the images are transmitted immediately to the PC. The lightning unit is triggered by one of the monochrome cameras. For configuration and monitoring of the image acquisition process, a software application (IggGeotagger.Ext) has been developed fulfilling two main tasks: the communication task handles the communication between the control unit of the PHENObot and the image acquisition computer; the image acquisition task controls the cameras and the image transport and storage. The application is also used for visualization of the images and for setting the camera parameters (screenshot in Figure 1). A single image acquisition cycle performs several steps (see Figure 2). The communication task waits for a message from the PHENObot control unit. As soon as the PHENObot has reached a predefined position, it sends a specific message containing the position, the orientation and the corresponding plant ID to the computer. Then the communication task starts the image acquisition task, which triggers all cameras, receives the images, generates the filenames for the images (plantID_camera_cameraID_datetime) and saves them to the hard drive. Additionally, the position and orientation information is written directly into the file header of the image. When the image acquisition task has finished, the communication task sends an acknowledgment message to the PHENObot control, signaling that it can move to the next position. One hundred forty grapevines have been assessed to verify the image section: (1) includes the whole bunch area of each grapevine assessed, and (2) remains the same when repeatedly approached. The PHENObot was stopped at the surveyed position of the grapevine and under the consideration of the training direction (trained to the south or north, respectively). Moreover, the 140 grapevines have been approached 4 times in a row.

Figure 1. Graphical user interface of the *IggGeotagger.Ext*. The software manages the communication between the control unit of the *PHENObot* and the image acquisition PC, triggers the cameras and controls the image transport and storage. It is preferentially used for the visualization of captured images and for setting the camera parameters.

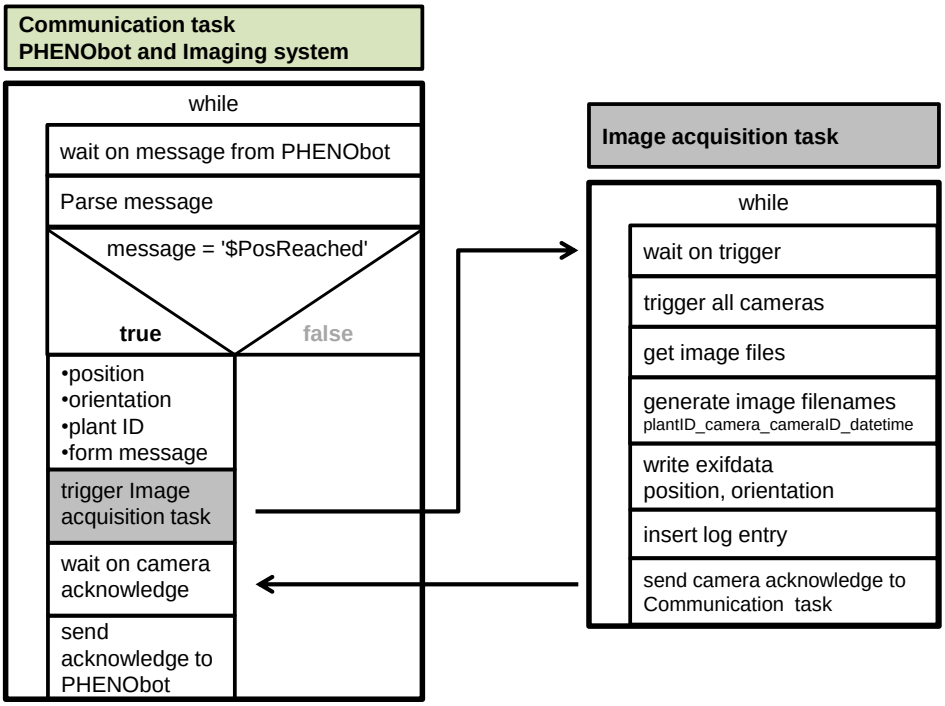

Figure 2. Communication and image acquisition task within the *IggGeotagger.Ext* software. The communication task handles the communication between the control unit of the *PHENObot* and the image acquisition PC; the image acquisition task controls the cameras and the image transport and storage.

2.3. Data Management

All 2700 grapevines of the genetic repository have been surveyed using a RTK (real-time-kinematic)-GPS system (Trimble® SPS852, Geo Systems GmbH, Jena, Germany) with 2-cm accuracy. The geo-information of each grapevine and the associated plant ID is stored in the central database, *PLA* (*Plant Location Administration*)—A common management tool for experimental areas in the Julius Kühn-Institut. All images delivered by the *IggGeotagger.Ext* are imported into the database, *IMAGEdata*. Based on the image names, which contain the plant ID, every image is uniquely assigned to a single grapevine. For this assignment, the *PLA* is used. *PLA*, as well as *IMAGEdata* work with geographical data (UTM). The aim of *IMAGEdata* is to have a powerful and easy to use tool for managing the images as a basis for further evaluation. These databases can be used by modern Web 2.0 interfaces and web services. Current technologies allow safe operation and offer modern user interfaces.

2.4. Image Analysis

Image analysis was conducted by using the MATLAB®-based tool, *BIVcolor* (Berries in Vineyards-color). Based on a one-class classification framework determining grapevine berry sizes, some slight modifications have been done (MATLAB 2012b and Image Processing Toolbox, The Mathworks, Natick, MA, USA) on the Berries in Vineyards (*BIV*) algorithm [34]. This was targeted to separately record mean RGB values of each single berry according to their color channels (RGB) and their position within the corresponding image. The data were written loop-wise into a tab-limited text file corresponding to the image file analyzed and finally stored in a SQL-database (Access 2010, Microsoft, Redmond, WA, USA). The known position of berries within a trait later on provides clustering to check berry patterns and outliers.

A set of 500 images, including 235 different accessions and n = 1,300,900 segmented single berries, was used for color information assessment. The mean of the RGB values of all berries detected in one image were used for statistical analysis. As reference data, the berry color was assessed as five classes (1 = black; 2 = red; 3 = rose; 4 = grey; 5 = green).

2.5. Statistical Analysis

Statistical analysis was conducted using the software R Version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria). Linear discriminate analysis (LDA) was performed to predict the berry color class using the RGB values as predictor variables.

3. Results and Discussion

3.1. Field Application of the Phenotyping Robot

A phenotyping pipeline has been set up and consists of the following components: (1) data acquisition; (2) data management; and (3) data analysis (Figure 3). Data acquisition was done automatically using the *PHENObot*. Each image was linked to one plant, respectively one genotype, without any post-processing. Applying the *PHENObot* image data from 2700 grapevines representing 970 grapevine accessions has been done. Automated data recording for these large set of plants was

completed within 12 h. The image acquisition of one grapevine took on average 15 s. Although the camera was equipped with a lightning unit, it was impossible to take standardized images on sunny days (Figure 4). Consequently, the image acquisition in the grapevine repository was done at night due to uniform light conditions. This has also been reported to work best for images taken in commercial vineyards to estimate yield [18].

Two pre-test drives consisting of 140 grapevines have been done. The first one to ascertain the image section comprises the whole bunch zone of each grapevine assessed and the second one to make sure the same image section is captured each time a grapevine is approached. The image section was best when the stopping position of the PHENObot was shifted 25 cm south or north in accordance with the training direction in order to enable one to see as much of the bunch zone as possible. The 140 grapevines were approached four times, and the image section stayed the same for each grapevine and all four repetitions. The comparison of the GPS position logged at the image acquisition point for the four drives showed a difference of 1–2 cm, which is within the accuracy of the GPS system.

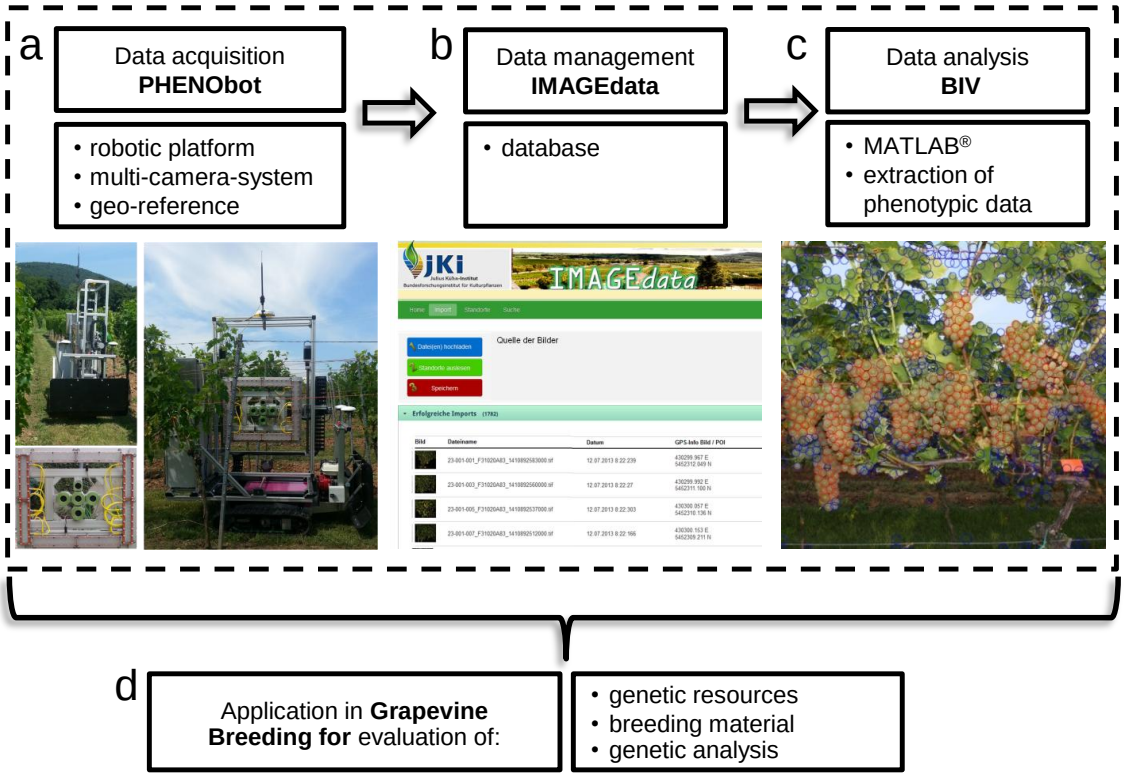

Figure 3. Phenotyping pipeline in grapevine breeding. (**a**) Data acquisition using the *PHENObot* consisting of a robotic platform, a multi-camera-system and a geo-information system; (**b**) data management of the sensor data is achieved by a database (*IMAGEdata*); (**c**) data analysis through the application of MATLAB®-based tools, e.g., *BIVcolor* (Berry in Vineyards-color), to extract the phenotypic data; (**d**) the phenotyping pipeline was developed for application in grapevine breeding. This enables the phenotyping of large sets of plant material from genetic resources or breeding material.

Figure 4. Comparison of images taken during the day and at night. Three examples of vines photographed on a sunny day and at night. All images were captured using the *PHENObot* with the lightning unit on. Image acquisition at night enables standardized conditions, which are very important for robust automated image analysis and comparable phenotyping results, e.g., with regard to the determination of berry colors.

3.2. Image Analysis

Images have been analyzed using the MATLAB®-based tool, BIVcolor. The tool enables the automated extraction of the phenotypic traits, berry size and color. The berry size is one of the most important fruit parameters integrated for seedling selection in breeding programs. The BIVcolor evaluated berry size ranging from 9.8 mm to 13.9 mm. The acquisition of the berry color is important for the characterization of genetic repositories or the phenotyping of mapping populations for genetic analysis. Initially, the color of grapes can be classified according to the presence or absence of anthocyanin in the berry skin, as either black or green. As a result of natural hybridization and human selection, the grape skin color is very diverse nowadays, ranging from green-yellow, grey, rose, red to black. The reference assessment for berry color in the set of 500 images showed a distribution of: 202 (Class 1 = black), 200 (Class 5 = green), 39 (Class 4 = grey), 37 (Class 2 = red) and 22 (Class 3 = rose) (Figure 5a). Linear discriminant analysis (LDA) using three predictor variables (red, green and blue color values) was used to predict the class of berry color. Table 1 shows the cross-validation of the real vs. predicted color class. The percentage of the correct prediction of black (197 berries; 97%) and green (178 berries; 89%) berries was very high. Some of the green berries were predicted as grey, but in most cases, grey berries were predicted as grey (28 berries; 71%). Thirteen images (59%) visually assessed as rose berries have been predicted as red. The difference between red and rose berries can be difficult to discern no matter whether one predicts the class doing visual estimations (Figure 5a) or if one uses RGB values (Figure 5b,c). Due to the fact that RGB values of these two classes are very similar and overlapping (Figure 5b,c), it was not possible to distinguish these two classes in our study. One can clearly distinguish between black, green, grey and red/rose berries, and this is exactly what can be used for the evaluation of genetic resources and breeding material, but also for the management of

grapevine repositories. Usually, three grapevines of one accession are planted next to each other, through the image-based color detection planting mistakes based on wrong berry color can be uncovered, for instance:

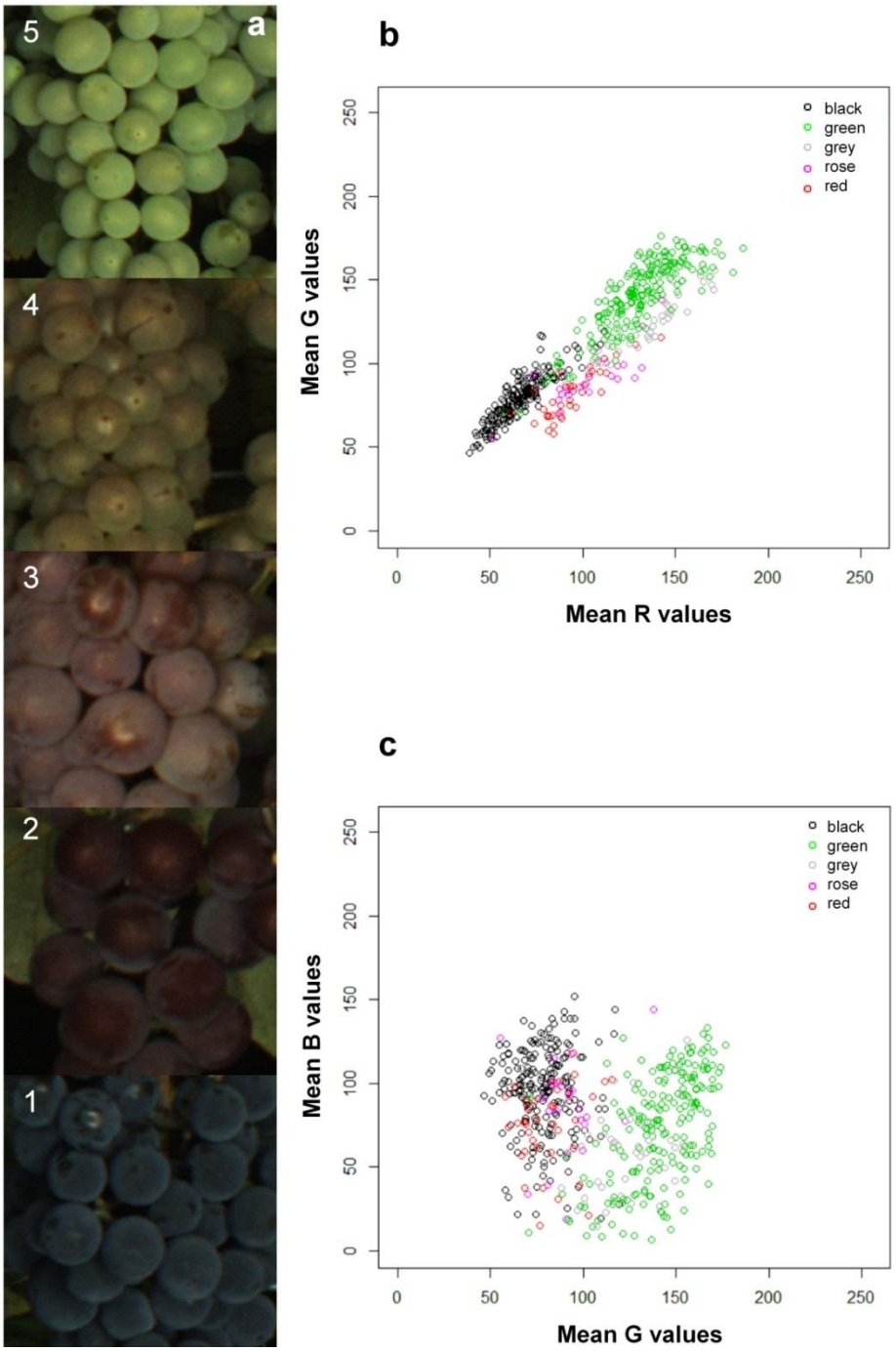

Figure 5. Distance plots of single RGB values indicating the fitness of the color model used for LDA. Prediction of berry color classes was done using the image-based detected RGB values. LDA used three parameters (red, green and blue color values) and, as the ground truth, the visually assessed berry color. (**a**) Berry color was visually assessed as five classes: Class 1 = black; Class 2 = red; Class 3 = rose; Class 4 = grey; Class 5 = green; (**b**) distance plot of R values *vs.* G values; (**c**) distance plot of G values *vs.* B values.

Table 1. Cross-validation of the real berry color classes assessed by visual estimation and the color classes predicted with the LDA.

	Real Color Classes				
Predicted Color Classes	**Black**	**Green**	**Grey**	**Red**	**Rose**
black	197	7	2	5	3
green	5	178	7	0	0
grey	0	15	28	2	3
red	0	0	1	26	13
rose	0	0	1	4	3

From previous work presented by Roscher *et al.* [34], it is known that the acquisition of images in the field and automated image analysis in order to determine berry sizes is about 24-times faster compared to the application of a caliper to measure the diameter of 50 berries per grapevine. The image analysis runs automatically and needs no user interaction after starting the program. Thus, the analysis can be performed simultaneously as daily work within the common breeding program. With the extension of the *BIV* tool [34] to *BIVcolor*, we gained information about an additional phenotypic trait that can be extracted from the images without losing any time for evaluations. Another advantage is that images can always be analyzed retrospectively when new tools come along.

3.3. Future Work

The phenotyping pipeline has been successfully tested in grapevine breeding. So far, only the RGB images are used for automated image analysis. The camera unit consisting of five cameras (one RGB, three monochrome and one NIR camera) offers more opportunities. It enables the generation of 3D information using the monochrome cameras [32]; furthermore, it is suitable to use the NIR information for vitality indices. In addition, it is conceivable that the sensor unit of the PHENObot is going to be extended by additional sensors, like lasers, multi- or hyper-spectral sensors. There are plans to connect the IMAGEdata database with other existing databases, like VIVC (Vitis International Variety Catalogue [35]) and the European Vitis Database [36], to complete the linkage of available information.

An important stage in grapevine development is the beginning of berry ripening, namely veraison. This is the time when the berries start to soften and colored cultivars start to change their color, e.g., from green to black. It is conceivable that BIVcolor can be used to detect that date if images are taken continuously throughout the growing period.

4. Conclusions

A setup of a phenotyping pipeline has been introduced for grapevine breeding and to support the management of a grapevine repository. A robotic platform, the PHENObot, was built to enable the automatic image acquisition directly in the field. In order to facilitate the management of the data gained by automated image acquisition, an image database was developed. Compared to human visual assessments, a larger set of grapevines can be screened automatically, and the data revealed are objective and precise.

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Author Contributions

A.K., K.H. and R.T. designed the study and coordinated it. A.K. and P.R. carried out the field trail. A.K. validated the results and drafted the manuscript. M.P. extended the image analysis script, carried out the image analysis and did the LDA. M.W. and H.K. developed the software IggGeotaggerEXT. S.K. developed the database. K.H., M.P., M.W., S.K. and R.T. helped to draft the manuscript. All authors read and approved the final manuscript.

Appendix

To be able to reproduce results and conclusions, a set of 100 RGB images and the associated results from *BIVcolor* were deposited and are freely accessible at the data repository of the Julius Kühn-Institut, the Federal Research Centre for Cultivated Plant in Germany (doi:10.5073/jki-data.2015.1).

Conflicts of Interest

The authors declare no conflicts of interest.

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6. General discussion

Plant phenotyping is typically known as the most laborious and technically challenging part in viticulture research. Screenings often need repetition across multiple environments or they have to be done regularly over a certain period of time, mostly for more than one season. In breeding applications phenotyping needs to be fast, cheap and simple often testing only for easy traits. Maintaining the important and valuable plant material is a precondition rather than using it for destructive tests. Therefore, current phenotyping methods are either to some extent subjective, visual estimations or they require destructive sampling. Furthermore, if the allelic variation needs to be screened e.g. for genotyping of mapping populations, this phenotyping work needs to be done much more precisely. Combining novel technologies like imaging, spectroscopy, image analysis, robotics and high-performance computer systems the phenotyping bottleneck can be addressed. Applying these novel techniques in controlled conditions is challenging but phenotyping at the field level is even more challenging in many respects compared to the controlled environments. For the perennial grapevine field phenotyping of the majority of traits is mandatory and the evaluation of quality traits requires conditions relevant for practice. In grapevine breeding it is essential to be able to assess the phenotype of vines on a single plant level.

6.1 Challenges of phenotyping grapevine

To assess grapevine yield parameters in the field using modern phenotyping methods several challenges were faced during this work: (1) variation of the trait, (2) light environment, (3) similar background and (4) occlusion of traits, which will be discussed in detail in the following section.

Figure 2 Variation of yield parameters. **A V**ariation of different vines captured directly in the field showing differences in colour, shape and size. **B-D** Variation acquired in the laboratory for analysis using different image analysing tool. Images shown in **B** and **C** can be analysed using the CAT (Kicherer et al., 2015c). The BAT (Kicherer et al., 2013) can be applied to analyse images from section **D**.

The phenotypic traits are highly variable depending on the genotype and the genotype-environment interaction. With regard to yield parameters the variation of the trait (Figure 2) can be challenging for the development of image analysis algorithms. Yield parameters for example can vary with regard to cluster size and architecture (Cubero et al., 2011; Kicherer et al., 2015c), berry size (Kicherer et al., 2013; Tardaguila et al., 2012; Wycislo et al., 2008), shape and colour (Kicherer et al., 2015a; Wycislo et al., 2008) (Figure 2). Furthermore, the colour of traits and the surrounding area can be very similar in terms of

early berry developmental stages prior to veraison for coloured (red, rose, black) varieties or throughout the whole season for white varieties. Therefore, an image analysis based only on colour features (Diago et al., 2012; Dunn and Martin, 2004) or shape (Rabatel and Guizard, 2007) or texture (Grossetete et al., 2012) can be insufficient. A combination of features is crucial (Nuske et al., 2014; Roscher et al., 2014).

The acquisition of sensor data can be influenced by the high variability of light quality and quantity and can therefore pose a challenge to image-based measurement systems in the fields. Light conditions are permanently changing from one recording to the next or within the screening of one set of vines (Figure3 A). Backlighting can be a problem, but even if the sun conditions are good or the light is diffuse the shading of the canopy (Figure3 A-2) can be a challenge for image analysis. As shown equally in these studies using a camera sensor system the light quantity and quality can be improved through an artificial light unit and the image acquisition at night (Font et al., 2014; Kicherer et al., 2015a; Nuske et al., 2014). Another approach to overcome the light problematic could be the use of a grape harvester or a tunnel sprayer as carrier platforms for a camera-based sensor system. With some adjustments the machines could be converted into more standardized field phenotyping carrier platforms equipped with artificial light units and different sensors, operating at any time of the day without being influenced through natural light environment changes and providing a standardized background at the same time. One disadvantage might be the aspect of plant movement while driving, therefore a stop and go approach must be implemented. However, compared to automated phenotyping robots (Kicherer et al., 2015a; Longo et al., 2011) at present the chances for automating these two approaches are rather low based on costs.

A standardized background is nonexistent for HT-field-phenotyping applications (Figure 3B) therefore the segmentation of an image into plant structure and background is a critical and difficult step in an image analysis framework. Phenotypic evaluations during early stages of development are particularly affected (Figure 3 B-1). This challenge can either be overcome by implying an artificial background either manually carried (Herzog et al., 2014; Kicherer et al., 2015a) or platform-based (harvester, tunnel sprayer) as mentioned before or through the application of computer vision-based methods (Herzog et al. 2014; Kicherer et al., 2015b; Klodt et al., 2015) extracting different levels of foreground and background of an image by using a depth map reconstruction.

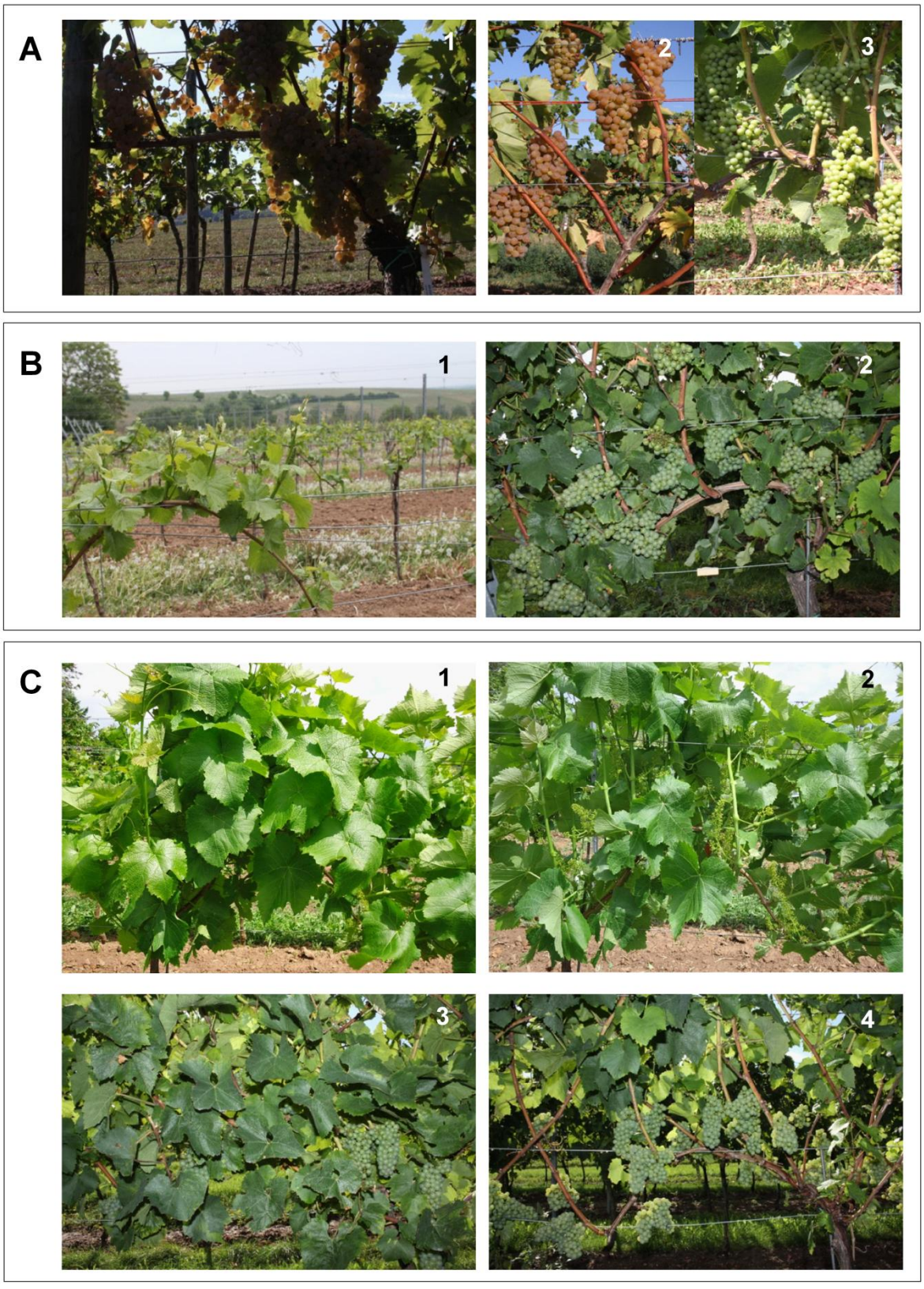

Figure 3 Challenges of image acquisition under field environment conditions. **A** quantity and quality of light (1=backlighting; 2=shading through the canopy). **B** similar background (1=early development stage BBCH 55; 2=later development stage BBCH 79). **C** occlusion through canopy or other grapes (1-2=early development stage BBCH 65; 3-4=later development stage close to ripening BBCH 85).

The detected amount of fruit using sensors can be negatively influenced because leaves and other grapes can mask the real amount of fruit on the vine (Figure 3 C). Approaches of a complete defoliation of the fruit zone enable a more accurate detection of the amount of fruits but on the other hand simultaneously increase the background problematic (Figure C-4). Such extreme defoliation in the fruiting zone is in any case artificial and not applicable to viticulture leaving a problem of masking of a certain amount of grapes.

Compared to phenotyping approaches under controlled environments some additional challenges of grapevine field phenotyping need to be considered. The establishment of grapevines for instance takes three to four years till first yield is measurable. Grafting of grapevine has an impact on its growth and development behaviour under field conditions which cannot be simulated under controlled conditions and should not be neglected. Finally quality trait considerations are only relevant if plants are grown under field conditions.

Field conditions represent the plants "natural" environment with daily and seasonally varying environmental parameters such as light, temperature, and water. Field conditions can be very heterogeneous (soil, fertilisation, light interception) and the inability to control environmental factors makes interpretation of results difficult. On the other hand results from controlled environment are far away from the influences plants will meet in the field. Therefore, the transmission of such results to plant breeding and plant production is also difficult (Araus and Cairns, 2014). Soil environment, pot size, water and nutrition limitation under controlled environments is one problem (Passioura, 2006; Poorter et al., 2012). Moreover, vines do not grow isolated in the field, they form a canopy and interact with each other. This further complicates the ability to mimic field conditions in a controlled setup and makes phenotyping on a field level very important. Since we are not able to control the soil and environmental conditions in the field, it is important to at least have comparable vineyard practice routines including plant protection, fertilisation, pruning and canopy management, to maximize standardization of field phenotyping. Winter pruning can have an influence on yield parameters such as clusters per vine. To be able to compare different genotypes within the screening process, vines need to be pruned to the same amount of buds per cane or the cane needs to be cut to same length as genotypes can have different internodes length. Canopy management can influence the vine balance, shading, and light interception during the season, therefore shoot trimming, desuckering and cluster thinning should be done consistently.

6.2 Approaches towards effective data acquisition

Effective data acquisition is influenced by the set up of a phenotyping pipeline. The choice of sensor is based on the trait that has to be evaluated. Besides the selection of the environment (growth chamber, greenhouse, foil tunnel, and field) in which the trait should be screened it is crucial to determine a time interval for the evaluation. Data acquisition can be done using different platforms (fully automated indoor applications and ground-based, aerial-based or satellite-based outdoor applications). Furthermore, data handling and the method for data analysis can influence the efficiency of data acquisition.

The main focus of this study was the use and development of modern phenotyping approaches taking phenotyping of yield parameters as an example. The first objective was the use of visible light sensors as cost-efficient and fast sensors. This was realized in two different approaches on a laboratory and a field level. In a first step towards HT phenotyping yield parameters were broken down using a controlled environment concept of RGB image analysis (CAT (Kicherer et al., 2015c) and BAT (Kicherer et al., 2013)) and in a second step a camera system was used on the field phenotyping platform. The general goal of this scientific work was the set up of a field phenotyping pipeline for grapevine breeding. The realization of this goal led to the first phenotyping pipeline for field application in grapevine breeding. Data acquisition was done automatically using the PHENObot. This phenotyping platform consists of a chain vehicle containing a control unit and a camera-light unit in combination with an industrial computer (Kicherer et al., 2015a). Every image is uniquely assigned to a single grapevine due to the geo-information and the associated plant ID. All images are stored in a database based on the image names, which contain the plant ID. Image analysis was done using the MATLAB®-based tool, BIVcolor (Berries in Vineyards-color) (Kicherer et al., 2015a).

Sensors

In our approach visible light sensors have been used to capture yield parameters. On the one hand a SLR camera has been used for the image acquisition in the laboratory under controlled environment (Kicherer et al., 2013; Kicherer et al., 2015c) on the other hand a multi-camera-system has been used on the PHENObot for image acquisition. The multi-camera-system consists of three monochrome (MC) cameras, one RGB camera and one NIR camera. To enable an adequate illumination for standardized image acquisition, a lightning unit containing eight LED bars was combined with the camera unit (Kicherer et al., 2015a). According to the desired application, the available sensors for effective data acquisition in modern plant phenotyping can have different advantages and disadvantages. Using VIS sensors enables a fast 2D–imaging at an affordable cost level, enabling not only the determination of traits such as size based on geometric information, but also radiometric information (MC, RGB,NIR). Therefore, the use of visible light sensors to evaluate yield parameters under controlled and field environments in grapevine are the best available. In addition to the acquisition of 2D information 3D depth information can be helpful.

3D-imaging based on the multi-camera-system on the PHENObot was used to remove the background within the automated detection of dormant pruning wood area (Kicherer et al., 2015b). It is conceivable that 3D depth information gained through a stereo vision approach can also be used to further improve the size evaluation of the BIV (Roscher et al., 2014), respectively the BIVcolor (Kicherer et al., 2015a). Besides the stereo vision approach, laser scanners provide a high 3D accuracy that enable the construction of plant parts and their modelling (Paulus et al., 2014). But on the other hand complex data reconstruction is required and for some laser instruments a specific illumination is needed, moreover long acquisition times are needed what makes field application even more difficult (Dhondt et al,. 2013).

Furthermore, the NIR camera within the multi-camera-system of the PHENObot could be used to remove objects with low reflectance and high absorbance in near-infrared in the image like the sky or the ground. The challenge is to match the two images from the different cameras due to the different size of their sensors. Nevertheless, the registration of both images is possible using a suitable matching algorithm and an interest operator to find

corresponding points in both images (McGlone et al., 2004). Moreover, if the relative orientation of both cameras is known beforehand, the images can be matched directly to each other.

Sensor platforms for field applications

Vineyards consist of perennial plants trained as vertical crops. Therefore, a ground-based evaluation of yield parameters is urgently crucial. The PHENObot (Kicherer et al., 2015a; Schwarz et al., 2013) consists of an automated, ground-based, tracked vehicle system. Tracked systems like that use one or more pairs of tracks, which rotate simultaneously. These are usually slower but have the ability to overcome obstacles like rocks or rough uneven grounds easier, which is crucial to operate in the vineyard. Due to the large surface contact area tracked systems have a high stability on the one hand and simultaneously a higher energy need. In return these systems are able to carry for instance 200-500 kg (Longo et al., 2011). Currently the PHENObot stops for image acquisition. The efficiency of data acquisition could be improved through a real-time image acquisition system capturing images or respectively videos while moving. Therefore, a more expensive hardware would be needed and software application would need to be adjusted to real time image capturing. Furthermore, it would be more difficult to link images to single vines as the platform would move during image acquisition.

Other existing designs for multi-terrain automated ground-based vehicles are wheeled (Nasa Facts, http://www.jpl.nasa.gov/news/fact_sheets/mars03rovers.pdf; Herzog et al., 2014b) and legged systems (Playter et al., 2006), each having their own strengths and weaknesses. For choosing the system with the highest efficiency to carry out the intended task, four main aspects need to be considered: (1) mobility, a combination of speed and manoeuvrability. Adequate speed to complete the task and manoeuvrability to avoid and overcame obstacles. (2) stability to keep the equilibrium when standing still and during movement. (3) power consumption of the system and (4) costs for installation.

Wheeled systems are the most popular systems because they are cheap and simple. Generally they have a higher speed than tracked or legged systems, while the manoeuvrability depends on the system. They are best suited for even terrains, whereas equilibrium can be a problem in rough terrains. Additionally, in an early stage of

automation (prototype stage) a tractor for instance (Herzog et al., 2014) can still pull a system.

Legged systems can be slow or fast depending on the system, their ability to overcome obstacles is high whereby the stability and the costs are dependent on the number of legs. The most commonly ground-based sensor systems can be tractor pulled like the BreedVision approach (Busemeyer et al., 2013) or just be mounted to an agriculture vehicle (Braun et al., 2010; Llorens et al., 2011; Mazzetto et al., 2010).

Ground-based phenotyping field platforms enable data acquisition at a plot level requiring little post-processing but simultaneous measuring of all plots at one time is not possible. Despite that they are considerably faster than a comparable manual data acquisition, thus being much more efficient and powerful. Compared to ground-based systems areal platforms enable rapid characterization of many plots within a short amount of time (only minutes). It always depends on the research question that needs to be addressed. For some traits like the yield evaluation in viticulture a top view of the whole vineyard is insufficient as the side view of individual vines is desired. For monitoring questions in vineyards like for specific diseases as e.g. Esca areal platforms might be very useful and efficient.

Yield parameters

One objective of this study was the determination of grapevine yield parameters, such as: vines per unit area, cluster per vine (determined through shoots per vine and clusters per shoot), berries per cluster, cluster and berry size, respectively weight, using RGB images and image analysis.

Approaches to dissect the yield parameters berry size and shape are mainly set in the laboratory using image-based methods (Kicherer et al., 2015c; Kicherer et al., 2013; Tardaguila et al., 2013; Wycislo et al., 2008). As a first step towards HT phenotyping of yield parameters in grapevine breeding, approaches like the CAT (Kicherer et al., 2015c) or BAT (Kicherer et al., 2013) provide lots of valuable detailed information on single yield parameters, even if destructive harvest of grape clusters is needed. Both tools work fully automatic, providing four (CAT: cluster length, cluster width, berry size and cluster compactness), respectively three parameters (BAT: berry number, size and volume) per image at once, therefore being faster compared to manual measurements. These precise and detailed data are particularly essential for genetic analysis to describe the variation

between different genotypes. Another procedure was introduced by Federici et al. 2009, using a laboratory terahertz imaging system to differentiate individual grape berries from stems, branches and leaves. With a time requirement of roughly 1 h to image a 7 cm² area (Federici et al., 2009), this method is way too slow.

The evaluation of yield variance of a set of vines is frequently used in precision viticulture (Clingeleffer et al., 2001; Nuske et al., 2014) and can be split into three main yield components:

- number of clusters per vine. Contributes approximately 60% of the variance.
- number of berries per cluster. Contributes approximately 30% of the variance.
- berry size. Contributes approximately 10% of the variance.

The assessment of the exact number of clusters per vine is difficult, no matter if sensor-based or visual estimation approaches are used, especially late in the season due to occlusion through leaves and other grape clusters. Some efforts have been made to detect this parameter although it was not based on counting the number of clusters but more on calculating the amount of fruit pixels (Dunn and Martin, 2004). Diago et al. 2012 estimated leaf and yield areas in different defoliation and yield thinning steps to calculate the amount of grapes on the vine. Font et al. 2014 counted the number of berries on individual red clusters, captured under field conditions.

Another approach is to combine the two parameters, number of clusters per vine and berries per cluster, into one measurement, counting the number of berries per vine (Nuske et al., 2011; Nuske et al., 2014). This method captures only the visible berries, missing out on the occluded ones. However, yield estimations in this study captured up to 75% of spatial yield variance with an average error between 3% and 11% of total yield (Nuske et al., 2014). Furthermore, some approaches have been conducted in the field using hand-held devices to detect the number of berries per single clusters (Rabatel and Guizard, 2007) whereas Grossetete et al. 2012 introduced a Smartphone application for the counting of flowers of single inflorescences in images taken at night. Diago et al. 2013 showed that counting flowers on an inflorescence is possible using a hand-held device with an artificial background and a camera unit. A next step would be the assessment of inflorescences on a whole vine level to gain information of the number of inflorescences per vine and the number of flowers per inflorescence. This should be done at an early stage of development like BBCH 53-55 and can then be used to forecast the number of clusters per vine, respectively yield per vine. Furthermore, a method like this could be very helpful for

thinning decisions in minimal pruning systems (Walg, 2014) where yield reduction using a grape harvester is essential in an early development stage.

Roscher et al. 2014 implemented the first automatic tool to detect the berry size out of images taken in the field. The characterization of multiple traits in a single pass is a crucial step to increase the sample throughput. Therefore this tool was extended to additionally assess the berry colour by Kicherer et al. 2015a.

As with most perennial plants, vineyard yield fluctuates significantly from year to year and is largely determined by bud fruitfulness. The induction and differentiation of inflorescences primordial for next year`s crop begins soon after bud-break of the current season (Cunha et al., 2010). The microclimate around and within the canopy could be related to fruitfulness, therefore Cunha et al. 2010 used a satellite-based remote sensing approach for the detection of NDVI in the previous season and used it to detect yield. The prediction model explained 77-88% of inter-annual variability in wine yield.

Although vine balance is not strictly a yield parameter it is indeed closely related to yield and was therefore considered in this thesis. Vine balance is defined as the relation between vegetative and generative growth. It can be expressed as relation between grape yield and the dormant pruning wood weight. The assessment of dormant pruning wood weight is traditionally a very time consuming and laborious task done manually in the field. For fast, low cost, and robust, high-throughput and objective acquisition of pruning weight a fast ground-based sensor method using cameras was developed by (Kicherer et al., 2015b). To avoid the use of an artificial background, a 3D stereo reconstruction and calculation of depth maps approach has been used. So far stereoscopic approaches have been used to construct plant models (Andersen et al., 2005; Biskup et al., 2007; Biskup et al., 2009), for reconstruction of aerial images (Kuschk and Cremers, 2013) or driver assistance systems (Ranftl et al., 2012). Capturing pruning wood in a vineyard images are not taken from above but within the row, with a bunch of disturbing, similar looking vines in the background. The application of stereo vision methods to remove the background was first introduced by Herzog et al. 2014 and Klodt et al. 2015 in an approach to acquire grapevine canopy dimensions. This method has been adopted to detect the much smaller parts of grapevine dormant pruning wood (Kicherer et al., 2015b). It was shown that the automatically image-based detected pruning area using depth segmentation was linearly correlated with the field measurements of pruning weight. Additionally, it was principally shown in this study that this new inexpensive and time saving method together with other selection criteria is suitable to validate the yield potential of seedling selections. Further

efforts have been made to detect the dormant pruning wood weight using remote sensing (Dobrowski et al., 2003) or laser scanner (Tagarakis et al., 2013). Whereas the resolution of 1 x 2 m in the remote sensing approach is too big to evaluate on a single vine level and the laser scanner approach is too time-consuming compared with the a stereo vision system used by Kicherer et al. 2015b.

6.3 Approaches towards effective data management and analysis

An additional important objective of this thesis was the automated data handling within the phenotyping pipeline. The application of the PHENObot (Kicherer et al., 2015a) within the grapevine repository at Geilweilerhof showed that the quantity of data that needs to be stored and the processing requirements need to be taken into consideration. A set of five images (one RGB, three MC and one NIR image) generates 30MB of data, screening around 3000 vines sums up to 90 GB for a onetime screening. This number can increase very fast by increasing the sample number, the frequency of screenings throughout the season, the amount of sensors or the type of sensors. For the organization of sensor data the linkage to the geo-reference in the field can be useful (Andrade-Sanchez et al., 2013; Hall et al., 2002; Llorens et al., 2011). It has been shown in this scientific work that using databases (IMAGEdata and PLA) to handle images and geo-references is the best way to link images to single vines in the field, respectively single genotypes (Kicherer et al.,2015a). Furthermore, it is planned to connect the existing databases, like *V*IVC (*Vitis* International Variety Catalogue; http://www.vivc.de/) and the European *Vitis* Database (http://www.eu-vitis.de/index.php), to complete the linkage of available information.

The development of wireless sensor networks to characterise the environmental conditions (climatic and soil moister status) to enable real-time monitoring would be helpful to increase the efficiency of data acquisition in the field (Araus and Cairns, 2014). As part of the CROP.SENSe.net project a quality management system for HT phenotyping experiments was established (http://www.fz-juelich.de/ibg/ibg-2/DE/Projekte/_bund/cropsense_net/cropsense_net_d1/D1_node.html) combining the basic data of the experiments, plant history as well as phenotypic and genotypic data.

The extraction of phenotypic traits from HT phenotyping experiments requires an automated image analysis framework in order to extract important relevant biological information from the images. In the best case scenario a huge number of phenotypic traits are quantified from a single image to increase the efficiency as shown for CAT (Kicherer et al., 2015c), BAT (Kicherer et al., 2013) and BIVcolor (Kicherer et al., 2015a). The most labour intensive step within the process of developing an automated image interpretation tool is the automation itself. Image processing usually includes four steps: (1) preprocessing, (2) image segmentation, (3) feature extraction, and (4) postprocessing. Preprocessing is done to make images from different cameras or acquired at different times throughout the development stage comparable by correction of the orientation, adjusting the brightness and irregular illumination, reducing the noises of images, and adapt different zoom changes (Klukas et al., 2014). Within the image segmentation step regions of interest are separated from the background. Based on these results different plant features like the size, shape, texture or colour are extracted from the image data. Postprocessing mainly contains summarizing of the results and statistic analysis.

A set of software applications has been developed to analyse at a whole plant level (De Vylder et al., 2012; Green et al., 2012; Hartmann et al., 2011; Klukas et al., 2014) or on base of different plant organs (Pound et al., 2013; Tanabata et al., 2012; Weight et al., 2008) in controlled environments. This software uses different algorithms to detect a wide range of plant architectural and physiological parameters from images captured with camera sensors. In field based phenotyping there is not that much software available. Most of the tools are designated to that special set up and question of one specific trait and are not yet able to detect as much different traits in one image than the ones developed for controlled environments.

Due to the variety of existing tools and the lack of a central repository it is challenging for researchers to identify, the software that is best suited for their research. Therefore an online database of image analysis software tools (Lobet et al., 2013) has recently been established comprising mainly tools to analyse images acquired under controlled environments (growth chambers, greenhouses). Furthermore an open source framework for high-throughput plant phenotyping, the Integrated Analysis Platform (IAP) has been published (Klukas et al., 2014). Comparable database or frameworks for sensor data (images, spectral data) acquired in the field is required. Although it is going to be difficult to provide an overall solution as different plant species require different setups to acquire different desired traits. A start could be the establishing of so-called “benchmarks” as done

the first time for a set of grapevine images by Kicherer et al. 2015a. A benchmark is a set of images and associated results that has been used to establish a new image-analysing tool, which is available for the scientific community. These benchmarks are commonly used within the image analysing community to compare and test different algorithms for a specific trait. If the same set of images is used to test different methods to extract a phenotypic trait the methods are more comparable and reliable and it is possible to decide which methods work best on this set of images.

The methods used need to be rapid and robust as well as the equipment. Instruments need to be robust to environmental conditions like temperature, humidity and dust. Data modelling and bioinformatics are becoming crucial to reduce the complexity of the phenotypic landscape and to generate new hypotheses (Fiorani and Schurr, 2013). A good data management routine is essential. For some of the numbers obtained through sensor-based approaches we do not even have a physical concept of what they mean in terms of plant performance (Cobb et al., 2013). Most of the data gained is just a mathematical transformation of numbers. Some linear combinations of them might have some significant correlations with important traits, for reasons we do not yet understand (Araus and Cairns, 2014). Multispectral imaging for example provides information about plant parts and physiological stage that is not visible to humans. For the validation of the biological utility of gained sensor data the acquisition of "ground-truth" data is therefore strongly required. This can be done by conventionally used instruments, laboratory evaluations or traditional visual estimations. Furthermore the standardization of the implemented phenotyping method is crucial to ensure reliability of the data collection.

Eventually, integrating competences through building interdisciplinary teams including plant biologist, physicists, mathematicians and engineers is very important to achieve a successful plant phenotyping set up.

6.4 Further applications for grapevine breeding

Another aim of this thesis was the interpretation and evaluation of such gained sensor data for grapevine breeding. The utilization of modern phenotyping methods has been shown to help evaluating the yield potential of seedling selections (Kicherer et al., 2015b) and the phenotyping pipeline can be used to assess a grapevine repository for the two important traits berry size and berry colour (Kicherer et. al., 2015a). Furthermore the information captured with CAT (Kicherer et al., 2015c) and BAT (Kicherer et al., 2013) can be used for QTL analysis. This section refers to the opportunity of further applications in grapevine breeding concerning additional sensors, different platforms and further traits.

Sensors

In addition to the extraction of the berry colour (Kicherer et al., 2015a) RGB images can be used to quantify the senescence, arising from nutrition deficiencies, toxicities or pathogen infection as shown in barley (*Hordeum vulgare* L.) (Schnurbusch et al., 2010). Although VIS offers no advantage in sensitivity over the detection of symptoms by eye, it provides an HT-technique to quantify areas of lesions or chlorotic areas of leaves. Compared to other sensors the physiological information is limited. In contrast the detection of fluorescence can provide more information on the photosystem II and photochemistry *in vivo* whereas fluorescence measurements on whole plants or shoot analyses can be complicated and pre acclimatization of plants is required. Whereas thermal and spectral imaging sensors passively acquire radiation and reflectance data, fluorescence is actively recorded at specific wavelength after induction by laser or light (Dhondt et al., 2013).

2D thermal-imaging enables a rapid measurement method to determine information on transpiration and heat dissipation but these measurements are influenced by numerous factors and sound physics based interpretation of results is needed.

A large amount of information is provided by generating spectral 2D information (NIR, multi- or hyperspectral). The sensors are capable of scanning wavebands of interest at a high resolution, in particular around the peak of green reflectance at 550 nm, water absorption bands in near-infrared and mid-infrared region (Ustin and Gamon, 2010), strong bands at 970 nm, 1,200 nm, 1,450 nm, 1,930 nm, and 2,500 nm (Knipling, 1970; Munns et al., 2010). Spectral information is often used to calculate vegetation indices reducing the multi-wave–band data at each image pixel to a single numerical value (index) to describe the vegetation vigour. The most commonly used index is the Normalized Difference Vegetation Index (NDVI) using the reflectance of the vegetation at red and near-infrared wavelengths. A disadvantage of this sensor is the extensive calibration needed, they are more expensive and they create a large set of image data, especially hyperspectral (more than 10 wavebands per pixel) data interpretation is very complex.

Other recently applied sensors in modern plant phenotyping like X-ray, MRI, and PET (Fiorani and Schurr, 2013) facilitate root analyses and the dissection of macroscopic traits with the possibility to link them to microscopic ones. These methods are very expensive and can only be used in controlled environments so far.

Sensor platforms for field applications

In addition to the ground-based approaches, represented through the PHENObot (Kicherer et al., 2015a), remote satellite-based and aerial concepts are available. Remote sensing traditionally describes measuring features on the earth surface using satellite and aircraft–mounted sensors (Hall et al., 2002). The size of the sensor (number of image-forming pixels) and the distance from the ground, respectively the object, contribute to determine the pixel size on the ground (object) and the overall displayed image section (Hall et al., 2002). For satellite-based platforms that means for example operating at a height of 705 km above the earth's surface (American Landsat satellite), recording a 185 x 185 km section with a 30 x 30 m pixel size. Compared to that the French SPOT satellite orbits at 832 km, generating full scenes of 60 x 60 km and a 20 m pixel or moreover high-resolution satellites can provide a 4 m resolution (IKONOS) (Hall et al., 2002). However, the cost of such data in most cases remains a significant disadvantage to its widespread use (Lamb et al., 2001).

Sensors on manned airborne platforms are used 3 km above the ground can therefore deliver 1-2 m pixels and scenes of 100 ha (Lamb, 2000) which is closer to desired resolution of grapevine management but not grapevine breeding where sensor data needs to be broken down to a single vine level. When using remote satellite information it is also important to reconsider how often data acquisition needs to be done as the typical commercial satellites have a revisit interval of 15-25 days, respectively (Hall et al., 2002). Aircraft or ground-based platforms have the advantage on the other hand that they can theoretically be operated at any time and have the added advantage to not being influenced by a high cloud base.

Remote sensing sensors placed on unmanned aerial vehicles (UAVs) could fill this gap, providing low-cost approaches with a greater flight control and autonomy (Araus and Cairns, 2014) and a better ground pixle size for grapevine breeding approaches of single vine monitoring.

Traits

One of the most important breeding goals in grapevine breeding is the resistance to different disease. Therefore, the screening for resistances is a main task within the breeding program. In a classical breeding approach (Figure 1) the resistance screening for *Plasmopara viticola* and *Erysiphe necator* are done in the greenhouses by inoculation and the following visual screening, besides MAS and MABC (marker assisted back crossing), for staging of resistance or defining crossing parents. At this stage of the breeding program it would be helpful to have a non-invasive and sensor-based method to quantify disease symptoms on the plants in the greenhouse. On the other hand, the evaluation of disease infection under field conditions would be interesting not only for breeding purposes but also for vineyard management decisions. Some efforts have been made to quantify *Erysiphe necator* and *Plasmopara viticola* infected leaves (Boso et al., 2004; Li et al., 2012) and leaf discs (Peressotti et al., 2011) using image analysis. In addition, Meunkaewjinda et al. 2008 proposed an image processing approach to classify infected grapevine leaves into three classes. Moreover, chlorophyll fluorescence has been used to detect pre-symptomatic *Plasmopara viticola* infection on potted grapevines (Cséfalvay et al., 2009). The detection of disease in the field is difficult as the different diseases can show different signs and symptoms depending on the grape variety, the stage of grape

development, and the severity of the disease. Furthermore, multiple diseases might be present at once and other symptoms like sunburn, nutrition deficit or other abiotic stresses might be masking the disease symptoms. Remote sensing approaches using the NDVI to monitor canopy health and vigour showed a good correlation to identify rows with *Plasmopara viticola* infections (Mazzetto et al., 2010). Methods to identify diseases should be able to take more than two diseases into account and at its best being able to distinguish between different diseases. Multi- and hyperspectral approaches are most likely the promising methods in that case.

Cluster architecture is another parameter not only influencing the yield but also responsible for the health status as *Botrytis*, grape bunch rot, is one of the biggest problems in viticulture linked to a especially high risk with compact cluster architecture. The evaluation of cluster architecture has only been done under controlled environments so far (Cubero et al., 2015; Kicherer et al., 2015c). With the utilization of CAT (Kicherer et al., 2015c) the compactness of a clusters can be fast and easily detected and used for genetic studies.

Quality assessment of grapevine is closely linked to yield parameters. Indicators of ripeness like the phenolics, flavonoids, sugars, acids and aroma compounds are important traits. By measuring chlorophyll fluorescence the amount of anthocyanins and therefore the different degrees of pigmentation in olives (Agati et al., 2005) and grapes (Agati et al., 2013) has been detected during ripening. The chlorophyll index measured on grapes with the Multiplex was inversely correlated in a linear manner to the total soluble solids (°Brix), Agati et al. 2013 suggested that it could, therefore, be used as a new index of so-called technological maturity.

Biomass, germination time, and growth rate depend on the seed mass (Fiorani and Schurr, 2013). The germination rate of grapevine seeds is around 50%, therefore, using sensor assisted selection the quantitative detection of germination, respectively seed mass, could be an interesting trait for grapevine breeding. Knowing which seeds will germinate and which won’t can save time and costs. Moreover, quantitative analyses of this trait may reveal that small differences in seed mass can explain variation in relative growth rates that would normally be interoperated otherwise as shown in *Arabidopsis* (Tholen et al., 2004).

The phenological stages of grapevines are influenced by several environmental factors and viticultural practice. Measuring the phenological stage of grapevines is important to

evaluate the effects of the local environment and climatic changes and subsequently wine quality. Identification of the developmental stage is basically done by visual estimation using the BBCH scale (Lorenz et al., 1995). Stages that would be interesting for breeding are the time of bud burst, flowering, veraison and ripening. Under controlled environments first steps have been made to determine ripening using fluorescence (Agati et al., 2013) and images of single berries (Rodríguez-Pulido et al., 2012). Kicherer et al. 2015a suggested that the BIVcolor could be used to detect the time of veraison in the field. A semi-automated approach to quantify bud burst in images acquired in the field was suggested by Herzog et al. 2015. In table grape production the variation of the time of ripening is important to staggering harvest along growing seasons, expanding production towards periods when fruits get higher value in the market. Furthermore, a variation in ripening can be used for a breeding adaption to climatic and geographical conditions. Identifying the genetic factors responsible for phenological and fertility variation may also help to improve the understanding of yield parameters.

6.5 Conclusion and outlook

In the context of high-throughput phenotyping, the present work implemented several valuable laboratory tools to break down yield parameters for genetic studies. Furthermore the first automated field phenotyping pipeline for grapevine breeding was developed. The pipeline consists of an automatic robotic platform to acquire geo-referenced images under field conditions, an image database to handle the data management, and an image analysis framework for the fully automated extraction of phenotypic traits. In particular, the berry size and colour has been assessed in a grapevine repository to show the functionality of the automated, precise and non-invasive phenotyping method and pipeline as a possible tool of sensor assisted selection.

In this particular setup the utilization of the PHENObot enables the assessment of 20 times more individuals compared to manual assessments of berry size. With this analysis proof of principle was demonstrated. The pilot pipeline provides the basis for further development of additional evaluation modules as well as the integration of additional sensors. The overall goal of effective data acquisition is to acquire several traits at once saving time. Therefore, the introduction of additional sensors to be able to detect different

traits within one run is a desirable goal. Through doubling the set of sensors to screen both wine rows at the same time, the efficiency of plant phenotyping in grapevine breeding could be improved even more. Field phenotyping of appropriated parameters starting at this point with using the tools introduced in this study should become an integral and key component in the grapevine breeding process to increase the efficiency of future breeding programs.

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7. All publications of the dissertation

7.1 International journals (peer-reviewed)

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7.2 Journals

Kicherer, A.; Roscher, R.; Herzog, K.; Simon, S.; Förstner, W. and Töpfer, R. 2013. BAT (Beeren Analyse Tool) - ein Werkzeug zur Phänotypisierung von Beerenmerkmalen der Rebe. Geilweilerhof aktuell, Band: 41 (1):6-13.

7.3 Presentations

Kicherer, A., Herzog, K. 2014. High-throughput and precise phenotyping of grapevine traits in vineyards by using non-invasive sensors and automation. 11th International Conference on Grapevine Breeding and Genetics, 28.07-02.08.2014, Beijing, CN.

Kicherer, A. 2013. High-Throughput Phenotyping of yield parameters in the vineyard – first steps. 6. Young Scientist Meeting of the Julius Kühn-Institut. 27-29.11.2013, Quedlinburg, DE.

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7.4 Poster

Kicherer, A. 2015. Application of an automatic phenotyping pipeline to characterize genetic resources. PLANT2030 Status Seminar, 04.-06.03.2015, Potsdam, DE

Kicherer, A. 2014. Setup of a phenotyping pipeline in grapevine breeding. 7. Young Scientist Meeting of the Julius Kühn-Institut. 26.-28.11.2014, Quedlinburg, DE.

Kicherer, A. 2014. Image based evaluation for the detection of cluster parameters in grapevine. 11th International Conference on Grapevine Breeding and Genetics, 28.07-02.08.2014, Beijing, CN.

Kicherer, A. 2014. Image based evaluation of yield parameters in grapevine. PLANT2030 Status Seminar, 31.03-02.04.2014, Potsdam, DE.

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Erklärung

Hiermit erkläre ich an Eides statt, dass die vorliegende Arbeit von mir selbst verfasst und lediglich unter Zuhilfenahme der angegebenen Quellen und Hilfsmittel angefertigt wurde. Wörtlich oder inhaltlich übernommene Stellen wurden als solche gekennzeichnet.

Die vorliegende Arbeit wurde in gleicher oder ähnlicher Form noch keiner anderen Institution oder Prüfungsbehörde vorgelegt.

Insbesondere erkläre ich, dass ich nicht früher oder gleichzeitig einen Antrag auf Eröffnung eines Promotionsverfahrens unter Vorlage der hier eingereichten Dissertation gestellt habe.

Hohenheim 27.04.2015

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