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 evaluation is crucial but time consuming. In the present study a high-throughput image interpretation tool was developed 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 using 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 long-term 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 environmental 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 (DYOSTEM 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

P l a n t   m a t e r i a l : 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.

I m a g e   a c q u i s i t i o n : A black perforated metal 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 derive 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
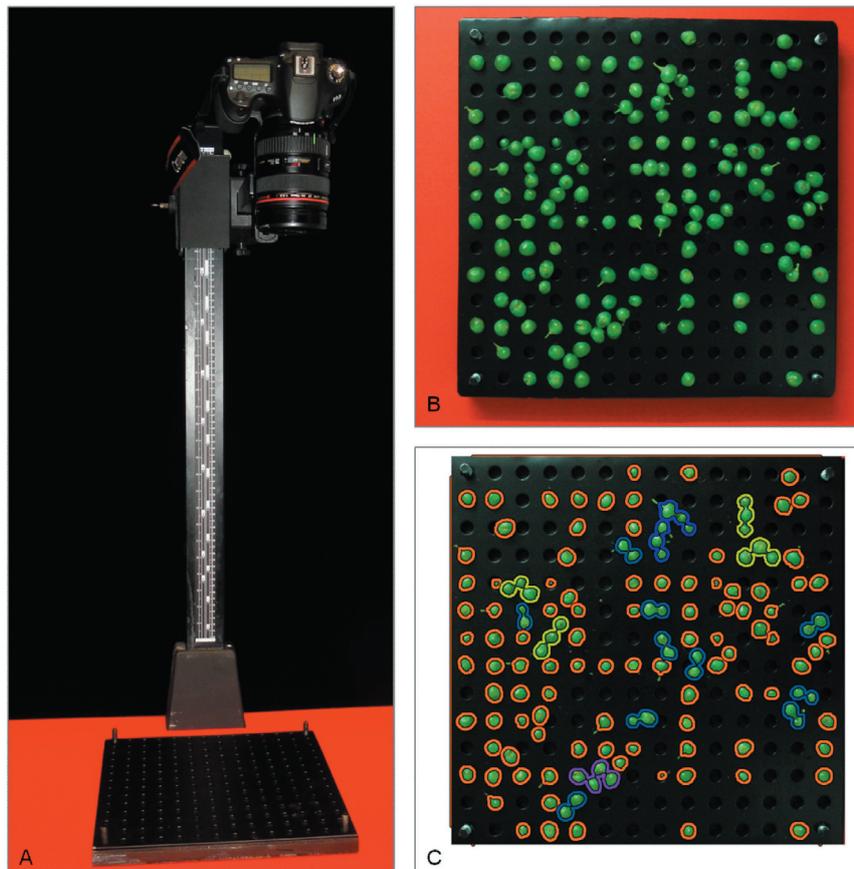

Fig. 1: Image acquisition, detection and quantification of grapevine berries using BAT algorithm. **A**: Image acquisition setup: Camera stand with a DSLR camera, black perforated metal plate (300 x 300 mm), black tray of equal size with bolts positioned in all four edges and the red background **B**: 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.

D a t a a n a l y s i s : Data sets of manually and software based values were analysed by Pearson correlation and ANOVA (Tukey Test). Statistical analyses were performed using SAS 4.3 (SAS Institute, Cary, NC, USA).

B AT (B e r r y a n a l y s i s t o o l) w o r k f l o w : 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_n, 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.

S t e p 1 : D e t e c t i o n o f t h e c o n s t r u c t i o n b o u n d a r y a n d t h e e l i m i n a t i o n o f t h e r e d b a c k g r o u n d : 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 one-dimensional 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 determine 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.

S t e p 2 : C l a s s i f i c a t i o n o f " b e r r y " a n d " b a c k g r o u n d " : 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 Transform (PENG et al. 2007) was used to acquire training data for the class "berry" by detecting distinctive, round objects.
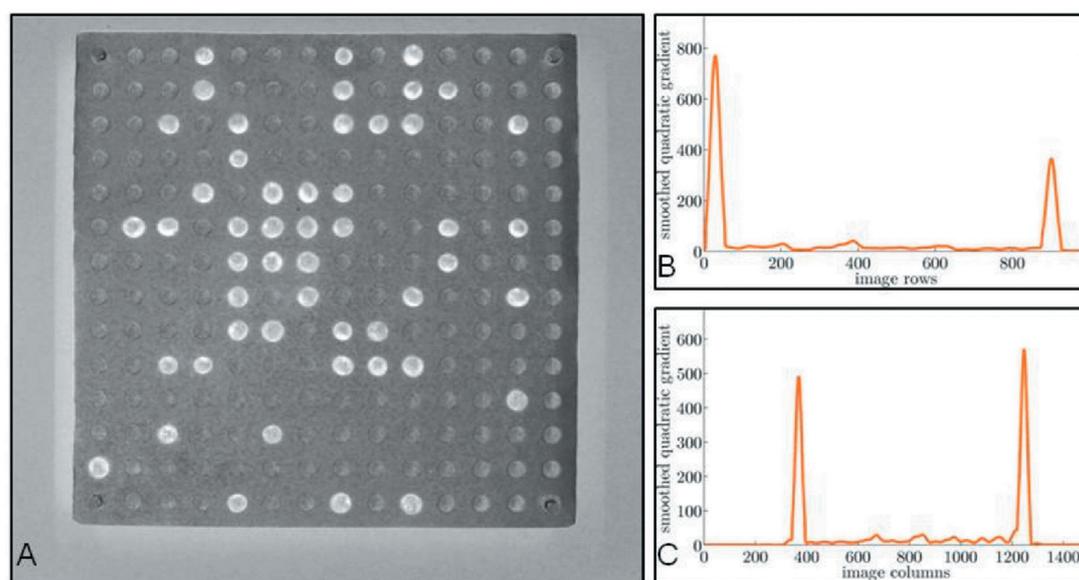

Fig. 2: Sum of the hue and saturation channel (**A**) and the obtained curves of the squared smoothed gradients-image rows (**B**) and image columns (**C**). The two largest values (Peaks) in each curve indicate the border of the metal plate. The difference between the largest values is the length l 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 to remove 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, insects 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 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 berries in mm and volume of individual berries in mL.

Validation of detected berry numbers and 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
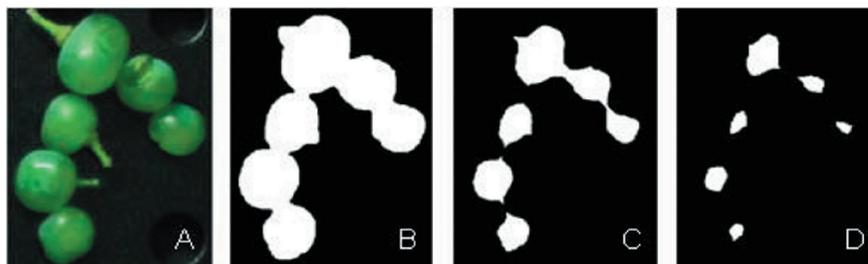

Fig. 3: Separation of single berries which are touched: Successive erosion with a disk-shaped structuring element of increasing size. **A**: Image detail of size 338 x 261 pixel of a detected region containing 6 berries. **B**: Classification result of the image detail after the opening. **C**: Erosion structuring element of size 15 pixel. **D**: Erosion structuring element of size 25 pixel, whereas all subregions $\mathcal{R}_s$ can be detected.
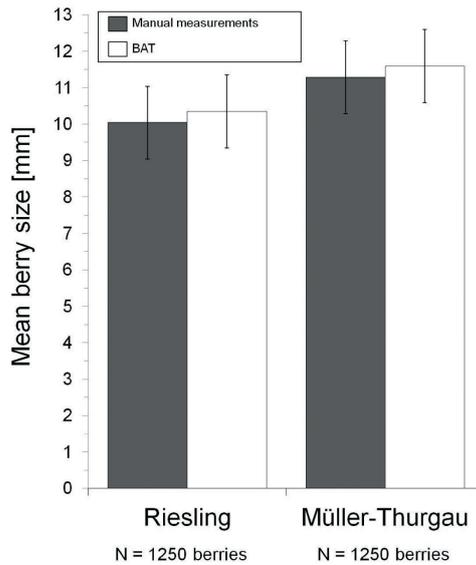
Fig. 4: Comparison of berry size (berry width) determined by manual measurements and by BAT. Error bars represent the standard deviation. An overestimation of 0.3 mm was observed. Difference of mean berry width between 'Riesling' and 'Müller-Thurgau' was 1.25 mm.

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 images: 1,500 grape clusters were destemmed and photographed. 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 overestimated. 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).
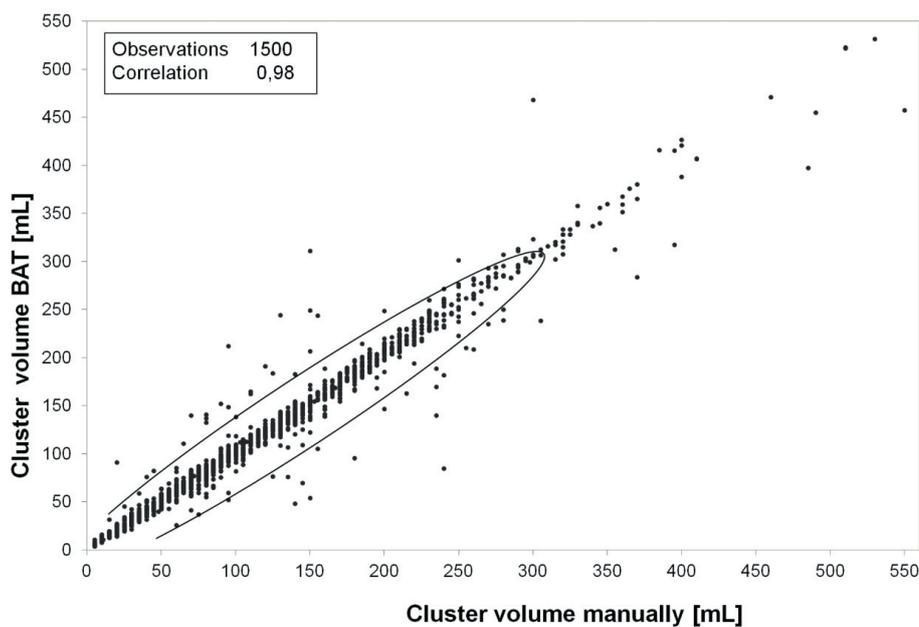

Fig. 5: Showing a correlation plot of the manually measured and BAT-calculated ellipsoid cluster volume (r = 0.98) from 130 F1 plants of the mapping population 'Gf.Ga 47-42' x 'Villard Blanc'. Each point represents the volume of one cluster/image. The density ellipse encompasses 95 % of the data points.

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-throughput. 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 advantage of BAT is the export of berry diameter and berry volume as numbers with dimensional units (mm or ml instead 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 requires 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 dipping 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 positioning 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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