

Genetic analysis of loose cluster architecture in grapevine

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Abstract. Loose cluster architecture is a well known trait supporting *Botrytis* resilience by permitting a faster drying of bunches. Furthermore, a loose bunch enables a better application of fungicides into the cluster. The analysis of 150 F1 plants of the superior breeding line GF.GA-47-42 ('Bacchus' x 'Seyval blanc') crossed with 'Villard blanc' segregating for compactness of the cluster was used for QTL analysis. Plenty of QTL were identified reproducibly for two years, QTLs stable over three growing seasons were identified for rachis length, peduncle length, and pedicel length. In a second approach 'Pinot noir' clones showing variation for cluster architecture were analyzed for differential gene expression. Grown in three different German viticultural areas, loose versus compact clustered 'Pinot noir' clones showed in gene expression experiments a candidate gene expressed fivefold higher in loosely clustered clones between stages BBCH57 and BBCH71.

1. Introduction

The phyto-sanitary status of grapevine fruits is positively influenced by a loose architecture of bunches (Thoma 1995). The extended distance between the berries leads to reduced physical stress on the berry cuticula. The effect of berries squeezing each other is avoided. In the epidermis of berries the formation of micro cracks induced by droppable water is reduced. Loose cluster architecture allows an accelerated air exchange and supports efficient and fast drying-off in the succession of high humidity or rain fall. In this way a physical barrier is established against humidity-affected diseases like *Botryotinia fuckeliana*, teleomorph of *Botrytis cinerea*. In addition, loose cluster architecture allows a better application of fungicides into the cluster.

Several options exist to achieve a loose cluster architecture. Shoot trimming (e.g., Bondada et al. 2016) is one option to be applied in existing vineyards. For new plantations, however, loosely clustered clones of a particular cultivar are a less labour consuming option. Several 'Pinot noir' clones are available and represent an excellent example for variation within clonal material (Thoma 1995). Consequently, during the selection of new grapevine varieties breeders evaluate cluster architecture and select individuals showing loose clusters. A very new example is the breeding strain Gf.1993-22-6 (denominated as 'Calardis blanc', https://www.julius-kuehn.de/media/Veroeffentlichungen/Flyer/Rebsorte-Calardisblanc_Gf.1993-22-6.pdf) which shows very good *Botrytis* resilience due to loose clusters and tight berry skin. Calardis blanc is a descendent of the selection GF.GA-47-42 bred by Gerhardt Alleweldt in 1964 which is used in this study (see below). To systematically improve the selection efficiency in breeding programmes we set to investigate the genetic determinants of loosely clustered grapevine phenotypes. For this purpose two different approaches are followed: (1) The cross of breeding line GF.GA-47-42 x 'Villard blanc'

resulted in 150 F1 individuals which segregate for the trait of cluster architecture. (2) Differential gene expression experiments with loosely and compactly clustered 'Pinot' clones grown in three different German viticultural areas.

2. Material and methods

150 F1-plants of GF.GA-47-42 crossed with 'Villard blanc' was used for phenotyping. Gf.GA-47-42 is an elite breeding line originating from 'Bacchus' x 'Seyval blanc' (Zyprian et al. 2016) showing loose clusters and excellent wine quality. F1-plants and the parents grown at Geilweilerhof were evaluated for cluster traits determining various length, size and volume parameter (Richter et al. in preparation). Evaluations were done for at least two years.

Clonal material as indicated in figure 1 of 'Pinot noir' was used. In all cases 125 AA was used as the rootstock and plants were at least 10 years old. In order to identify effects independent of the location and the season 'Pinot noir' clones grown at three places and for the seasons, 2015, 2016 were investigated. First location was at the vineyards of the JKI Institute for Grapevine Breeding Geilweilerhof (Siebeldingen, Pfalz), second location was at the vineyards of Antes Rebenverdelung (Heppenheim, Hessische Bergstraße), and third location was at Reben Sibbus GmbH (Sasbach-Jechtingen, Baden).

3. Results and discussion

'Pinot noir' clones showing differences in their cluster architecture grown at three different places in Germany as indicated in Fig. 2 were studied. The phenology was carefully monitored during the years 2015 and 2016. In parallel measurements were done to determine the length of rachis, peduncle pedicel, and to determine further subtraits of cluster architecture. It became evident that the growth gain measured in weekly intervals was higher in clones that show a loose cluster architecture (Fig. 3). In

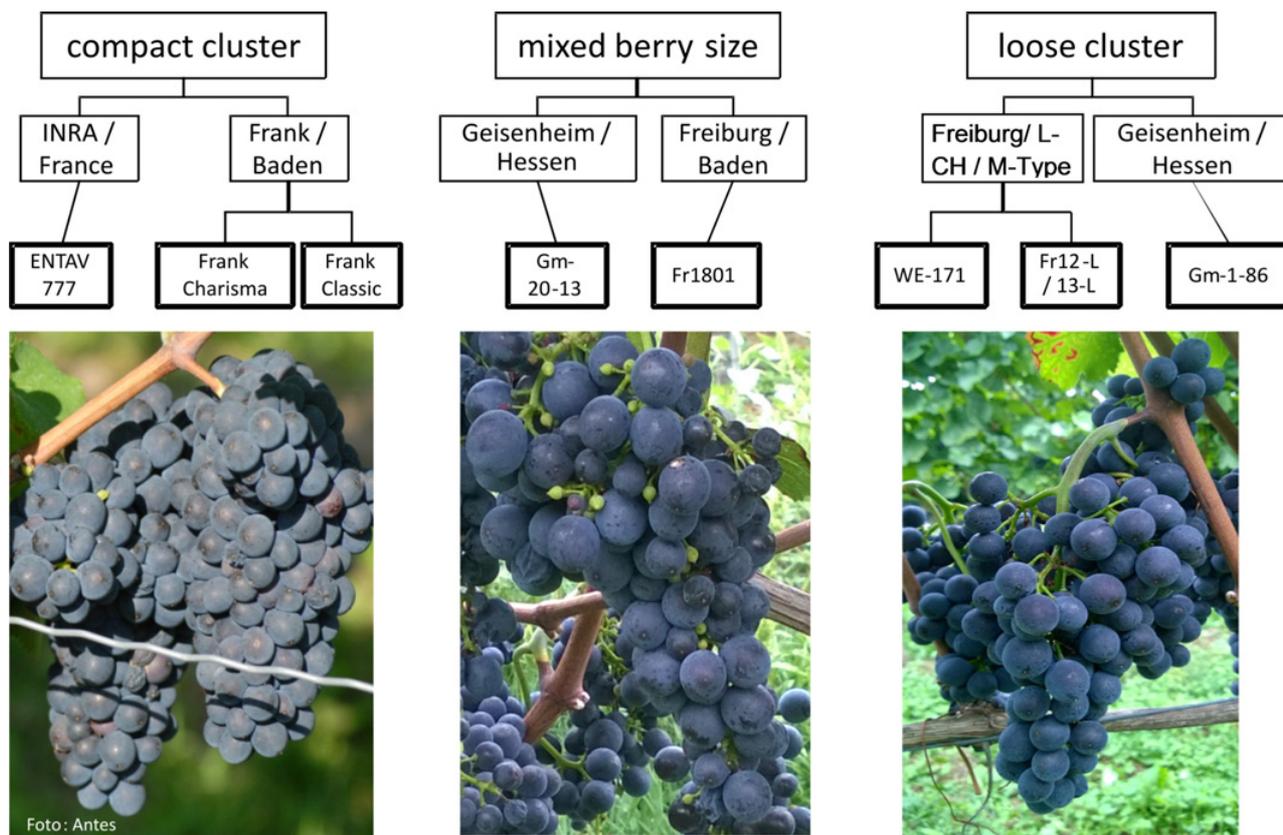


Figure 1. ‘Pinot noir’ clones used in this study and examples for their cluster architecture. The variation range is from compact clusters (left), to mixed sized berries (middle), and loose cluster architecture (right).

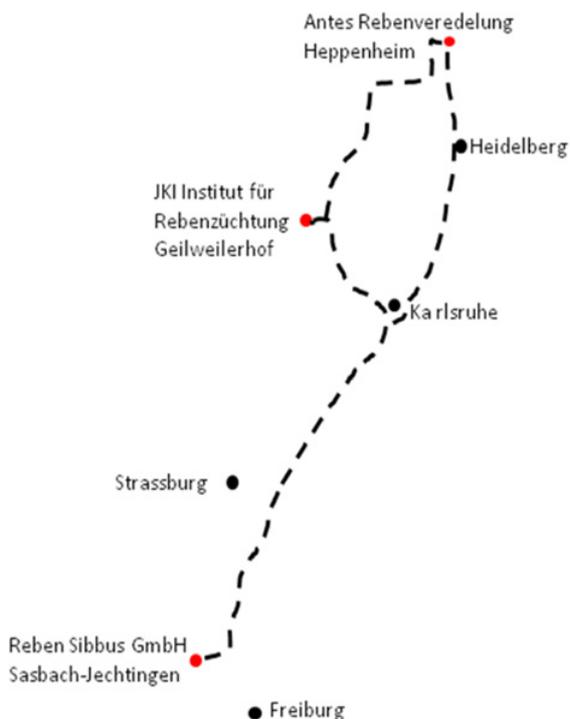


Figure 2. Locations (Antes, JKI, Sibbus) for sampling ‘Pinot noir’ clonal plant material. The distance between Heppenheim and Sasbach-Jechtingen is around 200 km.

2015 the rachis during June grew 40 to 45 mm within three weeks while in compact variants the growth gain was 20 to 25 mm. In the following year the rachis grew

about 35 mm within three weeks in loose cluster clones while the compact variants again showed a growth rate of about 25 mm in three weeks. Thus, in both seasons weekly measurements of the increase in length of the rachis revealed a reduced growth in compact and mixed berry size clones versus Pinot clones showing loose clusters (Fig. 3).

In a concomitant RNAseq approach (Rossmann et al. in preparation) experiments were performed on the ‘Pinot noir’ clones differing in their cluster architecture. Several differentially expressed genes were identified. One of the genes was investigated in more detail during the seasons 2015 and 2016. Elongation of the rachis was recorded as a function of time. The comparison of gene expression of the candidate gene identified in RNAseq studies at BBCH71 in reference to glycerolaldehydphosphate dehydrogenase (GAPDH) reproducibly revealed an altered expression pattern. The candidate gene was expressed higher in loose clusters compared to either compact ones or those that showed the mixed berry sized phenotype. Despite of the correlation further evidence needs to be generated to prove the causal link between candidate gene and phenotype. In a complementary approach a genetic analysis was initiated. For that purpose a F1 population GF.GA-47-42 x ‘Villard blanc’ was used (Zyprian et al. 2016). Several sub-traits of cluster architecture were phenotypically characterized each year in this population.

The phenotypic data were used for the computation of QTLs in the genetic map for GF.GA-47-42 x ‘Villard blanc’ which consists of 543 loci. So far more than 20 QTLs related to sub-traits of cluster architecture were reproducibly found. These QTLs were stable over two years and they are dispersed throughout the genome. QTLs

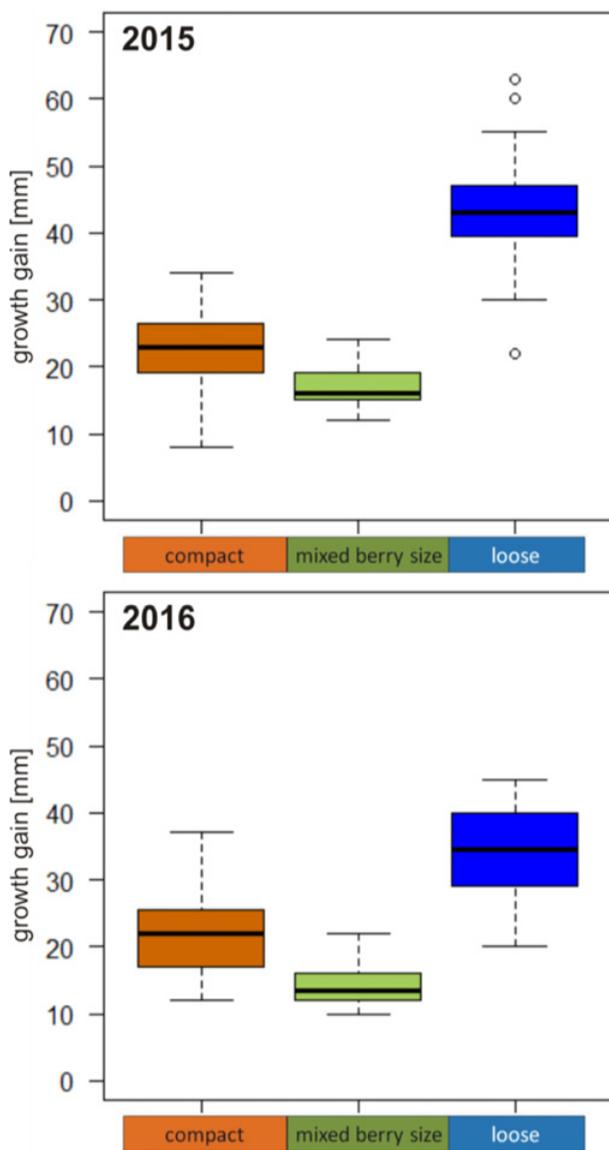


Figure 3. Growth gain of the rachis during June 2015 and June 2016, respectively. The rachis in loose clusters grows faster per week than that of compact clusters. As a consequence their rachis becomes longer than that of compact clustered clones.

stable over three growing seasons could be identified for the sub-traits rachis length, peduncle length, and pedicel length indicating the importance of these subtraits for cluster architecture in ‘Pinot noir’ clonal material.

The molecular markers flanking QTL will be used to project the corresponding genetic regions onto the physical map of the grapevine reference genome. These regions of the reference genome will further be screened for candidate genes.

References

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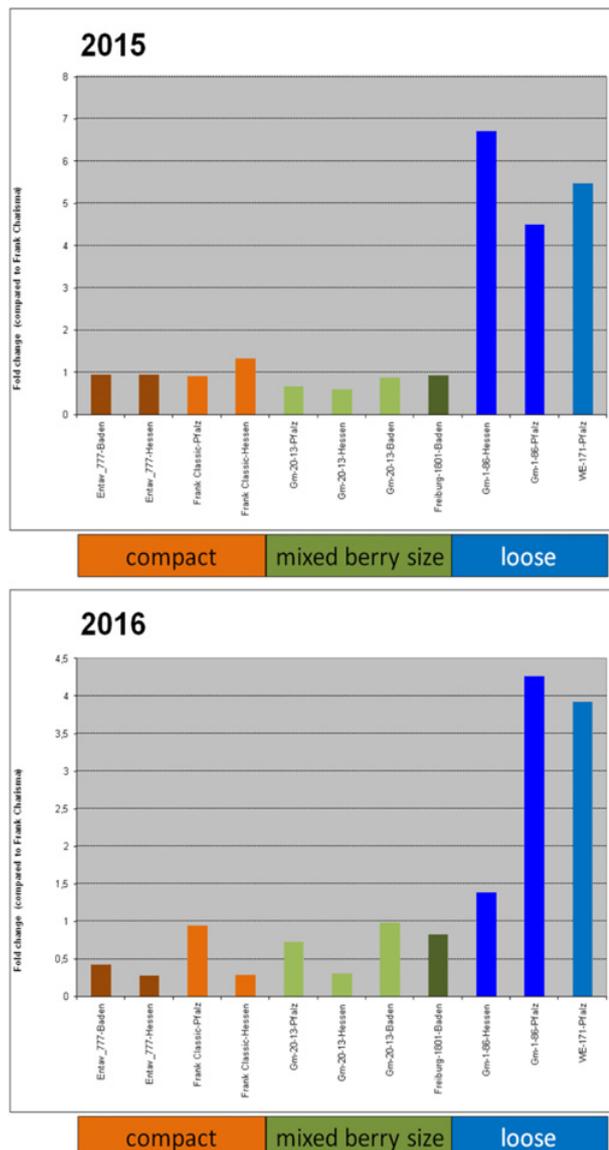


Figure 4. Expression pattern of a candidate gene at BBCH 71 in compact, mixed berry size and loosely clustered ‘Pinot noir’ clones. The candidate gene follows the grouping and is stronger expressed in loose clustered clones. It needs to be figured out whether or not there is a causal link between candidate gene and phenotype.

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