

Epigenetics: an innovative lever for grapevine breeding in times of climate changes

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Context: Climate change results in erratic weather conditions, which may lead for many crops including grapevine, to a reduced production and products of lower quality. Concerning grapevine, climate change results in shorter growing seasons and dates for budbreak, flowering and fruit maturity occur earlier in many regions. It also leads to an increase of various pests and diseases, as well as the vectors responsible for disease distribution (Mira de Orduña, 2010).

A major objective of this century's agriculture is therefore to generate crops able to face the consequences of climate changes. In grapevine, as for other crops, this problem can be tackled through plant classical breeding, which relies on genetic diversity for the development of more resilient crops. However, intense breeding has reduced genetic diversity for many crops therefore limiting the efficiency of classical breeding, and for others with a long reproduction phase, such as grapevine, it is an extremely long process. In this context, epigenetic diversity now emerges as a new source of phenotypic variations. Heritable epigenetic variations, which occur independently of DNA sequence changes, have been associated with modification in gene expression and stably inherited phenotypes (Pikaard and Scheid, 2014), and may provide a new lever for crop improvement by acclimating plants to stresses or by generating epigenetic allelic diversity (epialleles) (Gallusci *et al.*, 2017).

Objectives of the review: In this review, we briefly describe the stresses that climate changes impose to grapevines, before presenting the current knowledge of the epigenetic plant memories of stresses, and discuss how this can be used as a new lever to improve grapevine tolerance to stresses. We also discuss ways to generate epigenetic diversity by exploiting the specificity of grapevine, a grafted, perennial clonally propagated, woody plant. Finally, innovative approaches to develop Epi-breeding strategies in grapevine, which would allow directly using elite varieties to generate phenotypic diversity independently of sequence variations, are presented. These approaches will provide innovative and more rapid ways for grapevine improvement facing climate changes.

Epigenetic in grapevine: current state of the art: There has been limited study of epigenetic regulations in grapevines (Fortes and Gallusci, 2017). A first description of the fruit methylome has been performed, but did not reveal major changes in levels or distribution of DNA methylation

(Shangguan *et al.*, 2020). There is however accumulating evidence that epigenetic mechanisms participate in the complex dialogue established between graft partners (Rubio *et al.*, 2022), are involved in the phenotypic plasticity and clonal diversity (Varela *et al.*, 2021), and contribute to the memory of abiotic stresses such as UV-B or drought (Marfil *et al.*, 2019). In addition, the leaf DNA methylation landscape seems to be determined in part by the regions and vineyard management (Xie *et al.*, 2017), even though the contribution of the parental origin of plants is also likely.

Plant epigenetic responses and memories of stresses: It is clearly demonstrated that epigenetic regulations are central to the response of plants to both abiotic and biotic stresses. This includes DNA methylation remodeling that occurs in response to many different abiotic and biotic stresses but also involves histone posttranslational modifications (HPTMs) and histone variants. In addition, plants have also developed a memory of stress, which relies on cellular mechanisms such as metabolites accumulation, posttranslational modifications of regulatory proteins and epigenetic mechanisms (Crisp *et al.*, 2016). The latter may play a major role in this context as they embody important aspects of the memory of cells (Pikaard and Mittelsten Scheid, 2014). Indeed epigenetic marks are maintained during cell division, which allows a somatic memory of epigenetic imprints, including those generated by stresses. Furthermore, part of the stress-induced epigenetic changes is transmitted to the next generation, a process that however depends on the type of reproduction (Anastasiadi *et al.*, 2021). Transgenerational epigenetic heredity seems to be more efficient for agamous than for sexual reproduction. Finally, in grapevine, as for other perennials, plants may maintain their epigenetic landscape over years although environmental conditions may generate an epigenetic drift (Gallusci *et al.*, 2022).

Epigenetic aspects of plant priming: applications to grapevine: Plant stress somatic memory contributes to their acclimation to the environment, a process also called priming. Priming consists in the response of a plant to a first stress (biotic or abiotic) that will be in part memorized. This molecular memory will be maintained for some time during a recovery period and mobilized when the plant will face subsequent stresses (Mozgova *et al.*, 2019). In that sense, the plant is prepared to better respond to additional stresses. At the molecular level, priming is in part mediated by memory genes, the transcriptional state of which is determined and maintained by epigenetic processes (Bäurle, 2018). As far as grapevine is concerned, priming has been described in many different situations to acclimate plants to biotic or abiotic stresses (Delaunoy *et al.*, 2014). However, there is little analysis of the molecular responses and of the epigenetic determinants of priming. Studies evaluating the grapevine plant responses and memories using appropriate molecular approaches are now necessary.

Trans and/or intergenerational plant epigenetic memories: grapevine specificities: Works in arabidopsis have shown the stable transmission of epigenetic marks over generations after sexual reproduction. This is clearly established for intergenerational (one-generation) transmission of epigenetic information but is still unclear for the transgenerational (several generations) inheritance of epigenetic information generated by stress.

In clonally propagated plants such as grapevine, the progeny is generated by cutting. In this case, maintenance of epigenetic marks, mediated through mitosis in the stem cells located in meristems, (Latzel, Rendina González and Rosenthal, 2016). Of course generating cuttings may lead to re-juvenilization and reset some of the epigenetic imprints of the parental lines. However, recent work has shown that even when going through plant regeneration, which corresponds, to a major developmental reprogramming, the regenerated plant maintains part of the epigenetic imprints of the organ of origin (Wibowo *et al.*, 2018).

As far as grapevine is concerned there is little work investigating how growing conditions of mother plants affects the phenotypes and epigenomes of the progeny generated by cuttings. However, the

grapevine plant environment, in a broad sense, seems to be an important determinant of the plant methylome (Xie *et al.*, 2017). This suggests that epigenetic imprints, carried-over from the parental plants, may be transient in time or become erased by the new growing conditions of the progeny. In contrast, other works indicate that the DNA methylation patterns of grapevine clones are more dependent on clonal origin than location (Marfil *et al.*, 2019).

Obviously, there is a need for studies focusing on the transgenerational priming of clonally propagated plants as a new strategy for grapevine adaptation to climate changes.

Epi-breeding in grapevine: what strategies? Grapevine is a grafted and clonally propagated plant, which makes it unlikely that the phenotypic diversity observed in this plant is only due to genetic variations since genetic variants may remain hidden as heterozygous recessives. Epigenetic variants could therefore be critical in shaping phenotypic variations in this plant. An efficient strategy to investigate the contribution of heritable epigenetic variation to traits variation are the Epigenetic recombinant Inbred lines (EpiRILs), which takes advantage of the segregation and recombination between heritable DMRs to perform (epi)QTL mapping (Catoni and Cortijo, 2018). This strategy is based on sexual reproduction and cannot be developed in crops such as Grapevine that are clonally propagated and require extensive generation times. In this case, epigenome-wide association studies (EWASs) can be used to analyze populations of isogenic plants displaying epigenomic diversity as performed for palm oil trees (Ong-Abdullah *et al.*, 2015). We have generated a Pinot Noir Grapevine EpiClonal population, which is currently under vegetative propagation and will be used for methylome and phenotypic analyses. Alternatively, stress conditions may be used to generate an epigenetic drift. For example, using cuttings generated from a limited number of mother plants, it is expected to create different epigenomes by growing them in very contrasted conditions. These plants can be used to evaluate their tolerance to stresses and relate this to an eventual epigenetic drift. Finally parental priming of rootstocks and scions could provide new ways to develop grapevine propagation strategies better adapted to the climate changes building on the epigenetic changes existing between the graft partners.

Conclusion: There is an urgent need to develop innovative strategies to generate and use heritable epigenetic variations for crop improvement without relying on genetic diversity and sexual reproduction and /or to use epigenetic memories as a way to prime plants facing stresses. Such methods will undoubtedly accelerate grapevine breeding for stress tolerance, because elite varieties can directly be used to generate the required epigenetic diversity and for Epi-Breeding. For plant like grapevine with long reproduction phase and essentially multiplied asexually, epigenetics may provide a quicker and more efficient ways to generate cultivars more resilient to the combined stresses generated by climate changes.

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