

SPECIAL ISSUE ARTICLE

Exploring epigenetic variation for breeding climate resilient apple crops

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Abstract

Climate change with warmer winter and spring temperatures poses major challenges to apple fruit production. Long-term observations confirm the trend toward earlier flowering, which leads to an increased risk of frost damage. New breeding strategies are needed to generate cultivars that are able to stay largely unaffected by warmer temperatures. Recently, epigenetic variation has been proposed as a new resource for breeding purposes and seems suitable in principle for apple breeding. However, to serve as a new resource for apple breeding, it is necessary to clarify whether epigenetic variation can be induced by the environment, whether it can create phenotypic variation, and whether this variation is stable across generations. In this brief review, we summarize the impact of climate change on the timing of apple phenology, highlight how epigenetic variation can potentially support novel breeding strategies, and point out important features of epigenetic variation that are required for its application in breeding programs.

1 | MATERIAL AND METHODS

The two *Malus × domestica* Borkh. cultivars “Golden Delicious” and “Idared” were grown in the orchard of the Julius Kühn Institute at Dresden-Pillnitz (geographic coordinates: 50.999040, 13.886715). They were phenotyped for the beginning of flowering every spring between the years 1993 and 2017. At least 50 flowers were observed on plurannual wood daily, per genotype and year. The date was recorded as the beginning of flowering when 10% of flowers were open. The linear regression was determined using RStudio (RStudio Team, 2021).

2 | RESULTS AND DISCUSSION

Apples (*Malus × domestica* Borkh.) are the fourth most important fruit crop produced worldwide in temperate climates. This explains the

long breeding history and the high interest in cultivars suited for highly efficient apple production. The marked need for high quality and efficiency, however, also explains why the genetic diversity of modern cultivars is rather low, with only very few successful cultivars repeatedly used as progenitors of novel cultivars (Migicovsky et al., 2021). Besides excellent fruit quality, cultivar resistance to pathogens as well as resilience to changing climatic conditions is strongly needed for more sustainable cultivation practices. The impact of climate change on the annual life cycle of apple trees is already apparent, resulting in significant yield losses for many years. Especially warmer temperatures in spring disturb the tightly controlled timing of the alternating phases of winter dormancy, where buds are well protected by bud scales and can outlast strong frosts and the phase of plant growth and reproduction. As temperatures gradually rise in spring, bud break and flowering occur ever earlier and the risk of flower damage and yield losses from late frost events increases significantly. Long-term phenology observations revealed that the strongest

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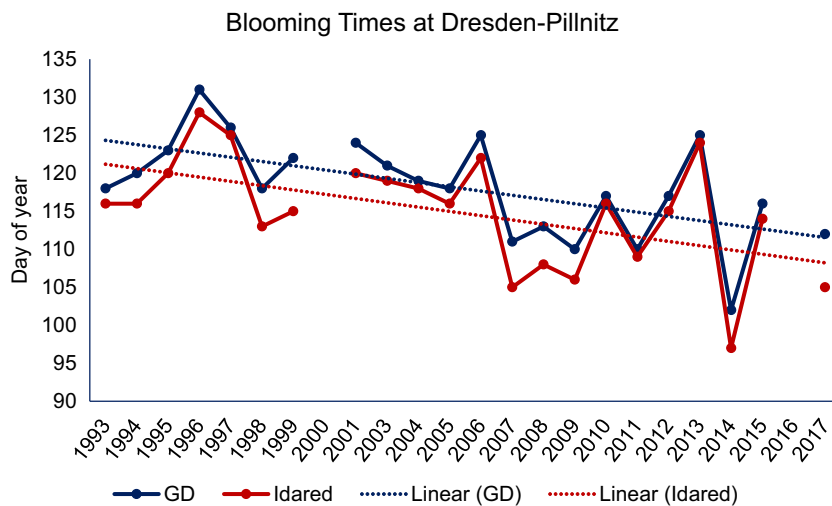


FIGURE 1 Presentation of the beginning of flowering for the two cultivars “Golden Delicious” (GD) and “Idared” at the JKI Dresden-Pillnitz between the years 1993 and 2017. The slope of the trendline by linear regression is -0.55 and -0.56 for “Idared” and “Golden Delicious,” respectively. Both regressions are significant with $p = 0.012$ for “Idared” and $p = 0.042$ for “Golden Delicious”

advance in leaf-out days occurred in the 1980s (Vitasse et al., 2022). Data on the flowering onset of two apple cultivars in Dresden-Pillnitz, Germany, over 24 years also show a clear trend toward earlier flowering onset (Figure 1). Both cultivars “Idared” and “Golden Delicious” show the same trend of 0.55 and 0.56 days earlier flowering per year. Kunz and Blanke revealed similar results; they compared the mean of two 30-year periods of blossom start and stated a 10 days earlier flowering of “Golden Delicious” from 1988 to 2017 compared to 1958 to 1987 (Kunz & Blanke, 2022). At the same time, however, the frequency of spring frosts during March and April did not decrease (Kunz & Blanke, 2022). The detrimental consequence of early flowering is a more frequent coincidence of susceptible flower bud stages and spring frosts, resulting in significant yield losses. In order to escape spring frosts, above-crown irrigation or frost candles can be applied. However, such measures are costly and not ideal.

There is a great need to generate a new generation of cultivars that are resilient to climate change, including cultivars that are able to delay flowering during spring in order to avoid frost damage. Novel breeding strategies are required to meet this need. Besides genetic variation, epigenetic variation has become a promising resource for generating phenotypic variation and for plant breeding (Gallusci et al., 2017; Kakoulidou et al., 2021; King, 2015; Latutrie et al., 2019; Springer, 2013). Epigenetic modifications can occur at histones or the DNA level via methylation and mostly affect genome accessibility, gene expression and the three-dimensional structure of chromatin (Pecinka et al., 2013; Schmitz & Ecker, 2012). It can be generated either by genetic control, at low levels it can occur stochastically, or it can be induced by environmental variation (Becker et al., 2011; Dubin et al., 2015; Luna & Ton, 2012; Rasmann et al., 2012; Richards et al., 2017; Wibowo et al., 2016). In order to use epigenetic variation as a novel resource for breeding climate-resilient apple varieties, certain criteria need to be met: epigenetic variation needs to be inducible by the environment, it needs to be able to create phenotypic variation in the timing of winter dormancy and flowering and it needs to be stable and inheritable across generations.

The inducibility of epigenetic variation by environmental conditions has been shown for different types of epigenetic variation. The fact that geographic origin is a major predictor of epigenetic variation in the model plant *A. thaliana* supports the hypothesis that environmental conditions can induce stable epigenetic variation (Dubin et al., 2015; Kawakatsu et al., 2016). Besides temperature, biotic and abiotic stresses represent conditions that can induce epigenetic variation that can modify gene expression and phenotype (Downen et al., 2012; González et al., 2016). Further support for the environmental inducibility of epigenetic variation offers the phenomenon of priming: Offspring of plants that experienced stress, for example, pathogen attack, show higher resistance to the applied stress compared to offspring of nonstressed plants (Conrath et al., 2015; Gully et al., 2019; Luna & Ton, 2012; Ramírez-Carrasco et al., 2017; Rasmann et al., 2012; Slaughter et al., 2012). Evidence exists that the underlying mechanism of such priming responses in plants can be based on epigenetic modifications, histone as well as DNA methylation (Conrath et al., 2015; Ramírez-Carrasco et al., 2017). Priming is a promising strategy to improve yield and productivity of commercial food production. A pre-treatment of seeds or mother plants with re-occurring stress—for example, temperature or water stress—will make offspring plants more resilient to the stress condition. Another example of environmentally induced epigenetic modification is the *A. thaliana* flowering time gene *FLOWERING LOCUS C (FLC)*, which is gradually silenced by low temperature via polycomb-mediated epigenetic silencing (Costa & Dean, 2019). Although there is no direct *FLC*-homolog in *M. domestica* (Kumar et al., 2016), expression levels of the MADS-box gene *MdFLC-like* can be influenced by or correlate with ambient temperature (Lempe et al., 2022; Porto et al., 2015; Takeuchi et al., 2018). Whether epigenetic marks are involved in the regulation of *MdFLC-like* needs to be determined. *MdFLC-like* is an important regulator of winter dormancy in apple (Falavigna et al., 2019; Lempe et al., 2022) and therefore, it is also a very promising candidate for a gene under environmentally induced epigenetic control that may alter flowering behavior in spring. Also, good candidates for environmentally and epigenetically controlled genes involved in the timing and

progression of winter dormancy and flowering are the *Dormancy-associated MADS-box (DAM)* genes. They appear to be under epigenetic control in peach, another species of the Rosacea family (Zhu et al., 2020).

The ability to induce phenotypic variation is another important feature of epigenetic variation as a breeding resource. This is true for *FLC*, which controls flowering time. However, many more examples exist for epigenetic variation underlying changes in phenotype. The first time this was shown was at the gene *cycloidea* in *Linaria vulgaris*, where high methylation levels and low expression of *cycloidea* are responsible for turning the bilateral symmetry of the flower into a radial one (Cubas et al., 1999). Since then, several other genes of diverse plant species have been identified (Kenchanmane Raju & Niederhuth, 2018). In apple, epigenetic variation at *MYB10* underlies variation in anthocyanin production and, therefore, in fruit color variation (Bucher et al., 2018; Daccord et al., 2017; El-Sharkawy et al., 2015; Telias et al., 2011). Apple, which is propagated clonally by grafting, “mutations” or so-called “sports” frequently occur, where only a single branch displays apples differing in color compared to apples of the rest of the tree. Farmers or breeders often select these types, without knowing the underlying molecular mechanism. Whether these selected sports are based on DNA sequence variation or epigenetic variation is currently unknown (Bucher et al., 2018).

Another property of epigenetic variation instrumental for its role in breeding processes is its stability. Although some experiments indicate little inheritance (Eichten & Springer, 2015; Pecinka & Mittelsten Scheid, 2012; Wibowo et al., 2016), other experiments provide good evidence for stable inheritance of DNA methylation patterns across generations (Hagmann et al., 2015; Johannes et al., 2009; Schmitz et al., 2013). The level of inheritance of epigenetic modifications is much lower in sexually reproducing plants, since during meiosis an epigenetic resetting occurs (Martienssen, 2008). In clonally derived specimens, such reprogramming mechanisms are bypassed and therefore, epigenetic variation is passed on to vegetatively propagated clones (Richards et al., 2012; Van den Broeck et al., 2018; Verhoeven & Preite, 2014). Therefore, asexual propagation of apple cultivars via scions is advantageous as there is no risk of losing induced epigenetic variation by meiotic resetting. Although there is a high potential for epigenetic variation to be transmitted to clonal offspring, how stable such epigenetic variation is across seasons and years is still an important question.

We conclude that epigenetic variation is a very promising resource to evaluate for breeding novel apple cultivars. As it is an out-crossing species, conventional breeding programs in apple are very time-consuming. Therefore, it would be very attractive to be able to modify cultivars with already excellent fruit qualities by environmental treatments without modification of the genomic sequence to obtain high quality crops that are resilient to environmental conditions. Clonal propagation practices will likely allow the transmission of epigenetic variation efficiently. However, more knowledge on epigenetic variation needs to accumulate on (1) the identity of existing epigenetic variation, (2) the environmental inducibility, (3) the traits it can affect,

and (4) the stability across seasons and time in asexually propagated apple cultivars.

AUTHOR CONTRIBUTION

All authors listed contributed to the work and approved it for publication.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as all new created data is already contained within this article.

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REFERENCES

- Becker, C., Hagmann, J., Müller, J., Koenig, D., Stegle, O., Borgwardt, K. et al. (2011) Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome. *Nature*, 480(7376), 245–249.
- Bucher, E., Kong, J., Teyssier, E. & Gallusci, P. (2018) Epigenetic regulations of fleshy fruit development and ripening and their potential applications to breeding strategies. In: *Advances in botanical research*. London: Elsevier, pp. 327–360.
- Conrath, U., Beckers, G.J., Langenbach, C.J. & Jaskiewicz, M.R. (2015) Priming for enhanced defense. *Annual Review of Phytopathology*, 53, 97–119.
- Costa, S. & Dean, C. (2019) Storing memories: the distinct phases of polycomb-mediated silencing of *Arabidopsis* FLC. *Biochemical Society Transactions*, 47(4), 1187–1196.
- Cubas, P., Vincent, C. & Coen, E. (1999) An epigenetic mutation responsible for natural variation in floral symmetry. *Nature*, 401(6749), 157–161.
- Daccord, N., Celton, J.M., Linsmith, G., Becker, C., Choise, N., Schijlen, E. et al. (2017) High-quality de novo assembly of the apple genome and methylome dynamics of early fruit development. *Nature Genetics*, 49(7), 1099–1106.
- Dowen, R.H., Pelizzola, M., Schmitz, R.J., Lister, R., Dowen, J.M., Nery, J.R. et al. (2012) Widespread dynamic DNA methylation in response to biotic stress. *Proceedings of the National Academy of Sciences of the United States of America*, 109(32), E2183–E2191.
- Dubin, M.J., Zhang, P., Meng, D., Remigereau, M.S., Osborne, E.J., Paolo Casale, F. et al. (2015) DNA methylation in *Arabidopsis* has a genetic basis and shows evidence of local adaptation. *eLife*, 4, e05255.
- Eichten, S.R. & Springer, N.M. (2015) Minimal evidence for consistent changes in maize DNA methylation patterns following environmental stress. *Frontiers in Plant Science*, 6, 308.
- El-Sharkawy, I., Liang, D. & Xu, K. (2015) Transcriptome analysis of an apple (*Malus × domestica*) yellow fruit somatic mutation identifies a gene network module highly associated with anthocyanin and epigenetic regulation. *Journal of Experimental Botany*, 66(22), 7359–7376.
- Falavigna, V.D.S., Guitton, B., Costes, E. & Andrés, F. (2019) I want to (bud) break free: the potential role of DAM and SVP-like genes in regulating dormancy cycle in temperate fruit trees. *Frontiers in Plant Science*, 9, 1990.
- Gallusci, P., Dai, Z., Génard, M., Gauffretau, A., Leblanc-Fournier, N., Richard-Molard, C. et al. (2017) Epigenetics for plant improvement:

- current knowledge and modeling avenues. *Trends in Plant Science*, 22(7), 610–623.
- González, A.P., Chrtek, J., Dobrev, P.I., Dumasová, V., Fehrer, J., Mráz, P. et al. (2016) Stress-induced memory alters growth of clonal offspring of white clover (*Trifolium repens*). *American Journal of Botany*, 103(9), 1567–1574.
- Gully, K., Celton, J.M., Degraeve, A., Pelletier, S., Brisset, M.-N. & Bucher, E. (2019) Biotic stress-induced priming and de-priming of transcriptional memory in Arabidopsis and apple. *Epigenomes*, 3(1), 1–20.
- Hagmann, J., Becker, C., Müller, J., Stegle, O., Meyer, R.C., Wang, G. et al. (2015) Century-scale methylome stability in a recently diverged *Arabidopsis thaliana* lineage. *PLoS Genetics*, 11(1), e1004920.
- Johannes, F., Porcher, E., Teixeira, F.K., Saliba-Colombani, V., Simon, M., Agier, N. et al. (2009) Assessing the impact of transgenerational epigenetic variation on complex traits. *PLoS Genetics*, 5(6), e1000530.
- Kakoulidou, I., Avramidou, E.V., Baranek, M., Brunel-Muguët, S., Farrona, S., Johannes, F. et al. (2021) Epigenetics for crop improvement in times of global change. *Biology-Basel*, 10(8), 766.
- Kawakatsu, T., Huang, S.C., Jupe, F., Sasaki, E., Schmitz, R.J., Urich, M.A. et al. (2016) Epigenomic diversity in a global collection of *Arabidopsis thaliana* accessions. *Cell*, 166(2), 492–505.
- Kenchanmane Raju, S.K. & Niederhuth, C.E. (2018) Chapter three – epigenetic diversity and application to breeding. In: Mirouze, M., Bucher, E. & Gallusci, P. (Eds.) *Advances in botanical research*. London: Academic Press, pp. 49–86.
- King, G.J. (2015) Crop epigenetics and the molecular hardware of genotype \times environment interactions. *Frontiers in Plant Science*, 6, 968.
- Kumar, G., Arya, P., Gupta, K., Randhawa, V., Acharya, V. & Singh, A.K. (2016) Comparative phylogenetic analysis and transcriptional profiling of MADS-box gene family identified DAM and FLC-like genes in apple (*Malus \times domestica*). *Scientific Reports-UK*, 6(1), 20695.
- Kunz, A. & Blanke, M. (2022) “60 years on”—effects of climatic change on tree phenology—a case study using pome fruit. *Horticulturae*, 8(2), 110.
- Latutrie, M., Gourcilleau, D. & Pujol, B. (2019) Epigenetic variation for agronomic improvement: an opportunity for vegetatively propagated crops. *American Journal of Botany*, 106(10), 1281–1284.
- Lempe, J., Peil, A. & Flachowsky, H. (2022) Time-resolved analysis of candidate gene expression and ambient temperature during bud dormancy in apple. *Frontiers in Plant Science*, 12, 803341.
- Luna, E. & Ton, J. (2012) The epigenetic machinery controlling transgenerational systemic acquired resistance. *Plant Signaling & Behavior*, 7(6), 615–618.
- Martienssen, R. (2008) Great leap forward? Transposable elements, small interfering RNA and adaptive Lamarckian evolution. *The New Phytologist*, 179(3), 570–572.
- Migicovsky, Z., Gardner, K.M., Richards, C., Chao, C.T., Schwaninger, H.R., Fazio, G. et al. (2021) Genomic consequences of apple improvement. *Horticulture Research-England*, 8(1), 9.
- Pecinka, A., Abdelsamad, A. & Vu, G.T. (2013) Hidden genetic nature of epigenetic natural variation in plants. *Trends in Plant Science*, 18(11), 625–632.
- Pecinka, A. & Mittelsten Scheid, O. (2012) Stress-induced chromatin changes: a critical view on their heritability. *Plant & Cell Physiology*, 53(5), 801–808.
- Porto, D.D., Bruneau, M., Perini, P., Anzanello, R., Renou, J.-P., Santos, H.P. et al. (2015) Transcription profiling of the chilling requirement for bud break in apples: a putative role for FLC-like genes. *Journal of Experimental Botany*, 66(9), 2659–2672.
- Ramírez-Carrasco, G., Martínez-Aguilar, K. & Alvarez-Venegas, R. (2017) Transgenerational defense priming for crop protection against plant pathogens: a hypothesis. *Frontiers in Plant Science*, 8, 696.
- Rasmann, S., De Vos, M. & Jander, G. (2012) Ecological role of transgenerational resistance against biotic threats. *Plant Signaling & Behavior*, 7(4), 447–449.
- Richards, C.L., Alonso, C., Becker, C., Bossdorf, O., Bucher, E., Colomé-Tatché, M. et al. (2017) Ecological plant epigenetics: evidence from model and non-model species, and the way forward. *Ecology Letters*, 20(12), 1576–1590.
- Richards, C.L., Schrey, A.W. & Pigliucci, M. (2012) Invasion of diverse habitats by few Japanese knotweed genotypes is correlated with epigenetic differentiation. *Ecology Letters*, 15(9), 1016–1025.
- RStudio Team. (2021) *RStudio: integrated development environment for R*. Boston, MA. Available from: RStudio, PBC. <http://www.rstudio.com/>
- Schmitz, R.J. & Ecker, J.R. (2012) Epigenetic and epigenomic variation in *Arabidopsis thaliana*. *Trends in Plant Science*, 17(3), 149–154.
- Schmitz, R.J., Schultz, M.D., Urich, M.A., Nery, J.R., Pelizzola, M., Libiger, O. et al. (2013) Patterns of population epigenomic diversity. *Nature*, 495(7440), 193–198.
- Slaughter, A., Daniel, X., Flors, V., Luna, E., Hohn, B. & Mauch-Mani, B. (2012) Descendants of primed Arabidopsis plants exhibit resistance to biotic stress. *Plant Physiology*, 158(2), 835–843.
- Springer, N.M. (2013) Epigenetics and crop improvement. *Trends in Genetics*, 29(4), 241–247.
- Takeuchi, T., Matsushita, M.C., Nishiyama, S., Yamane, H., Banno, K. & Tao, R. (2018) RNA-sequencing analysis identifies genes associated with chilling-mediated endodormancy release in apple. *Journal of the American Society for Horticultural Science*, 143(3), 194–206.
- Telias, A., Lin-Wang, K., Stevenson, D.E., Cooney, J.M., Hellens, R.P., Allan, A.C. et al. (2011) Apple skin patterning is associated with differential expression of MYB10. *BMC Plant Biology*, 11, 93.
- Van den Broeck, A., Cox, K., Brys, R., Castiglione, S., Ciatelli, A., Guarino, F. et al. (2018) Variability in DNA methylation and generational plasticity in the Lombardy poplar, a single genotype worldwide distributed since the eighteenth century. *Frontiers in Plant Science*, 9, 1635.
- Verhoeven, K.J. & Preite, V. (2014) Epigenetic variation in asexually reproducing organisms. *Evolution*, 68(3), 644–655.
- Vitasse, Y., Baumgarten, F., Zohner, C.M., Rutishauser, T., Pietragalla, B., Gehrig, R. et al. (2022) The great acceleration of plant phenological shifts. *Nature Climate Change*, 12(4), 300–302.
- Wibowo, A., Becker, C., Marconi, G., Durr, J., Price, J., Hagmann, J. et al. (2016) Hyperosmotic stress memory in Arabidopsis is mediated by distinct epigenetically labile sites in the genome and is restricted in the male germline by DNA glycosylase activity. *eLife*, 5, 1–27.
- Zhu, H., Chen, P.Y., Zhong, S.L., Dardick, C., Callahan, A., An, Y.Q. et al. (2020) Thermal-responsive genetic and epigenetic regulation of DAM cluster controlling dormancy and chilling requirement in peach floral buds. *Horticulture Research-England*, 7(1), 114.

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