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Experimental waterlogging of grape seeds, impact on seed shape and geometrical reversing for morphometric inference

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Highlights

- Chemical treatments to understand the impact of waterlogging on grape pip shape.
- Moderate shape changes were identified independently of the chemical.
- Deformation is restricted and primarily affect the beak of the pip.
- A reverse vector of shape changes is experienced to compensate for the deformation.
- The wild or domesticated status can be securely identified after waterlogging.
- Advices are provided for the use of waterlogged pips for morphometric analyses.

Abstract

While the impact of charring on grape seed morphology is well documented, waterlogged pips have up to now been considered in morphometric studies to undergo very limited changes and to be directly comparable to reference collections of fresh seeds. In this study we investigate the impact of waterlogging using different chemicals (H_2O_2 , HNO_3 , $NaOH$, KOH) on pips of various cultivars and wild grapevines. Pips are soaked individually in a given chemical solution for increasing periods of time, photographed at each stage, and their dorsal and lateral outlines analysed using Elliptic Fourier Transforms to quantify shape changes. We observe that the different products have comparable effects. The seeds start to swell and then return to their original shapes. Deformation is limited and primarily affects the beak which is slightly blunt and somewhat more pointed than on the original pips. These changes are reminiscent of the deformations observed in waterlogged archaeological seeds. Our results show that they are not strong enough to affect significantly the identification of wild and domesticated morphotypes by seed outline analyses. The effect is greater on the identifications at cultivars level but the accuracy can still be considered acceptable. We experiment the application of an average reverse vector of shape changes to compensate for some of the deformation of artificially waterlogged pips. This correction appears to have a positive effect on the accuracy of identifications for some cultivars. Our conclusion is that outline analysis can be confidently performed on archaeological waterlogged grape pips to identify wild or domesticated status and that it is more appropriate to aim at identifying groups of varieties than individual cultivars. Considering that deformations due to charring and waterlogging have differentiated effects on the identifications it is recommended to take into account both waterlogged and charred seed assemblages.

Keywords

Vitis vinifera ; Archaeobotany ; Elliptic Fourier Transforms; Anoxic preservation ; Chemical treatment; Taphonomy

1. Introduction

By allowing the precise quantification of the phenotype of archaeological animal and plant remains, geometric morphometrics (GMM) have taken studies on domestication, historical biogeography and spatio-temporal changes in crop and animal biodiversity to a new level (Evin et al., 2022). In archaeobotany, GMMs have so far been applied mainly to seeds and fruits (Portillo et al., 2020). The most common objectives are to distinguish close taxonomical levels, from neighbouring species (Bonhomme et al., 2017; Jesus et al., 2021; Roushannafas et al., 2022; Tarongi et al., 2021; Terral et al., 2012; Ucchesu et al., 2017); subspecies (Bonhomme et al., 2020; Gros-Balthazard et al., 2017; Orrù et al., 2013; Ros et al., 2014), especially in domestication studies to differentiate domestic forms from wild ancestors; and groups of cultivated varieties (Pagnoux et al., 2021; Terral et al., 2021, 2010). Nearly all of the studies in archaeobotany use 2D digital images and of outline analyses of the seeds/fruits, mostly using elliptical Fourier transforms (EFT) and polynomial fitting (Evin et al., 2022).

In Europe most of archaeobotanical remains are preserved by charring. Charring generally involves changes in shape and size of seed/fruit, which may be challenging for morphometric studies. The effect of charring on the preservation and morphology of seed/fruit has been extensively studied for various species through experimental charring, mostly with muffle furnaces (e.g. Berihuete-Azorín et al., 2019; Boardman and Jones, 1990; Braadbaart and van Bergen, 2005; Charles et al., 2015).

As concerns grape (*Vitis vinifera* L.), numerous experimental research have studied the effect of charring on the morphology of pips (Bouby et al., 2018; Mangafa and Kotsakis, 1996; Smith and Jones, 1990; Ucchesu et al., 2016). These studies agree that charring implies a reduction in size and some changes in seed shapes, leading the domesticated seeds to take a rounder shape, thus resembling the wild grapevine pips. However, the long beak distinctive of the domesticated pips is little affected by deformation and the discrimination between wild and domesticated grapevine remains possible after charring, especially if one applies outline analysis compared to traditional distance measurements. Although its discriminating power is reduced, EFT outline analysis can even still be used to distinguish pips from several grape varieties after charring (Bouby et al., 2018).

Waterlogged and desiccated seeds/fruits are often targeted for GMM studies as their morphology is considered to be only marginally affected by taphonomic processes and to be directly comparable to modern reference collections of fresh seeds/fruits (Burger et al., 2011; Jesus et al., 2021; Sabato et al., 2019; Terral et al., 2004). This is especially the case for grapevine (Bouby et al., 2021; Moricca et al., 2021; Orrù et al., 2013; Terral et al., 2010). Waterlogged anoxic conditions are remarkably favourable for the preservation of uncharred seeds and fruits. In anaerobic conditions most of the bacteria and fungi involved in the decomposition of organic matter cannot thrive. Aerobic decomposition can only occur under the water table until all the available oxygen is used up, or by seasonal or occasional dewatering (Kenward and Hall, 2000). However, under anaerobic conditions complex microbial processes will still result in some decomposition of organic matter through fermentation reactions and anaerobic respiration (Herndon et al., 2015; Keller and Takagi, 2013). On waterlogged plant remains these processes more especially involve bacteria over fungal species (Beccaccioli et al., 2023). Chemical factors also impact organic decomposition in combination with micro-organisms. Not all compounds of organic matter degrade at the

same pace and rate. Most sugars and amino-acids are quickly broken down while complex carbohydrates such as cellulose, and even more so lignin, are most resistant (Bleicher and Schubert, 2015). Lignin is hardly decomposed at all in anoxic contexts. As a consequence, seed/fruit remains mostly made of lignin, such as olive or plums endocarps, will be much less affected by decomposition than seed/fruits predominantly composed of non-lignified tissues. Grape seeds are composed of three main tissues: seed coat or testa, endosperm and embryo. The seed coat comprises three layers: the outer tegument composed of cells with fairly soft cellulose walls, and the middle and inner teguments, composed of strongly lignified elongated cells (Huglin and Schneider, 1998).

The composition of the seed coat is therefore liable to make grape pips resistant to decomposition in waterlogged environments. Unexpected results in the comparison of archaeological waterlogged pips to their modern counterparts however lead to question the possible effects of burial under anoxic conditions on their morphology. This is particularly the case of the high proportion of pips regularly assigned to the wild morphotype in archaeobotanical assemblages dating from the Roman and Medieval periods (Bonhomme et al., 2021b; Bouby et al., 2013, 2021). Based on the Roman site of Sauvian-La Lesse (Figueiral et al., 2015), the calculation of mean shapes of the archaeological pips assigned to the wild and domesticated morphotypes using seed outline analysis show some differences with the mean shapes of current wild and domesticated pips (Fig 1). In both morphotypes, the beak in particular is somewhat weathered and slightly more pointed than in the current pips. These differences could be the result of differential taphonomic processes.

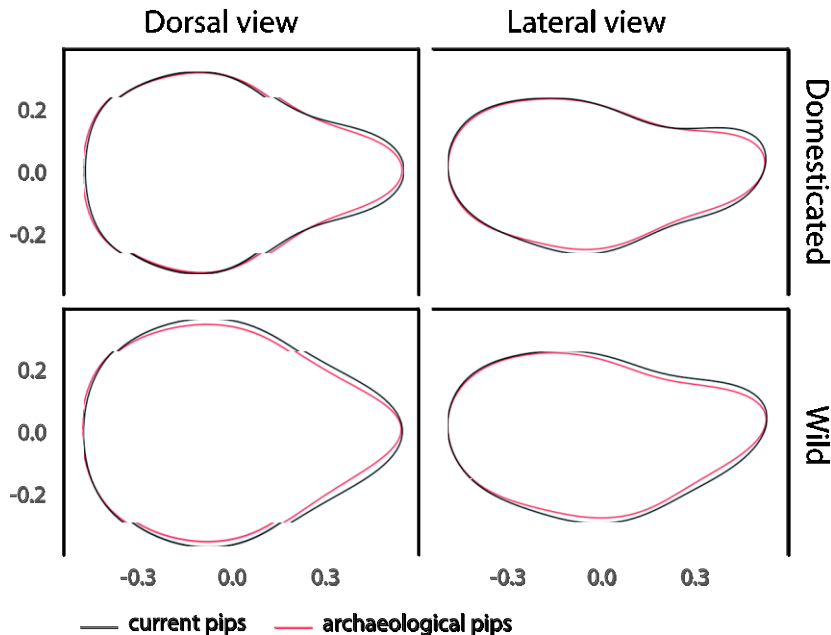


Fig. 1. Mean shapes visualization of morphological differences, in dorsal (VD) and lateral (VL) views, between current pips of wild and domesticated grapevines and pips from the Roman archaeological site of Sauvian-La Lesse (Figueiral et al., 2015) assigned to wild and domesticated morphotypes by EFT outline analysis.

It is difficult, not to say impossible, to experimentally reproduce long-term decay of waterlogged plant remains (Hall and Kenward, 2004). Simple chemical degradative

treatments can nevertheless be used to mimic the alterations and changes that may have occurred in sedimentary deposits (Mösle et al., 1997; Souto et al., 2016). In this study, we test the effect of four chemical treatments mentioned in the literature to artificially fossilize plant organs or to facilitate sieving of clay soils but causing an impact on the preservation of waterlogged macro-remains .

Our objectives are threefold: 1) to compare the respective effects of these products on the morphology of the seeds of various cultivated varieties and wild grapevines; 2) to test whether these effects have an impact on the capacity to identify the seeds of wild versus domestic grapevines and of the different grape varieties using outline analyses; and 3) to explore if a geometrical reversal of shape deformation could improve the latter identification accuracies.

2. Materials and Methods

2.1. Grape samples

The experiments were performed on samples of 10 seeds originating from 6 cultivated varieties ('Carignan', 'Clairette blanche', 'Grenache noir', 'Mourvèdre', 'Sideritis', 'Sliva') and 3 wild plants (Pic Saint-Loup H, Pic Saint-Loup 13, Venetikos 44). Seeds of cultivated varieties were collected from the French central ampelographic collection (INRA, Vassal-Montpellier Grapevine Biological Resources Center; <https://www6.montpellier.inra.fr/vassal>). Cultivated varieties were selected to be representative of the morphological diversity observed in previous studies (Bouby et al., 2021; Pagnoux et al., 2015) in domesticated grape seeds. The morphology of the 'Clairette blanche' and 'Grenache' pips is close to that of the wild grape pips, whereas the 'Sliva' and 'Carignan' pips are distant from it, with an elongated shape and a long beak. 'Mourvèdre' and 'Sideritis' show an intermediate morphology. All the samples were dried at 30°C for 72 h before the experiments began.

In order to examine the extent of shape changes in experimentally treated seeds we have observed them in a space representative of the morphological variability of wild and domesticated grape pips. For that purpose, we used a previously published modern reference collection composed of 38 traditional cultivars and 18 wild individuals of Euro-Mediterranean origins (Bonhomme et al., 2020).

2.2. Chemical treatments

Four chemical products, with their associated concentration values and heating conditions, were selected based on the literature (Fig 2): 1) 30% Hydrogen peroxide (H_2O_2) with Acetic acid glacial ($C_2H_4O_2$) 1/1, heated at 40°C (Mösle et al., 1997), 2) 65% Nitric acid (HNO_3) (Kerp, 1990), 3) 10% Potassium hydroxide (KOH), heated 40°C (Vandorpe and Jacomet, 2007), 4) 8% Sodium hydroxyde (NaOH), heated 40°C (Souto et al., 2016). For each chemical, the seeds were soaked in 5 ml of solution in individual beakers for increasing times. It was important to separate each seed into a beaker so that it could be clearly identified and photographed between each soaking period. In between soaking times, the seeds were systematically cleaned with a 5% Sodium hypochlorite solution and washed with water. In a

first phase we used the 4 products with only two grape varieties and soaking times ranging from 5 min to 24 h. In a second phase, we used only two products (HNO₃ and NaOH) with the full range of grapevine accessions and soaking times from 5 to 360 min (Fig. 2).

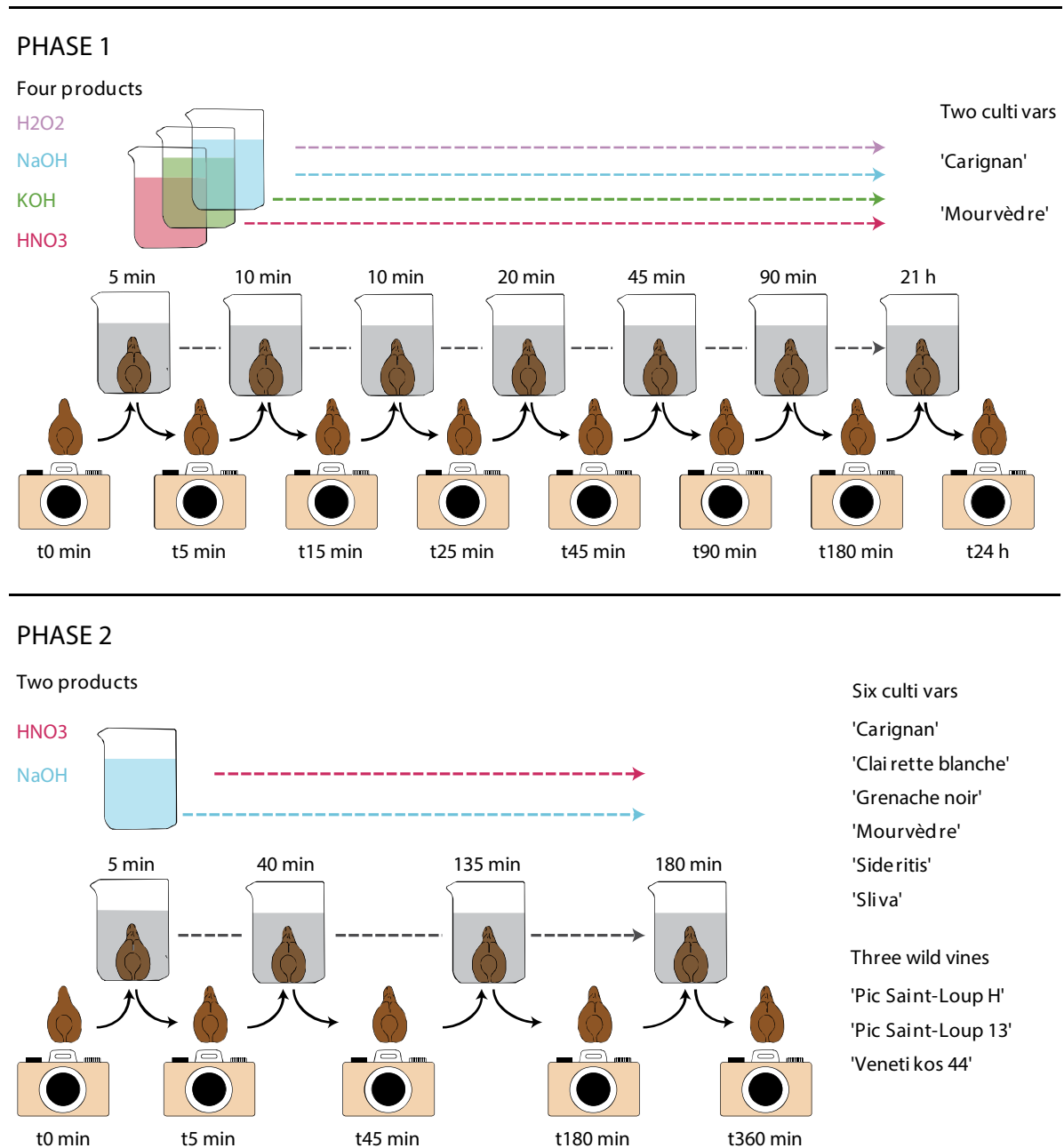


Fig. 2. Experimental procedure.

2.3. Morphometric method

Pip outline analyses were performed using the same EFT method described elsewhere (Bonhomme et al., 2021a; Bouby et al., 2018; Terral et al., 2010). Pips were photographed in orthogonal dorsal (VD) and lateral (VL) views by one single operator, using an Olympus SZ-ET stereomicroscope and an Olympus DP camera. The photos were converted to black masks

and outline coordinates (x ; y) were extracted from the images. Outlines were then normalized for their position, rotation, size and first point. Then outline analyses, namely elliptical Fourier transforms (EFT), were used to turn outline geometries into quantitative variables of shapes (Fourier coefficients). Five harmonics were used for both VD and VL views and were enough to gather 95% of the total harmonic power (Bonhomme et al. 2014). With four coefficients per harmonic, 40 Fourier coefficients were obtained and used as quantitative variables describing the shape for all subsequent analyses. Pip length was measured numerically on aligned pips (VD view), rescaled from pixels to millimeters. Pip length was shown to be the best predictor of all other lengths measured on pips (Bonhomme et al., 2020) and here helped to analyse form, that is the shape plus size.

2.4. Quantitative and statistical procedures

All statistical analyses were done in the R 4.1.3 (R Core Team, 2023) environment, using the packages from the tidyverse for general data tidying, manipulation and visualization (Wickham et al., 2019), the package Momocs for everything morphometrics (Bonhomme et al., 2014) and the package MASS (Venables et al., 2002) for linear discriminant analyses (LDAs).

To visualize the intensity of deformation induced by different chemical products and durations, we calculated two indices, one for shape and one for length change. For shape change, we calculated the euclidean distance on Fourier coefficients between each original pip and the same pip along different treatments and durations. To make more sense of these unitless index, we standardized the differences obtained by the median euclidean distance between all pips within a given accession. This way, changes >1 would indicate that a given pip had more shape changes than the median variability that exists between pips within a particular accession. For length changes, we simply calculated the relative change in pip length, ie $(L_i - L_0)/L_0$, so that 0 would indicate no change in length, 1 a doubling, -0.5 a halving, etc.

To visualize the direction and magnitude of the deformation induced by the different chemical products and soaking times, we first reduced the dimensionality of the dataset using only the first two axes of a principal component analysis (PCA) calculated on a balanced (500 wild / 500 domesticated pips) from the modern reference collection. We then reprojected all pips involved in Phase 2 chemical treatments. The initial and the final convex hulls are shown on Fig. 7 to visualize their respective occupancy on this morphological space. For all duration increment, we also calculated the mean PC1:PC2 score and represented it using a segmented arrow.

In order to assess the impact of deformation on the characterization of the pips using EFT we applied two LDAs, one to identify the status (LDA_{status} ; wild vs. domesticated) and one to identify the six cultivars considered in the experiments (LDA_{cv}). The pips involved in Phase 2 experiments were classified in the two LDAs using their outlines at t_0 and t_{360} min. The pips from wild accessions were excluded from LDA_{cv} . For LDA_{status} , 30 balanced dataset with 500 wild and 500 domesticated were obtained from the modern reference collection (Evin et al., 2013); for LDA_{cv} the sample sizes within accession were already balanced yet too small ($N=30$) to allow meaningful permutations.

In addition, to attempt to compensate pip deformation we aimed at applying the inverse vector of shape changes to soaked pips. We first calculated the mean difference of Fourier coefficients between fresh and t_{360} min pips. To temper the differential change and the unbalanced sample sizes between compartments (domesticated/wild pips), and also within accession (varieties/wild individuals), we calculated the median change vector within accession, then the median of these vectors (ie within compartment) and finally the median change of these two vectors. Then, we simply added this "reversing" vector to the values of Fourier coefficients obtained for each pip from the Phase 2 dataset. These pips were classified in LDA_{status} and LDA_{cv} and we present the accuracies obtained at correctly predicting either status and cultivar for fresh, t_{360} min and reversed t_{360} min pips.

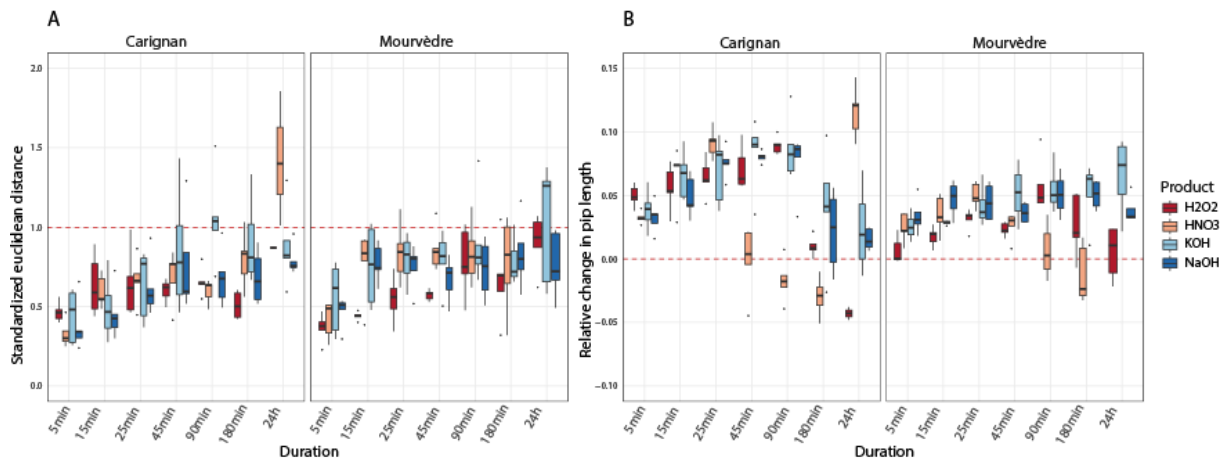


Fig. 3. Intensity of pip deformation induced by four chemical products and different soaking times on the pips of two grape varieties 'Carignan' and 'Mourvèdre'. Shape changes (A) are calculated using the euclidean distance on Fourier coefficients between the original pip and the same pip after treatment. The resulting differences were standardized by the median euclidean distance between all pips within a given accession (red dotted line). Length changes (B) were calculated as follows: $(L_t - L_0)/L_0$, so that 0 would indicate no change in length and 1 a doubling.

3. Results

3.1. Effect of chemicals as a function of soaking time and varieties

A first experimental phase was carried out using two varieties, four chemicals and increasing soaking times up to 24h. The different products have a consistent effect on length and more especially on the shape of the seeds (Fig. 3). The two bases, KOH and NaOH, always have a very similar effect. The effect of HNO_3 can diverge noticeably from that of the bases when referring to length. Whatever the product, the effect on shape increases with soaking time but tends to reach a maximum fairly rapidly and stabilise. The effect after 24 hours of soaking shown on the graph is not reliable because most of the pips are badly damaged, often broken, when exposed to chemicals for such a long time. For this reason, the maximum soaking time in phase 2 of the experiment was reduced to t_{360} min, a time after which only few seeds are broken and most are still usable for morphometrics.

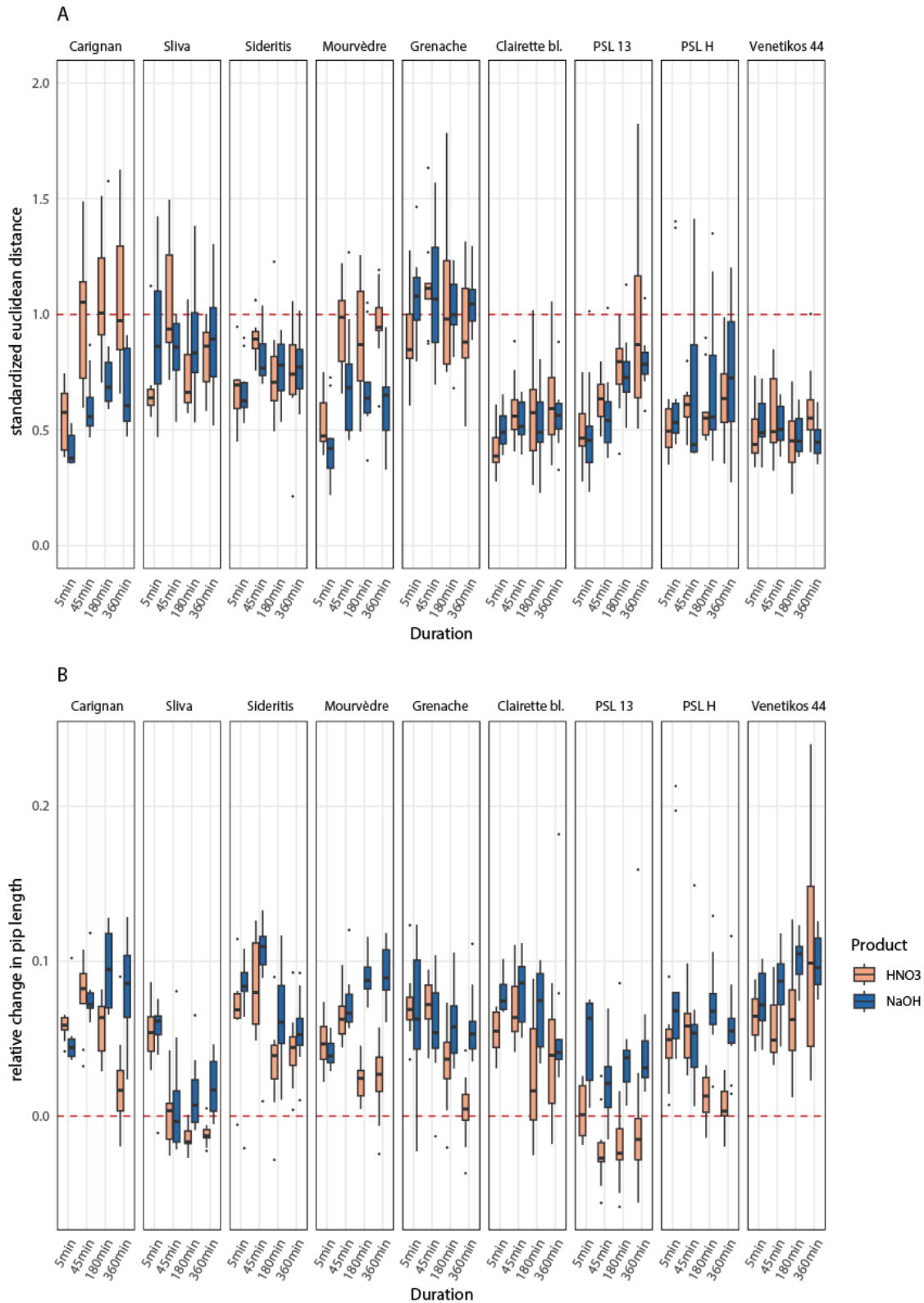


Fig. 4. Intensity of pip deformation induced by two chemical products and different soaking times on the pips of six grape varieties and three wild accessions. Shape changes (A) are calculated using the euclidean distance on Fourier coefficients between the original pip and the same pip after treatment. The resulting differences were standardized by the median euclidean distance between all pips within a given accession (red dotted line). Length changes (B) were calculated as follows: $(L_t - L_0)/L_0$, so that 0 would indicate no change in length and 1 a doubling.

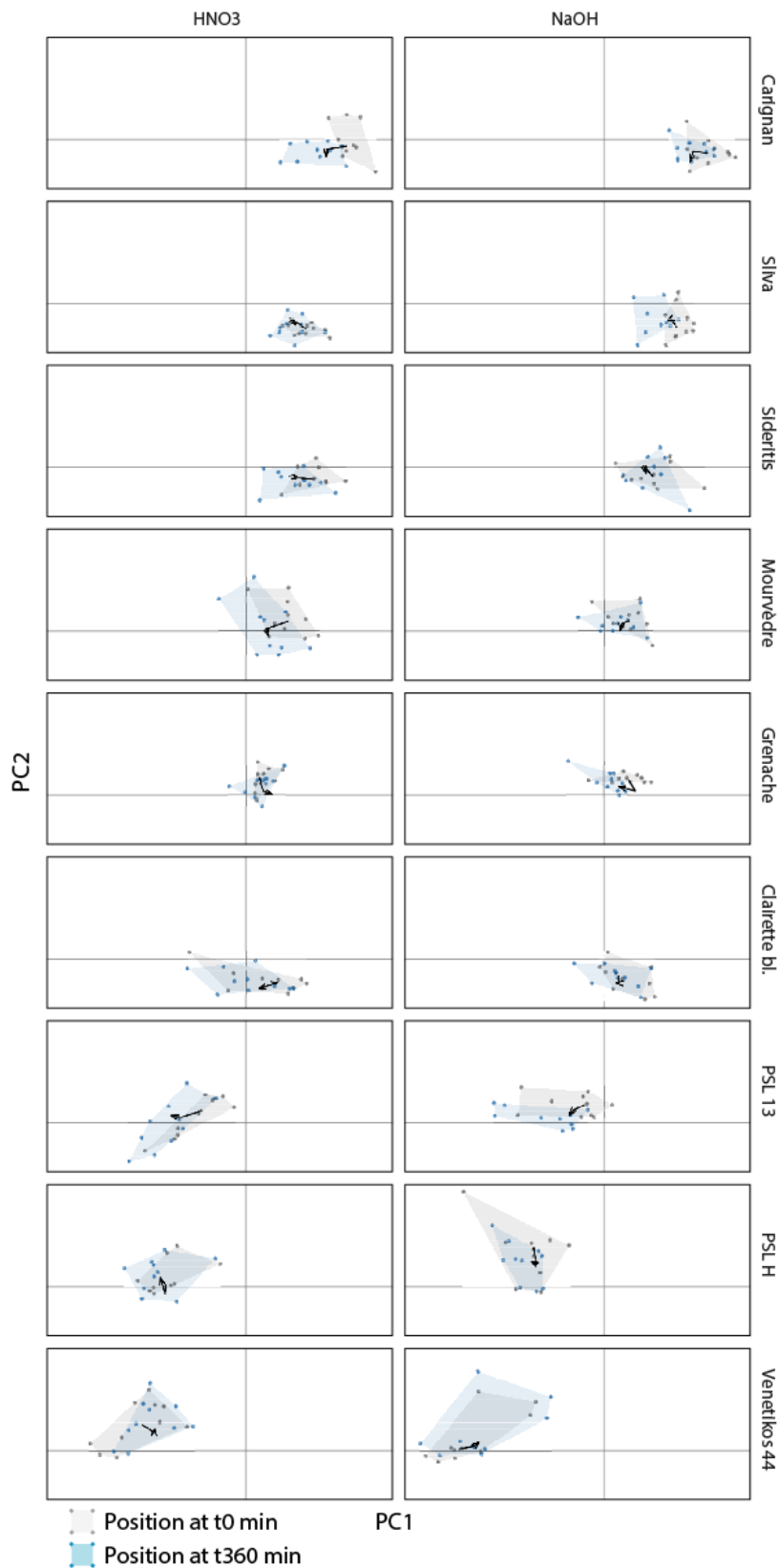


Fig. 5. First biplot of a PCA (62% & 12%) performed on a balanced sample (500 wild / 500 domesticated pips) from the modern reference collection (active observations not shown on the graph). All pips included in Phase 2 experiments are projected as supplementary observations at all soaking times. Only the initial (t_0 min: grey convexhull) and final (t_{360} min: blue convexhull) are fully represented. The segmented arrows show the deformation trajectories from t_0 min to t_{360} through all intermediate soaking times represented as the mean PC1:PC2 scores of all pips involved in the experiment at t_i .

For this second phase, we repeated the experiments with only two products, HNO_3 and NaOH , but this time using a greater number of grape accessions, six varieties, chosen to have seeds of different shapes, and three wild accessions (Fig. 4, Supp. Fig. 1). The two products chosen are an acid and a base, representing the most divergent effects among the four chemicals tested in phase 1. Despite differences between accessions, this experiment confirms that, with the exception of the PSL13 wild grapevine, the seeds of all the accessions reach a maximum deformation around t_{45} min of soaking, then the deformation reaches a plateau or decrease slightly.

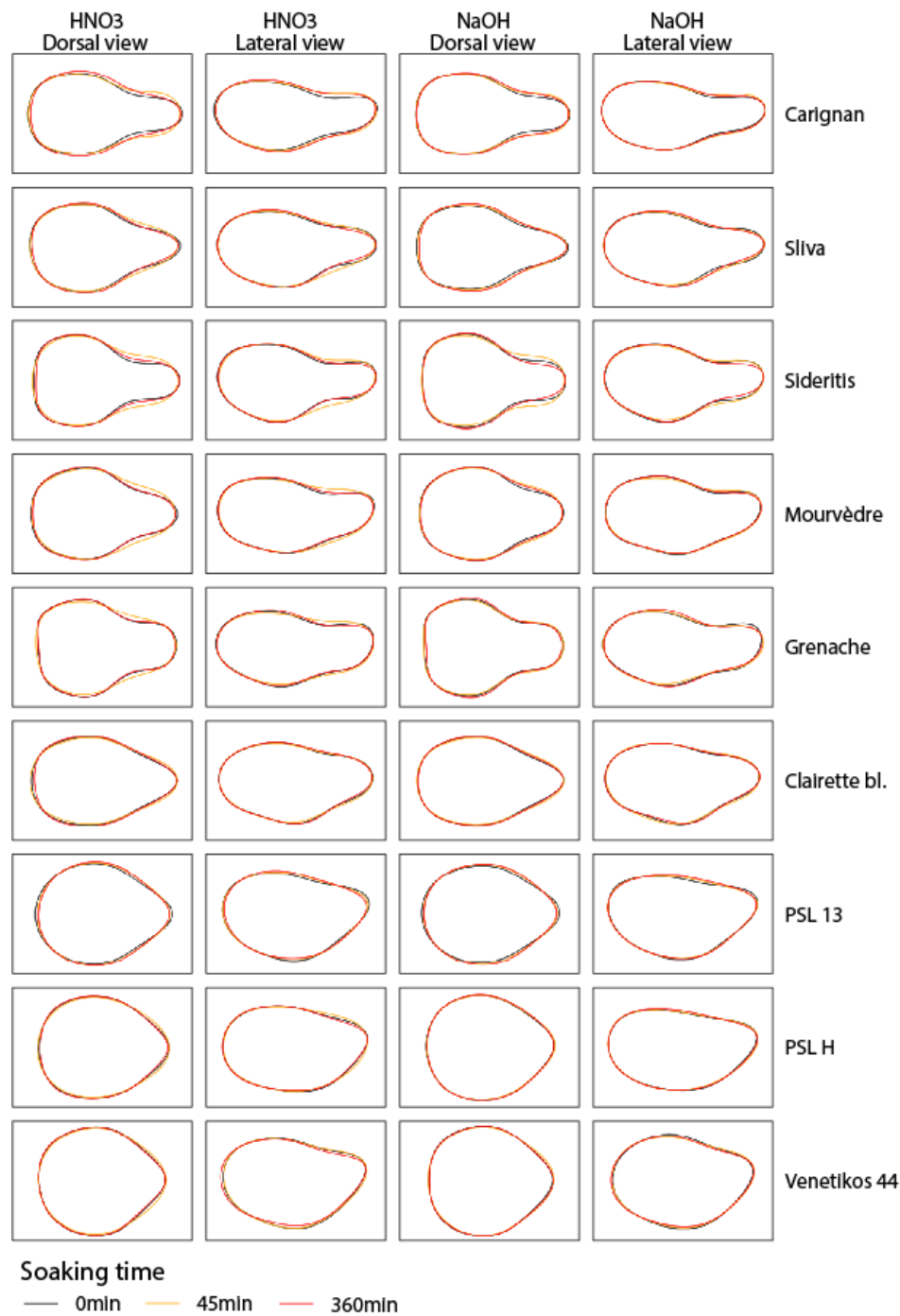


Fig. 6. Visualization of morphological changes according to chemical products (HNO_3 and NaOH), soaking times and Vitis accessions in Phase 2 experiments. Mean shapes were calculated for each view at t_0 min, t_{45} min and t_{360} min, using the inverse elliptical Fourier transformation.

The effect on length is more variable from one accession to another. It usually reaches a maximum at t_{45} min and then decreases to tend towards the original size.

3.2. Pip deformation in relation to wild and domesticated morphotypes

The projection of all pips involved in Phase 2 experiments on the first biplot of a PCA performed on the modern reference collection shows that seed deformation follows a rather erratic trajectory according to the different soaking times for all accessions and both products (Fig. 5). However, after 360 min of soaking the accessions are systematically shifted on PC1 towards the morphological space of wild grapevines. The actual seeds of wild grapevines are also slightly shifted on PC1 and thus take on an even wilder character. In fact, the more the seeds have a shape typical of the domesticated grapevine (elongated shape, long beak), the more the accession is shifted on axis 1. The same overall pattern is identifiable whether the experiment was performed with HNO_3 or NaOH . On the contrary, no clear and consistent pattern can be observed on PC2 for any product.

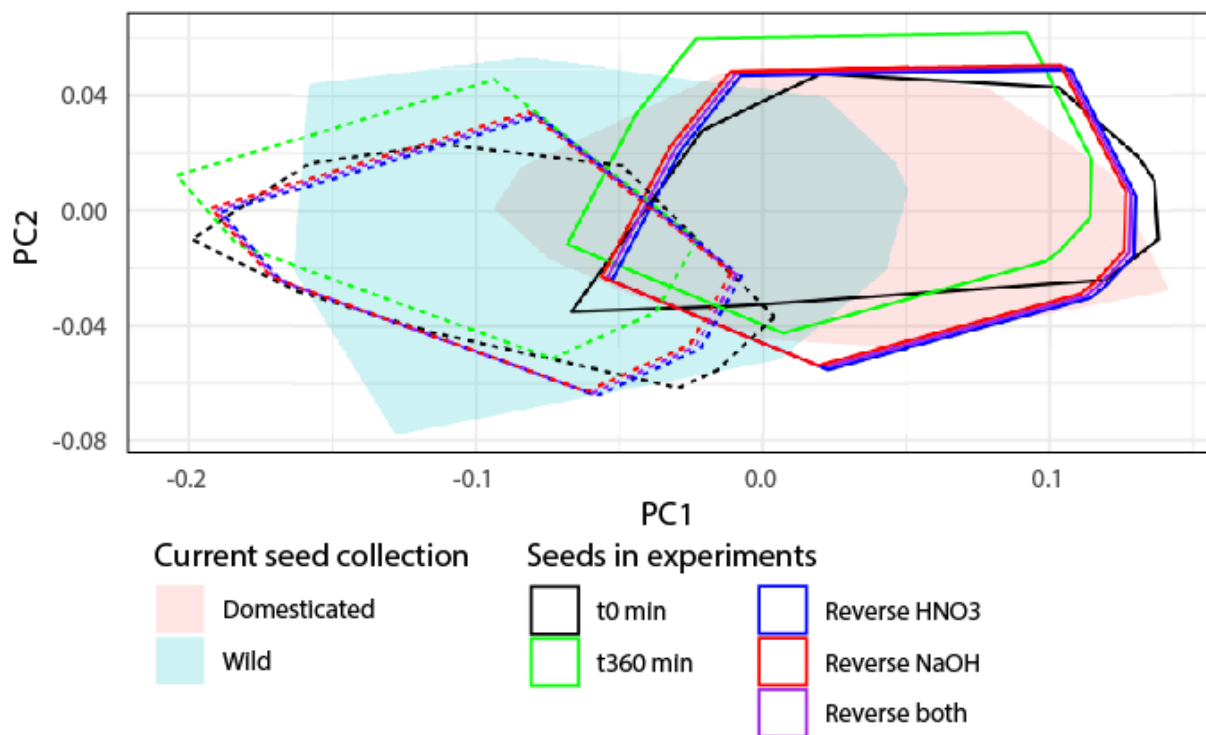


Fig. 7. Geometrical reversing of pip deformation according to status and chemicals. Projection as supplementary observations of the pips included in Phase 2 experiments in the PCA (biplot PC1 & PC2) performed on a balanced sample (500 wild / 500 domesticated pips) from the modern reference collection. Only the convex hulls are shown for the pips of wild and domesticated accessions at t_0 min and t_{360} min. In addition, the position of the pips soaked for 360 min is corrected by the application of a reverse vector to the Fourier coefficient by distinguishing the seeds processed with HNO_3 and NaOH and by considering all the seeds as a single group.

The calculation of mean shapes for dorsal and lateral views show that at t_{45} min, when pip deformation is usually the most pronounced, the seeds generally swell in the beak area and at

the interface between the beak and seed body (Fig. 6). At t_{360} min the pips undergo reverse morphological changes. The mean shape is in most cases closer to the one at t_0 min or even goes beyond it. Pip shape at t_{360} min tends to be more similar to shapes observed in archaeological pips, with the beaks taking on a blunter and more pointed appearance.

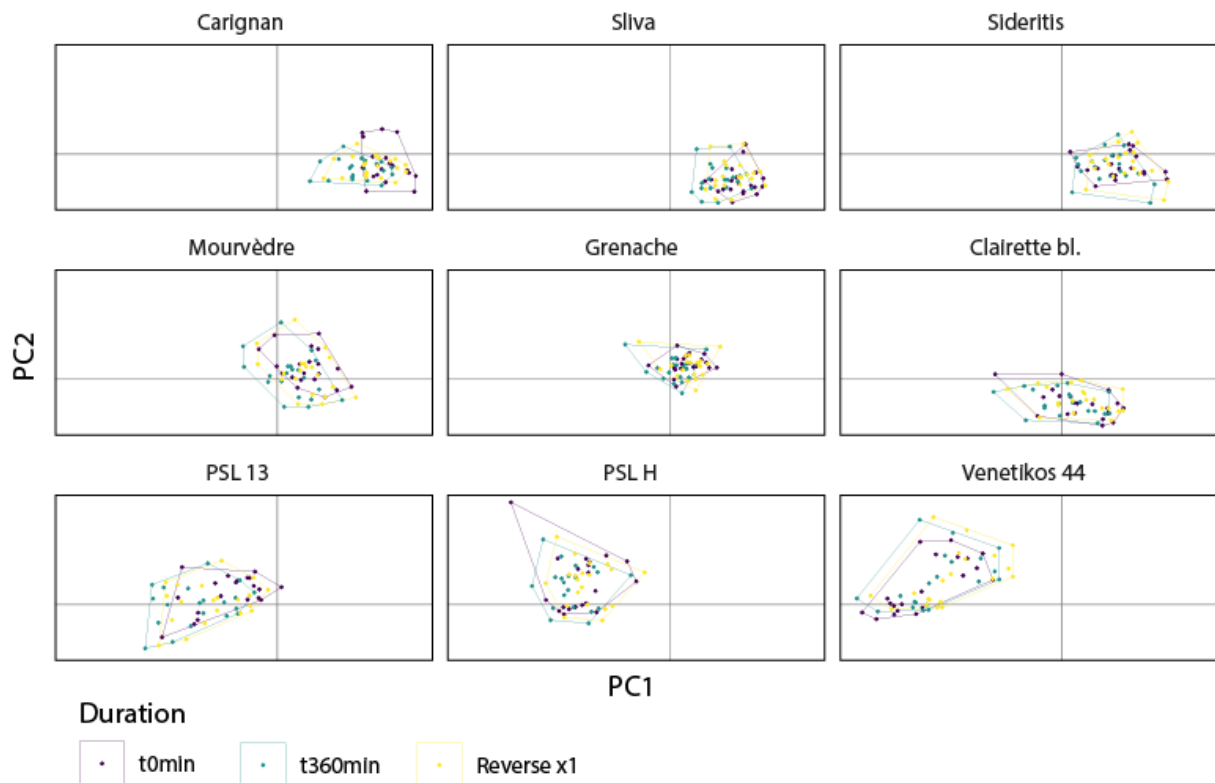


Fig. 8. Geometrical reversing of pip deformation according to cultivars. Projection as supplementary observations of the pips included in Phase 2 experiments in the PCA (biplot PC1 & PC2) performed on a balanced sample (500 wild / 500 domesticated pips) from the modern reference collection. The position of the pips involved in Phase 2 experiments is shown at t_{360} min and after correction by application of a reverse vector to the Fourier coefficients.

3.3. Geometrical reversing of pip deformation

We sought to geometrically compensate for the deformation of pip outlines caused by chemicals and soaking by applying an average reverse vector of shape changes. Still using the projection of the pips from Phase 2 experiments on the first biplot of the PCA performed on the modern reference collection, one can see from the position of the convex hulls how pip outlines at t_{360} min are shifted towards the morphological space of wild grapevine (Fig. 7). The addition of the reverse vector to the Fourier coefficient of each t_{360} min pip bring back the convex hull close to its position at t_0 min. The application of this method by distinguishing between the seeds soaked in HNO_3 and NaOH shows that the two groups have a very comparable response. This confirms that the two chemicals have a similar effect and allows us to consider the seeds soaked in both products as one group. The correction is efficient for both wild and domestic grapevine seeds. If we consider individual accessions, the correction is similarly efficient in each case, whether the accessions are of wild or domesticated grapevines. In this last case however, the correction seems less important for the cultivars most typical of the domesticated morphotype, in particular for ‘Carignan’ (Fig. 8).

3.4. Possibility to correctly identify the status and variety of soaked pips

The LDA_{status} performed on the modern reference collection allows to correctly classify most of the pips from domesticated and wild grapevines involved in Phase 2 experiments (Fig. 9). The accuracy is above 98%, except only for the cultivars ‘Mourvèdre’ and ‘Clairette blanche’. This last cultivar was selected for the experiment specifically because its seeds are close in shape to the seeds of the wild grapevine. After 360 minutes of soaking the prediction results are still very good, in many cases still close to 100%. The impact of chemical treatments on the possibility to correctly identify the status of the domesticated accessions is the strongest on pips that have a morphology close to that of wild grapevine seeds and on the wild grapevine pips. In the case of ‘Clairette blanche’, the most serious, more than 60% of the pips are erroneously assigned to the wild subspecies. It should be also taken into consideration that soaking into chemicals for 360 min also has a noticeable impact on the possibility to identify the status of seeds from wild grapevines. The proportion of correctly classified pips is slightly reduced for PSL-13 and Venetikos-44. The application of a reverse vector of shape changes to compensate for pip deformation does not provide a noticeable improvement of the performance of LDA_{status}.

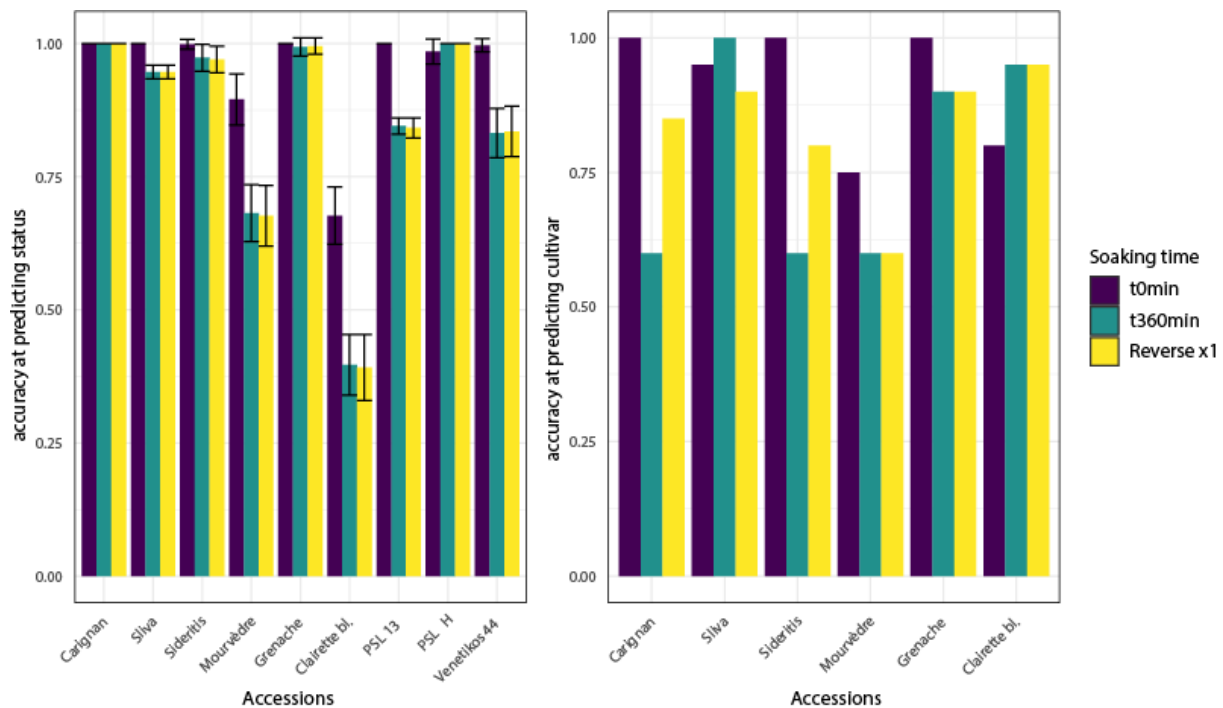


Fig. 9. Impact of deformation of the pips induced by soaking into HNO₃ and NaOH on their characterization using EFT. Classification of the pips involved in Phase 2 experiments in the LDA_{status} and LDA_{cv}. The pips are classified at t₀ min, t₃₆₀ min and t₃₆₀ min after compensation of pip deformation by applying the reversing vector of shape changes based on the mean differences of Fourier coefficients at t₀ min and t₃₆₀ min. Pips from the experiments using HNO₃ and NaOH are considered as a single group.

The LDA performed to identify cultivars (LDA_{cv}) is also very powerful, which was expected given the results achieved in previous studies (Bouby et al., 2021; Terral et al., 2010), and even more so here as only 6 different grape varieties are used. From 75% to 100% of the seeds are correctly classified depending on the variety. The impact of deformation due to

chemicals is somewhat higher in this case. At t_{360} min the percentage of correctly identified pips is usually reduced compared to t_0 min, except in two cases. For two cultivars ('Carignan' and 'Sideritis') the reverse pip deformation partially corrects the effects of soaking and allows to achieve percentages of correctly classified pips closer to the results obtained at t_0 min. These cultivars are among those with the most elongated overall shape and beak. For the other cultivars the correction has no noticeable effect on the accuracy of identification.

4. Discussion and conclusion

Applied to current seed collections, outline analysis based on EFT is a powerful tool to identify wild and domesticated grapevines, and even to identify individual cultivars or groups of cultivars (Bonhomme et al., 2021a; Terral et al., 2010). Using a modern collection composed of 280 cultivars, leave-one-out cross-validation allows the correct classification of 77.2% of the pips (Bouby et al., 2021). Such models build on modern reference material are consequently of great interest to identify archaeological seeds, provided taphonomic issues are taken into consideration. The preservation processes of plant macroremains can indeed have an impact on their shape. This is well known for charred seeds and fruits. Changes in the shape of grape pips due to carbonization have been the topic of various experimental studies. It was concluded that, in spite of these changes, charred pips from wild and domesticated grapevines can still be distinguished by morphometry (Mangafa and Kotsakis, 1996; Uccesu et al., 2016). Charring of the pips at low temperature (250° C) in reducing conditions even still allow their characterization at cultivar level with good reliability, although classification error becomes significantly more important at higher temperatures (450 °C) (Bouby et al., 2018). As a consequence, waterlogged pips were often prioritised for detailed morphometric studies as they are assumed to undergo only minor shape changes during burial. However, some decomposition of organic remains is still occurring under anoxic conditions.

As though degradation of waterlogged plant remains is impossible to reproduce experimentally we tested here the effects of different chemicals as proxies of this long-term decay. The choice to process each seed individually allows us to observe very variable outcomes from one pip to another within one single accession, probably in relation to differences in size, shape and pip composition. General trends can nevertheless be observed. In this way, the four products that we tested had comparable effects. The magnitude of the impact on shape increases over time until about 45 min of soaking. After swelling during this first phase, the seeds tend to return to their original shape, with however small differences in the beak area. The beak is slightly blunter and somewhat more pointed than on the original specimens, whether they come from domesticated or wild grapevines. This situation is reminiscent of the differences in morphology observed between archaeological pips assigned by morphometrics to the wild and domesticated morphotypes and their current counterparts. This confirms that soaking in chemicals does to some extent replicate the effect of long-term waterlogging. In our experiments we have retained 360 min as the limit of soaking time because after that an increasing proportion of the seeds are destroyed or too damaged to allow morphometric analyses. The fact that degradation is more pronounced in the beak area can be explained by anatomical factors. In this area there is a greater development of the outer tegument of the seed coat, which is composed of cells with soft cellulose walls (Huglin and

Schneider, 1998). This tegument is therefore more vulnerable to decomposition in anoxic contexts than the middle and inner teguments, composed of strongly lignified cells.

The morphological changes that have been observed are not, however, strong enough to affect significantly the identification of wild and domesticated morphotypes by seed outline analyses. After soaking during 360 min into chemical most of the pips still can be correctly classified into the correct compartment. However, errors are made for a small number of wild pips and for a proportion of pips from grape varieties whose morphology is close to that of wild grapevine pips. The accuracy of identifications at cultivar level is also still acceptable for artificially waterlogged pips, even though the effect is greater than on status identifications.

The application of an average reverse vector of shape changes to soaked pips allows to geometrically compensate for some of their deformation. However, it does not particularly improve the accuracy of classification at the status level. Geometric compensation for deformation, on the other hand, has a positive effect on classification accuracy at cultivar level for certain varieties whose seeds have an elongated general shape and beak. However, more accessions and a higher number of pips per accession would be needed to better assess the accuracy of identifications according to the variability in seed shape of the cultivars.

This first experimental study on the deformation of waterlogged grape pips provides valuable insights for the morphometric analysis of archaeological seeds. First and foremost, it shows that if deformations exist they are not sufficient to substantially confuse the identification of wild and domesticated morphotypes. The stubby and relatively short-beaked pips, which are close in shape to wild grapevine, are the most affected by these errors among domesticated pips. If we make the assumption that these seeds come from comparatively low selected varieties, closer to the wild grapevine, and that such forms were more common in early phases of viticulture and grape domestication, the number of domesticated seeds erroneously assigned to wild grapevine may be somewhat higher in archaeological assemblages. However, the experiments show that, in the opposite direction, degradation cause a small proportion of wild grapevine seeds to be incorrectly identified as domesticated. In the end, the errors generated by deformations are not liable to skew enough status identifications to explain the large proportion of waterlogged archaeological pips allocated to the wild morphotype in Roman sites. This is consistent with the fact that charred seeds also allow the identification of an important proportion of seeds belonging to the wild morphotype (Bouby, 2014).

Identification at cultivar level also appears to be still feasible despite the distortions caused by waterlogging, especially if a reverse correction vector is applied. The errors are however more important. For this reason, and also because it may be questionable to compare archaeological seeds with present-day varieties that may not have existed at the time, it is preferable to seek to identify large groups of varieties related to their primary use (i.e. table vs wine) or geographical origin (Bonhomme et al., 2021a). The major eco-geographical groups of varieties relate to different stages in the process of domestication and diffusion of cultivated grapevine (Bacilieri et al., 2013; Dong et al., 2023; Magris et al., 2021).

As with charring, waterlogging leads to some deformation of grape seeds. However, these shape changes do not impact the seed in the same way and are less serious in most cases, depending on charring temperature. While the changes mainly relate to the beak area in waterlogged seeds, they comparatively affect more severely the body of the pip, which swells under heating (Bouby et al., 2018; Mangafa and Kotsakis, 1996; Smith and Jones, 1990;

Uccesu et al., 2016). Deformations due to charring and waterlogging will consequently have differentiated effects on the identifications based on morphometric analyses. When investigating the evolution of grapevine in a given area based on pip morphology it is therefore recommended to aim at taking into account both waterlogged and charred seed assemblages.

Finally, the numerical method of shape reversing is simple and generic and can easily be parameterised, tested and used in other conservation context, and beyond grape. Given the universal taphonomical bias one has to consider when it comes to archaeobotanical macroremains further study will help clarify if and how it can help archaeobotanical inference.

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Supplementary Fig. 1. *Examples of pips of the different accessions used in the experiments at different soaking times (t_0 min, t_{45} min and t_{360} min) in the two chemicals HNO_3 and $NaOH$.*

Declaration of interests

The authors declare no competing interests.

Authors contribution

Laurent Bouby: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Supervision; Visualization; Writing - original draft; Writing - review & editing.

Vincent Bonhomme: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Visualization; Writing - original draft; Writing - review & editing.

Sarah Ivorra: Data curation; Formal analysis; Investigation; Writing - review & editing.

Thierry Pastor: Data curation; Methodology; Investigation; Writing - review & editing.

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