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FINAL REPORT 2024

SA WINE number: P04000060-2019

1. PROGRAMME & PROJECT LEADER INFORMATION

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2. PROJECT INFORMATION

Project title	Testing Pinotage mutation plants
Short title	Screening for novel ‘Pinotage’ clones

Fruit kind(s)	Wine grapes		
Start date (mm/yyyy)	2019-01	**End date** (mm/yyyy)	2023-12

Key words	Field screening, Selection

	CFPA	RAISIN SA	HORTGRO	SATI	SA WINE	ARC	OTHER
TOTALS All years	R 0	R 0	R 0	R 0	R 2126984	R 710746	R 0

Total cost of the entire project	R 2837730

3. EXECUTIVE SUMMARY

Objectives and Rationale

The ultimate goal of this project was to generate more Pinotage clones, given that only very few commercial clones are available for this important cultivar and that the available clones did not display prominent variation in viticultural and oenological traits. In the first part of this multi-phase project (2017-2018), three widely used Pinotage clones were subjected to gamma irradiance, whereas this second phase aimed to screen the resulting population, select some individuals for field testing and provide a first analysis of vegetative and reproductive traits observed in the population.

Methods

In the previous funding cycle, gamma irradiation was used to stimulate somatic mutations in *in vitro* cultured plants of three Pinotage clones. Over the course of this project, the resulting plants were hardened off and grown in tunnels where vegetative traits were scored. The population of approximately 1500 plants exhibited a diverse collection of traits, and a sub-population was selected for field evaluation and characterisation. A detailed characterisation of the diversity observed in the vegetative characteristics of 500 plants in a field trial was completed using morphometrics, and the first scoring of grape cluster and berry characteristics was possible for a subset of vines that yielded some bunches in the last year of this phase of the project.

Key Results

The initial similarity and dissimilarity scoring of the 1500 plants allowed the selection of 90 controls (30 per clone, based on similarity with the unirradiated controls), and the rest of the irradiated plants that exhibited dissimilar features (to the controls). Phenological progression of the field trial population indicated that budding time and onset of senescence were altered in many of the population of plants (earlier and later). Moreover, the morphometrics of leaves and petioles confirmed that the irradiance caused increased variation in most of the observed traits. The description of the growth habits (plant architecture) and initial bunch and berry characteristics in the mutant population during the final study year also provided confirmation that the population contained individuals with altered phenotypes, compared to the controls.

Key Conclusion of Discussion

The phenotyping of the mutant population indicated that increased diversity was achieved in several parameters analysed, confirming the potential of gamma irradiance to induce variation. The altered phenological development rates, with some vines displaying earlier or later ripening compared to existing clones holds potential as a parameter in further selection criteria in the population. Similarly, the wide diversity in bunch architecture, berry size, and skin and flesh colours observed is interesting and needs to be studied in more depth in the population. The broad range of canopy sizes and growth habits observed, from very bushy to upright, also warrants further investigation and confirms that the irradiance caused more diverse phenotypes regarding these parameters compared to the controls.

Take Home Message for Industry

This project concludes with successfully demonstrating the efficacy of gamma irradiance to induce plant phenotypic variation in several important vegetative and reproductive traits that could support clonal diversification for Pinotage. The progress made in characterising the mutant population in this project provides the base for the next steps in the project, which would be the preselection of some candidate plants for further evaluation in a clonal selection

process.

4. PROBLEM IDENTIFICATION AND MOTIVATION

Problem Identification

Clones (sometimes new cultivars) normally arise through natural mutations, especially in very old cultivars that were widely grown under diverse conditions. Obvious pronounced differences (i.e., berry colour and different leaf shape) are usually regarded as new cultivars, while more subtle variations in traits (i.e., looser clusters or earlier ripening) are regarded as clones. Well-known examples are the Pinot group (mutations for berry colour and leaf hairiness) and the Chasselas group (mutations for leaf shape, berry colour and aroma compounds). Clonal selection has been central to plant improvement strategies for wine grapes for years, given the predominance of a few Vitis vinifera cultivars grown globally.

Relatively young cultivars, such as ‘Pinotage’, typically have fewer clones, since they have not been as widely and extensively planted under varying conditions to allow for natural mutations. Only a few commercial clones of ‘Pinotage’ exist, and given the growing importance of the cultivar, a more diverse set of clones to choose from is desirable. In a pilot project, three ‘Pinotage’ clones were used to optimise a method to induce mutations through irradiance of in vitro plants. The aim of this project is to screen the resulting plants to identify mutations and potentially useful ‘Pinotage’ clones.

Motivation

This project falls within the theme of plant improvement and uses a method to advance clonal diversity in Pinotage. The new knowledge generated through this project relates to the approach to use gamma irradiance as a means of clonal diversification. The outcomes of the project would be new plant materials, either in the form of novel Pinotage clones, or potentially new cultivars (for example, a white Pinotage), or breeding lines with novel characteristics that can be used in the ongoing traditional breeding programme. All of these outputs will directly benefit the wine industry.

5. ACCUMULATED PROGRESS TABLE

Objectives	Milestones (Significant event or stage in a project)	Date Achieved
Objective 1: Pre-screening of the irradiated and control populations in tunnels/ greenhouses to select a sub-population for the field screening	M1.1 Numbering and recording of all plants in the untransformed control groups, as well as the irradiated plants that survived the hardening off process. M 1.2 Pre-screening of the control and irradiated populations for off-types, growth habit, and morphological characteristics	2020-04-30
Objective 2: Selection of sub-population for field trial analysis	M2.1 Create similarity/dissimilarity score for each irradiated vine based on the pre-screening done during M1.2. M2.2 Selection of sub-population for field trial based on similarity/dissimilarity scoring.	2021-04-30

Objective 3: Screening of the selected sub-population under field conditions (500 plants)	M3.1 Establish infrastructure for the field plot and planting of the selected individuals [ALL PLANTS MOVED TO FIELD SITE IN JUNE 2022] M3.2 Phenological and Vegetative profiling of plants established in the field trial using reference methods M3.3 Vegetative profiling of plants established in the field trial using higher-throughput methods such as morphometrics M3.4 Identification of individual plants for further evaluation as potential clones Extension request to continue for a further season was granted (new target date: 2023-12-31). The first grapes were present in the 2022/2023 season, and scoring for cluster and berry traits was completed for the 2022/2023 season. New project application filed in 2023. Data collection and analyses continued on new funding starting in 2024.	2023-12-31
Objective 4: Maintenance and further phenotyping of residual vines under greenhouse conditions	M4.1 Greenhouse screening completed in April 2022; Plants were all moved to the field site in June 2022 and have been maintained under field conditions.	2022-06-30

6. WORKPLAN (MATERIALS AND METHODS)

This project followed from a pilot study where the method to perform gamma irradiance was optimised and then applied to three clones of Pinotage (clone 7, 45 and 48) on *in vitro* plants. The irradiated nodes were excised and grown into a population of plantlets that were hardened off and maintained in growth tunnels (as the output from the pilot study). Over the course of this project, the plants (1416) were pre-screened on vegetative traits to select plants for field screening, with the first pre-screening done in the 2020-21 growth season. After pre-screening, a population of 500 plants were selected for field screening, and these plants were transferred to the field site.

The methods used in the pre-screening, planting the field plot and screening of plants in the field plot for vegetative, crop and phenological traits for the first two seasons will be presented.

Objective 1: Pre-screening of the irradiated and control populations in tunnels/ greenhouses to select a sub-population for the field screening.

By the end of February 2020, all plants at Nietvoorbij were hardened off from *in vitro* conditions, transplanted to large (8 litre) growth bags and grouped by clone and irradiation dose in the tunnels.

Individuals in the population of 1416 plants were numbered, labelled and pre-screened on visible vegetative traits and compared against the non-irradiated controls of the three clones of Pinotage that form part of the study. Irradiated plants were compared with the non-irradiated controls to identify any obvious off-types (such as dwarf mutants) to exclude, as well as select a subset of plants showing low to moderate dissimilarity with the controls to be evaluated more comprehensively for the presence/phenotypic expression of favourable somatic mutations in the irradiated population.

During the pre-screening, the following attributes were looked at: growth stage, growth habit, internode lengths, whether the stem of the vine had naked patches, the presence of pests and diseases, general leaf appearance and leaf-, petiole- and vein colouration.

Objective 2: Selection of sub-population for field trial analysis.

M2.1 Create similarity/dissimilarity score for each irradiated vine based on the pre-screening done during M1.2.

To perform comparative screening of the 1416 plants according to the range of phenotypes, in-house scales were established (for example, a Leaf blade colour scoring scale, a Leaf vein colour scoring scale and a Petiole colour scoring scale). These scales represent the diversity of phenotypes that were present in the population of plants. In each of the scales, the typical appearance of the controls (non-irradiated plants) was also recorded and compared against that of the irradiated plants.

M2.2 Selection of sub-population for field trial based on similarity/dissimilarity scoring.

Based on all the scoring combined, the vines were then each scored for their overall similarity/dissimilarity compared to the non-irradiated clones, with a score ranging from 1 (completely dissimilar) to 9 (identical to the controls).

Based on these ratings, the 500 vines for the field trial were selected. Irradiation dose was not the main criterion for the selection; the individual vines' dissimilarity rating was the main criterion for selection and inclusion in the field trial. The control vines with the highest homology with other individuals in the control group were selected, whilst for the irradiated groups, vines with the lowest rating (most dissimilar) were selected as long as they did not present any unwanted/negative phenotypes.

Objective 3: Screening of the selected sub-population under field conditions.

M 3.1 Establish infrastructure for the field plot and planting of the selected individuals.

The field site was chosen to minimise the risk of virus infection as the foremost consideration. Thus, potential risks posed by nearby vineyards, as well as the history of the block, were taken into account when block B3 was selected for the field site. Block B3 was previously planted with various table grape cultivars, but all vines were removed in 2014, and since then, no vines have been planted (korog or oats were sown for soil conditioning).

Soil preparation: Done in autumn 2016 by ripping (1.2 m depth) and cross-ripping (500-600 mm).

Soil fumigation: Strip-fumigation of soil was done in October 2017.

The adjacent blocks were planted with seedlings from table grape crosses planted on their own roots after soil fumigation (B4) and a block that lay fallow for a number of years (B2). The first few rows in Block B3 were planted with certified (foundation status) Ramsey rootstocks. Two seedling populations, used for mapping or other studies and seedlings selected for breeding parents were grafted on these after being tested for leafroll.

Layout of the experimental plot

The 500 pre-selected vines (including controls) were planted in September 2021 with a spacing of 2.15 m between rows and 0.5 m between vines. Each row consists of 11 parcels ("vakkies") in total. The first 10 parcels have 9 vines in each, with the last parcel having 10 vines, thus 100 vines per row with 5 rows in total. As each irradiated vine is a unique individual, the 500 vines were planted with a randomised plot layout. Since very few plants

flower in tunnels, the rest of the plants were moved to the field plot in June 2022 after they were screened for another season in the greenhouse in March/April 2022.

M 3.2 Phenological and Vegetative profiling of plants established in the field trial using reference methods.

During the 2021/2022 season, basic phenological scoring using the EL-scoring system was used to track the vegetative developmental patterns of the vines under field conditions.

M 3.3 Vegetative profiling of plants established in the field trial using higher-throughput methods.

Leaf samples were collected from the main shoots of each vine and stored in individually labelled plastic bags. These were then transported in a cooler with ice packs to stop the leaves from getting dehydrated prior to scanning, as this may influence the colour of the leaves. Three representative mature leaves and three young leaves were collected per grapevine, where possible, as some grapevines had very few leaves due to their young age. All these steps were based on the process followed during the morpho-colourimetric analysis conducted by Fuentes, Hernández-Montes, Escalona, et al. (2018).

The selection criteria for mature leaves were fully expanded leaves, at least five node positions from the growth tip and eight-node positions from the base of the shoot. Whilst young leaves were defined as leaves that have not yet fully expanded, but are entirely detached from the shoot tip and positioned in the first three leaves, separate from the shoot tip (Goussard, 2008).

The following was also considered when selecting leaves for analyses (Goussard, 2008): Leaves should not be damaged; leaves should not be shaded; no atypical leaves should be selected if this atypicality was caused by vineyard herbicide/fungicide treatments, insects or disease, and leaves from water shoots and lateral shoots were not selected. Images were then analysed using two custom codes written by Prof C. Poblete-Echeverría on MATLAB® R2021b.

Morpho-colorimetric Analysis.
The petioles were detached from the collected leaves, and the leaves and petioles were scanned using a Hewlett-Packard DeskJet Plus 6475 (Hewlett-Packard Software Company, Palo Alto, CA, USA) printer scanner at 600 dpi.

Images were scanned with a reference black square with known dimensions (10 mm x 10 mm) to determine the pixel count in a known area and relate that to the area of the leaf scanned. The codes analysed both the leaves and the petioles to determine morphometric and colour parameters within the scanned images. These parameters and their descriptions can be found in **Table 1**.

Table 1: Parameters evaluated during morpho-colourimetric analysis and their descriptions (The MathWorks, n.d.).

Parameter's indication	Parameters	Units	Descriptions
Size	Area	cm^2	The actual number of pixels in the region.
Size	Convex Area	cm^2	The number of pixels in Convex Image is returned as a scalar.
Size	Equivalent Diameter	cm	Diameter of a circle with the same area as the region.
Size	Filled Area	cm^2	Number of pixels in the region where all holes have been filled in.
Size	Major Axis Length	cm	Length of the major axis of the region.
Size	Minor Axis Length	cm	Length of the minor axis of the region.

Size & Form	Perimeter	cm	Distance around the boundary of the region
Form	Eccentricity	-	Eccentricity of the ellipse that has the same second moments as the region
Form	Extent	-	Ratio of pixels in the region to pixels in the total bounding box, returned as a scalar. Computed as the Area divided by the area of the bounding box.
Form	Orientation	degrees	Angle between the x-axis and the major axis of the ellipse that has the same second moments as the region,
Form	Solidity	-	Proportion of the pixels in the convex hull that are also in the region,
Form	Fractal dimension (Average and SD)	-	A quantification of the complexity of the region.
Colour	RGB Max	-	A scale used to measure colour where R = red, G = green and B = blue colours
Colour	RGB Mean	-	A scale used to measure colour where R = red, G = green and B = blue colours
Colour	L lightness	-	A measure of the relative degree of black or white mixed into a given hue (L = 0 yields black and L = 100 indicates white)
Colour	a green–red	-	Ratio of green to red (where negative values indicate towards green and positive values indicate towards red)
Colour	b blue–yellow	-	Ratio of blue to yellow (where negative values indicate towards blue and positive values indicate towards yellow)
Colour	Green Leaf Index (GLI)	-	The relation between the reflectance in the green channel compared to the other two visible light channels

A multivariate data analysis approach was implemented for the data obtained from the Morpho-colorimetric Analyses, using principal component analysis (PCA) on the SIMCA® multivariate data analysis software (Sartorius AG Company, Göttingen, Germany). Basic statistical analyses were also performed to determine the mean, minimum, maximum and standard deviations of the parameters obtained in specific groupings and individual grapevines with the use of Statistica® (Statistica Software Company, Hamburg, Germany).

M3.4 Identification of individual plants for further evaluation as potential clones will be based on all the data collected during the growing season.

This was an ongoing action to identify any unique characteristics in the population that could warrant selection for a deeper analysis. An initial reproductive profiling for the vines that started fruiting in the 2022/2023 season was performed, using basic viticultural descriptors and berry characteristics.

Objective 4: Maintenance and further phenotyping of residual vines under greenhouse conditions.

All plants were moved to the field site in June 2022. Maintenance of the field site includes following a preventative spraying program for disease and pest control.

7. RESULTS AND DISCUSSIONS

This project aimed to describe and characterise the extent of and details of the variations in the

morphology of the vegetative characteristics of the gamma-irradiated Pinotage collection under greenhouse and field conditions.

Objectives 1 and 2 involved the initial pre-screening of the mutant collection under growth tunnel/greenhouse conditions to select 500 plants to establish a field trial that was planted in 2021.

Objective 1: Pre-screening of the irradiated and control populations in tunnels/ greenhouses to select a sub-population for the field screening

This objective entailed the numbering, labelling and initial screening of the entire grapevine population. During this process, a total of 1562 vines were counted initially, but 1416 vines formed part of the subsequent pre-screening after discarding 146 vines that died during the acclimation process. The breakdown of the population in terms of the number of non-irradiated plants for the three different Pinotage clones, as well as the population sizes of irradiated materials (radiation dosages, expressed in Gray (Gy) units from 10 to 30 Gy), is presented in Table 2.

Table 2: Recorded population size of control and irradiated grapevines according to Clone and radiation dosage received.

Population size						
Clone	No irradiation	Irradiation				Total
	Control (0 Gy)	10 Gy	15 Gy	20 Gy	30 Gy	
7	42	112	146	181	0	481
45	35	119	140	193	2	489
48	41	61	186	158	0	446
Total*	118	292	472	532	2	1416

*146 dead vines where discarded (9% of the total population).

During the pre-screening, the following attributes were looked at: growth stage, growth habit, internode lengths, whether the stem of the vine had naked patches, the presence of pests and diseases, general leaf appearance and leaf-, petiole- and vein colouration.

Interesting observations:

Many interesting phenotypes have been observed throughout the screening process of this project (refer to Figure 1 A – E for a few examples). The easiest phenotypes to see were those that affected the nodal structure (Fig 1A), or the leaves of the vines, which ranged from colour extremes of the blade, veins and petioles (Fig. 1B and C), or leaf shape (Fig. 1D). Indications of early senescence (Fig 1 B) could also be observed, although a phenological progression over the whole season was not performed due to different ages of the vines being evaluated – this aspect will be evaluated more closely in the field trial. Furthermore, several of the vines displayed early leaf loss at the base of the shoots, leaving naked patches over the lower part of the stems with a few leaves only forming at the top end of the shoots (Fig. 1E).

Figure 1: Some Interesting phenotypes that were observed. A) Atypical node morphology. B) Early senescence/leaf blade reddening C) Bleached leaves. D) Atypical leaf morphology. E) Naked patches on stems of vines due to leaf loss on the basal parts of the shoots.

Objective 2: Selection of sub-population for field trial analysis

To perform comparative screening of the 1416 plants according to the range of phenotypes, in-house scales were established as outlined in **Figure 2** (Leaf blade colour scoring scale), **Figure 3** (Leaf vein colour scoring scale) and **Figure 4** (Petiole colour scoring scale). These scales represent the diversity of phenotypes that were present in the population of plants. In each of the scales, the typical appearance of the controls (non-irradiated plants) is indicated with a red border around the specific image/number in the scale.

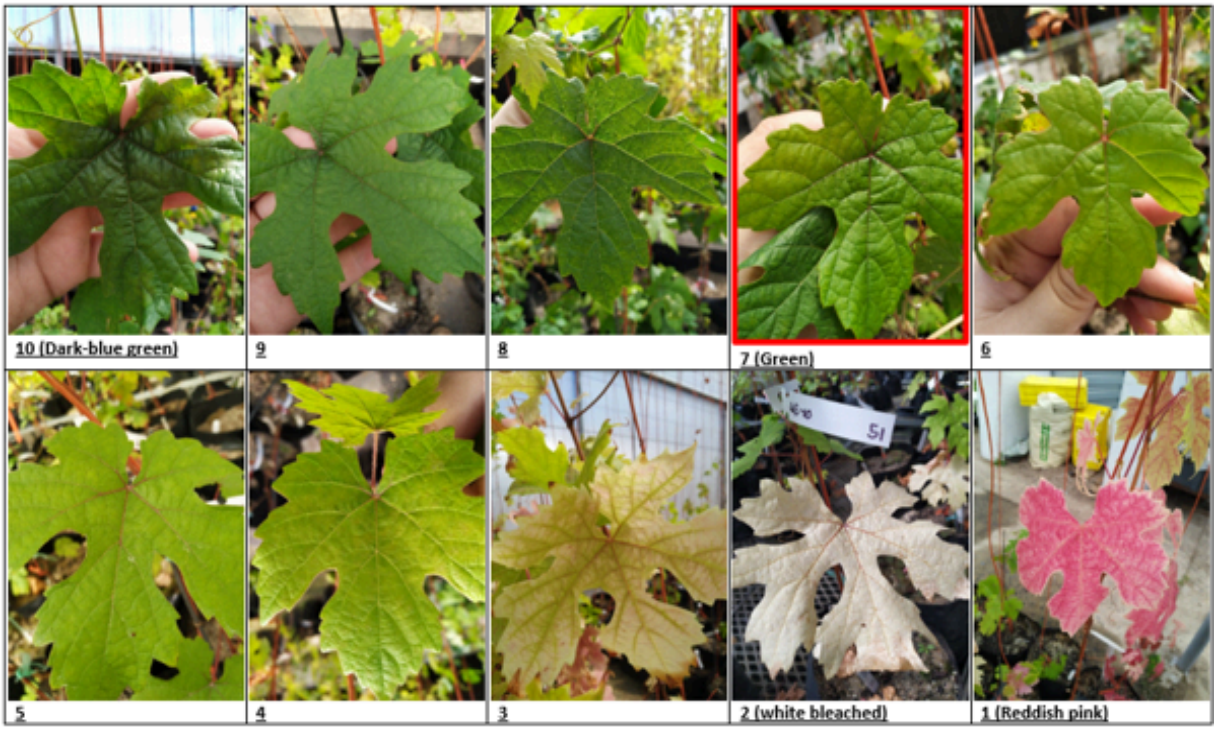

Figure 2: Leaf blade colour variation classification scale. The colour classification shows the entire range of colours observed in the grapevine population. The image (and associated number in the scale) with a red border indicates the score of the non-irradiated controls, also representing the colours mostly observed within the population.

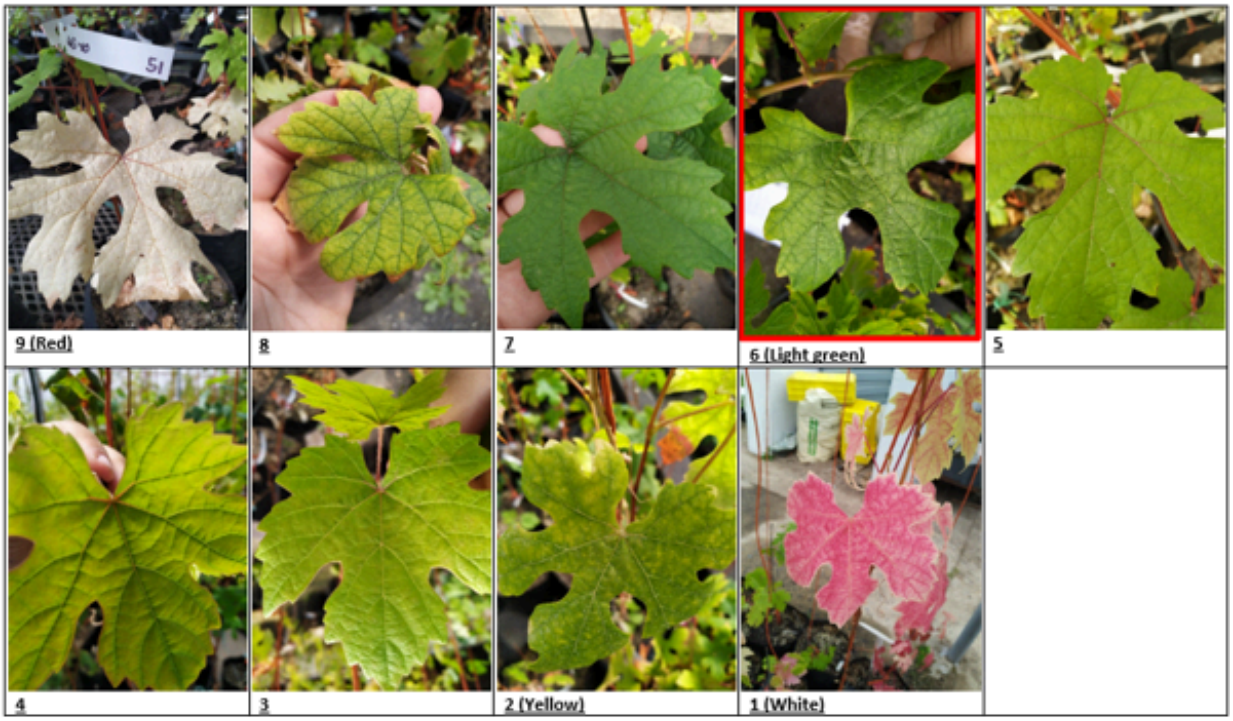

Figure 3: Leaf vein colour variation classification scale. The colour classification shows the entire range of colours observed in the grapevine population. The image (and associated number in the scale) with a red border indicates the score of the non-irradiated controls, also representing the colours mostly observed within the population.

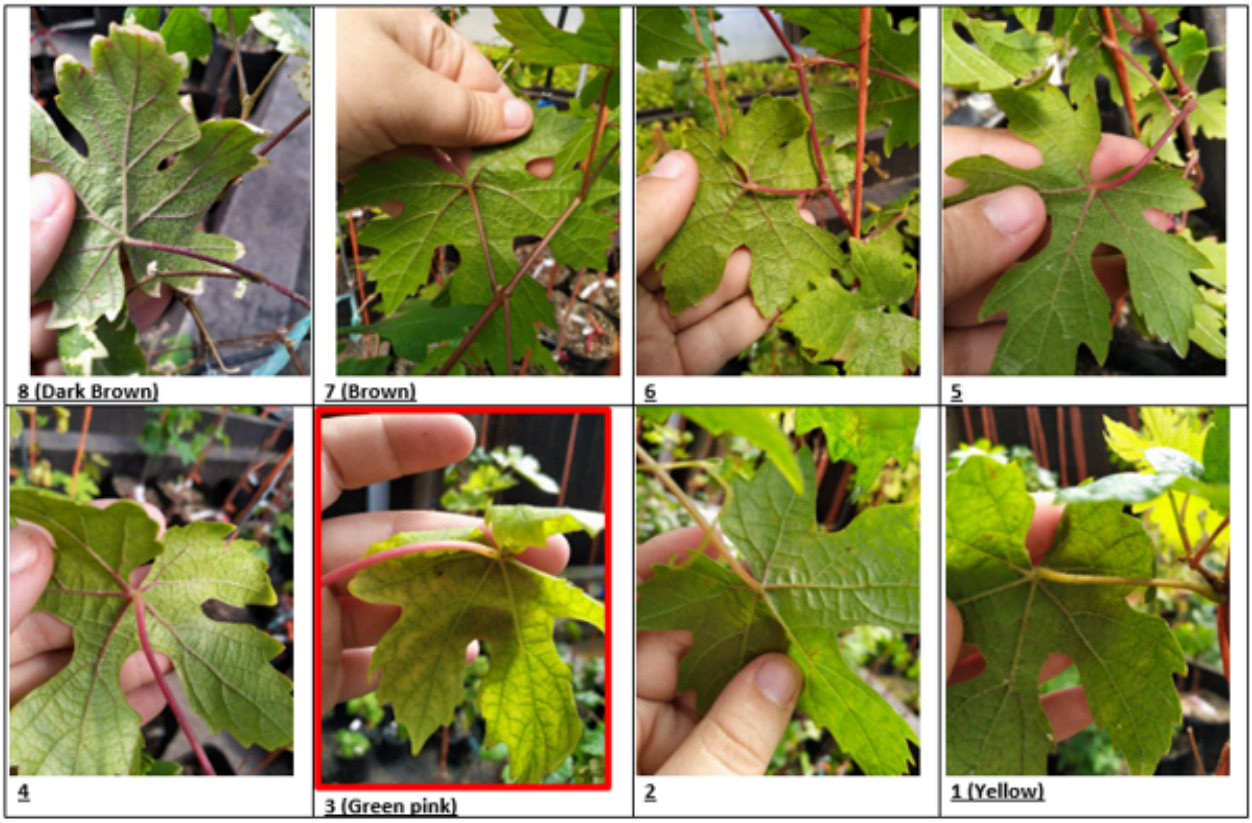

Figure 4: Leaf petiole colour variation classification scale. The colour classification shows the entire range of colours observed in the grapevine population. The image (and associated number in the scale) with a red border indicates the score of the non-irradiated controls, also representing the colours mostly observed within the population.

Based on all the scoring combined, the vines were then each scored for their overall similarity/dissimilarity compared to the non-irradiated clones, with a score ranging from 1 (completely dissimilar) to 9 (identical to the controls). An example of the distribution curves obtained is provided in **Figure 5** (for the irradiated population of Pinotage clone 48). The red blocks indicated the vine numbers that were selected for inclusion in the field trial.

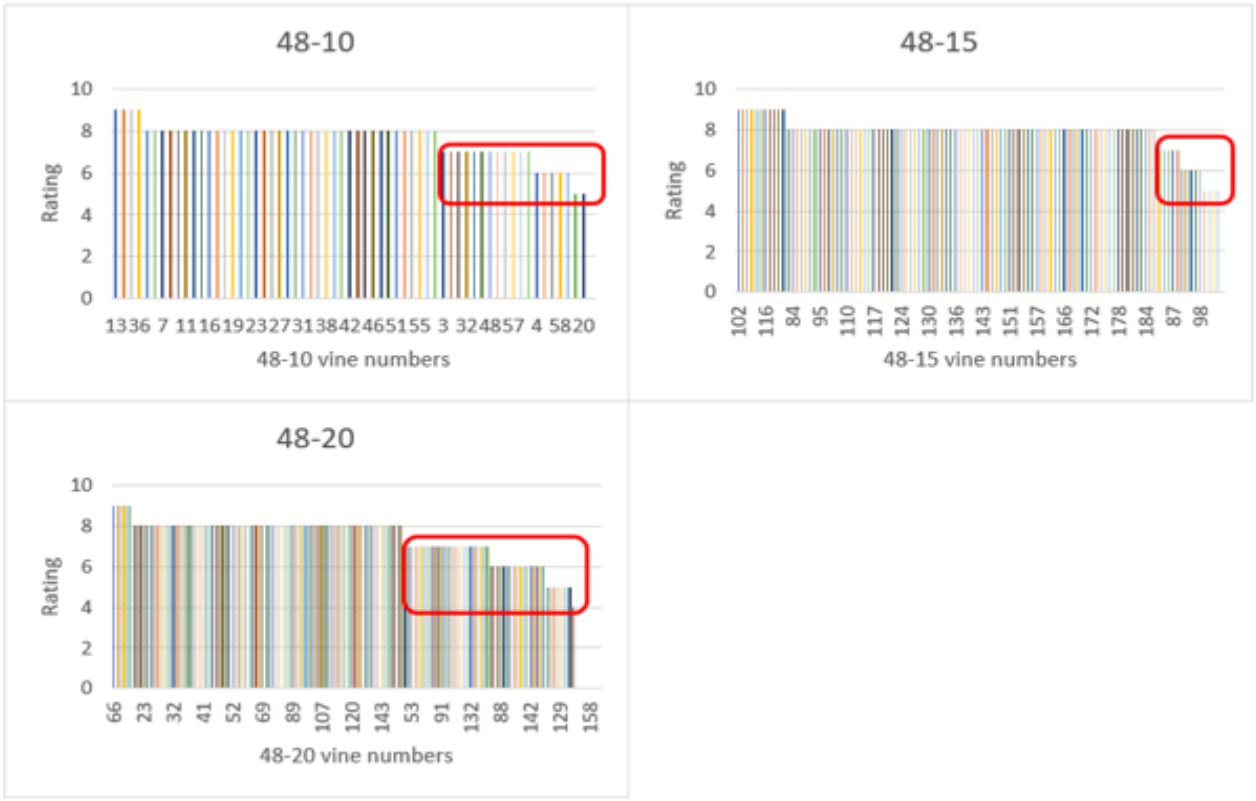

Fig. 5. Distribution curves of the irradiated Clone 48 vines presented per irradiance level (10, 15 and 20Gy). The red boxes indicate from which scoring sector the vines for the field trial were selected.

Based on these ratings, the 500 vines for the field trial were selected. Irradiation dose was not the main criterion for the selection; the individual vines' dissimilarity rating was the main criterion for selection and inclusion in the field trial. The control vines with the highest homology with other individuals in the control group were selected, whilst for the irradiated groups, vines with the lowest rating (most dissimilar) were selected as long as they did not present any unwanted/negative phenotypes (such as poor growth, dwarfism, etc.). The number of plants per clone and irradiation dose selected for field trial evaluation are summarised in **Table 3.**

Table 3. A summary of the composition of the field trial population.

Population size for field trial						
Clone	No irradiation	Irradiation				Total
	Control (0 Gy)	10 Gy	15 Gy	20 Gy	30 Gy	
7	30	36	66	63	0	195
45	30	32	36	42	2	142
48	30	27	46	60	0	163
Total	90	95	148	165	2	500

Objective 3: Screening of the selected sub-population under field conditions.

M3.1 Establish infrastructure for the field plot and planting of the selected individuals.

Completed and reported on in the 2021/2022 annual reports; details are shown in the Materials and Methods section of this final report.

M3.2 Phenological profiling of plants established in the field trial using reference methods.

The phenological progression of the entire collection was recorded using the Eichhorn-Lorenz scoring system growth stages, whereas internode sizes and trunk circumferences were measured as indicators of plant vigour. It was found that for the phenological development, the grapevines treated with radiation showed an overall decrease in phenological development compared to their control counterparts on average, but that there were individuals in the population that had earlier or later budding, as well as earlier or later onset in senescence. (detailed data shown in the 2022 progress report, as well as the MSc thesis of Pohl, 2022). This effect of the radiation could also be observed in decreased internode lengths and trunk circumferences of the treated grapevines overall (detailed data shown in the 2022 progress report, as well as the MSc thesis of Pohl, 2022). These aspects were scored during the first year since planting out and need several more seasons for deeper analysis and confirmation of observed phenotypes.

M3.3 Vegetative profiling of plants established in the field trial using higher-throughput methods.

During the initial evaluation, leaf morphology was the most frequent and easily spottable variable in the irradiated vines. Therefore, further investigation focused on analysing the leaf morphology of the field trial grapevines using scanned leaf images and analysing these images through custom code written on MATLAB in a process known as morpho-colourimetric analysis (refer to the details of the methodology as outlined in the work plan section of this report).

Morpho-colourimetric analysis
An increase in variability was observed for the treatment grapevines for the basic descriptive statistics of themorpho-colourimetric parameters, as well as using multivariate analyses of the morpho-colourimetric parameters (**Figures 6 and 7**, as examples). This increased variability of the treatment groups serves as an indicator that the radiation treatments were indeed successful in inducing mutation in the treatment vines.

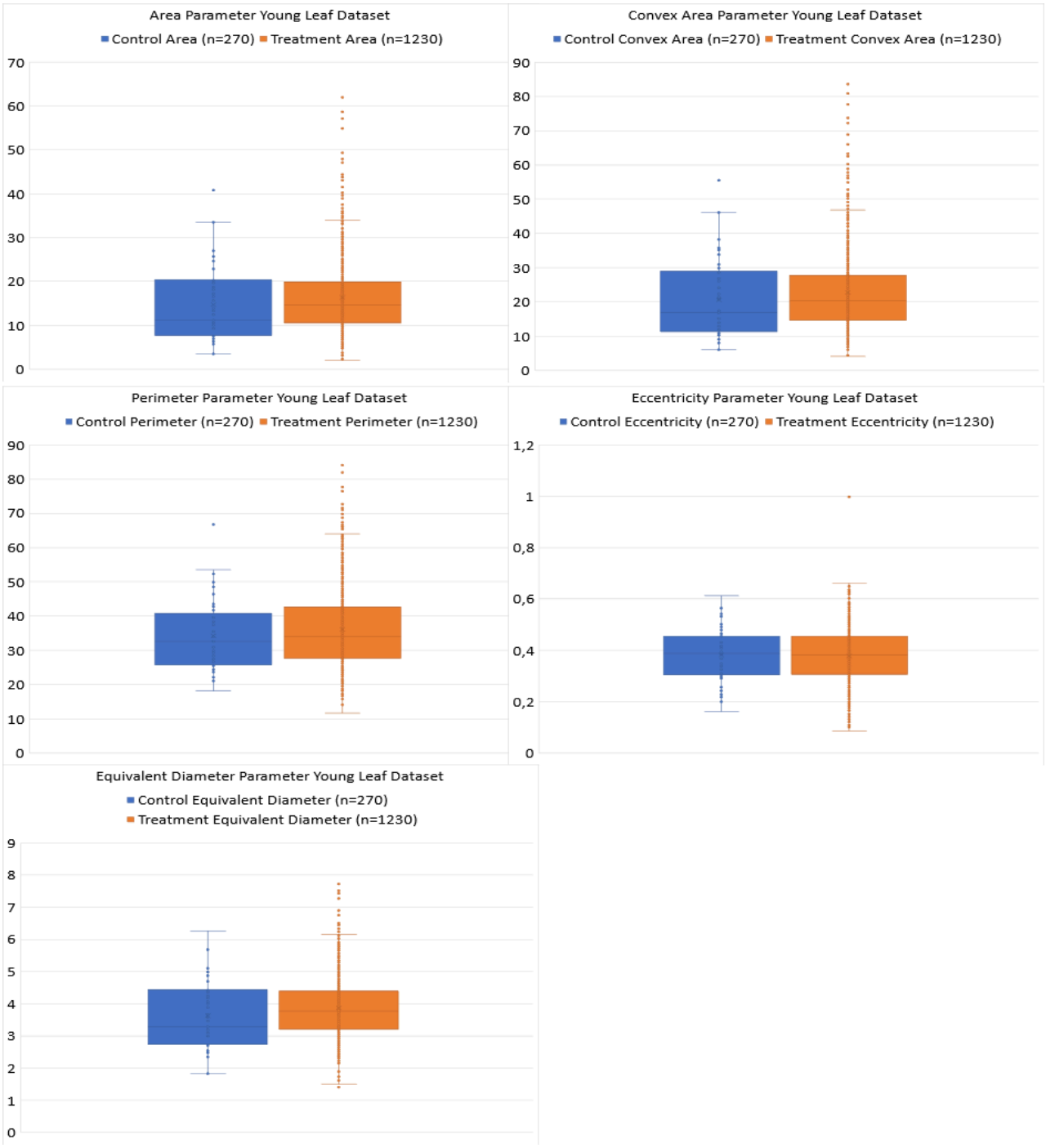

Figure 6. Box and whisker plots for a select few of the leaf parameters analysed (leaf area, convex area, leaf perimeter, eccentricity, equivalent diameter are shown– refer to Table 1 for a description of these parameters- to compare the control and treated (irradiated) grapevines of young leaves. **The data clearly show that the leaves from the irradiated plants are more variable regarding all the parameters measured.**

Given the large number of scans and the complexity of the dataset, a multivariate analysis was performed using the morpho-colourimetric data generated. A score (samples) PCA graph showing all the leaf samples scanned, irrespective of leaf age, is shown in **Figure 7** (Panel A is coloured by control/treatment, whereas Panel B is coloured by leaf age). Principal component 1 (t[1]) and principal component 2 (t[2]) explains 57.8% and 11.1%, respectively, of the variability of

the data, with a total of 68.9% of the variability of the data explained by these two components alone.

Figure 7. Principal component analysis (PCA) score (samples) graph of morpho-colourimetric parameters of all scanned leaf images. **A)** The different colours depict the sample distribution based on the Control and Treatment groups. **B)** The different colours depict sample distribution based on leaf age. The only clear separation observed within this sample dataset was based on leaf age. The mature leaf samples (green) are more spread out along the x-axis (principal component 1) and shifted to the right of the plot, whilst the young leaf samples (blue) are less spread out and shifted to the left of the plot.

Based on these observations from **Figure 7**, a decision was made to analyse the mature and young leaf samples separately.

PCA scores plots were prepared to compare the data for the three clones (non-irradiated) and the

treatment plants with the samples coloured according to the control and treatment samples (**Figure 8** shows the results for the young leaves, as an example, but similar results were obtained for the mature leaves). There was no clear separation between the treatment and control groups, but the treatment samples show substantially increased variability, especially along the Y-axis. Principal component 1 (t[1]) and principal component 2 (t[2]) explains 49.5% and 14.0%, respectively, of the variability of the data; a total of 63.5% of the variability of the data is explained by these two components alone (**Figure 8**).

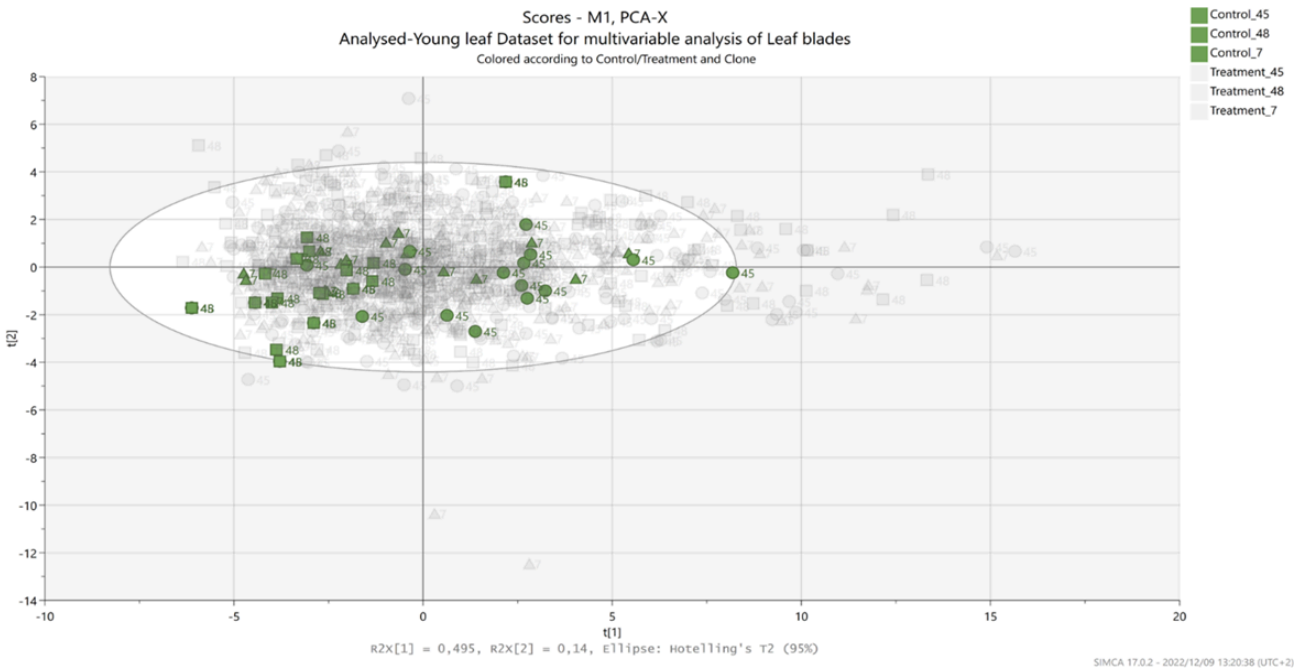

Figure 8. Principal component analysis (PCA) score (samples) graph of morpho-colourimetric parameters of **scanned young leaf images**. The different colours depict sample distribution based on Control and Treatment groups, with triangles indicating clone 7, circles indicating clone 45, and squares indicating clone 48. **The treatment samples show definite increased variability, especially along the Y-axis. Although no separation was observed on the clonal level, further analyses per clone were undertaken to see if any observations could be made in the smaller subsets (results not shown), but no additional information was evident from this analysis.**

From the various scoring and analyses, it was clear that the inherent variability was increased in the plants that originated from material exposed to gamma irradiance, as seen in **Figures 7 and 8**. This is in line with what was previously documented for gamma irradiance as a mutagen and confirmed that the treatment was successful in increasing variability in the mutant collection (Atay, Atay, Lauri, et al., 2018; Hasim, Shamsiah & Hussein, 2021; Islam, Islam & Hasan, 2015; Munir et al., 2015; Oualkadi A, Sbaghi & Mouhib, 2018).

First analysis of bunch and berry characteristics in the 2022/2023 season:

Given the fact that the analyses were done on young plants that were either just hardened off after generating them from nodal cuttings post-treatment or after establishing the vines in a field trial, the main limitation was that only vegetative characteristics could be used in the first sets of analyses. During the past season, 34% of the vines in the field trial yielded bunches (170/498 plants – refer to **Table 4**), with an average of 13 bunches per vine (minimum of 2 bunches and maximum 68 bunches per vine recorded).

Table 4. Composition of fruit-bearing vines in the 2022/2023 season amongst the population of 500* plants in the field trial.

Clone	Number of unirradiated vines bearing fruit/total	Number of irradiated vines bearing fruit/total	Total number of vines bearing fruit per clone/total
P7	**16**/ 30	**54**/ 165	**70** / 195
P45	**13**/ 30	**40**/ 111	**53**/ 141
P48	**12**/ 30	**35**/ 132	**47**/ 162
Total	**41**/ 90	**129**/ 408	**170**/ 498

***Note:** 2 plants were found to be missing from the field (likely did not survive).

A number of characteristics regarding plant growth habit, plant size, bunch characteristics, as well as berry shape, skin and flesh colour, as well as seed number and appearance were recorded and scored according to the OIV descriptors. All the data for the population were analysed with basic and multivariate statistics, and a few general observations will be shared here (**Figures 9 and 10**):

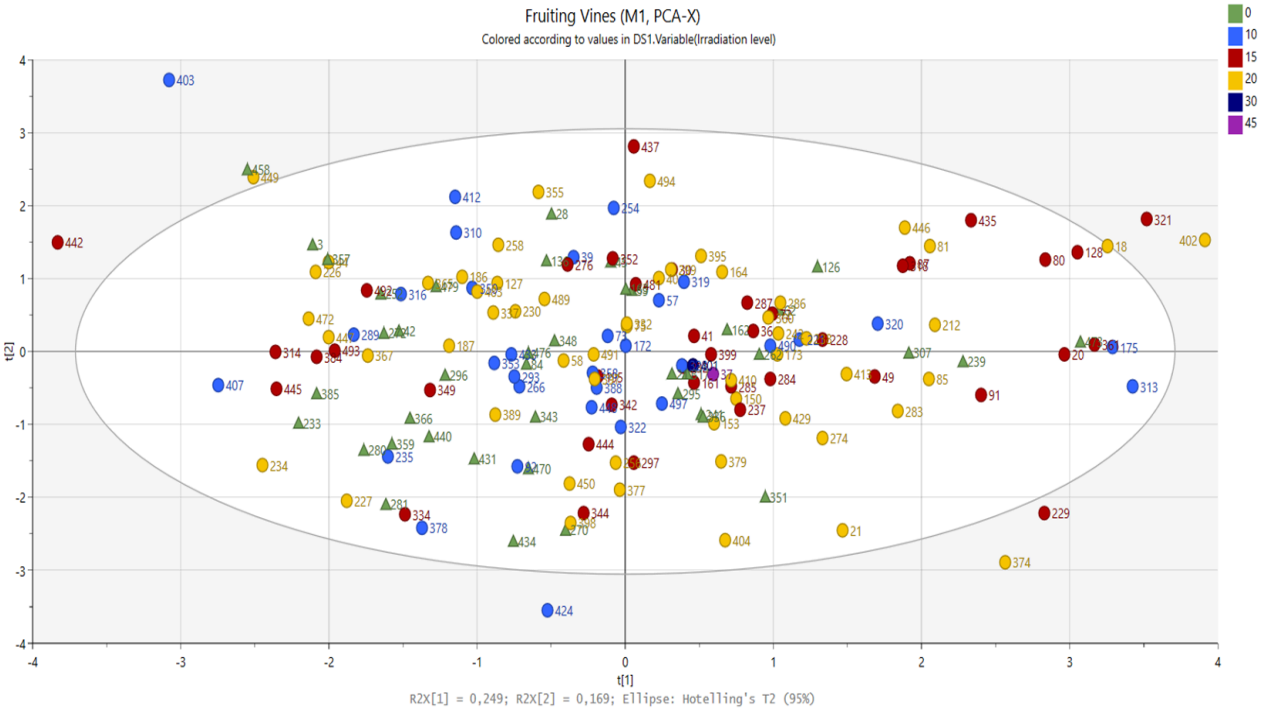

Figure 9: PCA plot of the fruiting population coloured by irradiation level. The unirradiated controls are indicated using a green triangle symbol. The irradiated population did not separate by irradiance level or clone (not shown), but **the irradiated population is spread over all quadrants of the plot, wider than the distribution of the controls, confirming increased variance.**

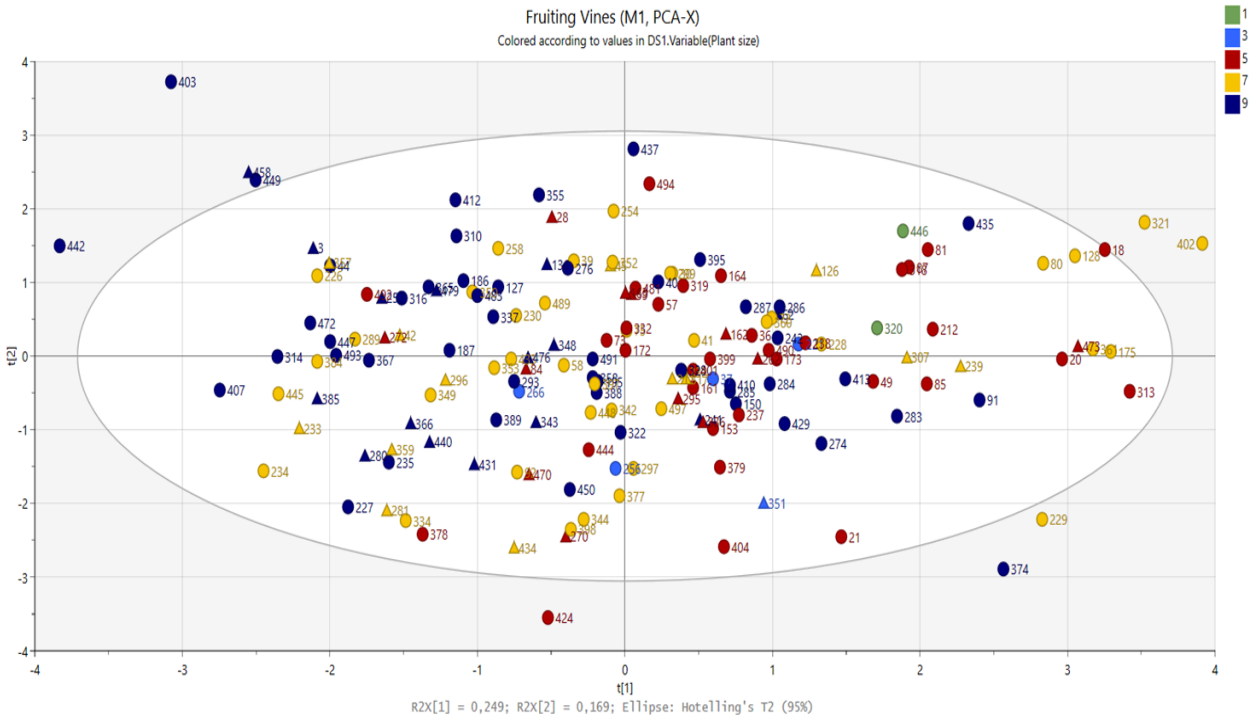

Figure 10: PCA plot of the fruiting population coloured by plant size. The unirradiated controls are indicated using a triangle symbol. **Only a few of the fruiting plants displayed smaller phenotypes.**

As was seen with the vegetative characteristics analysed, the multivariate analysis of the measurements of the fruiting populations did not separate between the unirradiated controls and the irradiated plants for any of the traits, but the irradiated populations consistently contained increase diversity in all of the traits observed (**Figure 9** shows a PCA coloured by irradiance level, whereas **Figure 10** displays the same PCA, but now coloured for plant size.

Figure 11 is a composite figure summarising the analysis of the fruit-bearing subpopulation in terms of berry cluster architecture. **Figure 11A** shows some representative samples of very compact to very loose clusters, and the corresponding rachis structures when the berries were removed are shown in **Figure 11B**. From the PCAs, it is clear that there is a proportion of irradiated plants which presented with very loose clusters, whereas very few unirradiated vines (controls) had loose clusters and none had very loose clusters (**Figure 11C and 11D**).

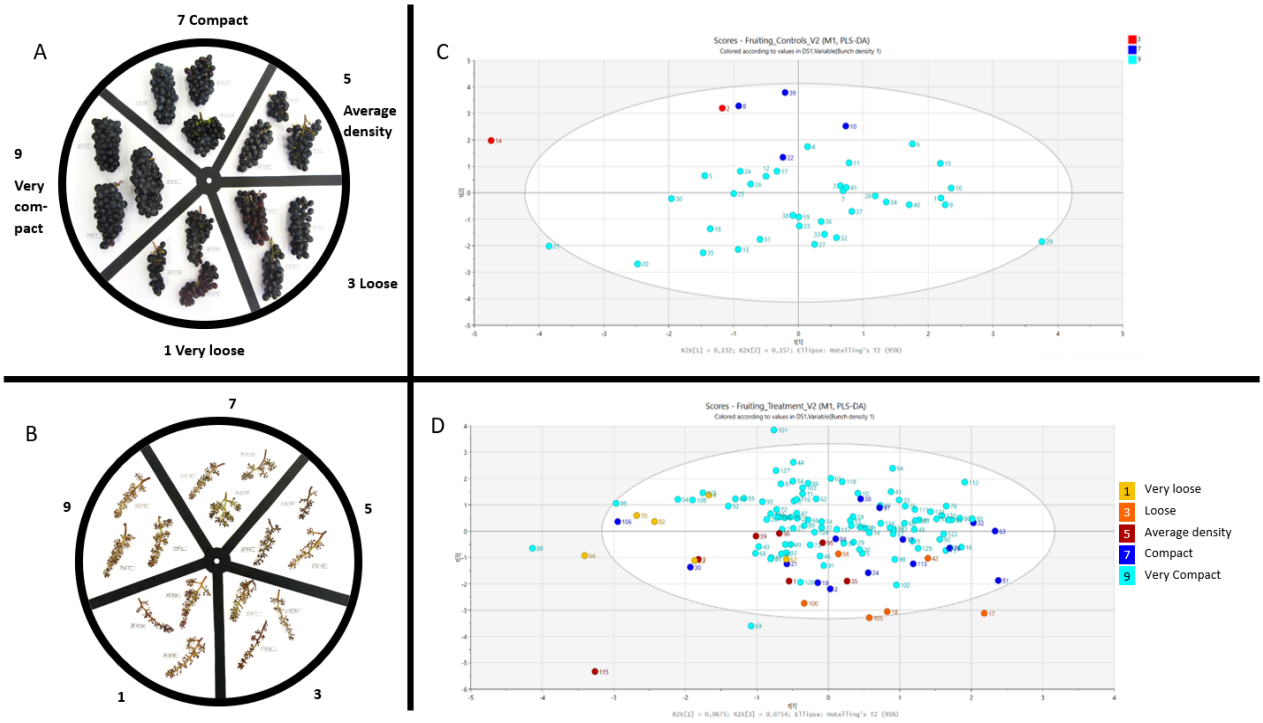

Figure 11: Composite figure showing the bunch density numerical scoring system in panel A, rachis architecture of representative bunch densities in panel B, and PCA plot coloured by bunch density scores in panel C for the unirradiated (control) vines, and panel D for the irradiated vines. **Several individuals in the irradiated fruiting population displayed significantly less compact bunches.**

Figures 12 and **13** are composite figures that showcase the variance observed in terms of berry skin and flesh colour in the population. In the irradiated population, there were individuals that showed significant changes to the normal skin and flesh colour, particularly the emergence of fruit with highly pigmented flesh, which could be due to a higher anthocyanin accumulation in the flesh (like that of teinturier grapes). There was, however, some fruit in the unirradiated population which had lightly or moderately pigmented berry flesh, which may be the result of somaclonal variation.

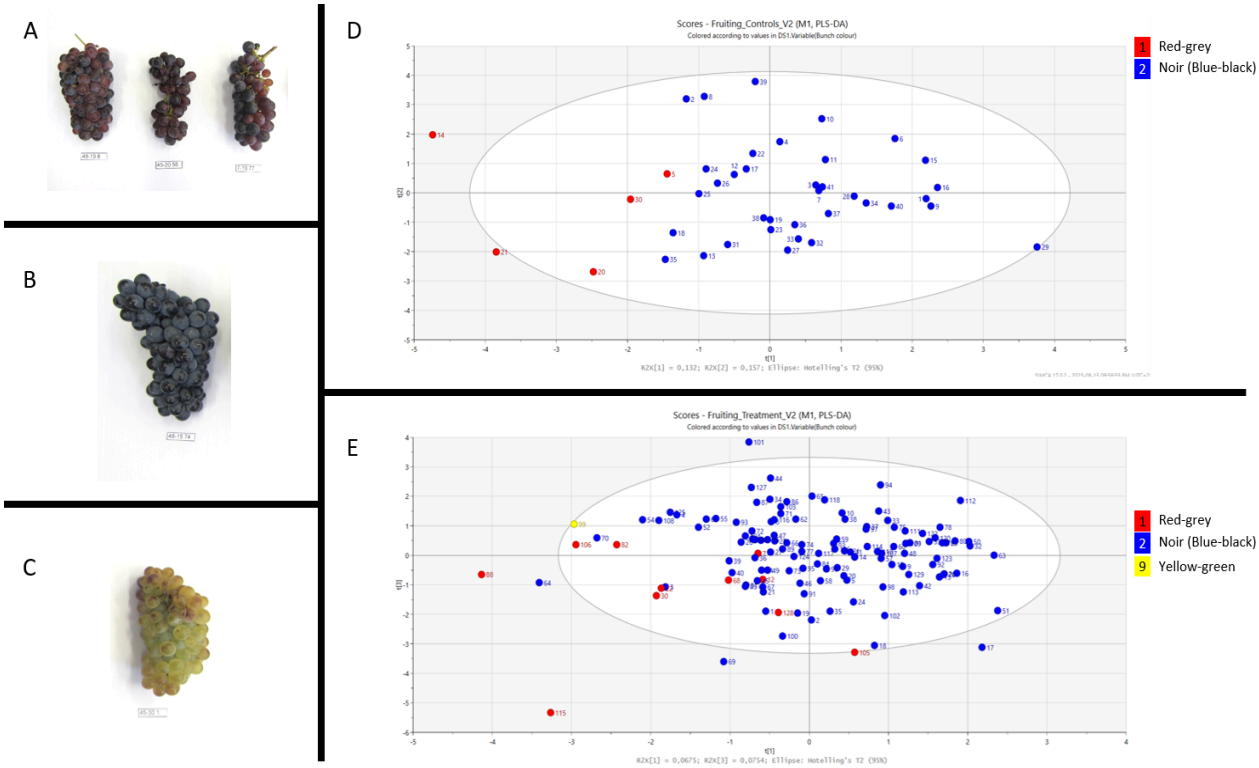

Figure 12. Composite figure showing the observed bunch colourations. In panel A, the red-grey, variable bunches are represented. In panel B, the wild-type noir (blue-black) bunch phenotype is indicated, and in panel ,C the abnormal yellow-green bunch phenotype is shown. Panel D is the PCA plot for the unirradiated (control) vines, while panel E represents the irradiated vines.

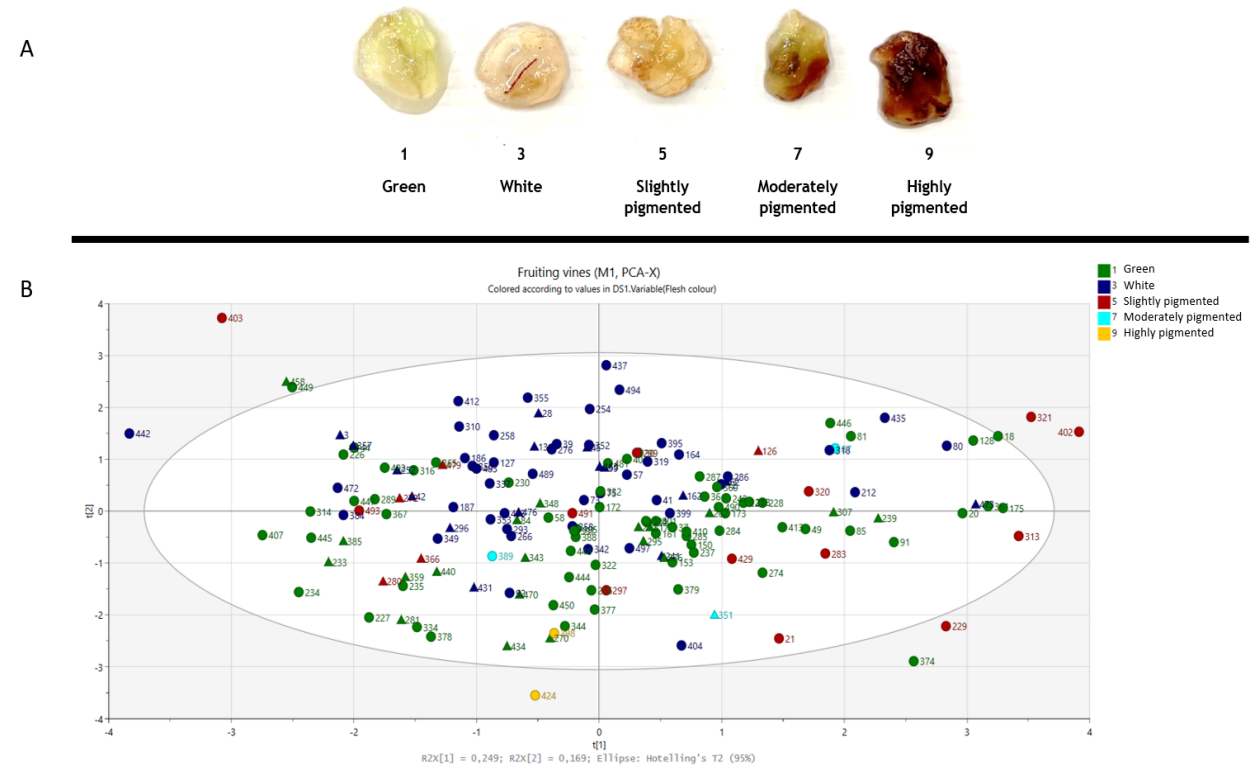

Figure 13. A composite figure indicating the observed flesh colouration observed in the Pinotage population. Panel A represents the flesh colour scoring system, while panel B is a PCA plot showing the

spread of the data points amongst the irradiated vines and the unirradiated vines (indicated as triangles).

M3.4 Identification of individual plants for further evaluation as potential clones will be based on all the data collected during the growing season.

These promising results provide motivation for a more in-depth analysis of the detailed phenology for the population, as well as time-course analysis of the ripening progression of the grapes and chemical analysis of the berry ripening indicators in the coming season. All these aspects form part of the next phase of analyses of the project that would be geared at evaluating the population to identify candidate individuals that can be further selected for more in-depth analyses after being grafted and propagated for further analysis on the plant and wine level (new application submitted to South Africa Wine in 2023; next phase of project initiated in 2024).

8. CONCLUSIONS AND RECOMMENDATIONS

A field trial of 500 plants (including controls) was successfully planted (in 2021) and evaluated over two seasons, whereas the residual plants were also planted out in the field (2022) for further analyses. The study's main findings were as follows:

(i) In general, a tendency of decreased rate of phenological development of irradiated grapevines compared to non-irradiated vines was observed, although there were differences between clones and variation around budding times and onset of senescence (earlier and later in both instances);

(ii) Significant phenotypic variation was evident in the population, involving several traits of economic importance;

(iii) Overall increased variation in parameters analysed in the irradiated populations as an indication of mutations caused by the gamma irradiation treatment; and

(iv) The first bunch and berry characteristics observed also point to an interesting mix of potentially valuable phenotypes, particularly with regard to bunch architecture and overall growth and fruitfulness of the plants.

The data obtained corroborate existing literature that indicates that gamma irradiance can be an effective mechanism to induce mutations in plants.

The data gathered on the collection, therefore, provides a resource and first description for the significant further research that still needs to be conducted on the collection in the scope of the bigger project this work forms part of (new phase of the project commenced in 2024).

9. PLANNED OUTPUTS

a) TECHNOLOGY DEVELOPMENT, PRODUCTS AND PATENTS

The project has the potential to yield novel plant materials that could be protected as new clones or cultivars. The project is still in the “screening” phase, and “selections” will only be possible in the next phase of the project (new project commencedin 2024).

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

A press release was made available via SA Wine during 2024 (https://sawine.co.za/unlocking-the-future-of-pinotage/).

An industry panel was held during 2023 (coordinated with the help of SAWine) to discuss the project progress and discuss the industry needs in terms of potential new Pinotage clones. This

type of interaction was very useful, and similar events would be useful.

Could the project perhaps form part of Pinotage-directed events to celebrate the development of Pinotage during 2025?

Popular papers could be prepared, and industry days at the field site can be organised.

c) HUMAN RESOURCES DEVELOPMENT / TRAINING (STUDENTS)

Student Name and Surname	Student Nationality	Degree (e.g., Hons, MSc)	Level of studies in the final year of the project	Total Bursary Cost for Industry for the entire project
Honours				
B Bruiners	South African	HonsBSc	Completed	R 0
M Litabe	South African	HonsBSc	Completed	R 0
Masters				
Tanika Padyashi	South African	MSc	1st year	R 43000
Monje Pohl	South African	MSc	Completed	R 0
Alexandra Bald	South African	MSc	1st year	R 110000
PhD				
Postdocs				

d) LIKELY PUBLICATIONS (POPULAR, PRESS RELEASES, SCIENTIFIC)

Ms Monje Pohl obtained an MScAgric (Viticulture) in March 2023 with the thesis titled: “Phenotypical Analysis of the Vegetative Traits of a Putative Mutant Collection Generated with Gamma Irradiation Applied to Three Pinotage Clones”.

The literature review from the thesis, as well as the research chapter, are in preparation to submit for publication in peer-reviewed journals.

A popular paper can be prepared after the completion of the current phase of the project (final report due in 2024).

e) PRESENTATIONS/PAPERS THAT COULD BE DELIVERED

No conference presentations have been made yet on this project, but Grape Breeding conferences would be appropriate for this type of work.

10. PROJECT OUTCOME AND IMPACT

New Knowledge	Benefits of the Supply Chain	Direct Grower Application	Direct Packhouse/Winery/ Cellar Application	Other
X		X		

Other is:

None

The Value of the project to the industry

The ultimate outcome will be to release new 'Pinotage' clones with superior attributes for commercial planting. It is too early, though, to identify such clones (first fruit 2022-23 season), but the analyses achieved to date indicate significant scope in terms of a range of important parameters. A new phase of the project commenced in 2024.

11. PERSONS PARTICIPATING IN THE PROJECT:

INITIALS AND SURNAME	HIGHEST QUALIFICATION	RACE (B, C, I, W)	GENDER (M, F, X)	INSTITUTE DEPARTMENT	POSITION	TOTAL COST TO PROJECT
RESEARCH PERSONNEL						R 106448
A Zietsman	PhD	W	F	SAGWRI/SUN	Researcher	R 106448
P Burger	MSc Agric	W	F	ARC INF-NVB	Researcher	R 0
MA Vivier	PhD	W	F	SAGWRI/SUN	Professor	R 0
SUPPORT PERSONNEL						R 340772
A Schmidt	Nat Dipl Agric	W	M	ARC INF-NVB		R 0
I Faro	Grade 11	C	M	ARC INF-NVB		R 0
M Korkie	Grade 12	C	F	SAGWRI/SUN	Research Assistant	R 340772

POSITION: Co = Co-worker (other researcher at your institution)
Coll = Collaborator (participating researcher that does not receive funding for this project from industry)
PF = Post-doctoral fellow
PL = Project leader
RA = Research assistant
TA = Technical assistant/ technician

12. TOTAL COST OF PROJECT

TOTAL ANNUAL COSTS (ALL YEARS)	CFPA	RAISINS SA	HORTGRO	SATI	SA WINE	ARC	OTHER	TOTAL
2019 and before	R 0	R 0	R 0	R 0	R 309222	R 132827	R 0	R 442049
2020	R 0	R 0	R 0	R 0	R 413440	R 165086	R 0	R 578526
2021	R 0	R 0	R 0	R 0	R 480124	R 164492	R 0	R 644616
2022	R 0	R 0	R 0	R 0	R 544188	R 162144	R 0	R 706332
2023	R 0	R 0	R 0	R 0	R 380010	R 86197	R 0	R 466207
2024	R 0	R 0	R 0	R 0	R 0	R 0	R 0	R 0
TOTAL	R 0	R 0	R 0	R 0	R 2126984	R 710746	R 0	R 2837730