Bud fertility of Cypriot grape varieties Maratheftiko and Xynisteri (*V.vinifera*) and comparison with five international grape varieties

Characteristics of the Seed Germination and Seedlings of Cypriot Grape Variety Maratheftiko (*V. vinifera*)

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Resumo

As implicações das alterações climáticas nas regiões vitícolas a nível mundial e em particular no Mediterrâneo exigem medidas de adaptação. Uma dessas medidas é a investigação de variedades de uvas nativas de climas quentes, como as encontradas em Chipre. O interesse por essas variedades, em particular *Vitis vinifera* Xynisteri e Maratheftiko, tem se expandido durante a última década, com foco particular em suas características de tolerância à seca e potencial adequação como medida de adaptação contra as mudanças climáticas. No entanto, a fertilidade de gemas de variedades cipriotas ou o comportamento da germinação de sementes para fins de seleção ainda não foram investigados e pouco se sabe sobre o desempenho dessas variedades em um contexto vitícola. Este estudo tem dois objetivos principais (1) avaliar a fertilidade das gemas das variedades cipriotas *V. vinifera* Maratheftiko e Xynisteri e compará-las com variedades bem estabelecidas e (2) avaliar estratégias para a germinação de sementes da variedade cipriota Maratheftiko (*V. vinifera*) e avaliar os diferentes fenótipos produzidos a partir das sementes. A fertilidade das gemas das castas cipriotas foi medida e comparada com as castas Shiraz, Cabernet Sauvignon, Mataro, Sangiovese e Nero D'Avola. As medidas de dissecção de gemas revelaram que Maratheftiko apresentou maior número de primórdios de inflorescência (2,04) e Xynisteri maior área de seção transversal de primórdios de inflorescência (0,098 mm2) em relação às demais variedades avaliadas. A germinação de sementes de Maratheftiko (*V. vinifera*) foi estudada e dois protocolos ótimos foram identificados, para atingir uma taxa de germinação superior a 50%: 1) extração manual + imersão em NaOCl por 10 minutos + lavagem com água destilada + secagem em poucas horas + 84 d estratificação + plantação em sementeira e 2) extração manual + lavagem com água destilada + secagem em poucas horas + 98 d estratificação + plantação em sementeira. Este estudo estabelece as bases preliminares para caracterizar a fertilidade de gemas e germinação de sementes de variedades cipriotas e permitirá otimizar outros protocolos relacionados à seleção e fenotipagem da videira.

Palavras-chave

primórdios de inflorescência, necrose de gemas, estratificação, fenotipagem, cultivares cipriotas

Abstract

The implications of climate change on the wine growing regions globally and in particular the Mediterranean are calling for adaptation measures. One of those measures is the investigation of grape varieties indigenous to hot climates, such as those found in Cyprus. Interest in these varieties, in particular *Vitis vinifera* Xynisteri and Maratheftiko has been expanding during the last decade with particular focus in their drought tolerance traits and potential suitability as an adaptation measure against climate change. However, bud fertility of Cypriot varieties or their seed germination behaviour for selection purposes have not yet been investigated and little is known about the performance of these varieties under a viticultural context. This study has two major aims (1) to assess bud fertility of the Cypriot *V. vinifera* varieties Maratheftiko and Xynisteri and compare them to well established varieties and (2) to assess strategies for seed germination of the Cypriot variety Maratheftiko (*V. vinifera*) and evaluate the different phenotypes produced from the seeds. Bud fertility of the Cypriot grape varieties was measured and compared to grape varieties Shiraz, Cabernet Sauvignon, Mataro, Sangiovese and Nero D'Avola. The bud dissection measurements revealed that Maratheftiko had a greater inflorescence primordia number (2.04) and Xynisteri a greater inflorescence primordia cross-sectional area (0.098 mm^2) compared to the other varieties assessed. Seed germination of Maratheftiko (*V. vinifera*) was studied and two optimum protocols were identified, to achieve a germination rate greater than 50 %: 1) manual extraction + soak in NaOCl for 10 mins + distilled water wash + dry over few hrs + 84 d stratification + planted in seed raising mixture and 2) manual extraction + wash with distilled water + dry over few hrs + 98 d stratification + planted in seed raising mixture. This study sets the preliminary ground to characterize bud fertility and seed germination of Cypriot varieties and will permit to optimize further protocols related to grapevine selection and phenotyping.

Keywords

inflorescence primordia, bud necrosis, stratification, phenotyping, Cypriot cultivars

Resumo

O impacte das alterações climáticas nas regiões vitivinícolas a nível global exigem medidas de adaptação por parte do sector. Uma das medidas passa pela investigação de variedades de videira nativas de climas quentes, como as encontradas na ilha de Chipre. O interesse por essas variedades, em particular *Vitis vinifera* Xynisteri e Maratheftiko, tem vindo a expandir-se durante a última década, com um foco particular na sua tolerância à secura e como uma medida de adaptação face ás alterações climáticas. No entanto, a fertilidade das variedades cipriotas ou a sua propagação por via seminal encontram-se ainda pouco estudadas. Pouco se sabe sobre o desempenho dessas variedades num contexto vitícola assim como os fatores que afetam esse desempenho ou o seu desenvolvimento a partir da semente, incluindo a variabilidade ao nível das sementes.

Os dois objectivos principais deste estudo foram (1) avaliar a fertilidade em gomos de duas castas cipriotas, a Maratheftiko e a Xynisteri (*Vitis vinifera* L.) e compará-la com as castas Shiraz, Cabernet Sauvignon, Mataro, Sangiovese e Nero D'Avola (*Vitis vinifera* L.) e (2) avaliar as estratégias de germinação das sementes da variedade Maratheftiko e avaliar os diferentes fenótipos produzidos por via seminal . As mudas serão avaliadas individualmente através de uma série de atributos relacionados com uma avaliação precoce da tolerância à secura e ao calor, nomeadamente como o crescimento radicular e vegetativo.

A dissecação microscópica dos rebentos pode ser usada na avaliação precoce da fertilidade em videira através da fertilidade dos gomos , antes da poda de Inverno na vinha. Na região de Marananga, no vale de Barossa, no sul da Austrália, o material vegetal foi colhido em maio de 2022 para medir a fertilidade dos botões das variedades da casta cipriota e compará-la com outras cinco castas. A fertilidade da gema, medida como o número de primórdios da inflorescência (IP) por nó e a incidência de necrose primária da gema (PBN) foram monitorizadas através de cortes transversais das gemas e as imagens obtidas foram posteriormente analisadas para medição dos primórdios da inflorescência (IP) transversalmente área. Os cortes das gemas, usadas para orientar a poda de inverno e como indicador precoce do rendimento potencial mostraram que as castas cipriotas possuiam maior número e área de primórdios de inflorescência comparativamente com as restantes castas avaliadas. Em particular, a casta Maratheftiko apresentou o maior número de primórdios de inflorescência e Xynisteri apresentou a maior área transversal de primórdios de inflorescência. Esta resposta foi significativamente dependente da cultivar e, no futuro, pode ser interessante investigar a frutificação real dos botões observada durante a ântese, bem como o rendimento antes da colheita, particularmente para Maratheftiko, uma variedade hermafrodita que apresenta baixa fertilidade em Chipre.

O interesse na germinação de sementes decorre principalmente da melhoria da eficiência na propagação, acelerando o processo de seleção recorrente intraespecífica. Neste estudo, a germinação de sementes foi realizada para optimizar o protocolo de germinação de sementes da casta Maratheftiko, e foi seguida por uma investigação qualitativa na variabilidade de sementes para avaliação precoce de atributos de tolerância à seca e ao calor, como crescimento e radicular vegetativo. O material vegetal foi coletado em maio e junho de 2022 na região de Marananga, no vale de Barossa, no sul da Austrália, e sujeito a diferentes tratamentos: tratamento do bago, tratamento de sementes, tratamento de pré-estratificação, estratificação, tratamento de pré-germinação e meio de germinação. Esta investigação identificou dois protocolos ideais que podem ser implementados para alcançar uma taxa de germinação superior a 50% para sementes de Maratheftiko, e que são 1) extração manual + imersão em NaOCl por 10 minutos + lavagem com água destilada + secagem durante 2 horas + estratificação de 84 dias + plantação em substrato e 2) extração manual + lavagem com água destilada + secagem por algumas horas + estratificação de 98 dias + plantado em substrato. As mudas de Maratheftiko foram então observadas ao nível dos seus estádios de desenvolvimento, e comparado com uma muda de Sémillon submetida a igual tratamento . A comparação revelou caracteristicas qualitativass sobre as sementes cipriotas que, em geral, pareciam crescer em um ritmo mais rápido, em particular o ao nível dos sistema radicular. Este estudo estabeleceu uma metodologia preliminar para a caracterização da germinação das sementes de Maratheftiko. No futuro, pode ser interessante colher bagos na colheita de 2023 e repetir o ensaio usaando os dois protocolos optimizados e bem-sucedidos, com o objetivo de aprofundar a investigação sobre o desenvolvimento das sementes de Maratheftiko de uma forma quantitativa, em vez de qualitativa, envolvendo medições do sistema radicular e crescimento do caule.

Palavras-chave

Primórdios de inflorescência, necrose de gemas, estratificação, fenotipagem, cultivares cipriotas

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List of Abbreviations

3SH – 3-sulfanylhexan-1-ol

3SHA – 3-sulfanylhexyl acetate

4MSP – 4-methyl-4-sulfanylpentan-2-one

ABA – Abscisic Acid

AHC – Agglomerative Hierarchical Clustering

ANOVA – Analysis of Variance

BM – Benzyl Mercaptan

CK – Cytokinin

cv. – cultivar

D.f. – Degrees of Freedom

DS – Drought Stress

GA – Gibberellin

GA3 – Gibberellic Acid

GC-FID – Gas Chromatography Flame Ionization Detector

GC-MS – Gas Chromatography Mass Spectrometry

Hac – Acetic Acid

HPLC-DAD-ESI/MS – High Performance Liquid Chromatography with Photodiode Array Detection-Mass

HPLC-MS/MS – High-Performance Liquid Chromatography–Tandem Mass Spectrometry

HS – Heat Stress

IP – Inflorescence Primordia

IRMS – Isotope-ratio Mass Spectrometry

ISO protocols – International Organization for Standardization

LCA – Life Cycle Assessment

LC-MS – Liquid Chromatography Mass Spectrometry

LSD – Fishers Least Significant Difference

PBA – 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine

PBN – Primary Bud Necrosis

PCA – Principal Component Analysis

PDO – Protected Designation of Origin

PEBPs – Phosphatidylethanolamine Binding Proteins

PFC – Product Carbon Footprint

RATA – Rate-All-That-Apply

SIDA – Stable Isotope Dilution Assay

SNIF-NMR – Site-specific Natural Isotope Fractionation by Nuclear Magnetic Resonance

UP – Uncommitted Primordia

1. Introduction

1.1. Cypriot Viti viniculture – A historical overview

Cyprus is theorised as one of the oldest vine growing regions of the world with an oenological history of over 5500 years. The history of wine and the history of the island have been interlinked and nowadays Cyprus is considered to be amongst the first countries to cultivate grapes and produce wine (Robinson & Johnson, 2019). Aristidou (1990) argues that Cyprus acquired fame initially for the excellent wine produced in the island and indeed modern excavations have established that during the Hellenic Age most of the wealth and prosperity in the island was attributed to wine production and trading. Archaeological artifacts – wall mosaics, sculptures, pots – discovered throughout the Cypriot history demonstrate the historical importance of wine in Cyprus over the Roman period as well as the consequent ages of the Lusignans (1149- 1489) and Venetians (1489-1571). During the Middle Ages, the island became renown for Commandaria, a dessert wine produced up to this day (Kythreotou, 2003) however, the Turkish Ottoman occupation of the late 16th century halted the development the progress of viticulture and wine production in Cyprus. For 300 years, wine production was neglected and only in the last 50 years have efforts recommenced to develop viticulture and oenology.

1.2. Current Overview of Cypriot Viticulture

One of the remaining phylloxera-free areas, vines are almost exclusively own-rooted with both autochthonous and international varieties (*Vitis vinifera* L.) being cultivated (Vickers, 1993). The current vineyard area is estimated at 7,623 hectares (Stylianou, 2022). The dominant varieties are the indigenous varieties, the white Xynisteri (ca. 30 % of the total vineyard area) and the red Mavro encompassing *ca.* 40 %, the former increasing rapidly in plantation frequency (Chrysargyris et al., 2018). The main varieties cultivated are shown in **Table 1**. Cypriot producers focus on low production volumes of high-quality wines with protected designation of origin (PDO) or protected geographical indication (PGI). Commandaria, a PDO dessert wine is produced from the above stated indigenous varieties and has been praised for the unique sensory profile (Constantinou et al., 2017). Wine comprises one of the major export products of the island, continuously developing in a demanding and competitive local market.

Table 1. An overview of the current vineyard area in Cyprus and the main varieties being cultivated. Adapted by Stylianou (2022).

	Area (ha)				Area (%)
Grape Variety	**2017**	**2018**	**2019**	**2020**	**2020**
Others	823.4	829.5	825.7	820.6	10.7
Sultanina	477.4	471.9	475	471	6.2
Maratheftiko	166	168	169.6	173	2.3
Cabernet Franc	134.5	123.8	118.4	114.1	1.5
Cabernet Sauvignon	188.2	174.4	168.5	160.2	2.1
Shiraz	334.7	341.4	343.6	338.9	4.5
Mataro	215.5	222.3	230	236.8	3.1
Carignan	170.2	168	165.2	167.9	2.2
Xynisteri	2166.7	2187.5	2179.1	2162.5	28.4
Mavro	3017.8	3022.3	3004	2978	39
Total	*7694.4*	*7709.1*	*7679.1*	*7623*	*100*

1.3. Cypriot Grape Varieties and Climate Change

The effects of climate, observable more than ever, is a crucial topic for current and future wine making. The wine regions of the world, historically present for centuries, are expected to undergo considerable changes in the upcoming decades due to reduced rainfall, increasing temperatures, berry composition changes and earlier, heat-induced harvest seasons (Hannah et al., 2013; Schultz & Jones, 2010; van Leeuwen et al., 2013, 2019; Webb et al., 2013). One of the adaptation measures for mitigation and adaptation to climate change effects involves research into heat and drought-resistant varieties, indigenous to hot Mediterranean wine growing regions, including Cyprus. Described as one of the cradles of viticulture by Evans (2008), the climatic conditions of the island are gradually becoming drier and hotter. Over the millennia farmers have been empirically hand-selecting indigenous cultivars resistant to drought and heat (Fraga et al., 2016; Patakas et al., 2005) to overcome the annual environmental stresses during summer – extreme winds, heatwaves and water deficit (Beis & Patakas, 2012; Chrysargyris et al., 2018). The indigenous varieties discussed are well-adapted to drought and hence, require lower amounts of water and fertilisers in comparison to international varieties, making their cultivation a potential adaptation measure to climate change (Litskas et al., 2017).

1.4. Current Research

To date the studies carried out on Cypriot viti-viniculture are limited, despite the rich history of the island. Studies have focused on various aspects, from sun-drying techniques for the production of Commandaria (Ioannou-Papayianni et al., 2011), to the impact of soil management practices on the bacterial soil communities and the use of isotopic and elemental markers for authenticating Cypriot wines (Kokkinofta et al., 2003; 2017) to promote Cypriot wine

characterization and preserve its unique identity. More recent studies involve the response of Cypriot cultivars to different irrigation regimes and their germplasm characterization via microsatellite primers. Recent research is expanded upon subsequently.

Sensory profiling of Cypriot wines and acceptability to Australian consumers

This study by Copper et al. (2019a) in which the sensory and chemical characteristics of Cypriot wines were profiled and contrasted with Australian wines, focused on commercial Cypriot wines produced from Xynisteri, Maratheftiko and Giannoudhi. The wines, detailed in **Table 2.** included five Xynisteri wines, one Australian Pinot Gris and Chardonnay for the whites and three Maratheftiko, one Giannoudhi and one Australian Shiraz for the red wines. Prices ranged from 5-20€ for the Cypriot wines and $20-$25 AUD for the Australian wines. The sensory profiling was established using A Rate-All-That-Apply (RATA) method (n=56 panellists on Xynisteri and n=60 on Maratheftiko and Giannoudhi). Chemical analysis for aroma compounds was carried out quantitatively by gas chromatography mass spectrometry (GC-MS) and phenolic compounds were profiled with liquid chromatography mass spectrometry (LC-MS).

Table 2. Basic chemical, oak treatment and other information of wines used in sensory, consumer acceptance and chemical analysis. Adapted from Copper et al., (2019a).

Code	Wine	Ph	TA	Alc %	Oak	Other
M1	Maratheftiko 2015	3.43	5.86	14.8	Yes	
M2	Maratheftiko 2013	3.62	5.45	13.2	Yes	
M3	Maratheftiko 2015	3.44	5.88	14.5	Yes	
SH	Shiraz 2015	3.57	6.13	14.5	Yes	
Yia	Giannoudhi 2015	3.65	5.5	13.4	Yes	
CH	Chardonnay 2017	3.33	7.35	12.9	No	
PG	Pinot Gris 2017	3.54	6.65	12.5	No	
X1	Xynisteri 2016	3.21	5.93	12.8	No	
X2	Xynisteri 2015	3.26	5.94	12.8	Yes	
X3	Xynisteri 2016	3.22	5.52	13.7	No	
X4	Xynisteri 2016	3.35	5.44	12.8	No	5 % Muscat
X5	Xynisteri 2016	3.16	4.72	12.6	No	
X6	Xynisteri 2016	3.42	5.02	12.6	No	

The RATA study was analysed with Principal Component Analysis (PCA) which identified the sensory characteristics of Xynisteri as: citrus, apple/pear, grassy, stone fruit, herbaceous, dried fruit, confectionary, creamy, vanilla, buttery, toasty and wood. Wines from Maratheftiko wines were identified as dried fruit, woody, chocolate, confectionary, herbaceous, sweet, jammy, and full bodied. Wine from Giannoudhi wine was identified as dried fruit, woody, chocolate and full

bodied. Regarding phenolic and volatile compounds, 15 and 21 were identified in the white wines respectively, 17 and 26 in the red wines. The chemical composition was correlated to the RATA and consumer hedonic responses to determine the drivers for consumer preference, using Agglomerative Hierarchical Clustering (AHC) and PCA. It was concluded that Cypriot wines were liked similarly to Australian wines. (Copper et al., 2019a)

Preliminary investigation of potent thiols in Cypriot wines

In light with the previously outlined research by Copper et al. (2019a), the polyfunctional thiols, considered as key aromatic compounds in Sauvignon Blanc and Chardonnay, of several wines were investigated. Specifically, five thiols outlined in **Table 3** with their corresponding aromatic expression, were investigated in wines produced from in Xynisteri, Maratheftiko, Giannoudhi, Pinot gris, Chardonnay and Shiraz. The wines were analysed with Stable Isotope Dilution Assay (SIDA) with derivatisation and High-Performance Liquid Chromatography–Tandem Mass Spectrometry (HPLC-MS/MS).

Table 3. The varietal thiols investigated with their corresponding aromatic expression. (Copper et al., 2021)

Thiol	Expression
4-methyl-4-sulfanylpentan-2-one (4MSP)	boxwood" and "cat urine"
3-sulfanylhexan-1-ol (3SH)	grapefruit/tropical fruit
3-sulfanylhexyl acetate (3SHA)	passionfruit
benzyl mercaptan (BM)	smoke and meat
benzyl mercaptan (BM)	roasted coffee

Whilst all the white wine sampled contained 3SHA, 3SH and BM only three of the Xynisteri wines and Pinot Gris were shown to contain 4MSP. Conversely, FFT was observed in four of the Xynisteri wines and Chardonnay. Interestingly, the authors observed that the thiols found were at higher concentrations than their respective aroma detection thresholds, particularly 3SH. The results of this investigation although preliminary, provide an insight into the chemical composition of Cypriot wines with respect to thiol presence, compounds important in the expression of "citrusy, fruity and tropical" notes in wines. (Copper et al., 2021)

Response of *V. vinifera* L. cv. Xynisteri to different irrigation regimes and its comparison to cvs. Maratheftiko, Shiraz and Sauvignon Blanc

Australian producers in search of drought-resistant varieties have been focusing on cultivars from Spain, Portugal, Greece and Cyprus. Copper et al. (2022) evaluated the performance of Cypriot autochthones *V. vinifera* L. cvs. Xynisteri and Maratheftiko under different irrigation regimes against cultivars Shiraz and Sauvignon Blanc. Irrigation was established on three different levels

(full, 50 %, 25 %) and trials measuring vine growth and vine physiology, were carried out in 2019 in Cyprus and 2020-2021 in Australia. The results summarised in **Table 4.** indicate that Xynisteri shows a more vigorous growth under lower irrigation levels in comparison to international varieties Shiraz, Chardonnay and Sauvignon Blanc. Specifically, the 2019 trial in Cyprus demonstrated that Xynisteri had the highest stomatal conductance, stem water potential, biomass and leaf chlorophyll contents under all three irrigation regimes. Similarly, in 2020-2021 Xynisteri showed a greater biomass than the other cultivars including Maratheftiko. The results of this study are in tandem with the mitigation measure for adaptation to climate change and demonstrate the possibility of cultivating Xynisteri, hypothesized to possess better cultivar-specific growth traits, in areas with water limitations. According to the authors further research into the biomass and root structure of Xynisteri in field-cultivated vines could elucidate the mechanism of drought tolerance shown by the cultivar and the role of roots in that mechanism (Copper et al., 2022).

Table 4. Stomatal density, fresh root, shoot and leaf mass for potted trials in seasons 2019 and 2020/2021.

Season	Variety	Stomatal density	Root Mass (gm)	Shoot Mass (gm)	Leaf Mass (gm)
201920	XCV	238.6 ^{a}	n/a	n/a	n/a
	XK	227.5 ^{a}	387 ^{ab}	112 ^{b}	102 ^{ab}
	XM	233.2 ^{a}	486 ^{a}	180 ^{a}	156 ^{a}
	SBC	139.8 ^{b}	182 ^{b}	63 ^{c}	48 ^{b}
	Pr > F	<0.0001	<0.0001	0.0002	0.0001
2020/2021	XP	206.1 ^{a}	892 ^{a}	342 ^{a}	259 ^{a}
	MP	189.0 ^{b}	539 ^{b}	296 ^{ab}	201 ^{ab}
	SZ	170.5 ^{c}	320 ^{c}	238 ^{ab}	137 ^{b}
	SBA	151.4 ^{d}	443 ^{d}	206 ^{b}	140 ^{b}
	Pr > F	<0.0001	<0.0001	0.029	0.001

XCV: Xynisteri Cyprus Vineyard, XM: Xynisteri Mandria, XK: Xynisteri Kathikas, XP: Xynisteri Paphos, MP: Maratheftiko Paphos, SBC: Sauvignon Blanc Cyprus, SBA: Sauvignon Blanc Adelaide, SZ: Shiraz. Stomatal density – number of stomata per mm^2. Different letters next to the measures indicate significant differences $p < 0.05$.

Although, interest in Cypriot cultivars has been growing in recent years (see also **Table 5**), a gap in literature remains around their germination characteristics and bud fertility, which will therefore be the primary topic of this work.

Table 5. A summary of recent studies involving Cypriot grape varieties and wine, along with the key outcomes of the investigations.

Location	Summary Points	Key Outcomes	References
Australia/ Cyprus	The response of Xynisteri to different irrigation regimes was assessed and compared to cvs. Maratheftiko, Shiraz and Sauvignon Blanc. Irrigation was established on 3 different levels (full, 50%, 25%) and trials measuring vine growth and vine physiology, were carried out in 2019 in Cyprus and 2020-2021 in Australia.	Xynisteri demonstrated a more vigorous growth under lower irrigation levels in comparison to international varieties Shiraz, Chardonnay and Sauvignon Blanc. In the 2019 trial Xynisteri had the highest stomatal conductance, stem water potential, biomass and chlorophyll under all three irrigation regimes.	Copper et al. (2022)
Australia	Preliminary Investigation of the polyfunctional thiols in wines produced from in Xynisteri, Maratheftiko, Giannoudhi, Pinot gris, Chardonnay and Shiraz. The wines were analysed with SIDA with derivatisation and HPLC-MS/MS.	All the white wines sampled contained 3SHA, 3SH and BM but only three of the Xynisteri wines and Pinot Gris were shown to contain 4MSP. Conversely, FFT was observed in four of the Xynisteri wines and Chardonnay.	Copper et al. (2021)
Cyprus	The effect of irrigation and tillage on soil bacteria communities at different plant phenological stages was investigated, by examining the soil bacteria community of cvs. Xynisteri, Maratheftiko and Chardonnay at flowering, véraison and harvest.	The soil bacteria communities are impacted by both endogenous and environmental factors. Bacterial populations were shaped by cultivar, the phenological stage of the grapevine (endogenous), soil physiochemistry and soil management practices (environmental) as well as an interactive effect between these factors.	Vink et al. (2021)
Cyprus	Vine performance benchmarking of cvs. Xynisteri and Maratheftiko followed by a comparison to Shiraz and Sauvignon Blanc from dry grown vineyards in Cyprus, during 2017, 2018 and 2019. Measurements were taken at flowering, véraison and pre-harvest.	Xynisteri demonstrated the highest stomatal density, higher leaf water potential at harvest, more leaves and shoots, heavier bunches and a greater yield. The stomatal conductance of Xynisteri was equal to Maratheftiko, but greater than Shiraz and Sauvignon Blanc. Maratheftiko had the greatest chlorophyll content.	Copper et al. (2020)
Cyprus	The physiological and biochemical response of Xynisteri and Maratheftiko to short-term light stress, moderate drought stress (DS) and heat stress (HS) was evaluated via physiological and biochemical stress markers.	Overall, the performance of both cvs. was affected more by short-term DS than HS. In Xynisteri photosynthetic rate and leaf stomatal conductance decreased whereas, total phenols and antioxidant capacity increased. Conversely, in Chardonnay this response was absent and leaf damage with increased lipid peroxidation levels was observed. Xynisteri exhibited a better performance based on antioxidative activities and the damage index.	Tzortzakis et al. (2020)
Cyprus	The lineage of Cypriot cultivars was examined through a two-year collection of centennial grapevine cultivars considered to belong in the four indigenous variety clusters Mavro, Xynisteri, Maratheftiko, and Veriko and a consequent characterization with a universal microsatellite primer set. (164 accessions)	According to the genetic analysis, the indigenous Cypriot germplasm consists of a polyclonal structure containing a high degree of heterozygosity. The number of discrete genotypes discovered, is larger than expected indicating the potential existence of unexplored varieties or several lineages. The lineages of Cypriot grapevines were established, across epochs via clonal and sexual propagation.	Grigoriou et al. (2020)
Spain	The fermentation derived volatile compounds and the anthocyanin profile of two single-varietal wines made from Maratheftiko and Yiannoudi across vintages 2014, 2015 and 2016 were evaluated with GC-FID and HPLC-DAD-ESI/MS followed by a blind wine testing sensory evaluation.	The sensory analysis of younger wines (vintage 2016) showed a clear distinction in the anthocyanin profile; in Maratheftiko caffeoyl derivative monomers and polymeric pigments were mostly identified whereas Yiannoudi was strongly distinguished by Vitisins and vinylphenolics. In aged wines, varietal distinction was unsuccessful in both the analytical results and the blind wine tasting.	Tsiakkas et al. (2020)
Australia	Preliminary sensory and chemical profiling of Cypriot wines by gas chromatography mass spectrometry (GC-MS) for aromatic compound analysis and liquid chromatography mass spectrometry (LC-MS) for phenolic compound profiling.	Principal Component Analysis (PCA) identified the sensory characteristics and phenolic compounds of cvs. Xynisteri, Maratheftiko and Giannoudhi. A consequent Agglomerative Hierarchical Clustering (AHC) and PCA analysis revealed that Cypriot wines were liked similarly to Australian wines.	Copper et al. (2019)

Cyprus	The physiological response of Maratheftiko to short-term light stress, moderate drought stress (DS) and heat stress (HS) was evaluated via physiological and biochemical stress markers.	Overall, Maratheftiko performance is affected more by short-term DS than HS. Lead photosynthetic rate, leaf stomatal conductance and chlorophyll fluorescence decreased. Total phenols and flavonoids content and antioxidant capacity (FRAP and ABTS) increased as well as leaf hydrogen peroxide and lipid peroxidation.	Chrysargyris et al. (2018)
Cyprus	The effects of soil management practices, specifically tillage and irrigation on the yield and quality-associated characteristics of Maratheftiko were evaluated.	No tillage management is a potential adaptation strategy in the context of CC as the absence of tillage seemed to compensate for the negative effect of non-irrigation on yield. In addition, tillage increased total phenolics in both irrigated and non-irrigated plants whereas absence of irrigation and tillage increased total soluble solids and anthocyanins of berries.	Chrysargyris et al. (2018)
Cyprus	The effect of defoliation at véraison on the metabolites of fresh and dehydrated grapes of cvs. Xynisteri and Mavro, destined for the production of Commandaria PDO, were analysed using LC-DAD-qTOF-MS.	Defoliation led to the decrease of titratable acidity, soluble solids, phenolic compounds and aromatic potential in the must of both cvs. Conversely, dehydration led to the increase of all parameters in both cvs. In addition, the phenolic compounds in Xynisteri were decreased (from 66.73 to 44.15 mg L−1) whereas in Mavro they remained relatively constant (from 94.78 to 96.72 mg L−1) but with a different distribution among phenolic groups. Flavonols and flavan-3-ol concentration was greater in the must from dehydrated Mavro grapes.	Constantinou et al. (2018)
Cyprus	The comparison of postharvest dehydration methods on phenolic composition, aromatic potential, browning compounds and oenological parameters of musts from Xynisteri grapes. Methods: (a) traditional sun-drying method (TM) (b) multiple horizontal wires (MHW), (c) multiple vertical pallets (MVP), (d) low greenhouse (LGH) and (e) hot-air dryer treatment (HAD).	According to the existing legal framework, LGH demonstrated the greatest potential for the production of high quality must from dehydrated Xynisteri grapes. It led to a significant reduction of the dehydration period, it concentrated the total bound volatiles as well as induced the formation of brown pigments and led to a significant increase in total phenolic content in the must, based on the Folin-Ciocalteu index.	Constantinou et al. (2018)
Cyprus	The product carbon footprint (PFC) of indigenous and international cvs. in 90 vineyards across Cyprus was determined via the Life Cycle Assessment (LCA) based on ISO protocols for greenhouse gas emissions.	The highest PCF was observed for Soultanina (Thompson seedless) at 0.846 kg CO_2 eq./27 kg of grapes, whereas the lowest PCF was exhibited by Xynisteri at 0.283. The model under investigation demonstrated that the application of locally sourced animal manure and a reduction in tillage frequency can reduce carbon footprint by 40- 67% and PFC for Xynisteri can reach values close to zero.	Litskas et al. (2017)
Cyprus	The isotopic profiles and elements in Cypriot wines were assessed in relation to grape variety, environmental factors and provenance to provide tools for the lucid characterization of Cypriot wines.	ICP-AES assessed the elemental content of the wines. The distribution of the naturally occurring stable isotopes deuterium/hydrogen (D/H) ratios and carbon ($^{13}C/^{12}C$) in ethanol of wine and oxygen ratio ($^{18}O/^{16}O$) in wine water, were determined by SNIF-NMR and IRMS. Unsupervised PCA established the importance of grape variety and provenance, while supervised PCA established the importance of vineyard and vintage year.	Kokkinofta et al. (2017)
Cyprus	The characterisation of 12 wines from indigenous Cypriot cultivars was carried out in terms of anthocyanin composition, phenolic profile and antioxidant capacity determined by ferric reducing ability and radical scavenging.	Overall, cvs. Lefkada and Maratheftiko exhibited greater concentrations of o-diphenols, hydroxycinnamic acid derivatives, anthocyanins and flavonols in comparison to the rest of the cvs. However, a higher phenolic concentration did not necessarily reflect a greater antioxidant capacity of the wines, which is influenced by the antagonistic effect between the observed compounds.	Galanakis et al. (2015)

2. Bud and Cluster Development

The reproductive development of grapevines encompasses two growing seasons and hence two vegetative cycles for inflorescence and cluster formation (**Figure 1**). The mechanism that results in the development of reproductive structures in dormant compound buds, comprises of the induction and floral differentiation. These processes are categorised into three discrete stages: (1) formation of uncommitted primordia, known as anlagen, (2) inflorescence primordia differentiation occurring in the first season and (3) inflorescence development and flower formation in the second season (Magalhães, 2015). These stages, affected by both exogenous (environmental) and endogenous factors, are comprised of physiological, morphological and biochemical events.

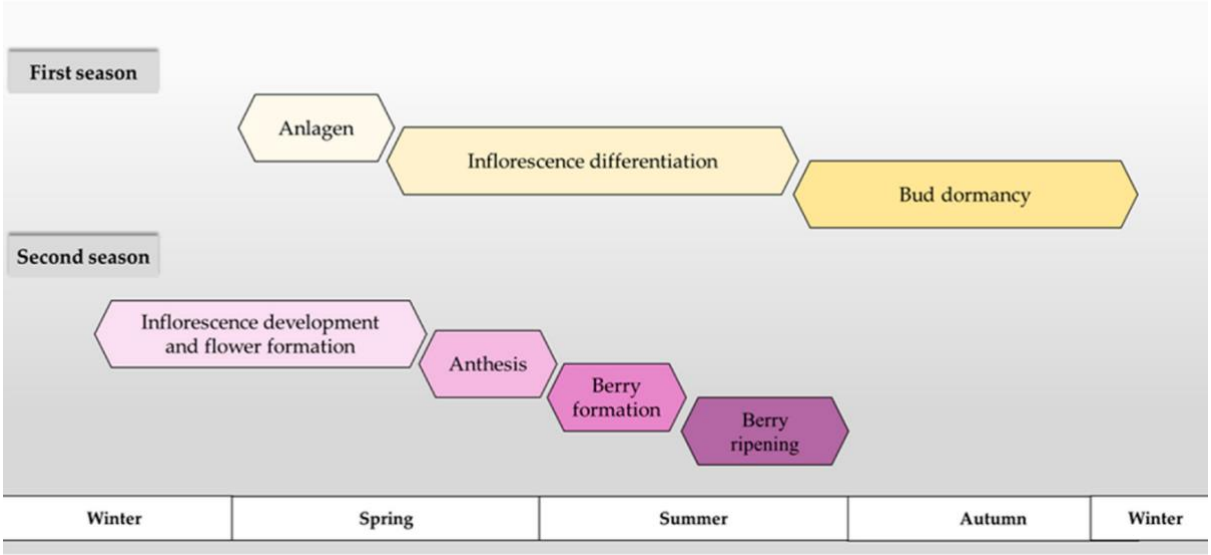

Figure 1. The reproductive cycle of grapevines focusing on the stages of cluster formation over two growing seasons (Monteiro et al., 2021).

The formation of inflorescence primordia dictates the number of potential clusters that will form in the next season and hence, viticulturists and winemakers can obtain a first yield prediction during bud dormancy by determining the bud fruitfulness (Collins et al., 2020; Dry, 2000). Bud fertility or fruitfulness defines the potential number of bunches that will form in the next season based on the number of inflorescences per bud and their size (Magalhães, 2015; Ramos, 1991). It depends on the cultivar, the type of bud as well as the positioning of the bud with lower bud fruitfulness observed in basal buds and reaching maximum values in the 4th or 5th bud (Khanduja & Balasubrahmanyam, 1972; Vasconcelos et al., 2009). When buds contain at least one inflorescence primordium they are classified as fruitful whereas, infertile buds are absent of any inflorescence primordia, or they contain only tendril primordia (Srinivasan & Mullins, 1980).

Bud fertility allows for adjustments in bud load to promote a balance between yield and fruit quality in addition to increasing commercial value. Therefore, the present review discusses the morphology and physiology of inflorescence primordia and the principal factors involved (biotic

and abiotic) in their formation as well as current techniques employed for the analysis of the compound buds for yield forecasting via the measurement of potential bud fertility.

2.1. Bud development

During the growing season, as shoots develop, bud formation occurs in the leaf axil at the petiole base (Vasconcelos et al., 2009). The first bud to form, the lateral bud, develops into the lateral shoot in the first growing season. The compound bud forms in the axis of this prophyll and develops at a slower rate. In normal ontogenesis compound buds undergo dormancy and thus, are referred to as dormant buds. The compound bud remains dormant until the next growing season due to hormonal effects on the lateral shoot apex (Jackson, 2014; Magalhães, 2015). This bud can produce 10 to 12 leaf primordia and 1 to 3 inflorescence primordia prior to dormancy, depending on the cultivar. Anatomically, the compound bud contains the primary, secondary and tertiary buds, enclosed by the lateral bud/shoot prophyll or the basal scale; these structures form the principal compound bud, referred to as eye, observed on the nodes of mature canes. It is a basal appendage of the lateral bud (or shoot), structures associated closely through the vascular tissue of young compound buds that lead to the lateral bud. **Figure 2** demonstrates a fully developed compound bud and a transversal cut of a dormant bud revealing the primary and secondary buds. In a normal growing season, the primary bud will develop into a fruiting shoot during spring, while the secondary and/or tertiary buds retain dormancy. However, if the primary bud undergoes damage, the secondary and tertiary bud can develop into a shoot to replace the primary bud (Lavee & May, 1997) but the fruitfulness will be lower in comparison to the primary bud. Whilst the secondary bud may form inflorescence primordia depending on the cultivar, the tertiary bud does not (Jackson, 2014; Srinivasan & Mullins, 1976).

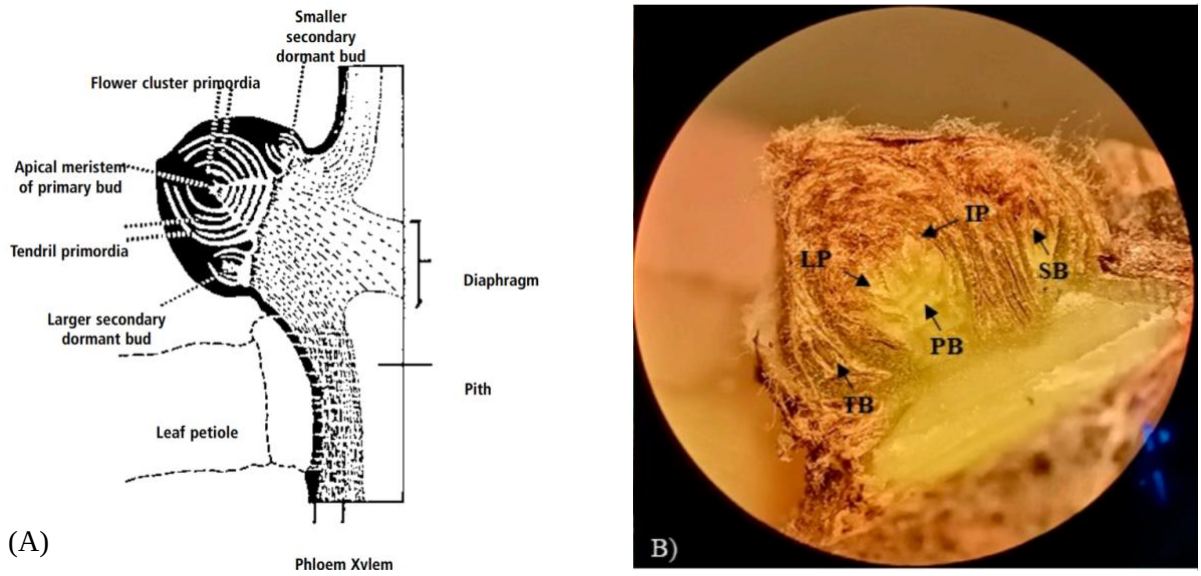

Figure 2. (A) Cross-section of compound bud showing the cluster, leaf and tendril primordia (Williams, 2000). (B) Longitudinal section of a dormant bud (Loureiro cv.) showing the bud organs: the primary bud (PB), the secondary (SB) and tertiary bud (TB), the leaf primordia (LP) and the inflorescence primordia (IP) (Monteiro et al., 2021).

2.2. Inflorescence primordia differentiation

The clusters for the crop of the following season commence forming concurrently with the leaf primordia formation inside the compound bud. The primary bud can produce 3-4 leaf primordia, depending on the cultivar. Inflorescence formation commences in dormant buds, and it is the result of three main phases which take place over two growing seasons (Srinivasan & Mullins, 1976). The first phase commences with the formation of uncommitted primordia (UP) or the anlagen (Lebon et al., 2008; Srinivasan & Mullins, 1980; Vasconcelos et al., 2009). Located opposite to the youngest leaf primordium, UP form from the bud apex and resemble club-shaped meristematic protuberances. Analgen formation is considered an indicator for the commencement of the inflorescence axis formation (Lebon et al., 2008; Srinivasan & Mullins, 1976;1979). Primarily, UP appear on the shoot basal buds and during the progression of the growing season, more toward the shoot apex. UP, as they develop, differentiate into either inflorescence or tendril primordia or seldomly an intermediate structure based on hormonal and environmental factors. In the second phase the UP divide into two unequal parts: the inner arm which is larger and the smaller outer arm. While both arms may develop into inflorescence or tendril primordia (Li-Mallet et al., 2016; Williams, 2000), the inner arm – the adaxial portion nearer to the apex – has more potential to develop into globular branch primordia and consequently the rachis. Conversely, the outer arm – the smaller abaxial part adjoining the bract – will form either large branches or a wing located at the top of clusters (Srinivasan & Mullins, 1976).

An inflorescence primordium that has fully developed resembles a grape bunch, where each berrylike branch primordium is a mass of undifferentiated tissue (Botelho et al., 2006). Both phases are critical in bud fruitfulness and signify the start of floral initiation which establishes the potential inflorescence number and thus cluster per bud in the subsequent growing season (Carmona et al., 2008). The third phase is flower differentiation. After formation of the reproductive organs compound buds undergo dormancy (endodormancy or deep organic dormancy) until the next spring when growth restarts to complete the development of flowers and berries (Carmona et al., 2007b). These buds are typically the most fruitful as they underwent a long differentiation process resulting in one to two inflorescences per bud based on the cultivar, the bud positioning on the shoot and the biotic and abiotic conditions.

2.3. Flower differentiation

Following the exit of the compound bud from dormancy, the flower differentiation on the inflorescence primordium begins during which the branch primordium undergoes division to produce the flower initials (Boss et al., 2003; Srinivasan & Mullins, 1978). The beginning of flowering is marked by the presence of a calyx on the primordium rim which appears as a

continuous ring of tissue. The calyx tissue covers the entire flower primordium and creates an incomplete cap (Mullins et al., 1992). In tandem with the calyx formation, petals develop and become lobed and appear through the incomplete calyx cap. As individual petals elongate cell formation occurs at the margins which interlock with adjacent cells on the nearest forming petal to form the calyptra (Meneghetti et al., 2006; Srinivasan & Mullins, 1979). The process precedes the start of the bud growth and continues after budbreak until the flowers are fully developed and anthesis occurs. The flower arrangement becomes visible right before anthesis when the inflorescences start elongating rapidly (Reynier, 1990).

2.4. Tendril Primordia Formation

During the reproductive development of grapevines, the UP which have the potential to develop into cluster or tendril primordia, start dividing into two branches, a critical stage of grapevine growth. During the first stage governed by coarse control, homologous meristematic structures form, and during the second stage governed by finer control, two-branched UP switch into either a tendril or cluster pathway. During the developmental pathway of UP, if they undergo branching repeatedly, inflorescences will form while, less rigorous branching results in tendril formation (May, 2000). The differentiation of UP into inflorescence or tendril primordia is regulated by both environmental and endogenous factors (e.g., hormonal) an interplay between these factors during UP differentiation determines the differentiation outcome (Boss et al., 2003).

3. Factors affecting induction and differentiation of inflorescence primordia

There have been multiple studies into the impact of environmental (abiotic) and endogenous (biotic) factors on the induction and differentiation processes of inflorescence primordia (Khanduja & Balasubrahmanyam, 1972; Li-Mallet et al., 2016; Srinivasan & Mullins, 1980; Vasconcelos et al., 2009). Environmental factors including light, temperature, water status and macronutrient availability and endogenous factors such as hormonal balance, genetics and source/sink regulation, impact flower formation both directly and indirectly (Carmona et al., 2008; Li-Mallet et al., 2016; Vasconcelos et al., 2009). As a result, during anlagen differentiation, these factors affect the development of inflorescence primordia and thus, fruitfulness.

3.1. Environmental Factors

Light

Light or irradiance is a crucial factor in the initiation of inflorescence and the development of dormant buds (Buttrose, 1969; Dry, 2000; May, 1965; May & Antcliff, 1963; Sommer et al., 2000). Buttrose (1969) reports that with increasing light intensity under otherwise controlled environmental condition, the number of primordia increased. Other findings support this and report an increase in the number of inflorescences with increased shoot exposure to solar

radiation in field; the cultivars assessed were Cabernet Sauvignon, Flame Seedless, Chardonnay and Thompson Seedless (Sánchez & Dokoozlian, 2005). Conversely, May and Antcliff (1963) report a reduction in bud fruitfulness following 70 % reduction in light intensity for a period of four weeks prior to anthesis. Thus, during initiation and differentiation total shading or reduced light intensity can reduce the inflorescence primordia number and size (Keller & Koblet, 1995; May, 1965; Srinivasan & Mullins, 1980). Direct radiation affects leaf photosynthetic activity and carbohydrate availability whereas, low light intensity causes a reduction in the photo assimilate availability which limits carbohydrates allocation to buds in development (Dry, 2000; Keller, 2015; Lebon et al., 2008; Vasconcelos et al., 2009). As a result, canopy management practices such as row spacing, trellis-training system, pruning and shoot control are essential to ensure adequate light exposure to prevent yield losses (Dry, 2000; Magalhães, 2015; Morgan et al., 1985).

Temperature

Several studies reported a positive correlation between high air temperature during the inflorescence primordia differentiation and the number of primordia formed in compound buds (Buttrose, 1969; Guilpart et al., 2014; Sommer et al., 2000; Srinivasan & Mullins, 1976). However, the effects are varietal dependent (Li-Mallet et al., 2016; Sánchez & Dokoozlian, 2005). The optimum air temperature range for inflorescence formation has been shown to be 20 to 35 °C (Buttrose, 1969). Temperatures above 30 °C for a minimum 4 to 5 h duration per day were sufficient to induce the development of the maximum inflorescences in the varieties Syrah, Riesling, Muscat of Alexandria, Almeria and Thompson Seedless. Conversely temperatures below 20 °C induce tendril development which in turn, reduced bud fruitfulness and yield (Buttrose, 1969; Srinivasan & Mullins, 1980). Although the mode of action of temperature has not been elucidated yet, hypotheses involving the biosynthesis of gibberellins (GAs) and cytokinins (CKs) have been proposed. The optimum temperature range for the formation of inflorescence primordia (25 to 35 °C) promotes the biosynthesis of CKs which is known to stimulate inflorescence differentiation (Jackson, 2014). Conversely, temperatures below 20 °C promote the biosynthesis of GAs which limit nutrient accumulation and stimulate vegetative growth. Notably, photosynthesis is also optimized at a temperature range of 25-30 °C based on the cultivar, phenological stage and pedoclimatic conditions. Above 35 °C stomata closure is induced and photosynthetic activity decreases. Other hypotheses involve the effect of temperature on enzymatic and respiratory activities (Keller, 2015; Moutinho-Pereira et al., 2007).

Water Status

Grapevine water status has a direct and indirect impact on the biochemical and biosynthetic reactions impacting the flower formation. Namely, it regulates photosynthetic activity, cell turgidity, photo assimilate and nutrient transport (Jackson, 2014; Li-Mallet et al., 2016; Osakabe et al., 2014; Vasconcelos et al., 2009; Williams, 2000; Chaves et al., 2010). Sufficient water availability improves the conditions of inflorescence differentiation. Conversely, water stress negatively impacts the inflorescence number and size, as demonstrated by Buttrose (1974) where increased grapevine water stress decreased the number and weight of Cabernet Sauvignon inflorescences progressively. Water stress conditions reduce photosynthetic activity and thus, carbohydrate production is insufficient to provide energy for inflorescence differentiation (Magalhães, 2015). In addition, water stress increases abscisic acid (ABA) in the stems and leaves and decreases the CK's levels in the xylem sap which creates a hormonal imbalance with detrimental effects in inflorescence differentiation (Vasconcelos et al., 2009). The increase of ABA in response to water stress induces the closure of stomata which in turn hinders photosynthesis (Osakabe et al., 2014; Chaves et al., 2010). Notably, moderate water stress has been proven to promote a balanced ratio between reproductive and vegetative growth by increasing bud fruitfulness due to reduced canopy density and improved light exposure, particularly in the renewal zone (Keller et al., 2005).

Macronutrient and Micronutrient Availability

Sufficient macronutrient availability is integral to the induction and differentiation of inflorescence and consistent bud fruitfulness. Nitrogen (N) is essential for the composition of amino acids which make up proteins and enzymes, the catalysts of biochemical processes in the grapevines. Nitrogen is also a fundamental element in the chemical structure of chlorophyl and plant hormones (Keller, 2015; Srinivasan & Mullins, 1976). Low level applications of nitrogen have been shown to increase the number of inflorescences (Guilpart et al., 2014; Mullins et al., 1992). However, excess nitrogen causes increased plant vigour and thus, bud shading which negatively impacts fruitfulness (Srinivasan & Mullins, 1980; Vasconcelos et al., 2009). Phosphorus (P) is another essential macronutrient which makes up phospholipids and nucleic acids, biomolecules functioning in metabolic reactions (Stigter & Plaxton, 2015). Skinner and Matthews (1989) report that the initiation and differentiation of primordia are sensitive to phosphorus deficiency. Potassium (K), being involved in multiple biochemical and physiological processes such as photosynthesis, is also essential for normal plant function (Rogiers et al., 2017). Srinivasan and Mullins (1978) found that during the first growth cycle, potassium fertilisation induced a 40 to 58 % increase in the inflorescence primordia size depending on the positioning of the bud. Zinc (Zn) is integral in chloroplast development and is primarily present in the protein-bound form

with synthesis proteins involved in gene regulation via DNA/RNA binding (Keller, 2015). Zn deficiency results in a reduction of protein synthesis ad starch production which is manifested as coulure in the grapevine and interveinal chlorosis as well as stunted cane growth (Bertoldi et al., 2011; Broadley et al., 2007; Holzapfel, 2021). Molybdenum (Mo) acts a cofactor in molybdoenzymes (Schwarz & Mendel, 2006) such as nitrate reductase and aldehyde oxidase involved in nitrogen conversion and hormone (auxin and ABA) production respectively (Schwarz & Mendel, 2006). Therefore, Mo deficiency results in nitrate accumulation and a reduction in amino acid synthesis (Currle et al., 1983). The plant undergoes reduced growth and yield as well as poor fruit set (Kaiser et al., 2005; Longbottom et al., 2010; Williams et al., 2004). Boron (B) is mostly present as borate bound to pectic polysaccharides and is involved in carbohydrate metabolism pathway (O'Neill et al., 2004). Boron toxicity or deficiency are manifested similarly (Currle et al., 1983; Takano et al., 2008) with the visual symptoms being millerandage and stunted shoot and root growth (Keller, 2015).

3.2. Endogenous Factors

Carbohydrate Reserves

Sugar availability and carbohydrate reserves are fundamental in the development of inflorescences. The reserves are accumulated in the previous growing season in the perennial plant organs and during the current growing season via photosynthesis (Howell, 2001). To evaluate the impact of carbohydrate availability, defoliation was carried out at various phenological stages of the plant and the effect on bud fruitfulness of the consequent year was studied (Bennett et al., 2002; Vasconcelos & Koblet, 1990). Bennett et al. (2005) observed the effects of defoliation post-flowering, at three different occasions at monthly intervals. They proposed that the root and overwintering trunk carbohydrate content significantly impacts the number of inflorescences as well as the flowers per inflorescence formed in the next growing season. This effect was more prominent in early defoliation, which led to a decrease in the number of inflorescences per shoot and flower number per inflorescence by up to 50 %. Similarly, defoliation at the véraison stage alters the carbohydrate distribution of the plant leading to a reduction in the starch content and an increase in the soluble sugar content, which confirms the important role of carbohydrate reserves in the sexual reproduction rate of the plant. Notably, grapevines can adjust the inflorescence number depending on the availability of carbohydrates in the perennial plant organs (Vaillant-Gaveau et al., 2014).

Hormonal Balance

The induction and differentiation of inflorescence primordia is regulated by the interaction and balance between two antagonistic hormones, Gibberellic Acid and Cytokinin (Srinivasan &

Mullins, 1980; Williams, 2000). GAs are produced in the leaves and they induce the formation of anlagen however, they later inhibit anlagen development into inflorescence and instead encourage tendril formation (Carmona et al., 2008; Srinivasan & Mullins, 1978). On the other hand, CKs are produced in the roots and transported through the xylem to the target sites where they interfere directly with primordia differentiation (Srinivasan & Mullins, 1976; Vasconcelos et al., 2009). Application of hormones and/or hormone inhibitors has been shown to interconvert anlagen into tendrils and vice versa (Srinivasan & Mullins, 1979, 1981). Specifically, GA applied exogenously in the form of gibberellic acid (GA3) converts young inflorescences into tendrils. On the other hand, applying a gibberellin inhibitor exogenously such as chlormequat promotes the formation of inflorescences from tendrils. When the synthesis of GAs is inhibited endogenous CKs increase in the xylem sap (Mullins et al., 1992). Conversely, the application of CKs at the shoot apex has been shown to induce inflorescence formation instead of tendril formation. Applying synthetic CKs such as PBA (6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine) in the tendril primordia (in-vitro culture isolation) induces their branching through their conversion into inflorescence (Srinivasan & Mullins, 1978, 1979, 1980).

Genetic Factors

The induction of inflorescence primordia and sequential differentiation are controlled genetically (Meneghetti et al., 2006). Molecular studies of *Vitis vinifera* showed that multiple genes which are expressed in flowering are expressed in compound buds during floral induction and differentiation. The induction and formation of floral organs happens via an intricate network of gene and protein, non-linear interaction. Several species have demonstrated such interactions such as *A. thaliana* (Poupin et al., 2011). Specifically, the family gene of the flowering locus T (FT)/terminal flower 1 (TFL1) has important action as the flowering signal integrator. The homologous gene family of *A. thaliana* encodes similar proteins to PEBPs (phosphatidylethanolamine binding proteins) which promote and repress flowering (Carmona et al., 2008, 2006; Wickland & Hanzawa, 2015; Yoo et al., 2010). During inflorescence development, phylogenetic analysis of the dormant bud identified members of the grapevine gene family of FT/TLF1, in three subfamilies: TFL1-like, FT-like and MFT-like. Notably, an FT orthologue, the VvFT gene has been shown in compound buds during the first stages of inflorescence formation (Carmona et al., 2008); both VvFT and VvMFT are involved in organ differentiation and meristem determination, by promoting flowering. Conversely, the genes belonging to the TFL1-like family, VvTFL1A, VvTFL1B and VvTFL1C, are involved in vegetative growth and promote the meristem indetermination (Crane et al., 2011; Li-Mallet et al., 2016; Poupin et al., 2011). Therefore, premature flowering can be promoted when specific genes are over-suppressive. Another gene associated in flowering is the transcription factor, VvVFL (AtLEAFY orthologue) which is

expressed in the anlagen of compound buds in Tempranillo and Riesling grapes (Carmona et al., 2008). Tempranillo and Cabernet Sauvignon cultivars also display some families of genes associated with floral induction namely, the genes VvSOC1 and VvMADS8. VvSOC1 is a homologue of SOC1 in *A. thaliana* and VvMADS8 belongs to a subfamily of the MAFS-box gene which is positively regulated in the initial stages of floral development (Díaz-Riquelme et al., 2012, 2014). As discussed, the balance between GAs and CKs is essential for floral induction and differentiation. Genetic studies support the role of GAs in inhibiting inflorescence differentiation, through a mutation of the VvGAI gene giving rise to a mutant phenotype of grapevine that is insensitive to gibberellin. In this phenotype, the tendril primordia differentiated into inflorescences, supporting the hypothesis of a GA-dependent signal-transduction pathway which inhibits inflorescence differentiation (Boss et al., 2003; Carmona et al., 2007b; Poupin et al., 2011). Further research on transcriptional analyses shows that tendrils and inflorescences share a common transcriptional program initially, associated with cell growth (Díaz-Riquelme et al., 2014). In later stages of development tendrils display transcription of genes more related to hormonal signalling, photosynthesis and secondary metabolism whereas, inflorescences display gene transcription of factors in the MADS-box family.

4. Analysis Techniques of Bud Fruitfulness

4.1. Bud Dissection and Histological Analysis

The identification and quantification of bud fertility during dormancy, is difficult to conduct and requires specialised laboratory procedures and techniques to evaluate the inflorescence primordia (Martínez de Toda, 1991; Ramos, 1991). The dissection of buds in tandem with their histological analysis allow the quantification of the inflorescence primordia in each bud and the analysis of bud viability, namely the diagnosis of tissue necrosis and the extent of the lesion in the tissue (Andreini et al., 2010; Rawnsley & Collins, 2005). The method of the dormant bud dissection involves the use of a stereomicroscope for the observation and identification of the inflorescence primordia (Martínez de Toda, 1991; Ramos, 1991). A scalpel is used to dissect the bud and consequently, tweezers are used to easily transfer it onto the stereomicroscope slide. Nonetheless, this procedure is time-consuming and requires careful handling of the bud during the cutting and protective structure removal, to prevent damage of the inflorescence primordia. To achieve this, careful and small cuts are made from the top part of the bud towards the bottom base or longitudinally to reveal the bud interior. One of the major problems of this technique is the difficulty of removing the epidermal hairs lining the fragile primordia and meristems which hinder visualization. One of the advantages of the dissection technique is that the information on bud vitality and the number and size of inflorescence primordia is readily available following the anatomical cut of the bud. In Australia, bud dissection for estimating fruitfulness is a widely used technique, provided by commercial laboratories to assist winegrowers in decision making on

winter pruning (Rawnsley & Collins, 2005). For histological analysis, the bud is embedded in paraffin wax, sectioned, stained and lastly observed under an optical microscope (Jackson, 2014; Skinner & Matthews, 1989). The success of this technique depends on the fixation process of the plant material. Prior to the fixation the epidermal hairs and scales of the bud are removed to allow the paraffin wax and fixative solutions to act and preserve the integrity of the bud structure (Martínez de Toda, 1991). To increase the effectiveness of this method, the cuts made on the buds should be carried out sequentially to ensure the visualization of all the inflorescence primordia. Notably, this method is also time-consuming, and the reagents increase the overall operational costs.

Despite involving different technical procedures, these methods require adequate knowledge of the bud anatomy to unambiguously identify the structures and the primordia as a misidentification of inflorescence can result in inaccurate conclusions about the bud fertility or lack of thereof. The microscopic analysis of buds is a destructive method, therefore selected buds should be those removed at pruning and not destined for annual production (Martínez de Toda, 1991; Ramos, 1991). This introduces potential inaccuracies into the method especially for small buds such as the basal ones (Martínez de Toda, 1991). **Figure 3** shows longitudinal cuts of dormant buds under both microscopes.

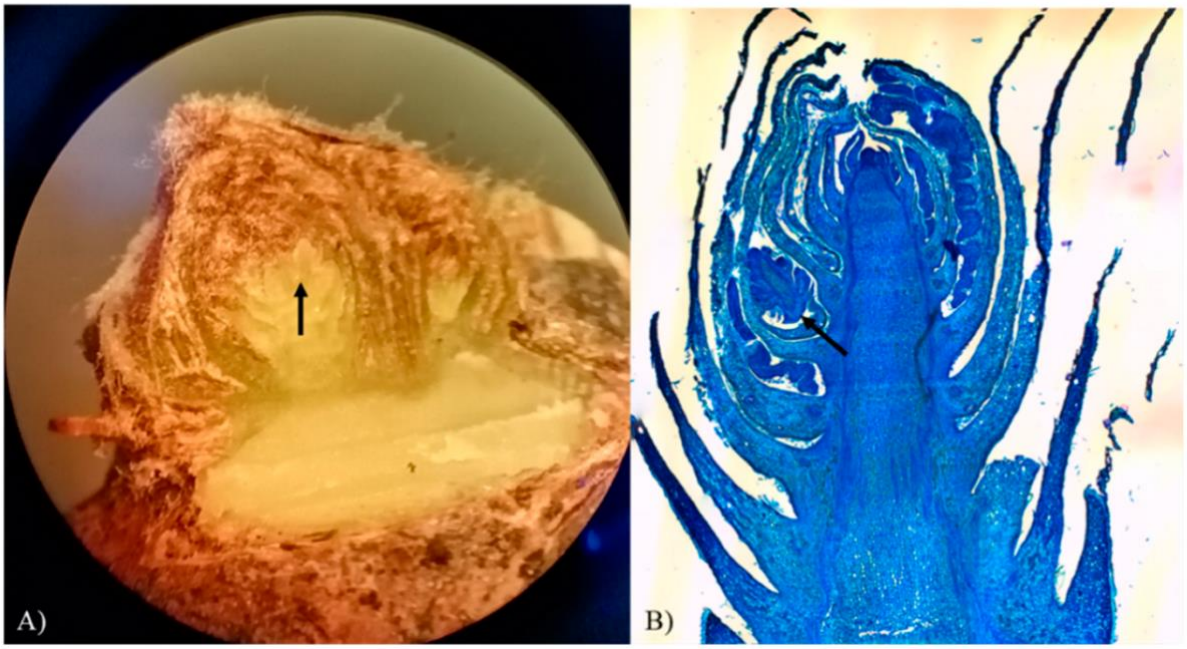

Figure 3. Dormant bud dissected longitudinally and observed under a stereomicroscope at 20x magnification (A). Dormant bud dissected longitudinally and observed under a light microscope (B). The black arrows indicate inflorescence primordia (Monteiro et al., 2021).

4.2. Bud Viability

Microscope techniques can also be applied to evaluate bud viability namely, the presence of damaged tissue commonly referred to as necrosis, which can impede bud development (Collins et al., 2006). One of the major causes that impacts yield detrimentally is primary bud necrosis (PBN); impacting bud fertility (Vasconcelos et al., 2009). PBN is a physiological disorder that results in the death of the primary bud during bud initiation (Collins et al., 2006; Dry, 2000; Rawnsley & Collins, 2005; Vasudevan et al., 1998). The necrotic dormant bud appears identical to a healthy bud therefore, the necrosis can only be diagnosed and identified by investigating the internal structure of the bud via anatomical dissection and histological analysis (Dry, 2000). PBN susceptibility seems to depend on the grapevine variety. For example, a high PBN incidence was reported in Shiraz (Collins et al., 2006), Riesling (Vasudevan et al., 1998) and Thompson Seedless (Sultana) varieties (Morrison & Iodi, 1990; Perez & Kliewer, 1990). Other factors that have been associated with PBN occurrence are rootstocks of American species of *Vitis* (Cox et al., 2012), high shoot vigour (Ziv et al., 1981), canopy shading (Perez & Kliewer, 1990), exogenous application of gibberellic acid (Rawnsley & Collins, 2005; Ziv et al., 1981) low bud carbohydrates content (Cox et al., 2012; Vasudevan et al., 1998) and excessive irrigation (Kliewer et al., 1994). Despite the secondary bud remaining healthy and developing normally, it is often less fruitful and lower yields are obtained. Therefore, early diagnosis of PBN is crucial to adjust winter pruning namely, the number of buds left and consequently, the yield predictions (Dry, 2000; Kavoosi et al., 2013; Rawnsley & Collins, 2005).

5. Propagation by seed and seed germination

Recent technological developments in viti-vinicultural research have facilitated breeding of different grape varieties for a range of reasons, including enhancing resistance of grapevines against diseases, tolerance to biotic and abiotic factors, the improvement of grape quality as well as modulating the ripening period (Wang et al., 2022; Wang et al., 2021). Although multiple breeding techniques and methods are being explored, successful breeding requires high-quality genetic material making seed selection an important aspect of the breeding process (Chai, 2005). *Vitis vinifera* is a primary breeding resource material (Huang et al., 2005; Wang et al., 2021) for its widespread use and economic value globally. However, *Vitis* seeds are characterised by low seed germination rates, unless endodormancy is overcome (Ellis et al., 1983). Therefore, improved germination and seedling formation rate are fundamental in the development of effective breeding strategies for intraspecific hybrids of *Vitis vinifera*.

5.1. Germination of *Vitis vinifera*

Seed dormancy in grapes is normally due to the tough and thick coat of the seed, considered a mechanical barrier to successful germination (Conner, 2008). To overcome seed endodormancy,

seeds undergo cold stratification for a period of 3 to 4 months although, this treatment still results in reduced germination percentages for *Vitis vinifera* L. cultivars (Ellis et al., 1983; Selim et al., 1981). The dynamics and rate of germination vary accordingly to the grape species; *Vitis labrusca* L. and *Vitis vinifera* L. demonstrate a germination rate lower than 30 % whereas, the germination rate of *V. riparia* Michx., *V. rupestris* Scheele and *V. acerifolia* Raf. ranges from 40 – 100 % (Rombough, 2002). However moderate germination rates can also be attributed to incomplete physiological maturity whereby the seed embryo and endosperm of the female parent plant are not fully developed at harvest time (Ma et al., 2014). Following the seed extraction from the harvested grapes, seeds can be subjected to improper humidity and temperature conditions during storage which results in either premature germination or seed decay (Boss et al., 2003). Moreover, the stratification period is a crucial parameter as removing the seeds from the cold treatment too early or too late can lead to the inhibition of the germination by the phenolic compounds contained in the endosperm (Gao et al., 2014). Finally, the germination conditions specifically the soil humidity, is difficult to regulate and as such impacts the germination rate; inadequate levels of humidity inhibit germination due to lack of water whereas, excessive humidity can lead to fungus formation (Lin et al., 2009). The consequent cultivation conditions following seed sowing can also inhibit the development of the seed (Lei et al., 2010).

5.2. Research Development

Previous studies investigated the dynamics of grape seed germination and seedling formation as well as the difference in germination rates depending on the cultivar, the cultivation and transplanting techniques. Wang et al. (2021) reported the physiological stages of germination in tandem with optimising the release from dormancy (Wang et al., 2022; Yang et al., 2021). Specifically, Chai (2005) reported the germination and seedling rate of *V. vinifera* and *V. labrusca* cultivars, with *V. vinifera* displaying lower germination rates than *V. labrusca* (Chai, 2005; Lin, Zhang, Huang, Peng, & Hu, 2009).

Pan et al. (2010) investigated the effects of GA3 on the germination characteristics of the wine grape *V. adenoclada* Hand. -Mazz, and table grape *V. davidi*, reporting that GA3 improves the germination rate significantly (Pan et al., 2010). Both the germination index of grape seeds and the germination potential increased, whereas the germination length was shortened (Pan et al., 2007; Ye, 2008; Zhang et al., 2008). Other chemical substances and their effects on seed germination rate were investigated including, Forchior fennron (CPUU), of 6-benzyl aminopurine (6-BA), polyethylene glycol, acetic acid (Hac), indole acetic acid, lime nitrogen, 2,4-dichlorophenoxyacetic acid and ammonium nitrate (Kong, 2018; Lan et al., 2011; Ma et al., 2014; Wu & Li, 1999).

Another study on the effects of direct sowing of the seeds in field or a greenhouse, in tandem with film mulching, observed the highest rate of hybrid seedling formation by seed sowing in the greenhouse with a hole disc method followed by transplanting the seedlings in field after 4-5 true leaves emerged, by late spring (Zhang et al., 2009). A study by Wang et al. (2022) determined the optimal pre-treatment methods for seed germination of six *V. vinifera* cultivars. The factors tested as well as the results, which varied in effectiveness depending on the variety are outlined in **Tables 6** and **7**. Generally, GA3 soaking and beak-cutting seemed to promote the germination and seedling rates of the seeds (Wang et al., 2022).

Table 6. A summary of the different experimental treatments and results in terms of germination and emergence rates for different varieties. Adapted by Wang et al. (2022).

Level	Experimental Factor			
	Stratification Method (A)	Chemical Substance (B)	Beak Cutting (C)	Pre-Germination Method (D)
1	chilling gauze-storage	GA_3	beak cutting	pre-germination in bean sprouter
2	chilling sand-storage	6-BA	no beak cutting	pre-germination in petri dishes
3		CPUU		
4		HAc		
5		CK		

Table 7. A summary of the results depicting the optimum combination for achieving the greatest germination rate per each variety. Adapted by Wang et al. (2022)

Varieties	Optimal Combination	Germination Rate	Emergence Rate
Ecolly	A2B1C1D1	94.21 ± 4.33	53.24 ± 4.19
Garanior	A2B4C2D2	92.18 ± 4.56	67.65 ± 5.34
Dunkelfelder	A2B1C1D2	79.99 ± 5.72	51.52 ± 4.24
Cabernet Sauvignon	A2B1C1D2	82.49 ± 7.01	56.17 ± 3.55

The data based on three replicates was represented as M ± SD. A, B, C, and D represent experimental factor of stratification method, chemical substance, beat cutting, and pre-germination method respectively. 1, 2, 3, 4, and 5 correspond to the level factors of each experimental factor.

For Dunkelfelder and Cabernet Sauvignon the treatment with optimal germination rate was chilling sand-storage + GA3 soaking seed + beak cutting + pre-germination in petri dishes. For the cultivar Meili it was chilling sand-storage + HAc soaking seed + beak cutting + pre-germination in petri dishes. For Ecolly it was chilling sand-storage + GA3 soaking seed + beak cutting + pre-germination in a bean sprouter and for Garanior it was chilling sand-storage + HAc soaking seed + no beak cutting + pre-germination in petri dishes. Lastly, for Marselan it was chilling gauze-storage + GA3 soaking seed + beak cutting + pre-germination in a bean sprouter (Wang et al., 2022).

Notably the study although conclusive, evaluated only the effects of exogenous treatments on germination rate; other factors such as the physiology of the seeds, the quality of the seeds following the exogenous treatments and the seed embryo vigour were not taken into consideration for the purposes of this study. The understanding of seed dormancy and germination as well as seedling formation is not elucidated and thus, more comprehensive studies are needed to manage irregular germination. Seed cultivation and sowing remain determinants of breeding efficiency (Wang et al., 2022) and contribute to the assessment of the phenotypes once germinated.

6. Materials and Methods

6.1. Experimental site and plant material

The experiments were conducted in 2022 at the Wine Innovation Central Building, located at the Waite Research Campus of the University of Adelaide. The plant material was collected from the commercial vineyard Georgiadis Estates, situated in Marananga (34.48° S, 138.94° E) in the Barossa Valley. The vineyard was planted in 1995 and spans over 13.2 ha with a planting density of 1851 vines/ha. The training system is vertical shoot positioning (VSP) with 1-year-old canes pruned back to eight nodes/vine. The vines are subjected to different pruning operations during summer such as shoot thinning, lateral removal and defoliation as well as common viticultural practices such as pest control, compost application and irrigation applied only at critical phenological stages to ensure physiological ripeness. The five cultivars used in the experiments and grown under the above conditions are own-rooted Shiraz, Cabernet Sauvignon, Mataro, Sangiovese and Nero D'Avola. The Cypriot vines Maratheftiko and Xynisteri, were planted in October 2021 in the Mataro Block and follow the same management.

The soil profile varies across the vineyard from red-brown loam over red clay, with ironstone to red-brown loam over clay and limestone. The climate is warm-summer Mediterranean (Csb Köppen) characterised by cool, wet winters and warm, dry summers. The vineyard altitude ranges over 250-290 m above sea level which protects the vines from severe frost events. Long-term averages (1996-2022) for annual temperature and precipitation are 15.4°C and 473 mm respectively. Annual air temperature and rainfall for the season 2021 is shown in **Figure 4**. January was the warmest and sunniest month of the year with a maximum temperature of 30°C and an average of 326 hours of sunshine. Conversely, the coldest month was July with an average maximum temperature of 16°C, while the wettest was June with 79 mm of rainfall. During 2022 plant material was collected in winter at pruning time (**Figure 5**) for two different experiments: (1) collecting grape bunches of Cypriot varieties to extract seeds for germination and (2) collecting canes from all seven varieties for bud fertility measurements.

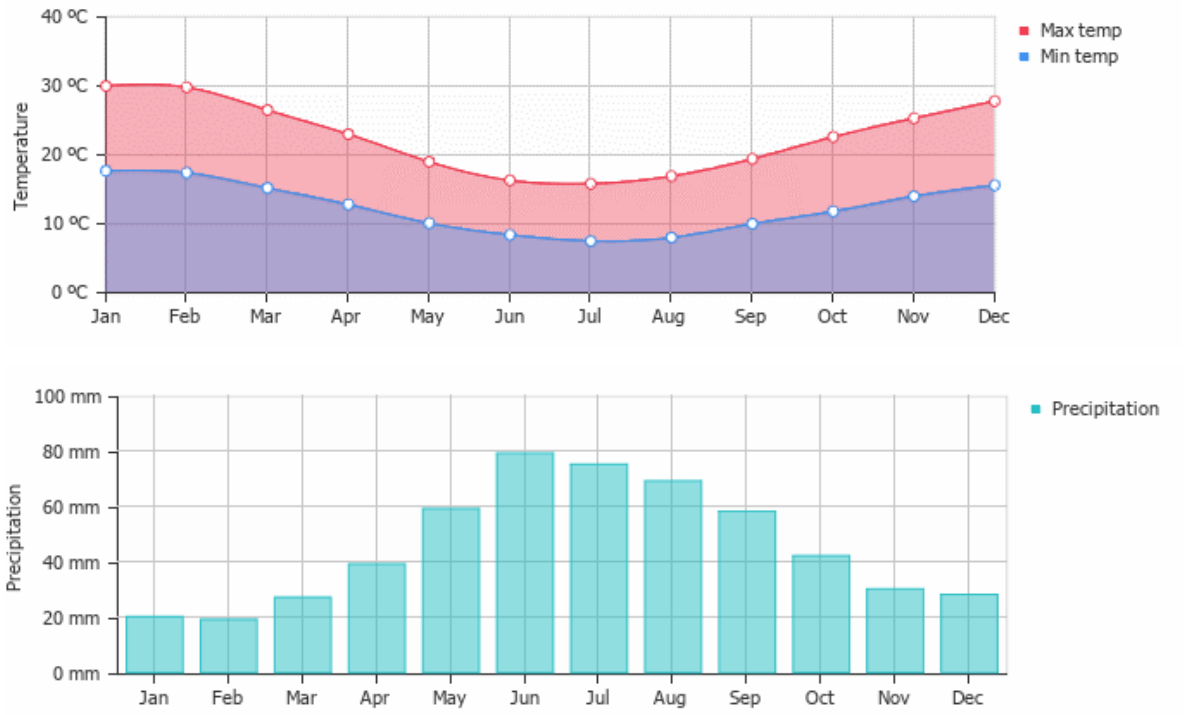

Figure 4. The mean minimum and maximum temperatures over season 2021 (up) and the mean monthly precipitation over season 2021 (down).

Figure 5. The vineyard in Georgiadis Estates pictured in June, after pre-pruning operations. The different varieties selected for cane collection: (A) Shiraz, (B) Sangiovese, (C) Nero D'Avola, (D) Mataro, (E) Cabernet Sauvignon, (F) Maratheftiko, (G) Xynisteri.

6.2. Bud Dissection

Plant material was collected from the experimental site at the end of May and June, after pre-pruning operations were carried out. Canes were collected from Sangiovese, Nero D'Avola, Shiraz, Mataro, Cabernet Sauvignon on the 28th of May and from Xynisteri and Maratheftiko on the 28th of June. Thirty canes from two rows (2 rows x 15 canes per variety) per cultivar were chosen randomly amounting to a total of 210 1-year old canes. The canes were transported to the Waite Research Campus and stored in transparent plastic bags at 2 °C until they were dissected at room temperature. Buds were dissected at node positions 3 to 7; node 3 classified as a basal position along the cane, nodes 4 to 6 classified as medium positions and node 7 classified as a distal position.

Bud dissections were performed at the Waite Research Institute in the University of Adelaide. Internode length from the third to the fourth node was measured with a manual caliper (Mitutoyo, Kawasaki, Japan). The buds were cross dissected and transverse sections were conducted with a single edged razor blade (Personna, Verona, VA, USA) and were assessed for IP number and PBN, using Leica MS5 Stereomicroscope (Leica, Wetzlar, Germany). If PBN was detected in the primary bud, the secondary bud was assessed for IP number (**Figure 6**). Consequently, IP images were obtained for all buds, using TLI Digital Eye-Piece MD500 (TLI, Illawong, NSW, Australia) and an iPad (Apple Inc., Cupertino, CA, USA) for the measurement of the cross-sectional IP area by ImageJ software (National Institutes of Health, Bethesda, MD, USA) (see **Figure 6**).

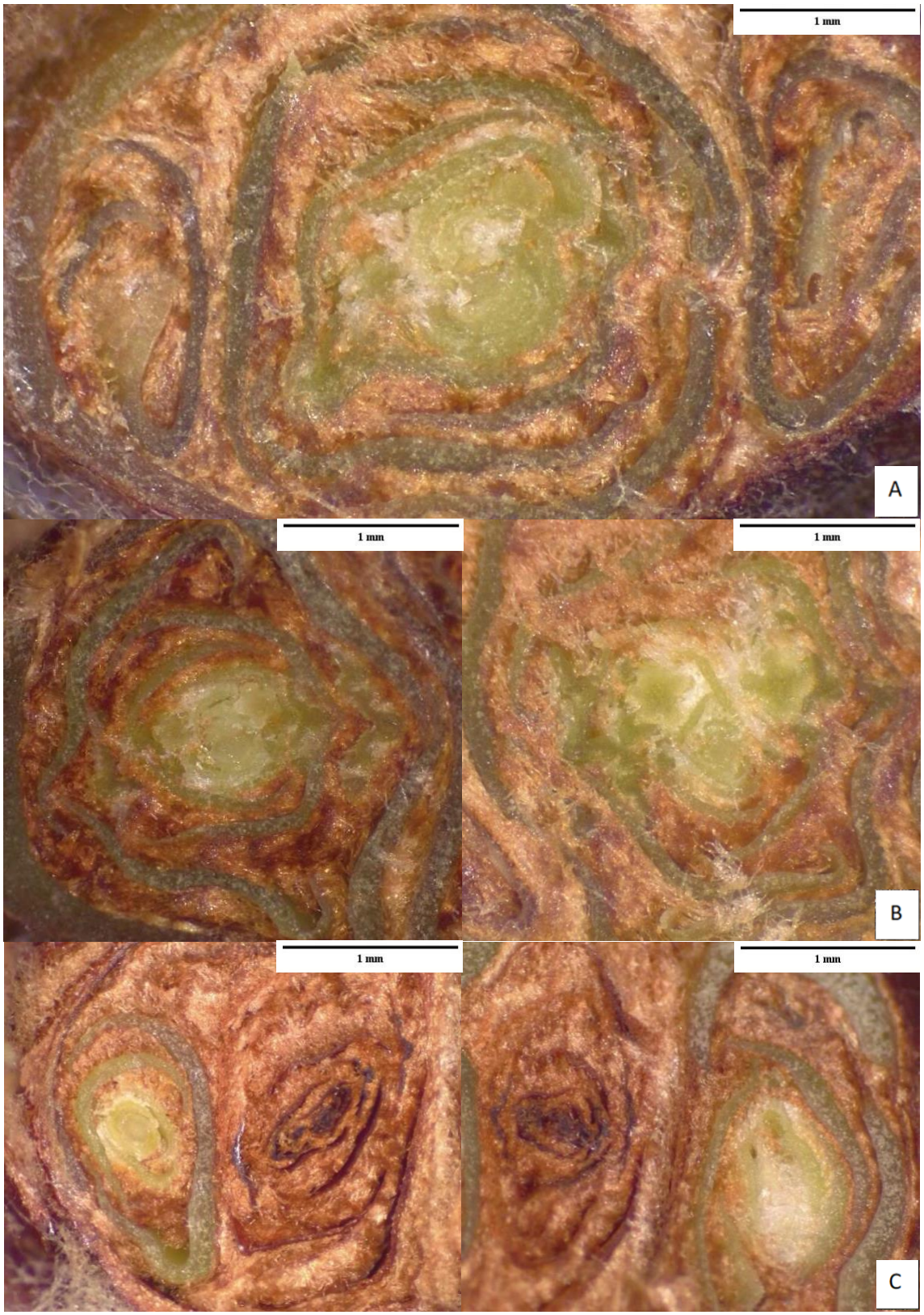

Figure 6. Bud Dissection to evaluate bud fertility and PBN. (A) Transverse section through a compound bud of Sangiovese, showing a healthy primary bud (middle), secondary bud (right) and tertiary bud (left). (B) Transverse section through the compound bud of Sangiovese (left) and Xynisteri (right), showing a healthy primary bud with two IP clearly depicted. (C) Transverse section through the compound bud of Shiraz showing full PBN with a healthy secondary bud which is assessed in this case.

6.2.1. Data Collected

Bud dissection facilitated the measurement of the following parameters: (1) potential bud fertility (number of IP per compound bud), (2) primary bud necrosis (percentage of primary necrotic buds), (3) IP cross-sectional area and internode length and diameter. Data was collected in Microsoft Excel, version 2206 (One Microsoft Way, Redmond, Washington, United States) and statistical analysis was performed with the software Rstudio, version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). Data for all the varieties was assessed with ANOVA and the Fisher Least Significant Difference (LSD) method to compare the bud fertility and the IP cross-sectional area between each variety and within each variety at different node positions.

6.3. Seeding

Grape bunches of the variety Maratheftiko were collected from the experimental site on 17th of May. Fifteen bunches were chosen randomly from two rows, and different bunches were selected from the upper, middle and lower parts of the vine. The bunches were transported to the Waite Research Campus and stored in transparent plastic bags at 2 °C until the seeds were extracted at room temperature. Seed number varied from 2 to 4 seeds per berry amounting to a total of 2034 seeds collected to be tested in the subsequent trials (**Figure 7**). Germination trials were set up at the Waite Research Institute in the University of Adelaide. The seeds were extracted manually using a single edged razor blade (Personna, Verona, VA, USA) to separate them from the skin and pulp. The extracted seeds were rinsed with tap water several times and rolled over a blotting tissue to remove any excess pulp followed by a wash over distilled water (**Figure 7**).

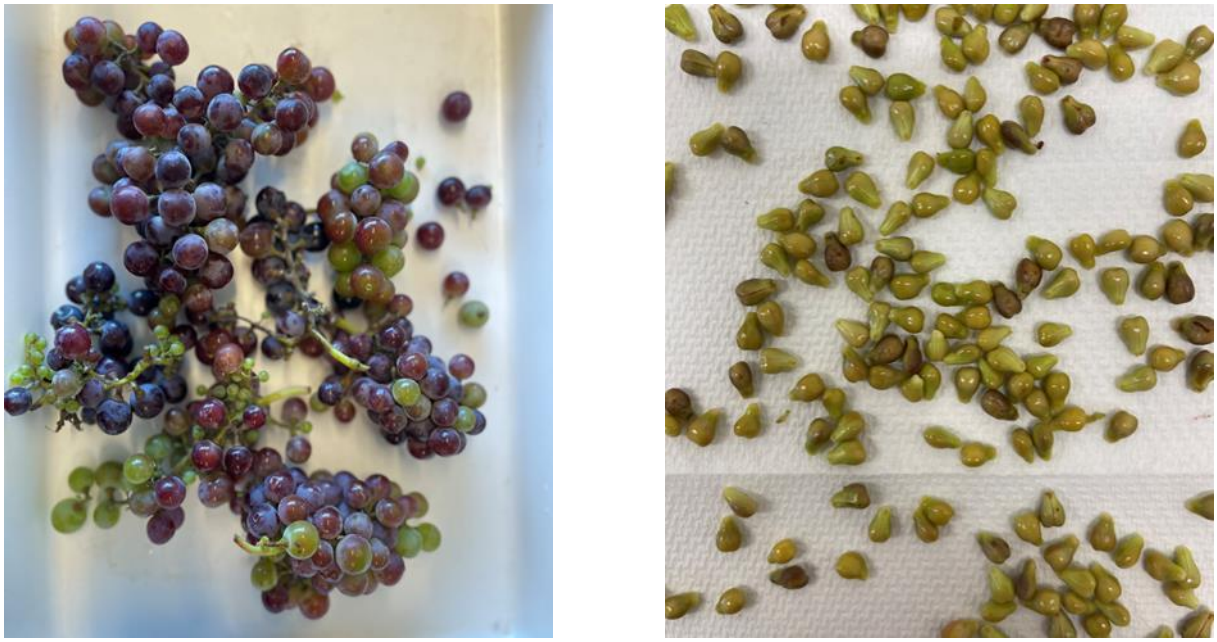

Figure 7. Maratheftiko (*V. vinifera*) grapes collected on 17th of May, prior (left) and after seed extraction (right).

6.3.1. Treatment method of seeds

Different experimental parameters were implemented at the following steps to assess the optimum germination protocol for Maratheftiko seeds: (1) grape treatment, (2) seed treatment, (3) pre-stratification treatment, (4) stratification, (5) pre-germination treatment and (6) germination medium (see **Table 8**).

(1) Grape Treatment: Manual Seed Extraction with blade

(2) Seed treatment: Wash with distilled water / Sterilise with NaOCl or $NaCO_3$ soak for 10 min followed by a wash with distilled water / Wash with cold water / Wash with hot water

(3) Pre-stratification treatment: Air-dry seeds over few hours / Air-dry seeds overnight / Leave moist

(4) Stratification: Cold (2 ± 1 °C) stratification in 70 ml, sterile, screw cap containers (Technoplus, Thermoline Scientific, NSW, Australia) for 14 d, 28 d, 42 d, 56 d, 70 d, 84 d, 90 d

(5) Pre-germination treatment: No treatment / Soak 24 hours in distilled water / Soak 48 hours in distilled water / Smash with pestle and mortar / Beak-cutting with razor

(6) Germination medium: Filter paper / Filter paper + cotton / Blotting tissue / Sterilized sand / Cotton / Seed Raising Mixture

(7) Germination Temperature: Controlled by warming tray (Ratek, Crown Scientific, Melbourne, Australia) at 30 °C

Table 8. The different experimental parameters tested to assess the optimal protocol for the highest germination rate of *V. vinifera* cv. Maratheftiko seeds.

Experimental Parameters					
1	**2**	**3**	**4**	**6**	**7**
Grape Treatment	*Seed Treatment*	*Pre-stratification Treatment*	*Stratification*	*Pre-germination Treatment*	*Germination Medium*
Manual seed extraction	Soak in distilled water	Dry over few hrs	14 days	No treatment	Filter paper
	Soak in $NaHCO_3$	Dry over weekend	28 days	Soak in distilled water for 24 h	Filter paper + cotton
	Soak in NaOCl	Keep wet	42 days	Soak in distilled water for 48h	Blotting tissue
	Wash w/ distilled water		56 days	Smash with pestle and mortar	Sterilized sand
	Wash w/ cold water		70 days	Beak-cutting with razor	Cotton
	Wash w/ hot water		84 days		Seed Raising Mixture
			90 days		

6.3.2. Sowing and Planting

Two kinds of sowing methods were tested: placing the seeds in petri dishes or plastic containers (Technoplus, Thermoline Scientific, NSW, Australia) containing 20 or 5 seeds respectively. The sowing containers were placed on a heating pad (Ratek, Crown Scientific, Melbourne, Australia) to facilitate germination at an air temperature of 30 °C with an air relative humidity of 60-65 % and a light period of 12 h/d. The seeds were routinely moistened to prevent seed dehydration. Germination was measured by emergence which is defined as the time the seedling develops a two-pieced of cotyledon. The seedlings were transplanted in a larger pot (15 cm x 15 cm) with seed raising mixture, after developing the first true leaf and they were moisturised routinely (ca. 2 times/ week).

Figure 8. Different seeding media. (A) Sterilised sand in petri dishes on heating pad. (B) Filter Paper (left), sterilised sand (middle) and cotton (right) in petri dished on heating pad. (C) Seed raising mixture in semi-sealed containers on windowsill prior to being placed on the heating pad.

7. Results

7.1. Bud Fertility

Inflorescence primordia number (IP) (see **Table 9**) varied between the different varieties but was consistently greater than 1.00. IP number was highest in Maratheftiko (2.04) and lowest in Nero D'Avola (1.14). Percent PBN varied from 0-20 %, with the highest value obtained in Shiraz. Notably, the lowest PBN values were obtained for the Cypriot varieties, especially Xynisteri.

Table 9. Average number of inflorescence primordia, incidence of bud necrosis and average inflorescence primordia area in the primary and secondary buds of the seven V. vinifera cultivars investigated, shown along the fruiting cane at node positions 3 to 7.

Variety	Measurement	Node				
		3	**4**	**5**	**6**	**7**
Shiraz	IP number	1.75 ^{ab}	1.75 ^{ab}	2.00 ^{a}	2.00 ^{a}	1.75 ^{abc}
	PBN (%)	17	20	3	4	0
	IP Area (mm^2)	0.039 ^{d}	0.040 ^{c}	0.047 ^{b}	0.055 ^{cd}	0.053 ^{bc}
Cabernet Sauvignon	Bud Fertility	1.63 ^{b}	1.63 ^{ab}	1.88 ^{ab}	1.94 ^{ab}	1.88 ^{a}
	PBN (%)	4	3	7	3	0
	IP Area (mm^2)	0.038 ^{d}	0.045 ^{c}	0.045 ^{b}	0.052 ^{d}	0.053 ^{c}
Mataro	IP number	1.59 ^{b}	1.82 ^{a}	1.82 ^{abc}	1.86 ^{abc}	1.77 ^{ab}
	PBN (%)	7	0	0	3	0
	IP Area (mm^2)	0.065 ^{c}	0.064 ^{bc}	0.060 ^{b}	0.065 ^{c}	0.072 ^{b}
Sangiovese	IP number	1.57 ^{bc}	1.71 ^{ab}	1.71 ^{abc}	1.43 ^{d}	1.57 ^{abc}
	PBN (%)	3	7	0	0	0
	IP Area (mm^2)	0.035 ^{d}	0.039 ^{c}	0.049 ^{b}	0.039 ^{d}	0.034 ^{c}
Nero D'Avola	IP number	1.14 ^{c}	1.48 ^{b}	1.48 ^{c}	1.57 ^{bcd}	1.33 ^{c}
	PBN (%)	3	5	0	4	3
	IP Area (mm^2)	0.073 ^{bc}	0.081 ^{ab}	0.084 ^{a}	0.074 ^{bc}	0.092 ^{a}
Maratheftiko	IP number	2.04 ^{a}	1.83 ^{a}	1.83 ^{abc}	1.70 ^{abcd}	1.52 ^{bc}
	PBN (%)	0	3	0	3	0
	IP Area (mm^2)	0.080 ^{ab}	0.089 ^{a}	0.087 ^{a}	0.076 ^{b}	0.075 ^{b}
Xynisteri	IP number	1.58 ^{b}	1.58 ^{ab}	1.54 ^{bc}	1.50 ^{cd}	1.50 ^{bc}
	PBN (%)	3	0	0	0	0
	IP Area (mm^2)	0.089 ^{a}	0.096 ^{a}	0.098 ^{a}	0.094 ^{a}	0.089 ^{a}

IP Number represents the number of inflorescence primordia per node and primary bud necrosis (PBN) is the percentage of necrotic buds per bud. IP Area (measured in mm^2) represents the measured area of inflorescence primordia per bud. For each parameter, different letters indicate statistical differences between the varieties at $p < 0.05$. Each data point is a mean of n = 30 samples, and the means were analysed by ANOVA using Fisher's (LSD) Analysis.

IP number of Shiraz ranged from 1.75 to 2.00, with the highest value in nodes 5 and 6. PBN in Shiraz was the highest observed among the seven varieties with 20 % PBN in node 4. Similarly, in Cabernet Sauvignon IP ranged from 1.63 to 1.94 with the highest value in node 6. IP in Mataro ranged from 1.59 to 1.86, the highest observed at node 6. Sangiovese showed the highest number of IP in nodes 4 and 5 at 1.71 and the lowest in node 6 at 1.43. Nero D'Avola showed the lowest overall IP number from 1.14 to 1.57, whilst Maratheftiko had the highest among the investigated varieties ranging from 1.52 to 2.04. In Xynisteri IP number was 1.54 to 1.58, with the lowest PBN (3 %) observed and only at node 3. The results for IP number at individual node positions per cultivar are shown in **Figures 9 and 10.**

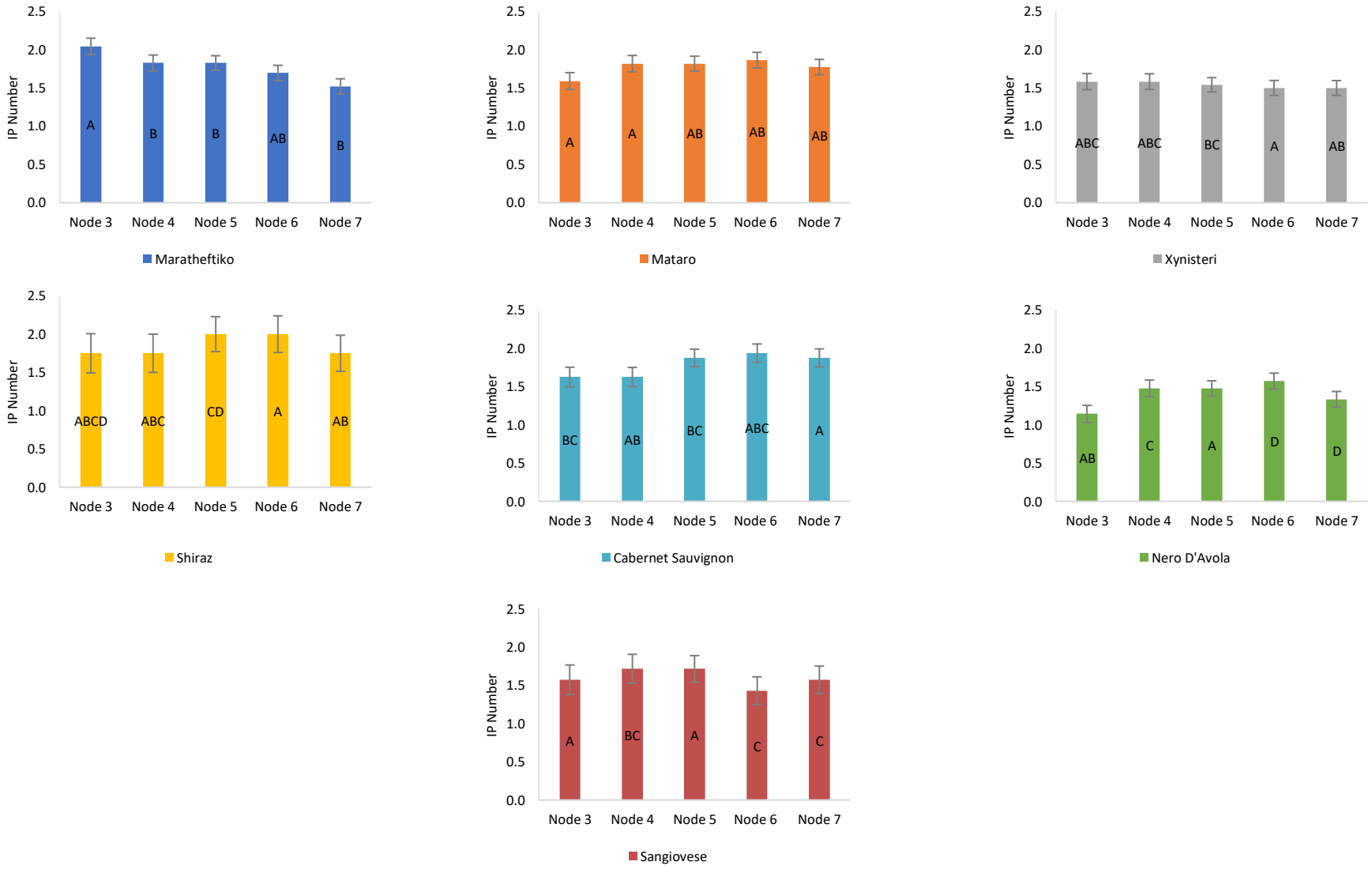

Figure 9. Bud Fertility measures for the seven cultivars investigated. Each data point is a mean of n = 30 samples separated by ANOVA using Fisher's (LSD) Analysis. Different letters indicate statistical differences at $p < 0.05$.

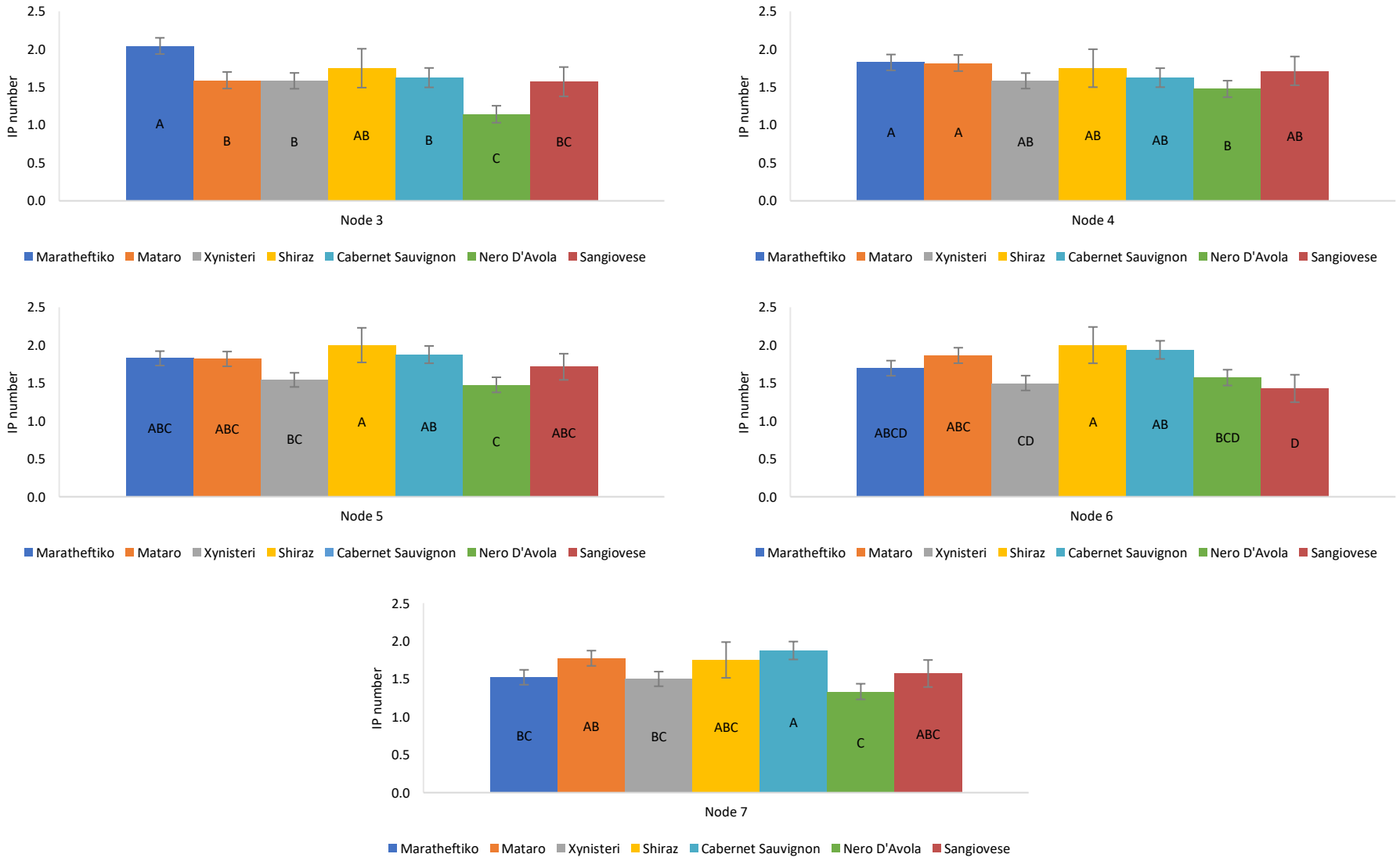

Figure 10. Bud Fertility measures for each node in the investigated cultivars. Each data point is a mean of n = 30 samples separated by ANOVA using Fisher's (LSD) Analysis. Different letters indicate statistical differences at $p < 0.05$.

7.1.1. Inflorescence Primordia Area

The values obtained for the average IP cross-sectional area (see **Table 9**) varied between the different varieties. Notably, the highest IP area values were obtained for the Cypriot cultivars Xynisteri and Maratheftiko as well as Nero D'Avola. Conversely, the lowest IP area values were observed for varieties Sangiovese and Cabernet Sauvignon.

The IP cross-sectional area of Shiraz ranged from 0.039 to 0.055 mm^2, with the highest value in node 6. In Cabernet Sauvignon the IP cross-sectional area ranged from 0.038 to 0.053 mm^2 with the highest value in node 7. The IP cross-sectional area in Mataro ranged from 0.060 to 0.072 mm^2, the highest observed at node 7. Sangiovese showed the lowest overall IP cross-sectional area in node 7 at 0.035 mm^2 whilst, the highest value observed for this variety was in node 5 at 0.049 mm^2. Nero D'Avola was amongst the highest in terms of IP cross-sectional area ranging from 0.073 mm^2 at node 3 to 0.092 mm^2 at node 7. In Maratheftiko the IP cross-sectional area ranged from 0.075 to 0.089 mm^2 whilst, Xynisteri had the highest one among the investigated varieties ranging from 0.089 to0.098 mm^2. The results for average IP cross-sectional area at individual node positions per cultivar are shown in **Figures 12 and 13**.

The difference in the IP cross-sectional area was confirmed visually during the analysis of each individual compound bud and is shown in **Figure 11**.

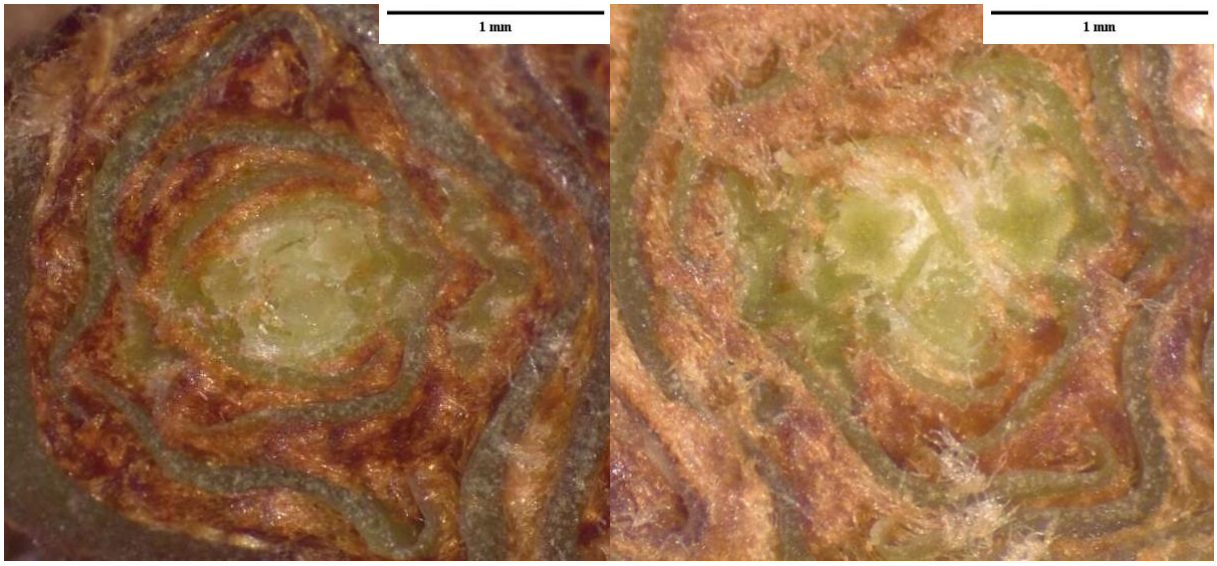

Figure 11. Transverse section through a compound bud of Sangiovese (left) and Xynisteri (right) showing a healthy primary bud with two IP clearly circled. Magnification was x20 for both dissections, clearly displaying the differences in IP cross-sectional area at the cultivar level.

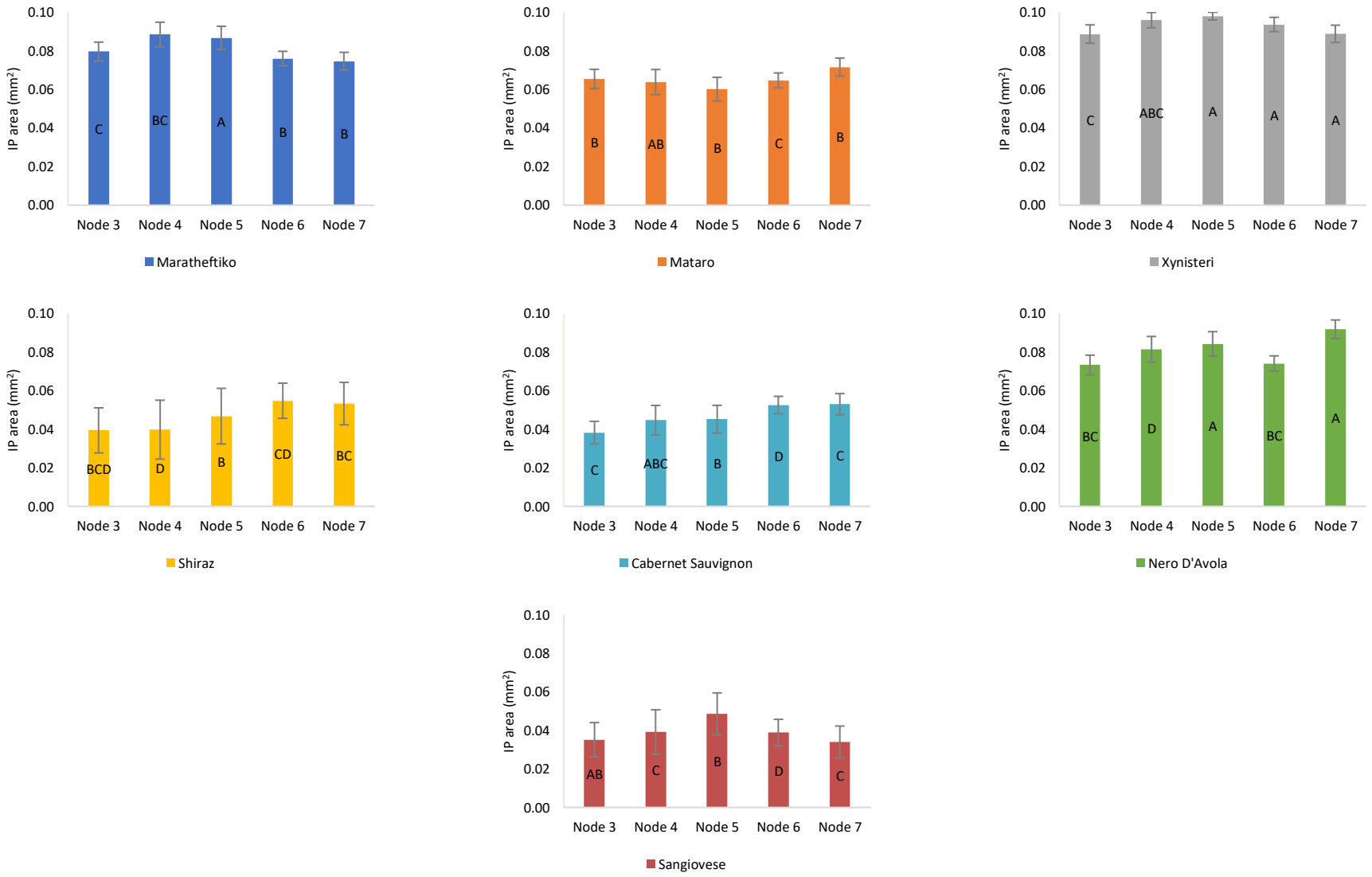

Figure 12. IP Area measures for the seven cultivars investigated. Each data point is a mean of n = 30 samples separated by ANOVA using Fisher's (LSD) Analysis. Different letters indicate statistical differences at $p < 0.05$.

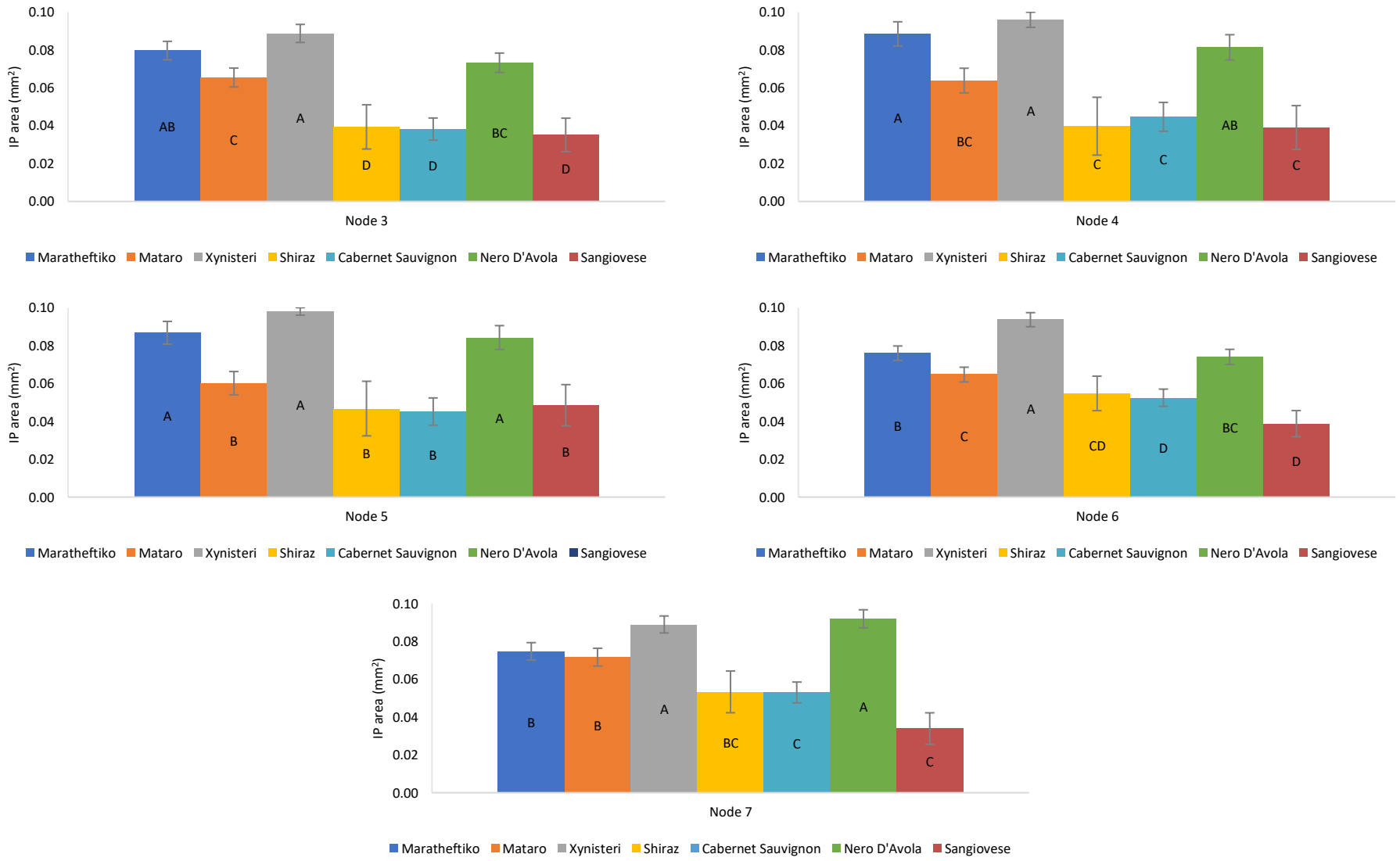

Figure 13. IP Area measures for each node in the investigated cultivars. Each data point is a mean of n = 30 samples separated by ANOVA using Fisher's (LSD) Analysis. Different letters indicate statistical differences at $p < 0.05$.

In tandem with IP cross-sectional area analysis per variety, internode length and cane diameter – measured between the third and fourth node consistently – were related to one another as shown in **Figure 14.** The correlation plot indicates an inverse correlation between IP area and internode distance for all cultivars, as internode distance increases, IP area decreases.

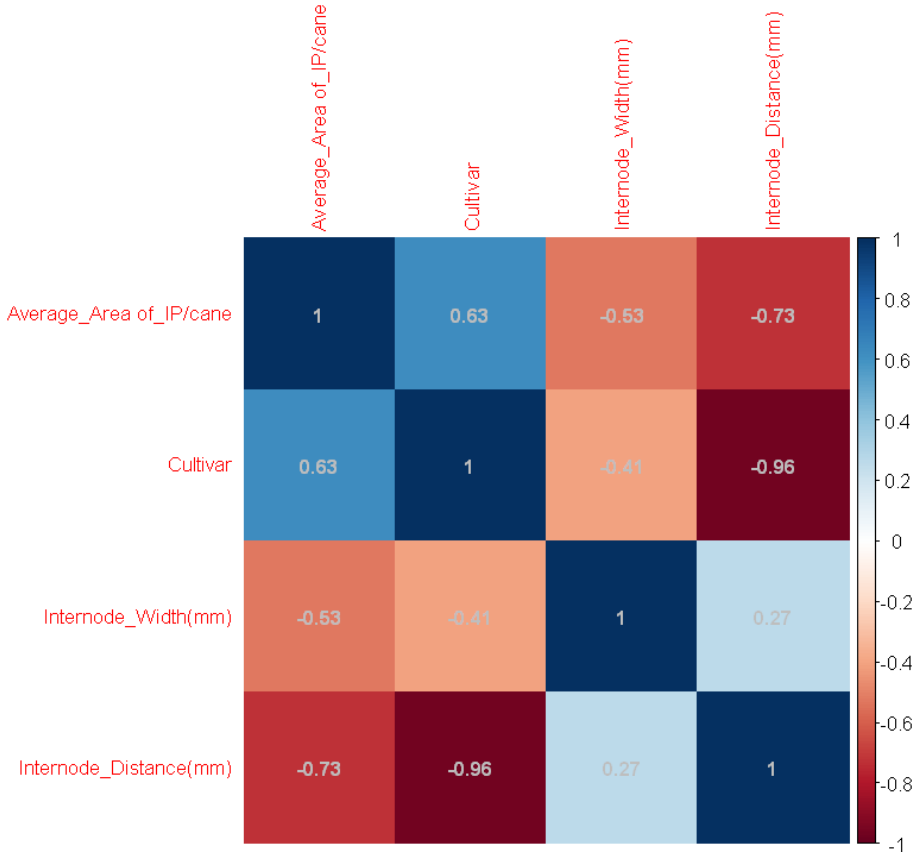

Figure 14. The correlation plot relating, cultivar, average IP cross-sectional area, internode width measured in mm and internode distance measured in mm.

To ensure the validity of the Fisher LSD test, an ANOVA test was conducted in RStudio on the number and area of IP per node for each cultivar. The results summarised in **Table 10** confirm the hypothesis that there is significant difference between the IP means. A two-tail prop test was carried out consequently to further confirm the result obtained in the ANOVA. Low *p*-values confirm the significant difference of the IP number and area between different cultivars.

Table 10. ANOVA and two-tail prop test applied to IP Number and Area measures.

Factor	**D.f.**	**F value**	***p*-value**
		ANOVA	
IP number	6	9.21	6.36e-09 ***
IP Area	6	14.96	3.77e-14 ***
		Two-tail prop test	
IP number	6	n/a	0.002712
IP Area	6	n/a	0.00483

Figure 15 summarises the results of the Bud Fertility analysis over cultivar and node, showing the trends discussed in this section.

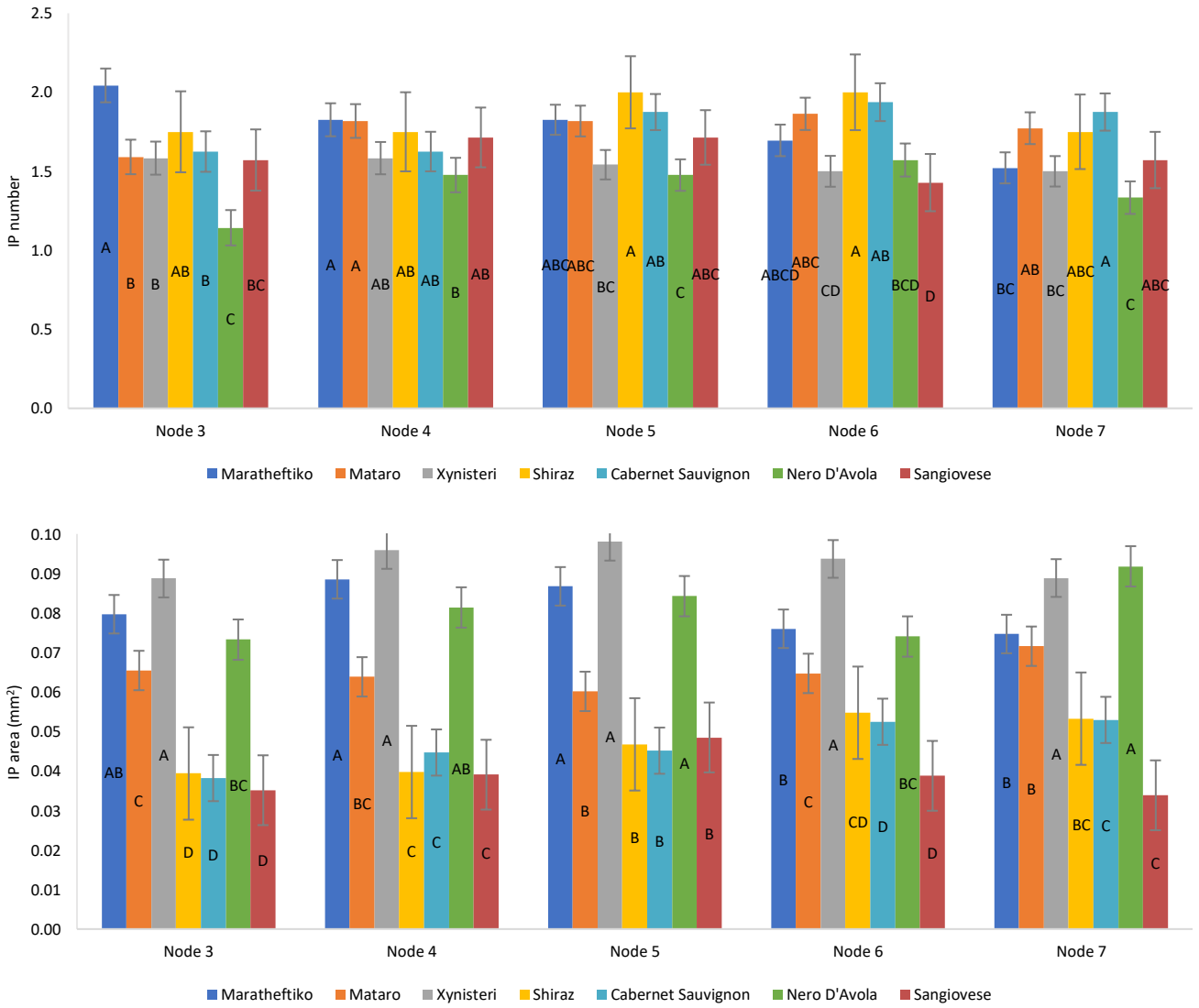

Figure 15. Total Bud Fertility and IP Area measures for the seven cultivars investigated at each node. Each data point is a mean of n = 30 samples separated by ANOVA using Fisher's (LSD) Analysis. Bars in scatter plots (higher) indicate standard error. Different letters in bar charts (lower) indicate statistical differences at $p < 0.05$.

7.2. Propagation by seed and seed germination

Results showed that seed germination was primarily under the control of stratification time, air temperature and thus, seed endodormancy. The lowest germination rate, corresponding to 0 %, was obtained in the seeds stratified for 0, 14, 28 and 42 days. The highest germination rate, corresponding to 60 % was observed in the seeds stratified for 56 days and over (**Tables 12 and 13**).

As it can be observed in **Table 11** multiple parameters seemed to have no significant effect on seed germination rate of Maratheftiko. The effects of seed treatment such as, washing the seeds with either cold or hot water, soaking the seeds in $NaHCO_3$ for 10 mins had no significant effect on the seed germination rate. Similarly, pre-stratification treatments such as drying the seeds overnight or keeping them wet did not promote seed germination. Stratification time below 56 days had no significant impact on germination rate, as well as any pre-germination treatment including soaking the seeds in distilled water for either 24 or 48 hours, lightly smashing them with a pestle and mortar or cutting the beak with a sharp razor. Regarding the germination media, filter paper, filter paper with cotton, blotting tissue, sterilized sand and cotton were all tested and found to have no impact on germination rate.

The main treatments and conditions that seemed to impact and favour germination, were a stratification period of over 56 days, the use of sodium hypochlorite or distilled water and using a seed raising mixture as the germination medium. Specifically, treating the seeds following extraction from the grapes with 2 % NaOCl, followed by a period of 84 or 98 days of stratification seemed to favour the germination of Maratheftiko seeds (**Tables 12 and 13**). Similarly, washing or soaking the seeds with distilled water followed by a period of 84 or 98 days seemed to increase the germination rate. According to the results obtained, the optimum protocol for germinating Maratheftiko seeds can be said to be two: 1) manual extraction + soak in NaOCl for 10 mins + distilled water wash + dry over few hrs + 84 d stratification + planted in seed raising mixture and 2) manual extraction + wash with distilled water + dry over few hrs + 98 d stratification + planted in seed raising mixture.

Table 11. The different experimental parameters tested to evaluate the optimum seeding protocol for Maratheftiko (*V. vinifera*) seeds.

Experimental Parameters						
Sample	*Grape Treatment*	*Seed Treatment*	*Pre-stratification Treatment*	*Stratification*	*Pre-germination Treatment*	*Germination Medium*
MSW	Manual seed extraction	Soak in DW x 10 mins	Dry over few hrs Dry over weekend Keep wet	Stratification for 14, 28, 42, 56, 70, 84, 90 d	No treatment Soak in DW for 24 h Soak in DW for 48h Smash with pestle & mortar Beak-cutting with razor	Filter paper Filter paper + cotton Blotting tissue Sterilized sand Cotton Seed Raising Mixture
MSB	Manual extraction	Soak in $NaHCO_3$ x 10 mins	Dry over few hrs Dry over weekend Keep wet	Stratification for 14, 28, 42, 56, 70, 84, 90 d	No treatment Soak in DW for 24 h Soak in DW for 48h Smash with pestle & mortar Beak-cutting with razor	Filter paper Filter paper + cotton Blotting tissue Sterilized sand Cotton Seed Raising Mixture
MWW	Manual extraction	Wash with DW	Dry over few hrs Dry over weekend Keep wet	Stratification for 14, 28, 42, 56, 70, 84, 90 d	No treatment Soak in DW for 24 h Soak in DW for 48h Smash with pestle & mortar Beak-cutting with razor	Filter paper Filter paper + cotton Blotting tissue Sterilized sand Cotton Seed Raising Mixture
MCW	Manual extraction	Wash with cold water	Dry over few hrs Dry over weekend Keep wet	Stratification for 14, 28, 42, 56, 70, 84, 90 d	No treatment Soak in DW for 24 h Soak in DW for 48h Smash with pestle & mortar Beak-cutting with razor	Filter paper Filter paper + cotton Blotting tissue Sterilized sand Cotton Seed Raising Mixture
MHW	Manual extraction	Wash with hot water	Dry over few hrs Dry over weekend Keep wet	Stratification for 14, 28, 42, 56, 70, 84, 90 d	No treatment Soak in DW for 24 h Soak in DW for 48h Smash with pestle & mortar Beak-cutting with razor	Filter paper Filter paper + cotton Blotting tissue Sterilized sand Cotton Seed Raising Mixture
MSH	Manual extraction	Soak in $NaHCO_3$ x 10 mins	Dry over few hrs Dry over weekend Keep wet	Stratification for 14, 28, 42, 56, 70, 84, 90 d	No treatment Soak in DW for 24 h Soak in DW for 48h Smash with pestle & mortar Beak-cutting with razor	Filter paper Filter paper + cotton Blotting tissue Sterilized sand Cotton Seed Raising Mixture

DW – Distilled Water, **MSW** – Maratheftiko seeds treated with distilled water, **MSB** – Maratheftiko seeds treated with sodium bicarbonate, **MWW** – Maratheftiko seeds washed with distilled water, **MCW** – Maratheftiko seeds washed with cold water, **MHW** – Maratheftiko seeds washed with hot water, **MSH** – Maratheftiko seeds treate**d** with sodium hypochlorite.

Table 12. The different experimental parameters tested, to evaluate the optimum seeding protocol for Maratheftiko (*V. vinifera*) seeds, with a germination rate of greater than 0 %.

Experimental Parameters							
Sample	*Grape Treatment*	*Seed Treatment*	*Pre-stratification Treatment*	*Stratification*	*Pre-germination Treatment*	*Germination Medium*	*Success*
1-MSW98	Manual extraction	Soak in distilled water x 10 mins	Dry over few hrs	Stratification for 98 d	No treatment	Seed Raising Mixture	2 %
2-MSW98	Manual extraction	Soak in distilled water x 10 mins	Dry over few hrs	Stratification for 98 d	No treatment	Seed Raising Mixture	20 %
1-MWW56	Manual extraction	Wash with distilled water	Dry over few hrs	Stratification for 56 d	No treatment	Seed Raising Mixture	20 %
2-MWW56	Manual extraction	Wash with distilled water	Dry over few hrs	Stratification for 56 d	No treatment	Seed Raising Mixture	3 %
1-MWW56 - DOW	Manual extraction	Wash with distilled water	Dry over weekend	Stratification for 56 d	No treatment	Seed Raising Mixture	11 %
2-MWW56 - DOW	Manual extraction	Wash with distilled water	Dry over weekend	Stratification for 56 d	No treatment	Seed Raising Mixture	20 %
MCW98	Manual extraction	Wash with cold water	Dry over few hrs	Stratification for 98 d	No treatment	Seed Raising Mixture	5 %
MHW98	Manual extraction	Wash with hot water	Dry over few hrs	Stratification for 98 d	No treatment	Seed Raising Mixture	10 %
MWW56 – BC	Manual extraction	Wash with distilled water	Dry over few hrs	Stratification for 56 d	Beak-cutting	Seed Raising Mixture	3 %
1-MSH84	Manual extraction	Soak in $NaHCO_3$ x 10 mins	Dry over few hrs	Stratification for 84 d	No treatment	Seed Raising Mixture	25 %
2-MSH84	Manual extraction	Soak in $NaHCO_3$ x 10 mins	Dry over few hrs	Stratification for 84 d	No treatment	Seed Raising Mixture	40 %
1-MSH98	Manual extraction	Soak in $NaHCO_3$ x 10 mins	Dry over few hrs	Stratification for 98 d	No treatment	Seed Raising Mixture	2 %
2-MSH98	Manual extraction	Soak in $NaHCO_3$ x 10 mins	Dry over few hrs	Stratification for 98 d	No treatment	Seed Raising Mixture	60 %

MSW – Maratheftiko seeds treated with distilled water, **MSB** – Maratheftiko seeds treated with sodium bicarbonate, **MWW** – Maratheftiko seeds washed with distilled water, **MCW** – Maratheftiko seeds washed with cold water, **MHW** – Maratheftiko seeds washed with hot water, **MSH** – Maratheftiko seeds treate**d** with sodium hypochlorite. Numbers **1** or **2** before the sample name denote trials in different containers. Numbers **56**, **84** and **98** after the sample name denote the period of stratification in days.

Table 13. The successfully germinated trials with dates of the time seeds were collected, stratified, planted and of seed germination, seedling emergence, growth of first true leaf, transplanting and the seedling stem colour.

Trial	Collected	Stratification period	Planted	Germination	Emergence	First True Leaf	Transplanting	Stem Colour/ Observations
1-MSW98	15/05/22	98 d	30/08	02/09	05/09	09/09	09/09	green
2-MSW98	15/05/22	98 d	30/08	16/09	19/09	23/09	23/09	red stem
2-MSW98	15/05/22	98 d	30/08	16/09	19/09	23/09	23/09	green stem
2-MSW98	15/05/22	98 d	30/08	16/09	23/09	26/09	26/09	red stem
2-MSW98	15/05/22	98 d	30/08	16/09	23/09	26/09	26/09	red stem
2-MSW98	15/05/22	98 d	30/08	16/09	23/09	26/09	26/09	green stem
1-MWW56	15/05/22	56 d	12/07	25/07	28/07	06/08	11/08	green stem turned red
2-MWW56	28/06/22	56 d	02/09	13/09	19/09	21/09	23/09	green stem
1-MWW56 – DOW	28/06/22	56 d	30/08	16/09	23/09	26/09	26/09	green stem
2-MWW56 – DOW	28/06/22	56 d	02/09	16/09	21/09	23/09	23/09	green/white stem
MCW98	15/05/22	98 d	30/08	16/09	19/09	21/09	23/09	green stem large cotyledons
MHW98	15/05/22	98 d	30/08	16/09	19/09	23/09	23/09	green stem
MHW98	15/05/22	98 d	30/08	16/09	19/09	26/09	26/09	red stem
MWW56 – BC	28/06/22	56 d	02/09	16/09	21/09	26/09	26/09	red stem
1-MSH84	15/05/22	84 d	09/08	02/09	05/09	13/09	13/09	green stem
1-MSH84	15/05/22	84 d	09/08	02/09	05/09	13/09	13/09	green stem
1-MSH84	15/05/22	84 d	09/08	06/09	09/09	17/09	17/09	red stem
2-MSH84	15/05/22	84 d	09/08	09/09	12/09	13/09	13/09	red stem turned green
2-MSH84	15/05/22	84 d	09/08	09/09	13/09	16/09	16/09	red stem
2-MSH84	15/05/22	84 d	09/08	09/09	13/09	-	-	dehydrated over weekend
1-MSH98	15/05/22	98 d	30/08	16/09	21/09	23/09	23/09	red stem one cotyledon
2-MSH98	15/05/22	98 d	30/08	16/09	21/09	23/09	23/09	red stem one cotyledon

MSW – Maratheftiko seeds treated with distilled water, **MSB** – Maratheftiko seeds treated with sodium bicarbonate, **MWW** – Maratheftiko seeds washed with distilled water, **MCW** – Maratheftiko seeds washed with cold water, **MHW** – Maratheftiko seeds washed with hot water, **MSH** – Maratheftiko seeds treated with sodium hypochlorite. Numbers **1** or **2** before the sample name denote trials in different containers. Numbers **56**, **84** and **98** after the sample name denote the period of stratification in days.

On average, germination was observed after 3-4 weeks of planting the seeds, followed by a growth period of three days until emergence was observed with the seedling developing two cotyledons (**Figure 16**). The first true leaf would usually start developing following three days of the emergence time and would then continue growing until fully developed a week after. Post third leaf development, growth slowed down while the seedling is developing a stronger root system. In a period of one month the seedling developed at least four true leaves and required transplanting in a bigger pot as the root system (seen in **Figure 17**) exceeded the capacity of the first pot. Notably, some of the seedlings would emerge encapsulated by the seed coat which would shed two days after and different phenotypes were observed, specifically in stem colour (**Figure 18**).

Figure 16. The developmental stages of Maratheftiko seeds (*V. vinifera*) sequentially depicting germination, seedling emergence and cotyledon establishment and growth of first, second, third, fourth and fifth real leaf.

Figure 17. The root development of a Maratheftiko (*V. vinifera*) seedling following one month of growth. The young, white frame roots and the smaller, permanent roots ranged in length from 5 to 45 cm.

Figure 18. The seed emerging with the seed coat attached to the cotyledons, usually shed after three growth days (left). A comparison between the two different stem colours observed, red (middle) and green (right).

In addition to the Maratheftiko seeds, a Sémillon trial was established simultaneously to compare the development of the two varieties**. Figure 19** depicts the growth of the two seedlings following the period of one month. Although, the pictures were taken at the same point Maratheftiko is double in size compared to Sémillon whilst the latter has developed two true leaves and the former is in the process of developing its fourth true leaf.

Figure 19. A comparison between the development of a Sémillon (*V. vinifera*) seedling and a Maratheftiko (*V. vinifera*) seedling after one month of growth. The seeds were planted concurrently, and the germination occurred in the same week.

8. Discussion

8.1. Bud Fertility

In the present study the bud fertility of seven *V. vinifera* cultivars was investigated as well as the cross-sectional IP area and the cane parameters internode length and cane diameter. The number of IP related to bud fertility, was found be significantly different in terms of different varieties, a trend shown by IP area as well, with a clear difference between individual node positions.

The fertility parameters examined are indicators of bud fertility, more specifically potential bud fertility measured prior to anthesis, which is impacted by cultivar-dependent responses as well as environmental conditions (Smart, 1985). To minimise the influence of exogenous factors, the cane material was selected from a single-vineyard site where the grapevines are exposed to comparable environmental conditions and are subjected to consistent vineyard management practices. Environmental conditions such as temperature, light intensity and water availability exert significant influence on IP initiation and development. Temperature in the previous growing season ranged from 16 to 30 °C (with optimal temperatures in the range 20–35 °C) promoting the differentiation of the IP in the primary and secondary buds (Monteiro et al., 2021). Both high temperature and high light intensity encourage cytokinin synthesis which favour the UP differentiation into IP instead of tendril formation (Jackson, 2014; Monteiro et al., 2021). Pruning level, which can regulate the number of potential fertile buds (Feitosa et al., 2018) was carried out consistently at 8 nodes/cane for all varieties assessed. In view of the genetic control exerted on the development and induction of IP, the significant difference in bud fertility values reported here can be said to be primarily cultivar dependent (Carmona et al., 2007a, 2008, 2006).

8.1.1. Fertility parameters for each variety

Bud fertility and consequently fruitfulness can be lower at the basal node positions 1 to 3, and it increases successively along the shoot from nodes 4 to 7, to decline from node 8 onwards. The spatial distribution of winter bud fruitfulness is cultivar dependent (Sánchez & Dokoozlian, 2005; Sommer et al., 2000) and further influenced by the trellis system. Shiraz, Cabernet Sauvignon, Mataro and Nero D'Avola demonstrated such spatial distribution with the highest bud fertility observed in nodes 5 and 6. Conversely, PBN incidence tended to be highest in basal node 3 and medium node 4 (Dry & Coombe, 1994; Kavoosi et al., 2013; Rawnsley & Collins, 2005; Ziv et al., 1981). In the present study Maratheftiko and Xynisteri demonstrated a higher bud fertility in the basal bud at node position 3 which consequently declined over node positions 4 to 7. The findings of this study can be supported by repeating the experiment in winter 2023 (not possible in this

project due to time constraints), as the Cypriot varieties have not been investigated for bud fertility prior to this study.

The bud fertility along the cane is a decisive factor in the pruning system adopted in the vineyard; cultivars with low fertility of basal count buds are well-suited to cane pruning and conversely, cultivars with medium to high basal bud fertility are suitable for spur pruning. Implementing one pruning method over the other, depending on the position of the most fertile buds, productivity can be increased via the ensuing increase in the IP number and fruitfulness. (Meneguzzi et al., 2020; Srinivasan & Mullins, 1981). This study suggests that the Cypriot cultivars may be more suited to spur pruning over cane pruning with the potential for increased productivity.

Shiraz and Cabernet Sauvignon are internationally grown cultivars which have shown great adaptability in various pedo-climatic conditions as well as different vineyard management operations – pruning, nutrition, irrigation and soil management (Wohlfahrt et al., 2019). However, multiple studies report these varieties to be susceptible to PBN (Rawnsley & Collins, 2005; Sánchez & Dokoozlian, 2005; Vasudevan et al., 1998; Wolf & Kay Warren, 1995), an observation confirmed in this study as these two varieties showed the highest incidence of PBN out of the seven cultivars. In particular, the results for Shiraz in the present study agree with previous research, in that PBN incidence was considerably higher in the basal nodes (Dry & Coombe, 1994; Morrison & Iodi, 1990). The remaining varieties, Sangiovese, Nero D'Avola, Mataro, Xynisteri and Maratheftiko have not been investigated sufficiently to elucidate the PBN incidence observed.

For all seven cultivars the bud fertility in primary buds was higher than that of secondary buds, the latter exhibiting less development and smaller IP than primary buds; consistent with what is reported in other studies (May, 2000; Rawnsley & Collins, 2005; Sánchez & Dokoozlian, 2005; Srinivasan & Mullins, 1981).

8.1.2. Cane parameters and IP cross-sectional area

The cane parameters measured, cane diameter and internode length, are often used as shoot vigour indicators and are dependent on the environmental conditions of the previous growing season as well as the cultivar response (Smart, 1985). Excess shoot vigour, manifested as increased cane diameter and internode length is associated with a high incidence of PBN and thus lower bud fertility (Dry & Coombe, 1994; Wolf & Kay Warren, 1995; Ziv et al., 1981). However, in this study no relation was observed between shoot vigour and PBN incidence. Instead, an inverse relation between internode distance (and width) against the IP cross-sectional area was observed for all cultivars. As a result, the viticultural practices adopted in the vineyard to control shoot vigour can impact bud fertility by impacting IP size; some of these practices include delayed

winter pruning, shoot thinning for more efficient light penetration in the foliar canopy. In particular shoot thinning, as a result of increasing light interception in the bud zone (Collins et al., 2020), increases carbohydrate reserves in bud tissues or cane tissues – xylem and phloem – utilized during inflorescence development and formation.

8.1.3. Cypriot cultivars / Maratheftiko

The Cypriot cultivars show promising results in this investigation, with Maratheftiko reporting the highest value obtained for bud fertility and Xynisteri having the highest overall cross-sectional IP area, followed closely by Maratheftiko. Maratheftiko, which is also locally known as Vamvakada due to the cotton-like coating on the back of Maratheftiko leaves; Vamvaki (βαμβάκι) > cotton, is distinct from the currently cultivated *V. vinifera* grape varieties. Unlike modern, domesticated cultivars Maratheftiko has a low self-pollination rate due to its stumpy male structures. Although the flowers of Maratheftiko have well-developed pistils, the stamens are reflexed, a fact which hampers its cultivation. Attempts to overcome this have been carried out by viticulturists whereby, Maratheftiko is co-planted in alternating rows with other varieties such as Xynisteri, to achieve fertilization and fruit development. Despite the co-plantation, fertilization rates remain low resulting in poorly developed bunches affected by millerandage (Grigoriou et al., 2020).

Both, the bud fertility and IP area of Maratheftiko measured in this investigation are amongst the highest compared to the other six cultivars (Collins & Rawnsley, 2005; Cox et al., 2012; Wohlfahrt et al., 2019). It is likely that the higher fertility parameters observed for this cultivar correlate to the low fertilization rate observed; the plant developed this response mechanism to compensate for the lower actual fruitfulness (Grigoriou et al., 2020). Further studies into the actual bud fruitfulness measured during anthesis and the bud fertility in the consequent growing season of 2023 can consolidate this hypothesis.

8.2. Propagation by seed and seed germination

The key factors influencing seed germination are endogenous factors, seed endodormancy and exogenous factors, temperature and humidity (Kawano et al., 2020; Lifang et al., 2020). Depending on the morphological structure of different varieties and the growth characteristics, different treatments may be more effective in promoting seed germination and thus, a universal protocol for growing grapevines from seeds is not yet applicable (Wang et al., 2022). Different varieties have different seed maturity, seed coat thickness, seed size, content of endogenous hormones and water as well as different stratification requirements to overcome dormancy (Wang et al., 2022; Zhang et al., 2009). This study demonstrated the most effective protocol for germinating the Maratheftiko seeds collected for this experiment.

In tandem with stratification for at least 2 months, treating the seeds with NaOCl was required to promote germination. Although, chemical treatments can alter the seed metabolic mechanism, overcome endodormancy and promote seed germination (Parera & Cantliffe, 2010) these chemical compounds are usually plant growth regulators such as, GA. In this case the chemical used, NaOCl acted as facilitator by overcoming the mechanical barrier to germination, the seed coat. The seed coat of Maratheftiko is considerably thick and tough whereas, humidity is low during the stratification period which hinders seed expansion resulting in a low germination rate (Wang et al., 2022; Xie et al., 2012). Sodium hypochlorite breaks down the seed coat which facilitates the seedling penetration when planted at the right temperature and humidity. In a similar fashion, using the seed raising mixture instead of the other germination media allowed for the immersion of the seeds in the mixture and thus, the seeds were maintained at the appropriate humidity and temperature level without drying out. Conversely, the other media were unsuitable as the seeds would dry out or develop fungal growth both of which seemed to hinder germination.

Although soaking the seeds with sodium hypochlorite showed an enhanced permeability of the seedling by reducing the seed coat barrier, mechanical reduction of the barrier via beak-cutting and lightly smashing the seeds showed contrasting results. Beak-cutting with a sharp razor or lightly smashing the seeds with a pestle and mortar is performed to release the embryo from the testa comprising of the outer and inner integument (**Figure 20**). However, depending on the positioning of the seed embryo and the thickness of the testa beak-cutting and smashing the seeds may have the opposite effect of reducing germination rate by damaging the embryo (Wu & Li, 1999). The germination process for Maratheftiko seeds averaged 3 weeks for all successful treatments and ranged from 2 to 5 weeks. Under this germination duration seeds are susceptible to fungal attacks and thus, it can be argued that wounding the seed through mechanical means exposes the embryo to fungal attacks under prolonged germination conditions. This was

consistent with the visual observations for smashed seeds or with a cut beak that had visible fungal growth particularly where exposed.

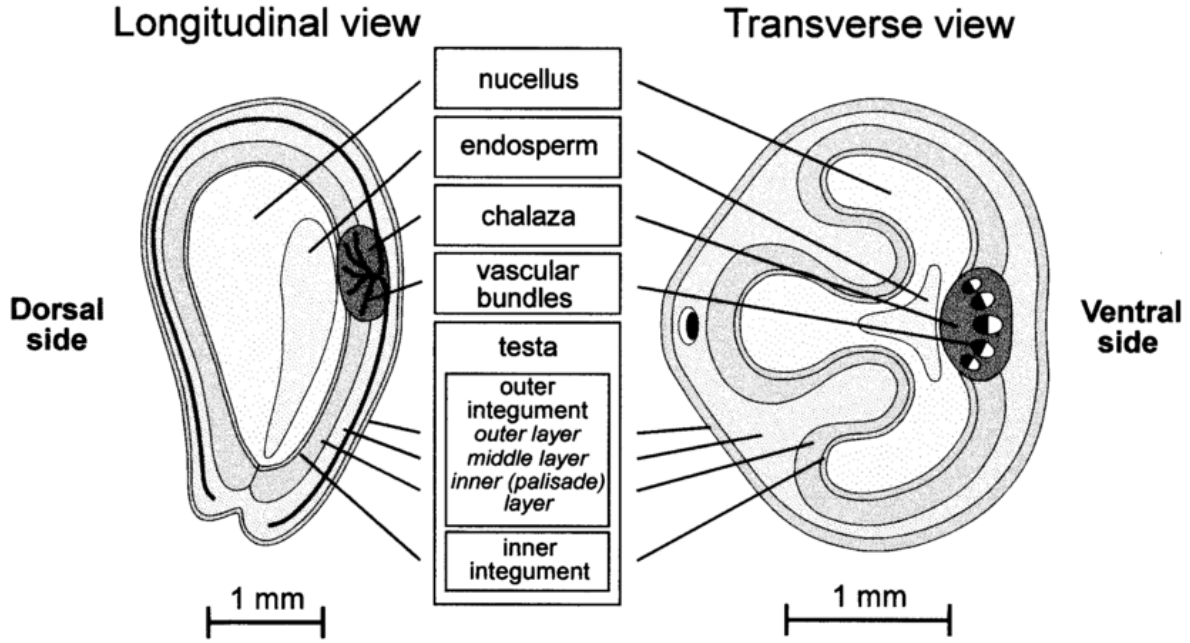

Figure 20. Diagrammatic structure of a grape seed 28 days after flowering (Walker et al., 1999).

Based on the findings of this study there are two optimum protocols that can be undertaken for achieving a greater germination rate for Maratheftiko seeds which are 1) manual extraction + soak in NaOCl for 10 mins + distilled water wash + dry over few hrs + 84 d stratification + planted in seed raising mixture and 2) manual extraction + wash with distilled water + dry over few hrs + 98 d stratification + planted in seed raising mixture.

8.2.1. Seedling Phenology

Seed germination is the first life stage where plants start perceiving external environmental conditions. It is also one of the most crucial stages of plant growth in terms of sensitivity to environment changes (Chen et al., 2003). Therefore, germination as well as germination conditions affect the seedling emergence and consequent growth and vigour (Ma et al., 2014), as seedling growth is key in the plant development and it is also one of the most vulnerable plant life stages it can drive seed screening and selection of high-quality seedlings based on seedling vigour and the phenology (Chai, 2005). Fully developed Maratheftiko grapevines are characterised by a white, cotton-like growth on the tip of the shoots. The young leaves of Maratheftiko are green-brown in colour and rough in texture, while the older leaves are dark green with trademark red veins at the tips. The grape bunches are medium-sized, with normally small, black berries – unless affected by millerandage – which give rise to the deeply-coloured wines. In this study, the seed

variability of Maratheftiko was investigated by observing the different anatomical characteristics in Maratheftiko seedlings. The most discerning difference was the stem colour, approximately 1:1 green to red-coloured stems were observed. Unlike fully-grown grapevines where shoot-redness is often associated with potassium deficiency, in seedlings the stem colour is associated with anthocyanin development, a trademark characteristic of Maratheftiko being its deeply pigmented grape skin.

A side-by-side comparison with germinated Sémillon seeds showed different growth rates in both stems and roots. Sémillon is considered a moderately vigorous, highly productive grape variety (Greer et al., 2010) but after a one-month growth Maratheftiko was double in size with a highly advanced root system; the roots of Maratheftiko ranged in length from 5 to 45 cm whereas, Sémillon had not produced roots thick and long enough to measure without damaging the fragile root-system. This observation is supported by previous findings for Cypriot varieties, where they are characterised as drought-tolerant and potentially could be used as adaptation measures against climate change (Chrysargyris et al., 2018; Constantinou et al., 2017; Constantinou et al., 2018; Copper et al., 2020, 2022). Further seed variability screening and comparison with other varieties could bring more insights into the development as well as the phenology of this variety and its possible adaptation. Similarly, the vegetative part of Maratheftiko was double the size of Sémillon, following a month's growth although both varieties had three true leaves. The stem-architecture of the two varieties was also considerably different; Maratheftiko showed an alternate phyllotaxis whereas, Sémillon showed a more spiral-phyllotaxis commonly observed for young vines. (Carmona et al., 2007b).

The germination success is also dependent on the ripening of the grapevines i.e., early-ripening varieties have a shorter growth cycle suggesting that the embryo development is incomplete and thus easily confined by the hard seed coat (Pan et al., 2010). Maratheftiko is a medium-ripening variety however, at harvest the berry development was heterogeneous whereby a single bunch contained both fully developed and under-developed berries. This could further explain the lower germination rate obtained for some of the trials.

9. Conclusion

The ever-prevalent effects of climate change in the wine growing regions of the world just like the Mediterranean Europe or Australia are calling for adaptation measures to preserve said regions and promote more sustainable viticultural practices and wine production. One such adaptation being explored in recent years, is the cultivation of cultivars acclimatised to more extreme climatic conditions, such as the indigenous cultivars from the eastern Mediterranean island of Cyprus, Maratheftiko and Xynisteri. Although, preliminary studies have been conducted with these grape varieties, reporting the phenolic profile, consumer perception as well as others, there is a discrepancy between the performance of these varieties in field. As part of improving the understanding and knowledge of the performance of these varieties two discrete experiments were carried out; the first one investigated the bud fertility of compound buds from Xynisteri and Maratheftiko canes and compared them to international varieties such as Shiraz, Cabernet Sauvignon, Sangiovese, Nero D'Avola and Mataro and the second one investigated the seed germination and development success of Maratheftiko seeds and compared them to the cultivar Sémillon.

For varieties Shiraz and Cabernet Sauvignon, bud fertility observed in this investigation was consistent with existing studies and supports previous findings. Interestingly, both Maratheftiko and Xynisteri demonstrated an increased basal fertility in node 3 and Maratheftiko in particular had the highest bud fertility amongst the varieties investigated. Xynisteri showed the highest IP cross-sectional area out of the varieties investigated. For Maratheftiko, this could be a response mechanism developed by the plan to overcome the low self-pollination rate due to the structure of its male flower organs. Notably, the data collected spanned half a growing season from April to October, due to time constraints and the entry restrictions to enter Australia, placed in order to limit the spread of covid-19. Nonetheless, extending this investigation by measuring fertility in the growing season of 2023 could provide more insight into the bud fertility of the Cypriot cultivars as well as the reasons behind the measured fertility, whether this is a cultivar-driven response or a pedo-climatic driven response.

The characterisation of Maratheftiko seeds although, qualitative provided some rudimentary insight into the germination of this variety and how to maximise the germination rate and consequent seedling development. The seeds require a minimum stratification duration of 56 days following their extraction and should be air dried prior to fridge storage. The optimum pre-germination treatment involves washing them with distilled water or soaking them in sodium hypochlorite to decompose the seed coat and facilitate the seedling penetration. Germination in seed raising mixture was preferred and usually takes 3-4 weeks for the seedling to emerge. The

timing of planting the seeds is also key as germination was observed starting September, the first month of spring in Australia.

10. Future Outlook

This research is directly related to climate change adaptation measures, and it sets the introductory ground for seed germination and bud fertility as well as the factors influencing them, specifically for the Cypriot autochthonous varieties Xynisteri and Maratheftiko. However, as it was undertaken as part of a Master's Thesis which constrained the timeframe of the project, the results in particular about seeding are of more qualitative nature.

Further research of these varieties can bring more insight into the potential bud fertility, measured as part of this study by measuring the actual fertility recorded during anthesis and inflorescence formation. A side-by-side comparison of potential and actual fertility for Maratheftiko in particular is an interesting extension point due to the cultivar's genetic morphology. Moreover, the inflorescence area measured during this investigation can be related to the bunch architecture observed at flowering as a method of bunch structure forecasting. Other possible extensions of this project could involve molecular analyses into the gene expression of the cultivars to study bud fertility more deeply and to differentiate varietal compared to other endogenous factors or environmental conditions that drive the bud fertility of these cultivars.

Regarding seed germination, an extended timeframe could allow for a deeper study of the Cypriot seeds. During vintage 2023 in Australia both Xynisteri and Maratheftiko grapes will be available for harvest; the seeds can be consequently extracted, stored in the fridge for 2-3 months which is the minimum stratification requirement and planted in spring 2023 while testing the two protocols which seemed to promote germination in this experiment. In order to elucidate the germination mechanism different air temperature or light/dark exposure can be tested to assess whether there is a thermosensitive or photosensitive component to germination. The seedlings can be monitored for growth stages and documentation of the phenological phases. Moreover, the seed propagation part of this study can be expanded by transplanting the seedlings from pots in field, growing them to carry out further studies involving vine ecology and physiology and/or vine management techniques, such as pruning.

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