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ORIGINAL ARTICLE

Screening of *Vitis vinifera* cultivars from the Grapevine Germplasm Bank of Aragon for susceptibility to *Botryosphaeria* dieback fungi

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Abstract

Grapevine trunk diseases (GTDs) are taking the forefront of winegrowers' concerns, as they cause considerable damage in vineyards not only quantitatively but also qualitatively. Furthermore, in the last three decades, an increasing incidence of the socioeconomic impact of these fungal pathologies has been observed. To date, no effective control strategies or curative treatments are available for these diseases that can replace the effectiveness offered by a series of chemical synthesis fungicides currently prohibited by European legislation. In this scenario, screening for less sensitive cultivars is regarded as a sustainable approach for GTDs management. In the study presented herein, the tolerance/susceptibility of 25 cultivars from the Grapevine Germplasm Bank of Aragon (Movera, Zaragoza, Spain) including commercial, local, or minority germplasm, was tested against two pathogens associated with *Botryosphaeria* dieback (viz. *Neofusicoccum parvum* and *Diplodia seriata*), which were inoculated in a detached cutting assay under open-air conditions. Based on lesion length development after eight months, significant differences were detected among the cultivars in the length of internal (vascular) necroses. In general terms, all cultivars were susceptible to fungal infection, but 'Macabeo' and one of the 'Garnacha Tinta' ecotypes under study (from Villanueva de Huerva, Zaragoza) would be the least susceptible white and red cultivars, respectively. On the other hand, 'Monegrina', 'Grumel', and 'Torcijón' would be among the least tolerant cultivars to fungal infection.

Keywords Black dead arm · *Botryosphaeriaceae* · Fungal pathogens · Grapevine · Natural resistance · Tolerance

Introduction

Among the different pests and diseases that compromise the viability and economic profitability of grapevine culture worldwide, grapevine trunk diseases (GTDs) cause great damage to plantations, decreasing productivity and reducing the useful life of the plant (Serra et al. 2021), with associated annual replanting costs estimated at over $1500 M (Hofstetter et al. 2012). GTDs caused by certain species of pathogenic fungi have in common that these taxa produce an internal alteration of the wood that they parasitize, producing phenomena of vascular necrosis, delayed growth, foliar symptoms, or root rot, leading to the death of the plant in an indeterminate period (from 1 to 30 years), but normally long (Luque i Font 2014). In recent years, alongside the classic pathologies associated with adult plants, a series of new phytosanitary problems have emerged, leading to the rapid decline and death of plants shortly after planting. Plantations have been observed with a non-negligible percentage of plants that do not sprout or die in the first year of planting (Barrios i Sanroma et al. 2004). Currently, this type of problem also triggers difficulties in the production of grafted plants in nurseries. Concerning the most frequent GTDs affecting young plants (i.e., those less than 8 years old), those are black-foot disease, Petri disease, and *Botryosphaeria* dieback, respectively. The latter is one of the

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most damaging and widespread diseases affecting vineyards in the main wine-producing regions (Chacon et al. 2020; Chacon-Vozmediano et al. 2021), and—among the different *Botryosphaeriaceae* species involved (belonging to the *Botryosphaeria, Neofusicoccum, Diplodia, Lasiodiplodia, Dothiorella, Spencermartinsia*, and *Sphaeropsis* genera (Gramaje et al. 2018))—*Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips (Laveau et al. 2009; Úrbez-Torres et al. 2006; Urbez-Torres 2011) and *Diplodia seriata* De Not (Rolshausen et al. 2010) stand out as the most usual choices for pathogenicity studies, given that the former one is particularly virulent and both are fast in terms of wood-colonization (Wallis 2021). In addition, their choice is especially interesting because they are polyphagous species, with a fairly wide range of hosts, capable of infecting and causing similar symptoms in woody crops in the Mediterranean area, which usually live next to the vineyard (Sakalidis et al. 2013).

The significant increase in these diseases in the last decades (David Gramaje et al. 2018) is regarded as the result of several factors, including the prohibition of phytosanitary products (e.g., sodium arsenite, benzimidazoles, and methyl bromide), the more intensive management methods that modern viticulture has adopted, and the increased demand for young plants from nurseries (Mondello et al. 2018). Given that, at present, there are no fully effective control measures against these diseases (David Gramaje et al. 2018), it is essential to investigate preventive and control strategies in an integrated pest management (IPM) framework, including biological control, nursery phytosanitary management practices, cultural methods, and screening for natural sources of resistance, among others.

In particular, the use of resistant/tolerant cultivars poses an attractive and sustainable solution to limit the impact of these diseases, aligned with the criteria of the European legislation currently in force (Article 14 in the European Directive 2009/128/EC). There is consensus that this strategy would be the cheapest, easiest, safest, most environmentally friendly, and most effective way to control these fungi (Martinez-Diz et al. 2019; Chacon et al. 2020; Chacon-Vozmediano et al. 2021; David Gramaje et al. 2018).

Although no *Vitis* spp. germplasm resistant to these diseases has been found yet (Bertsch et al. 2013), for winegrowers who plan to establish or replant vineyards and wish to lessen their reliance on fungicides and costs for managing GTD, knowledge of cultivar resistance to fungal trunk diseases is essential. In the case of grapevine wood pathologies in general, there is hardly any information available on the degree of tolerance/resistance of the different varieties and/or rootstocks commonly used commercially (Maldonado-González et al. 2018). Despite this, some evidence of the behavior of plant material against different wood pathologies has been reported: for instance, it has been verified that the 'Riparai Glorie' rootstock is susceptible to the black foot disease, while the 101–14 MGt rootstock has more tolerance to this type of fungal pathology. In addition, 'Garnacha tinta', 'Cabernet Sauvignon' and 'Syrah' cultivars show a higher incidence of eutypiosis, while 'Merlot', 'Riesling', 'Pinot noir', 'Sauvignon blanc', 'Chardonnay', and 'Semillon' are more tolerant to *Eutypa lata* (Pers.) Tul. & C.Tul. infection. The 'Sauvignon blanc' and 'Riesling' white grape varieties are more prone to developing foliar symptoms associated with esca, while the 'Pinot blanc' variety has fewer symptoms. In red grape cultivars, the 'Rebo' cultivar is more susceptible, while the 'Syrah' and 'Merlot' cultivars have fewer foliar symptoms associated with this syndrome. Moreover, cultivars grafted on SO4 present a higher incidence of symptoms associated with the mentioned esca than those grafted on 1103 P (Maldonado-González et al. 2018). In addition, 'Merlot', 'Malbec', 'Petit Verdot', 'Pinot Noir', 'Chardonnay', and 'Riesling' would also show some degree of tolerance against various GTDs (Ramirez et al. 2018; Chacon-Vozmediano et al. 2021; Sosnowski et al. 2022).

Concerning *Botryosphaeria* dieback, up to date some varieties and rootstocks more tolerant to *Botryosphaeriaceae* have been identified (Ramsing et al. 2021; Travadon et al. 2013; Billones-Baaijens et al. 2014; Chacon-Vozmediano et al. 2021; Chacon et al. 2020; Sosnowski et al. 2022; Serra et al. 2021; Ramirez et al. 2018; Guan et al. 2016). For instance, 'Bobal', 'Monastrell', 'Macabeo', 'Moscatel serrano', 'Caíño Longo', 'Caíño Tinto', 'Torrontés', 'Treixadura', and 'Dona Branca' cultivars have been reported to be more resistant to *N. parvum* than other varieties (Gramaje et al. 2020; Chacon et al. 2020). According to Sosnowski et al. (2022), 'Traminer', 'Sangiovese', and 'Grüner Veltliner' would be among the least susceptible to colonization by *D. seriata*.

The aforementioned phenotyping efforts have mainly focused either on mechanical artificial inoculations of the pathogenic fungi on cultivar cuttings or canes (under laboratory, greenhouse, or field conditions) or on field observations of natural infections (Chacon-Vozmediano et al. 2021). Given that the latter approach requires long-term studies, due to the latency period and the difficulties associated with the variable and discontinuous behavior of foliar symptoms, in the study presented herein the first approach—employing vine shoots previously inspected for their phytosanitary status—was chosen to screen for natural sources of resistance to GTDs. This work aimed to evaluate the susceptibility of 25 cultivars from the Grapevine Germplasm Bank of Aragon (Movera, Zaragoza, Spain) against two pathogens (viz. *N. parvum* and *D. seriata*) through pathogenicity studies conducted on rooted cuttings. This type of approach was selected for two reasons; on the one hand, rooting vine shoots from an experimental vineyard previously inspected (visually and by microbiological methods) made it possible to have non-grafted and healthy material from the phytosanitary point of view and, on the other hand, given the aggressiveness and virulence of the pathogens selected (especially *N. parvum*), it was considered adequate to establish a bioassay lasting one campaign, assuming high colonization rates for pathogens during that period and the imposition of a destructive sampling of the vine plants at the end of the test to assess the internal necrosis produced. These cultivars—mostly recovered from old vineyards—include unknown, local, and minority varieties; not all of them are cultivars authorized for their cultivation in Aragonese differentiated quality figures (Protected Designations of Origin, PDOs, and Protected Geographical Indications, PGI), but their agronomic behavior and susceptibility to diseases are being actively studied to valorize all this cultural heritage and explore their genetic potential for the identification of genes responsible for certain phenotypic traits or, as in our case, the tolerance to GTDs.

Materials and methods

Plant material

Mother plants came from the germplasm bank of the Aragon Government. Specifically, grapevine woody shoots used in the assays were sampled from an experimental vineyard and supplied by the Grapevine Technology and Improvement Unit of the Centro de Transferencia Agroalimentaria (CTA) (Movera, Zaragoza, Spain).

The twenty-five cultivars chosen to study the degree of susceptibility to *N. parvum* were 'Macabeo', 'Moscatel de Alejandría' (syn. = 'Moscatel de Málaga'), 'Moscatel Angües', 'Moscatel', 'Botón de Gato' (syn. = 'Negrilla'), 'Santanera' (syn. = 'St. Jaume de Queretes'), 'Gavina', two 'Greta' cultivars, 'Torcijón', 'Garnacha tinta', 'Mazuela Ara-52', 'Derechero Ara-90', 'Cadrete' (syn. = 'Santa Fe'), three 'Parrel' cultivars, 'Aubun' (syn. = 'Corvo'), 'Gonfaus', 'Almolda I', 'Monegrina', 'Vicibera', 'Grumel' and 'Salceño Negro'. Concerning the cultivars tested against *D. seriata* (n = 24), they were: 'Moscatel Morisco', 'Macabeo', 'Moscatel', 'Botón de Gato', 'Santanera', 'Gavina', 'Olivana', 'Torcijón', two 'Garnacha Tinta' cultivars, 'Mazuela Ara-52', 'Mazuela Ara-81', 'Cadrete', three 'Parrel' cultivars, two 'Aubun' cultivars, two 'Gonfaus' cultivars, 'Almolda I', 'Monegrina', 'Beturian' and 'Grumel'. Further information on each of the assayed cultivars is presented in Tables S1 and S2 [suppl].

It should also be noted that the sets of varieties screened were not exactly the same for the two pathogens studied; this situation was due to the fact that the sprouting was not uniform for the total number of cuttings available, so some of these cultivars with a low sprouting rate had to be destined in their entirety to be inoculated with one or another pathogen.

Seven shoots of each cultivar were cut in December 2021 before pruning. The shoots were then cut into at least 20 cm long fragments and stored in a cold room at 5 °C under optimum humidity conditions until planting. Previously, a visual inspection of the fragments was carried out to ensure that they were free of symptoms associated with some type of wood pathology (presence of vascular necrosis, sectoral rot, pitting, etc.) compatible with the pathogens to be artificially inoculated or others described as etiological agents of the most common and widespread grapevine trunk diseases (GTDs). In addition, asymptomatic samples (wood discs approximately 1 cm in diameter and 0.5 cm wide from 4–5 of the obtained fragments) of each detached cultivar were surface sterilized (EtOH 70% for 1 min, commercial sodium hypochlorite solution 3% for 4 min, and finally 4–5 washes in sterile bidistilled water), placed in PDA plates amended with 0.3 g/L streptomycin sulfate (to avoid bacterial contaminants) and incubated for 3–10 days at 26 °C in the dark to isolate and identify the different fungal endophytes inhabiting the aforementioned wood and discard the presence of pathogens associated with GTDs.

Fungal isolates and production of fungal inocula

The *Botryosphaeriaceae* fungi selected were an *N. parvum* and a *D. seriata* strain isolated from diseased Aragonese grapevine plants, preserved in the living collection of the Mycology Laboratory of the Department of Agricultural, Forest and Environmental Systems of the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA, Zaragoza, Spain). They were recovered from cryovials with 20% glycerol at a temperature of −80 °C as potato dextrose agar (PDA) subcultures, performing periodic replicates to each of the isolates to maintain optimal colonies.

Assays on rooted cuttings

In March 2022, grapevine cuttings were artificially inoculated with the pathogens. Seven replicates (cuttings) were arranged per variety and pathogen. Inoculations of the tested pathogens were carried out on the trunk of the shoots at two points located approximately 10 cm apart, although this distance was shorter in some cases due to the presence of internodes or other irregularities that made it impossible to place the wounds further apart. Agar plugs from fresh 3–5 days-old PDA cultures of each fungus were used as fungal inoculum. The slits (5 mm in diameter and 5 mm deep) were made using a manual nibbler. Then, the fungus-colonized agar plug (3 mm in diameter) was placed on the

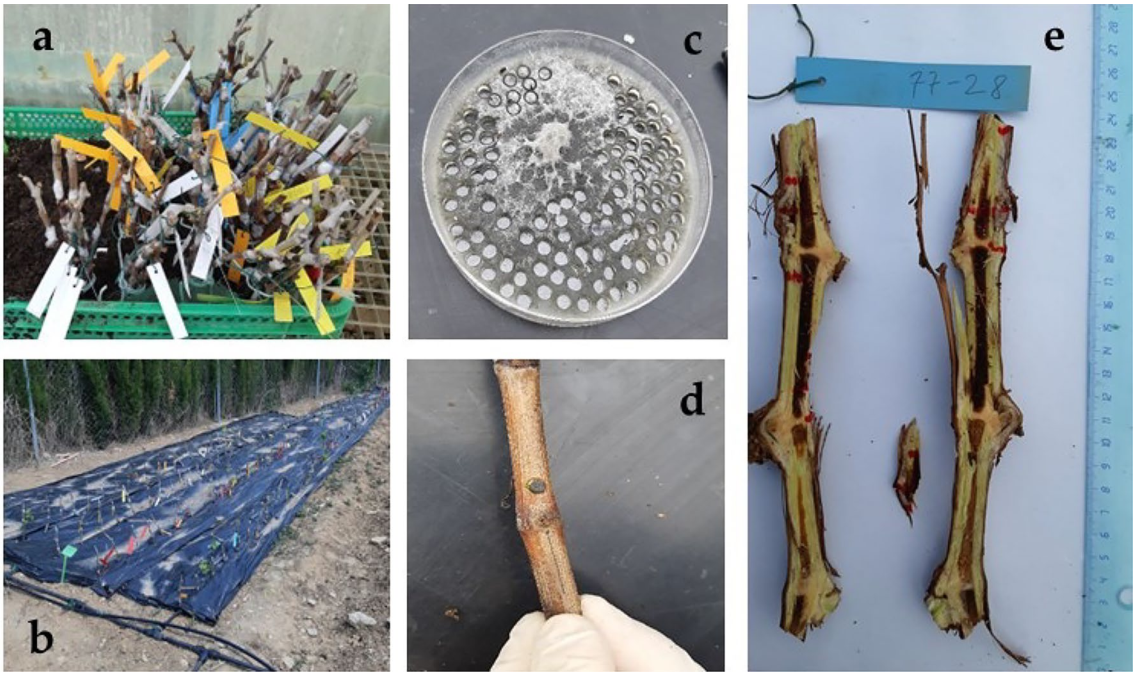

Fig. 1 Details of the artificial inoculation process: **a** grapevine cuttings of several of the analyzed cultivars; **b** overview of part of the bioassays; **c** Petri dish with *D. seriata* fungal inoculum in the form of rounded plugs; **d** slit with inserted *D. seriata* plug; **e** estimation of necrosis length

wound, covered with absorbent cotton moistened in sterile bidistilled water, and sealed with Parafilm™ tape (Fig. 1).

After inoculation, the cuttings were planted in a drip-irrigated plot at the Escuela Politécnica Superior, Universidad de Zaragoza (Huesca, Spain). Visual observation of the cuttings was carried out weekly to examine the different symptoms. Eight months after inoculation, the cuttings were removed from the plot, cut into sections, opened longitudinally, and the lengths of the wood necroses were evaluated. Lesions were measured longitudinally on both sides of the inoculation point in the upper and lower directions, taking an average of the four measurements as the necrosis length per slit. No overlaps between lesions were observed.

Statistical analyses

Since the normality and homoscedasticity requirements were not met, the Kruskal–Wallis nonparametric test was employed to examine differences among cultivars. Given that significant differences were found at a 5% significance level, a post hoc Conover–Iman test with Bonferroni corrections was conducted for multiple pairwise comparisons. The Conover-Iman test null hypothesis was to be rejected at a 5% significance level. All data analyses were conducted with the R statistical software v. 4.2.2 (R Core Team 2022), using the tidyverse (2.0) (Wickham et al. 2019), rstatix (0.7.2) (Kassambara 2023), and conover.test (1.1.5) (Dinno 2017) packages.

Results

Susceptibility to *N. parvum*

Based on the results obtained (Figure S1, [suppl]), it should be noted that none of the cultivars analyzed was tolerant to the infection by *N. parvum*. Thus, all the cultivars presented necrosis lengths to a greater or lesser extent after inoculation of the pathogen. Nonetheless, statistically significant differences were found in terms of the lengths of the vascular necroses as a function of the cultivar (Kruskal–Wallis test p value < 0.0001). As shown in Table 1, 'Garnacha Tinta' would be the most tolerant cultivar, followed by 'Macabeo', 'Moscatel Angües', and 'Greta'. On the other hand, 'Monegrina', 'Moscatel de Alejandría', 'Almolda I', 'Grumel', and 'Torcijón' would be the most susceptible cultivars to *N. parvum*. The median lengths of necrosis observed in the bioassay of inoculation with *N. parvum* were globally in a range from 5.7 mm for the 'Garnacha tinta' cultivar to 66.7 mm for the 'Torcijón' variety, both of which are red grape varieties. Considering only the white grape varieties, median necrosis lengths ranged from 7.8 mm ('Macabeo' variety) to 37.1 mm ('Moscatel de Alejandría' cultivar). It is worth noting that,

Table 1 Kruskal–Wallis test and multiple pairwise comparisons using the Conover-Iman procedure for the lengths of the vascular necroses for *N. parvum* ($p < 0.05$)

Cultivar	Mean of ranks	Groups		
Garnacha Tinta	33.000	A		
Macabeo	73.000	A	B	
Moscatel Angües	104.688	A	B	
Greta_2	114.875	A	B	
Moscatel	139.625	A	B	C
Greta_1	157.156	A	B	C
Gonfaus_2	157.650	A	B	C
Derechero Ara-90	169.625	A	B	C
Parrel_2	169.875	A	B	C
Cadrete	176.933	A	B	C
Gavina	191.100	A	B	C
Botón de Gato	193.672		B	C
Santanera	195.475		B	C
Salceño Negro	201.531		B	C
Vicibera	221.313		B	C
Aubun	223.875		B	C
Parrel_3	224.682		B	C
Parrel_1	225.750		B	C
Mazuela Ara-52	237.875		B	C
Monegrina	242.313			C
Gonfaus_1	253.781			C
Moscatel de Alejandría	262.250			C
Almolda I	302.313			C
Grumel	317.000			C
Torcijón	324.286			C

Table 2 Kruskal–Wallis test and multiple pairwise comparisons using the Conover-Iman procedure for the lengths of the vascular necroses for *D. seriata* ($p < 0.05$)

Cultivar	Mean of ranks	Groups	
Santanera	56.938	A	
Mazuela Ara-52	105.042	A	
Cadrete	120.354	A	
Macabeo	124.859	A	
Moscatel de Grano Menudo	143.125	A	B
Garnacha Tinta_2	150.967	A	B
Derechero Ara-81	161.646	A	B
Aubun	172.156	A	B
Beturian	172.571	A	B
Gonfaus_1	175.625	A	B
Almolda I	178.813	A	B
Parrel_3	192.742	A	B
Aubun_1	202.063	A	B
Botón de Gato	204.000	A	B
Olivana	216.000	A	B
Parrel_2	253.250		B
Grumel	258.188		B
Monegrina	265.250		B
Parrel_1	265.875		B
Torcijón	267.250		B
Moscatel	275.833		B
Garnacha Tinta_1	276.500		B
Gavina	292.750		B
Gonfaus_2	295.133		B

although not significantly different, noticeable differences in the necrosis lengths (median value of 15.3 compared to 29.1 mm) were registered for different cuttings belonging to or targeted with the same cultivar name (e.g., 'Gonfaus') that differed in their geographical origin (Table S1).

Susceptibility to *D. seriata*

Concerning the susceptibility to *D. seriata* (Figure S2 [suppl]), statistically significant (Kruskal–Wallis test *p* value < 0.0001) differences were also detected (Table 2). In this case, 'Santanera', 'Mazuela Ara-52', 'Cadrete', and 'Macabeo' were the least susceptible cultivars, followed by 'Moscatel de Grano Menudo' and the same 'Garnacha Tinta' ecotype from Villanueva de Huerva (Zaragoza) that showed the best behavior against *N. parvum*. Regarding the cultivars for which larger necroses were found, they included two of the 'Parrel' ecotypes, 'Grumel', 'Monegrina', 'Torcijón', one of the 'Garnacha Tinta' ecotypes (from Borja, Zaragoza), 'Gavina', and one of the 'Gonfaus' ecotypes (from La Almolda, Zaragoza). In the case of *D. seriata* artificial infection, median necrosis lengths ranged from 5.6 mm ('Santanera' variety) to 57.2 mm (exhibited by the 'Monegrina' cultivar). Considering only white grape cultivars, median length values ranged from the aforementioned 5.6 mm ('Santanera' variety) to 48.1 mm ('Moscatel' variety). When comparing red grape varieties, median lengths ranged from 7.4 ('Mazuela Ara-52' clone) to the abovementioned 57.2 mm for 'Monegrina' cultivar. Once again, the results showed, for different clones of the same variety, a differential behavior in terms of their tolerance/sensitivity against infection by *D. seriata*, as in the cases of the varieties 'Garnacha tinta', 'Parrel' or 'Gonfaus'.

Discussion

Although there is an increasing number of studies dealing with the screening and exploitation of natural sources of resistance in grapevine varieties against some of the most important and widespread etiological agents of GTDs, most of these are based either on commercial, worldwide distributed cultivars (Cardot et al. 2019; Feliciano et al. 2004;

Morales et al. 2012), local or minority grapevine germplasm exclusive from very specific wine-growing areas in some cases (Markakis et al. 2017), or even a mixture of both types of varieties (Foglia et al. 2022). These differences in the cultivars assayed, together with the employment of different etiological agents, makes it very difficult to compare and extrapolate results between studies.

In general, the results obtained showed that none of the sets of vine varieties and genotypes analyzed were fully tolerant to infection by both pathogens, observing a gradient in their response reflected in the values of median lengths of necrosis reported. In this way, the variation in these median necrosis length values was found to be continuous from the most sensitive to the most tolerant accession, which confirms the hypothesis of the existence of quantitative genetic control of the tolerance response, instead of a major tolerance gene. This is in agreement with what was reported by Martinez-Diz et al. (2019), who, in a study on the sensitivity of different varieties to infection by *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingf. & L. Mugnai) Crous & W. Gams, concluded that—in the absence of qualitative genetic control of resistance to this and other GTDs that avoid infection—all varieties are potentially susceptible to being infected, showing a gradient of sensitivity/tolerance and expression of symptoms, as in the case of the present study. Furthermore, Lemaitre-Guillier et al. (2020), in a study on the metabolomic profiles associated with the differential behavior of certain vine varieties against *Botryosphaeria* dieback, showed that there were specific patterns of each cultivar in the metabolic routes of synthesis and expression of certain phytochemicals and lipids, reinforcing the hypothesis of a complex and quantitative control of the tolerance to this type of fungal diseases in grapevine.

As previously mentioned, comparisons of these results with those of other studies on the behavior of different grapevine cultivars against several GTD pathogens should be taken with caution, given that the methodologies may be different, and other factors, such as plant material age, isolate virulence, cultivar sets, inoculum type and amount, or trial duration, could have an influence in determining disease resistance. For instance, there are studies like the one by Chacon-Vozmediano et al. (2021) that are based only on external symptoms observed in the vineyard. In that study, 'Garnacha Tinta' and 'Garnacha Tintorera' were among the 29 cultivars that did not show any symptoms, while in our study one of the ecotypes of 'Garnacha Tinta' ranked among the best cultivars, but showed symptoms. In turn, 'Macabeo' was among the cultivars that showed symptoms, but with less severe GTDs symptomatology (a result compatible with the one reported herein). Concerning other cultivars assayed in both studies, 'Moscatel de Alejandría' was identified in both studies as particularly sensitive; 'Moscatel de Grano Menudo' showed no symptoms in their study and was among the least sensitive to *D. seriata* in our assays; and a 'Mazuela' cultivar—which was the second least susceptible cultivar to *D. seriata*—did not show symptomatology in the tests conducted in La Mancha DO. Concerning this latter cultivar, it is worth noting that it is particularly sensitive to powdery mildew, so other factors—apart from susceptibility to GTDs—should be considered by the winegrowers when selecting a cultivar for planting. The same applies to other cultivars (Cabello Sáenz de Santa María et al. 2019).

Regarding studies of varietal sensitivity against fungi associated with *Botryosphaeria* dieback, in another study on Spanish red grape cultivars by Chacón et al. (2020) based on necrosis lengths after artificial inoculation, 'Garnacha Tinta' showed intermediate susceptibility to *N. parvum*, but—as noted above—noticeable differences between populations of this cultivar were also identified in this study. In a study based on the field evaluation of the sensitivity of three cultivars against several *Botryosphaeria* dieback-related fungi, Morales et al. (2012) found that all the varieties were equally susceptible to infection by *D. seriata*, without finding total tolerance to natural infection by this pathogen. Bellee et al. (2017) studied at the molecular level the response of different varieties to artificial infection by different Botryosphaeriaceous fungi (including the ones assayed herein), finding that *N. parvum*, the most aggressive species according to the lesions caused, had a differentiated metabolic profile from the rest of the pathogens, in such a way that none of the varieties tested showed tolerance against it, and that each grapevine variety presented a different transcript fingerprint, which in turn correlated with its response in terms of susceptibility/tolerance, reinforcing the idea of a complex control of the response to GTD fungal infection.

Ramirez et al. (2018), while studying the sensitivity of five vine varieties against artificial infection by *D. seriata* and *Diplodia mutila* Fries, found (as in our case) that all cultivars were sensitive to the action of the pathogen, with a gradient in the range of vascular lesions produced.

In addition, in an extensive study conducted by Sosnowski et al. (2022), which involved the visual field estimation of sensitivity to *E. lata* and *D. seriata* in 174 vine varieties, followed by the selection of varieties for inoculation in detached cuttings, a gradation in the sensitivity to infection by the latter fungus was reported. While no total tolerance was found in any of the varieties, the 'Muscadelle' variety (a white grape variety closely related to the different muscatels analyzed in this study) was identified as one of the most tolerant.

Going back to the results of the study by Martinez-Diz et al. (2019), lesion lengths caused by *P. chlamydospora* in 'Garnacha Tintorera' were high, in line with the behavior observed here for the 'Garnacha Tinta' ecotype from Borja, Zaragoza. These authors also noted that 'Moscatel Grano Menudo' was among the least susceptible cultivars to *P.*

chlamydospora in the EVEGA trial, in good agreement with our results.

Finally, regarding the presence in this study of several accessions with the same ampelographic identity but different geographical origin, the existence of a certain variability in the response (in terms of median length of necrosis) to artificial infection was verified. In this regard, it should be noted that cultivars such as 'Parrel' and 'Gonfaus' constitute an example that in old varieties, which have not been subjected to selection pressure and in which plant breeding programs have not been carried out, there may exist many circulating clones, representing most of them population variants. In other words, the ampelographic concept of these cultivars is based on the existence of a wide intra-varietal diversity in these ecotypes that, while maintaining the cultivar identity, provide genetic variability in terms of agronomic traits, yield, winemaking skills, and disease tolerance (Balda and Martínez de Toda 2017).

Conclusions

In this study, which aimed to screen for natural sources of resistance to certain *Botryosphaeria* dieback fungi, 25 cultivars from the Grapevine Germplasm Bank of Aragon were tested using artificial inoculations of the pathogens on rooted cuttings under open-air conditions. No cultivars that were fully resistant to either *N. parvum* or *D. seriata* were identified. These results are consistent with previous efforts to identify resistance to pathogens responsible for GTDs, in which no plant material showing full tolerance to infection by different species was identified. However, a certain degree of tolerance was detected in some cultivars, as significant differences in necrosis lengths were observed. In this sense, results suggested that 'Macabeo' and one of the 'Garnacha Tinta' ecotypes assayed (from Villanueva de Huerva, Zaragoza) would be the least susceptible white and red cultivars, respectively. The reported information may help winegrowers interested in reducing their reliance on fungicides and costs for controlling GTDs by facilitating their selection of plant material for new vineyards.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s41348-023-00741-9.

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Author contributions Conceived and designed the experiments: JC-G and VG-G. Performed the experiments: NL-L, VG-G, PM-R and JCG. Analyzed the data: JC-G and PM-R. Wrote the paper: NL-L, VG-G, PM-R and JCG. Supervised the work: VG-G and PM-R.

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Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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