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**FITNESS OF NATURAL HYBRIDS BETWEEN *DIMORPHANDRA EXALTATA* AND
*D. MOLLIS***

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*D. MOLLIS***

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RESUMO

A aptidão relativa dos híbridos em comparação com seus progenitores determina o destino das zonas híbridas. Neste estudo, foram avaliados os efeitos da aptidão híbrida no potencial evolutivo da hibridização interespecífica no gênero *Dimorphandra*, por meio da comparação da performance das espécies parentais, *D. exaltata* e *D. mollis*, e de seus híbridos, identificados como *D. wilsonii*, em uma zona híbrida localizada em uma área ecotonal entre o Cerrado (vegetação de savana) e a Mata Atlântica no sudeste do Brasil. Foram avaliados: (1) características morfológicas dos frutos e sementes, além da germinação em uma faixa de temperatura (12 – 40°C), para analisar a aptidão das espécies parentais, dos híbridos F1 e do híbrido retrocruzado (cruzamento: F1 × *D. exaltata*); (2) temperaturas cardinais, tempo térmico e margem de segurança térmica da germinação dos híbridos F1 e das espécies parentais sob temperatura média atual e em cenários futuros de aumento de temperatura; (3) a comparação do desempenho da progênie do híbrido F1 em relação às espécies parentais quanto à sobrevivência e crescimento em ambientes de pleno sol e sombra. A progênie do híbrido F1 apresentou aptidão predominantemente intermediária em relação às espécies parentais quanto à germinação, sobrevivência e crescimento, com desempenho geralmente inferior ao de *D. exaltata* e superior ao de *D. mollis*. Sua germinação, sobrevivência e crescimento foram elevados, sendo que a sobrevivência e o crescimento foram mais favorecidos em ambiente de plena incidência solar. Além disso, foi identificada a ocorrência de heterose nos híbridos F1 para algumas características. Seus frutos, em comparação com os das espécies parentais, foram quase um terço maiores, e suas sementes apresentaram massa maior do que as de ambas as espécies parentais. As sementes dos híbridos F1 também exibiram maior tolerância térmica e uma margem de segurança térmica mais ampla para a germinação do que as das espécies parentais. Por outro lado, os resultados de aptidão do híbrido de retrocruzamento são compatíveis com o desmoronamento do híbrido em gerações avançadas. O híbrido de retrocruzamento produziu um número substancialmente menor de sementes cheias por fruto, apresentou uma alta proporção de sementes malformadas em relação ao total de sementes por fruto e menor germinação do que as espécies parentais e os híbridos F1. Esses resultados indicam que os híbridos F1 combinam características das duas espécies parentais, o que pode possibilitar uma adaptabilidade a habitats parentais e de transição, com maior potencial de colonização em ambientes quentes e com alta incidência de luz. Além disso, a maior tolerância ao calor durante a germinação pode configurar-se em uma vantagem adaptativa diante do aquecimento global previsto. Ademais, esses achados sugerem que as barreiras pós-zigóticas

são inexistentes ou fracas para impedir o estabelecimento da progênie híbrida F1, embora uma barreira tenha sido identificada na geração de retrocruzamento. Isso evidencia o potencial das primeiras gerações de híbridos em contribuir para o fluxo gênico entre as espécies parentais, aumentando assim, a diversidade genética na zona híbrida das *Dimorphandras*.

Palavras-chave: desmoronamento híbrido, *Dimorphandra exaltata*, *Dimorphandra mollis*, *Dimorphandra wilsonii*, heterose, aptidão híbrida, zona híbrida, híbrido interespecífico, barreira pós-zigótica.

ABSTRACT

The relative fitness of the hybrids in comparison with their parents determines the fate of hybrid zones. Here we evaluate the effects of hybrid fitness on the potential evolution of interspecific hybridization in the *Dimorphandra* genus, through performance comparison of the parental species, *D. exaltata* and *D. mollis* and their hybrids, identified as *D. wilsonii*, in a hybrid zone from an ecotonal area between Cerrado (savanna vegetation) and the Atlantic Forest in southeastern Brazil. We evaluated: (1) morphological fruit, seed traits, and germination to a temperature range (12 – 40°C) to assess the fitness of parental species, F1 hybrids and backcross (crossing: F1 x *D. exaltata*); (2) cardinal temperatures, thermal time and thermal safety margin of germination for F1 hybrids and the parental species under current temperature and future scenarios of climate warming; (3) the performance comparison of F1 hybrid offspring with parental species for survival and growth under full sun and shade environments. The F1 hybrid offspring displayed predominantly intermediate fitness relative to its parental species in germination, survival, and growth, performing generally lower than *D. exaltata* and higher than *D. mollis*. Their germination, survival and growth were high, with survival and growth performing better under full sunlight environment. Additionally, we found the occurrence of heterosis in the F1 hybrids for some characteristics. Their fruits compared to that of their parents were almost a third larger, and their seeds were heavier than both parental species. The germination of F1 hybrid seeds also showed higher thermal tolerance and wider thermal safety margin than the parents. The growth of the F1 hybrid offspring was similar to or greater than that of either parent. Conversely, the fitness results for backcross hybrid are consistent with hybrid breakdown in further generations. The backcross hybrid produced a much lower number of filled seeds per fruit, with a high ratio of malformed seeds/total seeds per fruit, and a lower germination fitness than the parental species and F1. Together, our results imply that the F1 hybrid combines traits from both parental species, potentially enabling a balanced adaptability to parental and transitional habitats, with greater potential for colonizing hot environments with a high light incidence. Moreover, their higher heat tolerance in germination may confer an adaptive advantage to face the predicted global warming. Additionally, our findings suggest that the postzygotic barriers do not exist or are weak to prevent the establishment of the F1 hybrid offspring, despite having been identified in the backcross generation. This underscores the potential of the early generation of hybrids in contributing to gene flow between parental species, thereby enhancing genetic diversity in the *Dimorphandra* hybrid zone.

Keywords: *Dimorphandra exaltata*, *Dimorphandra mollis*, *Dimorphandra wilsonii*, heterosis, hybrid breakdown, hybrid fitness, hybrid zone, interspecific hybrid, postzygotic barrier.

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1 INTRODUCTION

It is well documented that hybridization plays an important role in the evolutionary process by the reinforcement or breakdown of reproductive barriers between divergent populations, in genetic adaptation and diversity through introgression, and in speciation through allopolyploidy or homoploid hybrid speciation (Abbott, 1992; Rieseberg, 1997; Rieseberg, 2006; Mallet, 2007; Abbott et al., 2013). Gene introgression can confer significant benefits to parental species by introducing genes that are more suited for a species habitat (Todesco et al., 2016) or even providing faster adaptation to the changing environment (Wong et al., 2022). This can be particularly advantageous for species with small population sizes and low genetic diversity (Chan et al., 2019; Wong et al., 2022). However, gene introgression also carries potential risks. The introduction of maladaptive alleles may reduce the overall fitness of a species, potentially leading to population decline or species extinction (Todesco et al., 2016; Edelman and Mallet, 2021). Hybrid speciation occurs when hybrids develop reproductive barriers that effectively isolate them from their parental species, becoming a reproductively isolated taxon (Mallet, 2007).

The potential role of natural hybridization in all these processes depends on the relative fitness of the hybrids with their parents. Access to hybrid fitness is essential for understanding the evolutionary significance of interspecific hybridization (Arnold et al., 2012). Hybrid fitness can be studied by comparing different phenotypic traits of the hybrids with their parental species (Arnold et al., 2012; Walter et al., 2020). Hybrids can exhibit high vigor (heterosis) across various traits, which consists of higher fitness than both parents (Hochholdinger and Baldauf, 2018). This phenomenon can arise from factors such as the masking of deleterious recessive mutations present in the parent species, heterozygote advantage at specific loci, or differences in gene expression (Hochholdinger and Baldauf, 2018). F₂ or subsequent generations of hybrids can show new phenotypes and/or new phenotypic combinations that outperform their parents, due to transgressive segregation. This occurs from the recombination of alleles with additive, complementary, or epistatic effects (Yakimowski and Rieseberg, 2014). Conversely, the hybrid breakdown can occur, it refers to a low hybrid fitness in the F₂ and/or subsequent generations compared to parental species. The hybrid breakdown occurs due to chromosomal or genic incompatibilities (Plötner et al., 2017; Xiong and Mallet, 2022; Mino et al., 2023). Thus, the fitness of hybrids can be lower, similar, intermediate, or even superior to that of their parental species, conferring them less, equivalent, or more fit in parental, intermediate to those of the parents or new habitats (Arnold and Martin, 2010; Arnold et al., 2012; Favre et al., 2017).

Characteristics associated with reproduction and vegetative growth, such as seed production, seed germination, seedling survival and growth are essential for the study of hybrids fitness (Arriola and Ellstrand, 1997; Jiménez-López et al., 2023). These processes could be affected by intrinsic genetic incompatibilities (intrinsic postzygotic barriers) or genotype-environment interactions (ecological or extrinsic barriers) (Johnston et al., 2003; Fritz et al., 2006; Lowry et al., 2008; Arnold et al., 2012; Karrenberg et al., 2019). This consists of postzygotic barriers, which operate after syngamy, acting on processes such as seed germination, seedling survival, flower, fruit and seed production, and vegetative growth, resulting in low hybrid viability, fertility and ecological inferiority (Lowry et al., 2008; Rundle et al., 2000; He et al., 2023; Jiménez-López et al., 2023). In addition, climate change brings new interests in this matter, as it can lead to the breakdown of spatial, temporal, ecological, or postzygotic reproductive barriers (Todesco et al., 2016; c). Thus, hybrid fitness can depend on the environment (Johnston et al., 2003; Fritz et al., 2006; Arnold et al., 2012). Plants can change their internal clock, metabolism, and gene expression from the influences of environmental factors such as light and temperature to regulate their growth, development and survival (Li and Liu, 2022). In this research, we focused on evaluating hybrid fitness based on traits related to seed production, seed germination and seedling establishment. We evaluated germination over a wide temperature gradient and the seedling establishment in two light conditions (sun and shade).

Temperature plays a fundamental role in several processes in the plant life cycle (Trudgill, 2005). Especially in the germination process, the time and rate of germination are strongly influenced by temperature (Garcia-Huidobro et al., 1982; Simão, 2010). Generally, the germination rate increases linearly with temperature from sub-optimal to optimal thermal conditions, beyond which it decreases linearly with increasing temperature (Garcia-Huidobro et al., 1982). The same occurs for germination percentage, since the fraction of seeds that germinate typically remains constant within a temperature range and decreases sharply on both sides of this range (Dürr et al., 2015). Light also plays a crucial role acting as an environmental cue for the adaptation of growth and development to the environment, besides providing energy for carbon fixation through photosynthesis (Kanojia, et al., 2023). During photomorphogenesis, plants remodel their growth and development according to light quantity and quality (De Wit et al., 2016). Thus, light influences processes such as seed germination, leaf and stem development and leaf senescence (Kanojia, et al., 2023). Morphological traits such as stem elongation and leaf size and thickness are influenced by light, besides physiological and biochemical (Mathur and Jajoo, 2018; Poorter et al., 2019; Yang and Jeong, 2022). Experiments

using seed production, seed germination, seedling survival, and initial growth have been widely used to access hybrid fitness, including tests under different environmental conditions (Johnston et al., 2003; Song, 2004; Barbour, et al., 2006; Kelly, 2006; Fritz et al., 2006; Sahoo et al., 2010; Marques et al., 2011; Kenzo et al., 2016; Jiménez-López et al., 2023). Such studies can contribute to understanding the evolutionary and ecological consequences of plant hybridization, as bring insights into postzygotic barriers and the potential for gene introgression.

The dynamic evolution of hybrid zones in the Neotropics is still poorly understood. Among the few neotropical hybrid zones being recently studied are the *Plathymenia* hybrid zone (Muniz et al., 2022) and the *Dimorphandra* (Muniz et al., 2020), both occurring in the same Cerrado/Atlantic forest ecotonal region in Minas Gerais, Brazil. These hybrid zones constitute a natural laboratory for ecological and evolutionary studies, which can bring insights about the importance of savanna/forest ecotonal areas in hybridization, lineage divergence and speciation. Here we attempt to contribute to advances knowledge related to the *Dimorphandra* hybrid zone. This hybrid zone is formed by interspecific hybridization between *Dimorphandra exaltata* Schott and *Dimorphandra mollis* Benth. that coexist in the ecotone Cerrado/Atlantic forest. The resulting hybrid (Muniz et al., 2020) was considered a species named *Dimorphandra wilsonii* Rizzini (Rizzini, 1969), and due to its rarity was listed in the IUCN Red List as a critically endangered species (Fernandes, 2006), also a National Plan of Action for its conservation (PAN faveiro-de-Wilson) has been conducted since 2014 (Plano de Ação Nacional para a Conservação do Faveiro-de-Wilson, 2025).

Dimorphandra wilsonii occurs as small populations only in a restricted ecotonal area between the Atlantic Forest and Cerrado (Fernandes and Rego, 2014). *D. exaltata* is also listed as a threatened species (Muniz et al., 2019; Fernandez and Moreira-Fernandes, 2021), its distribution occurs in seasonally semideciduous and ombrophilous forests (Atlantic Forest) in Southeast Brazil, where the climate is predominantly hot and humid (Alvares et al., 2013). In contrast, *D. mollis* Benth is widely distributed in the Cerrado biome (savannah), characterized by a tropical climate with a dry winter season (Aw) (Alvares et al., 2013). The *Dimorphandra* hybridization is homoploid. It was found that the hybrids are diploids, as their parents (Muniz et al., 2020). Until now, only hybrids of early generations were described, first generation F1 occurring in higher proportion, F2 (crossing: F1 x F1) and backcrosses (crossing: F1 x *D. exaltata*) with few specimens (Muniz et al., 2019). This cross with *D. exaltata* allows the gene introgression in this species (Muniz et al., 2020).

Therefore, we aimed to evaluate the effects of hybrid fitness on the potential evolution of interspecific hybridization in the *Dimorphandra* genus, by comparing the relative hybrid fitness with the parental species under different environmental conditions of temperature and light. For this purpose, we assessed the performance of the hybrids and their parents in fruit and seed production, seed germination under a wide gradient of temperature, and also seedling survival and growth under full sun and shade conditions. Understanding the performance of hybrids is critical to assess the potential postzygotic barriers separating the parental species, *D. exaltata* and *D. mollis*, and also the potential for gene introgression and speciation. This information can contribute to the conservation efforts of *D. wilsonii* and *D. exaltata*.

We subdivided this study in three chapters: In the chapter I, “Heterosis and hybrid breakdown in *Dimorphandra* hybrid zone in a savanna/forest ecotone”, we compare the fitness of two hybrid generations, F1 (crossing: *D. exaltata* x *D. mollis*) and a backcross (crossing: F1 x *D. exaltata*). The fitness was compared using morphological characteristics of the fruit and seeds and by comparing seed germination over a temperature gradient. In the chapter II, “Thermal safety margin and climate warming vulnerability for seed germination in a savanna/forest ecotone hybrid zone”, we compared the F1 hybrid with its parents regarding the thermal niche for seed germination. We obtained the cardinal temperatures of germination (base, optimum, and ceiling temperatures), the thermal time, and the thermal safety margin for germination. We also projected the thermal time and the thermal safety margin of germination for the temperature rise predicted in the climate change scenarios. In the chapter III, “Fitness of a natural interspecific hybrid in *Dimorphandra* genus: survival and growth during seedling establishment”, the F1 hybrid offspring and parental species were grown in a greenhouse under sun and shade conditions. We compared their performance in survival and initial growth. We also compared five progenies from each species and hybrid.

Chapter I

HETEROSIS AND HYBRID BREAKDOWN IN THE *DIMORPHANDRA* HYBRID ZONE IN A SAVANNA/FOREST ECOTONE

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HETEROSIS AND HYBRID BREAKDOWN IN THE *DIMORPHANDRA* HYBRID ZONE IN A SAVANNA/FOREST ECOTONE

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HETEROSIS AND HYBRID BREAKDOWN IN *DIMORPHANDRA* HYBRIDS

ABSTRACT

Premise

The relative fitness of the hybrids in comparison with their parents determines the fate of hybrid zones. Here we evaluated the fitness of the parental species, *Dimorphandra exaltata* and *D. mollis* and their hybrids, identified as *D. wilsonii*, in a hybrid zone in an ecotonal area between Cerrado (savanna vegetation) and the Atlantic Forest in southeastern Brazil.

Methods

We analysed biometry of fruit and seed traits and germination under a temperature range (12 – 40°C) to assess the fitness of parental species and F1 and backcross hybrids.

Key Results

We identified heterosis in F1 hybrids, since their fruits compared to their parents were almost a third larger, and their seeds were heavier than both parental species. The backcross hybrid showed a loss of vigor in fruit traits compared to F1. The backcross also produced a much lower number of filled seeds per fruit, with a high ratio of malformed seeds/total seeds per fruit, and a lower germination fitness than the parental species and F1. These results are consistent with a hybrid breakdown in further generations.

Conclusions

The heterosis found in the first hybrid generation coupled with the hybrid breakdown in further generations indicate that the hybrid zone is maintained by the recurrent crossing between *D. exaltata* and *D. mollis*. The rarity of advanced generations of hybrids can be explained at least partially by the intrinsic incompatibility of the recombinant hybrids. The results found here highlight the need for the conservation of this highly genetically diverse ecotonal region.

KEYWORDS: Atlantic Forest, Cerrado, *Dimorphandra exaltata*, *Dimorphandra mollis*, *Dimorphandra wilsonii*, fruit morphometry, heterosis, hybrid breakdown, interspecific hybrid, seed germination.

INTRODUCTION

Hybridization, occurring in almost 25% of plant species (Mallet, 2005), plays an important role in plant lineage evolution. It can generate novel phenotypes through genomic recombination, facilitate the colonization of new areas and lead to allopolyploidy or homoploid hybrid speciation (Abbott, 1992; Abbott et al., 2013; Mallet, 2007; Rieseberg, 1997). However, hybridization and genomic introgression can also lead rare taxa to extinction through genetic swamping due to the increase of alleles from one species into the genome of the other (Todesco et al., 2016).

Although hybridization among tree species has been extensively studied in temperate regions (e.g. Goicoechea et al., 2015; Kim et al., 2018; Leroy et al., 2020; Ortego et al., 2018), it is understudied among Neotropical tree species (Cavallari et al., 2010; Muniz et al., 2020, 2022, 2023; Resende-Moreira et al., 2017). Two hybrid zones, areas where the interbreeding between divergent lineages produces individuals of mixed ancestry, were recently identified among these few reports (Muniz et al., 2020, 2022). These two hybrid zones are located in an ecotonal area between the Brazilian savanna (Cerrado) and forest (Atlantic Forest) in southeastern Brazil.

Despite decades of studies in hybrid zones, the mechanisms that drive the fate of hybrids are not well understood and several models have been used to describe the process occurring in these areas. The tension zone is one of the most prominent models to describe hybrid zones (Barton & Hewitt, 1985). The model explains the maintenance of the hybrid zone based on the migration-selection balance between selection against hybrids and the dispersal of parental forms, which maintains the hybrid zone due to recurrent hybrid formation (Barton & Hewitt, 1985, 1989). The deleterious genomic incompatibilities would lead to hybrid inviability or reduced fitness leading to the endogenous selection against hybrids (Nikolakis et al., 2022). For instance, Bateson-Dobzhansky-Muller incompatibilities (BDMIs; Bateson, 1909; Dobzhansky,

1936; Muller, 1942) are shown empirically to be common. In BDMIs, the interaction between derived alleles of divergent populations produces a developmental and physiological breakdown of the progenies decreasing their levels of viability and fertility (Burke & Arnold, 2001). However, environmentally driven models of hybrid zones contrast the tension zone model focusing on the fitness of individuals due to exogenous selection in the intermediate habitats of hybrids (Endler, 1973; Moore, 1977). In these environmentally driven models, a stable hybrid zone with recombinant later-generation hybrids in the long term may be established in these habitats (Nosil et al., 2009; Rieseberg et al., 2003). Therefore, studying the differences in the hybrid's fitness and their parental species can give insights into the reproductive isolation between species and the fate of the hybrid zones.

In hybrid zones, the degree of divergence and demographic history impact the expectation of the strength and abundance of genomic incompatibilities and hybrid fitness (Abbott et al., 2013; Moran et al., 2022). For example, historically allopatric species that show gene flow upon secondary contacts could show large incompatibilities due to differentiation between specific genomic regions of parental species (Schumer et al., 2014). On the contrary, divergent species could show heterosis or hybrid vigor, i.e. the hybrid superiority in traits of early generation hybrid relative to their parental species, which can arise due to factors such as gene dominance, overdominance, and gene epistasis (Charlesworth & Charlesworth, 1987; Lynch, 1988; Whitlock et al., 2000). However, the heterosis in early-generation hybrids is generally transient and later-generation hybrids can show hybrid breakdown, i.e. the loss of fitness among the generations of hybridization relative to parental (Barton, 2001; Burke & Arnold, 2001). The hybrid breakdown in later-generation hybrids can be caused by the epistatic gene interaction which will break the coadapted gene complexes due to the recombination events of the genome (Johansen-Morris & Latta, 2006; Orr, 1995) such as that explained by BDMIs (Bateson, 1909; Dobzhansky, 1936; Muller, 1942).

Heterosis and hybrid breakdown may be expressed in different developmental stages. Thus, they can be evaluated by comparing fruit and seed traits and seed germination among the parental and different hybrid generations (Ackermann et al., 2008; Arriola & Ellstrand, 1997; McDade & Lundberg, 1982). The hybrid breakdown can be observed in seed failure and inviability which generally occur due to problems in endosperm development (Bachmann et al., 2019; Coughlan, 2023). The breakdown can be determined by evaluating the success of plant establishment, which is dependent on the timing and final percentage of germination, both strongly influenced by temperature (Duarte et al., 2018; Simão et al., 2010; Trudgill et al., 2005).

The hybrid zone constituted by *Dimorphandra* species (Leguminosae, Caesalpinioideae) was recently identified in an ecotonal area between the Atlantic Forest and Cerrado in the central region of the Minas Gerais state in southeastern Brazil by Muniz et al. (2020). This study showed that *Dimorphandra wilsonii* Rizzini described in 1969 (Rizzini, 1969) is in fact a population of interspecific hybrids (a nothotaxon) between *D. exaltata* Schott and *D. mollis* Benth., constituting the first report of hybrids previously described as a tree species in the Neotropics. *Dimorphandra wilsonii* was listed in the International Union for Conservation of Nature's (IUCN) Red List as a critically endangered species due to its rarity and the high levels of threats found in its habitats (Fernandes, 2006). *Dimorphandra exaltata* mainly occurs in seasonally semideciduous and ombrophylous forests (Atlantic Forest) of southeastern Brazil (Muniz et al., 2019; Silva, 1986) with its marginal distribution occurring in the patches of semideciduous forest in the ecotonal region between Cerrado and Atlantic Forest. The species was recently considered endangered due to its low genetic diversity and a small area of occupancy (Muniz et al., 2019) and included in the IUCN's Red List (Fernandez et al., 2021). *Dimorphandra mollis* has a widespread distribution in Brazil, occurring mainly in the savanna patches within the Cerrado and the ecotonal area between the Cerrado and Atlantic Forest. The

species' fruits are used for the rutin extraction, a flavonoid with anti-inflammatory usage having an economic importance (Silva, 1986).

Dimorphandra wilsonii populations are mainly diploid hybrids, classified as F1 (~85% of the population), with F2 and backcrossing appearing in less than ~10% of the populations. In addition, the detection of the backcross originated from the crossing between F1 and *D. exaltata* suggests the occurrence of gene flow into *D. exaltata* (Muniz et al., 2020). Knowledge about the fitness of different hybrid genotypic classes relative to the parental species can give insights into the evolutionary fate of *Dimorphandra* hybrid zone. However, there is still no information about fitness at any stage of the life cycle in this hybrid zone, and to our knowledge in any neotropical plant hybrid zone.

Here we evaluate fitness in fruit and seed traits and germination response to temperature of F1 and backcross hybrids (*D. wilsonii*) and the parental species *D. exaltata* and *D. mollis* in the hybrid zone to understand the process that shapes these species' evolution and the consequences of hybridization. Considering that the parental species are from contrasting habitats, *D. exaltata* from forest and *D. mollis* from savanna, differences in species' fruit traits, seed traits and their germination response to temperature would result from different adaptation strategies of the species for their respective habitats. A phylogenetic study showed low levels of haplotype differentiation based on cpDNA between *Dimorphandra* species, suggesting a recent origin for Cerrado and Atlantic Forest species (Rocha et al., 2024). In contrast, microsatellite data have found a high genetic distance between species and high inbreeding levels within *D. exaltata* (Muniz et al., 2019). A recent divergence between species would indicate that the genomic differentiation between species is low and the genomic incompatibilities have not developed yet. Furthermore, the species' differentiation would be driven by environmental factors in a few genes under natural selection.

Using biometry of fruit and seed traits and evaluating germination response to the temperature of *D. mollis*, *D. exaltata*, and their hybrids F1 and backcross the following hypotheses were tested: 1) considering that seedlings from large seeds are more competitive under shade conditions than those from smaller seeds (Leishman & Westoby, 1994), *D. exaltata* from forest would have larger fruits and seeds than the savanna species, *D. mollis*; 2) seeds of forest species could germinate in milder temperatures than those of the savanna species where the open habitat lead to higher temperatures; 3) considering that F1 are vigorous trees (personal observation), their fruits and seeds and germination could show heterosis or exhibit intermediate traits to parental species; 4) evidence of hybrid breakdown could be found in backcross hybrids since that in the hybrid zone only a few individuals are from posterior hybrid generations (Muniz et al., 2020). We also aimed to investigate if there are signs of intrinsic incompatibilities between the parental species. Finally, this study improves the knowledge about the evolution of hybrid zones in Neotropical areas and gives insights into the conservation of species of the ecotonal area between the Cerrado and Atlantic Forest.

MATERIALS AND METHODS

Study area and fruit sampling

We sampled fruits of *Dimorphandra exaltata*, *D. mollis* and *D. wilsonii* trees occurring in a Cerrado/Atlantic Forest ecotonal area at eight close municipalities in the central part of Minas Gerais state, southeastern Brazil (Fig. 1). The regional climate of the sampled area is subtropical humid with a hot summer (Cwa) according to Köppen classification (Alvares et al., 2013). The dry and rainy seasons are well-defined, with the dry season occurring from April to September and the rainy season from October to March (Alvares et al., 2013). The means of the minimum, average, and maximum daily temperatures recorded in 2018 – 2022 correspond to 14.7°C, 20.6°C, and 28.8°C, respectively. The average annual precipitation in this period

was 1722 mm (Appendix S1). The data are from a weather station maintained by the Instituto Nacional de Meteorologia - INMET in Florestal-MG.

In the field, the three taxa are distinguishable by the tree size, trunk morphology, and leaflet features. *Dimorphandra wilsonii* and *D. exaltata* are large trees that can reach 17 and 30m in height, respectively, with straighter trunks and thinner rhytidomes, similar to other forest-adapted trees. *D. mollis* is a small tree typical of Cerrado, with twisted trunks and a thicker rhytidome than both *D. exaltata* and *D. mollis* (Silva, 1986). *Dimorphandra mollis* has 6–14 leaflets with a dense indumentum, *D. wilsonii* has 6–12 leaflets with a moderately dense indumentum, and *D. exaltata* has 4–6 glabrous leaflets (Silva, 1986).

The genotypes of the trees were previously determined: *D. wilsonii* and *D. exaltata* individuals were genotyped for 11 SSR markers (Dmo5, Dmo7, Dmo13, Dmo20, Dmo21, Dw21, Dw33, Dw105, Dw28, Dw52, Dw103; Muniz et al., 2019; Muniz et al., 2020); and *D. mollis* for five out of the 11 SSR loci (Dmo5, Dmo7, Dmo13, Dmo20, and Dmo21; Souza et al., 2017). We sampled fruits of individuals previously identified as parental species (*D. exaltata* and *D. mollis*), and two hybrid classes (*D. wilsonii*), which were: F1 (*D. exaltata* x *D. mollis*) and backcross (BC) F1 x *D. exaltata*.

Fruit samplings (20 to 30 fruits per tree) were carried out between September and October. Fruits from 11 to 15 trees per species and F1 hybrids were sampled for fruit and seed measures. For BC fruit and seed measures, only five individuals were used since they were the only ones found in the field with fruits. For the germination experiments, the seeds of all individuals of each species or F1 were pooled to get enough seeds. The collected fruits from four BC individuals had many empty locules or seeds. So, for the germination assay of BC seeds from only one individual were used, which had more than a hundred of fruits sampled.

Fruit and seed traits

The sampled fruits, dried at room temperature, were measured and weighed. Five intact fruits per individual of each species/hybrid class were randomly selected. The length, width, and thickness of the fruits were measured with a calliper with a precision of 0.01 mm. The width and thickness were measured at the median region. The mass of each fruit was determined. The fruits were opened manually with a hammer and the numbers of filled seeds and malformed seeds (empty seeds) of each fruit were counted. The mean seed mass of filled seeds of each fruit was determined.

Germination assays

Filled seeds were disinfected by immersion in sodium hypochlorite solution (NaClO 2%) for 15 min, washed with deionized water, dried at room temperature, and stored in paper bags until use. To perform the germination assays, seeds were scarified on the extreme side opposite to the hilum using electric emery (Model MMI 50, Motomil®) and again disinfected with sodium hypochlorite solution (NaClO 5%) for 5 min and washed in deionized water. Afterward, they were sown in Gerbox-type plastic containers with paper moistened with a nystatin fungicide solution (Medley®, Brazil, 0.3%) and watered with this solution as necessary. Five replicates, each with 20 seeds, per species/hybrid class (*D. exaltata*, *D. mollis*, *D. wilsonii* F1 and *D. wilsonii* BC), were randomly arranged in a germination chamber (Thoth®, Brazil) with 12h photoperiod at a photosynthetic photon flux density of 50-70 $\mu\text{molm}^{-2}\text{s}^{-1}$ for each one of the following constant temperatures: 12, 15, 20, 25, 28, 30, 33, 35, 38 and 40°C $\pm$ 1°C. The experiment was conducted in two consecutive periods, using 5 germination chambers, each at a different temperature. Germinated seeds, showing a radicle of ~1mm, were counted daily for 30 days. The final germination percentage (G%) and germination time were calculated for each replicate according to Ranal & Santana (2006). Mean germination time (days), which is an index of germination speed (Soltani et al., 2015), was given by: $\bar{T} = \sum_{i=1}^k n_i t_i / \sum_{i=1}^k n_i$,

where n_i is the number of seeds germinated in the time i , t_i is the time from the start of the experiment to the i^{th} observation (day), and k is the time of last germination.

Statistical analyses

To compare fruit and seed traits among species/hybrids an one-way ANOVA was performed using the values of each fruit with *aov* function in R software version 4.4.2 (R Core Team, 2024). To evaluate the adherence of the data to ANOVA assumptions, the normal distribution of each seed and fruit trait was evaluated using *shapiro.test* and the homogeneity of variance among groups using the *bartlett.test* in R software version 4.4.2 (R Core Team, 2024). The pairwise differences among group means were compared by post hoc Tukey Honest Significant Differences (hereafter Tukey test) using the *TukeyHSD* function with a significant level at $p < 0.05$ in R software version 4.4.2 (R Core Team, 2024). To better visualize the differences among species/hybrids the mean and the standard deviations were plotted in a bar graph using the *ggplot2* version 3.5.1 package (Wickham, 2016).

Generalized linear models (GLM) were performed to investigate the relationship between the germination percentage and the germination time with the temperature range of germination (12°C to 40°C), species/hybrids, and their interaction using the R software (R Core Team, 2024). The best distribution of errors for the GLM was tested using the DHARMA package version 0.4.6 (Hartig, 2024). For the germination percentage, the best distribution of errors was the Gaussian. In contrast, for the germination time, the inverse Gaussian distribution was the best-fitted distribution (data not shown). The *anova* function in R software (R Core Team, 2024) was used to test the statistical significance of the association of the final germination percentage and time of germination with the predictors (species/hybrids and temperature range). A post-hoc Tukey test was used to compare the mean and confidence intervals of the model effects using the *emmeans* package version 1.8.9 (Lenth, 2025). Finally,

the mean and confidence intervals in each temperature were plotted for each species/hybrids using the *ggplot2* package (Wickham, 2016).

RESULTS

Fruit and seed traits of Dimorphandra species/hybrids

The ANOVA showed significant differences ($P < 0.0001$) among species/hybrids for all fruit morphological traits analysed (Appendix S2). Fruits of *Dimorphandra exaltata* had a lower length, higher thickness, and higher mass than those of another parental species, *D. mollis* (Figure 2A, B, C). Fruits of the F1 hybrid (*D. wilsonii* F1) were larger than the fruits of both parental species in terms of length, width, and mass (Fig. 2 A, B, D), and had a greater thickness than fruits of *D. mollis* (Fig. 2 C), indicating heterosis for these traits. The mean fruit mass of *D. wilsonii* F1 ($40.1\text{g} \pm 9.6$ SD) was 82% greater than the mean of *D. mollis* ($27.3\text{g} \pm 4.4$ SD) and 47% greater than *D. exaltata* ($22.1\text{g} \pm 9.0$ SD). Fruits of the backcross (*D. wilsonii* BC) showed a reduction in fruit length, thickness, and mass compared to F1 (Fig. 2 A, B, C). The BC showed two fruit traits similar to those of *D. mollis* (length and thickness), one trait similar to *D. exaltata* (fruit mass), and one similar to both species (fruit width) (Fig. 2).

Concerning seeds, *D. exaltata* produced heavier seeds (mean = $0.34\text{g} \pm 0.09$ SD) than *D. mollis* ($0.20\text{g} \pm 0.04$ SD), but a similar mean number of filled seeds per fruit and the same mean ratio of malformed seeds/total seeds as *D. mollis* (Fig. 3). The seed progenies of F1 were even heavier ($0.39\text{g} \pm 0.04$ SD) than *D. exaltata* (Fig. 3A). However, the F1 had similar mean number of filled seeds per fruit and the same mean ratio of malformed seeds/total seeds than parental species (Fig. 3 B, C). The BC had a mean number of filled seeds per fruit much lower than the parental species and F1, equivalent to 16-20% of the means of the parental species and F1 (Fig 3B), indicating hybrid breakdown. This low number of seeds filled per fruit from BC was accomplished by a great increase in the malformed seeds/total seeds ratio (0.67 ± 0.29 SD)

in comparison to parental seeds and F1 (Fig 3C). However, the filled seeds of BC had similar mass to *D. mollis* (Fig. 3A).

Effect of temperature on seed germination of Dimorphandra species/hybrids

The generalized linear model (GLM) showed that the seed germination percentages are dependent on the temperatures ($F_{9,145} = 82.4$, $P < 0.0001$) and species/hybrids ($F_{3,142} = 232.63$, $P < 0.0001$) as well as the interaction between species/hybrids and temperature ($F_{26,116} = 11.3$, $P < 0.0001$) (Appendix S3). The germination percentage of *D. exaltata* was significantly higher than *D. mollis* and the hybrids (*D. wilsonii* F1 and BC) at the range of 15°C to 28° (Fig. 4A). In addition, *D. exaltata*, a low germination percentage was observed in the species at 12°C and an abrupt increase was observed at 15°C where its values above 80% until 33°C (Fig. 4A). In *D. mollis* a gradual increase in germination percentage was observed from 15°C to 30°C, reaching values above 40% only from 25°C to 35°C, with the highest germination percentage observed (72.5%) at 30° (Fig. 4A).

For the seeds produced by F1 plants, the germination percentage increased from 12°C to 33°C ranging from 60 - 83% between 25 - 33°C. The germination percentage of F1 plants showed intermediate values between *D. mollis* and *D. exaltata* at 20°C, 25°C, 35°C and 38°C (Fig. 4A). In other temperatures seed progenies of F1 plants had germination percentage similar to one or both parental species. The progeny seeds of BC plants showed a low germination percentage, below 40%, for all temperatures, except at 33°C, which reached 70% (Fig. 4A). Compared to F1, the germination percentage of seeds produced by BC plants was lower in all temperatures between 20 and 35°C, except at 33°C. At 28°C and 30°C, the seeds from BC also showed a lower germination percentage than both parental species.

The GLM also showed significant differences in the mean germination time between temperatures ($F_{9,145} = 392.2$, $P < 0.0001$), species/hybrids ($F_{3,142} = 31.2$, $P < 0.0001$), and their interaction ($F_{26,116} = 1.69$, $P = 0.032$) (Appendix S3). The species/hybrids did not differ in the

mean germination time at low temperatures of 12°C and 15°C, however, some differences were observed at higher temperatures (Fig. 4B). Despite *D. exaltata* and *D. mollis* have shown different germination percentages at most temperatures, they were similar in terms of germination time in all temperatures, except at 40°C (Fig 4). Seeds of *D. wilsonii* F1 performed similarly to their parental species in mean germination time in most temperatures, but lower than *D. exaltata* at 30 and 33°C and lower than both parental at 38°C (Fig 4B). The mean germination time of seeds of *D. wilsonii* BC was similar to *D. exaltata* at all tested temperatures, as well to *D. mollis* at high temperatures, and higher than F1 at intermediary temperatures (Fig 4B). Thus, despite fewer seeds of BC germinating, those that germinated in general did not have an increase in mean germination time compared to parental species in most of the tested temperatures.

DISCUSSION

Dimorphandra exaltata, a forest species, showed larger fruits and seeds and germinated well at milder temperatures than *D. mollis*, a savanna species, according to our hypotheses. The F1 hybrid (*D. wilsonii* F1) showed superior values for fruit traits and seed mass than both parental species, indicating a superior fitness of the hybrids and the presence of heterosis. The backcross (*D. wilsonii* BC) showed a loss of vigor in fruit traits compared to F1 but showed similar values to one or other parental species. In addition, the backcross produced a much lower number of filled seeds per fruit and a high ratio of malformed seeds/total seeds per fruit, and the seed germination was lower compared to the parental species and F1. These results for backcross indicate a breakdown in later-generation hybrids, in accord with our hypothesis, suggesting that intrinsic factors would drive the loss of fitness in hybrids.

Fruit and seed traits of Dimorphandra species/hybrids

Our study showed that *Dimorphandra exaltata* and *D. mollis* can be differentiated by their fruit and seed traits. *Dimorphandra exaltata* has thicker and heavier fruits and heavier seeds than *D. mollis*. The fruit and seed trait values of *D. mollis* evaluated in our study are similar to those of Freitas et al. (2009) and Ferreira et al. (2001). The differences in fruit and seed traits between *D. exaltata* and *D. mollis* may be due to adaptation to forest and savanna habitats, respectively. The higher seed reserves of the forest species would provide a larger energy reserve to seedlings increasing survival and growth in the shaded forest environment (Leishman & Westoby, 1994). Furthermore, a positive relationship between plant size and seed mass has been described (Leishman et al., 1995; Moles et al., 2004), which further indicates that the divergence between *D. exaltata* and *D. mollis* is environmentally driven. There is a great difference in height between *D. exaltata* and *D. mollis* individuals and the difference in seed size confirms these findings.

Comparison of fruits and seeds among the *Dimorphandra* species, *Dimorphandra exaltata* and *D. mollis*, and *D. wilsonii*, identified as an interspecific hybrid only in 2020 (Muniz et al., 2020), has not been previously performed. Our data revealing that *D. wilsonii* F₁ has larger fruits and seeds compared to the parental species suggest higher fitness of F₁ indicating heterosis, a phenomenon that has been reported by several studies for fruit and seed sets in plant species (Arnold et al., 2012; Arnold & Martin, 2010; Klinger & Ellstrand, 1994). *Dimorphandra exaltata* is a rare species with a low effective population size and high levels of inbreeding in the ecotonal populations (Muniz et al., 2019). The ecotonal range is also a marginal area for *D. mollis* and these areas are considered to have less genetic diversity than the centre of the species distribution. Therefore, the causes of the heterosis could be related to the inbreeding levels of the parental populations. A genome-wide study would bring light to the genomic architecture of the heterosis in F₁ hybrids.

In contrast to F1 hybrids, the backcross (*D. wilsonii* BC) produced smaller fruits than F1 but showed similar size to one or both parental species. The BC hybrids produced the most malformed seeds and few filled seeds, indicating lower fertility than the parental species and the F1 hybrid. These results provide evidence of hybrid breakdown with a reduction of fitness in the advanced generation hybrids (Burton et al., 2013; Runemark et al., 2019). The low hybrid seed viability, as observed here, occurs when the ovules are fertilized, but the seeds do not reach maturation, usually due to defective endosperm (Coughlan, 2023). Hybrid breakdown has been observed in interspecific hybrids of many genera, such as in *Silene* (Favre et al., 2017) and *Typha*, where the F1 showed heterosis with a posterior hybrid breakdown in F2 and backcrosses (Bhargav et al., 2022). The hybrid breakdown is a type of postzygotic isolation, that can be explained by Bateson Dobzhansky Muller incompatibilities (BDMIs, Bateson, 1909; Dobzhansky, 1936; Muller, 1942). This model establishes that incompatibilities in hybrids result from epistatic interactions of recessive genes that have functionally diverged among the respective parents (Lowry et al., 2008; Rieseberg & Willis, 2007). Such epistasis-based recessive incompatibilities could result in partial reproductive isolation between *D. exaltata* and *D. mollis*, since BC hybrids are not completely inviable or lethal.

Temperature and seed germination of Dimorphandra species/hybrids

Despite its endangered status, the germination of *D. exaltata* is still poorly studied, with only one report showing that the germination percentage of freshly collected seeds was above 85% at 30°C (Matheus et al., 2017). In the present study, *D. exaltata* showed a high germination percentage (above 80%) at a wide temperature range. The seed germination percentage of *D. mollis* was lower than *D. exaltata*, only above 40% between 25- 35°C. Studies realized with *D. mollis* showed a high germination percentage ($\geq 80\%$) at temperatures around 25 - 35°C (Freitas et al., 2009; Pacheco et al., 2010), but these studies did not evaluate germination at temperatures below 20°C. Our data showed that germination is compromised in low temperatures in *D. mollis*

but not in *D. exaltata*, suggesting that the germination of these species may be related to their environment. The seeds of the forest species, *D. exaltata*, germinate in a shaded and humid understory that maintains a microclimate with a lower temperature, and the seeds of the species of savanna, *D. mollis*, germinate under higher solar radiation and therefore a higher temperature.

In contrast to fruit and seed morphological traits, the progeny of F1 (*D. wilsonii*) individuals did not show superior fitness to parental species in germination response to temperatures. The germination percentage of F1 was intermediate between the parental or equal to one of the parental species surpassing *D. mollis* in several thermal conditions tested. These results suggest that seeds of F1 hybrid can germinate in the original habitats of the parental species beyond the intermediate ecotonal habitat. Although it is known that hybrid fitness can depend on the environment (Arnold et al., 2012; Fritz et al., 2006), the progeny seeds of backcross trees (*D. wilsonii* BC) showed low germination in almost all the thermal conditions tested here, which suggest that the origin of the seed inviability is intrinsic, independent of the environment, consistent with intrinsic postzygotic reproductive isolation (Lowry et al., 2008).

The low fitness of later-generation hybrids can be one partial barrier to gene flow between species as it would decrease the opportunities for gene flow between species as well as the establishment of a later-generation hybrid population (Baack et al., 2015). The hybrid breakdown observed in the partial sterility of the backcross hybrids, due to low seed production and low germination could reinforce the reproductive barriers between species (Nosil et al., 2003; Ortiz-Barrientos et al., 2009). This mechanism can be an important factor in the evolution of reinforcement of lineage divergence after the secondary contact in hybrid zones such as the ecotone between the Atlantic Forest and Cerrado leading to the increase of the reproductive barrier between species (Calabrese & Pfennig, 2020). However, to better assess the strength of

reproductive isolation between *D. exaltata* and *D. mollis* it is important to consider the fitness at later developmental stages in BC or later generations hybrids.

Evolutionary and conservation considerations about the hybrid zone of the Dimorphandra genus

The low fitness of later-generation hybrids in seed set and germination would limit the long-term establishment of these hybrids, so the *Dimorphandra* hybrid zone probably shows a structure of a tension zone (Barton & Hewitt, 1989; Gompert et al., 2017). Therefore, the recurrent crossing between *D. exaltata* and *D. mollis* as well as the selection against later-generation hybrids would maintain the high frequency of F1 individuals in the hybrid zone. Currently, the ecotonal area between Atlantic Forest and Cerrado is a mosaic of forest, savanna, and anthropized environments, and hybrid trees can be found in all these habitats. This would make the *Dimorphandra* hybrid zone a model for the study of hybrid zones in Neotropical regions and the impacts of habitat fragmentation and climate change in the evolution of those areas. The occurrence of heterosis in F1 and the hybrid breakdown in later-generation hybrids provides insights into ecological and genomic determinants of the adaptive responses of species to contrasting environments, reproductive isolation between species, genetic divergence and ultimately speciation in ecotonal areas.

Dimorphandra exaltata is currently considered threatened of extinction by IUCN due to low area of occupancy, low population size, and low genetic diversity (Fernandez, 2021; Muniz et al., 2019). Given the low genetic diversity of the species, the adaptive introgression into *D. exaltata* may be a strategy to increase its genetic diversity. This strategy was suggested for several depauperate plant species (Suarez-Gonzalez et al., 2018). However, the low fitness of backcrosses between F1 and *D. exaltata* may suggest that the adaptive introgression into *D. exaltata* would lead to less adapted individuals. Furthermore, the increase of F1 individuals and the introgression of alleles into *D. exaltata* could lead to genetic or demographic swamping of

the *D. exaltata* increasing its extinction risk (Todesco et al., 2016). Since the germination of *D. exaltata* was high, the evaluation of the long-term survivorship of individuals in the field and common garden experiments would bring light to other environmental factors that decrease the species' survival in nature. These data would inform restoration and reintroduction projects for the species. Therefore, the evaluation of the risk the F1s and backcrosses impose on *D. exaltata* would also be useful for its conservation as well as for the conservation of Neotropical species in ecotonal areas.

In the Cerrado/Atlantic Forest ecotonal area where the hybrid zone of *Dimorphandra* occurs, the gene flow between two species of the *Plathymenia* genus, *P. reticulata* from Cerrado and *P. foliolosa* from the Atlantic Forest was also described (Muniz et al., 2022, 2023). These findings highlight the importance of active politics for conservation procedures for this geographic area of southeastern Brazil, which is under strong anthropic pressure mainly due to cattle farming, mineral exploration, and urbanization (Martins et al., 2014). In addition, the ecotonal area is gradually gaining attention as a unique vegetation type and studies have shown that it could be a stable climatic area (Costa et al., 2023). This study further highlights the ecotonal areas' capacity to generate and maintain high levels of biodiversity.

CONCLUSIONS

Our study using information about the genotypic composition of hybrids coupled with an evaluation of the fitness of fruits and seeds and germination brought insights into the dynamics of a recently identified hybrid zone in a Cerrado/Atlantic Forest ecotone. The parental species *D. mollis* from Cerrado and *D. exaltata* from Atlantic Forest show different fruit and seed traits and germination, probably adaptive to distinct habitats. The heterosis found on fruit and seed traits found in the F1 hybrid (*D. wilsonii*) coupled with the hybrid breakdown in later generation hybrids on seed set and germination indicate that the hybrid zone is maintained by

the recurrent crossing between *D. exaltata* and *D. mollis*. Thus, the rarity of advanced generations of hybrids can be at least partially due to the intrinsic incompatibility of the recombinant hybrids. *Dimorphandra exaltata*, a threatened species, can be subject to genetic and demographic swamping. The results found in this ecotonal region, which is highly disturbed but genetically diverse, highlight the need for its conservation to preserve the natural course of evolutionary processes maintaining the capacity of novelty generation in this area.

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AUTHOR CONTRIBUTIONS

A.B., JPL-F, and M.B.L. conceived the study and designed the experiments. A.B., A.C.M, T.V-B, and J.P.L-F identified the trees and collected fruits in the field. A.B. conducted the experiments. A.B., A.C.M and T.V-B analyzed the data. A.B., A.C.M, M.B.L., and J.P.L-F interpreted the data and wrote the manuscript. All authors read and approved the final version of the manuscript.

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FIGURE AND LEGENDS

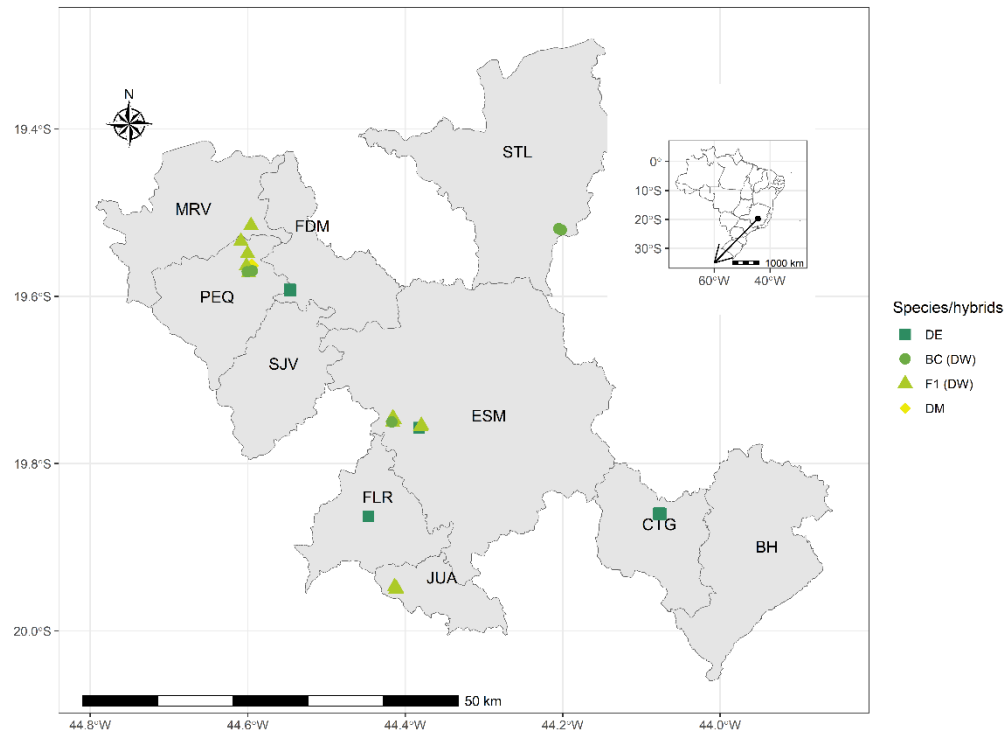


Fig. 1 Location of the sampled populations of *Dimorphandra* species and natural interspecific hybrids in a Cerrado/Atlantic Forest ecotonal area in the central part of Minas Gerais state, southeastern Brazil. BC (DW), *D. wilsonii* backcross; DE, *D. exaltata*; DM, *D. mollis*; F1 (DW), *D. wilsonii* F1. Municipalities: BH, Belo Horizonte; CTG, Contagem; ESM, Esmeraldas; FDM, Fortuna de Minas; FLR, Florestal; JUA, Juatuba; MRV, Maravilhas; PEQ, Pequi; SJV, São José da Varginha; STL, Sete Lagoas.

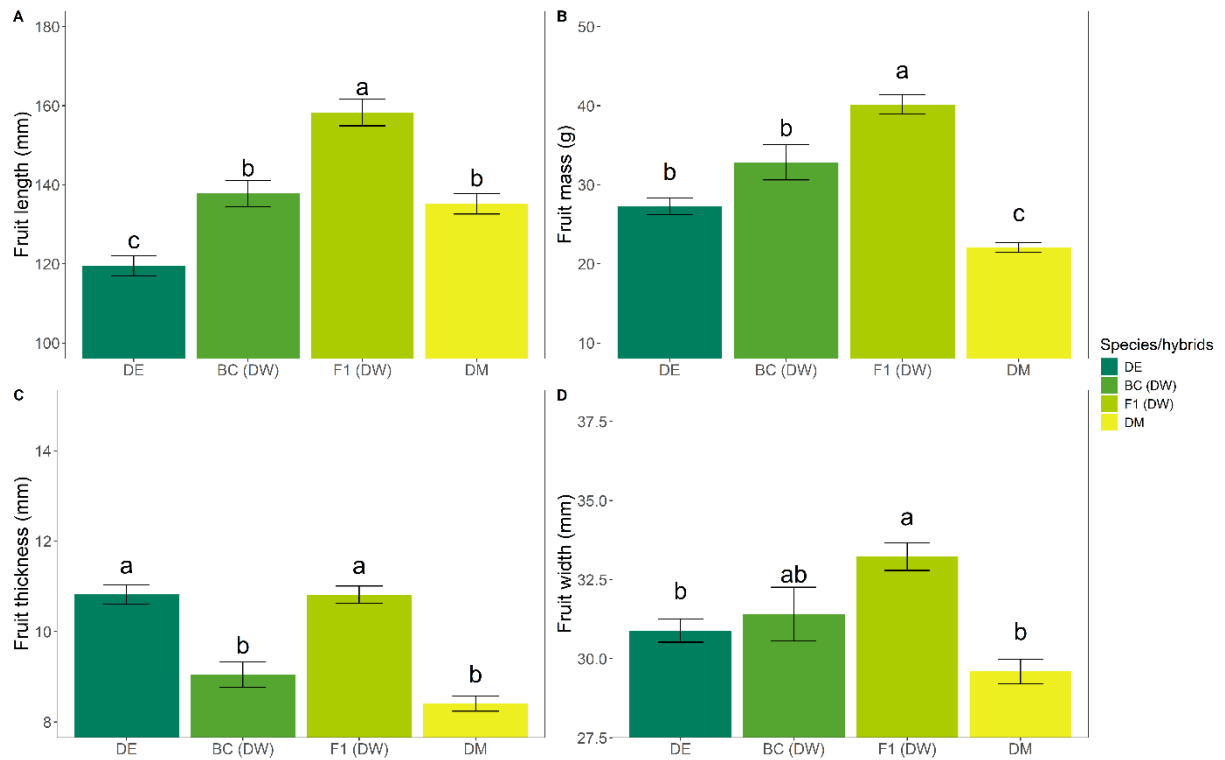


Fig. 2 Morphometry of fruits dried naturally for *Dimorphandra* species and natural interspecific hybrids. BC (DW), *D. wilsonii* backcross; DE, *D. exaltata*; DM, *D. mollis*; F1 (DW), *D. wilsonii* F1. Mean values and standard deviation were plotted. Data were analysed by one-way ANOVA and means compared by Tukey test ($p < 0.05$). Means followed by the same letters are not significantly different.

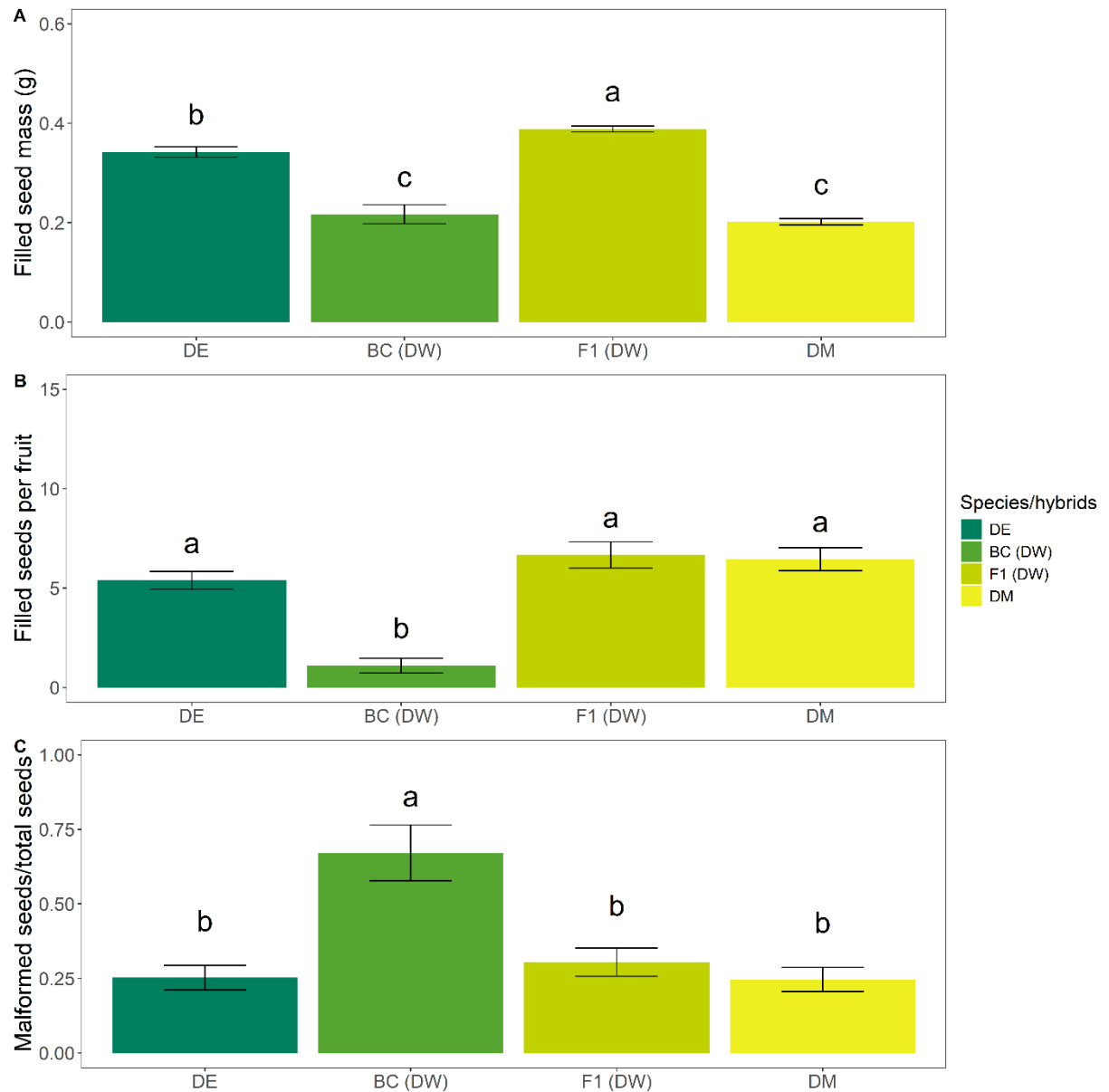


Fig. 3 Seed production in *Dimorphandra* species and natural interspecific hybrids. BC (DW), *D. wilsonii* backcross; DE, *D. exaltata*; DM, *D. mollis*; F1 (DW), *D. wilsonii* F1. Mean values and standard deviation were plotted. Data were analysed by one-way ANOVA and means compared by Tukey test ($p < 0.05$). Means followed by the same letters are not significantly different.

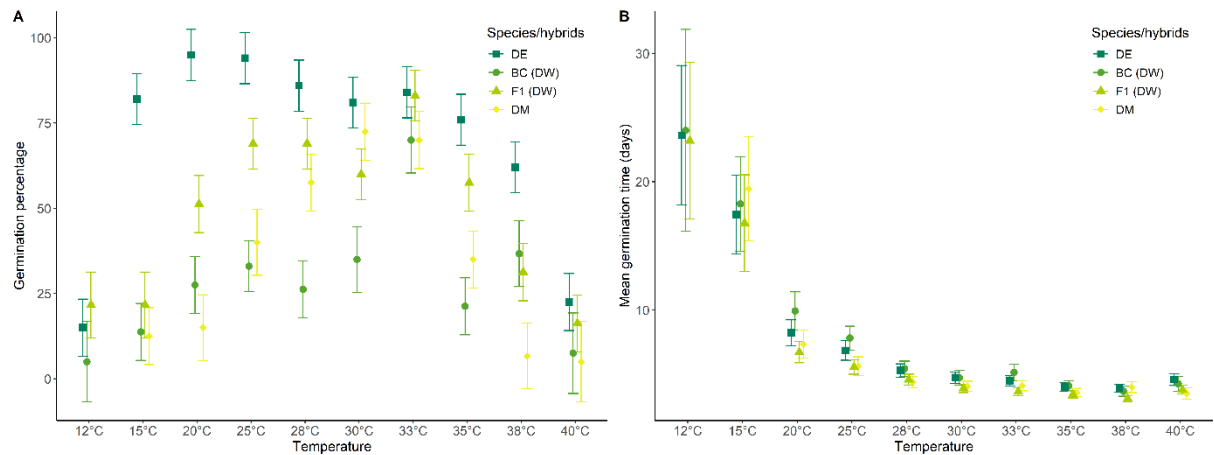
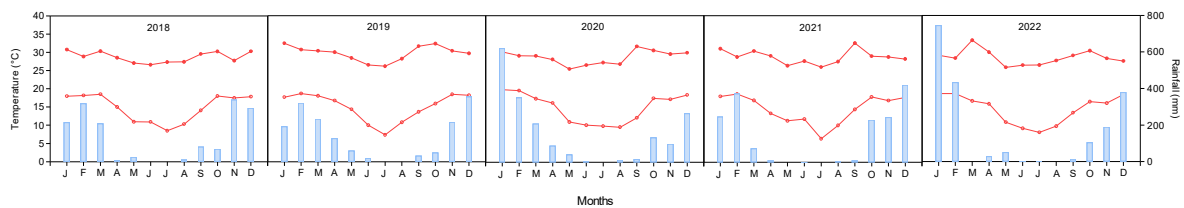


Fig. 4 Seed germination in *Dimorphandra* species and natural interspecific hybrids under different thermal conditions. (A) Final germination percentage. (B) Mean germination time. BC (DW), *D. wilsonii* backcross; DE, *D. exaltata*; DM, *D. mollis*; F1 (DW), *D. wilsonii* F1. Data were analysed by generalized linear models (GLM) followed by Tukey test ($p < 0.05$). Means followed by the same letters are not significantly different. *D. mollis* showed null germination at 12°C.

APÊNDICES

Appendix (S1) Climate data of de study area. Mean of the minimum (open symbols) and maximum temperatures (closed symbols), and total rainfall (bars) over the years 2018 - 2022.



Appendix (S2) ANOVA results of fruit and seed traits of species/hybrids of *Dimorphandra*.

Fruit lenght (mm)					
	Df	Sum Sq	Mean Sq	F value	P
Species/hybrids	3	41786	13929	27.81	< 0.0001
Residuals	164	82142	501		
Fruit thickness (mm)					
	Df	Sum Sq	Mean Sq	F value	P
Species/hybrids	3	242.4	80.82	34.01	< 0.0001
Residuals	203	482.4	2.38		
Fruit widht (mm)					
	Df	Sum Sq	Mean Sq	F value	P
Species/hybrids	3	337.4	112.48	10.66	< 0.0001
Residuals	164	1730.7	10.55		
Fruit mass (g)					
	Df	Sum Sq	Mean Sq	F value	P
Species/hybrids	3	10451	3484	48.30	< 0.0001
Residuals	203	14643	72		
Filled fruit number					
	Df	Sum Sq	Mean Sq	F value	P
Species/hybrids	3	303	101	5.28	0.0016
Residuals	197	3770	19.14		
Malformed seed/total seeds					
	Df	Sum Sq	Mean Sq	F value	P
Species/hybrids	3	1.666	0.555	4.49	0.00452
Residuals	196	24.247	0.124		
Filled seed mass (g)					
	Df	Sum Sq	Mean Sq	F value	P
Species/hybrids	3	0.9616	0.321	67.83	< 0.0001
Residuals	162	0.7655	0.005		

Appendix (S3) Generalized linear models showing the effects of temperature, *Dimorphandra* species/hybrids, and their interaction on the germination percentage and the germination time.

Germination Percentage						
	Df	Deviance Resid.	Df Resid.	Deviance	<i>F</i>	P
	154	13.1224				
Temperature	9	5.2741	145	7.8483	82.38	2.20E-16
Species/hybrids	3	4.9645	142	2.8838	232.63	2.20E-16
Temperature X species/hybrids	26	2.0586	116	0.8252	11.13	2.20E-16
Germination time						
	154	8.8292				
Temperature	9	8.2439	145	0.5852	392.2215	2.20E-16
Species/hybrids	3	0.2182	142	0.367	31.1496	7.63E-15
Temperature X species/hybrids	26	0.1023	116	0.2647	1.6855	0.03214

Chapter II

**Thermal safety margin and climate warming vulnerability for
seed germination in a savanna/forest ecotone hybrid zone**

Thermal safety margin and climate warming vulnerability for seed germination in a savanna/forest ecotone hybrid zone

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Abstract

Temperature is the most critical factor in seed germination apart from water availability. Accessing the thermal niche and thermal safety margin (TSM) for seed germination allows predicting where and when a species can germinate successfully and determining its vulnerability to climate warming. In the present study, we investigated the thermal limits and vulnerability to high temperatures for seed germination in a hybrid zone from an ecotonal area between savanna and forest, constituted of the parental species, *Dimorphandra mollis*, and *Dimorphandra exaltata* and its F1 hybrid, known as another species, *Dimorphandra wilsonii*. The supra optimum temperatures that reduce germination by 15% (T₁₅) and 50% (T₅₀), and the TSM defined as the difference between T₁₅ or T₅₀ and the germination temperature were determined. *D. mollis*, a species widely distributed in the Cerrado, exhibited the highest base temperature (T_b) and the narrowest range of temperatures for germination. The threatened species with restricted distribution in the Atlantic Forest, *D. exaltata*, had a lower T_b but a wider range of temperatures for germination. These results evidence that, more than the distribution range extension, the difference in species habitat was the greatest determinant of the differences in the thermal niche for germination among *Dimorphandra* species. The hybrid showed T_b and

ceiling temperature (T_c) intermediate to its parents. However, the hybrid showed higher T_{15} and wider TSM_{15} than the parents, suggesting a heterosis effect for these traits. The TSMs considering the high-temperature scenarios due to current climate changes were also wider for the hybrid suggesting their higher heat tolerance in germination which could be advantageous to face the predicted global warming. The wider TSM for seed germination of the F1 hybrid potentially can guarantee their continued establishment and expansion in ecotonal areas between the Cerrado and Atlantic Forest.

Keywords: cardinal temperatures, climate warming, *Dimorphandra exaltata*, *Dimorphandra mollis*, *Dimorphandra wilsonii*, heterosis, hybrid zone, seed germination, thermal niche, thermal safety margin.

Introduction

Successful establishment of plant species can be related to the timing for germination, particularly the rate and final percentage, which can be strongly influenced by temperature (Trudgill, 2005; Simão, 2010; Duarte et al., 2018). Generally, the germination rate increases linearly with temperature from sub-optimal to optimal thermal conditions, beyond which it decreases linearly with increasing temperature (Garcia-Huidobro et al., 1982). The same occurs for germination percentage, since the fraction of seeds that germinate typically remains constant within a temperature range and decreases sharply on both sides of this range (Dürr et al., 2015). Concerns about the effects of temperature on germination and the consequences of that on seedling establishment and species distribution have intensified with emerging climate change, which could raise the global average temperature between 1.5 – 5.7°C until the end of the 21st century (IPCC, 2023). In addition, climate models project an increase in the frequency of heatwaves, as well as an increase in their intensity, duration, and areas of occurrence (Domeisen et al. 2022).

The potential negative effects of the ongoing climate changes on seedling recruitment have been investigated by evaluating the thermal requirements for seed germination (Duarte et al. 2018; Cochrane 2020; Gillard et al., 2022; Filipe et al., 2023). In this sense, it is important to evaluate the thermal limits of germination, which consist of the optimum temperature range below and above which germination may be delayed or suppressed (Bewley et al., 2013). For this, the cardinal temperatures for germination must be determined, which consist in the base temperature (T_b), the optimal temperature (T_o), the ceiling temperature (T_c). T_b corresponds to the temperature below which germination does not occur, T_c corresponds to which above it the seeds do not germinate, T_o corresponds to the temperature at which germination percentage is greater and faster (Bradford, 2017). Once the T_b values are known, it is possible to determine the thermal time, which is the minimal total of accumulated degree days for a determinate

germination percentage (Garcia-Huidobro et al., 1982; Miller, 2018). Although commonly used to assess heat tolerance of the photosynthetic apparatus, the metrics that evaluate the critical temperatures that promote a decrease of 15% (T_{15}) and 50% (T_{50}), can also bring important information about the effects of high temperatures on germination.

Another metric that has recently been used to predict germination performance only in a few wild species under various climate change scenarios is the study of thermal safety margin for germination (Filipe et al., 2023). The thermal safety margin (TSM) for seed germination refers to the difference between the critical temperature at which germination begins to decline or fails and the optimal germination temperature. It can also be estimated as the difference between the upper and lower thermal limits within which germination can occur and the environmental temperature experienced by the seeds (Filipe et al., 2023). Species with a narrow thermal requirement for germination may be more affected by the increase in temperature, since their thermal requirements may be less present in the environment. The increase in temperature leads to a reduction in the upper safety margins, which, if exceeded, would affect the timing of germination and the plant phenology (Cochrane, 2019). Thus, the germination thermal niche can be a valuable indicator of the species vulnerability to climate change (Donohue et al., 2010; Cochrane, 2020), besides contributing to predicting where and when a species can germinate and establish successfully (Trudgill et al., 2005).

In this study, we evaluated the germination thermal niche and the vulnerability to climate warming of a recently identified hybrid zone in an ecotonal area between of Cerrado (Brazilian savanna) and forest (Atlantic Forest) (Muniz et al., 2020). This hybrid zone is compounded by two species, *Dimorphandra exaltata* Schott and *Dimorphandra mollis* Benth., and their hybrids, identified as another species (Rizzini, 1969), *Dimorphandra wilsonii* Rizzini. A recent study, however, showed that *D. wilsonii*, considered a threatened species (Fernandes, 2006) is a population of interspecific hybrids between *D. exaltata* and *D. mollis* (Muniz et al.,

2020). *D. exaltata*, also a threatened species (Muniz et al., 2019; Fernandez and Moreira-Fernandes, 2021), mainly occurs in seasonally semideciduous forests and in the ombrophylous forest (Atlantic Forest) of Southeast Brazil (Silva, 1986) in regions where the climate is mostly hot and humid (Alvares et al., 2013). *D. mollis* is a widespread species, occurring mainly in the Cerrado (Silva, 1986), where the typical climate is tropical with dry winter (Aw) (Alvares et al., 2013). These two species cooccur in an ecotonal area between the Atlantic Forest and Cerrado, where *D. wilsonii* Rizzini can be found in a restricted area of the ecotone (Fernandes and Rego, 2014), constituting a hybrid zone (Muniz et al., 2020).

In the *Dimorphandra* hybrid zone only hybrids of early generations were described, F1 occurring in higher proportion, F2 with few specimens, with backcrosses between F1 and *D. exaltata* being rare (Muniz et al., 2019). The hybridization and gene introgression can be beneficial for the species by introducing genes in a species that are more suited to the environment (Todesco et al., 2016), enhancing the resilience of species facing rapid environmental changes, especially species with small populations and low genetic variability (Chan et al., 2019; Wong et al., 2022). On the other hand, gene introgression can contribute to a reduction in species fitness by introducing genes that are less or not adapted, which can even lead to the species extinction (Todesco et al., 2016; Edelman and Mallet, 2021). These aspects are especially relevant for the *Dimorphandra* hybrid zone that harbors the rare and threatened species, *D. exaltata*.

The increase in temperature by climate change may favor certain phenotypic traits in detriment of others in one or more taxa involved in hybridization, resulting in the spatial displacement of the hybrid zone towards more suitable habitats for its development and changing the levels of gene introgression (Buggs, 2007; Todesco et al., 2016). The dynamic evolutionary of hybrid zones in Neotropics is still poorly understood. Besides the *Dimorphandra* hybrid zone, in the same Cerrado/Atlantic forest ecotonal region another tree

hybrid zone (*Plathymenia* genus) was also recently identified (Muniz et al., 2022). These hybrid zones constitute a natural laboratory for ecological and evolutionary studies, which can bring insights about the importance of savanna/forest ecotonal areas in hybridization, lineage divergence and ultimately speciation.

Seed germination is one of the most critical stages for recruitment of new individuals, allowing the population persistence and colonization in a changing climate (Filipe et al., 2023), and spite of this importance it rarely has been investigated in hybrid zones. The germination niche of the hybrids can be more similar to one of the parents, be intermediate to both, or even overlap the niches of one or both parents (Arnold and Martin, 2010; Arnold et al., 2012; Favre et al., 2017). This would lead to several different evolutionary outcomes, such as the hybrid zones persistence with recurrent F1 formation or hybrid zones with several hybrid types in new habitats (Barton and Hewitt, 1985). A previous study evaluating morphological traits of *Dimorphandra* fruits and seeds in the *Dimorphandra* hybrid zone found evidence for the occurrence of heterosis in the F1 generation and hybrid breakdown in the backcross generation (under review manuscript). Heterosis consists of the higher fitness exhibited by the hybrid compared to both parents (Birchler et al., 2010).

Herein, we compared the various metrics of thermal niche requirements and heat vulnerability of seed germination of the *Dimorphandra* parental species and the F1 hybrid aiming better understanding of the evolution of the hybrid zone under future scenarios of climate warming. Considering the differences in the range extension of the geographic distribution, and the distinct habitats of occurrence of species we hypothesized that *D. mollis*, a widely distributed in the Cerrado presents a wider thermal niche and higher resilience of germination to high temperature than *D. exaltata*, a threatened specie with a restrict distribution range in the Atlantic Forest. We also investigated if *D. wilsonii* F1 hybrid, in the same way as observed for morphological characters of fruits and seeds, would also exhibit a heterosis effect

expressing higher tolerance to heat exposition with a wider temperature safety margin (TSM) for germination than the parental species. The results of the present study may contribute to the understanding of the evolutionary dynamics in hybrid zones in the savanna-forest ecotone in the face of ongoing climate change.

Materials and methods

Study area, species, and seed collection

The study was conducted with individuals previously genotyped occurring in Cerrado/Atlantic Forest ecotone in the central part of Minas Gerais, Brazil. The genotyping data are from three published papers and were obtained with the same protocols in the same laboratory: *D. wilsonii* individuals were genotyped at 11 SSR markers (Dmo5, Dmo7, Dmo13, Dmo20, Dmo21, Dw21, Dw33, Dw105, Dw28, Dw52, Dw103; Muniz et al., 2020); *D. exaltata*, at the same 11 loci as *D. wilsonii* (Muniz et al., 2019); and *D. mollis* at five out of the 11 SSR loci analysed in *D. wilsonii* (Dmo5, Dmo7, Dmo13, Dmo20 and Dmo21; Souza et al., 2017). We sampled individuals identified as parental species (*D. exaltata* and *D. mollis*), and F1 hybrids (*D. wilsonii*). Fruits of genotyped individuals of hybrids and parental species were sampled between September and October 2021 at proximal locations in the ecotonal area between savanna (Cerrado) and forest (Atlantic Forest) in the Minas Gerais state, Brazil: *D. exaltata* sampled in Florestal and Contagem municipalities; *D. mollis* in Pequi; *D. wilsonii* (F1) in Juatuba.

The climate of the region is humid subtropical with a hot summer (Cwa) (Alvares et al. 2013). The dry and rainy seasons are well defined. The first from April to September and the second from October to March (Alvares et al., 2013). Meteorological data for 2018-2022, from a weather station maintained by the Instituto Nacional de Meteorologia (INMET) in Florestal-MG, were used to calculate the average temperatures and the average precipitation of the region

where the seeds were collected. We also use data from INMET to obtain the average daily temperature of the heatwaves. For this purpose, we considered the average air mean temperature values of the seven heatwaves between September 2023 and March 2024 in the seed collection site. We considered as a heatwave period when the recorded average temperature exceeded the common averages for the same time of year. The means of the minimum, average and maximum daily temperatures recorded in that period were 14.7°C, 20.6°C and 28.8°C, respectively (Fig.1 A), and the average annual precipitation was 1722 mm. The average daily temperature during the heatwave periods was 25.5°C, with maximum daily temperatures recorded between 33°C and 37°C (Fig.1 B).

Germination assays

The fruits were opened manually with a hammer, and seeds with a healthy appearance were selected. Seeds from at least 10 individuals per group (*D. exaltata*, *D. mollis*, and *D. wilsonii* (F1 hybrid) were mixed for the germination assay. Seeds were disinfected by immersion in sodium hypochlorite solution (NaClO, 2%) for 15 min, followed by washing with deionized water (Matheus et al., 2017). Then, they were dried at room temperature (~25°C) and stored in paper bags until use. To perform the experiment, seeds were scarified on the extreme side opposite to the hilum (Oliveira et al., 2008) using electric emery (Model MMI 50, Motomil®) and again disinfected with sodium hypochlorite solution (NaClO, 5%) for 5 min and washed in deionized water (Bicalho et al., 2017). Afterward, they were sown in Gerbox-type plastic containers with paper moistened with a nystatin fungicide solution (Medley, Brazil, 0.3%) and watered with this solution as necessary (Cruz et al., 2018). Five replicates per group, each with 20 seeds, were randomly arranged in germination chambers (Thoth®, Brazil) with 12h photoperiod at photosynthetic photon flux density of 50-70 $\mu\text{molm}^{-2}\text{s}^{-1}$ under constant temperatures of 12, 15, 20, 25, 28, 30, 33, 35, 38 and 40°C $\pm$ 1°C. Germinated seeds, showing a radicle of ~1mm, were counted daily for 30 days.

Cardinal temperatures and thermal time for germination

Three cardinal temperatures were determined: base temperature (T_b), optimum temperature (T_o), and ceiling temperature (T_c), using a quadratic polynomial regression of the final germination percentages against the germination temperatures with 95% confidence intervals (Fig 2). The thermal time requirement for germination (degree days, DD) was calculated from the number of degrees accumulated in each day (above T_b) for each of the five replicates from each species/hybrid, as follows: $DD = \sum (T_G - T_b)n$, where T_b is the base temperature for germination, T_G is the germination temperature, and n is the number of days. The thermal time value for 50% germination (θ_{50}) was determined from a sigmoidal regression of germination percentage against DD.

Using the values of θ_{50} , the number of days required to achieve 50% of germination at the current temperature (mean air temperature), and under the projected temperatures in the context of climate change were determined. For the current temperature, we used the mean daily temperature of the seed collection site as T_G (22.8°C) obtained from INMET for the years 2018 - 2022 for the six months after *Dimorphandra* seed dispersal, October to March, (Bizerril et al., 2005). And, for climate change context, we added to that value, the global average temperature predicted by Intergovernmental Panel On Climate Change report (IPCC, 2023) for five greenhouse gas emission scenarios between the period of 2040 and 2100: very low (SSP1-1.9), until 1.5°C; low (SSP1-2.6), until 2°C; intermediate (SSP2-4.5), until 3.5 °C; high (SSP3-7.0), until 4.6 °C; and very high (SSP5-8.5), until 5.7 °C. Then, for each *Dimorphandra* species and the hybrid, a linear regression was performed between the average air temperatures (current and predicted) and the time for germination (days).

Thermal safety margin

The quadratic polynomial regression used for determining T_b , T_o , and T_c was also used to determine the supraoptimum temperatures that promote a 15% and 50% decrease in

germination percentage, T_{15} and T_{50} , respectively (Fig 2). With the values of T_{15} and T_{50} , the Thermal Safety Margins were calculated as $TSMT_{15} = T_{15} - T_o$ and $TSMT_{50} = T_{50} - T_o$. We also determined the TSMs using the mean daily temperature of the seed collection site (22.8°C) and also simulated the TSMs considering the five possible scenarios of climate change, adding 1.5°C, 2°C, 3.5°C, 4.6°C and 5.7°C to the mean daily temperature, as described in the previous topic. In addition, we simulated TSM_{15} and TSM_{50} for heatwaves using the mean daily temperature of heatwave periods (25.5°C).

Results

Cardinal temperatures for germination

D. exaltata achieved higher maximum seed germination (95%) than *D. mollis* (72.5%). *D. wilsonii* F1 recorded maximum germination intermediate to the parental species (83%) (Fig. 2). The highest temperature base value (T_b) for germination was found for *D. mollis* (15.3°C), 6.7°C higher than that for *D. exaltata* (8.6°C). The *D. wilsonii* F1 showed intermediary T_b values (11.4 °C) (Table 1; Fig. 2). *D. exaltata* and *D. mollis* had different optimum temperatures for seed germination (T_o), 25.6°C and 27.8°C, respectively, and *D. wilsonii* F1 had intermediary values (Table 1; Fig. 2). The value of ceiling temperature for germination (T_c) was lower for *D. mollis* than *D. exaltata*, with *D. wilsonii* F1 also with intermediary value between them (Table 1; Fig. 2). Regarding the temperature range for germination ($\Delta = T_c - T_b$), *D. exaltata* and *D. mollis* had a great difference, 34.4°C and 24.7°C, respectively. The hybrids F1 presented the temperature range for germination closest to that observed for *D. exaltata* (Table 1; Fig. 2). So, the cardinal temperatures for seed germination of the hybrid F1 were intermediary to those of their parental species.

Evaluating the supraoptimum temperatures that decrease 15% and 50% in germination percentage, T_{15} and T_{50} , respectively, *D. exaltata* and *D. mollis* had a similar T_{15} , 29.3°C and

30.3°C, respectively. The hybrid F1 showed T_{15} at least 3°C above the values of their parents. *D. exaltata* showed a higher T_{50} than *D. mollis*, 38.1°C and 36.4°C, respectively, and *D. wilsonii* F1 showed a T_{50} equal to *D. exaltata* (Table 2; Fig. 2).

Thermal time for germination at the current temperature and the climate change scenarios

The thermal time to reach 50% of germination (θ_{50}) was higher for *D. exaltata* than *D. mollis*, 90.2 and 50.8 degree days, respectively, and the *D. wilsonii* F1 hybrid showed an intermediary value (78.2) (Table 1; Fig. 3). The number of days for to reach 50% of germination at the current mean air temperature at the seed collection sites (22.8°C) was similar for *D. exaltata*, *D. mollis* and *D. wilsonii* (7 days) (Fig. 4 A). The simulation for the climate change scenarios predicted that the progressive increase in air temperature cause a progressive reduction in germination time for the species and the hybrid, as evidenced by the negative correlation between time for germination and the increase of mean air temperature (Fig. 4 B, C D, E). The two *Dymorphandra* species showed a reduction of one day in the most optimistic scenario (+1.5°C), whereas the F1 hybrid was not affected (Fig. 4 A). The simulation for the second scenario (+2°C) showed an additional reduction of one day in germination time for the parental species and for *D. wilsonii*. In the third scenario (+3.5°C) *D. exaltata* and *D. wilsonii* F1 maintained a reduction of one day, while *D. mollis* underwent a decrease of two days (Fig. 4 A).

Effect of the predicted temperature increase on the thermal safety margin

The thermal safety margins experimentally determined considering the supraoptimum temperature that reduces by 15% the germination observed at T_o (TSM₁₅) for seed germination was wider in *D. wilsonii* F1 hybrid than in both parental species, *D. exaltata* and *D. mollis* (Table 2). *D. mollis* showed slightly narrower TSM₁₅, about 1°C, compared to *D. exaltata*. A wider TSM₁₅ for the hybrid compared with their parentals was also observed for the current mean air temperature and all the scenarios of temperature increase, including

heatwaves (Table 3). These results indicate that the *D. wilsonii* is more resilient to temperature increase than both parents.

Regarding the thermal safety margin that promoted a 50% decrease in germination (TSM₅₀), *D. exaltata* showed a wider TSM₅₀ than *D. mollis* and similar to F1 hybrid (Table 2). The F1 hybrid also showed a higher TSM₅₀ than *D. mollis* in all scenarios of climatic change (Table 3). The narrower TSM₅₀ indicate that the seed germination of *D. mollis* is potentially less able to withstand high-temperature stress than *D. exaltata* and the F1 hybrids.

Discussion

Cardinal temperatures and thermal time for germination

The forest species, *D. exaltata* showed a wider thermal range for germination with lower base temperature (T_b) and higher ceiling temperature (T_c) than *D. mollis*, contradicting the premise that the species with the wider distribution (*D. mollis*) would also present a wider niche range for germination. The F1 hybrid (*D. wilsonii*) was intermediate to the parents in terms of T_b , T_c , and germination temperature range ($\Delta = T_c - T_b$), and were more similar to *D. mollis* for optimum temperature for germination (T_o).

Despite the differences in these cardinal temperatures for germination, the values observed for the *Dimorphandra* species and F1 hybrid are consistent with findings for other tropical trees, except for the T_b found for *D. mollis* (15.3°C), which is higher than the range reported in a global study, between 8 - 13°C (average = 10°C) (Maleki et al., 2024). The difference in T_b values between *D. exaltata* and *D. mollis* may be related to their germination environment. Consistent with forest habitat, *D. exaltata* seeds can germinate in a shaded and humid understory that maintains a rally germinate under a more open canopy of savanna characterized by higher light incidence and higher temperature. The intermediary results of the cardinal temperatures for *D. wilsonii* F1 hybrid suggest that both, savanna and forest habitats

would not impose limitations on the initial stage of recruitment of the hybrids in the savanna/forest ecotone with possibility of them moving towards the Cerrado and the Atlantic Forest. So, the differences in thermal niches between *Dimorphandra* appear more influenced by habitat than the width of the geomicroclimate with a lower temperature. On the other hand, seeds of the Cerrado species, *D. mollis* generaphic range of the species.

The higher temperature above the optimal that promotes a 50% decrease in germination (T_{50}) of *D. exaltata* indicates higher seed germination resilience to high temperatures compared to *D. mollis*. On the other hand, *D. mollis* showed a lower thermal time to reach 50% germination (θ_{50}), which according to Maleki et al., (2024) indicates an adaptation to fast germination at moderately high temperatures. A lower T_b resulted in a higher θ_{50} in *D. exaltata*, while a higher T_b resulted in a lower θ_{50} in *D. mollis*, while in *D. wilsonii* F1 hybrid, these variables were intermediary. This trade-off between T_b and degree days is common and is related to temperature adaptation (Trudgill et al., 2005) and may be associated with greater fitness at low temperatures in *D. exaltata*, and high temperatures in *D. mollis*.

Differences in seed size might also explain the differences in the responses of germination to temperature among the *Dimorphandra* species. Studies have shown that heavier seeds can have better germination performance under warming temperatures (De Frenne et al., 2012; Filipe et al., 2023). It was also reported that seed mass is positively correlated with the temperature of the species' habitat (Soper Gorden et al., 2016). In the case of *Dimorphandra*, the species with larger seeds, *D. exaltata* ($0.34g \pm 0.09$), presented a higher T_{50} and θ_{50} values than *D. mollis*, species with smaller seeds ($0.20g \pm 0.04g$). Differences in germination time may be important for competition during the critical period of germination and emergence (Linhart, 1976, Gioria, et al., 2016), once, earlier emergence results in a higher position in the dominance hierarchy in a plant community (Ross and Harper, 1972; Cochrane et al., 2015). In this respect, we can infer that the F1 hybrid's progenies would be at an intermediate competitive advantage

compared to their parents' progenies. Under the current temperature, the time (days) required for 50% germination for *D. wilsonii* F1 hybrid was similar to the parentals. In our simulation, the increases in temperature in the optimistic scenarios (+2°C) caused a decrease of one day in the germination thermal time for *D. exaltata* and *D. mollis*, with a continuous reduction in more pessimist scenarios. It has been reported that the increase in temperature caused by ongoing climate change may not be detrimental to seed germination (Gillard et al., 2022). In the case of *Dimorphandra* species from the ecotonal region between forest and savanna, the reduction in germination time under warming scenarios indicates that the germination process may be favored by the increase in temperature.

Effect of the predicted temperature increase on the thermal safety margin

As expected, the progressive increase in temperature gradually promoted a narrowing of the thermal safety margin (TSM) for germination but differences were observed among *Dimorphandra* species/hybrid. Species with broader thermal niches tend to be less vulnerable to temperature increases (Cochrane, 2020). This is the case of *D. exaltata* from forest habitat that besides having presented a broader thermal niche also presented higher TSM than *D. mollis*. The only study evaluating TSM on seed germination reported until the moment (Filipe et al., 2023) compared two co-occurring species in Australia and found differences in TSM associated to different habitats. This also appears to be the case for the TSM between the two *Dimorphandra* species, *D. mollis* from savanna and *D. exaltata* from the forest habitat.

In contrast to germination studies, TSM is frequently used to determine the tolerance of photosynthetic apparatus to heat by chlorophyll fluorescence measurements. Substantial differences in TSM of photosystem II thermotolerance was observed among tropical rainforest and savanna species during heatwaves, with the forest species, even under lower environmental temperature, showing higher TSM than savanna species, exposed to higher environmental temperature (Kitudom et al., 2022). So, savanna species can be more vulnerable to heatwave

events as well as climate warming (O'Sullivan et al., 2017; Kitudom et al., 2022). The present study is the only one that compares the TSM values for germination between savanna and forest species and therefore it is necessary to increase the number of species evaluated to confirm the trend of TSM similarity of photosynthesis and germination.

Our results indicated a better performance concerning high temperatures for the F1 hybrid (*D. wilsonii*), which showed higher supra optimum temperature that reduced germination by 15% (T15) than their parentals, *D. exaltata* and *D. mollis*, evidencing the heterosis effect. *D. wilsonii* also demonstrated heterosis effect for TSM15. The TSM50 of F1 hybrid was like the forest species (*D. exaltata*) and wider than that of the Cerrado species. Considering the current temperature and those simulating the global warming, *D. wilsonii* F1 hybrid also showed higher T15 and TSM15 than their parents for germination. The higher T15 and TSM15 for germination of the F1 hybrids compared to their parents can result in a better resilience of germination to high temperature, and this can be interpreted as an expression of heterosis for these physiological traits (Kong et al., 2024.).

Conclusions

In the present study was evidenced that, more than the distribution range extension, the difference of habitat was the greatest determinant of the differences in the thermal niche for germination among *Dimorphandra* species. For *D. wilsonii* F1 hybrid, most of the cardinal germination traits evaluated presented intermediate values or like one of the parents. However, higher T15 values, and a wider thermal safety margin (TSM) were observed for the germination of seeds of the F1 hybrid individuals. This result points to the effect of heterosis for these traits and suggests that the hybrids would be more resilient to temperature increases than their parentals, *D. mollis* and *D. exaltata*. These findings suggest that hybridization may play a pivotal role in enhancing thermal adaptability, potentially conferring a fitness advantage in

response to global warming. The wider TSM for seed germination of the F1 hybrid, even in the worst warming scenario, potentially can guarantee their continued establishment and expansion in ecotonal areas between the Cerrado and Atlantic Forest. Our results also demonstrate that *D. exaltata*, although threatened due to low genetic variability, also has favorable characteristics to face rising temperatures. This suggests that the gene flow from hybrids to *D. exaltata* might continue, even in the climate warming context.

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TABLES

Table 1 Cardinal seed germination temperatures and thermal time to reach 50% germination in *Dimorphandra* species and their natural hybrids. *D. wilsonii* F1: *D. exaltata* x *D. mollis*. T_b : Base temperature. T_c : ceiling temperature. T_o : optimum temperature. $\Delta = T_c - T_b$: germination temperature range. θ_{50} : thermal time to reach 50% germination.

Species/hybrids	T_b (°C)	T_o (°C)	T_c (°C)	$\Delta = (T_c - T_b)$ °C)	θ_{50} (degree days)
<i>D. exaltata</i>	8.6	25.6	43	34.4	90.2
<i>D. mollis</i>	15.3	27.8	40	24.7	50.8
<i>D. wilsonii</i> F1	11.4	27.1	41.5	30.1	78.2

The cardinal temperatures were obtained using a quadratic polynomial regression between the final germination percentage and temperature. θ_{50} was obtained using a sigmoidal regression between the final germination percentage and degree days.

Table 2 Temperatures above the optimal (T_o) that promote a decrease of 15% and 50% (T_{15} and T_{50}), and thermal safety margin experimentally determined (TSM_e) for seed germination in *Dimorphandra* species and their natural hybrid. *D. wilsonii* F1: *D. exaltata* x *D. mollis*. TSM_{e15} = $T_{15} - T_o$; TSM_{e50} = $T_{50} - T_o$.

Species/hybrids	T_{15} (°C)	T_{50} (°C)	TSM _{e15} (°C)	TSM _{e50} (°C)
<i>D. exaltata</i>	29.3	38.1	3.7	12.5
<i>D. mollis</i>	30.3	36.4	2.5	8.6
<i>D. wilsonii</i> F1	33.2	38.2	6.2	11.1

The values of T_{15} and T_{50} were obtained using a quadratic polynomial regression between the final germination percentage and temperature.

Table 3 Thermal safety margin for seed germination in *Dimorphandra* species and their natural hybrids. *D. wilsonii* F1: *D. exaltata* x *D. mollis*. TSM₁₅ and TSM₅₀: The thermal safety margin was estimated by the difference between using the critical temperatures that decrease 15% (T₁₅) and 50% (T₅₀) using the mean air temperatures for the current time and for different scenarios predicted by IPCC (2023) and the mean air temperatures of the heatwaves.

Species/hybrids	Scenarios (mean temperature)						Heatwaves (25.5°C)
	Current (22.8°C)	SSP 1 - 1.9 (24.3°C)	SSP 1.0 - 2.6 (24.8°C)	SSP 2 - 3.4 (26.3°C)	SSP 3 - 7.0 (27.4°C)	SSP 5 - 8.5 (28.5°C)	
TSM ₁₅ (°C)							
<i>D. exaltata</i>	6.5	5	4.5	3	1.9	0.8	3.8
<i>D. mollis</i>	7.5	6	5.5	4	2.9	1.8	4.8
<i>D. wilsonii</i> F1	10.4	8.9	8.4	6.9	5.8	4.7	7.7
TSM ₅₀ (°C)							
<i>D. exaltata</i>	15.3	13.8	13.3	11.8	10.7	9.6	12.6
<i>D. mollis</i>	13.6	12.1	11.6	10.1	9	7.9	10.9
<i>D. wilsonii</i> F1	15.4	13.9	13.4	11.9	10.8	9.7	12.7

Current temperature refers to the mean daily temperature of the seed collection site. SSP refers to the Shared Socioeconomic Pathway predicted by the Intergovernmental Panel on Climate Change report (IPCC, 2023). To obtain the mean temperature in each scenario, we added the expected temperature increase in each scenario (SSP) and heatwave to the mean daily temperature of the seed collection site.

FIGURES

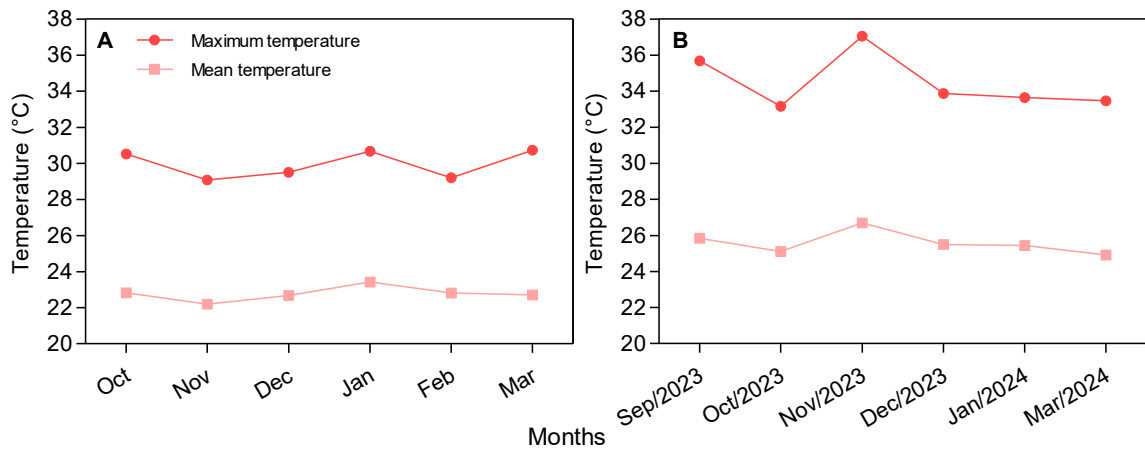


FIGURE 1 Temperature data of the study area. **A** Maximum and mean temperature of the rainy months after the *Dimorphandra* seeds dispersal, monthly average over the years 2018 - 2022. **B** Maximum and mean temperature of the heatwave periods between 2023 and 2024. Data from Florestal-MG, Brazil meteorological station (INMET - Instituto Nacional de Meteorologia).

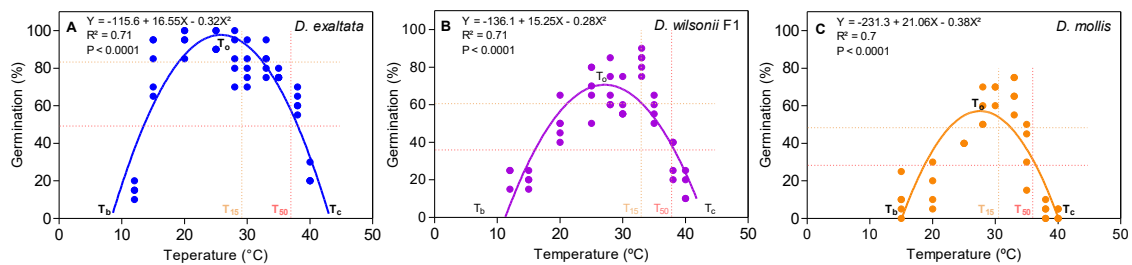


FIGURE 2 Cardinal temperatures of seeds germination in *Dimorphandra* species and their natural hybrid, *D. wilsonii* F1: *D. exaltata* x *D. mollis*. Base temperature (T_b), optimum temperature (T_o), ceiling temperature (T_c), temperature above T_o that result in a 15 % and 50% decrease in germination (T_{15} and T_{50}). Non-linear quadratic polynomial regression was performed.

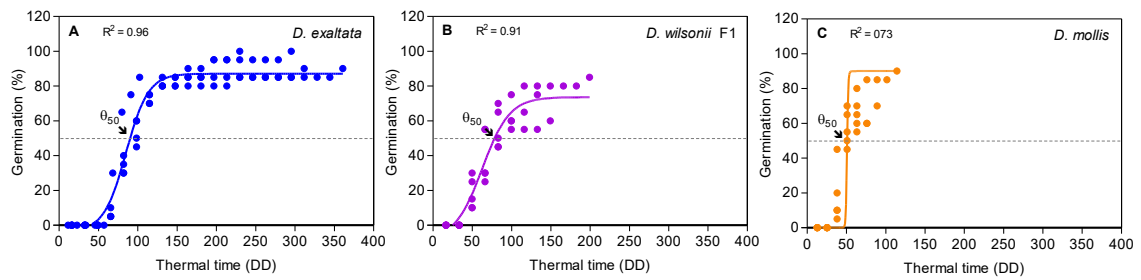


FIGURE 3 Thermal time requirements for seeds germination in *Dimorphandra* species and their natural hybrid, *D. wilsonii* F1: *D. exaltata* x *D. mollis*. Seed germination (%) as a function of thermal time requirements (DD). A sigmoidal dose-response regression was performed. Horizontal dashed line indicates the thermal time required to reach 50% of germination (θ_{50}).

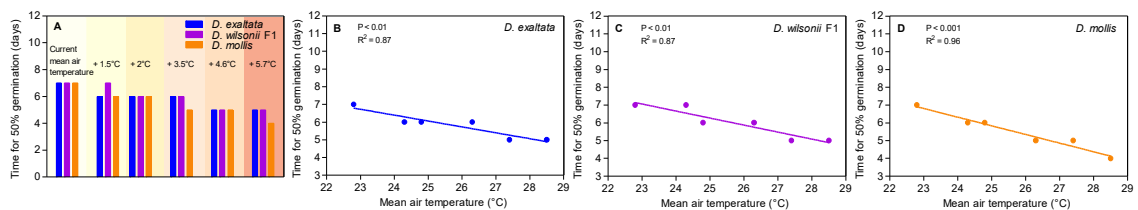


FIGURE 4 A Predicted time required for 50% (days) of seeds germination in *Dimorphandra* species and their natural hybrid, *D. wilsonii* F1: *D. exaltata* x *D. mollis*, at current mean air temperature and climate change scenarios. B, C, D Predicted time required for 50% of seeds germination as a function of increasing air temperature (linear regression). The values of temperature increase predicted for five climate change scenarios (1.5; 2; 3.5; 4.6 and 5.7°C) were added to the current average daily temperature (22.8°C) of the seed collection site (Florestal – Minas Gerais, Brazil).

Chapter III

**Fitness of a natural interspecific hybrid in *Dimorphandra* genus:
survival and growth during seedling establishment**

Fitness of a natural interspecific hybrid in *Dimorphandra* genus: survival and growth during seedling establishment

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Abstract

The evolutionary fate of natural hybridization is determined by the hybrid fitness. Specifically, their survival and growth could be affected by intrinsic genetic incompatibilities or genotype-environment interactions. Here, we evaluate the effects of hybrid fitness on the potential evolution of interspecific hybridization in the *Dimorphandra* genus, through performance comparison of F1 hybrid offspring with parental species for survival and growth traits during seedling establishment under full sun and shade environments. Both parent species, *D. exaltata* and *D. mollis* showed survival and growth traits consistent with their original habitats, Atlantic Forest and Brazilian Cerrado (savannah), respectively. The F1 hybrid offspring displayed predominantly intermediate fitness relative to its parental species, performing survival and growth generally lower than *D. exaltata* and higher than *D. mollis*. However, their fitness varied, being similar to or greater than that of either parent, depending on the light environment. In addition, the hybrids showed higher survival and final growth under sunlight. We also identified a weak extrinsic postzygotic barrier acting on the establishment of the F1 offspring under low radiation conditions, whereas, a postzygotic barrier was not identified for the high radiation environment. Our results imply that the hybrid combines traits from both parental species, potentially enabling a balanced adaptability to parental and transitional habitats, with

greater potential for colonizing environments with a high light incidence. This underscores the potential of the early generation of hybrids in contributing to gene flow between parental species, thereby enhancing genetic diversity in the *Dimorphandra* hybrid zone.

Keywords: *Dimorphandra exaltata*, *Dimorphandra mollis*, *Dimorphandra wilsonii*, ecotone, growth, hybrid zone, homoploid hybridization, postzygotic barriers, seedling establishment, survival.

Introduction

Interspecific hybridisation has played an important role in adaptation and diversification of plants through introgression and speciation (Rieseberg, 2006; Mallet, 2007; Abbott et al., 2013). Gene introgression can improve adaptation of parental species by introducing genes more suited for their habitat (Todesco et al., 2016) or even providing faster adaptation to the changing environment (Wong et al., 2022). This can be particularly advantageous for species with small population sizes and low genetic diversity (Chan et al., 2019; Wong et al., 2022). However, gene introgression also carries potential risks by introducing maladaptive alleles, reducing the overall fitness of a species, potentially leading to population decline or species extinction (Todesco et al., 2016; Edelman and Mallet, 2021). Interspecific hybridization occurs when some barrier to reproductive isolation is removed, which can occur due to natural or anthropogenic disturbance (Vallejo-Marín and Hiscock, 2016).

The evolutionary and ecological consequences of hybridization is determined by the relative fitness of hybrids, which is estimated by the performance of hybrids in comparison with their parents (Arnold et al., 2012; Walter et al., 2020). Hybrids can exhibit fitness lower, similar, intermediate, or even superior to that of their parental species, conferring them less, equivalent, or more fit in parental, intermediate to those of the parents or new habitats (Arnold and Martin, 2010; Arnold et al., 2012; Favre et al., 2017). In this sense, early establishment characteristics are essential to access hybrid fitness (Arriola and Ellstrand, 1997; Jiménez-López et al., 2023). Survival and vegetative growth experiments have been widely used for this purpose, including tests under different environmental conditions to study environmental effects on fitness (Johnston et al., 2003; Song, 2004; Barbour, et al., 2006; Sahoo et al., 2010; Marques et al., 2011; Kenzo et al., 2016). Such studies can bring insights into postzygotic barriers and the potential for gene introgression in plant hybridization.

Hybrids can exhibit heterosis, which is higher fitness than both parents (Hochholdinger and Baldauf, 2018). This phenomenon can arise from factors such as the masking of deleterious recessive mutations present in the parent species, heterozygote advantage at specific loci, or differences in gene expression (Hochholdinger and Baldauf, 2018). F2 or subsequent generations of hybrids can show new phenotypes and/or new phenotypic combinations that outperform their parents, due to transgressive segregation. This occurs from the recombination of alleles with additive, complementary, or epistatic effects (Yakimowski and Rieseberg, 2014). Conversely, postzygotic isolation barriers can be manifested during seedling establishment. This stage could be affected by intrinsic genetic incompatibilities (intrinsic barriers) or by genotype-environment interactions (extrinsic barriers) (Johnston et al., 2003; Fritz et al., 2006; Arnold et al., 2012; Karrenberg et al., 2019; He et al., 2023). Thus, at this stage, hybrid seedling lethality or inviability (Xiao et al., 2021; Deng et al., 2019; Mino et al., 2022), hybrid weakness (Shiragaki et al., 2020) and hybrid breakdown (Plötner et al., 2017; Zhang et al., 2021; Xiong and Mallet, 2022; Mino et al., 2023) can occur (Rundle et al., 2000; He et al., 2023; Jiménez-López et al., 2023). Hybrid breakdown refers to a low hybrid fitness in the F2 and/or subsequent generations compared to parental species (Plötner et al., 2017).

Among the environmental factors, light plays a crucial role acting as an environmental cue for plant adaptation in growth and development to the environment, besides providing energy for carbon fixation through photosynthesis (Kanojia, et al., 2023). During photomorphogenesis, plants remodel their growth and development according to changes in the light quantity and quality (De Wit et al., 2016). Thus, light influences processes such as seed germination, leaf and stem development and leaf senescence (Kanojia, et al., 2023). Morphological traits such as stem elongation and leaf size and thickness are influenced by light, besides physiological and biochemical processes (Mathur and Jajoo, 2018; Poorter et al., 2019; Yang and Jeong, 2022). Despite its great importance, the effect of light on hybrid fitness during

seedling establishment has been rarely studied (Emms and Arnold, 1997; Johnston et al., 2003; Kenzo et al., 2016).

Here we attempt to contribute to advances knowledge related to the *Dimorphandra* hybrid zone. The dynamic evolution of hybrid zones in the Neotropics is still poorly understood. Among the few neotropical hybrid zones being recently studied are the *Plathymenia* hybrid zone (Muniz et al., 2022) and the *Dimorphandra* (Muniz et al., 2020), both occurring in the same Cerrado/Atlantic forest ecotonal region in Minas Gerais, Brazil. These hybrid zones constitute a natural laboratory for ecological and evolutionary studies, which can bring insights about the importance of savanna/forest ecotonal areas in hybridization, lineage divergence and speciation. The *Dimorphandra* hybrid zone is formed by interspecific hybridization between *Dimorphandra exaltata* Schott and *Dimorphandra mollis* Benth., which coexist in the ecotone Cerrado/Atlantic forest. The resulting hybrid was considered a species (Rizzini, 1969) critically endangered (Fernandes, 2006), *Dimorphandra wilsonii* Rizzini. A recent study showed that *D. wilsonii* is a hybrid (Muniz et al., 2020). *D. exaltata* is also listed as a threatened species (Muniz et al., 2019; Fernandez and Moreira-Fernandes, 2021), its distribution occurs in seasonally semideciduous and ombrophilous forests (Atlantic Forest) in Southeast Brazil, where the climate is predominantly hot and humid (Alvares et al., 2013). In contrast, *D. mollis* Benth is widely distributed in the Cerrado biome (savanna), characterized by a tropical climate with a dry winter season (Aw) (Alvares et al., 2013). The *Dimorphandra* hybridization is homoploid. It was found that the hybrids are diploids, as their parents (Muniz et al., 2020). Until now, only hybrids of early generations were described, first generation F1 occurring in higher proportion, F2 (crossing: F1 x F1) and backcrosses (crossing: F1 x *D. exaltata*) with few specimens (Muniz et al., 2019). This cross with *D. exaltata* allows the gene introgression in this species (Muniz et al., 2020).

In this study we aimed to assess the fitness of the F1 hybrid, *D. wilsonii*, concerning the parental species, *D. exaltata* and *D. mollis*, detect the presence of a postzygotic barrier in the seedling establishment stage, and also estimate the genetic variation between hybrid and parental progenies. For this purpose, we evaluated the seedling establishment in a common garden under full sun and partial shade by the seedling survival and initial growth traits. We hypothesized the following: (1) Consistent with the habitats of *D. exaltata* and *D. mollis*, the first will achieve better fitness in the shaded environment, while the second in the full sun environment; (2) Considering that this is a homoploid hybridization, we expect the F1 offspring to show intermediate, similar or superior fitness to the parental species in both light environments; (3) Considering that hybridization can increase genetic variability in hybridized populations, we expect the hybrid progenies will present greater genetic variability than parent progenies.

Materials and methods

Study area, species/hybrid, and seed collection

We sampled fruits of *Dimorphandra* species and hybrid trees occurring in a Cerrado/Atlantic Forest ecotonal area in the central part of Minas Gerais state, southeastern Brazil, at different municipalities but proximally located: *D. exaltata* collected in Florestal and Contagem; *D. mollis* in Pequi; *D. wilsonii* (hybrid F1) in Juatuba. The regional climate of the sampled area is subtropical humid with a hot summer (Cwa) according to Köppen classification (Alvares et al., 2013). The dry and rainy seasons are well-defined, with the dry season occurring from April to September and the rainy season from October to March (Alvares et al., 2013).

The genotypes of the trees were previously determined, which were obtained with the same protocols in the same laboratory for other studies already published: *D. wilsonii* and *D. exaltata* individuals were genotyped for 11 SSR markers (Dmo5, Dmo7, Dmo13, Dmo20,

Dmo21, Dw21, Dw33, Dw105, Dw28, Dw52, Dw103; Muniz et al., 2019; Muniz et al., 2020); and *D. mollis* for five out of the 11 SSR loci (Dmo5, Dmo7, Dmo13, Dmo20, and Dmo21; Souza et al., 2017). We sampled fruits of individuals previously identified as parental species (*D. exaltata* and *D. mollis*) and hybrid F1 (*D. exaltata* crossed with *D. mollis*), *D. wilsonii*.

Experimental design in common garden

The fruits were collected between September and October of 2021 from 5 - 6 trees (progenies) per species and F1 hybrids. They were opened manually with a hammer to obtain the seeds. The visually well-formed seeds were selected, disinfected by immersion in sodium hypochlorite solution (NaClO 2%) for 15 min, washed with deionized water (Matheus et al., 2017), dried at room temperature, and stored in paper bags until use. For the germination process, seeds were scarified on the extreme side opposite to the hilum (Oliveira et al., 2008) using electric emery (Model MMI 50, Motomil®) and again disinfected with sodium hypochlorite solution (NaClO 5%) for 5 min and washed in deionized water (Bicalho et al., 2017). Afterward, they were sown in Gerbox-type plastic containers with paper moistened with a nystatin fungicide solution (Medley®, Brazil, 0.3%), and watered with this solution as necessary (Cruz et al., 2018). Seed germination was carried out in a germination chamber (Thoth®, Brazil) with 12h photoperiod at photosynthetic photon flux density of 50-70 $\mu\text{molm}^{-2}\text{s}^{-1}$ under constant temperatures of $28^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

On the fourth day after germination began, the germinated seeds were transplanted into vases containing 3L of vegetable soil and sand (3:1). The experimental design consisted of five progenies of each species and hybrid, except for *D. mollis*, which had six progenies due to its higher mortality rate, observed in previous tests. 12 replicates of each progeny were randomly arranged in sun and shade conditions in a common garden under semi-controlled conditions. The plants in the sun treatment were exposed to full sun and the plants in the shade treatment were placed in half shade, light data was collected using a quantum sensor (Li-COR 250A,

Germany) every 30 minutes during one day (Fig.1). Watering was carried out to field capacity as required, usually on alternating days.

Survival and growth measurements

The seedlings survival was recorded weekly until day 180 of the experiment. The values obtained were transformed as percentages. The growth measurements, height, stem diameter and leaf number were determined in each treatment replicate at seven times during growth: T1 (40 days), T2 (55 days), T3 (70 days), T4 (85 days), T5 (100 days), T6 (115 days) and T7 (200 days). The height was measured from the base near the soil to the apical shoot using metric tape. The stem diameter was measured at the base of the stem, near the soil, with a caliper at 0.01 mm precision. The leaf area was measured once, with seven months of growth. Leaves at the same nodal position were collected and scanned. The images were used to obtain the leaf area using Carl Zeiss (AxionVision 4.8.2 SP3 08-2013). The slenderness index, an indicator of stem elongation relative to stem diameter, was obtained as height/base diameter. The relative growth rate (RGR) was calculated as $RGR = [\ln(x_1))\ln(x_6)] / t_6)t_1$, where $\ln$ is the Neperian logarithm, x_1 and x_6 are seedling height, number of seedling leaves, or stem diameter at times t_1 (40 days) and t_6 (115 days), respectively. The growth rate of the progenies was obtained from the height and leaf number data, since the N of the diameter data was not sufficient.

The experiment was concluded after 20 months, when the measures of final growth were obtained. The plants were measured for stem diameter, and their roots were washed under flowing water and photographed. The images were used to obtain the root and shoot lengths using Carl Zeiss (AxionVision 4.8.2 SP3 08-2013). The plants were dried in a forced circulation oven at 70°C to obtain the total mass, root mass and shoot mass using a balance with precision of four digits. The final slenderness index was also calculated.

Data analyses

The seedlings survival was compared using a non-linear regression between survival and time comparing the curves at 95% confidence intervals. To evaluate progenies, the progenies of each species or hybrid were compared using a quadratic polynomial regression. To compare the species/hybrids, the means of the progenies obtained in each data collection were used to perform a non-linear sigmoidal dose-response regression. The growth variables (height, stem diameter, slenderness index, and leaf number) were analyzed using a simple linear regression at 95% confidence intervals to compare the progenies of each species/hybrid, and to compare the species and hybrids between them. We also compared the species and hybrids for these variables at each growth time (T1, T2, T3, T4, T5, T6 and T7) using two-way ANOVA (species/hybrid * light) followed by Tukey test with a significant level at $P < 0.05$. The leaf area, the final growth and the relative growth rate (RGR) were also analyzed using two-way ANOVA followed by Tukey test with a significant level at $P < 0.05$. The progenies of each species/hybrid were compared in the sun and shade environments (progeny * light), as were the species and hybrid (species/hybrid * light). All data compared with ANOVA showed normal distribution when submitted to the Shapiro-Wilk test, and homogeneity of variance in the analysis of the residuals in ANOVA test.

Results

Survival and growth of the progenies

The survival of progenies (Fig. 2) from species and the hybrid was influenced by light conditions. For *D. exaltata* the survival percentages appear to be slightly higher under shade than under sun. There was a difference in the survival of its progenies in both light environments (Fig. 2 A, B). For the hybrid, *D. wilsonii*, except for progeny 4, survival was higher in the sun. There was a difference in the survival of the progenies in both light conditions (Fig. 2 C, D).

For *D. mollis*, survival was also higher in the sun, with a difference between the progenies in both light conditions (Fig. 2 E, F). For both species and the hybrid, the range of variation in survival between progenies was higher in the shade. This amplitude was higher for *D. mollis* than for *D. exaltata* in both light environments. *D. wilsonii* showed a range of variation between progenies intermediate to its parents in both light environments (Fig. 2).

The height of progenies (Fig. 3) for *D. exaltata* depended on the light incidence, there was a difference between its progenies only under sun (Fig. 3 A, B). For *D. wilsonii*, there was a slight difference between the progenies in both light environments (Fig. 3 C, D). For *D. mollis*, there was difference between the progenies under both, sun and shade (Fig. 3 E, F). The stem diameter also differed between the progenies and among the progenies in the different light environments for the species and for the hybrid (Fig. 4). For the progenies of *D. exaltata*, the slenderness index performed differently between the light environments, with a difference between the progenies only under shade (Fig. 5 A, B). For *D. wilsonii* and *D. mollis*, there was no difference between the progenies, independent of the light environment (Fig. 5 C, D, E, F). For the leaf number, there was a difference between the progenies of *D. exaltata* only under sun, while for *D. wilsonii* and *D. mollis*, the progenies differed in both light environments (Fig. 6). The leaf area of progenies (Fig. 7) in *D. exaltata* differed between them only in the sun environment. Two of its progenies (P3 and P4) showed higher leaf area under sun, compared to shade (Fig. 7 A). For the progenies of *D. wilsonii*, only progeny 5 differed from the others, showing greater leaf area under sun (Fig. 7 B). For *D. mollis*, there was a difference in leaf area between progenies 2 and 5 under shade, and between light environments for progenies 3 and 5 (Fig. 7 C). The relative growth rate did not differ between the progenies of the species or the hybrid (These data were not shown in figures or tables).

Survival and growth of the species and the hybrid

The survival was high for both, *D. exaltata* and *D. mollis*, and for the hybrid, *D. wilsonii*, until the 50th day of growth (Fig 8 A, B). This highlights the importance of evaluating this variable over a longer period, as we did. The survival analysis over 180 days revealed great differences in survival patterns between the two species under sun and shade environments (Fig 8 A, B). *D. exaltata* demonstrated a higher survival percentage under both light conditions, with survival curves showing a decrease over time, but maintaining a higher percentage of surviving individuals compared to *D. mollis*, particularly under shade exposure, where *D. exaltata* maintained a high survival percentage, while *D. mollis* showed more pronounced mortality (Fig 8 A, B). The hybrid, *D. wilsonii*, displayed intermediate survival relative to its parents, particularly in sun environment. Under sun exposure, *D. wilsonii* showed a decline in survival that was less severe than *D. mollis*, but did not reach the resilience observed in *D. exaltata*. Under shade conditions, the survival of *D. wilsonii* was intermediate to its parents at the beginning of the evaluated period, and lower than that of both parents at the end of this period (Fig 8 A, B). After 18 months of growth, the survival under the sun was 26% for *D. exaltata*, 38.5% for *D. wilsonii* and 30.4% for *D. mollis*, under shade was 79.6% for *D. exaltata*, 21.6% for *D. wilsonii* and 26.7% for *D. mollis*. Therefore, under sun the hybrid showed slightly higher survival than its parents, and under shade, lower than *D. exaltata* and similar to *D. mollis*.

The results for growth shown in Figure 8, from the regression analysis, are corroborated by the ANOVA analyses shown in Table 1. They reveal consistent patterns in the growth of *D. exaltata*, *D. mollis* and *D. wilsonii*. Comparing the initial growth of *D. exaltata* and *D. mollis*, *D. exaltata* maintained higher growth in terms of height, stem diameter and slenderness index in the sun environment during all the evaluation period, and until 100 days, in the shade environment. *D. exaltata* still maintained higher growth for height and stem diameter at 115 days, and for stem diameter at 200 days in the shade environment (Fig. 8; Table 1). Leaf number

also varied between species. In both light environments, *D. exaltata* produced more leaves at the beginning of growth, while *D. mollis* surpassed *D. exaltata* at the end (T4 and T5). However, in the sun environment, around the middle of the experiment (T4 and T5), *D. mollis* maintained a similar number of leaves than *D. exaltata*, while in the shade environment this occurred at times T2, T3, T4 and T5 (Fig. 8; Table 1).

The hybrid, *D. wilsonii*, exhibited predominantly intermediate growth characteristics between its parental species, showing growth lower than *D. exaltata* and higher than *D. mollis* (Fig. 8; Table 1). Until T5 (100 days), *D. wilsonii* showed height intermediate between its parental under both light conditions. From T6, *D. wilsonii* remains lower in height than *D. exaltata*, but becomes similar to *D. mollis*, except at T7, when species and hybrid are similar in height (Table 1). The stem diameter was also intermediate for *D. wilsonii* between its parents (Fig. 8 E, F) predominantly in the sun at T1, T2, T4 and T5. And was, at most of the times evaluated (Sun: T6 and T7; Shade: T2, T3 and T5), similar to *D. mollis*, and only at T3 under, sun similar to *D. exaltata* (Table 1). The slenderness index of *D. wilsonii* also supports its intermediate status under sun until T5. At other moments it oscillated, showing similarity to *D. mollis* in the sun, and predominantly to *D. exaltata* in the shade (Fig. 8 G, H; Table 1). Leaf production in *D. wilsonii* was sometimes similar to its parents, other times closer to *D. mollis* (Fig. 8 I, J; Table 1).

The results of leaf area (Fig. 7 D) show that under both sun and shade conditions, *D. exaltata* exhibits higher leaf area than *D. mollis*. The leaf area of *D. exaltata* depended on the light environment, being higher in the sun, while *D. wilsonii* and *D. mollis* showed similar values in both light conditions. *D. wilsonii* demonstrated an intermediate leaf area between *D. exaltata* and *D. mollis* growing under sun condition, smaller than *D. exaltata* and larger than *D. mollis*. Under shade, *D. wilsonii* maintains a larger leaf area than *D. mollis*, but similar to *D. exaltata*.

The relative growth rate was higher for *D. exaltata* than *D. mollis* in height under sun and shade conditions (Fig. 9 A), and in stem diameter only under shade (Fig. 9 B). In leaf number, *D. mollis* achieved a higher RGR than *D. exaltata* (Fig. 9 C). For height, *D. wilsonii* has a lower RGR than *D. exaltata* and similar to *D. mollis* in both light conditions (Fig. 9 A). This pattern was the same for stem diameter under shade, but the RGR did not differ between hybrid and both parental under sun for this variable (Fig. 9 B). While for leaf number, the hybrid does not significantly differ from either parental under both light environments (Fig. 9 C).

Comparing the survival and growth of each species and the hybrid between the light environments, *D. exalta* showed greater stem elongation under sun conditions, maintaining greater height (~ 1 cm) in the sun than in the shade throughout the period evaluated. It also maintained a higher slenderness index in the sun at most of the times evaluated (T2, T4, T5, T6 and T7) (Table 1). However, its growth rate was generally higher in the shade (Fig. 9 A, B), and its survival was superior under shade. On the other hand, *D. mollis* did not show much variation in survival and growth between the light environments. It did not differ in height under both light environments. Its slenderness index was higher in the sun during T1-T3. Its number of leaves, in contrast, was higher from T1 to T5 in the sun (Table 1). *D. mollis* showed a similar RGR under sun and shade conditions (Fig. 9 A, B, C). The survival and growth of *D. wilsonii* differed slightly between the light environments. Its survival was slightly higher under sun. Its height was higher under sun at times T2, T3, T4, its stem diameter was higher under sun only at T2 and T4. Its leaf production was higher in the sun between T1 and T6 (Table 1). Its RGR did not differ between light environments (Fig. 9 A, B, C).

Final growth of of the species and the hybrid

The results of the final growth (Table 2) showed that, after 19 months and 20 days, *D. exaltata* grew more than *D. mollis* in both light conditions. *D. exaltata* showed higher values of mass for root and shoot, and total mass, also higher values of shoot length and stem diameter.

D. exaltata showed a higher slenderness index in shade conditions. In terms of proportion between root and shoot, under shade conditions, *D. exaltata* showed higher investment in mass for the shoot relative to total mass. In contrast, *D. mollis* showed higher investment in mass for root and lower in shoot relative to total mass. *D. mollis* also invested more in root mass and length concerning the shoot than *D. exaltata* in both light conditions (Table 2).

Compared to its parents, the hybrid *D. wilsonii* showed intermediate growth in some aspects. In sun condition, *D. wilsonii* showed higher values in root mass, total mass and stem diameter than *D. mollis*, and similar to *D. exaltata*. In shade condition, *D. wilsonii* showed lower values in root mass, shoot mass, total mass, shoot length, stem diameter and slenderness index than *D. exaltata*, with the same variables being similar to *D. mollis*. In the other cases, *D. wilsonii* was similar to both parents. Comparing the final growth between the light environments, *D. exaltata* showed similar growth under sun and shade, except for the slenderness index, which was higher in the shade. *D. mollis* showed similar growth under both light environments, except for root length, which was higher in the sun. *D. wilsonii* accumulated approximately twice as much mass under sun than under shade, both for root and total mass.

Discussion

Survival and growth of the progenies

The results showed that the *D. mollis* progenies had a higher range of variation in survival percentage than *D. exaltata* progenies, regardless of the light environment, under full light or shade. *D. wilsonii* F1 progenies showed a range of variation intermediate to parental species (*D. mollis* and *D. exaltata*) in both light environments. This finding corroborates with genetic results obtained for these species, in which *D. mollis* shows greater genetic variability than *D. exaltata*, and the genetic variability of *D. wilsonii* was high as that of *D. mollis* (Muniz et al., 2019). For *D. wilsonii*, an interspecific hybrid, high genetic variability is expected due to

genetic inheritance from both parents, heterozygosity, and gene recombinations (Abbott and Brennan, 2014; Wong et al., 2022).

In *D. exaltata*, the variation of growth between progenies depended on the light environment. The height, leaf number, and leaf area differed between progenies and varied widely only under sunlight. The stem diameter had a higher amplitude of variation under sunlight. As it is a typical forest species, full light may represent light stress (Costa et al., 2025). Since there is no evidence, until now, of gene introgression in the *D. mollis* population of the ecotone where hybridization occurs, the high variation among the progenies of this species must be explained by its wide distribution, probably adapted to a broad environmental gradient (Gonçalves et al., 2010). For *D. wilsonii* there was also great variation in the performance of the same progeny between the sun and shade environments, especially in terms of survival. For the progenies that we tested, with the exception of only one, the shade environment led to high mortality. This demonstrates the genotype-environment interaction, indicating the existence of a weak extrinsic postzygotic barrier dependent on light conditions (Karrenberg et al., 2019). This high mortality under shade may be associated with a reduced photosynthetic rate in this environment (Arntz et al., 2000).

Survival and growth of the species and the hybrid

The survival patterns between the *Dimorphandra* species are consistent with their original habitats. It showed that *D. exaltata*, from forest, achieved greater survival under shade, while *D. mollis*, from Cerrado (Brazilian savannah), under sun, suggesting that *D. exaltata* has greater adaptability to shaded environments, although it also shows high survival in the sun, while *D. mollis* to environments with high light incidence. The growth comparison between these two species supports their distinct ecological strategies, reflecting their forest and savannah habitats, respectively. *D. exaltata* consistently outperformed *D. mollis* in terms of height, stem diameter, and slenderness index during the initial growth stages under both, sun

and shade conditions, also evidenced in the RGR results. In general, *D. exaltata* grew more under shade, while *D. mollis* under full sun. Compared to the shade environment, under high-light condition tropical tree species that demand more light display greater survival and growth, which are associated with greater CO₂ assimilation capacity in this condition (Cifuentes and Moreno, 2022). While the survival of shade-tolerant species in high-light conditions is associated with the ability to dissipate the excess of light, such as high non-photochemical quenching and thicker leaves (Cifuentes and Moreno, 2022).

Regarding leaf number, *D. exaltata* produced more leaves at the beginning of growth, while *D. mollis* surpassed *D. exaltata* at the end, obtaining a higher RGR for this trait. However, the leaf area of *D. exaltata* was higher in both light environments. Besides growth, the results for leaf area also show that *D. exaltata* does not present an adaptive strategy for this trait, presenting greater leaf area growing in high-light environment, which leads to higher water loss through transpiration (Markesteijn, et al., 2007). In contrast, *D. mollis* maintained a similar leaf area in both light environments, showing a fixed adaptation to avoid water loss, typical of Cerrado trees (Bucci et al., 2005). These traits are consistent with the adaptation of *D. exaltata* to forest ecosystems, where competition for light in the shaded understory drives large leaf area (Poorter and Rozendaal, 2008; Niinemets et. al., 2015), elongation and slenderness, conferring faster growth and greater height to the trees than in the Cerrado, where the incidence of light is high (Pearcy, et al., 2005). Conversely, *D. mollis*, a savannah species, demonstrated a distinct growth pattern with increased leaf production, particularly under sunlight, in which it achieved the greatest growth. This suggests an emphasis on maximizing photosynthetic capacity to deal with the high light intensity and seasonal water limitations of the Cerrado (Chaves et al., 2002; Pearcy, et al., 2005).

The intermediate survival of *D. wilsonii* F1 offspring relative to the parental species implies that this hybrid combines survival traits from both parents, as found in many

interspecific hybrids (Glombik et al., 2020; Wong et al., 2022), potentially enabling a balanced adaptability to varying light conditions. However, the higher survival of *D. wilsonii* F1 offspring under sun exposure than under shade suggests that this hybrid is better suited to environments with a high light incidence. The better survival of *D. wilsonii* F1 offspring under high radiation coincides with greater growth under this light environment. Whenever there was a difference in growth parameters between the two light environments for *D. wilsonii* F1 offspring, the values were higher under the sun environment, which reinforces the probable relationship between better survival and growth performance with higher photosynthetic rates under high light.

The initial growth of *D. wilsonii* F1 offspring was predominantly intermediate between its parental species, being lower than *D. exaltata* and higher than *D. mollis*. Another pattern observed in *D. wilsonii* growth was its greater similarity to *D. mollis*, in this case, showing less growth than *D. exaltata* and similar to *D. mollis*. An exception to this last pattern was the leaf area, with intermediate values in the sun and similar to *D. exaltata* in the shade. Together, these findings indicate that *D. wilsonii* expresses a balanced combination of growth characteristics from both parental species. This potentially confers to the *D. wilsonii* F1 offspring the aptitude to establish in different light environments, such as those with direct light incidence, like savannas, and with variable light, such as forests, and also transitional habitats, such as forest-savannah ecotones. However, the greater similarity of *D. wilsonii* to *D. mollis* in terms of growth patterns, as well as the better growth in the sun, suggests that this hybrid may be better suited to environments with a high light incidence. The intermediate leaf area and similar leaf number observed in *D. wilsonii* F1 offspring related to its parents, also suggests ecological adaptability to varied light environments by balancing photosynthetic and water use efficiency (Chaves et al., 2002), also expanding the ecological niche of the hybrids.

Final growth of the species and the hybrid

The results of the final growth showed that *D. exaltata* grew more than *D. mollis* under both light conditions, achieving higher dry mass than *D. mollis*, especially under shade. This is coherent with these species size in adulthood, according to their habitats. *D. exaltata* reaches a height of around 9 - 15 m (Fortes et al., 2020), while *D. mollis* of 3.5 - 10 m (Silva, 1986). The proportion of mass and length investment between root and shoot, are also consistent with the specializations of these species for their respective habitats. *D. exaltata* exhibits a growth strategy characterized by substantial resource allocation to shoot development, an adaptation to forest environments where intense light competition under the canopy necessitates investment in shoots to maximize light capture, while water availability is generally not a limiting factor (Costa et al., 2025; Niinemets and Valladares, 2006). On the other hand, *D. mollis* prioritizes root development over shoot growth, a common adaptive strategy among Cerrado species (Abdala et al., 1998). In this biome, light is abundant, but water scarcity during prolonged dry seasons imposes significant physiological constraints. Enhanced root investment facilitates water acquisition improving drought resilience and ensuring survival under arid conditions (Qi et al., 2019; Guillemot et al., 2022).

The final growth fitness of *D. wilsonii* F1 offspring depended on the light conditions. Under full sun, its growth was closer to that of *D. exaltata*, while under shade it was closer to *D. mollis*. Concerning the proportion of mass and length between root and shoot, *D. wilsonii* F1 offspring was similar to both parents. These results suggest that *D. wilsonii* F1 offspring showed plasticity in adapting to the different light environments it was submitted to. And again, these results reinforce that *D. wilsonii* F1 offspring may have a high capacity to adapt to the habits of both parental species, as well as, intermediate habitats. And, the fact that *D. wilsonii* F1 offspring has accumulated approximately twice as much mass under full sun than the shade environments reinforces that this hybrid must be better suited to environments with a higher

light incidence. This final growth expressively higher under high radiation coincides with the higher final survival under this environment, as occurred for the initial growth results.

The growth of *D. wilsonii* F1 offspring was similar or greater than that of either parent, reaching, for example, total mass similar to *D. exaltata*, which was higher than *D. mollis*. This points out that the F1 progenies could be fertile. There is a strong positive correlation between biomass and fecundity, such as greater flower, fruit, and seed production, which increases the likelihood of leaving viable offspring (Younginger et al., 2017). This also shows, together with survival results, that the hybrids have high relative fitness at seedling stages under full sun and shade conditions, as observed for F2 hybrids between *Iris brevicaulis* and *Iris fulva*, (Johnston et al., 2003), although it depends on the light environment, performing better under full sun. These results are consistent with other studies showing that relative hybrid fitness can vary depending on the environment (Arntz et al., 2000; Johnston et al., 2003; Kenzo et al., 2016; Fritz et al., 2006; Yorkston and Daehler, 2006). Thus, the action of a weak extrinsic postzygotic barrier, dependent on the environment (Karrenberg et al., 2019; Bjerkan et al., 2020) was observed in the *D. wilsonii* F1 offspring during seedling establishment only in shaded conditions. For the full sun environment, was not identified a barrier to preventing survival, establishment and growth of the *D. wilsonii* F1 offspring, as observed in several cases of interspecific hybridization (Marques et al., 2011; Wong et al., 2022; Sohn et al., 2022).

Conclusions

Our results corroborated the initial hypotheses. *D. exaltata* exhibited superior survival and growth under shade environment, while *D. mollis* under full sunlight. Also traits such as leaf area and root: shoot ratio support their distinct ecological strategies in their original habitats, forest and Cerrado (savannah), respectively. The *D. wilsonii* F1 offspring displayed predominantly intermediate fitness relative to its parental species, performing survival and

growth generally lower than *D. exaltata* and higher than *D. mollis*. However, the fitness of these hybrids has varied, being similar to or greater than that of either parent, depending on light exposure. In addition, the hybrids showed higher survival and better final growth under sunlight. We also identified a weak extrinsic postzygotic barrier acting on the establishment of the *D. wilsonii* F1 offspring under low radiation conditions, whereas, for the high radiation environment, no postzygotic barrier was identified at this stage of the life cycle.

These findings imply that the hybrid combines traits from both parental species, potentially enabling a balanced adaptability to varying light conditions, with greater potential for colonizing environments with a high light incidence. This indicates that the F1 offspring have aptitudes for establishing themselves in parental or transitional habitats, such as forest, savannas, and Cerrado-Atlantic Forest ecotones. Additionally, the absence of significant postzygotic barriers to the establishment of the hybrids early generation underscores their evolutionary potential in contributing to gene flow between parental species, thereby enhancing genetic diversity and possibly expanding ecological niches. Future studies are needed to investigate the reproductive potential and long-term fitness of subsequent hybrid generations to better understand the persistence and evolutionary implications of *Dimorphandra* hybridization.

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TABLES

Table 1 Initial growth over time (T) of *Dimorphandra* species and their natural hybrid (*D. wilsonii*) in a greenhouse over 200 days (mean $\pm$ standard deviation, n = 30 - 50)

	Sun			Shade			P value	F value	Df
	<i>D. exaltata</i>	<i>D. wilsonii</i>	<i>D. mollis</i>	<i>D. exaltata</i>	<i>D. wilsonii</i>	<i>D. mollis</i>			
T1 (40 days)									
Height (cm)	6.52 ± 1.29a	5.19 ± 1.29bc	3.65 ± 0.73d	5.44 ± 1.17b	4.76 ± 1.52c	3.36 ± 0.74d	< 0.0001	64.26	5
Stem diameter (mm)	2.16 ± 0.25a	2.08 ± 0.36ab	1.7 ± 0.33d	1.96 ± 0.31bc	1.89 ± 0.38bc	1.82 ± 0.25cd	< 0.0001	16.2	5
Slenderness index (cm/mm)	3.02 ± 0.7a	2.53 ± 0.74b	2.17 ± 0.61c	2.81 ± 0.59ab	2.55 ± 0.9b	1.83 ± 0.47d	< 0.0001	24.31	5
Leaf number	3.89 ± 0.3a	3.74 ± 0.6ab	3.48 ± 0.64b	3.64 ± 0.62ab	3.13 ± 0.77c	2.9 ± 0.79c	< 0.0001	18.43	5
T2 (55 days)									
Height (cm)	7.59 ± 1.17a	5.72 ± 1.31c	3.75 ± 0.79e	6.4 ± 1.05b	4.95 ± 1.63d	6.62 ± 0.75e	< 0.0001	108.33	5
Stem diameter (mm)	2.2 ± 0.25a	1.91 ± 0.31b	1.6 ± 0.3c	2.24 ± 0.31a	2 ± 0.42b	1.93 ± 0.41b	< 0.0001	27.87	5
Slenderness index (cm/mm)	3.45 ± 0.64a	2.95 ± 0.66b	2.35 ± 0.58d	2.89 ± 0.53bc	2.55 ± 0.91cd	1.89 ± 0.54e	< 0.0001	38.48	5
Leaf number	5.58 ± 0.89a	4.97 ± 1.21b	4.6 ± 0.8b	3.91 ± 0.27c	3.6 ± 1.16c	3.63 ± 0.92c	< 0.0001	39.25	5
T3 (70 days)									
Height (cm)	8.42 ± 1.58a	6.18 ± 1.4c	3.87 ± 0.76e	7.48 ± 1.24b	5.4 ± 1.57d	3.62 ± 0.67e	< 0.0001	129.84	5
Stem diameter (mm)	2.29 ± 0.35a	2.1 ± 0.37a	1.53 ± 0.26c	2.28 ± 0.28a	1.83 ± 0.36b	1.68 ± 0.37bc	< 0.0001	48.97	5
Slenderness index (cm/mm)	3.7 ± 0.69a	3.01 ± 0.7b	2.59 ± 0.7c	3.29 ± 0.53ab	2.97 ± 0.92bc	2.21 ± 0.61d	< 0.0001	29.22	5
Leaf number	6.28 ± 0.71a	5.73 ± 1.03ab	5.55 ± 1.33b	5.2 ± 0.93bc	4.13 ± 1.48d	4.71 ± 1.17cd	< 0.0001	20.7	5
Time 4 (85 days)									
Height (cm)	8.66 ± 1.62a	5.9 ± 1.07c	3.9 ± 0.87e	7.67 ± 1.16b	5.08 ± 1.44d	3.68 ± 0.8e	< 0.0001	161.46	5
Stem diameter (mm)	2.16 ± 0.35a	1.87 ± 0.25b	1.52 ± 0.23c	2.27 ± 0.26a	1.93 ± 0.32b	1.59 ± 0.31c	< 0.0001	57.41	5
Slenderness index (cm/mm)	3.93 ± 0.86a	3.26 ± 0.81b	2.61 ± 0.71c	3.37 ± 0.58b	2.73 ± 0.96c	2.35 ± 0.61c	< 0.0001	30.64	5
Leaf number	7.27 ± 0.71a	7.23 ± 1.34a	6.95 ± 1.71a	6.1 ± 0.7b	5.02 ± 1.79c	5.76 ± 1.27bc	< 0.0001	20.6	5
T5 (100 days)									
Height (cm)	9.47 ± 1.74a	6.3 ± 1.07c	4.25 ± 0.94d	8.62 ± 1.3b	5.84 ± 1.14c	4.04 ± 0.97d	< 0.0001	143.86	5
Stem diameter (mm)	2.18 ± 0.42a	2 ± 0.44bc	1.55 ± 0.23d	2.4 ± 0.36ab	1.77 ± 0.35cd	1.53 ± 0.25d	< 0.0001	44.31	5
Slenderness index (cm/mm)	4.47 ± 1.24a	3.32 ± 1.03b	2.8 ± 0.68cd	3.63 ± 0.54b	3.37 ± 0.73bc	2.67 ± 0.61d	< 0.0001	24.78	5
Leaf number	7.91 ± 0.99a	8.23 ± 1.73a	8.39 ± 1.77a	6.81 ± 1b	6.21 ± 1.67b	6.93 ± 1.3b	< 0.0001	13.22	5
T6 (115 days)									
Height (cm)	9.49 ± 1.93a	6.06 ± 2.4b	6.4 ± 1.2b	9.23 ± 1.24a	6.15 ± 1.46b	5.79 ± 2.48b	< 0.0001	27.82	5
Stem diameter (mm)	2.22 ± 0.4a	1.94 ± 0.36bc	1.98 ± 0.78bc	2.58 ± 0.34b	1.85 ± 0.38bc	1.77 ± 0.44c	< 0.0001	12.9	5
Slenderness index (cm/mm)	4.28 ± 0.83a	3.42 ± 0.85bc	3.08 ± 0.45c	3.59 ± 0.55b	3.38 ± 0.8bc	3.21 ± 0.8bc	< 0.0001	12.25	5
Leaf number	7.8 ± 1.25bc	9.05 ± 1.66ab	9.4 ± 1.77a	7.36 ± 0.85c	7.04 ± 1.64c	9.36 ± 3.42a	< 0.0001	9.65	5
T7 (200 days)									
Height (cm)	15.5 ± 4.32a	10.85 ± 2.94b	9.25 ± 1.48b	11.8 ± 2.1b	10.44 ± 2.67b	8.3 ± 3.11b	< 0.0001	11.42	5
Stem diameter (mm)	3.55 ± 0.94a	3 ± 0.55bc	2.9 ± 0.53abc	3.89 ± 0.43ab	3.21 ± 0.7abc	2.38 ± 0.64c	< 0.001	4.87	5
Slenderness index (cm/mm)	4.26 ± 0.6a	3.61 ± 0.73b	3.24 ± 0.4b	3.5 ± 0.55b	3.03 ± 0.5b	3.47 ± 0.85b	< 0.0001	8.74	5
Leaf number	6.13 ± 3.29c	9.64 ± 2.25a	10.11 ± 1.16ab	6.82 ± 3.05bc	10.64 ± 3.22a	10.57 ± 4.42a	< 0.0001	8.13	5

Data were compared by two-way ANOVA followed by Tukey test ($p < 0.05$). Means followed by the same letters are not significantly different.

Table 2 Final growth and of *Dimorphandra* species and their natural hybrid (*D. wilsonii*) in a greenhouse over 20 months (mean $\pm$ standard deviation, n = 6)

Traits	Sun			Shade			P value	F value	Df
	<i>D. exaltata</i>	<i>D. wilsonii</i>	<i>D. mollis</i>	<i>D. exaltata</i>	<i>D. wilsonii</i>	<i>D. mollis</i>			
Root mass (g)	9.85 $\pm$ 1.7 ^{ab}	7.85 $\pm$ 0.11 ^b	3.35 $\pm$ 1.04 ^c	11.09 $\pm$ 1.35 ^a	3.81 $\pm$ 1.46 ^c	2.2 $\pm$ 0.47 ^c	< 0.0001	41.07	5
Shoot mass(g)	8.34 $\pm$ 2.24 ^{ab}	5.57 $\pm$ 3.48 ^{bc}	2 $\pm$ 1.54 ^c	12.36 $\pm$ 4.09 ^a	1.64 $\pm$ 0.41 ^c	1.37 $\pm$ 0.44 ^c	< 0.0001	16.7	5
Total mass (g)	18.2 $\pm$ 3.6 ^{ab}	14.65 $\pm$ 4.11 ^b	5.35 $\pm$ 2.5 ^c	23.46 $\pm$ 5.34 ^a	4.95 $\pm$ 1.28 ^c	4.16 $\pm$ 1.84 ^c	< 0.0001	29.44	5
Root: total mass ratio	0.53 $\pm$ 0.04 ^{ab}	0.63 $\pm$ 0.09 ^{ab}	0.65 $\pm$ 0.12 ^a	0.48 $\pm$ 0.05 ^b	0.58 $\pm$ 0.12 ^{ab}	0.65 $\pm$ 0.06 ^a	0.0169	3.35	5
Shoot: total mass ratio	0.45 $\pm$ 0.04 ^{ab}	0.4 $\pm$ 0.12 ^{ab}	0.31 $\pm$ 0.1 ^b	0.51 $\pm$ 0.05 ^a	0.41 $\pm$ 0.12 ^{ab}	0.34 $\pm$ 0.06 ^b	0.0132	3.56	5
Root: shoot mass ratio	1.07 $\pm$ 0.22 ^b	1.32 $\pm$ 0.5 ^{ab}	1.69 $\pm$ 0.63 ^a	0.95 $\pm$ 0.2 ^b	1.32 $\pm$ 0.52 ^{ab}	2 $\pm$ 0.55 ^a	0.0087	3.93	5
Shoot length (cm)	45.76 $\pm$ 10.25 ^a	32.85 $\pm$ 12.05 ^{ab}	23.08 $\pm$ 9.96 ^b	47.88 $\pm$ 8.98 ^a	26.78 $\pm$ 7.87 ^b	17.48 $\pm$ 3.01 ^b	< 0.0001	9.83	5
Root length (cm)	21.95 $\pm$ 1.09 ^{ab}	21.8 $\pm$ 1.02 ^{ab}	28.13 $\pm$ 2.93 ^a	22.63 $\pm$ 1.91 ^{ab}	22.6 $\pm$ 3.88 ^{ab}	20.48 $\pm$ 5.71 ^b	0.0349	2.87	5
Root: shoot length ratio	0.49 $\pm$ 0.09 ^b	0.69 $\pm$ 0.27 ^{ab}	1.34 $\pm$ 0.64 ^a	0.49 $\pm$ 0.12 ^b	0.87 $\pm$ 0.17 ^{ab}	1.21 $\pm$ 0.43 ^a	0.0011	5.78	5
Stem diameter (mm)	7.62 $\pm$ 1.66 ^a	7.12 $\pm$ 2 ^{ab}	4.88 $\pm$ 1.22 ^c	7.24 $\pm$ 1.12 ^a	5.47 $\pm$ 0.8 ^{bc}	4.11 $\pm$ 0.98 ^c	< 0.0001	14.35	5
Slenderness index (cm/mm ⁻¹)	5 $\pm$ 0.47 ^b	4.09 $\pm$ 0.97 ^{ab}	4.03 $\pm$ 0.98 ^b	6.23 $\pm$ 1.14 ^a	4.47 $\pm$ 0.82 ^b	4.47 $\pm$ 0.81 ^b	< 0.0001	7.47	5

Data were compared by two-way ANOVA followed by Tukey test ($p < 0.05$). Means followed by the same letters are not significantly different.

FIGURES

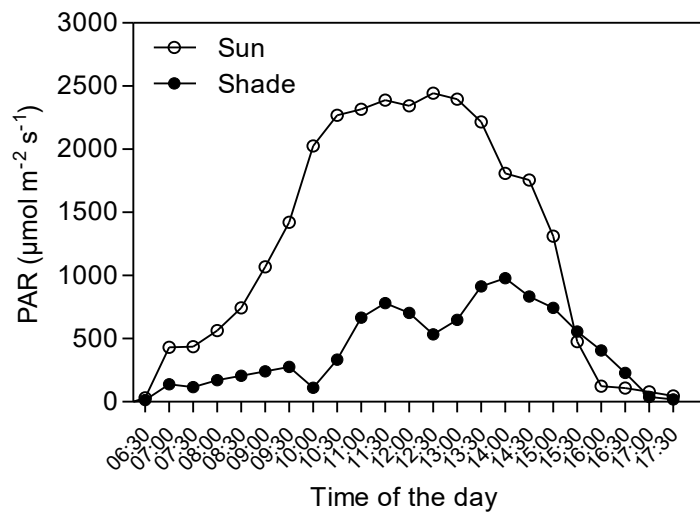


FIGURE 1 Photosynthetic active radiation (PAR) in the full sun and shade environments at the common garden where the experiment was conducted. The measurements were carried out every 30 minutes for one day.

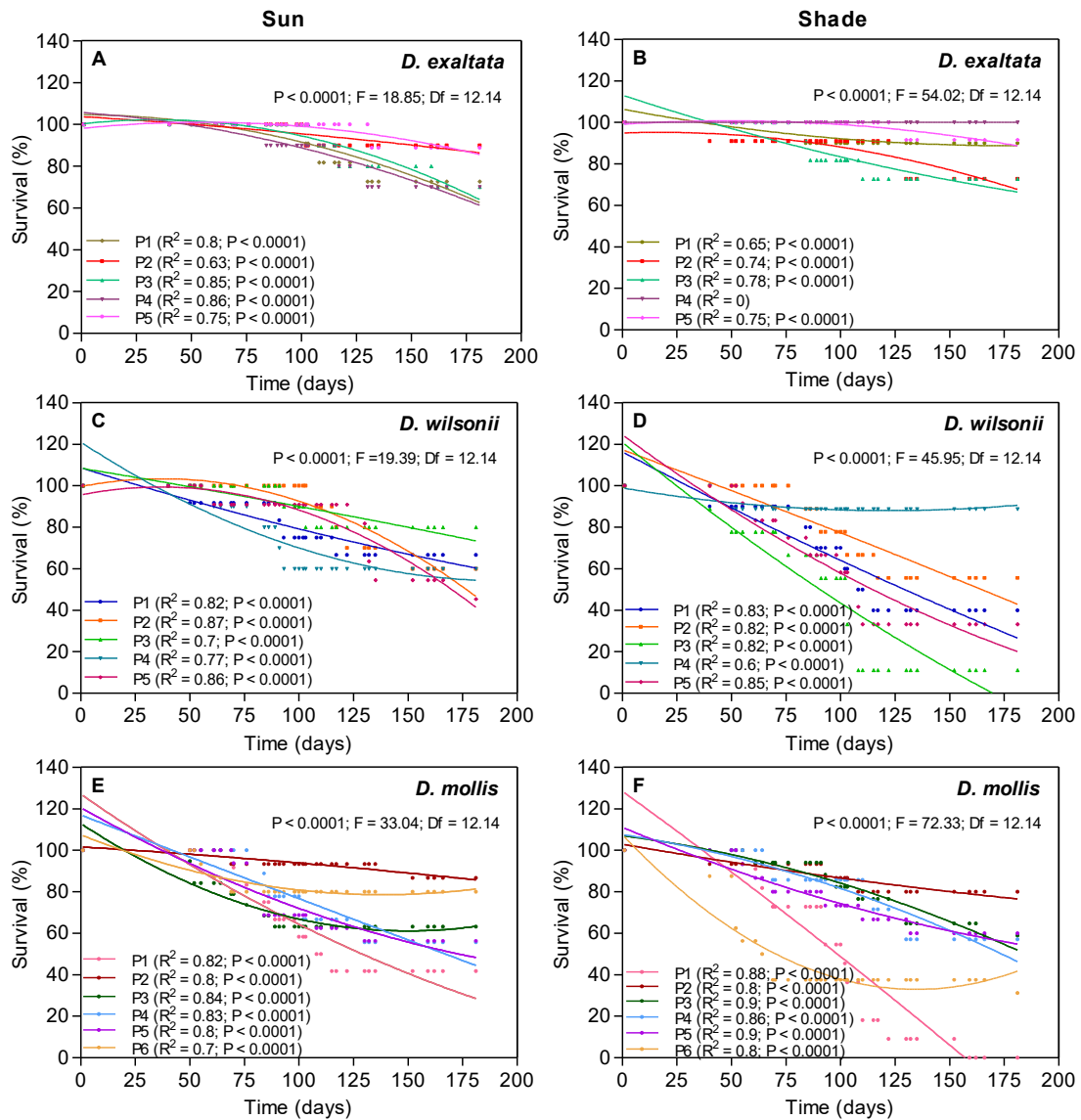


FIGURE 2 Survival of progenies (P) of *Dimorphandra* species and their natural hybrid (*D. wilsonii*) grown under sun and shade conditions until 180 days. The curves were performed and compared using a non-linear quadratic polynomial regression at 95% confidence intervals. P, F and Df values refers to the comparison between the curves.

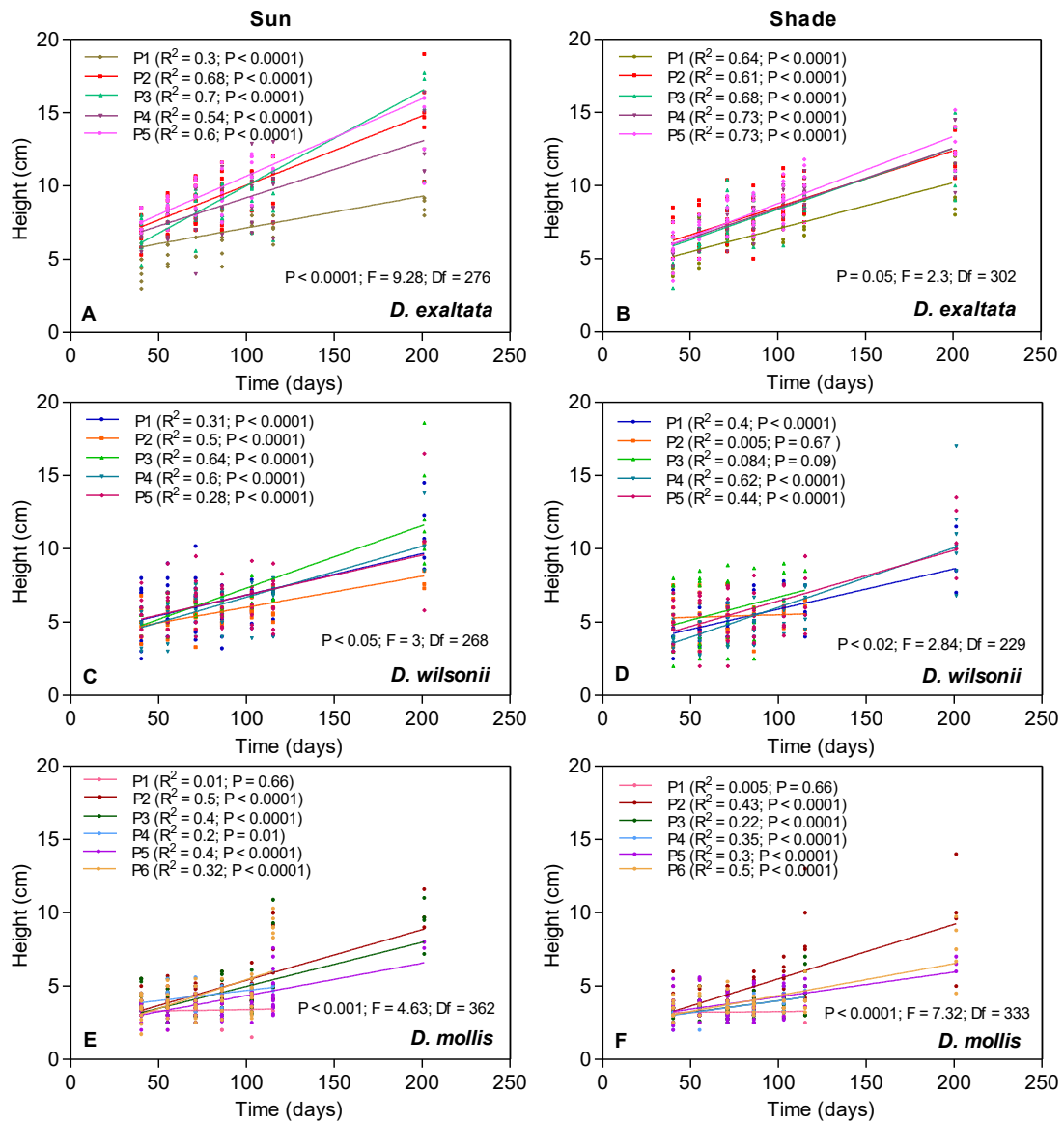


FIGURE 3 Height of progenies (P) of *Dimorphandra* species and their natural hybrid (*D. wilsonii*) grown under sun and shade conditions until 200 days. The regression lines were performed and compared using a simple linear regression at 95% confidence intervals. P, F and Df values refers to the comparison between regression lines.

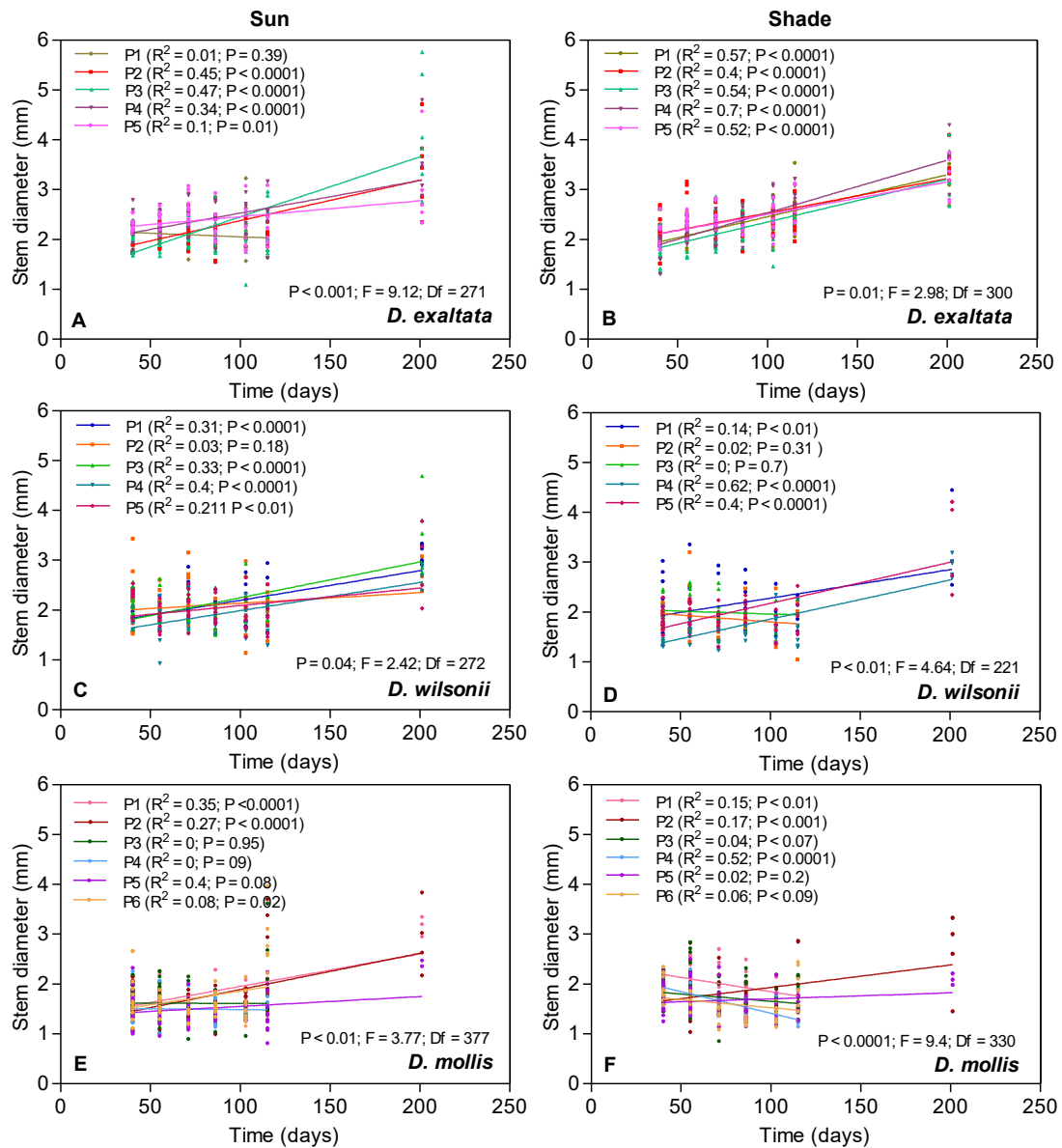


FIGURE 4 Stem diameter of progenies (P) of *Dimorphandra* species and their natural hybrid (*D. wilsonii*) grown under sun and shade conditions until 200 days. The regression lines were performed and compared using a simple linear regression at 95% confidence intervals. P, F and Df values refers to the comparison between regression lines.

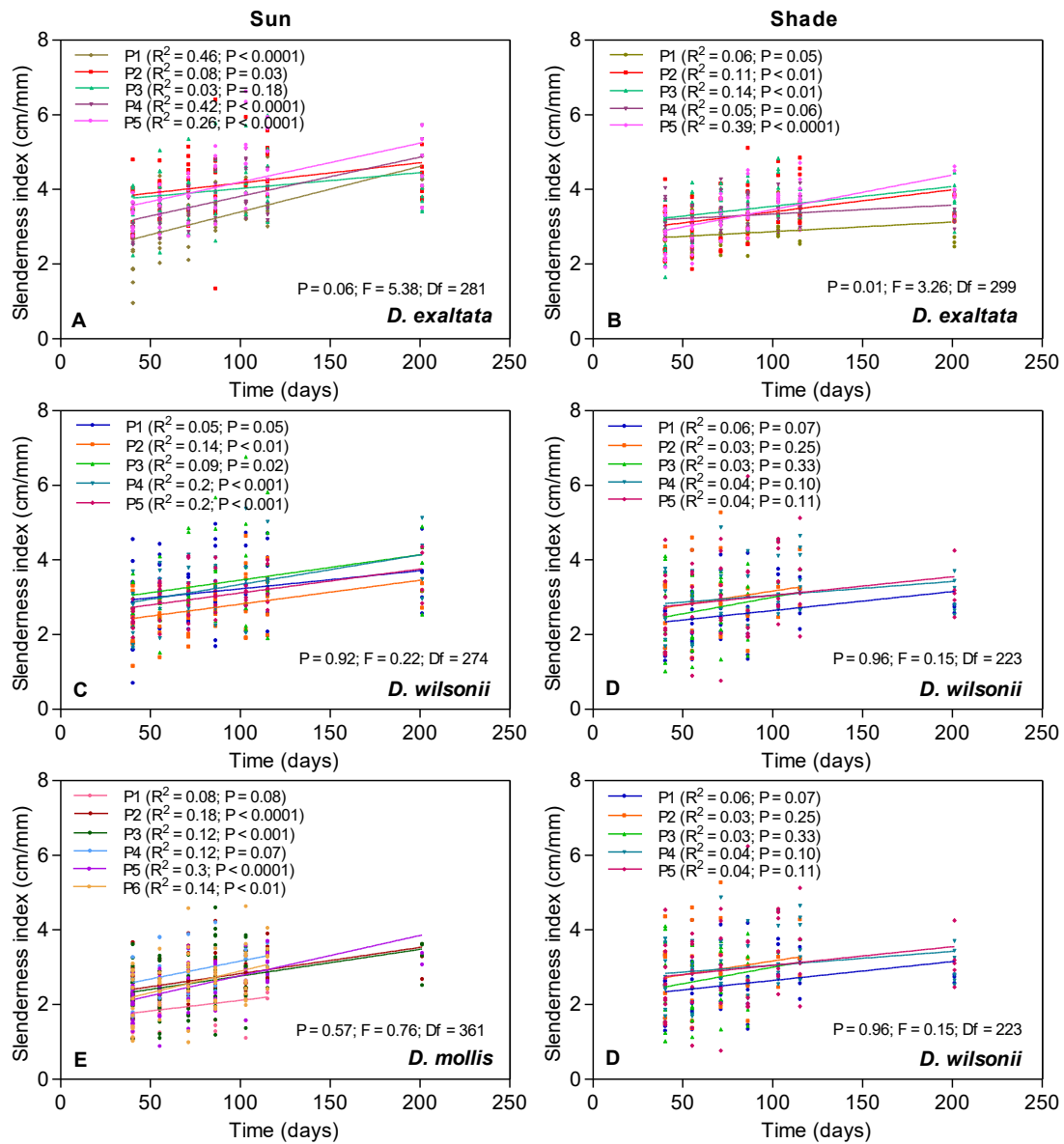


FIGURE 5 Slenderness index of progenies (P) of *Dimorphanthra* species and their natural hybrid (*D. wilsonii*) grown under sun and shade conditions until 200 days. The regression lines were performed and compared using a simple linear regression at 95% confidence intervals. P, F and Df values refers to the comparison between regression lines.

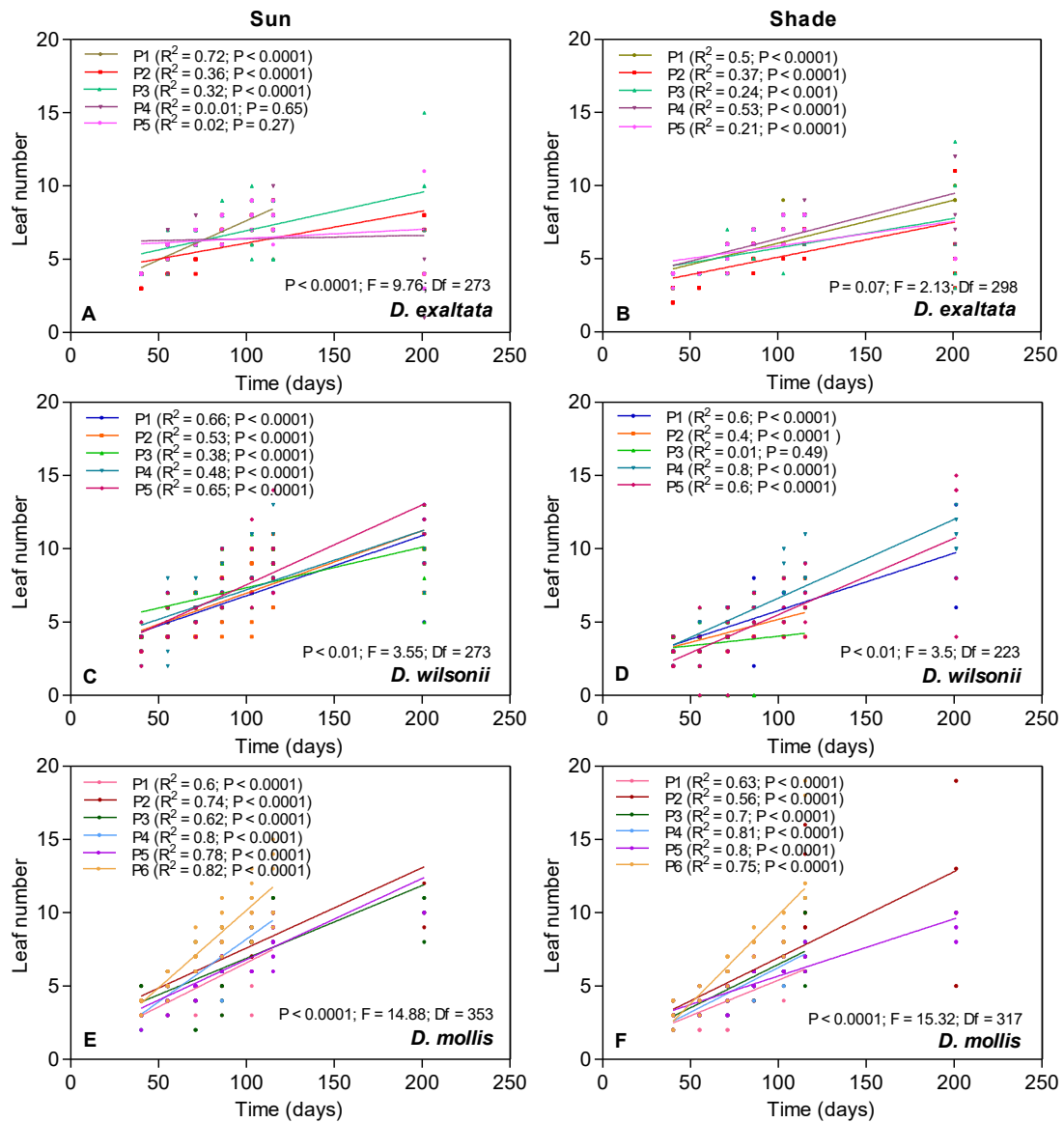


FIGURE 6 Leaf number of progenies (P) of *Dimorphandra* species and their natural hybrid (*D. wilsonii*) grown under sun and shade conditions until 200 days. The regression lines were performed and compared using a simple linear regression at 95% confidence intervals. P, F and Df values refers to the comparison between regression lines.

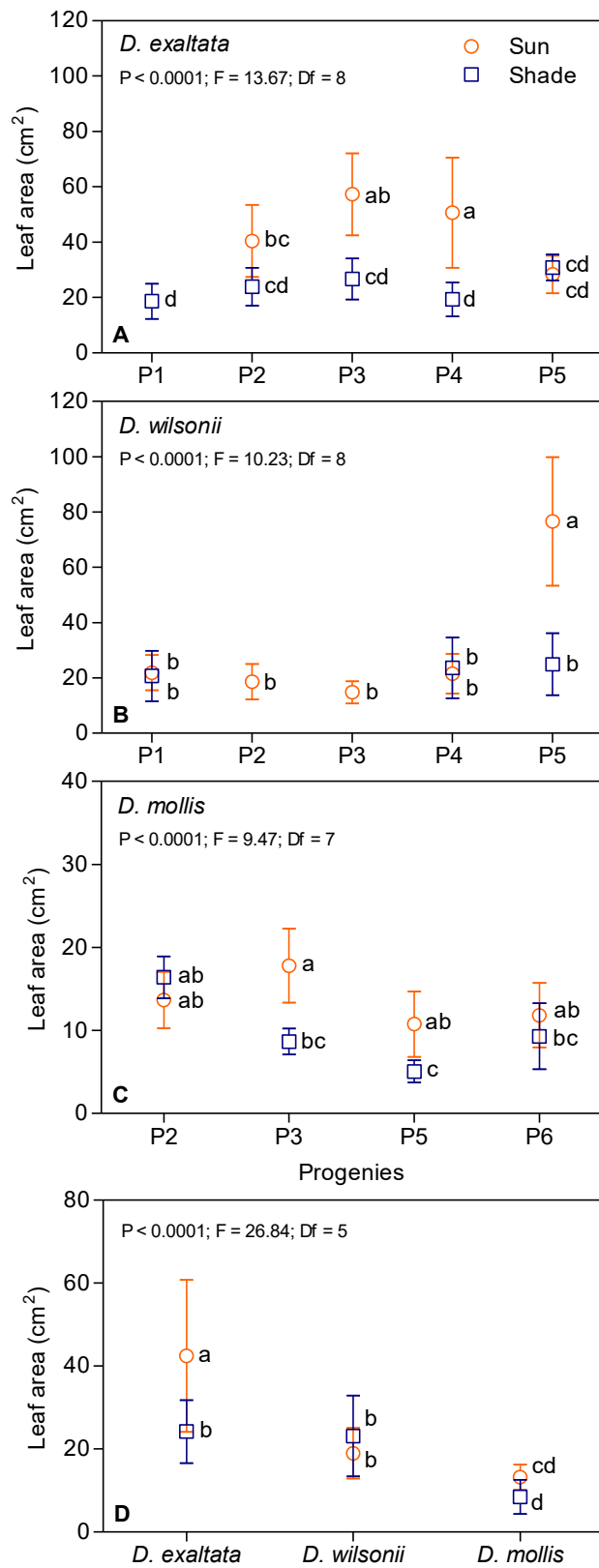


FIGURE 7 Leaf area of *Dimorphandra* species and their natural hybrid (*D. wilsonii*) grown under sun and shade conditions until seven months. A, B, C comparison between progenies (n = 4 – 6). D comparison between species/hybrid (n = 25 - 35). Mean and standard deviation were plotted. Data were compared by two-way ANOVA followed by Tukey test ($p < 0.05$). Means with the same letters are not significantly different.

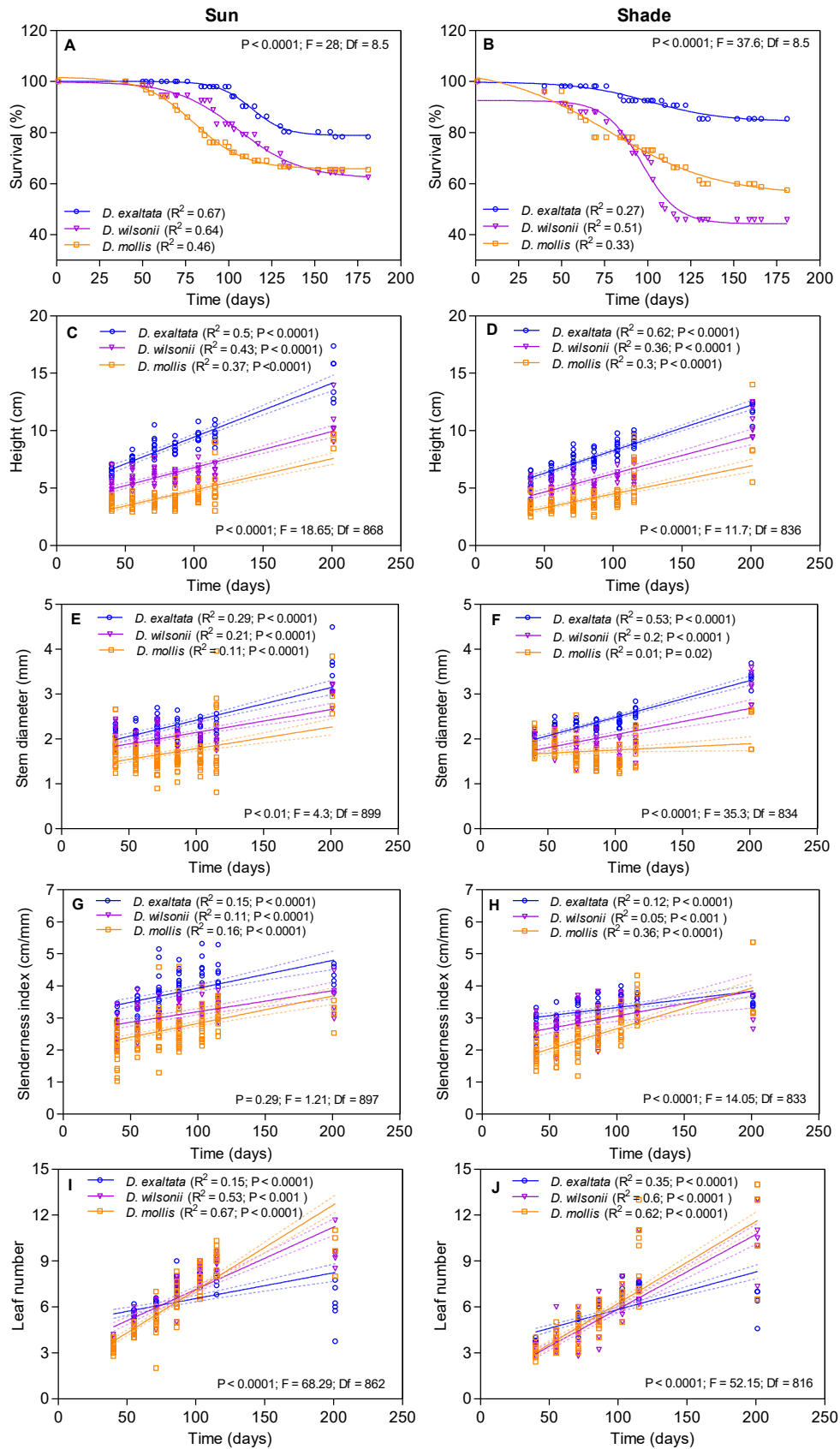


FIGURE 8 Survival and growth of *Dimorphandra* species and their natural hybrid (*D. wilsonii*) grown under sun and shade conditions until 180 (survival) and 200 days (growth). The means of the progenies of each species and hybrid were plotted. The survival curves (A - B) were performed and compared using a non-linear sigmoidal dose-response (variable slope) regression at 95% confidence intervals. The regression lines were performed for height (C - D), stem diameter (E - F), slenderness index (G - H), and leaf number (I - J). They were compared using a simple linear regression at 95% confidence intervals. P, F and Df values refer to comparing curves or lines.

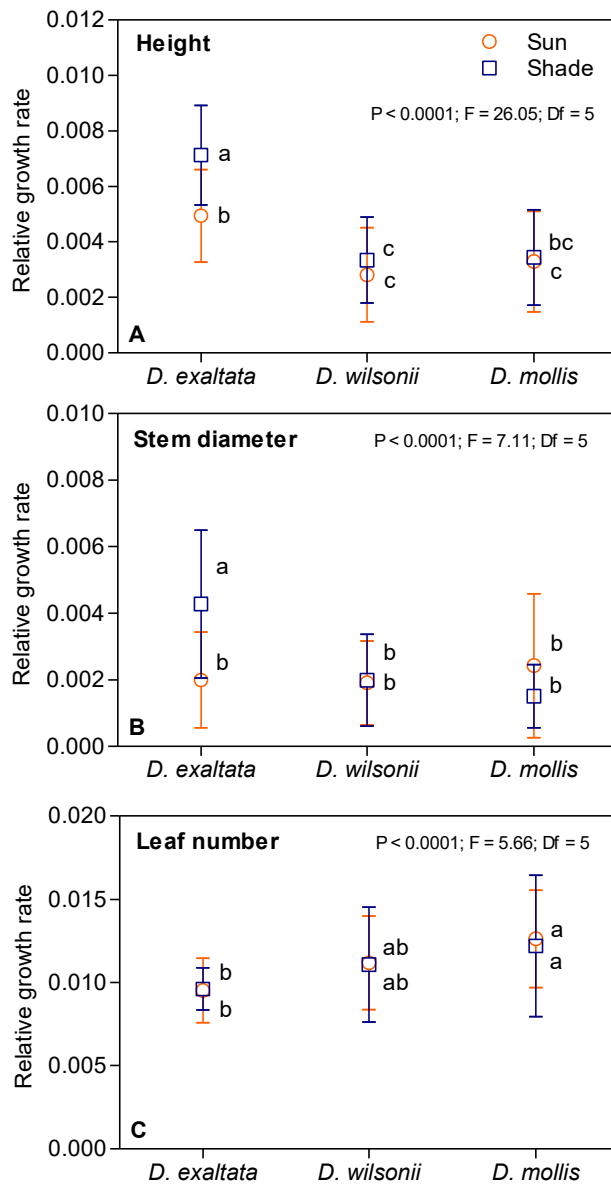


FIGURE 9 Relative growth rate of *Dimorphandra* species and their natural hybrid (*D. wilsonii*) grown under sun and shade conditions at an interval of 75 days. Mean and standard deviation were plotted ($n = 15 - 25$). Data were compared by two-way ANOVA followed by Tukey test ($p < 0.05$). Means with the same letters are not significantly different.

5 FINAL CONSIDERATIONS

Our results contribute to advances knowledge about the species that originated the *Dimorphandra* hybrid zone, highlighting the importance of the information provided here for *D. exaltata*, a threatened species and poorly studied. *D. exaltata* and *D. mollis* showed traits consistent with their original habitats, Atlantic Forest and Cerrado (savannah), respectively. *D. exaltata*, had a lower base temperature and a wider temperature range for germination. *D. exaltata* also showed superior survival and growth under shade environment. While *D. mollis* exhibited the highest base temperature and the narrowest temperature range for germination, and exhibited superior survival and growth under full sunlight.

The offspring of F1 hybrid displayed predominantly intermediate fitness relative to its parental species in germination, survival, and growth, performing generally lower than *D. exaltata* and higher than *D. mollis*. Their germination, survival and growth were high, with survival and growth performing better under full sunlight exposure. Additionally, we found the occurrence of heterosis in the F1 hybrids for some characteristics. Their fruits compared to that of their parents were almost a third larger, and their seeds were heavier than both parental species. The F1 hybrid also showed higher thermal tolerance and wider thermal safety margin for seed germination than the parentals. The fitness for growth of F1 offspring was similar to or greater than that of either parent. Conversely, the fitness results for backcross hybrid are consistent with hybrid breakdown in further generations. The backcross hybrid produced a much lower number of filled seeds per fruit, with a high ratio of malformed seeds/total seeds per fruit, and a lower germination fitness than the parental species and F1.

Together, our results imply that the hybrids combine traits from both parental species, potentially enabling a balanced adaptability to parental and transitional habitats, with greater potential for colonizing hot environments with a high light incidence. Moreover, their higher heat tolerance in germination may confer an adaptive advantage to face the predicted global warming. Additionally, our findings suggest that the postzygotic barriers do not exist or are weak to prevent the establishment of the F1 hybrid offspring, despite having been identified in the backcross generation. This underscores the potential of the early generation of hybrids in contributing to gene flow between parental species, thereby enhancing genetic diversity in the *Dimorphandra* hybrid zone. Future studies are needed to investigate other aspects of hybrid fitness, a deeper comparison of phenotypes with hybrid genotypes, as well as the reproductive potential and long-term fitness of subsequent hybrid generations to better understand the persistence and evolutionary implications of *Dimorphandra* hybridization. However, the

insights already provided about this hybrid zone, highlight the need for its conservation to preserve the natural course of evolutionary processes.

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