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English plantain deploys stress tolerance mechanisms at various organization levels across an altitudinal gradient in the Pyrenees

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Abstract

High-mountain plants must withstand high solar irradiation and low temperatures during winter. Furthermore, climate change is increasing drought events, which pose an additional threat to plants. Here, we studied the stress tolerance mechanisms at various levels of biological organization in English plantain (*Plantago lanceolata* L.), focusing on photoprotective and antioxidant responses. The response of populations from three different altitudes in the Eastern Pyrenees (1030, 1380, and 1660 m. a.s.l.) was compared during both autumn and winter. Results showed that plants not only suffered from photoinhibition due to very low temperatures at the highest elevation during winter, but also from mild drought stress at the lowest altitude during autumn. Individuals growing at the highest elevation showed reductions in the maximum photochemical efficiency of PSII (F_v/F_m ratio), which might be caused by the lack of an increased induction of tolerance mechanisms at the highest elevation compared to the intermediate one. Although most leaves died at the highest elevation, plants could withstand stress at the organism level by generating new leaves once the stress ceased. Drought at the lowest elevation during autumn caused mild stress with small decreases in the F_v/F_m ratio, along with an increase in abscisic acid and jasmonic acid content. This study underlines the great capacity of English plantain to adapt to high elevation by activating not only photo- and antioxidant protection mechanisms and adjustments in stress-related phytohormones, but also by fully regenerating its aboveground biomass through renewed growth once the stress has ceased.

1 | INTRODUCTION

Low temperatures and high solar irradiation during winter characterize high-mountain environments. Plants affected by these abiotic stresses have evolved several morphological, physiological, and molecular responses (Körner, 2003). Regarding physiological defensive strategies, a combination of elements allows plants to avoid and/or tolerate damages produced by these abiotic stresses (Banerjee & Roychoudhury, 2019; Germino & Smith, 2000; Marothia et al., 2020). One of the processes highly affected is photosynthesis (Gururani et al., 2015), a key biological process that determines growth and development, and ultimately the vigor of plants at every development stage. The photosynthetic machinery is likely to be affected by environmental conditions that can cause an energetic imbalance in chloroplasts leading to photoinhibition, which is the decrease of the photosynthetic activity due to an excess of absorbed light energy (Molina-Montenegro & Cavieres, 2010; Verhoeven, 2013), causing a reduction of photosynthetic efficiency (Banerjee & Roychoudhury, 2019). More specifically,

the production of singlet oxygen in chloroplasts can increase under excess light conditions and lead to photoinhibition due to the combination of high solar irradiation and cold stress, both limiting the photosynthetic electron transport and the capacity of the Calvin cycle to consume ATP and reducing power (Buchner et al., 2017).

Frequently, in response to the damages originated by these abiotic factors, high mountain plants respond by altering the content of some cellular compounds to avoid and defray the energetic imbalances (Molina-Montenegro & Cavieres, 2010). Consequently, a reduction of total chlorophyll (Chl) content is related to a photoprotective strategy that aims to reduce the amount of energy absorbed, ultimately reducing the photo-oxidative damage. Moreover, together with this Chl reduction, an increase of the expression of ROS-related genes and an increase of the content of nonenzymatic antioxidant compounds such as carotenoids (Car), phenylpropanoids (anthocyanins [Ant] and other flavonoids), and tocopherols (or vitamin E) have been described (Cotado et al., 2018; Cotado & Munné-Bosch, 2020; Fernández-Marín et al., 2017).

Cold stress is the main factor that decreases productivity at high altitudes in mountainous zones worldwide (Banerjee & Roychoudhury, 2019). Plants that are not well adapted to cold can survive punctual stress but are incapable of surviving prolonged cold stress and present various signs of cold-induced damage such as chlorosis, wither leaves or necrotic tissues. Exposure to cold generates different types of damage in different plant tissues, most of them related to membrane damage of different cellular compartments (Banerjee & Roychoudhury, 2019). Cold stress changes the lipid membrane composition and triggers the production of compounds such as proline, sugars, or polyphenols, as well as the synthesis of enzymatic and nonenzymatic antioxidants. If these mechanisms are inefficient or insufficient, sustained lipid peroxidation, and irreversible damage to the membranes can occur (Banerjee & Roychoudhury, 2019). Exposure to low temperatures causes the formation of ice crystals, which distorts the chloroplast membrane integrity due to a reduction in their fluidity (Levitt, 1980). This creates membrane ruptures as well as alterations in the Chl content, photosynthetic enzymes, electron transport chain and thylakoid structure. Thus, cold stress decreases the maximum photochemical efficiency of PSII (or F_v/F_m ratio), which is generally an indication of photoinhibition (Cotado et al., 2018).

Climate change increases drought events in several ecosystems, having an important impact on high-mountain plants, in part due to the severity of drought stress caused by the combined high solar irradiation and extreme temperatures seen in high-mountain ecosystems (Cotado & Munné-Bosch, 2020). Drought events, in particular when combined with extreme temperatures and high solar radiation, can lead to a loss of cell turgidity and cell growth arrest, osmotic stress, photosynthesis inhibition, and photo-oxidative damage in leaves (Buchner et al., 2017), thus having a large negative impact not only at the organ level, but also on the growth and development of the entire plant (Isah, 2019).

The activation of effective responses against stresses requires the investment of a considerable amount of energy and resources by plants. Thus, when faced with multiple stresses, plants must optimize their defensive strategies, and there is a trade-off between enhancing some to the detriment of others. In high-mountain plants such as *Saxifraga longifolia*, antioxidant compounds related to the protection from abiotic stress (Car, Ant, and tocopherols) increase with altitude, while biotic stress defensive phytohormone compounds (such as jasmonic acid [JA] or salicylic acid [SA]) decrease with altitude, thus indicating a trade-off favoring responses against abiotic stresses above those related to biotic stresses as the altitude increases (Cotado & Munné-Bosch, 2020). Furthermore, abscisic acid (ABA) plays a role in adapting plants to low temperatures (Rahman et al., 2020). Besides, the concentration of this inhibitory growth hormone increases with altitude due to an increase in solar irradiation and osmotic stress. In general, an increase in ABA content under cold stress conditions entails a decrease in plant growth (Rahman et al., 2020). In addition, to promote abiotic stress tolerance, ABA suppresses SA signaling, one of the hormones related to biotic stresses. This hormonal crosstalk between ABA and SA is a clear example of a mechanism that prioritizes abiotic stress responses in front of biotic stress responses and allows a better adaptation potential in conditions where the abiotic stresses are the severest. However, it is unknown whether this mechanism is advantageous or not in conditions where the severity of both biotic and abiotic stresses is similar (Berens et al., 2019). SA takes part in the defense against biotrophic pathogens, and it plays an antagonistic role in JA synthesis (Nguyen et al., 2016). Besides, SA is involved in stomatal closure together with ABA under drought stress conditions, allowing maintenance of the water content (Venegas-Molina et al., 2020). The action of these hormones is interconnected in conditions of both biotic and abiotic stresses. While systemic acquired resistance (SAR), which is a defensive response triggered by the detection of biotrophic pathogens, is mediated by SA, induced systemic resistance (ISR) is effective against necrotrophic pathogens and herbivory, and it is mediated by JA and ethylene (Venegas-Molina et al., 2020). Thus, the interactions between ABA, JA, and SA not only regulate plant trade-offs (Nguyen et al., 2016), but also take part in the prioritization and enhancement of the most suitable defensive strategy for the environmental condition (Ku et al., 2018).

Ribwort of English plantain (*Plantago lanceolata* L.) is a species of the Plantaginaceae family found on all continents. In the Iberian Peninsula, this hemicryptophyte is found from sea level to almost 2400 m. a.s.l.. In the Pyrenees, it grows in all types of soils along the submontane, montane, and subalpine ecosystems (Castroviejo et al., 1986). English plantain has huge phenotypic plasticity and thus, has a great capacity to adapt to environmental changes. It can be found in very different environments and is an invasive plant in its non-native range across the world (Smith et al., 2020). In this study, we aim to discern and understand which mechanisms are involved in the adaptation to altitude in *P. lanceolata*. Until now, a reduction of biomass with altitude has been described in this species (Pötsch & Schellberg, 2018), and an increase of ABA content with altitude has been noticed in another species of the same genera *Plantago major* L. (Rahman et al., 2020). However, these studies have not focused on the study of multiple stress markers throughout an altitudinal gradient during

winter (cold stress). We study the cold tolerance mechanisms along an altitudinal gradient in this species. Furthermore, we explore the possible trade-offs between abiotic and biotic stress defenses. We hypothesize that individuals living at increasing altitudes will show enhanced activation of cold tolerance mechanisms. Furthermore, we also hypothesize that individuals will invest more energy and deploy a larger set of mechanisms to overcome abiotic stresses at increasing altitudes, decreasing their resources to cope with biotic stresses.

2 | MATERIALS AND METHODS

2.1 | Study sites, experimental design, and sampling

Three different English plantain (*Plantago lanceolata* L.) populations were established throughout and altitudinal gradient in the Pyrenees (altitudes: 1030, 1380, and 1660 m. a.s.l.; coordinates: N42° 28.086′ E1° 29.978′; N42° 31.964′ E1° 30.745′; and N42° 31.624′ E1° 29.103′, respectively) during October 2020. The study was performed on 30 individuals of each population, which were individually labeled and identified in situ by GPS, and their geolocalization were transferred to ArcGis software to facilitate their monitoring throughout the study. Leaf samples used for physiological and biochemical analyses were collected at midday during two different sampling dates, the first during autumn and prior to the coldest yearly period (November 7th, 2020), and the other one during winter (February 14th, 2021). Two fully expanded young leaves from each plant were collected at midday (between 12:00 and 14:00 local time). One of them was used for the determination of some stress markers (leaf biomass, relative leaf water content [RWC], and the F_v/F_m ratio), while the other was immediately immersed in liquid nitrogen *in situ* and transported to the laboratory where they were stored at −80°C until subsequent analyses. Furthermore, population censuses were performed approximately every 40 days from the start of experiments and until mid-April (after winter season ends and early spring was) to follow the mortality rate in each population. Aside from stress marker and biochemical analyses and the demographic censuses, meteorological data were obtained from the *Servei Meteorològic del Govern d'Andorra*. Climatic data confirmed that the lowest altitude population was the driest and warmest during autumn, while the highest altitude population was the coldest over autumn and winter, but particularly during winter (Figure S1).

2.2 | Stress markers

To determine leaf biomass, RWC, and F_v/F_m ratio, leaves collected *in situ* were individually placed into Falcon tubes protected from light and immediately weighed to obtain the fresh weight (FW). Then, chlorophyll fluorescence was measured by using a portable mini-PAM (Walz) after adapting leaves to darkness for at least 1 h, and then the F_v/F_m ratio was calculated. Afterward, the same leaves were individually placed inside Falcon tubes containing distilled water to estimate the turgid weight (TW) 24 h later. Finally, the dry weight (DW) was measured after oven-drying the leaf at 60° for 72 h. RWC was calculated as 100 × (FW − DW)/(TW − DW).

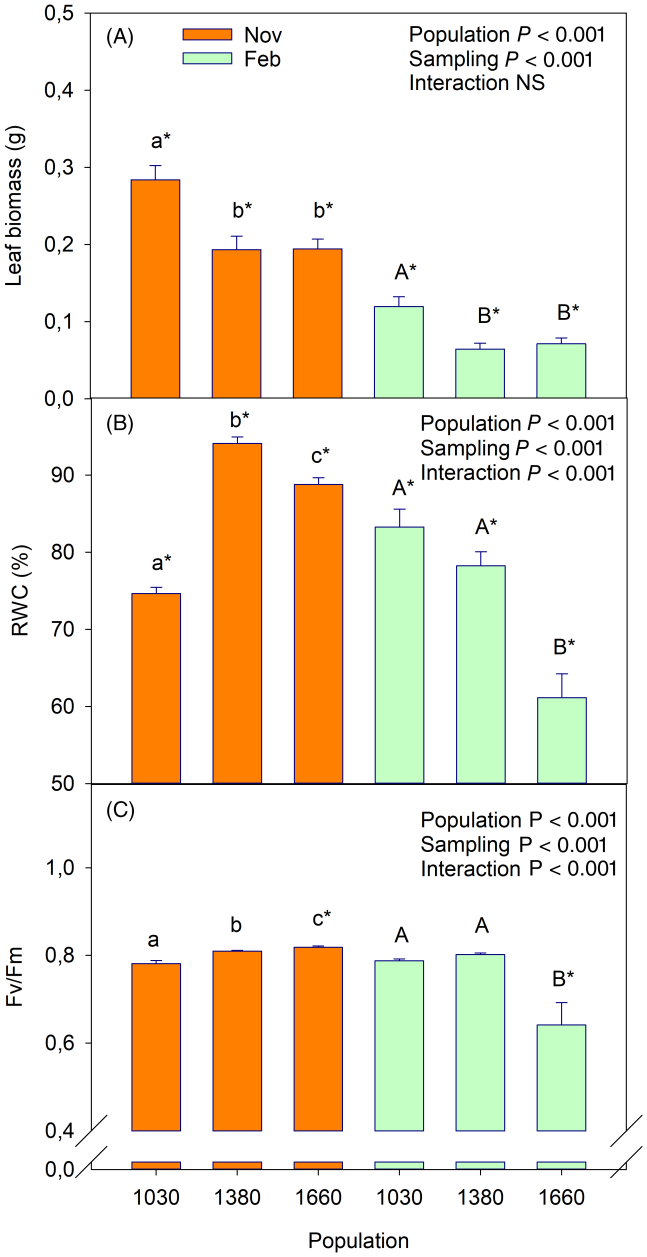


FIGURE 1 Variations in (A) leaf biomass, (B) relative water content (RWC), and (C) the maximum photochemical efficiency of PSII (F_v/F_m) between populations during autumn (November) and winter (February). Data show the mean ± SE of $n = 30$. Statistical differences were analyzed by nonparametric ART ANOVA. Different letters indicate significant differences (P <0.05). Small letters indicate differences between populations during autumn, and capital letters indicate differences between populations during winter. Asterisks indicate differences in the same population between both samplings. Feb, February; Nov, November

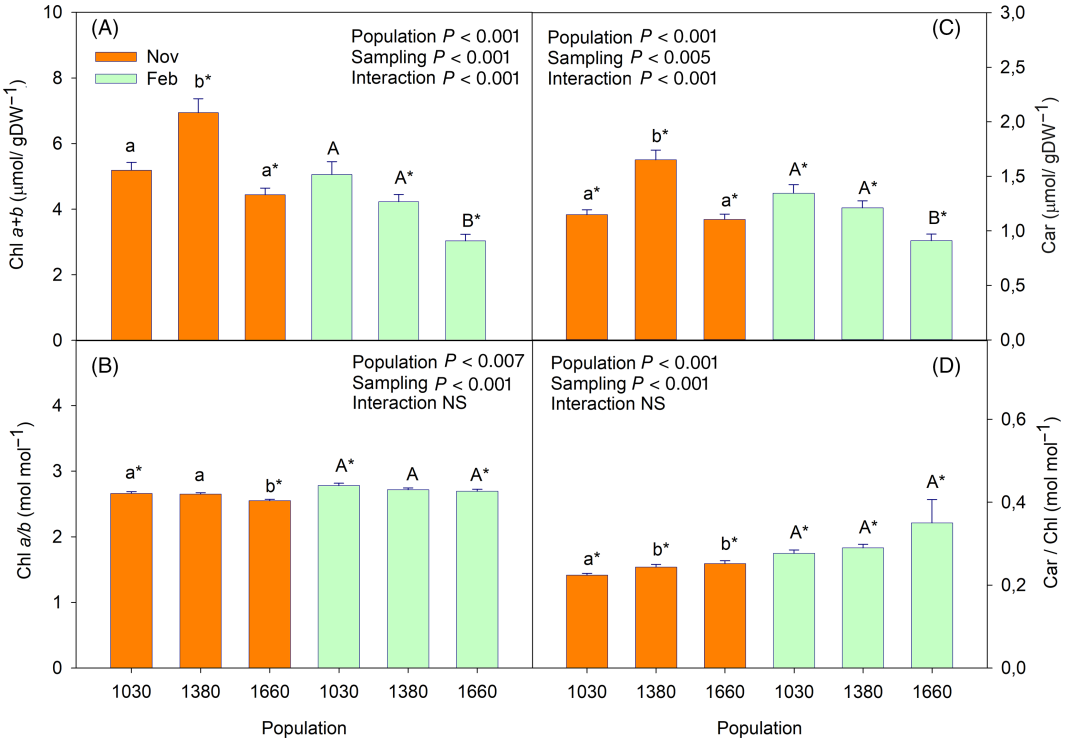


FIGURE 2 Variations in (A) total chlorophyll (Chl) content, (B) the Chl a/b ratio, (C) total carotenoids (Car) content, and (D) the Car/Chl ratio between populations during autumn (November) and winter (February). Data show the mean ± SE of $n = 30$. Statistical differences were analyzed by nonparametric ART ANOVA. Different letters indicate significant differences (P <0.05). Small letters indicate differences between populations during autumn, and capital letters indicate differences between populations during winter. Asterisks indicate differences in the same population between both samplings. Feb, February; Nov, November

2.3 | Biochemical analyses

Stress-related phytohormones (ABA, SA, and jasmonates), foliar pigments (Chl, Car, and Ant), vitamin E (α-tocopherol) and lipid hydroperoxides were all measured using the same methanolic extract. After grinding the samples with liquid nitrogen, we introduced 450 µl of methanol 0.01% BHT + 50 µl of deuterated (60 ppb) phytohormones inside each Eppendorf tube. Afterward, we vortexed the Eppendorf tubes and placed them in an ultrasonic bath for 30 min. We removed the balls used during the grinding, and the extract was centrifuged for 10 min at 4°C. The supernatant was collected, and the entire procedure was repeated for a re-extraction of the pellet. Finally, both supernatants were pooled and used for subsequent analyses.

For α-tocopherol analyses, the resulting extract was diluted with methanol (1:4, v/v), vortexed, and filtered before filling the HPLC vials. For HPLC analyses, an isocratic method with *n*-hexane and 1,4-dioxane (95.5:4.5, v/v), normal phase column and fluorescence detection were used, as described by Amaral et al. (2005). Standards from α-tocopherol (Sigma-Aldrich, Steinheim, Germany) were used for calibration.

For estimation of Chl, Car, and Ant contents, another aliquot of the resulting extract was diluted with methanol (1:4, v/v), vortexed, and centrifuged before spectrophotometric analyses. Chl and Car were measured in methanolic extracts following Lichtenthaler and Wellburn (1983), while Ant was measured in acidified methanolic extracts as described by Gitelson et al. (2001).

Oxylipins estimation was measured through lipid hydroperoxide contents, which were determined using the FOX-2 assay as described by Bou et al. (2008), and jasmonates, a product of lipid peroxidation. Jasmonates quantification, including JA, its precursor 12-*oxo*-phytodienoic acid (OPDA), and its conjugated, active form jasmonoyl-isoleucine (JA-Ile), were determined by ultrahigh performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), as described by Müller and Munné-Bosch (2011). The other stress-related phytohormones measured in this study (ABA and SA) were also determined by UHPLC-MS/MS, as described by Müller and Munné-Bosch (2011), using deuterated standards for their quantification.

2.4 | Statistical analyses

Since data did not comply with normality of residues and homogeneity of variance, the nonparametric approach Aligned Rank Transform (ART), and Spearman rank's correlation analyses were used. All statistical tests were performed using R-Studio, whereas all graphs were performed using SigmaPlot 11 (Systat Software Inc.).

3 | RESULTS

3.1 | Leaf biomass, water contents, F_v/F_m ratio, and photosynthetic pigments

Leaf biomass was higher in the first sampling compared to the second one, showing a generalized decrease in this parameter during winter (February) compared to autumn (November; Figure 1). The lowest population showed a higher value than the other two populations, both during autumn and winter. On the other hand, the lowest population also displayed a lower relative water content (RWC) and F_v/F_m ratio during November (Figure 1). However, only the highest population showed a severe decrease in the RWC and F_v/F_m ratio during winter (Figure 1). The decrease in the RWC and F_v/F_m ratio was more pronounced in the highest population during winter than in the lowest population during autumn (Figure 1).

The two highest populations showed a decrease in Chl content during winter compared to autumn, while in the lowest population, it did not vary significantly (Figure 2). The highest population showed the lowest Chl content during winter, which was not accompanied by changes in the Chl *a*/*b* ratio. Indeed, the Chl *a*/*b* did not differ among populations during February, while the lowest population showed the lowest Chl *a*/*b* ratio during November (Figure 2). In addition to the decrease in Chl content during winter, there was also a decrease in Car content in the highest population. Although the Car/Chl ratio did not differ between populations during winter, it increased in all three populations in winter compared to autumn. On the other hand, the lowest Car/Chl value was observed in the lowest population during autumn (Figure 2). Finally, we noticed a decrease in the Ant content during winter in the highest population compared to the other two, despite the Ant/Chl ratio did not differ among populations during both samplings (Figure 3).

3.2 | Vitamin E and oxylipin contents

During winter, the α-tocopherol content did not vary among populations. However, the α-tocopherol content was lower in the

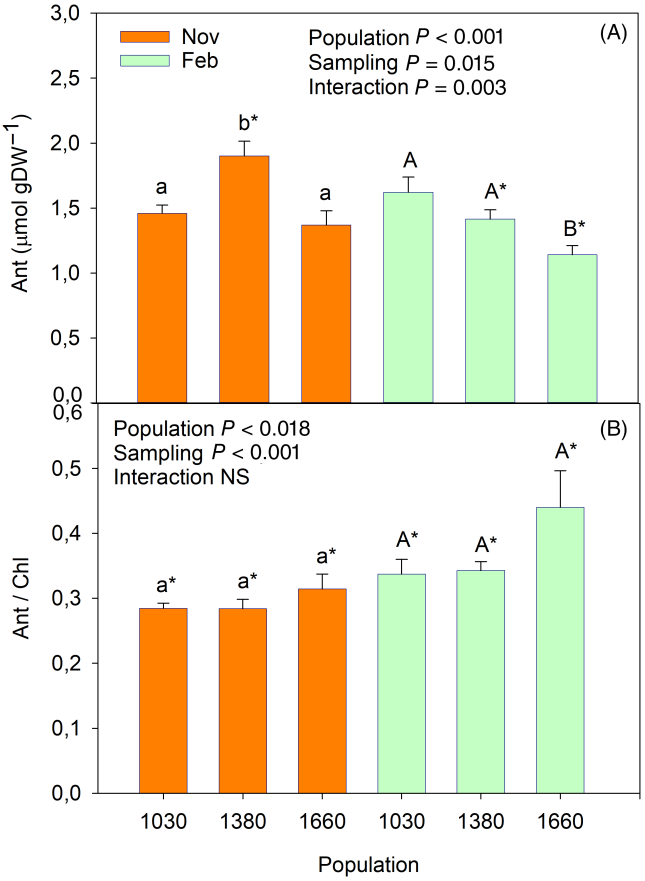


FIGURE 3 Variations in (A) the total anthocyanins (Ant) content and (B) the Ant/Chl ratio between populations during autumn (November) and winter (February). Data show the mean ± SE of $n = 30$. Statistical differences were analyzed by nonparametric ART ANOVA. Different letters indicate significant differences (P <0.05). Small letters indicate differences between populations during autumn, and capital letters indicate differences between populations during winter. Asterisks indicate differences in the same population between both samplings. Feb, February; Nov, November

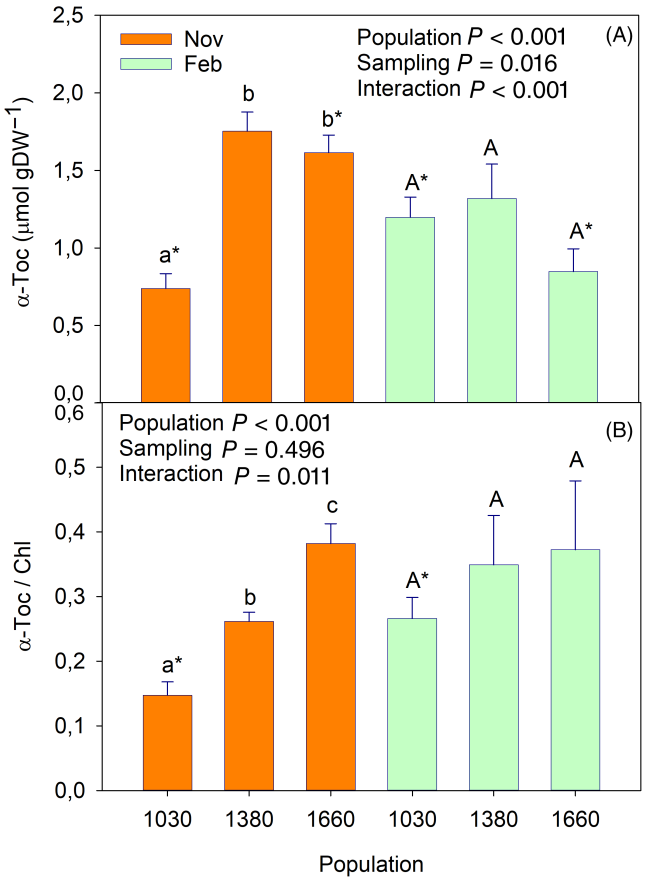


FIGURE 4 Variations in (A) α-tocopherol (α-Toc) content and (B) the α-Toc/Chl ratio between populations during autumn (November) and winter (February). Data show the mean ± SE of $n = 30$. Statistical differences were analyzed by nonparametric ART ANOVA. Different letters indicate significant differences (P <0.05). Small letters indicate differences between populations during autumn, and capital letters indicate differences between populations during winter. Asterisks indicate differences in the same population between both samplings. Feb, February; Nov, November

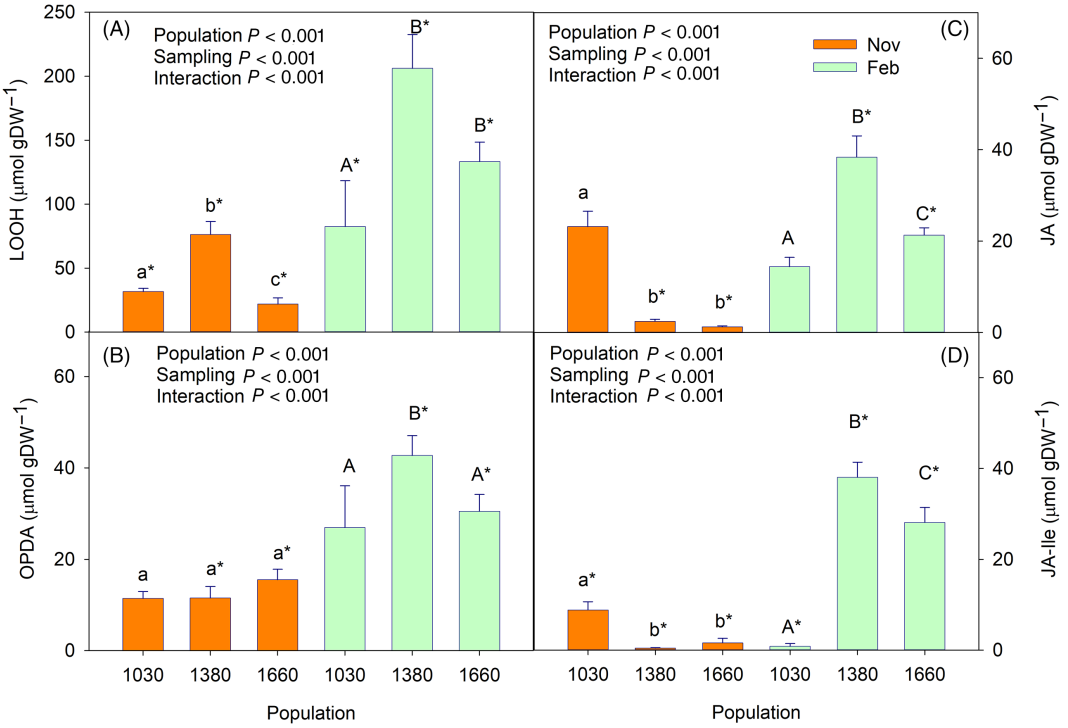


FIGURE 5 Variations in the contents of (A) lipid hidroperoxides (LOOH), (B) 12-*oxo*-phytodienoic acid, (C) jasmonic acid (JA), and (D) jasmonoyl isoleucine between populations during autumn (November) and winter (February). Data show the mean ± SE of $n = 30$. Statistical differences were analyzed by nonparametric ART ANOVA. Different letters indicate significant differences ($P < 0.05$). Small letters indicate differences between populations during autumn, and capital letters indicate differences between populations during winter. Asterisks indicate differences in the same population between both samplings. Feb, February; Nov, November

lowest population compared to the other two populations during autumn (Figure 4). The α-tocopherol/chlorophylls (α-Toc/Chl) ratio did not differ between populations in February but increased with altitude during November (Figure 4). Lipid hydroperoxides and jasmonates content increased in the two highest populations during winter (Figure 5). A notorious increase in JA and JA-Ile content was noticed in the lowest population during autumn (Figure 5).

On the other hand, during February, the highest population had a lower jasmonate content than the intermediate population, but it was higher than that of the lowest population (Figure 5). OPDA was the only jasmonate where there were no differences between the lowest and the highest populations during February. During winter, the two highest populations showed an increased lipid hydroperoxides content. This situation was different during autumn, where the intermediate population had the highest content followed by the highest and lowest populations, respectively.

3.3 | ABA and SA contents

During November, ABA content increased in the lowest population compared to the other two populations. This same population presented a decrease of this hormone during February compared to November. Importantly, the two highest populations exhibited a sharp increase in ABA content during winter, with no variation among them (Figure 6). In this respect, both populations manifested very low content of ABA during November and a very sharp increase of this hormone during February (Figure 6).

SA content was inversely proportional to altitude during winter (Figure 6). During autumn, the intermediate population showed the highest amount of this compound, followed by the highest and lowest populations, respectively. Irrespective of the population, SA content increased during winter compared to autumn (Figure 6).

3.4 | Mortality census and plant survival

The evolution of the demographic census and the phenotypic status of individuals throughout the study showed important differences among populations. Huge differences in the survival rates were observed depending on whether lost individuals were included or excluded from the analysis (Figure 7, Table S1). When lost individuals were considered dead, survival rates decreased as a function of altitude, so that survival decreased as the altitude of the population increased. However, mortality was negligible when lost individuals were excluded from the analysis. In other words, among individuals that were initially marked and that could be directly traced visually throughout the study, we did not observe any case of death (except for only one individual that died during April in the intermediate population, Figure 7 and Table S1).

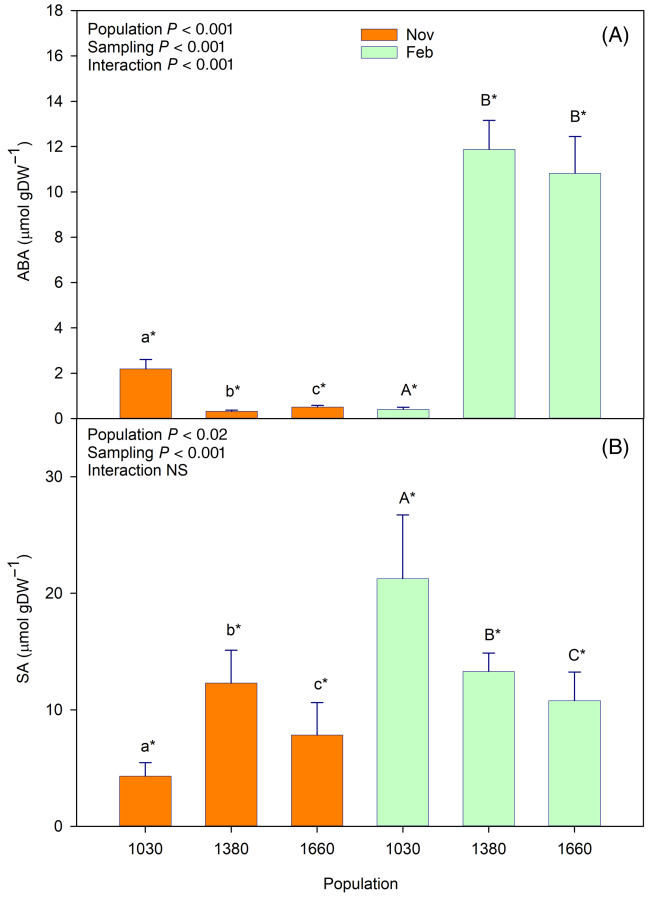


FIGURE 6 Variations in the endogenous contents of (A) abscisic acid (ABA) and (B) salicylic acid (SA) between populations during autumn (November) and winter (February). Data show the mean ± SE of $n = 30$. Statistical differences were analyzed by nonparametric ART ANOVA. Different letters indicate significant differences ($P < 0.05$). Small letters indicate differences between populations during autumn, and capital letters indicate differences between populations during winter. Asterisks indicate differences in the same population between both samplings. Feb, February; Nov, November

Major differences were observed in the phenotype of plants among populations. From late December to mid-February, some individuals of the highest population lost a large part of their leaves, even resulting in a complete loss of their rosette. This was not observed in the intermediate or lowest populations (Figure 7). During March, the physiological status of the three populations improved compared to winter. Surprisingly, those individuals of the highest populations that had been stressed during the winter recovered from the stress by generating new leaves. Indeed, the improvement of the visual physiological state of the individuals continued during April, when all visually traced plants seemed to be fully recovered from winter stress (Figure 7).

4 | DISCUSSION

Plant stress tolerance can be deployed at various organization levels, not only including changes at the biochemical and molecular levels, but also by the activation of defense responses that operate at the whole-plant and population levels. Phenotypic plasticity of ribwort or English plantain (*P. lanceolata*) has been described previously, with a reduced number of leaves per individual seen at the coldest temperatures (Orians et al., 2019) and a reduction in leaf biomass and area with altitude (Pötsch & Schellberg, 2018), which is in agreement with our study. Furthermore, we found that the highest population had a huge ability to replace the aboveground biomass after winter stress, which was essential to prevent mortality in all visually traced individuals. Regarding the adaptability of this species to the different conditions along this altitudinal gradient, our results show that English plantain uses different strategies to cope with winter stress at high altitudes, including not only changes at the biochemical level, but also at the whole-plant and population levels, compared to lower altitudes. Furthermore, our study shows that drought events may influence plant response at the lowest altitudes and that English plantain is adapting rather well to these conditions, at least in its native range in the Pyrenees.

Reductions in the RWC and F_v/F_m ratio in the lowest population during autumn (November) indicates that the population was stressed during this period, probably due to the drought persisting from summer (Figure S1). Otherwise, the severe decrease in these parameters in the highest population during winter (February) indicates that this population became more stressed during the winter period (Figure 1). The reduction in both RWC and F_v/F_m ratio is a clear marker of desiccation and photoinhibition due to abiotic stresses, such as cold and high solar irradiation as both type of stresses increase with altitude during winter. Besides, the postcold recovery in such short period of time (a few months only) of the individuals with lower F_v/F_m ratio indicates that regrowth in this species allows acclimation to high mountain conditions (Ugarte et al., 2019). This fact corroborates the idea that *P. lanceolata* has a huge plasticity.

As a photoprotective response, individuals of the most stressed population, the highest population during February, showed a decrease in the Chl content due to the higher solar irradiation and lower temperatures. This phenomenon is a symptom of stress, but it can also be an adaptive mechanism to absorb less light and protect the photosynthetic apparatus. On the other hand, in contrast with what occurs in other high mountain species (Banerjee & Roychoudhury, 2019; Cotado & Munné-Bosch, 2020; Fernández-Marín et al., 2021; Molina-Montenegro & Cavieres, 2010), the more stressed *P. lanceolata* individuals at the highest altitude did not show an increased Car content (neither when expressed per DW or Chl unit). This indicates that the photoprotective capacity of English plantain did not increase in relation to the amount of received light. Previous studies have shown that the Car/Chl ratio correlates well with survival in English plantain growing in tropical non-native populations. Indeed, DPS and the contents of carotenoids (including β-carotene, lutein and zeaxanthin) per unit of Chl were lower four months in advance in dead than in alive plants (Morales et al., 2021). Furthermore, in other Pyrenean plant species, such as *S. longifolia*, an increase in the Car content has also been described as a stress tolerance marker (Cotado & Munné-Bosch, 2020). Car contents decreased in

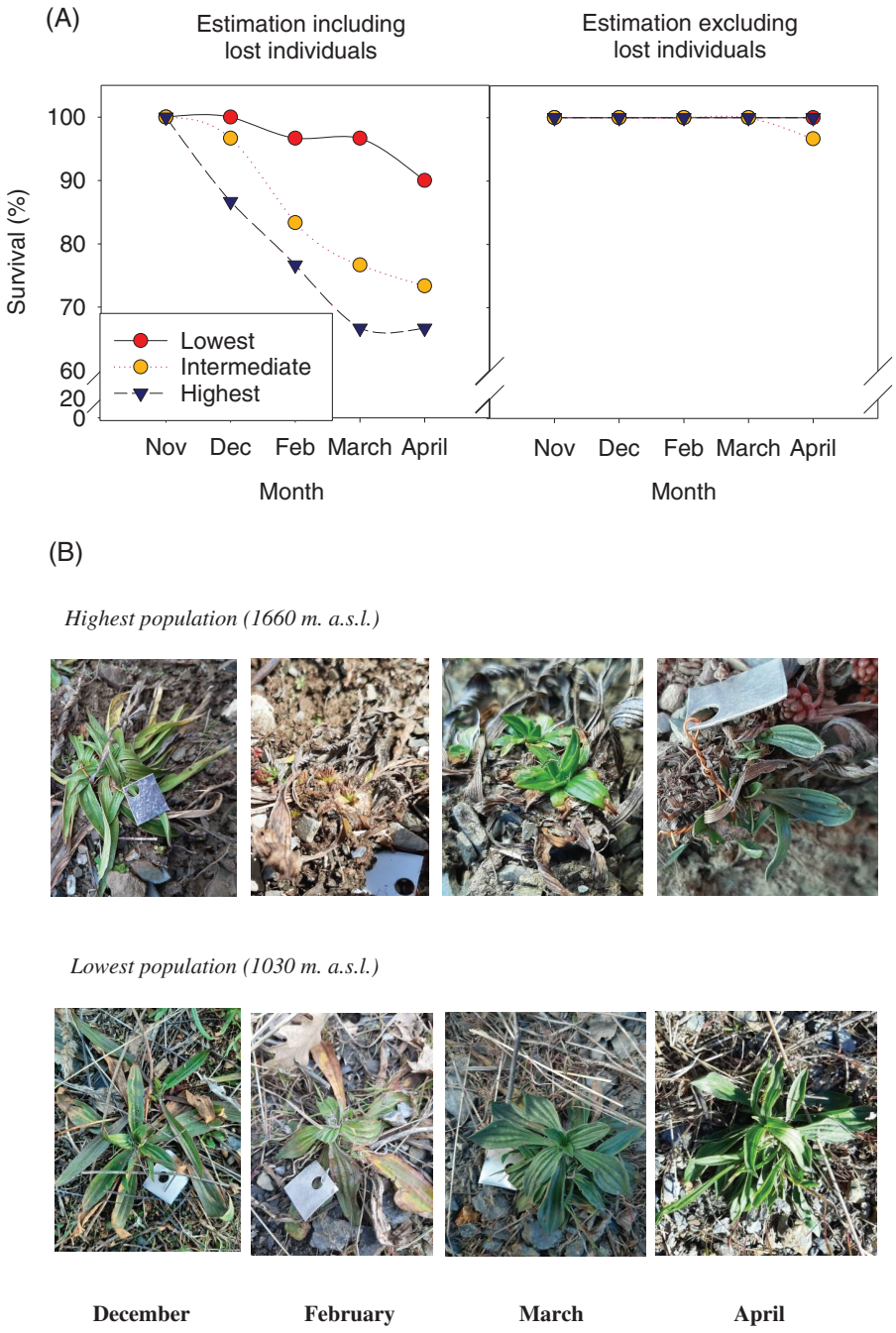
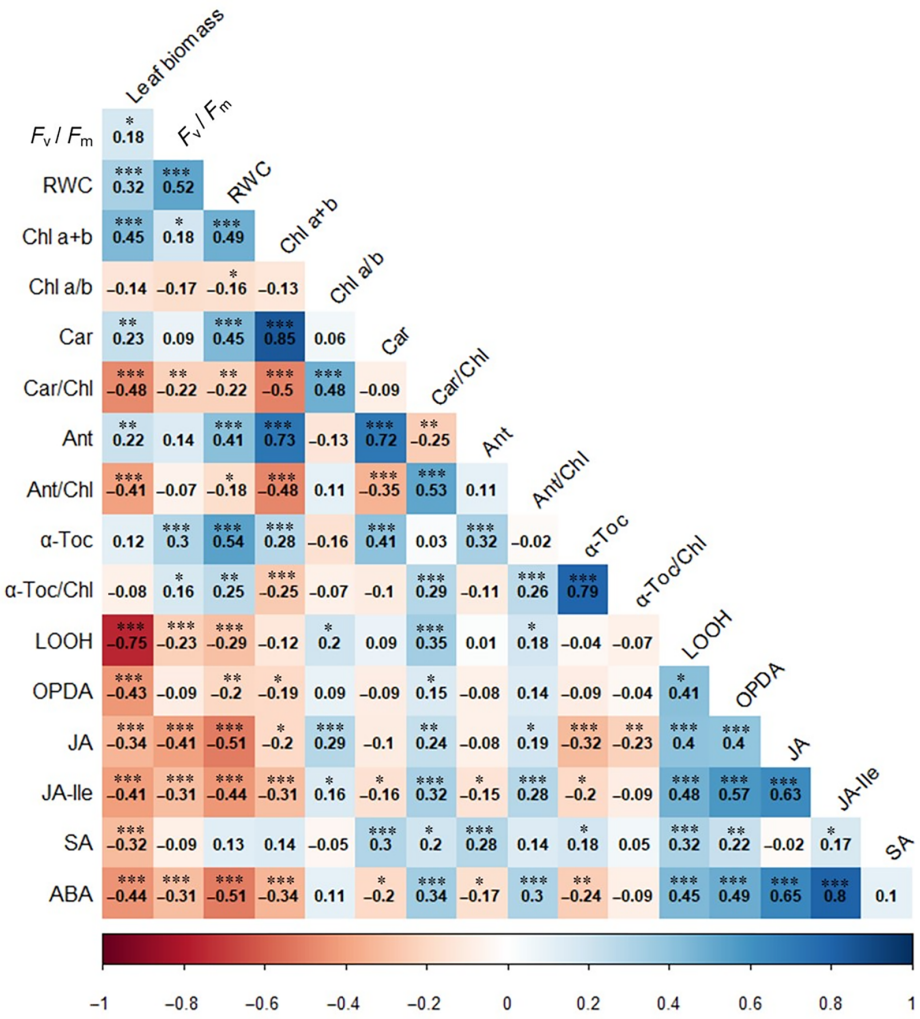


FIGURE 7 (A) Survival rates of the studied populations, considering data both including (left) and excluding (right) lost individuals during the demographic census. (B) Phenotypic evolution of the same stressed representative individuals of the highest (above) and lowest population (below) during mortality census sampling dates

P. lanceolata at the highest altitude, thus indicating some degree of stress susceptibility at the leaf level. Furthermore, Ant content and the Ant/Chl did not increase in the most stressed populations. Previous studies have described that the Ant content increases in response to the degree of incidence of multiple abiotic stresses in anthocyanin-accumulating species (Cotado et al., 2018; Fernández-Marín et al., 2017). Thus, English plantain did not activate a typical photoprotective response in terms of photoprotective pigments accumulation at the highest altitude.

The decrease of the F_v/F_m ratio during November in the lowest population might be related to the reduction of α-tocopherol content, since it is known that this antioxidant helps protect PSII from photoinhibitory damage (Munné-Bosch, 2005). This situation is the opposite of what has been noticed in drought-tolerant species, where abiotic stress induces α-tocopherol accumulation as well as the biosynthesis of other nonenzymatic antioxidants (Casadesús et al., 2021; Fernández-Marín et al., 2017). On the other hand, the reduction of F_v/F_m ratio in the highest population during February was not linked to a reduction in α-tocopherol content, suggesting that, while photoinhibition during autumn may be associated with drought-induced oxidative stress, photoinhibition during winter is not linked to photooxidative stress, but is most likely a direct effect of low temperature stress causing direct damage to the D1 protein, as reported in other species (Mattila et al., 2020).

TABLE 1 Spearman rank correlation matrix showing rho values between all the variables studied



Note: One, two, and three asterisks indicate *P* values below 0.05, 0.01, and 0.001, respectively. Color gradation ranges represent level of correlation from red (positive) to blue (negative).

Abbreviations: ABA, abscisic acid; Ant, anthocyanin; Car, carotenoid; Chl, chlorophyll; F_v/F_m, maximum photochemical efficiency of PSII; JA, jasmonic acid; JA-Ile, jasmonoyl isoleucine; LOOH, lipid hydroperoxides; OPDA, 12-*oxo*-phytodienoic acid; RWC, relative water content; SA, salicylic acid; α-Toc, α-tocopherol.

When it comes to coping with drought conditions, the role of jasmonates has been described in some plant species (Cotado et al., 2018; Orians et al., 2019). The increase of JA content in the lowest population during autumn might play such a role and appeared to be correlated with an increase in ABA content. Although the major role of jasmonates is established in responses against biotic stresses, previous studies have already shown a link between jasmonates and abiotic stresses, such as cold (Casadesús et al., 2021; Cotado et al., 2018; Müller & Munné-Bosch, 2021). The results obtained in winter support the implication of jasmonates in response to cold stress in *P. lanceolata*. Besides, the fact that the content of JA-Ile, the most active form among jasmonates, increases alongside the JA content, further supports this contention. On the other hand, the highest population showed lower jasmonates content than the intermediate population but higher values than the lowest one during winter. These results point out to the idea that the increase in jasmonates content is induced between 1030 and 1380 m. a.s.l., but this induction stops between 1380 and 1660 m. a.s.l.. This lack of induction of defensive responses in the highest population under more stressful conditions might explain the fact that a large part of leaves of this population end up dying during winter.

ABA is a plant hormone widely known to play a key role against multiple abiotic stresses, being an excellent stress marker in plants. In *P. major*, ABA content increased with cold stress at increasing altitudes in the Himalaya (Rahman et al., 2020), which agrees with our results. At the same time, the increasing levels of this inhibitory growth hormone explains the leaf biomass decrease noticed in the individuals of these two populations during winter. On the other hand, the ABA increase of the lowest population during the month of November is a clear drought stress marker. The increase in ABA content supports the idea that the highest populations during winter and the lowest population during November were the most stressed

populations in our study. At the same time, ABA is a positive regulator of both drought and cold stress tolerance, thus enhanced ABA contents under these conditions may partly explain the tolerance of this species to these abiotic stresses (Müller & Munné-Bosch, 2021). However, the highest population had a lower ABA content than expected considering the altitude, equal to those of the intermediate population. This fact, combined with a decreased content in Car and Ant, together with the lack of induction of α-tocopherol and jasmonates, may explain the death of most of the leaves in the highest population during winter.

A matrix correlation analysis, taking all data from all variables, revealed interesting relationships between various parameters (Table 1). Underlining correlations with *rho* values above 0.5 and *P*-values below 0.001 in the results of the Spearman rank's correlation analyses, it was shown that leaf negatively correlated with ABA and jasmonate contents, and most particularly with lipid hydroperoxide contents, thus suggesting an inhibitory role for these hormones and oxidative stress on leaf growth. Furthermore, RWC negatively correlated with ABA and jasmonates, while it positively correlated with the F_v/F_m ratio, and Chl and α-tocopherol contents. This suggests that leaf desiccation favors photoinhibition and both pigment and vitamin E degradation in this plant species, which is consistent with the aforementioned drought sensitivity for *P. lanceolata*. Also of interest was the positive association between Chl content and both Ant and Car contents, which suggests a higher need for photoprotection when Chl levels are high, and the positive relationship between JA-Ile and its precursors (lipid hydroperoxides, OPDA, and JA), and between ABA and jasmonates, the latter showing a coordinated action against abiotic stress in this plant species.

5 | CONCLUSIONS

In conclusion, a severe decrease of the F_v/F_m ratio, Chl, and Car contents combined with an insufficient induction of jasmonates and ABA accumulation might result in a higher leaf mortality in the highest population during winter (February). Despite that, death occurred in most of the leaves at the highest elevation, plants could withstand stress at the organism level by generating new leaves once the stress has ceased during next spring, thus showing a huge plasticity. Drought at the lowest elevation during autumn caused a mild stress with small decreases in the F_v/F_m ratio and Chl contents, as well as increased contents in ABA and JA. This study underlines the great capacity of English plantain to adapt to high elevation by activating not only some photo- and antioxidant protection mechanisms and adjustments in the endogenous contents of stress-related phytohormones, but also by fully regenerating its aboveground biomass through renewed growth once the stress has ceased.

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DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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