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RESEARCH

Selenium and Arbuscular Mycorrhizal Fungi improve growth, physiological traits, and forage yield of *Cichorium intybus* cv. Cropmate under water limit conditions

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Abstract

This study evaluated the effects of selenium (Se) application rates (0, 25, 50, and 75 mg/m²) and arbuscular mycorrhizal fungi (AMF) inoculation on growth, forage yield, physiological responses, and tissue Se accumulation of *Cichorium intybus* cv. Cropmate under controlled water limit conditions. A 4×2 factorial pot experiment was conducted with drought stress imposed from 14 days after sowing. Morphological traits were recorded weekly, while forage yield, root traits, chlorophyll content, relative water content, proline, total phenolics, flavonoids, and tissue Se content were determined at harvest. Both Se and AMF significantly affected leaf number, plant height, and stem diameter ($P<0.05$), although leaf width remained unaffected. At week VI, the 75 mg Se+AMF maximized height plants, exceeding the control by ~63.5%, whereas the 50 mg Se+AMF yielded the highest leaf number (14.40) and stem diameter (15.20 mm). The same combination resulted in the greates fresh forage and crude protein yields, with increases of ~51.9% and ~170%, respectively, relative to the control ($P<0.05$). Selenium application and AMF inoculation together enhanced chlorophyll and relative water content, while lowering proline, phenolic, and flavonoid levels, indicating improved drought tolerance. Tissue Se content increased with increasing Se dose, reaching 0.65 µg/g at the highest rate, while AMF appeared to modulate Se translocation. We conclude that a moderate Se dose (50 mg/m²) combined with AMF offers a promising strategy for chicory biofortification under drought conditions. Field validation and Se speciation analyses are warranted before farm-scale adoption can be recommended.

Keywords Abiotic stress · Biofortification strategy · Micronutrient · Mycorrhizae helper organism · Plant agronomic

1 Introduction

Climate variability and recurring dry seasons in many tropical regions increase demand for forage species that combine high nutritive value with drought resilience. *Cichorium intybus* L. (*C. intybus* L.) is a promising candidate because of its high crude protein, deep rooting, and rapid regrowth, which together support forage productivity under limited water supply (Li et al. 2023; Niderkorn et al. 2019). Chicory production has been shown to reach 16–19 tons/ha/year in non-irrigated and more than 22.6 tons/ha/year under irrigation (Neal et al. 2009). Chicory contains up to 29.6% crude protein and has a digestibility value of around 74% (Nwafor et al. 2017), thus classifying it as a high-quality feed crop. In Indonesia, recent initiatives have led to the forage cultivation of *C. intybus* cv. Chico, yielding promising biomass yields (Umami et al. 2017, 2022).

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Drought stress is a significant barrier to global crop productivity, with implications for food security and agricultural sustainability. It is clear that plants have evolved complex defense mechanisms to cope with water deficits; however, these mechanisms are often inadequate under severe or prolonged drought, necessitating urgent, innovative strategies to improve crop drought tolerance. Selenium (Se), a microelement traditionally considered non-essential for higher plants, has emerged as a powerful modulator of plant stress responses. Selenium has been linked to improved plant stress tolerance and enhanced nutritional quality, but it is widely distributed at 0.05–0.09 mg/kg (Rodriguez et al. 2005). Recent research shows that Se biofortification can significantly improve plant tolerance to drought by modulating antioxidant systems, osmotic adjustment, and stress signaling pathways, making Se a promising tool in climate-resilient agriculture (Ali et al. 2023; Fan et al. 2022; Song et al. 2024). Biofortification is a sustainable strategy that improves the nutritional quality of crops to address micronutrient deficiencies. This process involves incorporating essential minerals, vitamins, and other nutrients into plants using various methods. This includes adding Se to enhance the nutritional quality and antioxidant content of plants, thereby facilitating optimal plant growth (Ogundare et al. 2026). Moreover, biofortification can be integrated into existing agricultural systems by providing Se from natural food sources, which are often more readily absorbed. This method can also incorporate other phytochemicals and micronutrients, thereby enhancing nutritional value (Yadav et al. 2020).

In plants, Se is absorbed via sulfur assimilation pathways, incorporated into organic compounds such as seleno-amino acids, and contributes to antioxidant protection, delays senescence, and can stimulate biomass production (Gupta and Gupta 2017; Umami et al. 2022; Li et al. 2023). In livestock, Se is an essential micronutrient that is incorporated into selenoproteins that support antioxidant defense, thyroid function, and immunity (Rayman 2012). Previously, Se biofortified chicory has been reported to improve Se bio-accessibility, enhance forage quality, and confer health benefits when used as livestock feed (Li et al. 2023). However, the authors conducted their studies under normal conditions, without drought or water shortage, whereas studies under water restriction conditions remain exceedingly rare.

Arbuscular mycorrhizal fungi (AMF) have attracted considerable interest due to their symbiotic association with host plants, which enhances the uptake of both macronutrients and micronutrients. Through the formation of root symbiosis, AMF improve nutrient and water acquisition, modify root morphology, and increase plant tolerance to drought and salinity (Nadeem et al. 2017). For example, the *Capparis spinosa* (*C. spinosa*) plant is estimated to exhibit an AMF dependency of approximately 58%, highlighting its substantial reliance on AMF for biomass production under nutrient-deficient, semi-arid conditions (Bouskout et al. 2025a). Mycorrhizal in plants enhances stomatal regulation and nutrient acquisition, thereby sustaining productivity under abiotic stress (Augé et al. 2015). In addition to increasing plant resistance to drought stress, AMF also enhances the plant's ability to absorb Se and regulate its uptake to prevent toxicity (Osman et al. 2025). Several studies have reported that the combination of AMF and Se positively affects on plant performance (Bamberg et al. 2019; Chen et al. 2020; Antoniou et al. 2021; Liu et al. 2024b). Nevertheless, research on the combined effects of Se and AMF in chicory remains limited, especially under drought stress conditions.

Notwithstanding these advancements, most studies have focused on Se application in non-stressed environments or have failed to consider the role of AMF in modulating Se uptake under drought stress. Additionally, there is a paucity of research on how AMF inoculation may influence the biofortification potential of Se and its accumulation in plant tissues under water-limited conditions. The majority of studies have investigated Se and AMF in isolation, leaving a dearth of knowledge about their combined effects on plant growth, nutrient uptake, and drought resilience. This study aims to address this knowledge gap by investigating the synergistic effects of Se and AMF inoculation on the growth, physiological traits, and forage yield of *C. intybus* cv. Cropmate under drought stress conditions. This research offers significant insights into the collaborative effects of Se and AMF, elucidating their potential to enhance drought tolerance and forage quality. These findings contribute to the development of sustainable agricultural practices in water-scarce environments.

Therefore, we hypothesize that the combined application of Se and AMF will work synergistically to improve drought resilience in *C. intybus* cv. Cropmate enhances water and nutrient uptake, improves antioxidant defense, modulates secondary metabolite production, and regulates Se biofortification. The underlying mechanisms of these effects include enhanced photosynthesis, alleviation of oxidative stress, improved root architecture, and safe Se accumulation. Collectively, these factors will result in enhanced growth, increased biomass yield, and improved forage quality under water-limited conditions. To the best of our knowledge, this is the first comprehensive evaluation of this approach in Indonesia, and it contributes to

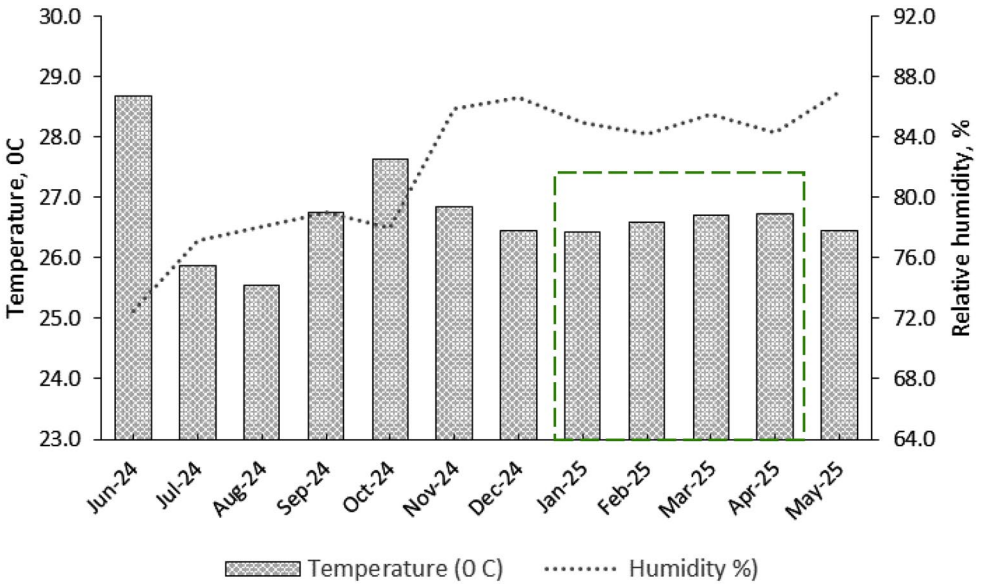


Fig. 1 The temperature (°C) and relative humidity (%) of the research location are based on data from the Meteorology, Climatology, and Geophysics Agency in Sleman-Yogyakarta, Indonesia. Month of research implementation

Table 1 Chemical content of soil as planting media

Parameters	Value	Criteria	Score
pH (H_2O)	7.2	Neutral soil	(6.6–7.3)[a]
C-Organic, %	2.41	Medium	(3.0–4.75)[a]
Organic matter, %	5.61	High	(6.16–15)[a]
Nitrogen total, %	0.35	Medium	(0.15–0.25)[a]
C/N ratio	6.8	Low	(5–10)[a]
Selenium, µg/100 g	7.14	Low	(10^5 – 10^8)[b]

[a] (Hazelton and Murphy 2016), [b] (De Temmerman et al. 2014)

the development of sustainable forage production strategies for tropical environments.

2 Methodology

2.1 Research location

The experiment was conducted in a greenhouse at the Faculty of Animal Science, Universitas Gadjah Mada, Indonesia (7.769238° S, 110.386085° E), under mild, stable conditions (26.4–27.7 °C; 84.2–85.5% RH), which were conducive to plant growth (Fig. 1).

2.2 Experimental design

A pot experiment was conducted using a factorial Completely Randomized Design (CRD) with two factors: Se dose and AMF inoculation. Selenium was applied at four levels (0, 25, 50, and 75 mg/m²), and AMF treatment was used at two levels (–AMF and +AMF). Each treatment combination had seven replicates, totaling 56 experimental units. Water restriction was imposed to simulate tropical dry-season conditions, beginning two weeks after planting. Irrigation was adjusted based on daily evaporation and soil moisture measurements to maintain controlled drought stress throughout the experiment.

2.3 Planting medium preparation

The soil selected for cultivation is classified as a regosol. The soil was mixed with compost at a 2:1 ratio to achieve a C/N ratio of 14. The prepared planting media are left to stand for 7 days before seed planting. The soil characteristics and physicochemical properties (Table 1) had a neutral pH (7.2) and high organic matter content (5.61%), indicating good fertility. Total nitrogen was moderate (0.35%), and the C/N ratio was low (6.8). Selenium content was low (7.14 µg/100 g), potentially limiting crop nutritional quality. The soils used were previously characterized for mineral content using *X-ray fluorescence* (Table 2).

2.4 Pot design and water retention calculation

We used plastic pots (13 × 45 cm) as experimental containers, each with an average surface area of 135 cm² and filled with 5.5 kg of planting medium. The water retention capacity of the medium was determined by saturating each pot with water until drainage ceased. The pots were then weighed to record the final weight. The difference between the saturated and drained weights used to calculate the water retention capacity corresponds to a maximum field capacity of approximately 900 mL.

2.5 Selenium application and AMF inoculation

Before planting, the growing medium was inoculated with AMF using a commercial inoculant, MycoGrow®

Table 2 Elemental analysis of soil as planting media

Elements	Concentration	Elements	Concentration
Na, %	0.66	Cu, ppm	239.4
Mg, ppm	86.2	Zn, ppm	391.7
Al, %	2.96	Ga, ppm	82
Si, %	8.50	As, ppm	37.4
P, %	0.20	Br, ppm	39.9
S, ppm	78.7	Rb, ppm	82.6
Cl, ppm	81	Sr, ppm	997.7
K, %	0.67	Y, ppm	96.8
Ca, %	1.89	Zr, ppm	520.1
Ti, %	0.77	Nb, ppm	18.7
V, ppm	295.3	Sn, ppm	363.2
Cr, ppm	63.7	Te, ppm	143.5
Mn, %	0.29	Ba, %	0.11
Fe, %	12.86	Eu, ppm	74.2
Co, ppm	773.9	Yb, ppm	82.9
Ni, ppm	36.2	Pb, ppm	64.2

Macro- and micro-mineral content was analyzed by *X-ray fluorescence* at the Radiation Laboratory, National research and innovation agency,Yogyakarta, Indonesia

(Agrofarm Nusa Raya, Indonesia). This product contains 33 spores/g and 300 live propagules/g. These comprise a mixed consortium of endomycorrhizal fungi, including *Glomus manihotis*, *Glomus intraradices*, *Glomus aggregatum*, *Acaulospora* sp., and *Gigaspora* sp. The Glomus species belong to the genus *Glomus*, while *Acaulospora* sp. and *Gigaspora* sp. are classified under the genera *Acaulospora* and *Gigaspora*, respectively. All of these belong to the class *Glomeromycetes*. This product is enriched with zeolite granules and organic complexes. A total of 30 g of inoculant per pot (~1,000 spores) was applied and incorporated into the growing medium at a depth of 15 cm. This placement was intended to ensure proximity and direct contact between the inoculum propagules and the roots of chicory seedlings, thereby facilitating effective colonization and symbiotic establishment.

Selenium was supplied as a slow-release Se source (2% selenium prill, Selprill Double®, Ravensdown Company, New Zealand) at 0, 0.038, 0.068, and 0.113 mg/pot, equivalent to 0, 25, 50, and 75 mg/m². Selenium was pre-mixed with fine soil as a carrier and incorporated into the potting substrate in the second week after planting by burying it 2 cm below the soil surface.

The Se application rates (0, 25, 50, and 75 mg Se/m²) were selected to represent a gradient from low to moderately high dose, enabling a comprehensive assessment of Se effects on *C. intybus* cv. Cropmate under drought stress. The 50 mg Se/m² dose rate corresponds to the manufacturer's recommended dose (Selprill Double®, Ravensdown, New Zealand) for standard agronomic conditions and has been reported to enhance crop performance without inducing toxicity (Curtin et al. 2008). The inclusion of 25 mg Se/m² represents a lower, biologically active level, while 75 mg Se/m² allows evaluation of the upper threshold of Se application and its interaction with AMF. Collectively, the 25–75 mg Se/m² range was chosen to balance the beneficial and adverse effects of Se, as low-to-moderate concentrations can enhance plant growth and drought tolerance by activating antioxidant systems that mitigate oxidative stress, whereas excessive doses may induce phytotoxicity. This range also ensures agronomic relevance for biofortification by promoting the accumulation of Se in bioavailable forms, thereby improving forage quality while maintaining safe limits for livestock consumption, and capturing the transition from beneficial to potentially harmful effects.

2.6 Planting, maintenance, and water restriction period

Cropmate chicory seeds (Cropmark Seed Company, New Zealand) were sown at five seeds per 1-cm-deep pot and thinned to one healthy seedling after germination. Regular weed removal throughout the study period is necessary to maintain cleanliness and avoid competition for nutrients, water, and sunlight. During the research period, no inorganic fertilizers or insecticides were applied. During the initial growth period of the seedlings, which extends up to two weeks, watering should be conducted regularly in the morning and afternoon to maintain adequate soil moisture, facilitate robust root development, and provide optimal early-stage growth conditions before water-restriction treatments begin.

Drought stress was initiated two weeks after sowing to simulate water-limited conditions typical of tropical dry seasons. Soil moisture in each pot was carefully monitored and maintained at ≤40% of field capacity, representing moderate drought stress that limits water availability without causing severe desiccation. After the establishment phase, irrigation was reduced and controlled volumes were maintained to maintain the target moisture level. Approximately 300 mL of water was supplied per pot as needed to achieve the desired soil moisture condition (≤40% of field capacity, equivalent to ≤60% reduction relative to total water-holding capacity). Additional watering was applied only when plants exhibited visible wilting to maintain consistent drought stress without over-irrigation. Soil moisture was measured every 2 days (Medyouni et al. 2021), using a portable intelligent soil detector with a probe sensor (Paqimolut brand) to ensure uniform and controlled water restriction.

The soil water retention capacity was determined by saturating the pots until drainage ceased and measuring their weight before and after saturation. This method yielded an estimated maximum water-holding capacity of approximately 900 mL per pot. This value was used to regulate

water application throughout the drought period. In addition to instrumental monitoring, visual indicators such as leaf wilting, color changes, and reduced turgidity were recorded daily to assess stress progression. Physiological parameters, including relative water content (RWC) and proline concentration, were measured to confirm plant responses to drought. The drought lasted for 6 weeks, from 2 weeks after sowing until harvest. This enabled the evaluation of the effects of Se and AMF on plant growth, physiology, and forage yield under controlled water-limited conditions.

2.7 Measuring plant morphological and forage production

The plant morphological measurements comprised plant height, leaf width and length, stem diameter, leaf area index, and root traits. Morphological traits were recorded weekly for six weeks. In week six, leaf area index was measured using the Petiole Pro mobile app with a medium calibration pad (Pad No. 10) placed 15 cm below the setup. Leaf color measurements were taken on the third or fourth leaf from the tip that had fully expanded to ensure samples were uniform. Observations were conducted in the morning under even, indirect natural lighting conditions to minimize visual distortion. Leaf color was determined by aligning the leaf surface with the royal horticultural society (RHS) color chart for plant and comparing it to the available scale until the closest color was found. Observations were conducted on all plant units within each treatment. Root traits were assessed at 60 days, following Mangwe et al. (2020), with root length measured from the base to the tip. Fresh root weight and forage yield were obtained using an analytical balance after removing adhering growth media. *Cichorium intybus* cv. Cropmate was analysed for dry matter (DM), organic matter (OM), and crude protein (CP) contents to calculate their respective yields. All variables were expressed as grams of fresh weight per plant.

2.8 Leaf chlorophyll measurements

Approximately 1 g of leaf sample was extracted using pre-cooled acetone. The leaves were initially macerated in a mortar, then 10 mL of cold acetone was added. The extraction process involved incubating the crushed material with the solvent at ambient temperature for 12 h. This was done to ensure complete solubilization of chlorophyll. Following the incubation period, the mixture was centrifuged at 2,000 rpm for 10 min. This procedure was undertaken to facilitate the separation of the liquid extract from the solid residue. Subsequently, the clear medium was utilized for spectrophotometric analysis to ascertain the concentrations of chlorophyll a, chlorophyll b, and total chlorophyll (Jiang et al. 2017). Absorbance was measured with a UV–Vis spectrophotometer at 663 and 645 nm. The concentrations of chlorophyll (mg/g) were calculated as delineated by Gu et al. (2016).

2.9 Relative water content and proline concentration measurements

Relative water content (RWC) was determined according to the method of Mwendia et al. (2013). Leaf segments were collected, stored in tubes within a cooler, and weighed fresh (A). They were then soaked in deionised water for 4 h at room temperature, blotted dry, and weighed to obtain turgid weight (B). Samples were subsequently oven-dried at 80 °C for 24 h to obtain dry weight (C). Relative water content was calculated as follows:

$$\mathrm{RWC}\% = [(\mathrm{A} - \mathrm{C})/(\mathrm{B} - \mathrm{C})] \times 100.$$

Proline concentration was measured according to the method of Bates et al. (1973). Leaf samples (0.5 g) were subjected to crushing and homogenization with 10 mL of 3% sulfosalicylic acid. The filtrate (2 mL) was then reacted with ninhydrin and glacial acetic acid. The test tube was wrapped in aluminum foil and heated in a water bath at 100 °C for 1 h. Subsequently, the test tube was placed in ice water. Subsequently, 4 mL of toluene was added to the mixture, which was vortexed for 15–20 s. The chromophore was then separated and placed in a cuvette for measurement at 520 nm using a Genesis 10s UV-Vis spectrophotometer.

2.10 Measuring total phenol and flavonoid concentration

A modified Folin–Ciocalteu method, as described by Singleton et al. (1999), was used to determine total phenol content. Two grams of the sample were dissolved in 70% ethanol and extracted for 30 min using an ultrasonic bath. A standard solution of gallic acid at concentrations of 0–30 mg/L was prepared for the calibration curve. Mixed 1 mL of the extracted sample or standard solution with 0.5 mL of Folin-Ciocalteu reagent (1 N) and 2.5 mL of 20% Na_2CO_3 solution. The mixture was incubated for 40 min at room temperature, then the absorbance was measured at 725 nm using a UV-Vis spectrophotometer. The total phenol content was expressed as gallic acid equivalents (mg GAE/g sample).

Total flavonoid content was determined using a UV–Vis spectrophotometric assay modified from Chang et al. (2002). Two grams of the sample were dissolved in 70% ethanol and extracted for 30 min in an ultrasonic bath. A quercetin calibration curve (5–80 mg/L) was prepared to obtain a calibration curve. Then, 1 mL of the extract sample

or standard was mixed with 0.1 mL of 10% $AlCl_3$ and 0.1 mL of 5% acetic acid, and then distilled water was added to a final volume of 2.5 mL. After 30 min of incubation at room temperature, absorbance was read at approximately 415 nm, and results were reported as quercetin equivalents (mg QE/g).

2.11 Measuring the tissue selenium content

Cropmate chicory samples were analysed for Se content by inductively coupled plasma mass spectrometry (ICP-MS) following the method of Thomas (2004). Torch position and ion lens settings were first optimised in standard mode through stability tests with a 1 µg/L tuning solution. The collision cell was then purged with a He–H_2 gas mixture for 30 min, after which a second optimisation of torch position and ion lens parameters was performed. Both optimization steps used mass-scanning mode. Multi-element calibration standards (2–100 µg/L) were prepared according to relative sensitivity factors. Selenium was quantified at m/z 80 in CCT mode using an internal standard mixture (^{70}Ga, ^{89}Y, ^{115}In) to correct short-term deviations across the m/z range. The alternative isotope ^{82}Se was measured in standard mode, with automatic correction applied to m/z 82.

2.12 Statistical analysis

The data were analyzed using two-way analysis of variance (two-way ANOVA), except for leaf color, which was analyzed descriptively. Before analysis, the Shapiro–Wilk test was employed to verify the assumption of normality, whilst Levene's test was used to verify the assumption of homoscedasticity. The Duncan's Multiple Range Test (DMRT) was employed to compare the means of all parameters in pairs, provided that the two factors (S × M) interacted significantly at $P<0.05$. All analyses were conducted in R (version 4.4.0) using the *agricolae* package (R Core Team 2022).

3 Result and discussion

3.1 Plant morphological characteristics

Morphological characteristics of *C. intybus* cv. Cropmate, including leaf number, plant height, plant length, and stem diameter, were significantly influenced by the treatments. In contrast, leaf width remained unaffected (Fig. 2). The current study, significant differences in leaf number were observed from weeks IV and VI onward with 50 mg Se+AMF (13.00 and 14.40 leaves, respectively), having the highest values significantly differing from all other treatments ($P<0.05$; Fig. 2A). Although leaf width did not differ significantly among treatments (4.58–7.64 cm (Fig. 2B).

Plant height was significantly influenced by treatment, particularly during the final observation period (week VI). The highest plant height was observed at 75 mg Se+AMF (37.04 cm), although a comparable value was recorded without AMF (37.18 cm). These values represent an increase of ~63.5% relative to the control (22.66 cm; Fig. 2C). From week IV ($P<0.05$), a substantial increase in plant height was observed, with the 75 mg Se treatment reaching 35.00 cm, which is nearly 1.5 times the height of the non-AMF control (22.20 cm; Fig. 2D). Similarly, stem diameter responded substantially to treatment ($P<0.05$), with the largest values observed under 50 mg Se+AMF (15.20 mm) and 75 mg Se (15.08 mm), compared to 9.72 mm in the non-AMF control (Fig. 2E).

The significant response of morphological traits to the combined application of Se and AMF in this study can be attributed to complementary physiological and biochemical mechanisms that enhance plant growth. Selenium has been demonstrated to enhance plant metabolism, particularly principally by means of lowering oxidative stress (Handa et al. 2017; Hasanuzzaman et al. 2010). This enhancement facilitates cell division and elongation, consequently increasing plant height, leaf number, and stem diameter. Additionally, Se improves chlorophyll stability and photosynthetic efficiency, hence enhancing assimilate production to support vegetative development, as observed in this study. Several researchers have also reported positive responses of Se to improved plant morphology under drought stress, such as increased plant height in *Okra* (Ali et al. 2020), maintaining and increasing leaf number in *Sesame* plant (Thuc et al. 2021), and *chicory* (Zaini et al. 2021; Umami et al. 2022), and positive effects on plant length and stem diameter in *Oryza sativa* (Luo et al. 2021). As the primary photosynthetic organs, leaves directly influence photosynthetic capacity, nutrient uptake, and productivity (Chondrogiannis and Grammatikopoulos 2016), while greater leaf abundance supports post-harvest regeneration, which is essential for sustainable forage systems (Mganga et al. 2021).

Concurrently, AMF play a role in increasing plant morphological traits by facilitating more efficient water and nutrient uptake. Under hydric stress, AMF have been found to support plants by modulating soluble sugar levels and photosynthetic pigments, so helping them deal with drought (Guo et al. 2024; Wang et al. 2025c). The present work reveals that modulation is visible, as indicated in Table 6, and this ultimately leads to a large increase in morphological responses. The findings of Bouskout et al. (2022) have consistently demonstrated that implementing AMF resulted in increased plant height, node number, and stem collar diameter in *C. spinosa*, irrespective of irrigation scenario. In their

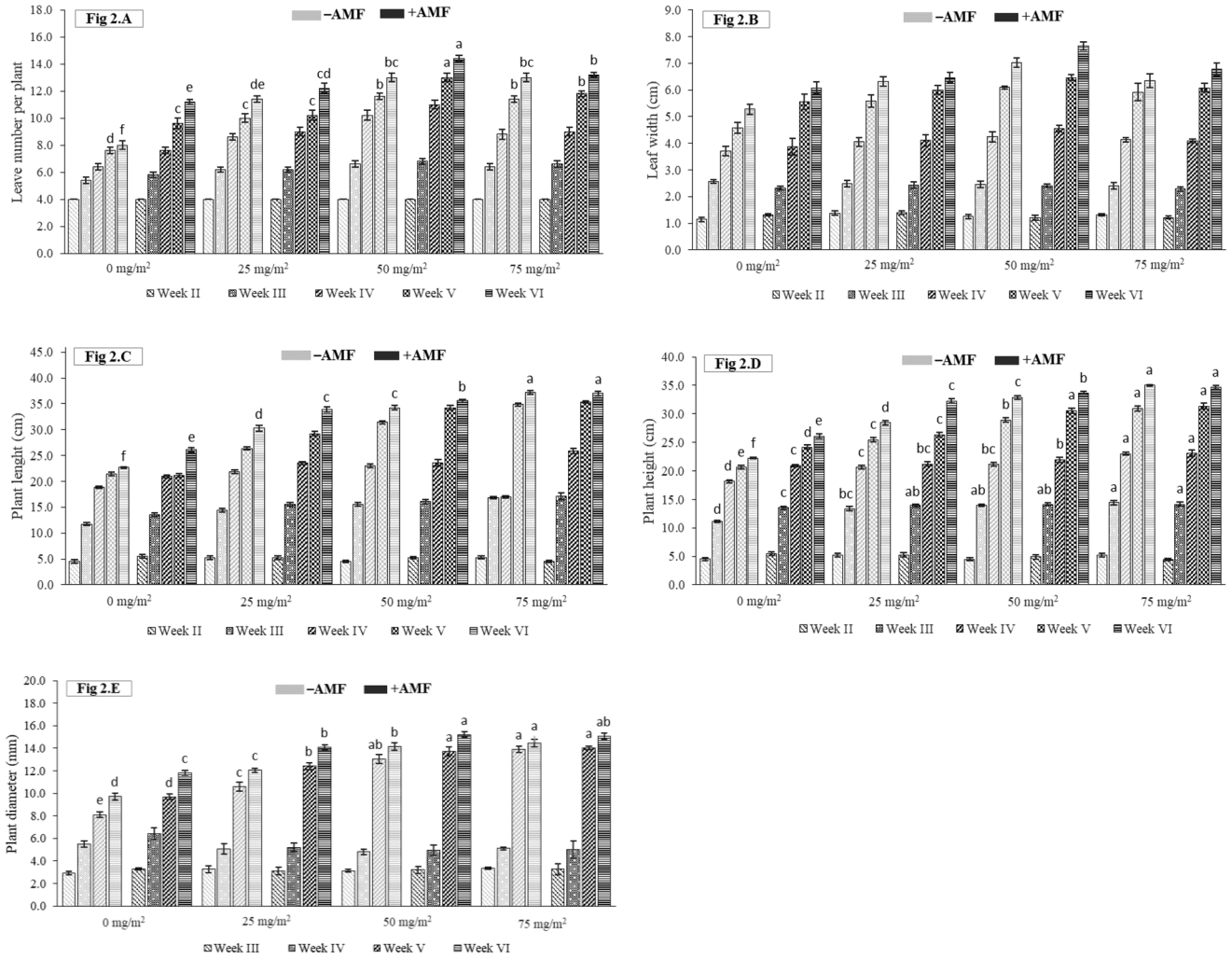


Fig. 2 Morphological characteristics of *C. intybus* cv. Cropmate under different application rates of Se and mycorrhizal inoculation across observation periods from Week II to Week VI. The bars represent the mean ± SD of $n = 7$ replicates. Different lowercase letters on the bars indicate significant differences among treatments based on DMRT post hoc test at $P < 0.05$. Bars without superscript letters within each week indicate that the interaction effect between factors was not statistically significant ($P > 0.05$)

investigation, the number of leaves in the AMF treatment was much larger than in uninoculated seedlings, increasing by ~172% and ~117% at 50% and 25% FC, respectively, for leaf and node counts.

Regarding synergistic effects, AMF facilitates the uptake and translocation of Se, whereas Se supports the antioxidant system, hence improving the symbiotic activity of AMF. This interaction has been found to promote nutrient utilization efficiency, hormonal regulation, and overall metabolic activity, consequently promoting vegetative growth. Synergistic effects of Se and AMF on growth have also been observed in *Brachiaria grass* (Ananias et al. 2025; Bamberg et al. 2019), with AMF aiding resource allocation during drought stress (Hashmi et al. 2010). However, the absence of a substantial response in leaf width show that this feature is likely more driven by genetic factors or assimilate allocation strategies, in which plants prioritize greater height and number of leaves over leaf area expansion. These findings reveal a synergistic effect of Se and AMF in inceasing vegetative development under water-limited settings, highlighting their agronomic potential for drought-prone regions.

Selenium application significantly altered leaf area index (LAI) ($P < 0.001$), although mycorrhizal inoculation and its interaction with Se did not (Table 3). The LAI values increased with increasing Se dose up to 50 mg/m², then declined at 75 mg/m² ($P < 0.001$). Although there was no statistically significant combined impact, the highest LAI was recorded in the 50 mg Se+AMF inoculation treatment, demonstrating an increase by ~52.5% compared to the control group that did not receive AMF. This was followed by the 25 mg Se+AMF treatment, which increased by ~51.8%. Conversely, the lowest LAI values were reported in the control treatment (0 mg/m²), in both the –AMF and +AMF treatments. The decline in the 75 mg Se+AMF

Table 3 Leaf area index (LAI) of *C. intybus* cv. Cropmate biofortification with selenium and AMF inoculation

Treatments		Leaf area index
Selenium application	Mycorrhizal inoculation	(cm^3)
0 mg/m^2	–AMF	50.77 ± 3.23
	+AMF	51.49 ± 2.40
25 mg/m^2	–AMF	70.69 ± 7.56
	+AMF	77.05 ± 3.63
50 mg/m^2	–AMF	72.24 ± 9.64
	+AMF	78.55 ± 8.64
75 mg/m^2	–AMF	63.90 ± 4.51
	+AMF	65.39 ± 13.1
Selenium application (S)		***
Mycorrhizal inoculation (M)		ns
S × M		ns

Mean value (n = 7) ± SD for LAI, for non-mycorrhizal (–AMF) and mycorrhizal (+AMF) inoculation between all dose selenium application that do not have the same lowercase letter are significantly different based on DMRT post hoc test at $P < 0.05$. Asterisks indicate the significance level of the effects of selenium application (S), mycorrhizal inoculation (M), and their interaction (S × M) on measured parameter as determined by a two-way ANOVA (***, $P < 0.001$; ns = no significance, $P > 0.05$)

Table 4 Leaf color of *C. intybus* cv. Cropmate biofortification with selenium and AMF inoculation

Treatments		Leaf color*
Selenium application	Mycorrhizal inoculation	
0 mg/m^2	–AMF	Medium green slightly faded - Greyish olive-green A (NN 137)
	+AMF	Medium green slightly faded - Greyish olive-green A (NN 137)
25 mg/m^2	–AMF	Medium green - Moderate olive-green B (137)
	+AMF	Medium green - Moderate olive-green B (137)
50 mg/m^2	–AMF	Medium green - Moderate olive-green B (137)
	+AMF	Medium green - Moderate olive-green B (137)
75 mg/m^2	–AMF	Medium green slightly faded - Greyish olive-green A (NN 137)
	+AMF	Medium green - Moderate olive-green B (137)

* Leaf color was measured using Royal Horticultural Society (RHS, sixth edition) color charts for plants

treatment was 6.9% compared to the peak at 50 mg Se/m². Moderate Se doses (25–50 mg) provided the highest LAI values (77.05–78.55 cm³) in inoculated plants, but excess Se (75 mg) caused a decrease in these values. In this case, the addition of AMF further boosted leaf expansion, suggesting improved carbon assimilation and nutrient acquisition (Abdelaal et al. 2024). Enhanced leaf shape, including increased leaf length, improved palatability and nutritional value by lowering the stem-to-leaf ratio, which is known to diminish forage quality (Akakpo et al. 2022). The combination of Se and AMF further promoted plant leaf expansion, indicating improved photosynthetic efficiency and greater carbon assimilation capacity, thereby supporting forage production. These improvements can be attributable to the combined effects of Se and AMF, which promote nutrient uptake and photosynthetic activity. Previous investigations confirmed that AMF improve nutrient acquisition and photosynthetic traits, while Se administration amplifies such benefits (Antoniou et al. 2021; Chen et al. 2017; Hamed-Far et al. 2025; Huang et al. 2024; Shi et al. 2020; Xu et al. 2016).

Leaf color indicates a clear improvement in greenness when Se and AMF are applied. At 0 mg Se –AMF, the leaves were graded as medium green, with moderate fading. At 25 and 50 mg Se treatment, plants revealed deeper pigments, shifting to medium olive green. At a dose of 75 mg Se, the leaves recovered to a slightly faded green, showing a threshold effect in which excessive Se administration may impair leaf coloration. Excessive Se appears to harm chloroplasts, as demonstrated in *celery* and *strawberries*, where high Se doses lowered pigments despite early stimulation (Antoniou et al. 2021; Huang et al. 2024). This pattern suggests an optimal Se dose for pigment biosynthesis that corresponds with findings of Se increasing chlorophyll production under moderate stress (Ravello et al. 2021). The detailed results for plant leaf color are shown in Table 4.

3.2 Root growth parameters

The data presented in Table 5 demonstrate that both Se application and AMF inoculation considerably altered C. *intybus* cv. Cropmate root traits parameters. Even though the interaction between factors was not significant, the root weight at 50 mg Se+AMF was ~83.3% higher than the control. Similarly, root length increased by ~70.2%, from 31.27 cm in the control to 53.23 cm under the same treatment, with both Se and AMF individually showing highly significant effects ($P < 0.001$).

These findings align with those of Bouskout et al. (2025a), who reported similar increases in root growth of *C. spinosa* under AMF inoculation. Moreover, Bouskout et al. (2025b) studied the beneficial effects of AMF on root growth of *C. spinosa* under abiotic stress, such as salinity pressure. Their findings demonstrated that plantlets inoculated with AMF consistently exhibited higher root fresh weight (RFW) and root dry weight (RDW) than non-mycorrhizal plants across all salinity levels. For instance, under non-stress conditions, +AMF plants exhibited RFW and RDW ~ 60.9% higher, and at low salinity levels, the increase could reach ~ 154–158%. Regarding Se application, several studies consistently confirm the favorable benefits of Se on root growth under drought conditions. In wheat seedlings, exogenous

Table 5 Root quantification characteristics of *C. intybus* cv. Cropmate biofortification with selenium and AMF inoculation

Treatments		Root weight	Root length	Root diameters		
Selenium application	Mycorrhizal inoculation	(g/plant)	(cm)	Top (mm)	Mid (mm)	Bottom (mm)
0 mg/m^2	–AMF	13.35±2.90	31.27±3.64	8.22±0.77	5.32±1.27	2.18±0.41
	+AMF	14.54±3.04	35.75±2.43	7.54±1.31	4.79±1.38	2.76±0.52
25 mg/m^2	–AMF	16.23±5.97	37.73±4.58	8.60±0.75	5.04±0.95	2.72±0.56
	+AMF	20.25±6.96	46.16±8.65	9.90±1.23	5.68±1.36	7.95±0.23
50 mg/m^2	–AMF	16.00±1.89	46.13±8.18	9.66±1.46	5.52±0.82	3.02±0.08
	+AMF	24.48±5.34	53.23±3.95	11.20±1.67	6.70±1.19	3.68±0.70
75 mg/m^2	–AMF	14.68±5.09	41.50±4.09	9.54±0.85	5.40±1.21	3.16±0.84
	+AMF	18.86±3.89	48.02±4.52	10.36±0.65	6.28±0.81	3.90±0.62
Selenium application (S)		*	***	***	ns	***
Mycorrhizal inoculation (M)		**	***	ns	ns	**
S × M		ns	ns	ns	ns	ns

Mean value ($n=7$)±SD for each root quantification characteristics, for non-mycorrhizal (–AMF) and mycorrhizal (+AMF) inoculation between all dose selenium application that do not have the same lowercase letter are significantly different based on DMRT post hoc test at $P<0.05$. Asterisks indicate the significance level of the effects of selenium application (S), mycorrhizal inoculation (M), and their interaction (S × M) on measured parameter, as determined by a two-way ANOVA (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ns = no significance, $P>0.05$)

Se treatment improved root length, root number, and both fresh and dry biomass during water-deficit stress (Chen et al. 2020). In pot marigold, seed priming with low Se concentrations improved root length and total biomass (Bayat and Aminifard 2021), while in quinoa, foliar Se application (25–50 mg/L) increased root length under drought circumstances (Sadak and Bakhoum 2022). Collectively, our findings demonstrate that Se plays a vital role in stimulating root development and biomass accumulation under drought stress, hence boosting plant tolerance to water limitation. The observed effects of AMF are consistent with early research, which demonstrate that AMF boosts root biomass by improving micronutrient uptake and increasing carbon allocation to belowground organs under abiotic stress conditions (Etesami et al. 2021).

Our findings also demonstrate that root diameter varies across root zones, with diverse responses among treatments. Top root diameter increased by ~36.3% under 50 mg Se+AMF compared to the control, with a significant effect of Se ($P<0.001$) but no significant effect of mycorrhizal inoculation. Notably, bottom root diameter showed the most substantial response, increasing by ~264.7% at 25 mg Se+AMF relative to the control, with both Se ($P<0.001$) and AMF ($P<0.01$) having significant impact. Such spatial variation in root thickening has been reported previously and is associated with functional differentiation along the root axis, particularly under conditions demanding greater mechanical stability and water absorption (Freschet et al. 2021; Lynch 2019).

Although no statistically significant interaction was detected between Se and AMF for any root traits parameter, across Se doses, AMF consistently enhanced root biomass, highlighting its key role in nutrient absorption and water utilization, as established in recent AMF studies under abiotic stress conditions (Abdelaal et al. 2024; Ouahmane et al. 2007). Moderate Se dose (25–50 mg Se/m^2) combined with AMF promote root elongation, biomass, and distal thickness, whereas high Se (75 mg Se/m²) decreased these benefits. This response pattern supports a dose-dependent role of Se in root development, with moderate concentrations encouraging cell proliferation and antioxidant protection, whereas excessive Se may disrupt root metabolic balance (Gupta and Gupta 2017; Kaur et al. 2024). Across all Se doses, AMF consistently enhanced root biomass and elongation, thereby highlighting its central role in improving nutrient acquisition and water-uptake efficiency through extensive hyphal networks and enhanced rhizosphere exploration (Begum et al. 2019; Hamed-Far et al. 2025; Smith and Smith 2012). The observed improvement in root system architecture increases root resistance and expands the effective absorption zone. This improves the efficiency with which water and nutrients are absorbed, resulting in amplifying the synergistic role of Se–AMF in enhancing plant resistance and productivity under drought stress.

3.3 Forage production

Selenium application and mycorrhizal inoculation significantly affected forage yield parameters, including fresh forage yield, DM, OM, and CP yield ($P<0.001$) (Table 6). A significant interaction between Se and AMF was observed for fresh forage and CP yield ($P<0.05$), whereas no interaction effect was detected for DM and OM yield. Fresh forage yield was significantly influenced by both Se and AMF ($P<0.001$) and by their combination ($P<0.05$). The largest fresh forage yield (74.02 g/plant) was observed at 50 mg Se+AMF, followed by 75 mg Se+AMF (65.14 g/plant). In comparison, the lowest result was obtained in the control

Table 6 Forage yield of *C. intybus* cv. Cropmate biofortification with selenium and AMF inoculation

Treatments		Fresh forage	Dry matter	Organic matter	Crude protein
Selenium application	Mycorrhizal inoculation	(g/plant)	(g/plant)	(g/plant)	(g/plant)
0 mg/m^2	–AMF	35.58 ± 4.83^{f}	32.34 ± 5.09	28.68 ± 4.45	6.45 ± 1,29^{f}
	+AMF	45.36 ± 4.95^{e}	40.27 ± 4.18	35.73 ± 3.68	8.61 ± 0.68^{e}
25 mg/m^2	–AMF	52.84 ± 5.73^{d}	48.42 ± 5.94	43.79 ± 5.36	11.03 ± 1.70^{d}
	+AMF	57.52 ± 5.32^{cd}	54.33 ± 4.77	47.13 ± 3.40	12.78 ± 1.55^{cd}
50 mg/m^2	–AMF	57.86 ± 3.77^{cd}	53.31 ± 3.45	46.57 ± 3.08	12.72 ± 1.01^{c}
	+AMF	74.02 ± 5.70^{a}	69.35 ± 4.85	59.72 ± 4.73	17.47 ± 1.42^{a}
75 mg/m^2	–AMF	62.10 ± 5.73^{bc}	55.09 ± 3.56	45.71 ± 3.25	13.95 ± 0.72^{bc}
	+AMF	65.14 ± 3.67^{b}	60.75 ± 3.36	51.59 ± 3.07	15.39 ± 0.71^{b}
Selenium application (S)		***	***	***	***
Mycorrhizal inoculation (M)		***	***	***	***
S × M		*	ns	ns	*

Mean value (n = 7) ± SD for each forage yield, for non-mycorrhizal (–AMF) and mycorrhizal (+AMF) inoculation between all dose selenium application that do not have the same lowercase letter are significantly different based on DMRT post hoc test at $P < 0.05$. Asterisks indicate the significance level of the effects of Se application (S), mycorrhizal inoculation (M), and their interaction (S × M) on measured parameter, as determined by a two-way ANOVA (*, $P < 0.05$; **, ***, $P < 0.001$; ns = no significance, $P > 0.05$)

treatment without AMF, which yielded 35.58 g/plant. Overall, across Se doses, AMF enhanced fresh forage yield by ~16.2%. These results indicate that the presence of AMF enhanced the effect of Se on fresh biomass production. The elevated yield observed at the moderate dose of Se in combination with AMF is presumably indicative of enhanced leaf hydration and diminished oxidative stress, a phenomenon attributed to augmented antioxidant enzyme activity such as SOD and CAT (Zhong et al. 2024), and to AMF-induced enhancements in water absorption, retention, and soil aggregation (Fall et al. 2022; Kuila and Ghosh 2022).

Dry matter and organic matter production were significantly affected by both Se application and mycorrhizal inoculation ($P < 0.001$), although their interaction was not significant. Both parameters demonstrated a similar response pattern, increasing significantly with Se application up to 50 mg and declining slightly at 75 mg. The highest DM yield (69.35 g/plant) and OM (59.72 g/plant) were recorded at 50 mg Se + AMF inoculation. Lower values were observed in the control and at the highest Se dose, indicating a consistent response to Se application regardless of AMF presence. The absence of interaction suggests that Se and AMF contributed independently to the accumulation of both dry and organic matter. Previously, Bouskout et al. (2022) clearly documented that biomass production of non-mycorrhizal plants is significantly lower than that of plants inoculated with AMF complexes across all levels of water stress. Although in our study the combined effect of Se application and AMF inoculation did not result in a statistically significant increase in either parameter, substantial numerical increases in DM and OM production, at 18.8% and 17.9%, respectively, suggesting a potential synergistic effect between Se and AMF. This phenomenon is evidenced by increased Se uptake via extraradical hyphae and rhizosphere modification by AMF (Peng et al. 2020; Zhang et al. 2022). A comparable synergistic response has been documented in forage grasses (Bamberg et al. 2019), in which AMF enhance biomass while reducing Se toxicity (Kaur et al. 2024).

Crude protein yield is one of the key forage agronomic parameters. The present study showed that CP yield was significantly affected by Se, AMF, and their interaction ($P < 0.001$ for main effects; $P < 0.05$ for interaction). The highest CP yield (17.47 g/plant) was observed at 50 mg Se + AMF, followed by 75 mg Se + AMF (15.39 g/plant). The lowest value was observed in the control without AMF, indicating an increase of ~170% at 50 mg Se + AMF compared to the control. The significant interaction indicates that AMF amplified the effect of Se on protein accumulation. Although the CP yield decreased slightly at 75 mg Se + AMF, it remained higher than in all non-mycorrhizal treatments, reflecting that AMF enhanced nutrient acquisition, particularly nitrogen, and the role of Se in protein metabolism through its incorporation into amino acids such as selenomethionine and selenocysteine (Liu et al. 2024b; Ndeko et al. 2024; Qin et al. 2023). The observed interaction suggests that AMF facilitate both the uptake and biotransformation of Se into bioavailable organic forms, thereby optimizing protein synthesis, improving forage quality, and enhancing drought tolerance. In line with these findings, analogous synergistic effects of AMF and Se have been documented across a wide range of crops, including legumes and forage species, where their application elevates protein content and overall CP yield (Elsafy et al. 2025; Golubkina et al. 2020). The results of this study demonstrate the potential of integrating Se application and AMF inoculation within sustainable agricultural systems to optimize forage production. Enhancing the nutritional quality of forage has been shown to improve livestock performance, thereby supporting better animal health and productivity (Dahlanuddin et al. 2024;

Kariyani et al. 2021; Putra et al. 2025; Sutaryono et al. 2025). Moreover, this strategy contributes to environmentally sustainable practices by reducing reliance on synthetic fertilizers while maintaining or improving crop yields.

3.4 Plant physiological traits

To better understand the physiological and biochemical responses underlying the observed treatment effects, plants were evaluated using key indicators, including photosynthetic pigments (chlorophyll a, chlorophyll b, and total chlorophyll), RWC, proline accumulation, and secondary metabolites (phenolics and flavonoids). Our result showed that Se application exhibited a significant effect on all measured physiological traits ($P<0.001$). In contrast, mycorrhizal inoculation significantly influenced most physiological trait parameters ($P<0.05$–0.001), except chlorophyll b and flavonoids (Table 7). A substantial interaction effect between Se and AMF was observed for all parameters.

Chlorophyll plays a central role in photosynthetic efficiency but declines under stress. Our findings showed that chlorophyll content (chlorophyll a, chlorophyll b, and total chlorophyll) increased with Se application, with the highest values generally recorded at 50 mg Se+AMF inoculation (2.73, 0.76, and 3.49 mg/g, respectively), representing increases of ~35–45% compared to the untreated plant. The chlorophyll content at the 25 mg Se treatment was significantly higher than that in the control group ($P<0.05$), yet lower than that at the 50 mg Se treatment ($P<0.05$). This indicates a clear dose-dependent response. A decline of ~5–10% was observed at 75 mg Se/m² compared to the peak at 50 mg Se/m², although values remained significantly higher than the control ($P<0.05$). These results indicate a synergistic interaction between Se and AMF in enhancing plant photosynthetic capacity, as reflected by the increased chlorophyll content. This effect was particularly evident in chlorophyll a and total chlorophyll, suggesting improved photosynthetic efficiency under combined treatments. In addition, the substance demonstrated a dose-dependent response, with the most pronounced effect observed at 50 mg/m². At this particular dosage, Se appeared to play a pivotal role in promoting chlorophyll biosynthesis and stabilizing photosynthetic pigments, thereby facilitating optimal chlorophyll accumulation. The findings emphasize the efficacy of moderate Se application, particularly in combination with AMF, in enhancing plant photosynthetic capacity. Consistent findings have been documented by Bouskout et al. (2022), who established that in *C. spinosa*, the levels of photosynthetic pigments in +AMF seedlings exhibited a marked increase in comparison to –AMF seedlings under all levels of water stress. Moreover, the study demonstrated that at a field capacity of 25%, AMF-colonized plants exhibited approximately twice the carotenoid content of uncolonized plants. In addition, reports in *Amaranthus tricolour*, corn, and green beans also show synergistic effects of Se and AMF on enhancing chlorophyll accumulation and stress tolerance (Alam et al. 2019; Kaur et al. 2024; Lu et al. 2024). The differential responses of chlorophyll a and b suggest distinct regulatory mechanisms, as observed in onion (Hamed-Far et al. 2025), whereas excessive Se may impair chloroplast integrity, as reported in celery and strawberries (Antoniou et al. 2021; Huang et al. 2024). At 75 mg Se treatment, chlorophyll declined regardless of AMF presence, indicating a possible inhibitory threshold.

The observed improvements in chlorophyll content under moderate Se and AMF treatments highlight their

Table 7 Physiological traits of *C. intybus* cv. Cropmate biofortification with selenium and AMF inoculation

Treatments		Chlorophyll a	Chlorophyll b	Total chlorophyll	RWC	Proline	Phenolic	Flavonoids
Selenium application	Mycorrhizal inoculation	(mg/g)	(mg/g)	(mg/g)	(%)	(µmol/g)	(mg GAE/100 g)	(mg QE/100 g)
0 mg/m^2	–AMF	1.40 ± 0.14^{f}	0.47 ± 0.10^{d}	1.87 ± 0.23^{d}	56.76 ± 1.36^{d}	2.73 ± 0.47^{a}	698.09 ± 0.58^{g}	0.14 ± 0.007^{d}
	+AMF	1.92 ± 0.18^{e}	0.62 ± 0.05^{c}	2.54 ± 0.21^{c}	62.85 ± 1.88^{c}	1.30 ± 0.17^{b}	903.65 ± 0.89^{c}	0.14 ± 0.010^{d}
25 mg/m^2	–AMF	2.24 ± 0.09^{d}	0.76 ± 0.11^{ab}	2.99 ± 0.19^{b}	65.45 ± 2.46^{bc}	1.00 ± 0.26^{b}	977.10 ± 1.48^{a}	0.15 ± 0.007^{d}
	+AMF	2.33 ± 0.07^{cd}	0.75 ± 0.05^{ab}	3.08 ± 0.08^{b}	67.10 ± 1.85^{b}	0.38 ± 0.09^{c}	849.53 ± 1.26^{f}	0.14 ± 0.006^{d}
50 mg/m^2	–AMF	2.45 ± 0.19^{bc}	0.73 ± 0.10^{abc}	3.18 ± 0.13^{b}	68.37 ± 1.40^{b}	0.31 ± 0.12^{c}	900.93 ± 1.12^{c}	0.17 ± 0.009^{c}
	+AMF	2.73 ± 0.09^{a}	0.76 ± 0.11^{ab}	3.49 ± 0.20^{a}	75.66 ± 1.84^{a}	0.39 ± 0.06^{c}	886.13 ± 5.60^{d}	0.21 ± 0.011^{b}
75 mg/m^2	–AMF	2.37 ± 0.07^{cd}	0.77 ± 0.05^{a}	3.14 ± 1.11^{b}	67.95 ± 2.67^{b}	0.39 ± 0.10^{c}	873.85 ± 6.80^{e}	0.24 ± 0.007^{a}
	+AMF	2.56 ± 0.12^{ab}	0.64 ± 0.04^{bc}	3.20 ± 0.16^{b}	73.05 ± 2.77^{a}	0.40 ± 0.08^{c}	949.39 ± 0.25^{b}	0.23 ± 0.013^{a}
Selenium application (S)		***	***	***	***	***	***	***
Mycorrhizal inoculation (M)		***	ns	***	***	***	***	ns
S × M		*	*	**	*	***	***	**

Mean value ($n=7$)±SD for each physiological trait, for without-mycorrhizal (–AMF) and mycorrhizal (+AMF) inoculation between all dose selenium application that do not have the same lowercase letter are significantly different based on DMRT post hoc test at $P<0.05$. Asterisks indicate the significance level of the effects of selenium application (S), mycorrhizal inoculation (M), and their interaction (S × M) on measured parameters, as determined by a two-way ANOVA: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ns = no significance, $P>0.05$

complementary roles in maintaining photosynthetic performance under drought stress. Selenium, as a beneficial micronutrient, has been shown to enhance photosynthesis by improving chlorophyll content, photosystem stability, and photosynthetic enzyme activity. For example, Se prevents pigment destruction, sustains enzymatic activity, and improves stomatal function (Hasanuzzaman et al. 2022; Zhao et al. 2023), and increases the photosynthetic pigments such as chlorophylls and carotenoids, which are critical for efficient photosynthesis (Araujo et al. 2023; Cunha et al. 2024). Also, Se reduces ROS accumulation, thereby preserving photosynthetic structures and sustaining chlorophyll content (Shafi et al. 2019). In addition, Se promotes the synthesis of osmolytes, such as proline, which contribute to osmotic adjustment and protect cells, thereby helping maintain cellular hydration under drought stress (Khan et al. 2015; Luo et al. 2019; Zhong et al. 2024).

Concurrently, AMF play a crucial role in improving plant water status and physiological resilience. Through their extensive hyphal networks, AMF enhance water uptake from deeper soil layers, thereby increasing water availability under deficit conditions. They also contribute to stomatal regulation and water-use efficiency through complex interactions involving abscisic acid (ABA) modulation, osmotic adjustment, and stress signaling pathways. Their role in facilitating stomatal closure and reducing transpiration water loss (Chareesri et al. 2020; Khan et al. 2015; Liu et al. 2016). Furthermore, AMF symbiosis enhances root hydraulic conductivity and osmotic adjustment, thereby supporting water movement and reducing transpiration water loss during stress (Zhu et al. 2015). These combined application of Se and AMF therefore exerts a synergistic effect, enabling plants to better withstand drought stress by maintaining photosynthetic capacity, protecting chloroplast integrity, and improving overall plant hydration.

The RWC was measured to evaluate plant water status and the potential role of Se application and AMF inoculation in improving water retention under the experimental conditions. The relative water content was significantly enhanced by both Se ($P<0.001$) and AMF inoculation ($P<0.01$), reaching a maximum of 75.6% at 50 mg Se+AMF, representing an increase of ~20–25% compared to the control. The RWC at 25 mg Se was significantly higher than in the control group ($P<0.05$), but lower than in the 50 mg Se treatment group ($P<0.05$) (Table 7). Meanwhile, the 75 mg treatment group exhibited a slight but significant~5% reduction compared to the peak value ($P<0.05$). In a manner analogous to chlorophyll content, RWC increased with Se application and declined at the highest dose. This finding suggests that the combination of Se and AMF exhibits a synergistic effect in improving plant water status. The increased RWC reflects improved hydration and cell stability, aligning with studies showing that Se enhances water retention (Alsamadany et al. 2023; Rady et al. 2020; Sajedi and Madani 2018), and AMF-mediated improvements in root architecture and osmotic regulation (Rasouli et al. 2023).

Conversely, proline concentration showed an opposing trend, declining substantially in response to elevated Se application ($P<0.001$), suggesting reduced in osmotic stress. In accordance with this observation, the highest proline concentration (2.73 µmol/g) was found in the control treatment without AMF inoculation, which was significantly higher than all Se-treated plants ($P<0.05$). The lowest values were recorded at 50 mg Se, indicating a~40–50% reduction compared to the control group. This finding suggests a significant decrease in stress-related metabolites. The 75 mg Se treatment demonstrated a modest increase of ~10–15% in comparison with the 50 mg Se treatment, yet remained substantially lower than the control group ($P<0.05$). Proline concentration decreased markedly under Se and AMF treatments in treated plants, indicating reduced osmotic stress and improved physiological balance, as similarly reported in *Vigna radiata* and *Allium cepa* (Alam et al. 2019; Hamed-Far et al. 2025). Collectively, these results indicate that Se and AMF synergistically improve plant water status while alleviating physiological stress, as reflected by reduced proline accumulation.

Furthermore, phenolic content showed significant responses to Se ($P<0.001$), AMF ($P<0.01$), and their interaction ($P<0.05$). The highest recorded value, 977.10 mg GAE/100 g, was observed in the 25 mg Se –AMF treatment. The phenolic content in the 50 and 75 mg Se groups was lower, showing a decrease of ~10–20% from the peak at 25 mg Se –AMF treatment, but was still significantly higher than the control ($P<0.05$), indicating a peak response at a moderate Se dose. Moreover, the flavonoid content also exhibited a substantial interaction between Se and AMF inoculation ($P<0.05$). Flavonoid levels increased progressively with Se application, with the highest values recorded at 75 mg Se under both AMF and non-AMF inoculation. However, the magnitude of increase differed between treatments, with AMF inoculated plants generally showing slightly higher values at moderate Se doses (25–50 mg/m²). Conversely, the disparities between AMF and non-AMF treatments became less pronounced at 75 mg Se/m². Compared with the control, flavonoid content increased by ~25–35%, demonstrating a dose-dependent response with stronger accumulation at higher Se doses.

The results of this study indicate that Se is the primary factor in increasing flavonoid levels. In contrast, the increase in phenolic compounds is influenced by a more complex interaction between Se and AMF. The concomitant application of Se and AMF inoculation led to substantial

increases in phenolic and flavonoid accumulation, which are pivotal antioxidants that help scavenge ROS and support stress adaptation (Saggu et al. 2014). This is consistent with reports in strawberries, onions, and other crops showing enhanced antioxidant metabolism under combined micronutrient and microbial treatments (Antoniou et al. 2021). Selenium biofortification in plants not only promotes phytochemical synthesis but also strengthens defense responses against oxidative and environmental stress. At the same time, transcriptomic and metabolomic studies confirm that AMF enhances Se uptake and activates flavonoid biosynthetic pathways in a dose and species-dependent manner (Wang et al. 2025a). Overall, these results indicate that the Se-AMF combination enhances chlorophyll biosynthesis, water balance, and secondary metabolite production, thereby increasing antioxidant capacity and overall plant resilience under drought conditions.

3.5 Plant tissue selenium content

The findings of the study demonstrated that tissue Se content in *C. intybus* cv. Cropmate increased with increasing Se application, both in combination with AMF and without AMF inoculation (Fig. 3). The tissue Se content revealed a significant increase in concomitance with the increasing dose of Se, reaching a peak at the 75 mg Se–AMF treatment ($P<0.05$). A comparison of treatments suggest that the effect of AMF inoculation is dependant on the Se dose. At 0 mg Se dose, no significant difference was observed between –AMF and +AMF. At the 25 mg Se dose, the AMF treatment (26.81 µg/100 g) tended to be higher than the non-AMF treatment (24.19 µg/100 g), although the difference was not yet significant. However, at the 50 mg Se dose, AMF significantly increased the Se content from 35.28 µg/100 g (–AMF) to 44.03 µg/100 g (+AMF) ($P<0.05$). Interestingly, at a dose of 75 mg Se, the presence of AMF resulted in a considerable decrease in tissue Se content compared to the inoculated plant at the same high dose, with ~8.11% reduction. These findings indicate an interaction between Se dose and AMF inoculation, in which AMF act effectively at moderate doses but is less effective, or even inhibits Se accumulation, at high doses.

As expected, tissue Se content increased consistently with increasing Se doses, and AMF can modulate Se uptake and partitioning, optimizing Se biofortification at moderate doses. Similar interactions between AMF and Se uptake have been observed in other crop systems, where AMF enhanced Se accumulation while mitigating toxicity by restricting excessive translocation to shoots (Kaur et al. 2024). Our results indicate that Se application and the presence of AMF substantially impact Se biofortification at moderate doses, and that their combination is also significant ($P<0.05$). The current findings align with recent research on *C. intybus* cv. Chico (Umami et al. 2022) and signal grass (Ananias et al. 2025; Bamberg et al. 2019). In these studies, the combined application of Se and AMF promote Se accumulation, improved antioxidant status, and increased thermotolerance by activating enzymes such as guaiacol peroxidase (GPX), catalase (CAT), and glutathione reductase (GSH) (Hawrylak-Nowak et al. 2018). Mycorrhizal has been demonstrated to promote Se uptake and its conversion into stable organic forms, such as selenomethionine, which is regarded safer for animals. Furthermore, AMF have been proved to extend root absorption areas and improve micronutrient solubility by organic acid exudation (Etesami et al. 2021). These complementary pathways boost Se bioavailability and contribute to plant resistance under stress circumtances.

No visual indications of Se toxicity were observed, and the tissue Se content (18–65 µg/100 g) remained within the safe range for non-accumulator species. As proven in the relevant literature, biofortification can substantially increase Se levels in plant tissues (Gupta and Gupta 2017),

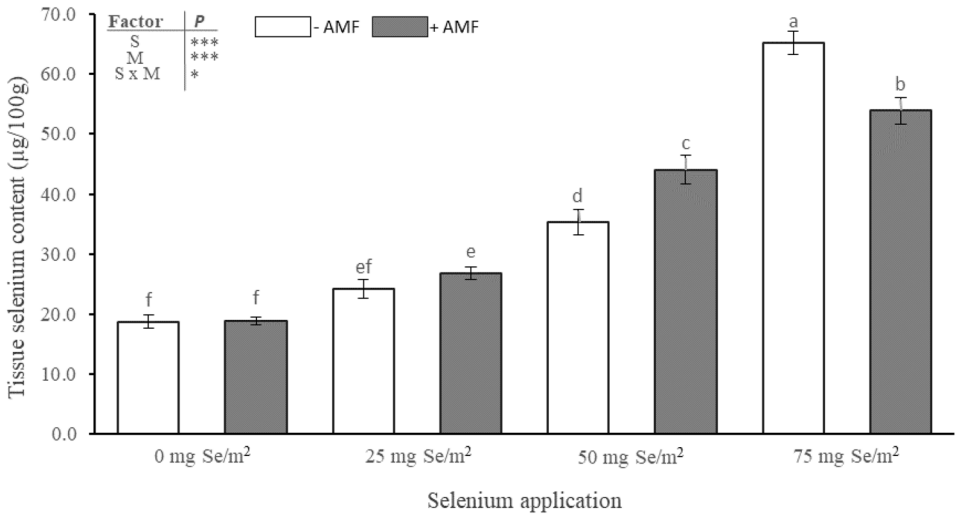


Fig. 3 Tissue selenium content of *C. intybus* cv. Cropmate biofortification with selenium and mycorrhizal inoculation. Different lowercase letters on the bars indicate significant differences among treatments based on DMRT post hoc test at $P<0.05$. The bars represent the mean ± SD of $n=7$ replicates. Asterisks indicate the significance level of the effects of selenium application (S), mycorrhizal inoculation (M), and their interaction (S × M) on measured parameters, as determined by a two-way ANOVA: *, $P<0.05$; ***, $P<0.001$; ns = no significance, $P>0.05$

offering a potential strategy to ameliotare Se deficiency. There is ample documentation of variation in Se accumulation among different plant species. For example, primary Se accumulators, such as *Astragalus* spp., have been shown to retain 100–1000 mg Se/kg DM without toxicity (Brown and Shrift 1982). In contrast, most forage and crop plants, including grasses, are non-accumulators and typically contain less than 25 mg Se/kg DM, with amount seldom 100 mg Se/kg even on seleniferous soils (Terry et al. 2000). These results verify *C. intybus* cv. Cropmate classification as a non-accumulator, yet highlight its potential for safe Se enrichment by AMF-assisted biofortification.

In agreement with Bouskout et al. (2022), AMF-complex inoculation in *C. spinosa* resulted in significantly greater accumulation of both macro-and micronutrients in aboveground tissues across all water deficit levels, compared to plants grown without mycorrhizal colonization. Building upon this evidence, the present study demonstrates that the Se content of *C. intybus* cv. Cropmate also increases significantly in response to AMF inoculation, particularly at moderate dose of Se application, suggesting that AMF symbiosis enhances Se uptake efficiency and its subsequent translocation to aboveground tissues. This increase can be explained by AMF expanding the absorption area through a hyphal network that can penetrate small soil pores and reach nutrients beyond the root-depletion zone (Jin et al. 2024), thereby increasing Se availability around the root system as root length increases in this study. Furthermore, mycorrhizal have been shown to enhance the activity of enzymes and genes involved in regulating nutrient transport in the host plant (Li et al. 2022; Peng et al. 2020), thus improving Se uptake efficiency.

Beyond improving Se uptake, AMF play an essential role in regulating Se dynamics within the plant and limiting its potential toxicity through integrated physiological and biochemical mechanisms. Mycorrhizal have been demonstrated to modify root system architecture, including increasing root surface area, specific root length, and root tip density (Sun et al. 2022). In addition, AMF have been demonstrated to induce the expression of Se transporter genes, hence boosting the uptake of both selenate and selenite from the soil. Furthermore, AMF contribute to the biotransformation of inorganic Se into less harmful forms, including elemental Se and volatile compounds such as dimethyl selenide, which can be released from plant tissues as part of a detoxification process (Kaur et al. 2025). This transformation is accompanied by increased activity of important enzymes involved in Se metabolism, such as adenosine triphosphate sulfurylase and cysteine methyltransferase (Wang et al. 2025b). Moreover, AMF regulate the internal distribution of Se by adjusting its translocation from roots to shoots, thereby preventing excessive accumulation in aboveground tissues. As Sun et al. (2021) stated, the restriction is associated with the downregulation of genes involved in long-distance Se transport, ultimately reducing the risk of Se toxicity, particularly in edible plant portions.

In accordance with these mechanisms, our results showed that AMF-inoculated plants exhibited better tolerance to Se toxicity than non-inoculated plants. This improved tolerance appears to be associated with AMF ability to regulate Se absorption and limit the accumulation of hazardous inorganic forms. In particular, AMF-treated plants showed a more efficient conversion of inorganic Se species, such as selenate, into less toxic organic forms, including selenomethionine, which likely contributed to improved growth and physiological performance. Similar results have also previously been reported that AMF increased the accumulation of organic Se forms in rice (Chen et al. 2020), garlic (Larsen et al. 2006), maize (Kaur et al. 2025), as well as in forages (Bamberg et al. 2019; Ananias et al. 2025) combined with Se fertilization. Besides that, AMF inoculation strengthened the antioxidant defense system, thereby mitigating oxidative damage induced by elevated Se levels. In contrast, non-inoculated plants displayed more pronounced symptoms of Se toxicity, including reduced growth, indicating a lower capacity to cope with Se-induced stress, acting as a pro-oxidant, and causing oxidative stress (especially at 75 mg Se/m^2 without AMF inoculation). Overall, these findings highlight the role of AMF in enhancing Se tolerance by biotransforming inorganic Se into bioavailable, less toxic organic forms, thereby enhancing Se biofortification in crops.

Arbuscular mycorrhizal fungi also promote the compartmentalization of Se within fungal structures such as vesicles and spores, effectively sequestering the element away from sensitive plant tissues. This sequestration mechanism provides an additional layer of protection by isolating potentially dangerous Se concentrations (Ananias et al. 2025). Moreover, AMF increase the plant antioxidant defense system, increasing the activity of enzymes such as peroxidases and upregulating genes involved in glutathione metabolism, so collectively lowering oxidative stress induced by excess Se. In parallel, AMF can influence the nature of the soil microbial community, including reducing populations of Se-mobilizing or Se-absorbing microorganisms, thereby indirectly avoiding excessive Se uptake (Sun et al. 2022). Through these coordinated mechanisms, AMF contribute to a balanced Se homeostasis that supports plant growth while minimizing toxicity risks.

3.6 Biochemical mechanisms of Se and AMF-mediated drought tolerance

As illustrated in Fig. 4, Se and AMF play essential roles in increasing plant drought tolerance. Selenium, a

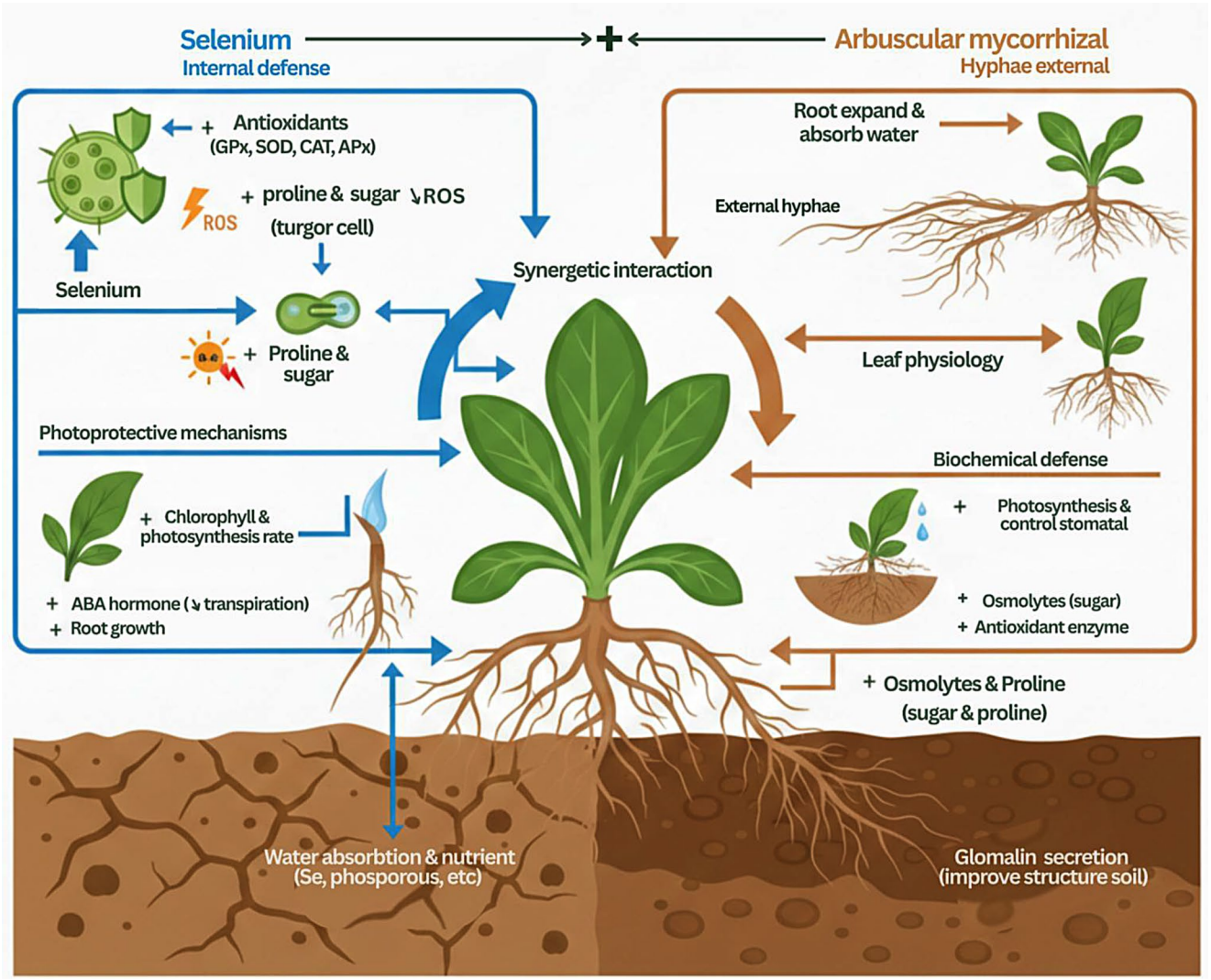


Fig. 4 Mechanisms underlying Se and AMF drought tolerance effects in the plant

micronutrient, has been shown to enhance plants' antioxidant defense systems. It is absorbed by plants and transformed into selenoproteins, which include antioxidant enzymes such as GPX, CAT, SOD, and APX. These enzymes are crucial for neutralizing ROS produced during stress (Kong et al. 2025; Nath et al. 2026). The heightened activity of these enzymes has been shown to mitigate oxidative damage to plant cells, which allows the plant to exhibit greater resistance in drought-exposed environments. Furthermore, Se has been found to enhance the accumulation of proline and other osmolytes, which play a crucial role in maintaining osmotic balance within cells during periods of drought (Luo et al. 2019; Zhong et al. 2024). Additionally, Se has been reported to promote water-use efficiency by regulating stomatal opening and closing, hence optimizing plant water relations (Zhong et al. 2024). On the other hand, AMF has been observed to enhance plant drought tolerance by expanding the plant root network through fungal hyphae, thereby enabling the plant to access water and nutrients from deeper soil layers. Mycorrhizal have also been demonstrated to enhance soil water retention capacity and soil structure, thereby facilitating water absorption by plant roots. Similar to Se, AMF also contribute to the accumulation of osmolytes, such as proline and sugars, which function in osmotic adjustment during drought. Furthermore, AMF have been demonstrated to influence the production of hormones such as ABA, which plays a pivotal role in regulating the plant drought response, including stomatal closure and the activation of drought-tolerant genes. Futhermore, AMF contribute to soil quality through multiple interrelated mechanisms. These substances have been shown to enhance glomalin production, which, in turn, increases soil carbon and nitrogen storage (Busso and Busso 2022; Wilkes et al. 2021). Additionally, glomalin production has been observed to reduce Se immobilization and enhance its bioavailability (Li et al. 2022). Moreover, AMF have been

shown to promote soil aggregation by forming stable macroaggregates, thereby enhancing soil structure and resistance to erosion (Ji et al. 2019). Their activity also enhances soil water-holding capacity and porosity, thereby supporting better water retention. Furthermore, they play a significant role in carbon sequestration by contributing to soil organic carbon (SOC) storage and long-term soil health (Busso and Busso 2022; Liu et al. 2024a).

Consequently, when Se and AMF are combined, their synergistic effects further enhance plant drought tolerance. Selenium and AMF have been shown to collaborate to enhance plant antioxidant capacity, mitigate oxidative damage, and optimize osmotic regulation, water, and nutrient uptake. The combination of these elements has been shown to enhance root growth, increase photosynthetic efficiency, and improve stress tolerance. It has also been observed to enhance Se immobilization in plants, leading to an increase in biomass and improvement plant quality under drought conditions. Thus, the implementation of Se and AMF emerges as a promising strategy to enhance plant resilience to drought in water-limited agricultural environments.

3.7 Practical implications, limitations, and future directions

The combined application of Se and AMF provides a compelling strategy for improving forage production and drought resilience in water-limited areas. Under drought conditions, this treatment consistently boost plant growth, physiological performance, and forage quality, indicating a strong synergistic interaction between Se supplementation and mycorrhizal function. Increases in chlorophyll content, relative water status, and root development contribute directly to increased stress tolerance, while gains in biomass and CP yield enhance forage nutritional value. These combined result present Se–AMF biofortification as a realistic and scalable solution for farmers in drought-prone regions. Importantly, Se concentrations remain within safe levels for animal intake, suggesting its use without harming their health (Salles et al. 2017).

Despite these advantages, the long-term effectiveness of this approach remains conditional. Its efficacy depends on Se persistence in soil, the stability of AMF symbiosis, and larger soil health dynamics. The slow-release Se formulation employed in this study provides maintained plant availability for up to 12 months under the manufacturer's protocol, eliminating the need for recurrent applications while preserving physiological effect (Curtin et al. 2008). However, extended residence presents the possibility of progressive Se accumulation, particularly in poorly drained soils or those with elevated background Se levels. In parallel, AMF efficacy is intrinsically context-dependent. Variability in soil physicochemical properties and native microbial population may impede colonization, while recurrent Se exposure may trigger plant-level adaptation that change absorption efficiency. These characteristics underline the necessity for long-term monitoring to avoid hazards of Se accumulation, diminishing responsiveness, and microbial imbalance.

Soil heterogeneity and environmental variables also impact field performance. Key soil properties, including texture, pH, organic matter, and nutrient interactions, significantly influence the concentration, mobility, and bioavailability of Se. Sandy soils tend to promote Se leaching (Shedeed et al. 2023), but clay-rich soils may immobilize Se and inhibit plant uptake (Lessa et al. 2016). Soil pH exhibits a similarly significant role, where higher pH promotes Se mobility but can impair root uptake, whereas acidic conditions favor retention in less accessible form (Fan and Zhao 2018). Additionally, high phosphorus levels may suppress Se absorption by ionic competition (Wang et al. 2020). Climatic variability further complicates these dynamics. Although AMF can enhance plant performance during mild drought, severe precipitation and temperature extremes may suppress fungal activity and diminish symbiotic effectiveness (Basyal and Emery 2021). Moreover, drought periods that facilitate AMF colonization may be compensated by following rainfall events that accelerate Se leaching, hence limiting its availability across growth cycles.

We interpret these findings in light of many methodological restrictions. The study was conducted under controlled pot conditions with uniform soil, water, and fertilizer inputs, limiting its capacity to capture field-scale variability. Using a single forage species and a single AMF inoculum across a single growth cycle further limits the generalizability of the results. Such settings do not reflect the spatial heterogeneity, microbiological complexity, climate change, or repetitive harvesting typical of agricultural systems. In addition, the absence of Se speciation studies in plant tissues hinders the assessment of bioavailability and feed safety, as Se chemical forms determine these.

Future research should prioritize three main directions. First, multi-season field trials spanning dry and semi-arid tropical agroecosystems are necessary to confirm the resilience of this technique under realistic management and climatic conditions. Second, the Se speciation study in plant tissues is necessary to establish the safety of biofortified forage for animals. Third, thorough comparisons of AMF species and inoculum levels are needed to resolve their involvement in modifying drought resistance and nutrient uptake. These efforts must combine soil health management measures, including organic amendments, and must also analyze implications on native soil microbiomes. Economic considerations will also be crucial to determine the feasibility of large-scale adoption. Expanding this paradigm

across multiple crop–livestock systems and climate adaption scenarios will be important to establish the conditions under which Se–AMF biofortification can yield consistent agronomic and ecological benefits.

4 Conclusion

This study reveals that the combined application of Se and AMF is an effective strategy for improving the growth, physiological performance, and forage quality of *C. intybus* cv. Cropmate under water-limit conditions. Among the studied treatments, a moderate Se dose of 50 mg/m² combined with AMF proved best, resulting in significant increases in biomass production, crude protein yield, and important physiological traits associated with drought resistance. The favorable benefits of this combined treatment were principally linked to enhanced photosynthetic capability, as reflected by higher chlorophyll content, improved plant water status through better water regulation, and more efficient osmotic adjustment, evidenced by lowering proline accumulation. In addition, the modulation of secondary metabolites, including phenolics and flavonoids, implies a balanced stress-response system that supports cellular protection while avoiding significant metabolic costs. Importantly, Se accumulation in plant tissues increased with Se application but remained within safe thresholds for forage use, highlighting the feasibility of nutritional biofortification without compromising feed safety.

From an agronomic and ecological standpoint, these findings have substantial implications for forage production in dry tropical and semi-arid environtment, where drought stress and diminishing forage quality severely impede livestock productivity. Chicory plant's inherent adaptability, combined with the low-input nature of AMF inoculation and moderate Se supplementation, makes this strategy particularly suitable for resource-limited farming systems commonly found in dry tropical climates. The Se–AMF strategy could improve forage yield stability, enhance feed nutritional value, and support climate-resilient livestock production systems amid increasing water scarcity. Overall, the results confirm *C. intybus* cv. Cropmate potential as a high-quality forage crop and support the adoption of combined Se and AMF-based management strategies as a sustainable solution for drought-prone environments.

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Author contributions Ryan Aryadin Putra initiated, designed, and conducted the experiments; analysed and interpreted the data; and wrote the initial draft of the manuscript. Ali Agus and Nafiatul Umami supervised the study and participated in the revision and final drafting of the manuscript.

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Data availability Data will be made available upon specific request.

Declarations

Ethics statement Not required for this research.

Generative AI statement The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Competing interests The authors declare no competing interests.

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