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ACCLIMATIZATION OF *WITHANIA SOMNIFERA* (L.) DUNAL IN VÂLCEA COUNTY, ROMANIA: OVERWINTERING, REGROWTH AFTER REPEATED CUTTING, AND THE FOLIAGE AND ROOTS OF SECOND-SEASON PLANTS

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ABSTRACT

Withania somnifera (L.) Dunal (Solanaceae) is a frost-tender perennial subshrub that is cultivated as an annual crop, and no quantitative data on its overwintering in Central or Eastern Europe were found. At the applied study site of Asociația Comunitară Beica-Mădulari (Vâlcea County, Romania), plants raised from seed and planted in 30 L bags on 1 June 2025 were grown in a polyethylene tunnel and overwintered in 2025–2026 in two lots of 100 plants: one with thermostat-controlled soil heating (estimated minimum at the collar about +2 °C) and one without heating, under an agrotextile cover (about −2 °C). Survival was 80 % and 50 %, respectively (Fisher's exact test, $p < 0.001$; one lot per treatment, observational design); an unprotected outdoor plant did not survive. The plants regrew from the collar in March 2026, tolerated removal of the whole aerial part at about 10 cm every three months, and set seed with 60% emergence. After 16 months, plants lifted from the bags had roots at least 34–45 cm long, collar diameters of 21–25 mm and 23.5–36.5 g fresh mass, with signs of container restriction. Leaves formed after winter dormancy were harvested and dried for a proposed paired leaf–root withanolide analysis.

INTRODUCTION

A member of the Solanaceae family, *Withania somnifera* (L.) Dunal, known as ashwagandha, has a native range extending from southern Europe, the Canary Islands and Africa to central China and Myanmar (POWO 2026). Botanically it is a perennial subshrub (Zhang et al. 1994, Mirjalili et al. 2009, POWO 2026); in Italy it is known from Sicily and Sardinia, where it grows as an evergreen perennial shrub (Nicoletti 2001).

In India, the main production area, the cultivated types behave as annuals. The crop is sown in the late rainy (kharif) season as a rainfed crop, and the plants are uprooted for their roots 150–180 days after sowing, from January to March (TNAU Agritech n.d.a); the Indian cultivated germplasm, separated by some authors as *W. ashwagandha*, is itself described as annual (Kumar et al. 2011, Khanna et al. 2013, Mir et al. 2013). Root dimensions reported for this crop are 10–17.5 cm in length and 0.6–1.2 cm in thickness (Sarkar et al. 2023), and a late harvest, 210 days

after sowing, gave roots of up to 31.6 cm and 1.32 cm (Swati et al. 2026). The root data published so far therefore come from plants grown for a single season.

European trials follow the same annual model. In south-eastern Poland, seedlings were transplanted in spring and the roots lifted in autumn (Krochmal-Marczak et al. 2025), and the antioxidant activity of herb and root was measured, but not their withanolides (Pisulewska et al. 2025). Germination capacity there was 95.5% at 30 °C, while germination energy fell from 93.6% at 30 °C to 0.4% at 10 °C (Krochmal-Marczak et al. 2025). In the Republic of Moldova the species, introduced through *in vitro* culture, also develops as an annual (Kutkovski-Mushtuk et al. 2020); its leaves, aerial parts and roots were characterized for total alkaloids (Calalb et al. 2016). On the sub-Mediterranean Southern Coast of Crimea, plants were reported not to freeze out in some winters and greenhouse plants are grown as a perennial, but no survival data were given (Logvinenko et al. 2021). In Sardinia, plants were grown outdoors for 18 months through a mild winter, with a January mean of 8.7 °C (Maccioni et al. 2024). In Romania, the species has been mentioned in the press as under evaluation for drought tolerance at the Vegetable Research and Development Station Buzău (Zafiu 2023), but we found no scientific report of its cultivation.

Horticultural guidance gives USDA hardiness zones 8–12 for the species and advises bringing it indoors in cooler climates (NC State Extension n.d.). These limits appear to reflect the cultivation range rather than measured survival. Controlled cold studies on *W. somnifera* used only chilling temperatures: 8 °C (Kumar et al. 2012), and 4 °C on two-month-old seedlings, which became necrotic when exposure exceeded eight days (Mir et al. 2015). A recent temperature-stress study tested only warming (Singh & Mina 2025), and the only freezing test in the genus that we found concerns the arid congener *W. coagulans* (Zendedel et al. 2022). We found no quantitative data on the overwintering survival of *W. somnifera* in Central or Eastern Europe.

Overwintering also matters for leaf production, because the leaves are rich in withanolides. In hydroponically grown plants in growth chambers, leaves contained 10–40 times more withaferin A and 12–22 times more withanone than roots (Kaul et al. 2016). At the start of flowering, leaves of the NMITLI-101 line contained 22.3 mg g^{-1} DW withaferin A and 18.4 mg g^{-1} DW withanone (Chatterjee et al. 2010). Leaf composition differs between chemotypes (Abraham et al. 1968, cited by Nicoletti 2001) and with light quality (Kaul et al. 2016). It also changes with growth stage, although the direction is disputed: one study found the maximum before flowering, at 60 days (Nair & Praveen 2019), another in the reproductive phase (Thorat et al. 2022).

The analyses of leaves from plants older than one season that we found all come from warmer sites. Two-year-old plants in northern India had less than 1 mg g^{-1} DW withaferin A in the leaves in five cultivars (Singh et al. 2018). Plants 18 months old that overwintered outdoors in Sardinia had 17.7 mg g^{-1} DW (Maccioni et al. 2024). In Central Europe, withaferin A was identified, without quantification, in leaves of greenhouse plants propagated *in vitro* in Poland (Furmanowa et al. 2001). We found no quantitative analysis of leaf withanolides from soil-grown plants in Central or Eastern Europe, and none from plants that regrew after a frost-limited winter dormancy.

The aim of this study was to establish whether *W. somnifera* can be maintained as a perennial under protected cultivation in Vâlcea County, Romania. On a cohort of plants established in June 2025 and kept into a second growing

season, we recorded (i) overwintering survival with and without soil heating, (ii) regrowth after repeated removal of the whole aerial part, (iii) root development of second-season plants in 30 L bags, and (iv) seed set and emergence, and we prepared the foliage of these second-season plants as the basis for a paired leaf–root withanolide analysis.

MATERIAL AND METHODS

Study site and climate. The work was carried out at the applied study site of Asociația Comunitară Beica-Mădulari in Iacovile village, Mădulari commune, Vâlcea County, Romania (44.68 °N, 24.10 °E; about 405 m a.s.l.), in the hilly area west of the Olt valley (Figure 1). The climate is temperate-continental. At the nearest weather station, Drăgășani (280 m a.s.l., about 10 km east of the site), the mean annual air temperature was 10.4 °C and the mean annual precipitation 636.5 mm in 1962–2013 (Mărăcineanu et al. 2020). The 1991–2020 climate normals for Râmnicu Vâlcea (WMO station 15346, 237 m a.s.l., about 50 km north-north-east of the site) give a January mean of −0.4 °C, a July mean of 22.4 °C, 87.3 days per year with a minimum air temperature ≤ 0 °C (from October to April), 7.2 days per year with a minimum ≤ −10 °C, and absolute extremes of −22.9 °C and 40.7 °C (WMO 2021). In winter 2025–2026, the lowest air minima were −10.2 °C at Râmnicu Vâlcea on 19 January 2026 and −10.3 °C at Drăgășani on 3 February 2026; at Râmnicu Vâlcea, frost occurred on 50 days between 13 December 2025 and 2 March 2026 (Météociel 2026).

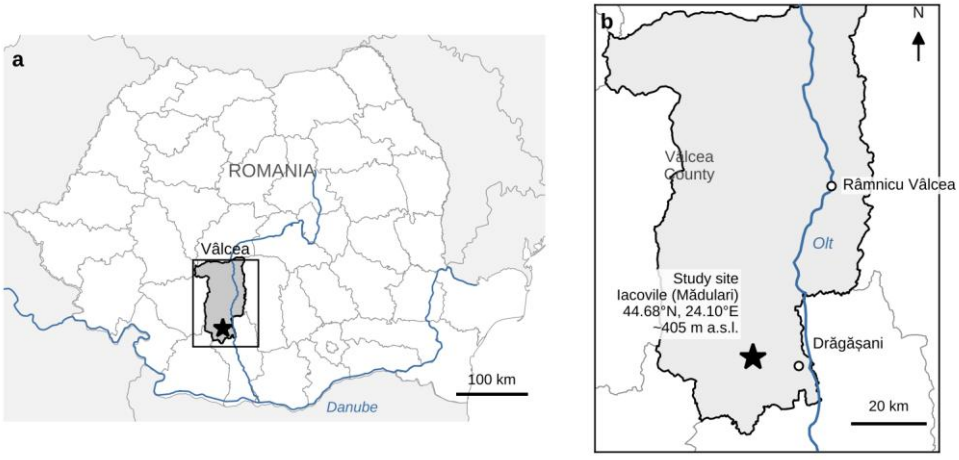


Figure 1. Location of the study site: (a) Vâlcea County in Romania; (b) the study site in southern Vâlcea County, with the nearest towns and weather stations (county boundaries after the Code for America “click_that_hood” open dataset; national borders and rivers after Natural Earth; processed by the authors)

Plant material and seedling production. Seeds were purchased from an accredited commercial supplier in the United Kingdom. The seed lot, with the supplier's name and the original packets, was declared to the Romanian phytosanitary authority under the association's registration no. RO/VL/071/PIDO. The seedlings were raised for about 45 days in cell trays filled with a peat–sand mixture and, on 1 June 2025, were planted singly, with one exception, into 30 L cultivation bags about 45–47 cm tall. The bags were filled with a sandy, free-draining

soil from the Olt river area of Vâlcea County; a smaller number of plants was grown in a humus-rich substrate for comparison, and growth was assessed visually. Within the cohort, two morphotypes were distinguished visually, in a ratio of about 70:30. Morphotype A had smaller, strongly scented leaves, shorter internodes and therefore denser foliage, and more abundant inflorescences; when left uncut, it put its growth mainly into flowering and fruiting rather than into new foliage. Morphotype B had more moderate but steady leaf growth and consistent root development.

Growing conditions. From planting onwards, the bags stood in a polyethylene tunnel at the study site (Figure 2) and the plants were grown in the bags for the whole period of the study; they were not held in the tunnel for later planting in the field. Part of the plants in the tunnel grew under a 50 % shade net. One additional plant was grown outdoors, in full sunlight and without winter protection, in a fenced area of the same site, as an outdoor comparison plant; it was not part of the overwintering lots. This plant did not survive the winter, and a new comparison plant was planted at the same place in May 2026 to follow development outdoors, in contrast to the protected, man-made growing environment. As a precaution for a non-native species under acclimatization, no plant material was distributed or planted outside the site.



Figure 2. Flowering plants of *W. somnifera* in the polyethylene tunnel at the study site (photo by the authors)

Cutting regime. The whole aerial part of each plant was cut at about 10 cm above the substrate at intervals of about three months, i.e. about twice per growing season; the last cut before winter was made on 20 October 2025, after which the

plants were treated with a 1 % stabilized copper sulfate product (Bordelais). The cut material was chopped and processed in the site's biofermenter.

Overwintering. In winter 2025–2026 the plants were held in the same tunnel as two overwintering lots of 100 plants each, placed in two zones without random allocation; both lots contained the two morphotypes in the same proportion. In the heated zone, an anti-frost system with resistive heating cable was controlled by a thermostat whose probe was placed in the soil at a depth of 20 cm; heating switched on at +4 °C and off at +8 °C. On this basis, the temperature at the collar and at the soil surface was estimated not to have fallen below about +2 °C, even on January 2026 mornings when the outdoor air at the site fell to about −14 °C (site readings). The unheated zone, in the middle of the tunnel, had no heating and was protected against frost only passively, with an agrotextile (non-woven) cover; its minimum at collar level was estimated at about −2 °C. These two values are estimates, not continuous measurements. In both zones watering was stopped during winter, and the tunnel was ventilated and treated repeatedly with the same 1% copper product to limit pathogen infection. The plants went dormant in November 2025. Survival was scored in March 2026 as the presence of regrowth.

Root sampling. Roots were sampled destructively twice. On 15 June 2026, about 12.5 months after planting, one plant was lifted to check root development. Excluding winter dormancy (November 2025–March 2026), this plant had about eight months of active growth (June–November 2025 and March–mid-June 2026). On 6 October 2026, about 16 months after planting, the bag coded ASH 001 was opened. It was the only bag holding two plants, coded ASH 001-A and ASH 001-B, both of morphotype B and from the heated overwintering zone. Bag ASH 002, opened the same day, held a single plant of morphotype B from the same zone, and bag ASH 003 a single plant of morphotype A from the unheated zone. A fifth plant, ASH 004 (morphotype B, unheated zone), was lifted from a 3 L pot about 22 cm deep; it was one of about 100 overwintered plants moved from their 30 L bags into such pots in May 2026, of which 90 established. Each root was measured with a tape from the collar to the tip; collar diameter was measured with a vernier caliper and fresh root mass with a kitchen balance (0.5 g resolution). The roots are being dried separately in the shade, at no more than 40 °C, for dry mass and chemical analysis.

Seed emergence test. Seed harvested from the cohort in 2026 was tested for emergence on a sample of 100 seeds.

Leaf material. Leaves of the second-season plants were harvested on 5 June 2026, dried in the shade with natural ventilation for 5 days at 26–32 °C, and packed in paper bags at about 10% moisture. Leaf size, density of foliage and pest presence were assessed visually.

Statistics. Survival in the two overwintering lots was compared with a two-sided Fisher's exact test. The odds ratio is reported with a log-based (Woolf) 95% confidence interval (CI), and the 95% CI of each proportion was calculated with the Wilson score method. Calculations were made in Python with SciPy (Virtanen et al. 2020). Because each treatment was represented by a single lot, the test compares the two groups of plants and does not by itself attribute the difference to heating. As plants were not allocated at random, the study is observational.

RESULTS AND DISCUSSIONS

Overwintering survival. The plants went dormant during the winter and resumed growth in March 2026. Survival was higher in the heated lot than in the unheated lot under the agrotextile cover (Table 1). The single plant left outdoors without protection did not survive the winter.

Table 1

Overwintering survival of *W. somnifera* in winter 2025–2026

Lot (estimated minimum at the collar)	Plants (no.)	Surviving (no.)	Survival (%)	95% CI of survival (%)
Heated (≈ +2 °C)	100	80	80	71.1–86.7
Unheated, agrotextile cover (≈ −2 °C)	100	50	50	40.4–59.6

Heated lot: resistive cable, thermostat probe at 20 cm depth, on at +4 °C, off at +8 °C. Collar minima are estimates. CI – confidence interval (Wilson score method). One lot per treatment.

The two lots differed significantly (Fisher's exact test, two-sided, $p = 1.4 \times 10^{-5}$; odds ratio 4.0, 95 % CI 2.1–7.5). Because each treatment was represented by a single lot, the test shows that the two groups of plants differed; it does not separate the effect of heating from other differences between the two zones.

In the heated lot, 80 % of the dormant plants survived at an estimated collar minimum of about +2 °C, well below the 10 °C that Indian cultivation guidance mentions as a tolerated low temperature (TNAU Agritech n.d.b). Even so, 20 % of these plants were lost. Without heating, at an estimated minimum of about −2 °C under the agrotextile cover, half of the plants were lost, and the unprotected outdoor plant died. Such losses at temperatures only slightly below 0 °C suggest that the USDA zones 8–12 given for the species (NC State Extension n.d.) should not be read as a measured hardiness limit.

Published cold experiments are not directly comparable. Two-month-old seedlings kept at 4 °C showed oxidative stress and became necrotic when exposure exceeded eight days, with a wild genotype less damaged than a cultivated one (Mir et al. 2015). These were young, actively growing plants exposed to low air temperature, whereas our plants were dormant and the temperatures given for them are estimates at the collar. We found no study that tested whole-plant survival of *W. somnifera* below 0 °C. In the arid congener *W. coagulans*, a short controlled freeze of potted seedlings gave a survival-based LT_{50} of −9.0 °C (Zendedel et al. 2022), which cannot be compared directly with season-long overwintering. In Romania, the outdoor overwintering of three tender ornamental taxa was recently assessed at the Bucharest Botanic Garden (Niță & Paraschiv 2025).

Both zones were in the same protected unit and held the two morphotypes in the same proportion, so the lots differed mainly in heating, in the agrotextile cover of the unheated zone and in the position of the zone. Plants were not allocated at random, however, so the comparison remains observational. The thermostat probe was at 20 cm depth, and the minima of about +2 °C and −2 °C at the collar are estimates; air temperature around the stumps was not logged and may have been lower. An estimated minimum also does not show how long the collar zone stayed below zero.

The causes of death were not diagnosed. Losses were concentrated in the unheated zone. Watering was stopped in winter, but from November until evaporation from the substrate ceased, condensation and the need for ventilation

were the main difficulty in the tunnel, and the risk of disease stayed high despite repeated copper treatments. Part of the losses may therefore come from disease in cold, damp conditions rather than from cold alone. The trial will be repeated in winter 2026–2027 with replicated zones, random allocation of plants, continuous logging of substrate and air temperature, and examination of the plants that die.

Regrowth under repeated cutting. After the winter most buds broke as new shoots at the collar, rather than on the old stems that had entered the winter. Regrowth thus came mainly from the collar, the part of the shoot closest to the soil and best protected from frost. The plants then tolerated further cuts of the whole aerial part at intervals of about three months. They flowered in 2026 and set fruit; 60 of 100 seeds harvested from the cohort emerged in the emergence test.

Under protected cultivation, the cohort thus completed the cycle from seed to seed in Vâlcea County within two growing seasons. In the open field in the Republic of Moldova, by contrast, the species develops as an annual (Kutkovski-Mushtuk et al. 2020). The 60 % emergence is lower than the 95.5 % germination capacity reported at 30 °C in Poland (Krochmal-Marczak et al. 2025). An emergence test in substrate usually gives lower values than laboratory germination, so the two figures are not directly comparable.

Both canopy-removal experiments on ashwagandha that we found were carried out at Anand Agricultural University, each with a single removal in an annual crop. Patel et al. (2015) compared the removal of 50 %, 75 % and the whole aerial part: removing 50 % of the canopy 75 days after sowing raised dry root yield from 562 to 705 kg ha^{-1} and root withanolides from 0.55 % to 0.63 %, and a 75 % cut gave a smaller gain. Removing 25 % of the leaves at 60 days, combined with growth-regulator sprays, gave the highest root yield, 847 kg ha^{-1}, with 0.75 % withanolides (Meena 2015). Our regime removes the whole aerial part repeatedly, on plants older than one year; we found no study of repeated cutting on such plants.

Roots are able to synthesize withanolide A *de novo* (Sangwan et al. 2008). Repeated cutting may therefore affect root withanolides at least partly through the carbon supply to the root. This remains to be tested by measuring root growth, starch and withanolides.

Root development. The root lifted on 15 June 2026, about 12.5 months after planting and after about eight months of active growth, had a taproot of about 33 cm against the tape; its length along the curves is somewhat greater. Fibrous lateral roots were concentrated 5–12 cm below the collar, where the taproot also showed a rounded swelling of undetermined origin at about 8–9 cm. The lower half of the taproot was sinuous rather than straight.

Root dimensions reported for the annual crop are 10–17.5 cm in length and 0.6–1.2 cm in thickness (Sarkar et al. 2023), with maxima of 31.6 cm and 1.32 cm after 210 days (Swati et al. 2026). The DUS guidelines for the species class a main root above 30 cm as long, and diameters as thin (< 18 mm), moderate (18–25 mm) or thick (> 25 mm) (PPV&FRA n.d.). With about eight months of active growth, our root falls in the long class and is comparable to the longest roots reported for annual crops harvested 210 days (about seven months) after sowing. We found no published measurements of root length or diameter from plants grown beyond one season; the two studies that harvested older plants reported only phytochemistry (Singh et al. 2018, Maccioni et al. 2024).

The roots of the four plants lifted from bags on 6 October 2026 were all at least as long as the June root (Table 2, Figure 3), including the two plants that shared

one bag, which is consistent with continued growth after June. Collar diameters of 21–25 mm fall in the moderate DUS class (18–25 mm) and are well above the root thickness of 0.6–1.32 cm reported for annual crops, although collar and root thickness are not measured at exactly the same point. The taproot of ASH 001-B was forked about 8 cm below the collar. ASH 002, the only plant in its bag, had a dense fibrous system of lateral roots, and when the bag was opened the outer surface of the soil ball was covered by fine white roots running along the bag wall. ASH 003, the only bag plant sampled from the unheated zone, was the largest: it divided at the collar into two roots of similar thickness, the longer reaching at least 45 cm, and both had reached the bottom of the bag and turned back. It was still in leaf, with fruiting calyces, when lifted. Root tips may have broken during lifting, and a thin root of ASH 002 continued through the bottom of the bag, so all lengths are minimum values. ASH 003 differs from the other plants in both morphotype and overwintering zone, and its larger size should not be read as an effect of either: only half of the plants survived in that zone, so the survivors may have been the most vigorous ones, and a single plant cannot separate these factors. Visual observation suggests, however, that the vigour of morphotype A depends on the cuts: with two cuts per growing season it regrew strongly, whereas uncut plants of this morphotype put their growth mainly into fruiting, while morphotype B grew more moderately above ground but developed its roots consistently. Whether repeated cutting favours root growth in morphotype A will be tested on more plants of both morphotypes.

Table 2

Roots of the five plants lifted on 6 October 2026, about 16 months after planting

Plant	Length of longest root (cm)	Collar diameter (mm)	Fresh root mass (g)	Form
ASH 001-A	≥ 34	23	26.0	single taproot, slightly curved
ASH 001-B	≥ 37	22	23.5	forked about 8 cm below the collar
ASH 002	≥ 35	21	26.0	dense fibrous laterals; fine roots along the bag wall
ASH 003	≥ 45	25	36.5	two roots of similar thickness from the collar, both turned back at the bag bottom
ASH 004 (3 L pot)	≥ 32	16	18.5	mostly fine laterals; roots matted at the bottom of the root ball

ASH 001-A and ASH 001-B (one bag) and ASH 002: morphotype B, heated zone; ASH 003: morphotype A, unheated zone; ASH 004: morphotype B, unheated zone, moved from its 30 L bag to a 3 L pot in May 2026. All lengths are minimum values.

ASH 004, moved in May 2026 from its bag into a 3 L pot about 22 cm deep, had the smallest root of the five: a collar diameter of 16 mm and 18.5 g fresh mass. Its longest root measured 32 cm, about 10 cm more than the depth of the pot, so it must have turned at the bottom. Most of its mass was in fine lateral roots, and the roots were matted at the bottom of the root ball. Two factors are confounded here, the smaller container and the transplanting; damage to the taproot at transplanting, which was not recorded, could alone produce the same pattern.

The sinuous and forked forms, the thin root of ASH 002 that grew through the bottom of its bag (Figure 3d), the fine roots covering the soil ball along the bag wall in ASH 002, and the roots of ASH 003 that reached the bottom of the bag and turned back suggest that the container restricted root growth. Rooting volume limits growth in general: across 65 studies, doubling pot size raised plant biomass by 43% on average (Poorter et al. 2012). A deeper container would show whether the taproot keeps a straight form. These data come from six plants, two of which shared a bag; further plants are being measured at the end of the 2026 season, and dry root mass will be determined after drying.



Figure 3. Plants lifted on 6 October 2026, about 16 months after planting: (a) the two plants of bag ASH 001 (morphotype B, heated zone), with tape measure and vernier caliper; (b) soil ball of ASH 002 (morphotype B, heated zone), covered by fine white roots along the bag wall; (c) root of ASH 003 (morphotype A, unheated zone), divided at the collar into two roots of similar thickness; (d) bag ASH 002, with fine roots grown through the bottom (photos by the authors)

Behaviour in containers. Two further observations, both from visual assessment, concern the bags. The plants were very sensitive to water deficit. They also grew better in sandy than in humus-rich substrate, in line with the recommended soils: light sandy loams with a pH of 7.5–8.0 and good drainage (TNAU Agritech n.d.a, NC State Extension n.d.). A possible explanation for both is the limited volume of the bags. A small water reserve dries quickly, while an organic substrate in the same volume may stay wet at the bottom; in either case the taproot cannot reach deeper, evenly moist soil.

Foliage of second-season plants. The leaves harvested on 5 June 2026 had all formed after the plants resumed growth in March 2026, on a root system more than one year old. Such leaves have not, to our knowledge, been analysed for withanolides.

Under the 50 % shade net the plants formed denser foliage, but smaller leaves than the outdoor comparison plant planted in May 2026 (visual assessment). Plants grown in low light usually invest relatively more in leaf area (Poorter et al. 2019), so the smaller leaves are unexpected. The polyethylene cover, the container, the repeated cutting and the plant density could each contribute, and the present data cannot separate them. Polyethylene covers and shade nets also reduce UV radiation, and UV supplementation increased leaf withaferin A in controlled experiments (Kaul et al. 2016). Leaves from protected cultivation may therefore differ in composition from field-grown material.

The main pests on the leaves were whiteflies and spider mites. Leaf samples for analysis should be scored for infestation, and infested and clean leaves kept apart. The two morphotypes will be described with the DUS descriptors (PPV&FRA n.d.), using leaves from a fixed node position.

The chemotype of our seed lot is not known. Plants from Sicily and Sardinia were assigned to chemotype III, characterized by withanolides J, E and S; withaferin A was not among the compounds isolated, and no withanolides were found in their roots (Nicoletti 2001). Cultivated plants in Sardinia, by contrast, had 17.7 mg g^{-1} DW withaferin A in the leaves (Maccioni et al. 2024). The first analysis should therefore establish the chemotype of each morphotype, with withaferin A, withanone, withanolide A, withanolide D and withanolide E as reference compounds and LC–MS for unidentified peaks.

Dried leaves of the second-season plants and a root from the same cohort are available. We propose to analyse leaf and root samples taken from the same plants, on the same day and at the same phenological stage, together with the Faculty of Horticulture, University of Craiova. Such paired data would show whether leaves formed after dormancy resemble the young leaves of an annual crop or the leaves of older plants, and how leaf and root composition relate within the same plant.

CONCLUSIONS

1. Under protected cultivation in Vâlcea County, *W. somnifera* could be maintained as a perennial: plants established on 1 June 2025 went dormant in winter, regrew in March 2026, completed a second growing season and produced seed, of which 60 % emerged.

2. Overwintering survival was 80 % with thermostat-controlled soil heating (estimated collar minimum about +2 °C) and 50 % without heating under an agrotextile cover (about −2 °C), one lot per treatment; an unprotected outdoor plant did not survive. These results suggest heating the root zone so that the collar stays

above 0 °C and limiting condensation, to be confirmed in a replicated trial; even with heating, 20 % of the plants were lost.

3. The plants tolerated removal of the whole aerial part at about 10 cm every three months and regrew after each cut; after winter, regrowth came mainly from new shoots at the collar.

4. In 30 L bags, the root lifted after 12.5 months was about 33 cm long, and roots lifted after 16 months were at least 34–45 cm long, with collar diameters of 21–25 mm; sinuous and forked forms, roots growing through or turning back at the bottom of the bag, and fine roots covering the soil ball along the bag wall suggest restriction by the container. A plant moved to a 3 L pot in May 2026 had a smaller, mostly fibrous root.

5. In bags, the plants grew better in sandy than in humus-rich substrate and were sensitive to water deficit (visual assessment).

6. The leaves harvested in June 2026 formed after winter dormancy, on a root system more than one year old. Together with the lack of quantitative leaf withanolide data from Central and Eastern Europe, this makes the foliage of second-season plants the first priority for chemical analysis.

The next steps are a replicated overwintering trial with random allocation and continuous logging of soil and air temperature at collar level, deeper containers, the description of the two morphotypes, and paired leaf–root analysis of withanolides, proposed to the Faculty of Horticulture, University of Craiova.

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