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Rooting patterns and aucubin content of *Plantago lanceolata* varieties

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Abstract:

This study investigated the potential of *Plantago lanceolata* as an alternative plant to mitigate the environmental impacts of agriculture in the face of climate change. Eleven genotypes of ribwort were compared under field conditions in northern Switzerland, focusing on the characteristics of the root system and the aucubin content in the leaves and roots. Aucubin, known to inhibit nitrification process in the soil, varied according to genotype and season. The content of secondary metabolite in leaves was higher than in roots, which may indicate the greater importance of leaves in reducing soil nitrification. Dry matter yield and for the first time root characteristics, such as length density and specific root length, were evaluated at two different depths, and at different positions in the experimental plots. The results showed that ribwort is well adapted in terms of rooting in topsoil. In addition, the plant's ability to root deeply after one year of cultivation under field conditions has been demonstrated, reaching depths of up to 1.88 metres. This allows the plant to effectively absorb water and nutrients from the subsoil layers.

I. Introduction

Globally expected climate changes will be associated with both an increase in heavy rainfall events and longer dry periods^{1,2}. Increasingly frequent dry periods can be counteracted in arable land and grassland management by cultivating plants that have a high water uptake capacity due to their deep root growth³. At the same time, these plants should also help reduce the emission of climate-impacting gases from agricultural land⁴. Nitrous oxide is one of the most emitted climate change gases, accounting for a high proportion of total global emissions from agriculture^{5,6}, and its impact on the greenhouse effect is 300 times higher than that of CO₂⁷. To reduce nitrous oxide emissions in crop production, plants that have a high capacity to take up nitrate and ammonium nitrate from the soil and release nitrification-inhibiting substances into the soil are needed. These above-average root properties also ultimately result in reduced pollution of ground and surface water by NO₃⁻, ultimately decreasing eutrophication, which has a negative effect on the environment^{8,9}.

Ribwort plantain (*Plantago lanceolata* L.), a plant that has not been commonly cultivated in arable farming thus far, apparently has a high ability to take up nitrate from the soil¹⁰ and to release nitrification-inhibiting substances into the soil¹¹. However, only a few studies have been published on the rooting patterns of ribwort plantain^{10,12}. Recently, a number of studies have been published showing the reduction of N₂O emissions by *P. lanceolata* under field conditions, especially in grassland stands^{13,14}. The objective of this study was to analyse and compare rooting patterns, rooting depth ability, lateral root growth and aucubin content in the roots and leaves of different ribwort plantain varieties grown under field conditions.

Materials and methods

Experimental sites

The first trial was established on 18 June 2021 in an experimental field belonging to the Research Institute of Organic Agriculture (FiBL) in Switzerland. The site (47°30'39.3"N 8°01'23.3"E) was located close to Frick at 348 m a.s.l. The mean annual precipitation and air temperature (2 m) were 1041 mm and 10.5°C, on average, from 2006 to 2022. In the period of the field experiment, the average air temperature and total precipitation were 11.0°C and 882 mm, respectively (weather station Frick). The experiment was conducted on clay loamy soil¹⁵ (pH_ CaCl₂: 7.3; P_CAL: 3.4 mg kg⁻¹; K_CAL: 6.3 mg kg⁻¹) and contained 44 plots (four replications) of 1.2 m² each in a randomised block design. The soil content in terms of stones was 17.5%.

The sowing density was 300 seeds per m². Plots consisted of two 2-m rows of manually seeded plants. The space between the rows was 30 cm, which was recommended for the cultivation of this plant¹⁶. The distance between each plot was 50 cm. The seeds were sown manually at a depth of 0.5 cm. No fertilisers or plant protection products were applied during the experiment. Before the trial was set up, the field was a non-permanent grassland. The commercially available genotypes used in the experiment were acquired through purchase and are presented in Table I.

Table I. Characteristics of the genotypes used in the field experiment

No.	Variety/Genotype Name	Origin of the Seeds	1000-Seed Weight [g]
1.	Lot: 14981a	Switzerland	2.92
2.	Lot: 90245	Switzerland	2.40
3.	Lot: 21583a	Switzerland	2.63
4.	Genotype A	Poland	1.39
5.	Genotype B	Poland	1.58
6.	Genotype C	Poland	1.16
7.	Libor	Czech Republic	1.32
8.	Libor	Czech Republic	2.57
9.	BIO	Germany	2.35
10.	Boston	New Zealand	1.35

11.	Tonic	New Zealand	2.07
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The second trial was established the first week of August 2020 in Struppen (Saxony, Germany; 50°55'38.3"N 14°00'42.3"E; 270 m a.s.l) on loess-derived soil. During the field experiment, the average air temperature and total precipitation were 11°C and 589 mm, respectively (weather station Struppen). The soil contained 6.4 mg P per 100 g (CAL) and 11.5 mg K per 100 g (CAL) and was characterised by a pH (H₂O) of 6.8. The sowing density of *P. lanceolata* (cv. Libor) was 300 seeds per m². The size of the plot was 7 m × 20 m.

Root sampling in the field

Root samples were collected from 19 to 24 October 2021 using a single root auger (model: 05.01) to minimise damage to the roots and determine their length. Because of the presence of a large amount of stones, clay in the soil and equipment limitations, samples up to 15 cm depth in the soil profile were collected. Samples were collected at five sites in each plot (Fig. 1) to a depth of 0.0–7.5 cm, and another five samples were collected at the same sites to a depth of 7.5–15.0 cm. Finally, 440 soil samples, each with a cylinder volume of 376.99 cm³ were packed in plastic bags and stored immediately in a cooling chamber (0.1°C) until the time of manual root extraction.

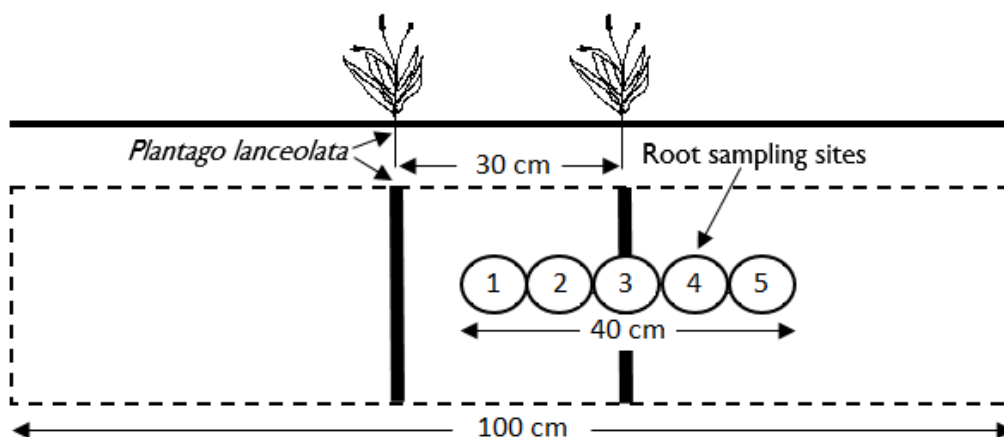


Fig. 1. Schematic representation of the experimental plot with the root sampling sites

Root measurements

Location Frick (Switzerland):

Roots were extracted manually from the soil samples using metal tweezers, and each sample was washed carefully with a stream of water and dried with a paper towel. Samples were placed separately in plastic string bags and stored in the refrigerator (-18°C) to avoid the loss of bioactive compounds. The root length analysis of each sample was evaluated by The Rhizo-II Root Biometrics Suite¹⁷ using a scanner (Toshiba, Tokyo, Japan, E-Studio 2010 AC). Each root sample was then lyophilised, and the dry mass was determined. From each plot, the root length density, specific root length and root dry matter (DM) distribution were determined. The dry mass of the roots was milled (0.1 mm gradation) and stored in a dark, dry place until further chemical analyses.

Location Struppen (Germany)

Root measurements were carried out on loess soil of the pseudogley pallidal type. Measurements of the root length density and rooting depth of *P. lanceolata* under field conditions were conducted according to Böhm¹⁸ using the profile wall method. A pit 250 cm deep was dug out on the shorter side of the experimental field using an excavator. For an even surface, the profile wall was smoothed with a spade. The root profile was then exposed to a depth of 0.5 cm with pressurised water using a hand sprayer. The next step on the suitably prepared surface was to apply and fix a sheet of acrylic glass with a pre-attached plastic sheet of mesh foil with 4 cm × 4 cm grids. The size of the acrylic sheet on which the measurements were taken was 100 cm wide and 200 cm deep. Points corresponding to 0.5 cm of root length were marked, and the value for each individual square was counted and recalculated for root length units. The root length density per soil volume [cm cm^{-3}] was counted separately for each of 25 squares per 4 cm soil depth by dividing the root length unit of each grid by 8 cm^3 (4 cm × 4 cm × 0.5 cm). The standard deviation was calculated using 25 individual measurements per 4-cm soil depth.

Collection of leaf samples

Harvesting the aboveground parts at Frick (Switzerland) was carried out on 3 November 2021 and 17 May 2022 and was performed by hand using hedge shears. In each plot containing two rows of 2 m length, 1.5 m of fresh leaf mass was cut from the centre of each row. The cutting height was 3 cm above the ground. The leaf yield from each plot was weighed, and 30 g of the harvested leaves from each plot were placed in string bags, deep-frozen (-18°C), freeze-dried

and milled (0.01 mm gradation). For each variety, the dry matter (DM) to fresh matter (FM) ratio (in %) was also calculated.

Aucubin measurements

For the analysis of the aucubin content in the leaves of *P. lanceolata*, 200 mg of DM was weighted for each sample and transferred into a 15 mL falcon tube. Then, 10 mL of methanol was added to the plant material. The suspension was vigorously mixed for 3 hours using a rotary laboratory mixer. Subsequently, 5 mL of extract was transferred into a 5 mL Eppendorf tube and centrifuged at 3000 rpm for 4 min. The extract prepared for analysis was transferred into a high-performance liquid chromatography (HPLC) vial. The same preparation procedure was performed for the extract obtained from the DM of the roots, but due to the limited amount of plant material, the volume was reduced to maintain an appropriate ratio of DM to methanol (100 mg of DM to 5 mL of methanol).

HPLC was performed on a chromatographic system consisting of a degasser, a quaternary pump (LC-20AD), a column oven (CTO-20AC), a PDA detector (SPD-M20A), and a triple quadrupole mass spectrometer (LCMS-8030) (All Shimadzu, Kyoto, Japan). A ReproSil-Pur 120 C18-AQ column (150 × 3 mm, i.d., 3 µm, Dr. Maisch, Ammerbuch, Germany) equipped with a guard column (3.0 × 10 mm) was used for separation. The mobile phase consisted of water (A) and acetonitrile (B). A gradient of 1 to 15% B was applied for 10 min at a flow rate of 0.5 mL/min. Detection was performed at 200 nm, and 5 µL was injected. Aucubin was eluted at 8.3 min. A total of 176 samples were analysed in triplicate for the presence of aucubin in the roots and leaves of *P. lanceolata*. For each analysis batch, a calibration curve ($R^2 > 0.985$) was prepared with a commercial sample of aucubin (Bioynth Ltd., Billingham, United Kingdom) dissolved in methanol (0.014 to 0.365 mg mL⁻¹ aucubin).

Plant Guidelines Statement

All experimental research and field studies on plants, encompassing both cultivated and wild specimens, including the collection of plant material, strictly adhered to pertinent institutional, national, and international guidelines and legislation. The methods employed in this study were carried out in accordance with ethical standards outlined by the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (CITES), as well as other relevant regulatory bodies and institutional protocols.

Results:

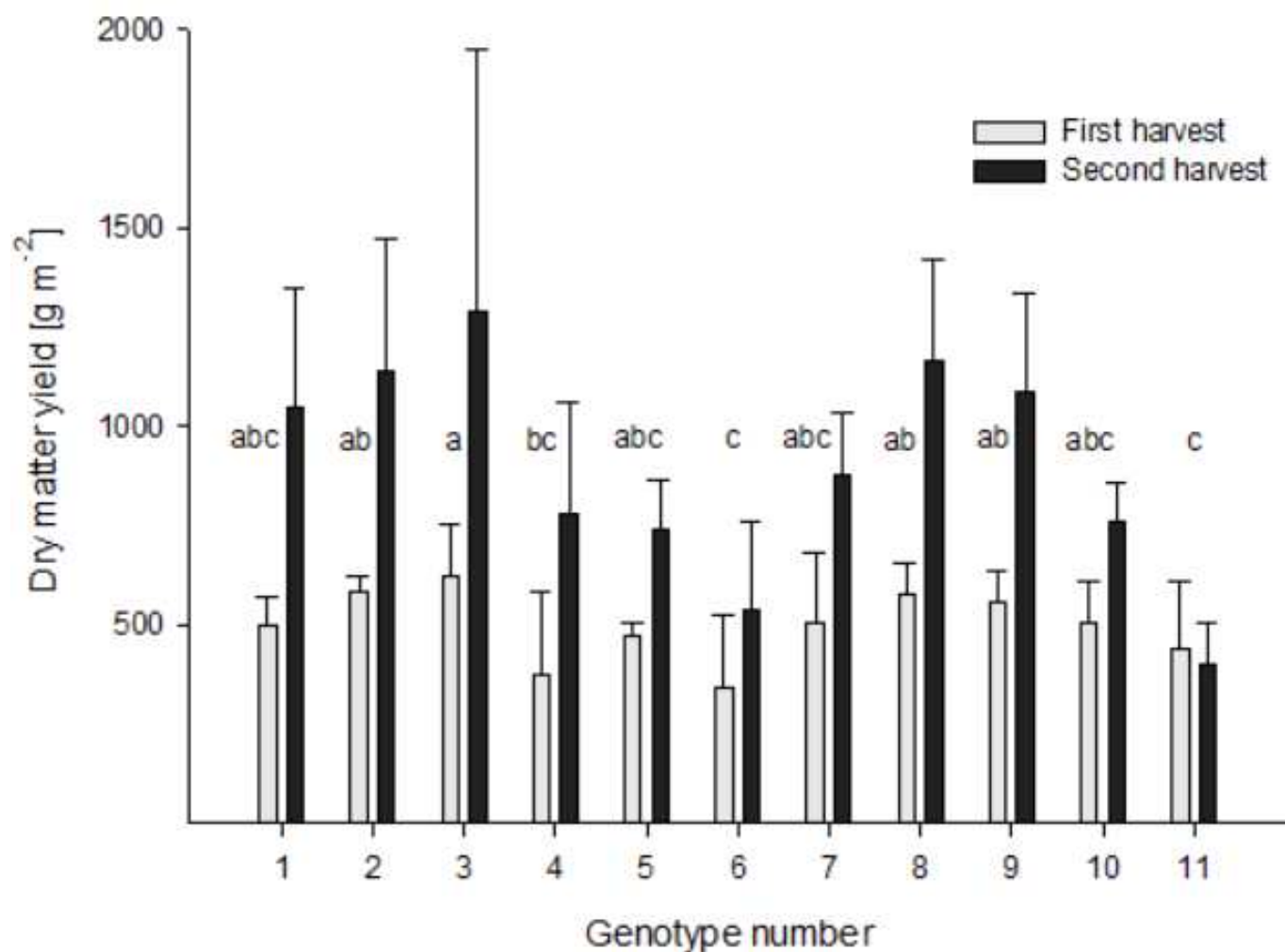


Fig. 2. Comparison of total leaf dry matter yield (g m^{-2} , + standard deviation) of ribwort plantain genotypes from two harvests (years 2021, 2022). The results of Tukey's test (0.05) applied to test for significant differences between varieties are indicated with different letters

During the first harvest carried out on 3 November 2021, the highest average dry matter yield was found in two genotypes of Swiss origin (Nos. 2 and 3) and a variety of Czech origin (No. 8) 624, 587 and 575 g DM m^{-2} , respectively (Fig. 2). The lowest dry matter yield was recorded in genotypes of New Zealand (No. 11) and Polish origin (Nos. 4 and 6; 443, 373 and 345 g DM m^{-2} , respectively). During the second harvest, 79% more dry matter, on average, was harvested from all tested genotypes compared to the first cut.

The highest average dry matter yield was found, as in the first harvest, in variety 3 of Swiss origin (1292 g DM m^{-2}), which was an increase of more than 107% compared to the first cut. The next most successful genotypes were, as in the previous year (2021), genotype Nos. 2 and 8, which had average dry matter yields of 1141 and 1166 g DM m^{-2} , respectively, an increase compared

to the first harvest of 94 and 103%, respectively. In 2022, the lowest yields were found in the genotypes of Polish (Nos. 5 and 6) and New Zealand origin. The average dry matter yields were 739, 539 and 403.1 g DM m⁻², respectively, resulting in an increase in dry matter of 57 and 56% and, in the case of the New Zealand variety, a lower DM yield of 9% (Fig. 3). Statistically significant differences were found for harvest time ($P = 0.0001$) and genotype ($P = 0.0001$) but not for their interaction ($P = 0.0675$).

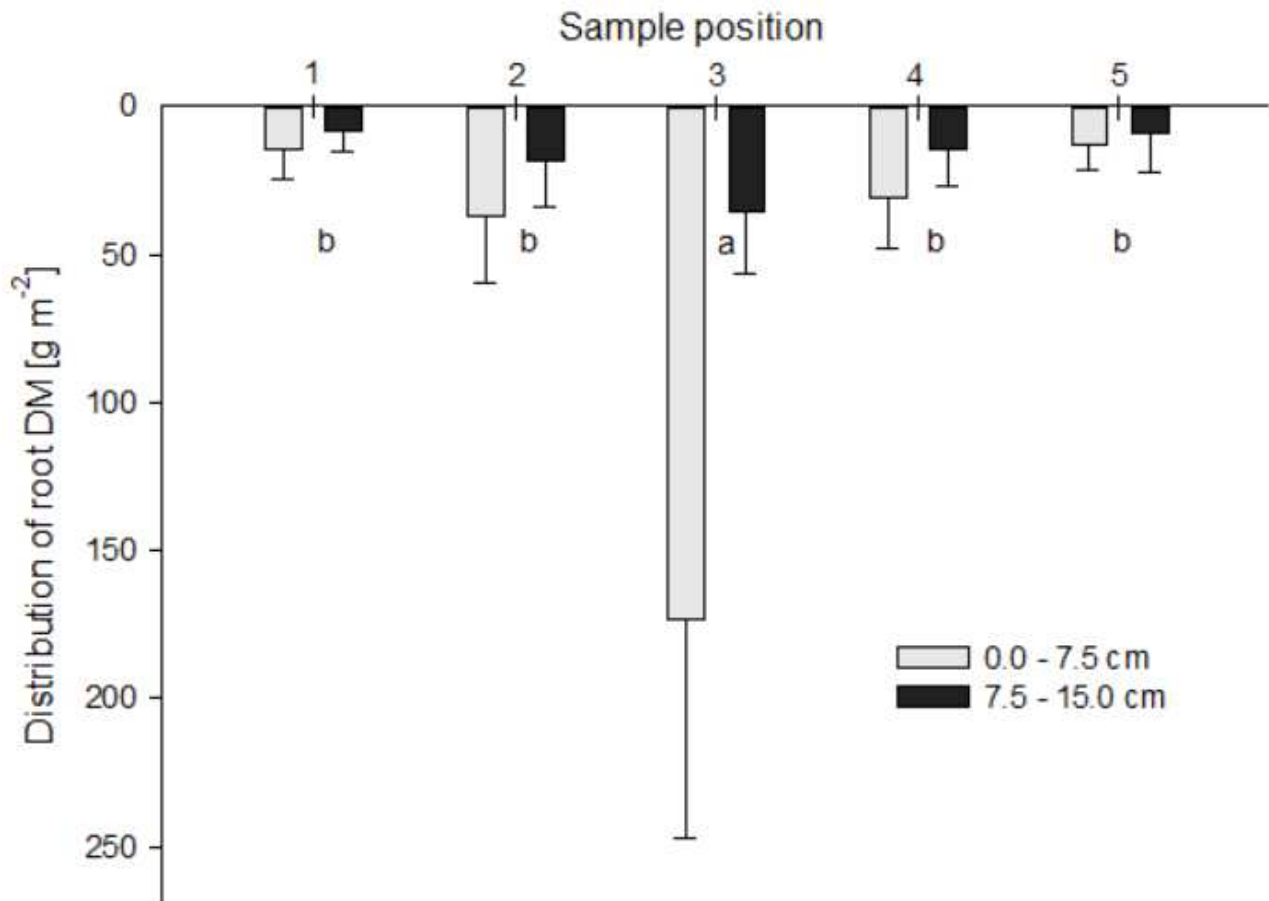


Fig. 3. Average distribution of root dry mass (g m⁻² + standard deviation) of tested *Plantago lanceolata* genotypes and 5 sampling positions (see Fig. 1). The results of Tukey's test (0.05) applied to test for significant differences in sample positions are indicated with different letters

The highest mean distribution of root dry mass of the tested genotypes was found directly under the plants (sampling position No. 3). This amounted to 173 g m⁻² for the soil layer 0–7.5 cm deep and 35 g m⁻² for the bottom layer (7.5–15 cm; Fig. 3). For the topsoil (0–7.5 cm), the mean values for samples taken between rows (sampling positions 1 and 2) from the outer part of the plots (sampling positions 4 and 5) were 14.7 and 36.8 g m⁻², respectively, and for sampling positions 4 and 5, 30.8 and 12.7 g m⁻², respectively. At a depth of 7.5–15 cm, the dry mass distribution for samples 1 and 2 was 8.5 and 18.7 g m⁻², while the mean values obtained on the outer side of the

experimental plots (sampling positions 4 and 5) were 14.8 and 9.1 g m⁻². The sum of the mean distribution of dry mass of the samples between the rows was 78.8 g m⁻², while on the outer side of the experimental plot, the value was 67.4 g m⁻², i.e. 16.9% lower (Fig. 3). Statistically significant differences were found according to sample position ($P = 0.0001$) and genotype ($P = 0.0093$) but not for their interaction ($P = 0.5371$).

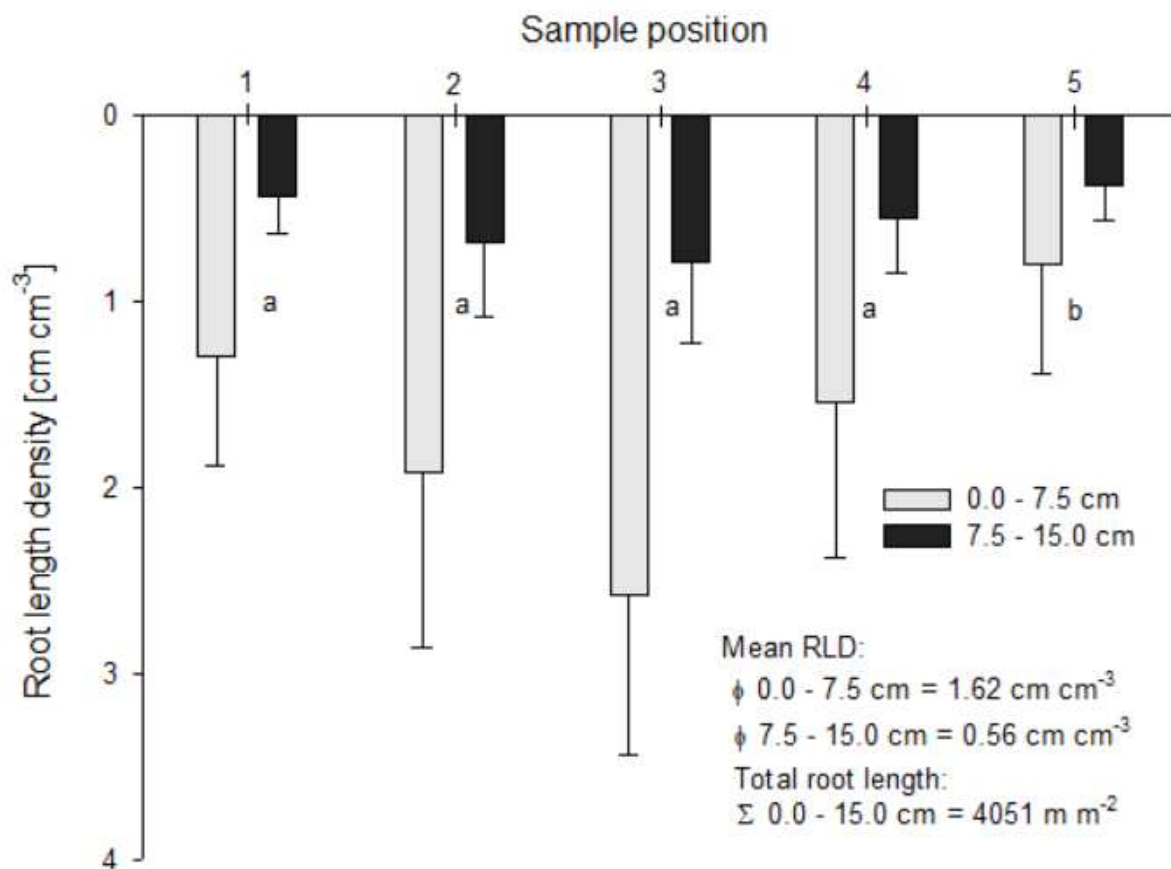


Fig. 4. Average root length density (cm cm⁻³ + standard deviation) of 11 genotypes of *Plantago lanceolata* and 5 sampling positions (see Fig. 1). The results of Tukey's test (0.05) applied to test for significant differences in sample positions are indicated with different letters

The highest mean total root length density per unit soil volume was determined directly under the plants (position No. 3, Fig. 1) at a depth of 0–7.5 and 7.5–15 cm at the line of the row in the experimental plots (2.57 and 0.78 cm cm⁻³, respectively; Fig. 4). Higher average root length density values were recorded in samples directed to the parallel row of the same genotype (Nos. 1 and 2), while lower values were found on the outer side of the experimental plots (Nos. 4 and 5). Comparing positions 2 and 4 at a depth of 0–7.5 cm between the two rows of one genotype, the average tested value was 1.91 cm cm⁻³, while from the outer side, it was 19% lower and

amounted to 1.54 cm cm^{-3} . In the deeper soil layer of 7.5–15 cm at position 2, the mean root length density was 0.68 cm cm^{-3} , while at position 4, it was 0.54 cm cm^{-3} (20% lower). The greatest differences were observed in the samples between the rows and those taken from the outer part of the experimental plot. Comparing the mean values of the samples in the layer at a depth of 0–7.5 cm, for position 1, it was 1.29 cm cm^{-3} , while for position 5, it was 0.8 cm cm^{-3} (38% difference). For the given samples at a depth of 7.5–15 cm, for sample No. 1, it was 0.43 cm cm^{-3} , while for sample No. 5, it was 0.37 cm cm^{-3} , resulting in a difference of 14%. The mean root length density for all tested genotypes for the depth of 0–7.5 cm was 1.62 cm cm^{-3} , while for the depth of 7.5 to 15 cm, it was 0.56 cm cm^{-3} . The mean total root length of 0 to 15 cm for the tested genotypes was 4051 m m^{-2} (Fig. 2.). Statistically significant differences in root length density were found in relation to sampling position ($P = 0.001$) and genotype ($P = 0.0269$) but not for their interaction ($P = 0.9379$).

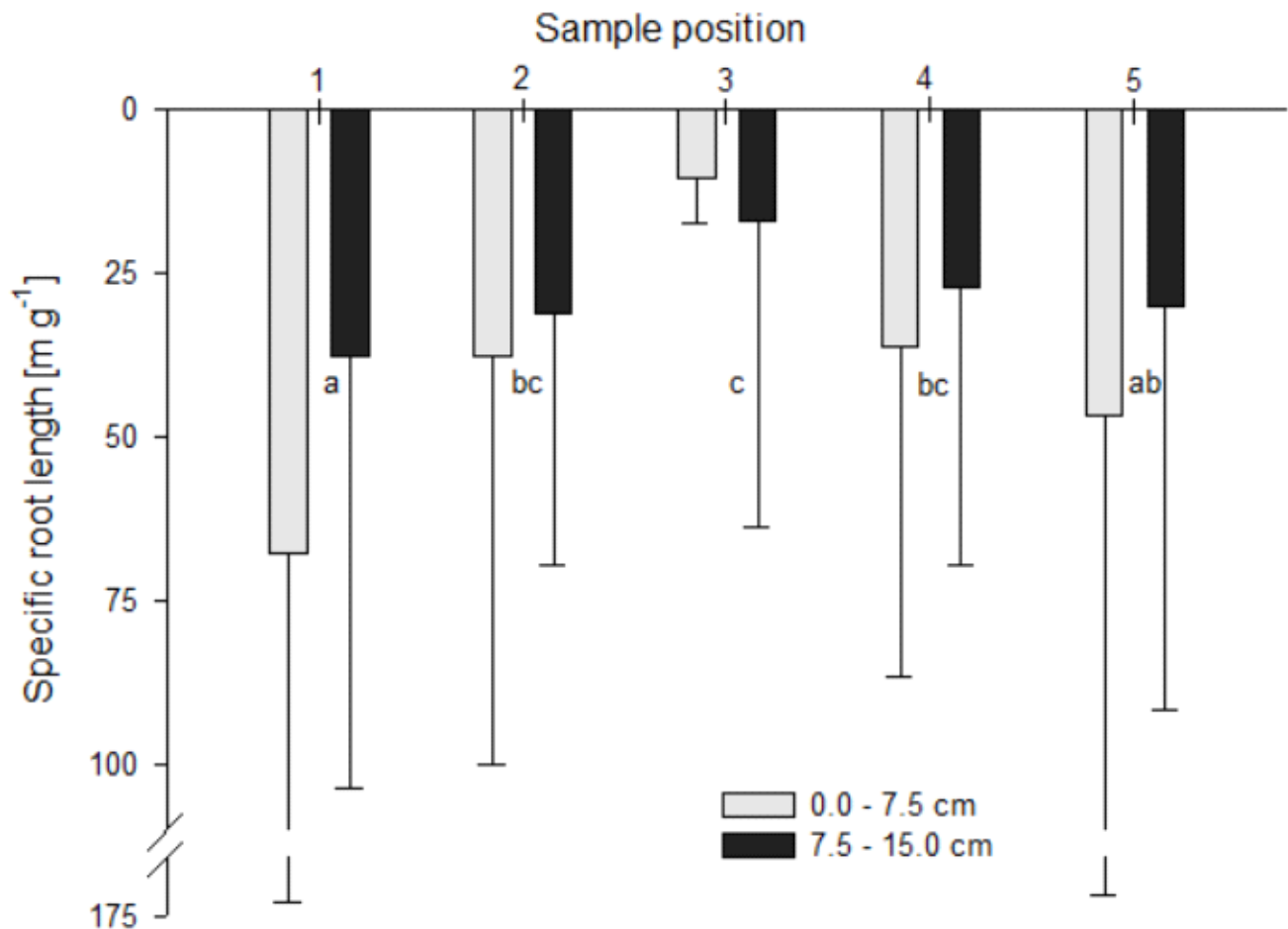


Fig. 5. Average specific root length ($\text{m g}^{-1} \text{ DM} + \text{standard deviation}$) of tested *Plantago lanceolata* genotypes and 5 sampling positions (see Fig. 1). The results of Tukey's test (0.05) applied to test for significant differences in sample positions are indicated with different letters

Maximum average specific root length – the finest roots – for the genotypes tested were found in samples taken from a depth of 0–7.5 cm located between the two rows of one genotype (sample position 1) and for samples from the outer parts of the experimental plots (sample position 5), and these values were 67.5 and 46.7 m g⁻¹, respectively (Fig. 5). In the case of samples taken from a depth of 7.5–15 cm, the highest average values were observed for samples taken from positions 1 and 2 (37.5 and 31.0 m g⁻¹, respectively). The averages of samples taken from the depth in question from positions 4 and 5 were 30 and 27 m g⁻¹, respectively. The comparable values shown in the topsoil for samples No. 2 and 4 were 37 and 36 m g⁻¹, respectively. The lowest mean values of specific root length were recorded in the sample taken from the middle of the row, directly under the plants, and for the depth of 0–7.5 cm; it was 10.5 m g⁻¹, and for the depth of 7.5–15.0 cm, it was 6.8 m g⁻¹ (Fig. 5). Differences in specific root length were significant in relation to sample position ($P = 0.0001$) but not for genotype ($P = 0.4591$) or their interaction ($P = 0.9878$).

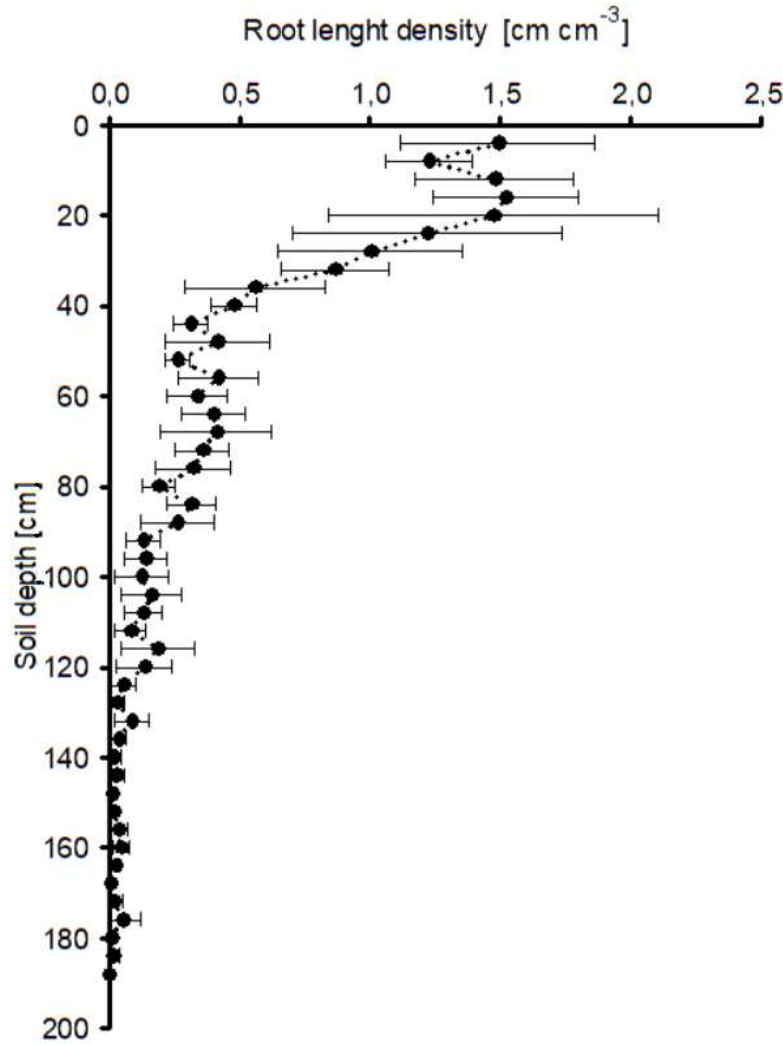


Fig. 6. Root length density ($\text{cm cm}^{-3} \pm$ standard deviation) as a function of soil depth (cm) of *Plantago lanceolata* genotype “Libor” after one year of cultivation under field conditions at Struppen

The highest root length density (cm cm^{-3}) was determined at a depth of up to 20 cm in the soil and had an average value of 1.44 cm cm^{-3} (Fig. 6). The maximum value was obtained at a depth of 16 cm and was 1.52 cm cm^{-3} . Subsequently, a decrease in root length density was observed in the soil profile and from 36 to 76 cm; it remained and ranged from 0.26 to 0.56 cm cm^{-3} . With a slight increase to a 76 cm depth into the soil profile, the root length density decreased steadily. The profile wall method showed that *P. lanceolata* could root up to 188 cm deep into the soil profile within one year (Fig. 6). The total root length, up to 188 cm, was 1417 m m^{-2} .

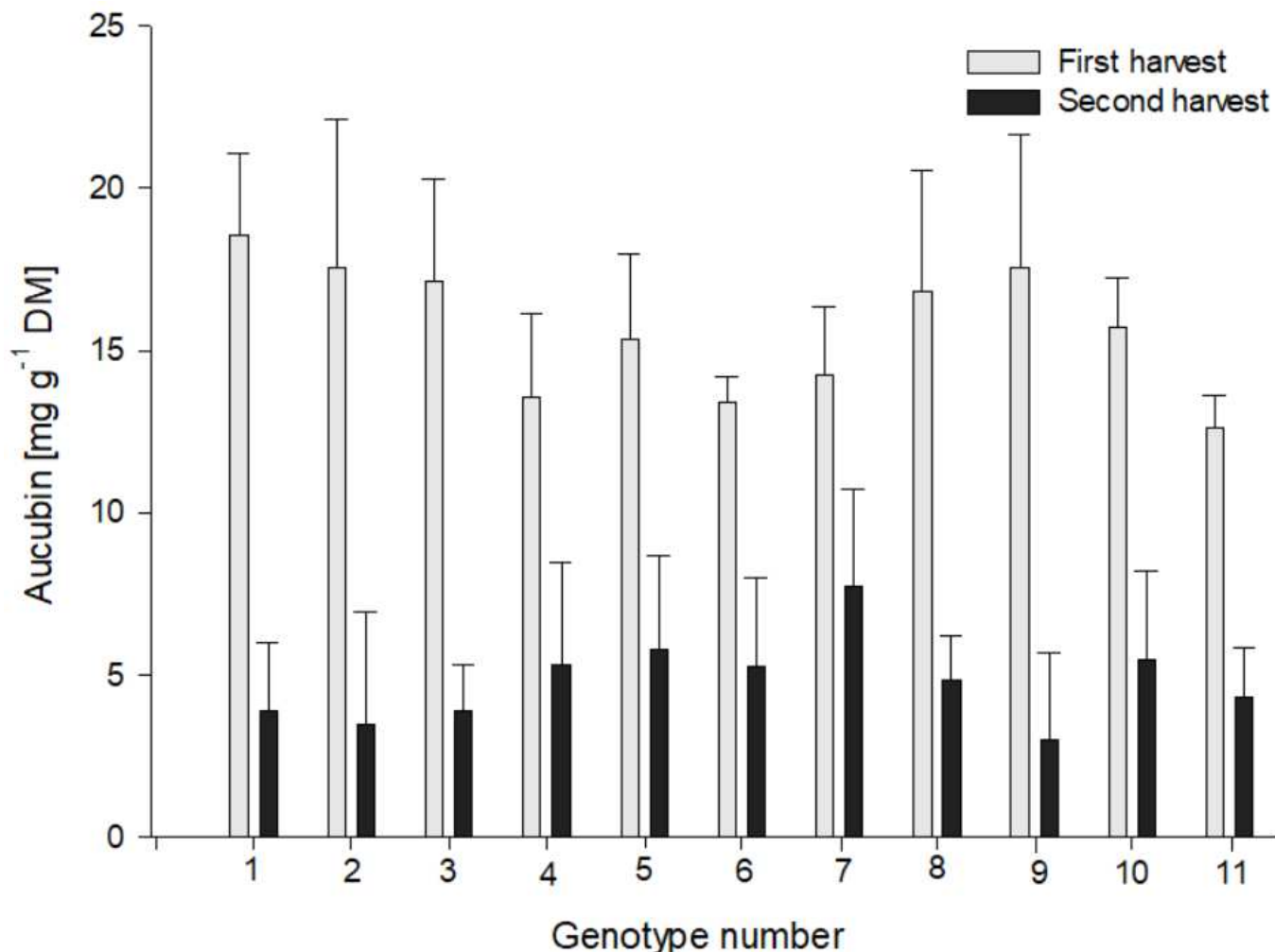


Fig. 7. Leaf aucubin content (mg g⁻¹ DM + standard deviation) of 11 *Plantago lanceolata* genotypes in the first and second years

In the first year, the highest average aucubin content (mg g⁻¹ DM) in the leaves was found in varieties 1, 2 and 9 (18.57, 17.57 and 17.56 mg g⁻¹ DM, respectively; Fig. 7). Aucubin content was slightly lower in genotype number 3 (17.12 mg g⁻¹ DM). The lowest average aucubin content at the first harvest was recorded in genotype Nos. 4 and 6 and cultivar No. 11, with values of 13.54, 13.40 and 12.62 mg g⁻¹ DM, respectively.

At the second harvest, the analytical results showed a significant decrease in aucubin content in the plant material. The highest average aucubin content was found in genotype Nos. 7 (7.73 mg g⁻¹ DM), 5 (5.77 mg g⁻¹ DM), 10 (5.45 mg g⁻¹ DM), 4 (5.29 mg g⁻¹ DM) and 6 (5.25 mg g⁻¹ DM). The lowest aucubin content in the leaves occurred in genotype Nos. 3 (3.88 mg g⁻¹ DM), 1 (3.87 mg g⁻¹ DM), 2 (3.49 mg g⁻¹ DM) and 9 (3.02 mg g⁻¹ DM). Statistically significant differences were determined for harvest date ($P = 0.0001$) and an interaction between harvest date and variety ($P = 0.0077$) was found but not for genotype ($P = 0.5732$). While the differences in aucubin content

between the first and second dates were particularly large in genotypes 1, 2, 3 and 9, this was smaller in genotype 7 by comparison.

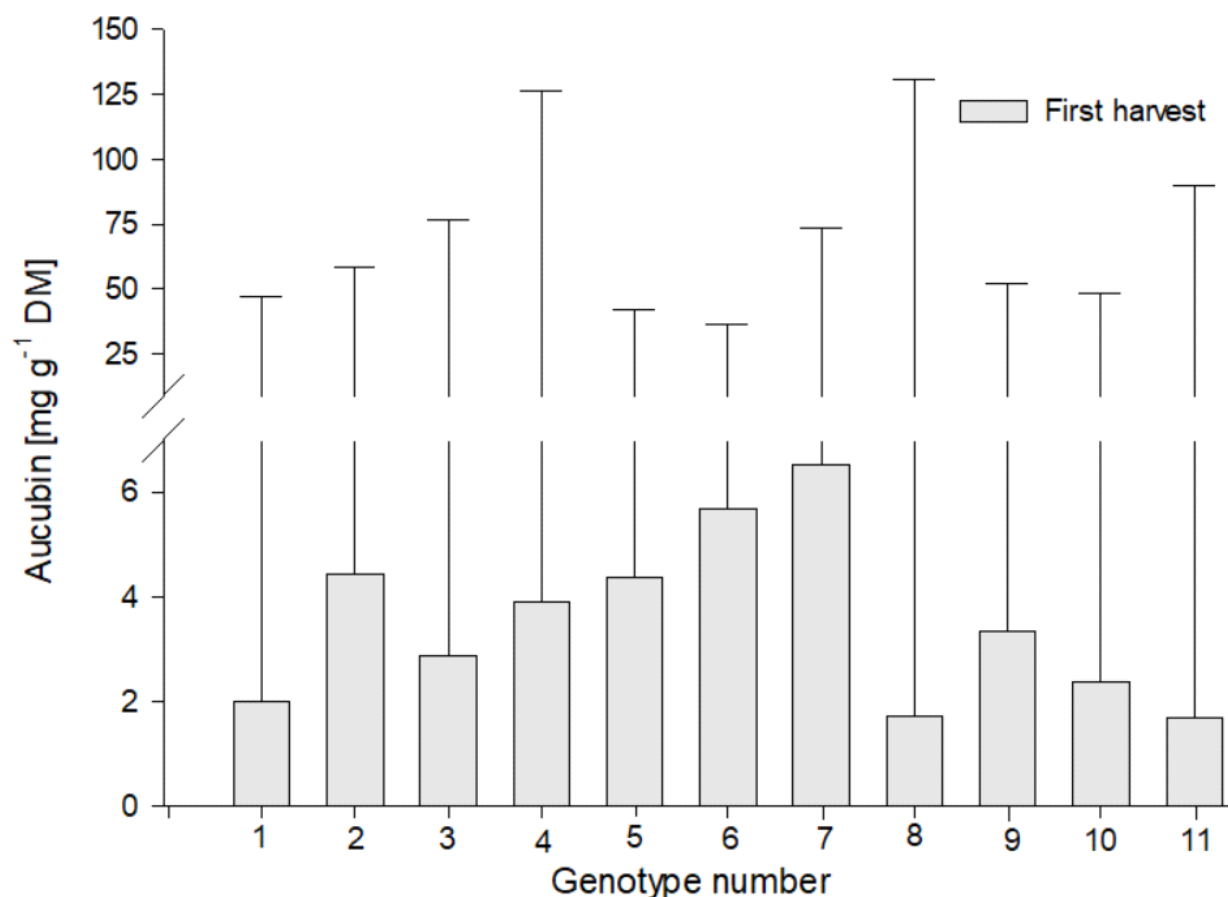


Fig. 8 Aucubin content ($\text{mg g}^{-1} \text{DM} \pm$ standard deviation) in roots of *Plantago lanceolata* genotypes at first harvest extracted from a soil sample containing the main root and part of the lateral roots

All root samples from the five sampling positions were collected at the same time at the first harvest of the aboveground parts of *P. lanceolata*. First, analyses of aucubin content in the underground parts were initially carried out only using plant material manually extracted from root sampling position 3, located on the row line from the topsoil surface (depth of 0.0–7.5 cm). In this phase of the study, 44 root samples were tested for aucubin content (Fig. 8). The extracted material consisted of the main root, with a negligible amount of lateral roots as part of a cylindrical sample volume of 376.99 cm³. The samples differed slightly in the proportion of main root to lateral roots. The highest average aucubin content was recorded in root samples of genotype Nos. 7 (6.53 mg g⁻¹ DM), 6 (5.66 mg g⁻¹ DM), 2 (4.44 mg g⁻¹ DM) and 5 (4.37 mg g⁻¹ DM). The lowest values were determined in genotype Nos. 1 (2.01 mg g⁻¹ DM), 8 (1.72 mg g⁻¹ DM) and 11 (1.69 mg g⁻¹ DM). Further analyses of roots extracted from soil samples for aucubin content in individual genotypes were carried out using only lateral roots. No measurable amounts of aucubin were detected in any of these samples. There were no statistically significant correlations found between the aucubin content of the leaves and roots ($P = 0.3108$).

Discussion

Aucubin content

The harvested aboveground parts analysed by HPLC for aucubin content collected during the first harvest on 03 November 2021 had a significantly higher aucubin content (average: 1.6% DM) compared to the aboveground parts collected during the second harvest on 17 May 2022 (average: 0.5% DM). In the results presented by Tamura and Nishibe obtained from the field experiment at the National Agricultural Research Center for the Tohoku Region, Morioka, Japan, two genotypes of *P. lanceolata*, Ceres Tonic and Grasslands Lancelot, were compared in terms of bioactive compounds¹⁹. In the tested varieties, the aucubin content increased in the leaves from late spring, reached a maximum value at the end of September and then decreased. In the cultivar Grasslands Lancelot, an aucubin content of approximately 2% DM was recorded in the aboveground parts in the second half of June, while for the cultivar Ceres Tonic, it was determined to be around 1% DM. The highest value at the end of September for the Grasslands Lancelot variety was around 5% DM, and for the Ceres Tonic variety, it was just under 3% DM. Ribwort plantain genotypes tested for the purposes of aucubin content in the current study also had significantly lower levels of the tested plant parts during the spring harvest (minimum 0.3% DM, maximum 0.8% DM) compared to the autumn harvest (minimum 1.3% DM, minimum 1.9% DM). This confirms the hypothesis that aucubin content in leaves is caused by seasonal changes^{20,21}. The wide differences in the results obtained for aucubin content in the leaves between Tamura and Nishibe 2002¹⁹ and the experiment conducted at Frick (Switzerland) are most likely due to environmental conditions. Further research conducted by Mieke-Steier et al. in a greenhouse concluded that phenotypic plasticity responding to environmental changes could be responsible for the variation in the aucubin content of the aboveground parts of ribwort plantain²². The authors obtained aucubin content results ranging from 1.6% DM to just over 1.8% DM under high light conditions (14 hours of light per day). The 12% difference in the content of the test ingredient was due to nutrient application. In the experiment carried out at Frick, no application of any fertiliser was performed, so it is possible that this also had an impact on the reduced amount of aucubin measured at the second harvest. The production of secondary metabolites, given the examples of other species, can also be affected by drought^{23,24}. In addition, the bioactive compound synthesis rate in the shoots may also be influenced by water availability, temperature fluctuations²⁵ and the presence of pests²⁶, although these were not observed during the field experiment in Frick. In the present study, the tested genotypes in Bowers et al. were characterised by different contents of aucubin at harvest²⁵. The three-way analysis of variance (ANOVA) performed by the authors clearly indicated significant statistical differences in terms of genotype, date, site, and genotype x site and genotype x date interactions. Tamura and Nishibe also found statistically significant differences due to the sampling date of aboveground parts of

the ribwort plantain¹⁹. This confirms the hypothesis that leaves differ in their aucubin content due to age. Additionally, Klockars et al. observed a significant positive correlation between the concentration of iridoid glycosides (including aucubin) and the aspect in discussion²⁷. The aucubin content increased from undetectable in older leaves to 9% DM in the newest leaves. In this study, the same amounts of green matter were collected from each plot for aucubin analyses, although they may have differed slightly in terms of the proportion of younger and older leaves. This probably resulted in an increased standard deviation between the samples tested. As in the leaves, the aucubin content of the roots is influenced, among others, by the plant's access to light. For aucubin analyses, root samples of the tested genotypes were collected in parallel with the first harvest. In an experiment under controlled environmental conditions, Miehle-Steier et al. showed the effect of light availability on the aucubin content in the belowground parts of ribwort plantain²². The aucubin content in the roots under good light conditions was 7 times higher (2.1% DM) than under poor light conditions (0.3% DM), and the hypothesis of an effect of nutrient availability on the aucubin content in the roots of *P. lanceolata* was ruled out. Values obtained from Frick showed the average aucubin content in the tested genotypes at the level of 0.4% DM. The obtained value is comparable to the results obtained by the cited author under low light conditions. The low aucubin content of the test component in the belowground parts of the plants may have been caused by the soil conditions and the availability of water during the experiment that was carried out. For the analyses of the roots extracted, the first samples were taken from the line of the row and contained varying amounts of lateral roots and the main root (0–7.5 cm deep into the soil profile). This may have influenced the final absence of statistical correlation between the samples analysed. Moreover, for the purposes of further research, lateral roots were analysed for aucubin content, and no measurable aucubin content was detected. Currently, there is no information in the literature on the various contents of aucubin in the different root parts of ribwort plantain; thus, further studies are required to better characterise the main root and lateral roots in terms of aucubin content.

Root growth

Due to the increasing incidence of periods of limited rainfall, to avoid drought stress for plants, plants with higher water uptake capacities from deeper soil layers should be grown²⁸. A deeper root system will also allow for more efficient carbon sequestration²⁹ and nutrient uptake³⁰, particularly nitrate¹⁰ and phosphorus³¹. These properties ultimately affect total crop productivity; thus, it is very important to choose the right crop and crop varieties adapted to fast-changing climatic conditions. The results obtained in the study clearly indicate the very high rooting potential of ribwort plantain. After one year of development, roots of the variety Libor were found at a soil depth of almost 190 cm. The above-average properties of plantain that make the plant attractive for agricultural use¹² may be competitive with some currently used varieties. For example, most grass species are successively becoming less and less drought-tolerant due to

limited possibilities of penetration of deeper soil layers by roots. Durand and Ghesquière, after analysing data obtained through neutron probe measurements, determined the maximum water uptake capacity for Italian ryegrass (*Lolium multiflorum* Lam.) under field conditions 80 cm deep in the soil profile³². Similar measurement results were obtained by Jacques³³ and Gibbs³⁴ where the deepest roots of annual ryegrass were also found at a depth of 80 cm. To make the best use of the properties of *P. lanceolata*, it would be beneficial to use it in agriculture in mixtures with plants that are similarly resistant to water deficiencies, i.e. lucerne (*Medicago sativa* L.). In areas at risk of drought, farmers and pasture managers should use a combination of forage species that is more efficient than monocultures (e.g. *Lolium perenne* L.) to mitigate the effects of water shortages³⁵. The use of *P. lanceolata* in pasture mixtures is currently popular in New Zealand due to its inhibition of soil N nitrification¹³. In addition, the presence of aucubin in the aboveground parts of the plant had a positive impact on the health of grazing animals³⁶.

Another *P. lanceolata* root indicator tested under field conditions was root length density (RLD). This value refers to the concentration or abundance of roots in a given volume of soil; thus, it is a measure of their distribution over a defined area. A high root density in a plant directly affects its overall growth and productivity through the efficient uptake of immobile soil resources, such as potassium and phosphorus. At a depth of 0–7.5 cm into the soil profile, the average root length density value was 1.62 cm³ for the tested genotypes 123 days after establishing the field experiment at Frick and was slightly higher than the value obtained after one year of cultivation under field conditions for the Libor variety, which averaged 1.36 cm³ at a depth of 0–8 cm. A large difference, in contrast, was observed in the average rooting value variety of Libor after one year of cultivation. At a depth of 8–16 cm, the average RLD was 1.40 cm³, while the average value for the genotypes tested at Frick at a depth of 7.5–15 cm was 0.56 cm³. The tested values may differ due to the length of growth of the plants tested and the physical soil conditions³⁷ occurring at the two experimental sites. In the experiment conducted in Frick, the stone content of the soil was comparatively high, which may have had a slight negative impact on the value in question. According to Liu et al., the value of the discussed coefficient was characterised for oilseeds, legumes and wheat at different depths in the soil profile³⁸. Up to a depth of 20 cm under low water conditions, the highest value (6.00 cm cm⁻³) discussed was recorded for spring wheat. Slightly lower values were recorded for pulses (chickpea, field pea, lentil) with 3.39 cm cm⁻³ and oilseeds (canola, flax, mustard) with 2.70 cm cm⁻³. Sugar beet has a lower RLD value; values obtained from a pot experiment by Camposeo and Rubino³⁹ in the form of a two-year average at harvest, depending on irrigation frequency, ranged between 0.1 ± 0.4 and 1.8 ± 0.3 cm cm⁻³. Our results showed that *P. lanceolata* could form an intermediate RLD compared to other crops.

A further root indicator examined in this work was the specific root length (SRL). It is a measure of root morphology that considers the weight and length of the roots⁴⁰. This parameter characterises the fineness of the root system, i.e. how much root dry mass the plant must provide to realise one metre of root length. This determines the plant's ability to acquire resources from

the soil, such as nutrients and water⁴¹. In this work, SRL was tested between and beyond the sown rows at 30 cm spacing (Fig. 1). The highest average value (67.5 m g^{-1}) was recorded between sown rows at a depth of 0.0–7.5 cm and a distance of 12 to 20 cm from sampling site 3 (Fig. 1), running in the centre of one of the two rows in the plot. For comparison, the root value discussed was also determined for bentgrass (*Agrostis capillaris* L.) and field sorrel (*Rumex acetosa* L.) by Leuschner et al. under permanent grassland conditions⁴². The SRL value was determined at a depth of approximately 20–30 cm, in which samples were collected with a spade around the target plant⁴². Depending on the amount of mineral fertilisation, the discussed mean values were 24 m g^{-1} for *R. acetosa* and 155 m g^{-1} for *A. capillaris* L. Slightly higher SRL values were obtained in a field experiment by Welbank et al., who compared semi-dwarf selections of wheat (*Triticum aestivum* L.) of European and Japanese origin (derived from variety Norin 10)⁴³. In an experiment established on 3 October 1970, soil samples containing roots were collected on five different dates during the growing season of the plants. The highest average SRL value was recorded at a depth of 0–15 cm on 16 March and was 199 m g^{-1} , which was also the highest recorded throughout the experiment. For the 15–25 cm depth, the highest value was obtained on 8 June 1971 (197 m g^{-1}). Similar analyses were carried out by the authors for an experiment sown slightly later, on 22 October 1970, and roots were collected for further analyses at four different time intervals. On 16 March 1971, the highest average SRL was obtained for the 0–15 cm depth for the tested selections and was 262 m g^{-1} . As in the experiment sown earlier, the highest value was determined at this depth. Additionally, on this sampling day (16 March 1971), the highest value was obtained for a depth of 15–25 cm (242 m g^{-1}). Similarly, the SRL for wheat and barley varieties in a study conducted under controlled environmental conditions was compared by Loes and Gahoonia⁴⁴. For the experiment conducted with soil conditions for barley, the value ranged from 182 (variety Herse) to 243 m g^{-1} (varieties SWE013 S, 2r and Olve, 2r), while for wheat, the SRL ranged from 173 (variety Brakar) to 197 m g^{-1} (varieties Reno and Børsum). For varieties grown in nutrient solution, for barley, the average SRL values ranged from 186 (cv. Fager) to 329 m g^{-1} (cv. Herta). For wheat, the lowest SRL value was determined for cv. Diamant and amounted to 255 m g^{-1} , while cv. NK97537 showed the highest value among the tested varieties (340 m g^{-1}). Much lower SRL values were obtained by Katsua and Takashi, who analysed maize (*Zea mays* L.) roots⁴⁵. This study investigated the morphological plasticity of roots in soil enriched by different phosphate (P) contents and patch sizes (containers filled with the soil). After 55 days of plant cultivation at different P doses and patch sizes, the SRL values obtained ranged from 27.7 to 78.7 m g^{-1} .

In this study, for the first time, the RLD and SRL parameters were determined for *P. lanceolata*. Comparing the results presented in the study to those cited by other studies showed that ribwort (*P. lanceolata*) is well adapted in terms of rooting abilities in the topsoil and deep into the soil profile. These properties are some of the basic growth characteristics of plants grown in nutrient-poor soils. High values for these specific parameters also have a positive effect on the plant's

ability to absorb water due to maximising its uptake and the ability of the roots to penetrate a larger soil volume. This demonstrates the high competitiveness of the plant, which affects its ability to establish itself in different environments. A high SRL also has a limiting effect on erosion by binding soil particles, which has a positive effect on the soil structure⁴⁶.

Conclusions

The results presented in this publication indicate that *P. lanceolata* is well adapted to water deficit conditions through its rooting properties. Furthermore, based on the values characterising roots (RLD and SRL), it can be concluded that the ribwort plantain has a good ability to take up immobile and mobile nutrients from the soil. Major differences in aucubin content between leaves and roots are related to the higher value of the aboveground parts of ribwort plantain, which is especially essential for inhibiting the nitrification process in the soil.

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Data availability statement

All data generated or analysed during this study are included in its supplementary information files.

References:

1. Calanca, P. Climate change and drought occurrence in the Alpine region: How severe are becoming the extremes? *Glob. Planet. Change* **57**(1-2), 151–160 (2007).
2. Jacob, D. et al. Klimaauswirkungen und Anpassung in Deutschland – Phase I: Erstellung regionaler Klimaszenarien für Deutschland (11/2008), *Climate Change* (2008).
3. McKenzie, B. M. et al. Deep rooting and drought screening of cereal crops: A novel field-based method and its application. *Field Crops Res.* **112**(2), 165–171 (2009).
4. Sandhu, N. et al. Biochemical and genetic approaches improving nitrogen use efficiency in cereal crops: A review. *Front. Plant Sci.* **12**(657629), (2017).
5. Bouwman, A. F. Direct emission of nitrous oxide from agricultural soils. *Nutr. Cycl. Agroecosyst.* **46**(1), 53-70 (1996).
6. De Klein, C. A. M. et al. Estimating a nitrous oxide emission factor for animal urine from some New Zealand pastoral soils. *Aust. J. Soil Res.* **41**(3), 381-399 (2003).

7. Nacry, P., Bouguyon, E. & Gojon, A. Nitrogen acquisition by roots: Physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. *Plant and Soil* **370**(1-2), 1-29 (2013).
8. Diaz, R.J. & Rosenberg, R. Spreading dead zones and consequences for marine ecosystems. *Science* **321**(5891), 926-929 (2008).
9. Galloway, J. N. et al. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* **320**(5878), 889-892 (2008).
10. Rauber, R. et al. Agronomic effects of underseeding ribwort plantain (*Plantago lanceolata* L.) in organically grown potatoes. *Pflanzenbauwiss* **12**, 32-40 (2008).
11. Dietz, M. et al. Inhibitory effects of *Plantago lanceolata* L. on soil N mineralization. *Plant Soil* **368**, 445-458 (2008).
12. Pol, M., Schmidtke, K. & Lewandowska, S. *Plantago lanceolata* – An overview of its agronomically and healing valuable features. *Open Agriculture* **6**(1), 479-488 (2021).
13. Gardiner, C. A. et al. Ruminant urine patch nitrification and N₂O flux: Effects of urine aucubin rate in a laboratory trial. *N. Z. J. Agric. Res.* **63**(1), 65-72 (2019).
14. Pijlman, J. et al. Can the presence of plantain (*Plantago lanceolata* L.) improve nitrogen cycling of dairy grassland systems on peat soils? *N. Z. J. Agric. Res.* **63**(1), 106-122 (2020).
15. Sans, F. X. et al. Tillage effects on weed communities in an organic winter wheat–sunflower–spelt cropping sequence. *Weed Res.* **51**(4), 413-421 (2011).
16. Sadowski, A. Uprawa ziół i możliwości ich wykorzystania. Wydawnictwo Uniwersytetu w Białymstoku (2013).
17. Shahzad, Z. et al. EZ-Root-VIS – A software pipeline for the visual reconstruction of averaged root system architecture. *Plant Physiology* **177**(4), 1368-1381 (2018).
18. Böhm, W. Profile Wall methods. *Methods of Studying Root Syst.* 48-60 (1979).
19. Tamura, Y. & Nishibe, S. Changes in the concentrations of bioactive compounds in plantain leaves. *J. Agric. Food Chem.* **50**(9), 2514-2518 (2002).
20. Grigore, A. et al. *Plantago lanceolata* L. crops – Source of valuable raw material for various industrial applications. *A. Agron.* **58**, 207-214 (2015).
21. European Medicine Agency. Assessment report on *Plantago lanceolata* L., folium, Draft, Community herbal monograph on *Plantago lanceolata* L. 2010, folium, (2011).
22. Miehe-Steier, A. et al. Light and nutrient dependent responses in secondary metabolites of *Plantago lanceolata* offspring are due to phenotypic plasticity in experimental grasslands. *PLoS ONE* **10**(9), e0136073 (2015).
23. Waterman, P. G. & Mole, S. Extrinsic factors influencing production of secondary metabolites in plants in *Insect-plant interactions* (ed. Bernays, E. A.) 107-134 (CRC Press, 1989).
24. Briske, D. D. & Camp, B. J. Water stress increases alkaloid concentration in threadleaf groundsel (*Senecio longilobus*). *Weed Sci.* **30**(1), 106-114 (1982).
25. Bowers, M. D. & Stamp, N. E. Chemical variation within and between individuals of *Plantago lanceolata* (Plantaginaceae). *J. Chem. Ecol.* **18**, 985-995 (1992).

26. Lynn, S., Adler J. S. & Bowers, M. D. Genetic variation in defensive chemistry in *Plantago lanceolata* (Plantaginaceae) and its effect on the specialist herbivore *Junonia coenia* (Nymphalidae). *Oecologia* **101**(1), 75-85 (1995).
27. Klockars, G. K., Bowers, M. D. & Cooney, B. Leaf variation in iridoid glycoside content of *Plantago lanceolata* (Plantaginaceae) and oviposition of the buckeye, *Junonia coenia* (Nymphalidae). *Chemoecology* **4**, 72-78 (1993).
28. Narayanan, S. et al. Variability of root traits in spring wheat germplasm. *PLoS ONE* **9**(6), e0100317 (2014).
29. Canadell, J. et al. Maximum rooting depth of vegetation types at the global scale. *Oecologia* **108**(4), 583-595 (1996).
30. Barley, K. P. The configuration of the root system in relation to nutrient uptake. *Adv. Agron.*, **22**, 159-201 (1970).
31. Otani, T. & Ae, N. Sensitivity of phosphorus uptake in changes in root length and soil volume. *Agron. J.* **88**(3), 371-375 (1996).
32. Durand, J. L. et al. Ranking of the depth of water extraction by individual grass plants, using natural ¹⁸O isotope abundance. *Environ. Exp. Bot.* **60**(1), 137-144 (2007).
33. Jacques, W. A. Root development in some common New Zealand pasture plants II. Perennial ryegrass (*Lolium perenne*) cocksfoot (*Dactylis glomerata*) and white clover (*Trifolium repens*). *N. Z. J. Sci. and Technol.* **A25**, 91-117 (1943).
34. Gibbs, R. J. Changes in soil structure under different cropping systems. PhD thesis, Lincoln College, Univ. of Canterbury (1986).
35. Hofer, D. et al. Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought. *J. Appl. Ecol.* **53**(4), 1023-1032 (2016).
36. Al-Marashdeh, O. et al. Integrating plantain (*Plantago lanceolata* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) into New Zealand grazing dairy system: The effect on farm productivity, profitability, and nitrogen losses. *Animals* **11**(2), 376 (2021).
37. Bengough, A. G. et al. Root responses to soil physical conditions; Growth dynamics from field to cell. *J. of Exp. Bot.* **57**(2), 437-447 (2006).
38. Liu, L. et al. Rooting systems of oilseed and pulse crops. II: Vertical distribution patterns across the soil profile. *Field Crops Res.* **122**(3), 248-255 (2011).
39. Camposeo, S. & Rubino, P. Effect of irrigation frequency on root water uptake in sugar beet. *Plant and Soil* **253**(2), 301-309 (2003).
40. Boot, R. A. & Mensink, M. Size and morphology of root systems of perennial grasses from contrasting habitats as affected by nitrogen supply. *Plant Soil* **129**, 291-299 (1990).
41. Weemstra, M. et al. The role of fine-root mass, specific root length and life span in tree performance: A whole-tree exploration. *Funct. Ecol.* **34**(3), 575-585 (2020).
42. Leuschner, C., Gebel, S. & Rose, L. Root trait responses of six temperate grassland species to intensive mowing and NPK fertilisation: A field study in a temperate grassland. *Plant Soil.* **373**(1-2), 687-698 (2013).

43. Welbank, P. J. et al. Root growth of cereal crops. *Rothamsted Exp. Station Rep. for 1973*, **2**(2), 26-66 (1974).
44. Løes, A. K. & Gahoonia, T. S. Genetic variation in specific root length in Scandinavian wheat and barley accessions. *Euphytica* **137**, 243-249 (2004).
45. Katsuya, Y. & Takashi, K. Root morphological plasticity for heterogeneous phosphorus supply in *Zea mays* L. *Plant Prod. Sci.* **8**(4), 427-432 (2015).
46. Vannoppen, W. et al. How do root and soil characteristics affect the erosion-reducing potential of plant species? *Ecol. Eng.* **109** Part B, 186-195 (2017).

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