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RESPONSES OF GERMINATION AND EARLY GROWTH OF SCORZONERA (*SCORZONERA HISPANICA* L.) TO pH, MINERAL DEFICIENCIES AND GROWTH SUBSTRATES

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Abstract

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In order to identify early constraints to scorzonera cultivation, seed germination under a range of pH values, the response of plantlet growth to six different mineral deficiencies and to different growth substrates were investigated in laboratory and outdoor pot experiments. Total germination, time needed for germination to begin and to finish and asymmetry of germination distribution over time was insensitive to pH within the range 5−10. Generalized and clear symptoms of mineral deficiency in plantlets younger than two months were only found in the absence of iron. Significant reductions in shoot and total biomass were found in the absence of magnesium or iron and in the later also in root biomass. Very young scorzonera plantlets seem thus to be able to rapidly accumulate enough mineral reserves to sustain subsequent growth during a considerable period. After seven months growing outdoors, scorzonera plants especially the roots, grew significantly better in a very light texture mixture of sand and vermiculite than in a heavier commercial growth substrate. Altogether, these results suggest that scorzonera is a species able to grow in less favourable environments, thus offering good prospects for its cultivation as cash crop in marginal and less productive soils.

Key words: germination; growth substrate; mineral deficiencies; pH; plant growth; *Scorzonera hispanica*

Introduction

Scorzonera, black salsify or Spanish salsify (*Scorzonera hispanica* L.) is a perennial Asteraceae that reproduces by cypselas. It possesses a long taproot, white, milky inside, and blackish outside, the root being its main interest as a crop (Nuez and Bermejo, 1994). Roots are rich in inulin (Vulsteke and Calus, 1990; Dolota and Dąbrowska, 2004a), the second most abundant storage carbohydrate in nature (Singh and Singh, 2010).

Scorzonera is distributed over central and southern Europe and in southern areas of the former USSR but is absent from Sicily, Greece, northwest Africa and southwest Asia (Nuez and Bermejo, 1994). Its roots, together with those of carrot, parsnip, skirret and others were popular foods in France during the 16th and 17th century. Roots can be processed and canned (Vulsteke and Calus, 1990) or eaten fresh (raw, seasoned or cooked) and because of their high levels of inulin roots are very appropriate for diabetic diets (Nuez and Bermejo, 1994). Leaves are rich in vitamin C but lack inulin (Dolota and Dąbrowska, 2004b) and are eaten, especially in salads.

In Southern Portugal, in the 1950s and 1960s sweets made of crystallised roots of scorzoneramainly harvested in the wild but also cultivated in gardens were priced gifts to offer close relatives. Increased agricultural mechanisation, overexploitation of wild populations and changes in consumption patterns are among the chief causes of the progressive disappearance of this practice which reduced the use of scorzonera in Portugal almost to nothing (CGA, 2010; SLF, 2012).

However to be successful, the revival of scorzonera use, either as sweet or in different ways has to rely for now in

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cultivated plants because of its present rarity and threatened status in the wild. In fact, scorzonera is a rare and threatened species highly susceptible to habitat fragmentation (Münzbergová, 2006; Münzbergová and Plačková, 2010) and its distribution in Portugal has drastically reduced in the past hundred years. Scorzonera was relatively frequent in Portuguese hinterland in the beginning of twenty century (Sampaio, 1946) but in the 1930s, it was only registered in a few places in northeast hinterland and a little more frequently in southern Alentejo (Coutinho, 1939). In the late 1980s a review of the genus *Scorzonera* in the Iberian Peninsula only refers its occurrence in very few places in southern Portugal (Guardia and Blanca, 1987), the same in a very recent survey in which scorzonera was only found in very few sites in southern Portugal (SPB, 2013).

Despite that some recent work on cultivated scorzonera exists for eastern Europe conditions, including the effect of cultivation on yield (Dolota and Dąbrowska, 2004a, 2007a, 2007b), weed infestation (Błażewicz-Woźniak and Konopiński, 2011) and susceptibility to pathogens (Patkowska and Konopiński, 2008) very little is known concerning the biology of early stages of scorzonera life cycle except in relation to the effects of constant and alternating temperature on seed germination and seedling growth (Dias et al., 2013).

Therefore, in the scope of a programme aimed at the identification of precocious constraints to scorzonera instalment and growth, we set out to investigate the responses of seed germination to pH and the effects of mineral deficiencies and of different substrates on scorzonera growth.

Materials and Methods

Plant material: Papus-free cypselas of scorzonera cv. Géant Noire de Russie (Semillas Batlle), referred to as seeds hereafter, were purchased at a specialized seed shop. Fifty seeds were randomly selected, their length, width and thickness individually determined to the nearest 0.01 mm and their mass to the nearest mg.

pH experiment: Four replicated 10 cm glass Petri dishes per treatment were fitted with Whatman No. 1 paper, sown with 25 seeds each and wetted with 5 mL of the appropriate solution. Solutions of pH 5, 6, 7, 8, 9 and 10 were prepared with distilled water and HCl 1 N or KOH 1 M, and checked with a pH Meter Metrohm 744 and kept at 25ºC until used. Seeds were incubated under 25ºC, constant dark, and were considered germinated when any part of the embryo, generally the root, was visible. Germination was registered twice a day during eight days and germinated seeds discarded.

Mineral nutrition experiment: Treatments consisted in growing plants in nutrient solutions where one element was absent. Elements absent were nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) and iron (Fe). A modified complete Hoagland solution was used as control. The composition of nutrient solutions is shown in Table 1. Seeds were sown in 10 cm glass Petri dishes fitted with Whatman No. 1 paper wetted with 5 mL of distilled water and incubated under alternating 10/20ºC, constant darkness. Germination was daily checked and germinated seeds were transferred to new Petri dishes and incubated under alternat-

Table 1
Composition of nutrient solutions, mgL^{-1}

		Deficiency					
Element	Complete	Nitrogen	Potassium	Calcium	Magnesium	Phosphorus	Iron
N (NO_3)	195.9	0.0	154.1	139.8	170.9	202.9	195.9
N (NH_4)	25.6	0.0	67.6	81.6	42.0	7.0	25.6
K	234.0	234.0	0.0	234.0	241.8	234.0	234.0
Ca	212.7	220.0	212.7	0.0	212.7	212.7	212.7
Cl	94.2	390.7	94.2	1.6	94.2	94.2	94.2
S	69.0	149.0	69.0	69.0	68.6	69.0	64.4
Mg	48.8	48.8	48.8	48.8	0.0	48.8	48.8
P	28.3	30.7	28.3	28.3	30.7	0.0	28.3
Fe	8.0	8.0	8.0	8.0	8.0	8.0	0.0

Micronutrients with the same concentration in all solutions: boron (1007.1 μgL^{-1}), manganese (999.4 μgL^{-1}), zinc (230.2 μgL^{-1}), molybdenum (106.6 μgL^{-1}), copper (59.6 μgL^{-1}). Stock solutions used to prepare nutrient solutions were $(NH_4)_2HPO_4$ 0.20 M; NH_4NO_3 0.50 M; $Ca(NO_3)_2 \cdot 4H_2O$ 0.80 M; $CaCl_2$ 0.26 M; $MgCl_2 \cdot 6H_2O$ 0.20 M; $Mg(NO_3)_2 \cdot 6H_2O$ 0.20 M; $MgSO_4 \cdot 7H_2O$ 0.40 M; KH_2PO_4 0.20 M; KNO_3 1.20 M; K_2SO_4 0.50 M; H_3BO_3 0.0466 M; $CuCl_2 \cdot 2H_2O$ 0.0005 M; $MnCl_2 \cdot 4H_2O$ 0.0091 M; $ZnCl_2$ 0.044 M; MoO_3 0.0006 M; $FeSO_4 \cdot 7H_2O$ 0.0712 M chelated with EDTA.

ing 10/20ºC and 16 h photoperiod. Nine days after sowing seedlings were planted outdoors in pots fitted with a mixture (3:1, v:v) of coarse sand and commercial growth substrate (Siro® Plant; pH in $CaCl_2$ 5.5−6.5, organic matter >70%, nitrogen 150−250 mgL^{-1}, phosphorus as P_2O_5 150−250 mgL^{-1}, potassium as K_2O 300−500 mgL^{-1}). Approximately 1.5 month after sowing healthy plantlets were carefully removed from the mixture, roots gently washed under tap water and distilled water, number of roots and number of leaves counted, length of the largest leaf and of the largest root measured to the nearest mm. Six plantlets were assigned at random to each treatment, all of them still keeping their long and narrow leaf like cotyledons and almost all with two leaves. Solutions were aerated continuously and plant containers refilled after seven days. Growth was carried indoors, with natural light (~12 h per day) supplemented during 8 h by two halogen lamps (500 W each) and one 12 LED lamp (1 W each, PAR ~300 µmol m^{-1} s^{-1}). Plantlets were daily inspected for visual symptoms of mineral deficiencies and measured again after 15 days at the end of the experiment. Shoots and roots were separately oven-dried at 60ºC during 72 h and their biomass determined to the nearest mg.

Growth substrate experiment: Seeds were sown in 10 cm glass Petri dishes fitted with Whatman No. 1 paper wetted with 5 mL of distilled water (pH~7.0) and incubated under 25ºC, constant dark. Germination was daily checked and germinated seeds were transferred to new Petri dishes and incubated during seven days under 25ºC and 16 h photoperiod. In April 2011 plants were transplanted to plastic pots 12 cm high, 14 cm top diameter, 8 cm bottom diameter and 4 drainage holes. Ten plants were transplanted to pots fitted with a mixture (1:1, v:v) of coarse sand and vermiculite and 20 plants transplanted to pots fitted with a commercial growth substrate (Siro® Plant). Pots were placed outdoors in the surroundings of Évora, southern Portugal, watered as needed with well water and with the commercial liquid fertilizer Nutriquisa 5-8-10®every three weeks. After seven months, in November 2011, mortality was recorded, leaves were counted, plants were carefully uprooted, roots counted, length and basal diameter of the largest root measured to the nearest mm and 0.5 mm respectively.

Data analyses: Volume of seeds was determined as VOL=πLWT/6 (Casco and Dias, 2008), where L is length, W is width and T is thickness. Shape of seeds was estimated as the population variance of L, W and T, all of them scaled so that L=1 (Bakker et al., 1996), seed density as M/VOL, where M is seed mass.

In the pH experiment, the germinative process over time was described by the three parameter Weibull equations (Weibull, 1951):

$$G = 1 - \exp - \{ [(T - l) / k]^{c} \}, \quad (1)$$

where G is the cumulative proportion of germinated seeds at time T, l is a location parameter estimating the latest time at which germination is strictly zero representing the lag of germination, k is a scale parameter with $l+k$ estimating the time at which the cumulative germination is approximately 63% and c is a shape parameter that evaluates the symmetry of the germination distribution with $3.25 \leq c \leq 3.61$ showing symmetry and representing a good approximation to a normal distribution, $c<3.25$ positive and $c>3.61$ negative asymmetry (Dias, 2001). Weibull equations were fitted by least squares nonlinear regression without replication using the Marquardt method (Marquardt, 1963). Fitted equations were only accepted after a consistency check of parameter estimates and germination predictions against original data. Duration of germination (D_{100}) was determined from fitted Weibull equations for each pH treatment. Data of final germination, l, D_{100} and c were compared by ANOVA after checking for homocedasticity using the two-tailed F distribution. Whenever the assumption of homocedasticity was rejected, ANOVAs were performed on data transformed by the Box-Cox family of transformations (Box and Cox, 1964).

In the mineral nutrition experiment, relative growth rates were calculated as the difference between final and initial values weighted by the initial value and number of days. Relative shoot growth was calculated as the ratio between shoot and total biomass. Treatments were compared with the control by exact or approximate one tailed Student's t tests (two tailed in relative shoot growth) after checking for homocedasticity using the two-tailed F distribution.

In the growth substrate experiment roots were assumed to be approximately cones and root volume was calculated as $\pi r^2 h/3$ where r is the basal radius and h is the length of the largest root. Number of leaves, number of roots, length, basal diameter and volume of the largest root of plants growing in sand plus vermiculite or in commercial substrate were compared by exact or approximate two-tailed Student's t tests after checking for homocedasticity using the two-tailed F distribution.

Statistical procedures were performed using Statgraphics Plus ver. 3.3 (Manugistics, Rockville, USA), except homocedasticity tests and Box-Cox transformations performed using Microsoft Excel® 2010 and BIOM (Applied Biostatistics, New York, USA) respectively. Significance levels of $P=0.05$ were used throughout and data is presented as mean ± standard error of the mean.

Results and Discussion

Plant material: Volume of scorzonera seeds ranged between 5.476 mm^3 (13.39×1.10×0.71 mm) and 32.299 mm^3

(14.69×2.32×1.81 mm) with a mean value (±SE) of 14.630±0.853 mm^3. The variance of linear dimensions of seeds ranged between 0.162 and 0.195 (0.180±0.001). Seed mass ranged between 6 mg and 23 mg (13.4±0.6 mg). Seeds density ranged between 0.588 and 1.461 (0.970±0.027) and 58 % of seeds had density below one. Using the equation of Bond et al. (1999), the maximum depth for successful emergence of plants in soil ranges between 49.6 mm and 77.8 mm (64.3±0.9 mm).

Thus, scorzonera seeds are large, non-spherical, highly elongated, and generally buoyant in water. Given their size, in all likelihood scorzonera seeds are able to germinate and produce viable plantlets even when deeply buried in soil.

pH experiment: Results of pH experiment are presented in Table 2. Final germination of scorzonera ranged between 90.2±3.0% and 96.6±2.1% but no significant differences among treatments were found and final germination pooled across all pH treatments was as high as 93.0±1.2%.

Weibull equations could always be fitted except in two samples, one in pH 6 treatment the other in pH 9 treatment. Mean R^2 of fitted equations was 0.725±0.040. Lag of germination l ranged between 0.99±0.29 day and 1.49±0.01 day, duration of germination D_{100} ranged between 3.66±0.50 day and 4.94±0.50 day, shape of germination c ranged between 1.69±0.11 and 2.97±0.90. However, no significant differences among treatments were found in any of these germination parameters and data of all pH treatments could be combined with a pooled mean for l of 1.38±0.06 day, a pooled mean for D_{100} of 4.26±0.22 day and a pooled mean for c of 2.25±0.25.

Despite that many species have high percentages of germination over a wide range of pH values, pH is known since long to affect seed germination of a high number of species in a variety of ways (Baskin and Baskin, 1998). However, given the wide range of pH values tested our results clearly show that scorzonera germination is remarkably insensitive to pH variation over the wide range 5 to 10. Insensitivity of scorzonera seeds to pH includes not only final germination but also the time needed for germination to start and the time needed for its completion afterwards, l and D_{100} respectively. This insensitivity to pH is more noticeable because the experiment was done on filter paper, a seedbed known to increase the sensitivity of seed germination to pH in various species (Shoemaker and Carlson, 1990).

Therefore, scorzonera germination starts very quickly and the final germination is not only relatively high but is attained in a short period almost regardless of pH value, and germination rates higher than 90% can be expected by day six after sowing. Shape of germination c was always lower than 3.25 meaning that the distribution of germination over time is positively asymmetric which in all likelihood means that factors that control the germination of scorzonera seeds act multiplicatively (Limpert et al., 2001). Because no significant differences were found among c values it also means that, whichever they are, those factors are insensitive to wide pH changes.

Altogether, these results suggest that pH is not a worrisome factor when designing a program aimed to cultivation of scorzonera, at least in what concerns seed germination.

Mineral nutrition experiment: Despite the complete absence of one element in each treatment, only plantlets of the treatment without iron showed clear symptoms of deficiency. Scorzonera plantlets growing in the nutrient solution without iron developed a uniform chlorosis, one of the most common symptoms of iron deficiency, which was almost evident by the 7th day, especially in younger leaves. By the 15th day a gradient of chlorosis was clearly in place, with younger leaves extremely pale, older leaves darker and the colour of the leaf like cotyledons unchanged, a typical response to deficiency of a low mobility element like iron, known to be required for the

Table 2
Effects of pH on germination of scorzonera

pH	Final germination, %	Lag of germination, day	D_{100}, day	c
5	92.7±3.6	1.46±0.01	4.49±0.65	1.87±0.22
6	90.6±2.6	1.47±0.01	4.94±0.50	1.69±0.11
7	91.7±4.5	0.99±0.29	4.18±0.47	2.97±0.90
8	95.8±1.7	1.49±0.01	3.87±0.16	2.00±0.08
9	90.2±3.0	1.47±0.02	4.70±0.97	1.90±0.36
10	96.6±2.1	1.47±0.01	3.66±0.50	2.85±0.96
$F_{(5,16)}$	0.779	1.506	0.748	1.411
P	0.578	0.243	0.600	0.273

Lag of germination is the time necessary to begin germination, D_{100} is the duration of germination and c measures the symmetry of germination distribution over time. Degrees of freedom in ANOVA of final germination are 5,18. Box-Cox λ in lag of germination was 11.596, in c was −2.278.

synthesis of some chlorophyll-protein complexes in chloroplasts (Taiz and Zeiger, 2006).

In all other treatments scorzonera plantlets had a normal appearance compared to control. Thus scorzonera appears to be able of precocious and fast accumulation of mineral reserves, allowing plantlets to delay or overcome deficiencies except when iron is involved.

Relative growth rate of leaves (Figure 1a) was significantly reduced in plantlets growing in nutrient solutions without nitrogen (P=0.043), without magnesium (P=0.041) and without iron (P=0.007) while relative growth rate of roots (Figure 1b) was significantly reduced in plantlets growing in nutrient solutions without potassium (P=0.010) and without iron (P=0.001). However, reductions of relative growth rates did

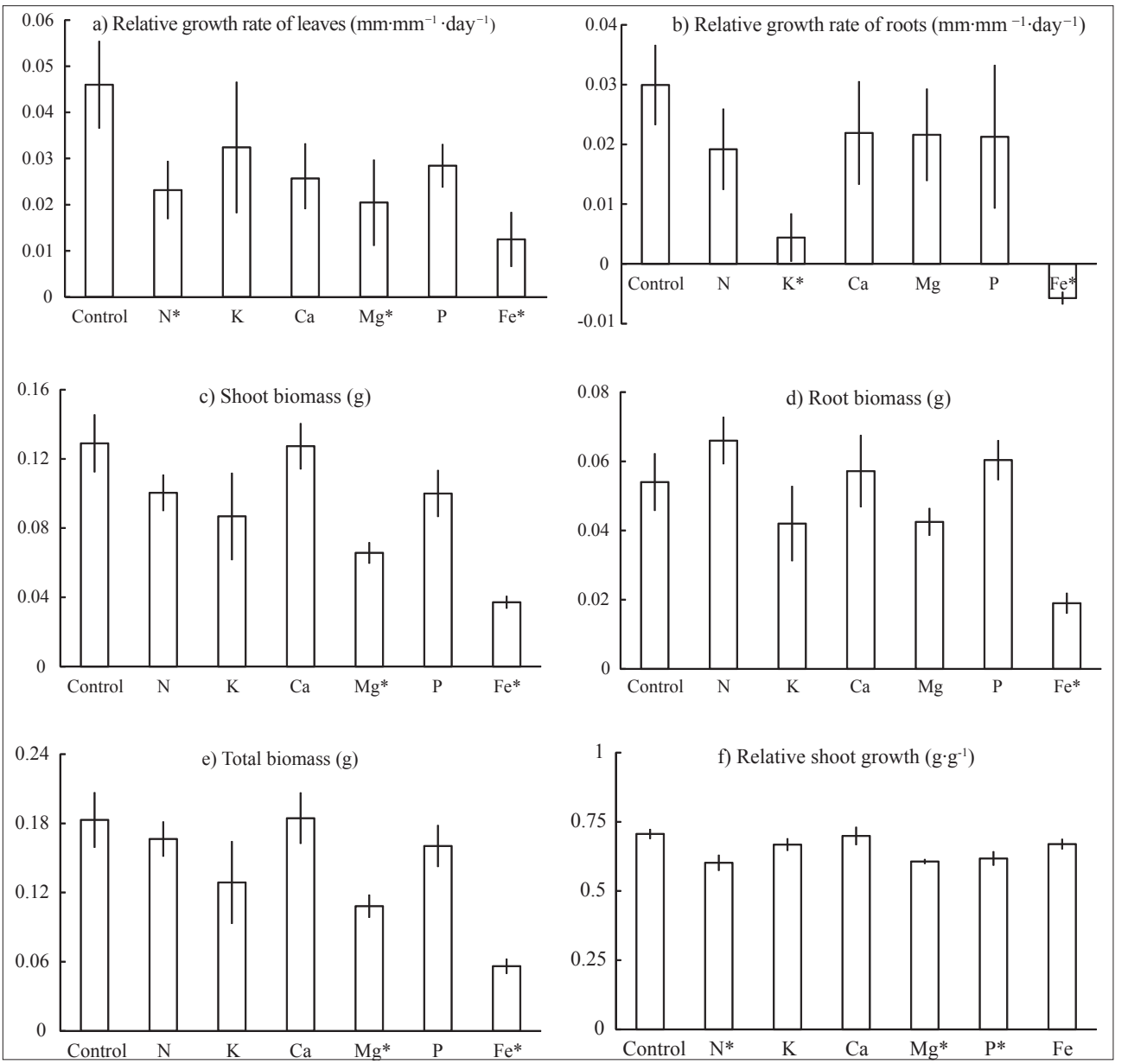

Fig. 1. Effects of mineral deficiencies on growth of scorzonera plantlets (means±standard errors)

Control refers to complete nutrient solution, letters to the element absent in each nutrient solution treatment. Relative shoot growth determined as the ratio shoot biomass/total biomass. Treatments with * have means significantly lower (significantly different in relative shoot growth) than the corresponding control (exact or approximate Student's t test, $P \leq 0.05$).

not translate to a diminished biomass production except in plantlets growing in nutrient solutions without magnesium or iron (Figure 1c, 1d).

In the absence of magnesium or in the absence of iron, shoot biomass (Figure 1c) was significantly reduced (P=0.005 and P=0.001 respectively) mirroring the significant reductions of relative growth rate of leaves. Total biomass (Figure 1e) was also significantly reduced only in plantlets growing in nutrient solutions without magnesium or without iron (P=0.008 and P=0.001 respectively). Therefore, reduction of total biomass of plantlets growing without magnesium results from shoot biomass reductions alone, because no significant differences were found in roots biomass. Conversely, plantlets growing without iron also had root biomass (Figure 1d) significantly reduced (P=0.003). Again, root biomass reductions mirror the effects either observed in relative growth rates of roots growing without magnesium or without iron, elements that in all likelihood did not accumulate in roots, especially iron, in amounts high enough to sustain healthy growth.

As could be expected from the above, relative shoot growth of plantlets (Figure 1f) was significantly affected in the absence of magnesium (P=0.0003) but not in the absence of iron (P=0.170). In addition relative shoot growth of plantlets was significantly affected also in the absence of nitrogen (P=0.009) or phosphorus (P=0.015). When the relative shoot growth was significantly affected, the relative investment in shoot biomass was always lowered. These results imply that the mineral environment of scorzonera in soil might affect biomass partition below and above ground, especially in response to nitrogen, magnesium and phosphorus. Further studies are needed to confirm it, but it appears that relatively low levels of these three elements might result in an increase of root growth at the cost of shoot growth, which could be advantageous in a species that is mainly cultivated for its roots.

Growth substrate experiment: The same mortality (30%) was observed in scorzonera plants growing in sand plus vermiculite or in commercial substrate. Also no significant differences (P=0.118) were found in the number of tuberous roots between plants growing in sand plus vermiculite and in commercial substrate (pooled number of roots 3.2±0.4). Conversely significant differences between the two groups of plants were found in number of leaves (P=2.4×10^{-5}), length (P=0.004), basal diameter (P=1.5×10^{-5}) and volume (P=5.4×10^{-7}) of the largest root, with plants growing in sand plus vermiculite exceeding those growing in commercial substrate between 1.5-fold in the length of the largest root and 4.4-fold in the volume of the largest root (Figure 2).

Because growth substrates have a light (commercial substrate) or very light (sand plus vermiculite) texture our results suggest that very light textures clearly favours early growth of scorzonera plants. In addition and as reported above, plantlets are able to rapidly accumulate essential nutrients in roots thus providing reserves to sustain growth.

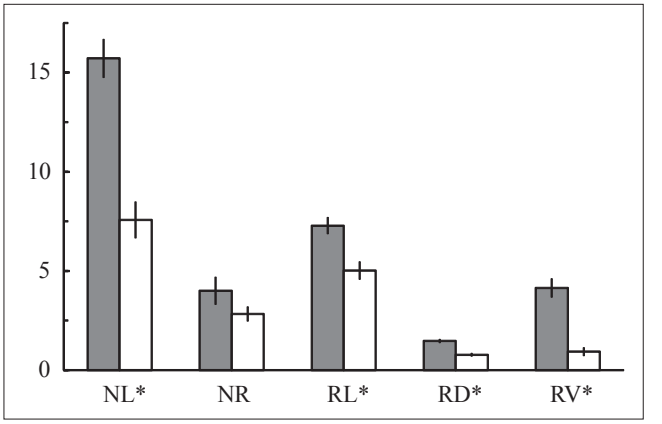

Fig. 2. Growth of scorzoneraplants (means±standard errors) in sand plus vermiculite (grey) and in commercial substrate (white)

Ordinate axis with numerical values only, units and scale depending on the variable. NL – number of leaves (×1); NR – number of roots (×1); RL – length of largest root (×10^{-1} mm); RD – basal diameter of the largest root (×10^{-1} mm); RV – volume of the largest root (×10^{-3} mm^3). Asterisks indicate significant differences between treatments (exact or approximate Student's t test, P≤0.05)

Conclusions

The results of this study show that (1) total germination, time needed to the beginning of germination, duration of germination, and asymmetry of germination distribution over time is insensitive to pH change in the range pH 5 to pH 10, (2) even very young plantlets grown in a mixture of coarse sand and commercial growth substrate for about 40 days are able to accumulate reserves in their roots at levels high enough to support growth for a considerable period in the absence of several mineral nutrients, (3) very light textures of growth substrate result in significant increases of leaf and root growth of young plants.

Therefore, scorzonera is a species able to withstand and grow in relatively unfavourable environments, suggesting that it may be a cash crop in marginal and less productive soils which might set up the basis to the cultivation of scorzonera and consequently the revival of its consumption as a delicacy. Assuming that the effects of pH, mineral deficiencies and texture on seed germination and plantlet growth of scorzonera growing in the wild are similar to those reported here for cultivated scorzonera, results of this work may

also provide guidelines for a parallel and much needed effort aimed at the conservation and recovery of natural populations of scorzonera.

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