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# Increasing Drought Tolerance of Forage Perennial Ryegrass by Improvement of Root Characteristics

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## ABSTRACT

**Perennial ryegrass is an essential cool-season forage grass with high-quality biomass, but with pronounced sensitivity to drought. This research was aimed to determine the shoot dry matter and root characteristics of perennial ryegrass populations (cultivar K-11 and breeding population LPb3). 20 randomly chosen individual plants per population were clonally divided into twelve small parts, remets, and transferred into the plastic root-screening tubes for root length and depth density evaluation. Also, shoot dry matter (SDM) was analysed. The experiment was designed as a two-factorial design, with population and watering regime as factors (2x4), in three replications. Analysed populations showed different root and shoot characteristics, especially in treatments with water reduction. Significant differences for most of the analysed traits between treatments were determined. Shoot dry matter was higher by 9% on average in population LPb3 than in K-11. Also, the dry matter yield of the total root dry matter (TRDM) was larger in LPb3. Treatments significantly affected total root dry matter, which dropped with water reduction. Dry matter of roots below 90 cm (RB90) was statistically significantly higher in LPb3 than in K-11. Broad-sense heritability and estimated genetic gain for most analysed traits were higher in population LPb3 than in K-11. The breeding of all investigated traits in this perennial ryegrass material is promising, especially in population LPb3.**

**Keywords:** breeding, genotypes, root/shoot ratio, root dry matter, shoot dry matter.

## INTRODUCTION

Global weather changes have led to an increase in droughts across the European continent, negatively affecting agricultural production (Spinoni et al., 2018). According to FAO, about 70% of the accessible freshwater has been used for irrigation (FAO and ITPS, 2015) and the increase of irrigation in these circumstances is quite questionable. The primary and most efficient way to bypass the problem is growing of drought-tolerant cultivars.

Perennial ryegrass is one of the most important forage grasses in northern climates, with many superior characteristics in fodder production (Jaškune and Statkevičiute, 2021), but it is drought-susceptible and less suitable for grassland mixtures in hot and dry summer climates (Huber et al., 2021). Therefore, selecting genotypes that show better performance and production in dry conditions is essential. Traditionally, forage grass plant breeding is focused on improving the economically most important traits (yield, dry matter quality, disease tolerance), which determine the overall sustainability of forage production. However, soil water deficit tolerance has become a crucial breeding criterion in recent years, particularly in cool-season forage perennial grasses, which are rain-fed crops (Bothe et al., 2018). Earlier attempts to develop more drought-tolerant cultivars of some grass species have concentrated on observation of longer field persistency (Sokolović et al., 2010) in dry conditions or determining the survivors after drought. Though these breeding procedures are initially quite successful, the later steps should involve criteria and methods to accelerate further selection and make it more efficient. Consequently, a lot of different breeding techniques and their combinations were developed to generate drought-tolerant genotypes (Wittmann et al., 2021) or

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genotypes with root improved characteristics (Thorup-Kristensen et al., 2020).

Generally, water balance maintenance depends on plant aboveground structure and covering tissue, but also on the development of the deep root system or root biomass distribution (Paustian et al., 2016). Despite plant potential for deeper rooting, the effective rooting depth, where major absorption of water and nutrients happens, of the most common Poaceae species is usually between 0.5 and 1.0 m (Fan et al., 2016). Rooting depth distribution is a species-specific characteristic. Perennial ryegrass develops most of the root system in the top 0.8 m and only some roots below (Thorup-Kristensen and Rasmussen, 2015). Deeper rooting, among other traits, is beneficial for plant production and survival, especially under water-limited conditions (Hoekstra et al., 2015). Also, drought tolerance of different forage species is closely related to the spatial distribution and penetration of root systems in the deeper portions of the soil (Lelievre et al., 2011). Plants must keep some root contact with ground water to survive (Thorup-Kristensen et al., 2020). Even a few deep roots may be needed to survive and maintain cell turgor (White and Kirkegaard, 2010). Variability of vertical root mass distribution and root/shoot ratios, as well as deeper roots, are useful in perennial ryegrass drought tolerance breeding (Sokolovic et al., 2012). The hypothesis is that genotypes that invest more dry matter into roots are more tolerant to drought. Generally, long term breeding goal would be to develop perennial ryegrass plants with improved architecture of roots, which can better exploit the available, deeper, soil water, while maintaining above ground biomass yield. The main question that needs to be answered is does perennial ryegrass persistence and drought tolerance come from deeper roots. Also, we need to know how these root characteristics will act in less available soil water, or more precisely, does root architecture change in drought conditions, since knowledge about patterns of root growth and their reaction to water stress is not enough (Bachmann-Pfabe et al., 2021).

Therefore, the objectives of this study were to determine root distribution and depth, production and root/shoot ratio and investigate possible changes of these parameters in water deficit conditions.

## MATERIAL AND METHODS

Two diploid perennial ryegrass populations (cultivar K-11 as a basic population and breeding population LPb3) were the objects in this research. The breeding population LPb3 was selected based on better root architecture in two breeding cycles from a basic population already characterized by improved persistency. Seed of both populations was sown in a glasshouse in April. Four weeks after seedlings emerged, 20 randomly chosen individual plants per population were transferred into the pots and grown there for three months. On the 15th of August, chosen individual plants were clonally divided into twelve small parts-remets, each with three tillers. All remets were leaf and root trimmed at a length of 2 cm and transferred in the plastic root-screening tubes.

The experiment for root length and depth density evaluation was set up as was developed in Bonos et al. (2004) and Crush et al. (2005). The trial was conducted in 1 m long polyvinyl chloride (PVC) tubes, 75 mm in diameter (2 mm wall thickness), in the rain-out shelter. All tubes were cut in half lengthwise and rejoined with adhesive tape. Tubes were put on 10 mm polythene foam on the floor, at an approximate angle of 25° from vertical, filled 5 cm from the bottom with gravel for drainage, and washed mortar sand progressively to the top using a spray of water for uniform packing. Mortar sand particles were from 0.5 to 2 mm in size. Remets were planted in the centre of each tube, and sand was covered with plastic foil to reduce passive sand evaporation. The experiment was designed as a two-factorial design, with population and watering regime as factors (2x4), in three replications. The experiment was carried out with 480 remets, i.e., new plants planted in the tube (20 started plants per population in 4 watering regimes in 3 replications).

During one month after transplanting the seedlings into the tubes, each plant received 100 ml of a low-strength complete nutrient solution per day for uniform root development (Blamey et al., 1991) in two separate doses, morning and evening. After one month of growth (September 15), soil water content reduction was gradually started. During the next 15 days (until October 01), irrigation with 100 ml of solution was maintained in the control treatment, while in the other treatments, irrigation was reduced to 75% (75 ml per day) of the amount of nutrient solution. This was followed by a new reduction over the next 15 days (until October 15) as follows: in the control, irrigation continued with 100 ml of nutrient solution per day, and in the treatment 75% with 75 ml per day, and in the other two treatments, irrigation was reduced to 50% (50 ml) of nutrient solution per day. Finally, on October 15, irrigation was reduced to the final scheme of four treatments: full-control treatment with 100 ml, 75% with 75 ml, 50% with 50 ml, and 25% with 25 ml of nutrient solution per day.

The shoots were trimmed twice. Firstly, without measures when final water reduction started in all treatments (October 15) and secondly, on November 30, with weighting of air-dried dry matter (DM), just before root analyses. The roots were extracted by laying the tubes down on a 5x5 mm plastic mesh. Sand-root columns were split into halves, rolled out, washed under a water shower and cut into 10 cm pieces. Plants with 1 cm of roots were weighed and planted in stock. Shoots and root parts were paper-dried and weighed, then air-dried at 65°C and weighed.

Analyses and results were presented in seven traits: shoots dry matter (SDM, g), total roots dry matter (TRDM, g), dry matter of roots in top 20 cm (RTOP20, g), dry matter of roots below 70 cm (RB70, g), dry matter of roots below 90 cm (RB90, g), total root/shoot ratio (TRSr) and deep root/shoot ratio (RB90Sr). The root/shoot ratio has been calculated as a ratio of total root dry matter and shoot dry matter. The deep root/shoot ratio has been defined as the dry matter of roots deeper than 90 cm and the shoot dry matter.

Differences in dry matter production and root characteristics between populations in all watering regimes were analysed. The effects of the population and watering regime on shoot dry matter, root depth and dry matter were tested using a mixed nested ANOVA. The fixed factors were population and watering regime, whereas the genotype (single plants) was a random factor, nested within the population. Tukey's HSD test, at a probability level 0.05, was performed as a post hoc test.

The genotypic variance was calculated as $\sigma^2_g = (MSq_g - MSq_{vg}) / vr$ where $MSq_g$ and $MSq_{vg}$ are mean squares of genotypes and interaction, v is the number of watering regimes, and g is the number of populations. Broad sense heritability ($h_b^2$) was calculated from the phenotypic variance ($\sigma^2_p$) and the genotypic variance ($\sigma^2_g$) as:

$$h^2_b = \sigma^2_g / \sigma^2_p$$

where $\sigma^2_p = \sigma^2_g + \sigma^2_{vg}/g + \sigma^2_{er} / rg$, and where $\sigma^2_{vg}$ is the variance of interaction, $\sigma^2_{er}$ the residual variance, g the number of populations and r the number of replications.

The estimated genetic gain per cycle of selection was calculated as:

$$\Delta G_e = kh_b^2\sigma_p$$

where k is the standardised selection differential (Halauer and Miranda, 1981), dependent on selection intensity (S%), and $\sigma_p$ is the standardised phenotypic deviation. In this research, S was 25% and k was 1.26. Correlation analysis, regression analysis and principal component analysis (PCA) were performed by software Statistica 8.0, StatSoft Inc.

## RESULTS AND DISCUSSION

Perennial ryegrass populations showed different root and shoot characteristics, especially in treatments with water reduction. The first analysis comprised root penetration depth and showed that the percentage of plants reaching deeper substrate layers was significantly increased with watering reduction (Table 1).

*Table 1*. The proportion of plants whose roots reached deeper substrate layers (%)

| | K-11 | | | | LPb3 | | | |
|---|---|---|---|---|---|---|---|---|
| Watering<br>Substrate layers | Full | 75% | 50% | 25% | Full | 75% | 50% | 25% |
| Below 70 cm in depth | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Below 90 cm in depth | 22.5 | 40.0 | 47.5 | 55.0 | 37.5 | 45.0 | 50.0 | 60.0 |

All roots reached substrate depth at 70 cm, but the number of plants with roots in layers below 90 cm was decreased. Furthermore, the proportion of plants that reached the deepest substrate layer was considerably higher in the breeding population LPb3 (Table 1) in all watering regimes. Analysis of variance revealed significant differences for most of the analysed traits (Table 2).

*Table 2*. Average values of investigated traits and significance of differences between treatments

| | SDM (g) | TRDM (g) | RTOP20 (g) | RB70 (g) | RB90 (g) | TRSr | RB90Sr |
|---|---|---|---|---|---|---|---|
| Population[#] | ns | ns | ns | ns | ** | ns | ns |
| K-11 | 1.19 | 2.09 | 0.99 | 0.182 | 0.006 b | 1.88 | 0.073 |
| LPb3 | 1.30 | 2.13 | 1.09 | 0.207 | 0.036 a | 1.66 | 0.089 |
| Irrigation[#] | ** | * | ns | * | * | ** | ** |
| Full | 1.35 a[$] | 2.14 ab | 1.07 | 0.176 b | 0.017 b | 1.63 b | 0.068 b |
| 75% | 1.36 a | 2.21 a | 1.09 | 0.221 a | 0.024 a | 1.65 b | 0.082 b |
| 50% | 1.33 a | 2.05 b | 1.03 | 0.197 ab | 0.027 a | 1.59 b | 0.078 b |
| 25% | 0.99 b | 2.04 b | 0.99 | 0.195 ab | 0.029 a | 2.10 a | 0.103 a |
| Interaction[#] | ** | ** | ns | * | * | ** | ns |
| K-11-Full | 1.36 ab | 2.05 ab | 1.03 | 0.173 bc | 0.0003 d | 1.56 cd | 0.066 |
| K-11-75% | 1.33 b | 2.24 a | 1.00 | 0.214 ab | 0.011 bc | 1.75 bc | 0.076 |
| K-11-50% | 1.13 c | 1.87 b | 0.94 | 0.145 c | 0.009 bc | 1.73 bc | 0.058 |
| K-11.25% | 0.95 d | 2.21 a | 0.97 | 0.195 ab | 0.005 c | 2.46 a | 0.093 |
| LPb3-Full | 1.34 b | 2.19 a | 1.07 | 0.178 bc | 0.027 b | 1.66 cd | 0.069 |
| LPb3-75% | 1.38 ab | 2.20 a | 1.14 | 0.225 a | 0.033 ab | 1.58 cd | 0.086 |
| LPb3-50% | 1.45 a | 2.17 a | 1.09 | 0.229 a | 0.038 ab | 1.51 d | 0.091 |
| LPb3-25% | 1.02 cd | 1.94 b | 1.02 | 0.195 ab | 0.044 a | 1.88 b | 0.108 |
| Genotypes[&] | ** | ** | ** | ** | ** | ** | ** |

[#] F test significance for the factors and the traits: ns - no significant differences, *significantly different (P<0.05), **significantly different (P<0.01); [&] F test significance for within-population variability between genotypes (plants); [$] the same letter - not significantly different (P<0.05) in LSD test.

Shoot dry matter (SDM) was higher by 9% on average in population LPb3, although statistically insignificant (Table 2). The highest SDM was recorded in the first level of reduction and full watering, and a statistically significant decrease was determined in the final irrigation reduction. Drought tolerance in LPb3 was higher since SDM was increased with water reduction till the reduction of 50%. Even though above-ground biomass production was expectedly reduced in the most severe water reduction, the SDM of LPb3, generally in all water regime treatments, was significantly better than in K-11. The variability of SDM between plants within populations was highly significant, which is important for future breeding. Eight of ten genotypes with the highest SDM were from population LPb3 with a maximum yield of over 2 g per plant. In the most reduced irrigation treatment, genotype L15 reached 1.85 g per plant (80% more than average), representing promising breeding material.

The dry matter yield of the total root system (TRDM) hasn't shown a statistically significant difference between populations (Table 2), but TRDM in LPb3 was a few percent larger than in K-11. On the contrary, different watering treatments significantly affected total root dry matter, which dropped with water reduction. Also, the interaction of the two trial factors significantly impacted differences in total root dry matter. Similar relations were detected between populations for dry matter of root in the top 20 cm of the substrate (RTOP20). Irrigation treatments and factor interactions didn't have a statistically significant effect on that perennial ryegrass trait, as well. Nevertheless, differences among genotypes (single plants) within the population for that trait, as well as for all other traits, were large and statistically significant (Table 2).

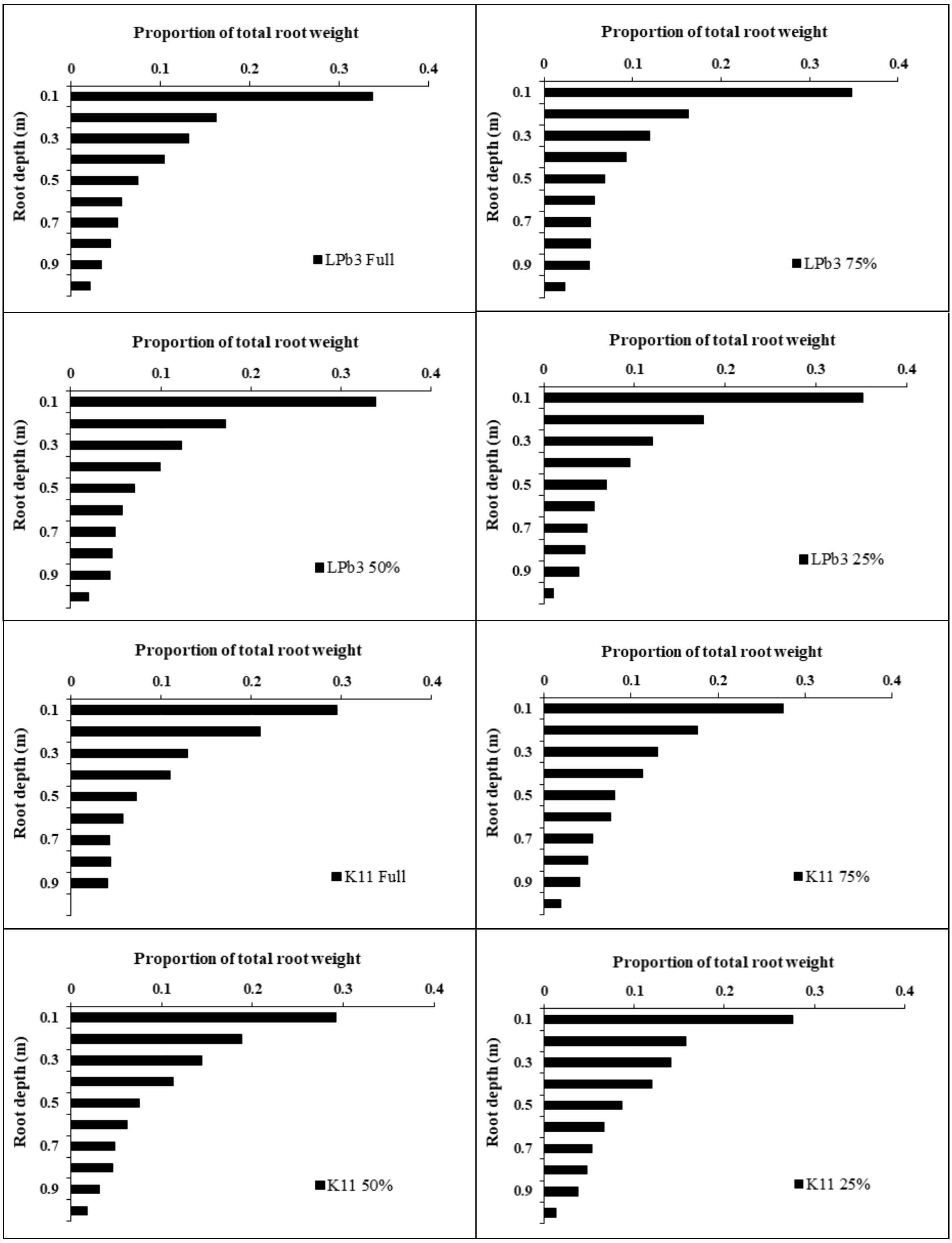

*Figure 1.* The proportion of dry matter root depth fraction within total root dry matter

The proportion of dry matter of the root depth fraction within total root dry matter is presented in Figure 1. It is noticeable that both populations showed similar proportions of root depth fractions. Even though differences of RTOP20 between populations weren't statistically significant, LPb3 showed a generally higher proportion of roots up to 20 cm in depth than K-11. If we consider deeper root fractions, the situation is quite different. DM of roots below 70 cm hasn't shown statistically significant differences between population K-11 and LPb3, but differences between different water regimes and the interaction of the two factors were statistically significant ($P<0.05$). The deepest root fraction (RB90) showed opposite values. Differences in dry matter of RB90 were statistically significant, more than five times higher in LPb3 than in K-11. Generally, the highest value of this trait was observed for the lowest irrigation level and was pronounced in population LPb3. Thirteen genotypes with the highest RB90 were from this population and their values exceeded genotypes from K-11 several times (Figure 2).

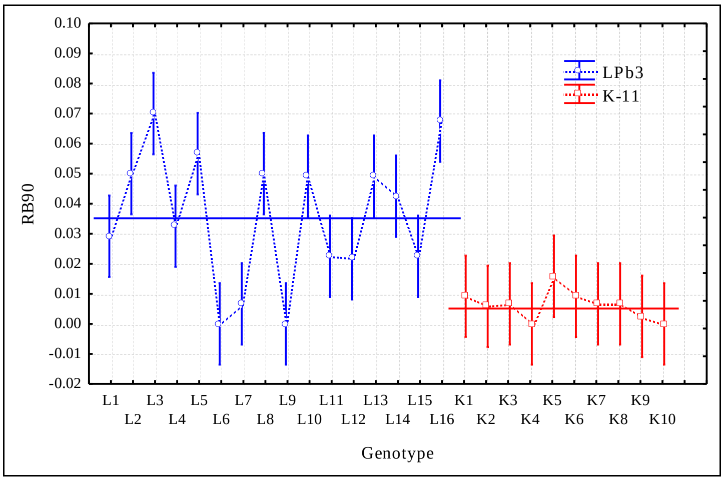

*Figure 2.* Dry matter of roots below 90cm (RB90, g) of individual genotypes (single plants) from both populations; L1-L16 - genotypes from population LPb3; K1-K10 - genotypes from population K-11

Differences between populations for root-to-shoot DM ratios were not statistically significant. It is noticeable that the relations of averages for TRSr and RB90Sr were opposite (Table 2) since differences between populations were higher several times for RB90 than for TRDM. These ratios showed a slight increase with irrigation reduction, probably due to a significantly higher drop of DMY than total root biomass or increasing deep root biomass in higher water reduction treatments.

*Table 3.* Genetic variance, broad sense heritability and estimated genetic gain per cycle of breeding

| Population | LPb3 | | | | | | |
|---|---|---|---|---|---|---|---|
| Traits | SDM (g) | TRDM (g) | RTOP20 (g) | RB70 (g) | RB90 (g) | TRSr | RB90Sr |
| Average | 1.298 | 2.125 | 1.085 | 0.207 | 0.0356 | 1.656 | 0.089 |
| $\sigma_g^{2*}$ | 0.083 | 0.558 | 0.120 | 0.009 | 0.0003 | 0.1297 | 0.0016 |
| $h^{2\#}$ | 80.83 | 94.05 | 91.99 | 87.71 | 54.43 | 85.38 | 79.75 |
| $\Delta G_e^{\&}$ | 0.327 (25.2%) | 0.913 (43%) | 0.419 (38.6%) | 0.111 (53.6%) | 0.0153 (43%) | 0.419 (25.3%) | 0.045 (50.6%) |
| Population | K-11 | | | | | | |
| Traits | SDM (g) | TRDM (g) | RTOP20 (g) | RB70 (g) | RB90 (g) | TRSr | RB90Sr |
| Average | 1.190 | 2.089 | 0.987 | 0.182 | 0.0063 | 1.881 | 0.073 |
| $\sigma_g^{2*}$ | 0.036 | 0.148 | 0.0422 | 0.001 | 0.00001 | 0.1708 | 0.0001 |
| $h^{2\#}$ | 85.29 | 82.53 | 85.58 | 45.24 | 49.75 | 87.96 | 7.74 |
| $\Delta G_e^{\&}$ | 0.221 (18.6%) | 0.440 (21.1%) | 0.239 (24.2%) | 0.028 (15.4%) | 0.0028 (44.4%) | 0.488 (25.9%) | 0.0023 (3.2%) |

[*] Genetic variance; [#] Broad sense heritability (%); [&] Estimated genetic gain per one cycle of selection, in brackets percent of average values.

Broad-sense heritability and genetic gain per selection cycle were calculated based on genetic variance within each population. Generally, heritability for all investigated traits was high, especially in population LPb3. Therefore, the estimated genetic gain for all traits was also higher in that population (Table 3). The lowest heritability was determined for the deep roots fraction in both populations. Nevertheless, the estimated genetic gain per breeding cycle for RB90 in both populations and TRDM and RB70 in population LPb3 was quite high (43-54.4%). Since perennial ryegrass is an outbred anemophily plant species, investigated populations showed high genetic variability of all analyzed traits important for drought-tolerant cultivars breeding.

Correlations of investigated traits showed different values in the studied populations (Table 4). While almost all coefficients of correlations in population LPb3 were statistically significant, in K-11 only correlations among TRDM and RTOP20 and other traits excluding RB90, and between SDM and TRSr in opposite direction, as well as CV between RB70 and RB90 ($p<0.05$) and RB90Sr ($p<0.01$) showed such significance.

*Table 4.* Correlation coefficients of investigated traits in populations LPb3 (below diagonal line) and K-11 (above diagonal line) (* significant at p<0.05; ** significant at p<0.01)

| Traits | SDM | TRDM | RTOP20 | RB70 | RB90 | TRSr | RB90Sr | K-11 |
|---|---|---|---|---|---|---|---|---|
| SDM | - | 0.18 | 0.11 | 0.28 | 0.16 | -0.67** | -0.092 | |
| TRDM | 0.70** | - | 0.91** | 0.74** | 0.16 | 0.54** | 0.54** | |
| RTOP20 | 0.73** | 0.96** | - | 0.50** | 0.01 | 0.52** | 0.34* | |
| RB70 | 0.48** | 0.81** | 0.71** | - | 0.30* | 0.28 | 0.82** | |
| RB90 | 0.18 | 0.45** | 0.38* | 0.70** | - | -0.04 | 0.22 | |
| TRSr | -0.02 | 0.67** | 0.59** | 0.66** | 0.48** | - | 0.48** | |
| RB90Sr | 0.08 | 0.52** | 0.42* | 0.84** | 0.77** | 0.73** | - | |
| LPb3 | | | | | | | | |

In LPb3 all root traits showed high correlations with above-ground biomass production, but the coefficient of correlation between SDM and RB90, which represent two traits of our main interest, showed quite low values. This is obviously because some plants haven't reached substrate depth below 90 cm and the value of RB90 for them was zero. If we exclude zeros (the plants that haven't reached the deepest layers), in population LPb3 the correlation was higher and the slope of the regression line was steeper (Figure 3) and statistically significant on level 99%. It indeed implies that SDM rises with increasing amounts of roots below 90 cm and it was determined in all plants that have these deep roots.

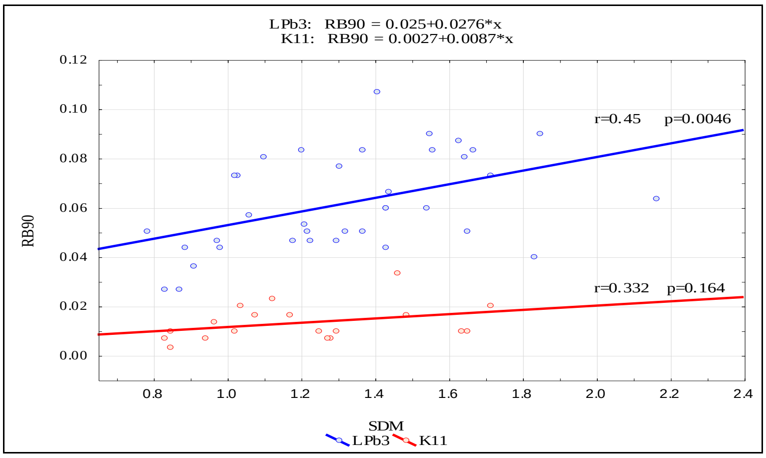

*Figure 3.* Regression graph (with regression equations) for RB90 and SDM in both populations (plants that have zero values for RB90 were excluded); r-coefficient of correlations; p-probability values (significant at the level 99% when p is less than 0.01).

Principal component analysis (PCA) was performed for all traits to determine their contribution to overall variability (Figure 4). The first two principal components describe 85% of available variability, and the biplot between them showed that most of the genotypes from LPb3 were located in the lower-left quarter of the graph. In that direction, most of the trait vectors were pointed, which means that genotypes from LPb3 (especially L05 and L08) are characterised by high shoot dry matter and dry matter of root below 90 cm at the same time. Most of the genotypes from population LPb3 (especially L03, L16, L04, L15, L13) are located in the direction of all root traits, which implies their better root characteristics, development of the root system, and ratio between shoots dry matter and roots below 90 cm. Genotypes K02 and K03 showed higher total root mass and shoot dry matter ratio.

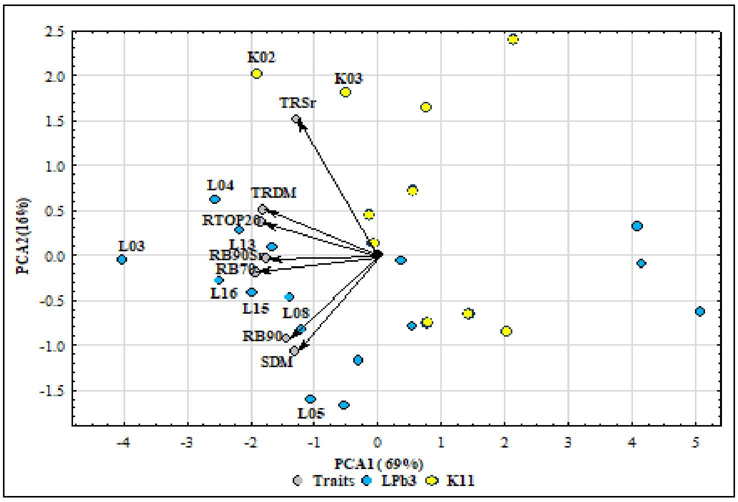

*Figure 4.* Biplot of principal component analysis with presented trait vectors

Phenotyping and breeding of root systems are quite difficult per se (Maeght et al., 2013), and previous research has shown that studying the root systems of perennial grasses in field conditions is laborious and time-consuming (Graham et al., 2024). Root characteristics in perennial grasses have been investigated and represent key breeding criteria (Bachmann-Pfabe et al., 2021) and there are indications that drought tolerance and nutrient uptake are significantly influenced by root architecture (Griffiths and Paul, 2017). We have previously attempted to determine whether the persistence of perennial ryegrass genotypes is partly due to deeper root development in a climate with droughty periods during the production season. In previous research with improved breeding populations of *Lolium perenne*, it is evident that populations bred for deeper roots exhibited better production and persistence (Sokolovic et al., 2012). In this experiment, the aim was to investigate how populations bred for improved drought tolerance and enhanced root characteristics respond to reduced water intake, specifically whether the root architecture of these genotypes changes under water limitation. There is some knowledge about seasonal patterns of grass root growth (Snyman, 2009), but the results of their reaction to water stress are interesting. There is a need to determine the genetic variation and heritability of traits describing biomass located under the soil surface and to define the impact of these traits on important agronomic traits, which are prerequisites for successful breeding of cultivars with enhanced drought tolerance. Since the maximum depth of rooting in the soil dominantly depends on the penetration ability and the root growth duration (Wang and Smith, 2004), we put genotypes in the same rooting conditions and in the same root growth time. In this study, roots were measured in wide and deep enough substrate columns, allowing relatively free root growth and avoiding root twisting at the bottom of the column. Therefore, the differences that are determined mostly belong to genotypes. The number of plants that reached deeper substrate horizons was higher for population LPb3 than for cultivar K-11 in all watering regimes. This percentage increased with irrigation reduction (Table 1). This means that breeding for deeper roots has shown results in dry conditions, which was the goal of breeding in the first instance. In the matter of fact, the plants with at least some deep roots would survive a pronounced drought period during the vegetation season, reaching deeper soil water (Thorup-Kristensen et al., 2020). It was demonstrated earlier that more well-drained soils require deeper root distributions to maintain optimal water use and that deep roots represent optimal root profiles in water-limited ecosystems (Rudd et al., 2014). In this research, the proportion of LPb3 plants that reached the deepest substrate layers below 90 cm (Table 1) was 10% higher than K-11 on the highest water reduction treatment. Therefore, this experiment has confirmed that root trait improvement obtained by breeding in optimal conditions can be retained in droughty conditions.

SDM and root traits variability remained very high within both investigated populations, even though both were exposed to the long breeding activities. According to the facts that *L. perenne* is out cross anemophily species and that root traits weren't common breeding criteria, high levels of heterozygosity and variability remained in perennial ryegrass genotypes after breeding. That represents a large source for further breeding of these traits. Nevertheless, a reduction in shoot dry matter of perennial ryegrass plants was expected as the water deficit increased. However, this reduction was significantly lower in the population selected for deeper roots (LPb3), especially with increased water reduction (Table 2). Population LPb3 in 50% reduction had the highest values for SDM, which could be the result of the successful previous selection of this population for drought conditions. The average SDM in LPb3 in 25% irrigation (1.02 g) is 23% reduced compared with SDM in full irrigation, but it is still 8% higher than in K-11 (0.95g), at the same level of irrigation.

It was also noticeable that SDM in the treatment with 75% irrigation was higher than in 100% irrigation. That was probably because full water saturation of the substrate inhibits plant development and growth. Similar values were also recorded in all other root traits.

The plant root systems architecture significantly affects crop growth and yield and plays an important role in the plant-soil-production relationship (Wang et al., 2014). The total root dry matter (TRDM) and dry matter of roots in the top 20 cm of substrate haven't showed significant differences between populations. On the contrary, differences in TRDM were significant between different irrigation treatments. Generally, TRDM was reduced with water limitation, but this was less pronounced in the LPb3 population. Also, some data root dry mass was reduced under drought in durum wheat genotypes (Raziuddin et al., 2010). Still, there were records of higher absolute root biomass under drought in barley and wheat cultivars (Ayad et al., 2010). Differences in shallow root DM weren't statistically significant, even between watering treatments. This may be because drop nozzles made the water addition at the substrate surface and shallow roots always received water first. However, in deeper root portions, where water remained longer, differences became statistically significant between populations and applied water levels.

The highest percentage of roots in the good and moderate water condition grasslands was found in shallow soil. The most productive grasses have 86 to 94% of their roots in the top 30 cm of soil (Snyman, 2009). Such high concentrations of roots could be due to increased nutrient proportions in these surface soil layers or even due to the high level of bedrock below the soil.

Recent research suggests that deep roots could be of pivotal importance in alleviating water stress in many crops (Gewin, 2010). LPb3, on average, produced five times more roots below 90 cm than K-11. RB90 was increased with the decrease of irrigation and reached the highest level in population LPb3 at 25% of added water. That value was more than eightfold higher than in K-11. Therefore, there is the possibility that in the population LPb3, drought induces deep-root growth, enabling survival and persistence. That could even allow a certain level of forage production and reduction of summer dormancy, which is always a problem during the summer dry period in moderate and full continental climates. Compensatory root growth at depth under water deficits has been previously reported in some cereals (Ayad et al., 2010).

Crush et al. (2005) noted value of 0.35 for the narrow-sense heritability for root system size and shape in perennial ryegrass half-sib families of an open pollinated breeding pool, which means that progress in breeding for root traits would be slow, though some results of progeny evaluation from a first cycle of mass selection for root system shape show that it is achievable. In our research (Table 3), the level of heritability for roots below 90 cm (54.43 in LPb3 and 49.75 in K11) also indicates that breeding of this trait is possible, but difficult and results are always uncertain. On the other hand, narrow-sense heritability for deep root growth in *Agrostis stolonifera* is relatively high (Lehman and Engelke, 1991) and selection for deeper root portions is likely possible. The estimated genetic gain of 43% of the average value in LPb3 and 44.4% in K-11 makes breeding for that trait promising.

Characteristics of perennial grasses aboveground biomass and the existence of the yield, are overall important since they are fodder plants. Nevertheless, although survival during drought is crucial, plants should have some DMY to justify the basic purpose of such production (Robinson et al., 2018). Root to shoot ratio is often used to indicate relative biomass allocation to below and above-ground biomass (Edwards et al., 2016). Therefore, an important factor to consider in root breeding is the root-to-shoot ratio (Mathew et al., 2018). The root-to-shoot ratio is usually an important selection criterion, as investment in roots should be accompanied by aboveground biomass production in forage species. Nevertheless, the selection criteria must not be restricted to ratios only but include a wide range of other traits. Our idea was that this ratio in perennial ryegrass dry matter yield and drought tolerance breeding should remain the same or be slightly increased, indicating that plants invest more in deep root production and improve genotype drought tolerance, but also produce more biomass at the same time. The differences in this ratio between populations weren’t statistically significant in this research, but differences between irrigation levels and interaction between treatments were statistically significant at the level of 99%. In many studies, the tendency is that root/shoot ratios decrease with plant age and over the season, these ratios are above 1.8 and as high as 5 on grass species (Ghebrehiwot et al., 2006). In our study, this ratio was above 1.8 in the control population K-11, although it was less productive, while in the LPb3 population, the TRSr was lower (1.66). The highest values of this parameter were determined in the highest levels of water reduction in both studied populations (2.46 in K 11 and 1.88 in LPb3). Thorup-Kristensen and Rasmussen (2015) noted an average root-to-shoot ratio at perennial ryegrass of 1.5. Although there are different relationships in annual species, some investigations on wheat described some adaptive characteristics of drought-induced increasing of root-to-shoot ratio under water stress during the vegetative period (Bacher et al., 2022). However, perhaps more important for drought tolerance and production is the ratio of roots below 90 cm (RB90) and SDM, which also increases with water reduction. This ratio is, on average, better in the population LPb3 selected for deep roots. Even population K-11 had a higher whole root/shoot ratio than population LPb3, the SDM and deep roots below 90 cm ratio was opposite and higher in LPb3. It is obvious that the ratio of roots, especially deep ones, should remain the same even though SDM is rising. It means that plants invest in forage production and deep roots production at the same time, without significantly reducing above-ground dry matter yield. These ratios increased with water reduction since above-ground biomass is more susceptible to drought than the root. In literature root to shoot ratio is generally reported to increase with drought in cereals (Hoad et al., 2001), probably due to a higher allocation of assimilated C to roots. In drought conditions, some wheat genotypes tend to increase root mass and raise the root to shoot growth ratio to extract more water from the soil while maintaining shoot production (Bacher et al., 2022). Although the correlation of root to shoot ratio and the yield is regularly negative (Mathew et al., 2018), in this research, it becomes positive in population LPb3, considering the deep roots relation with shoot biomass. That is also confirmed by steep regression line between SDM and RB90 with a statistically significant correlation coefficient on level 99%. It indeed implies that SDM rises with deep root increasing.

It is also important that there are a lot of single genotypes, especially in LPb3, which can be selected for both SDM and deep roots at the same time and which showed good characteristics in reduced watering treatments. The breeding of these traits is possible since vectors in PC analysis for both traits are directed in the same course.

## CONCLUSIONS

A highly significant variability was detected in both studied populations. Thirteen single genotypes with the highest dry matter of root below 90 cm were from the LPb3 population. These genotypes represent the basis of the next breeding cycle for improved production of deep roots and productivity. According to the genetic variability and the level of broad-sense heritability, it could be claimed that the breeding of all investigated traits in this perennial ryegrass material is feasible, especially in population LPb3. It is obvious that heritability for deep roots drops down to about 50%, but according to these values, breeding of these traits is still feasible. Generally speaking, the estimated genetic gain was much higher in the breeding population LPb3 than in K-11. Expected genetic gain per breeding cycle for shoot dry matter reached a quarter of the average value, while for dry matter of root below 70 cm and dry matter of root below 90 cm, it was even higher. Since above-ground dry matter production is the most important trait for forage perennial ryegrass, the plants that have the best shoot dry matter and dry matter of root below 90 cm at the same time are going to be selected.

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