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ROOT SYSTEM SIZE OF ALFALFA VARIETIES UNDER DIFFERENT PLANT DENSITIES

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Abstract

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The field experiment was established from pre-grown alfalfa sprouts in 2008. In 2009 and 2010, the harvest was carried out. The yield and the root size system (RSS) were evaluated in the total of five cuts. Two localities of diverse soil and weather conditions were selected. Žabčice is a dry location with a highly permeable sandy soil layer, but at the same time, with a considerable fluctuation of the underground water level; Troubsko, on the other hand, represented a location with a higher content of clay in soil suitable for growing alfalfa. The trial was established in two types of plant spacing – 25 × 25 cm (16 plants per m^2) and 50 × 50 cm (4 plants per m^2). Two alfalfa varieties were used – *Hodonínka* (an old variety) and *Oslava* (a new variety). The following factors were evaluated by statistical means: location, variety, plant spacing, and cut number. The effect of location on the above ground phytomass and the RSS value proved highly statistically decisive. The higher average values of above ground phytomass as well as RSS were achieved on the Troubsko location where the above ground phytomass was 19.57 $t.ha^{-1}$ and RSS was 2.71 nanofarad (nF). The impact of the variety was highly statistically significant only in respect to RSS. The effect of variety on the alfalfa above ground phytomass was not proven. The higher average RSS values were reached by the *Hodonínka* variety (2.59 nF). The effect of the plant spacing on both the above ground phytomass and the RSS values was highly statistically significant. The higher average above ground phytomass was achieved when the 25 × 25 cm plant spacing was employed (18.64 $t.ha^{-1}$). As for the RSS value, the case was exactly opposite – the higher average value was reached when the plant spacing was 50 × 50 cm (2.99 nF). The impact of the cut number on both the above ground phytomass and the RSS was highly statistically significant. The highest average above ground phytomass and root system size were measured in the third cut. The above ground phytomass was 30.36 $t.ha^{-1}$ and RSS was 3.86 nF.

alfalfa, root system size, above ground phytomass, variety, location, density, cut number

On the world scale, Alfalfa (*Medicago sativa* L.) is perhaps the oldest and, along with maize, also the most important forage crop (Chloupek, 2000). Cultivated alfalfa, a tetraploid (*Medicago sativa* L., 2n = 4x = 32), is the most important perennial forage crop grown in North America, often called queen of the forages (Barnes *et al.,* 1988). The root system is essential for nutrients and water uptake, and plant stability; several studies have discussed the use of the electrical capacitance method for measuring the root system size (Chloupek, 1977). In recent years, lack of water in soil has been the main stress factor. To eliminate water scarcity in soil, plants need to have a sufficiently developed root system that is able to receive the optimal amount of water and nutrients (Středa *et al.,* 2009). Therefore, the study is aimed at presenting the method of root system size (RSS) evaluation, and attempts to describe it in practical use. In particular, this work focuses on the study of the effect of alfalfa varieties, location, and plant density on the size of the root system.

The evaluation of root size is very difficult mainly by species with tap-root reaching deeper than arable layer. Root system size (RSS) can be determined using electrical capacitance measured in relation to the surrounding soil with the dry surface of

aboveground parts of the plants (Chloupek, 1972, 1977). The method is based on polarization of biological membranes in the root system. However, the measured values are "contaminated" by electric capacitance of the surrounding soil, wires etc., with the result that comparisons can only be made among plants of the same species measured in the same soil and under uniform moisture conditions. The "parasitic" capacitance much depends on the soil water, since water has a high dielectric value. A current must be lead to the whole root system. This may be a problem in older plants, which are sometimes disintegrated due to root rot (Chloupek *et al.*, 1999) or if a large portion of the root was naturally damaged (Kendall *et al.*, 1982). This method is used mainly for breeding selection of various cultural species, e.g. Chloupek *et al.* (1999) or Chloupek *et al.* (2003). According to Chloupek *et al.* (1999), progenies of lucerne plant with large RSS tended to have a large RSS and higher forage yield than progenies of plants with small RSS. The described differences within M. sativa complex are concerned mainly on traits of aboveground part of plants (e.g. Pelikán *et al.*, 2007).

MATERIAL AND METHODS

Two alfalfa varieties were used in the experiment (*Hodonínka* and *Oslava*). The *Hodonínka* and *Oslava* varieties were chosen to compare the effect of the process of breeding RSS. The *Hodonínka* variety is an old variety developed by crossing Moravian regional varieties with the *Grim* variety. The *Hodonínka* was registered in 1940, and is not grown anymore. It has got a partially erect tuft shape with low number of stems. Stem is medium. The number of inflorescence is mid-high; the color of flowers is purple. Unfortunately, no more details were found in regard to this variety as it had become old-fashioned many years ago. The seed was provided by the genetic bank in Prague. The *Oslava* variety is a synthetic variety bred for economic parameters and the higher symbiotic fixation of aerial nitrogen. The components of the synthetic population are clones 728 (*Pálava* x *Zuzana*), 730 (*Pálava* x *Zuzana*), 742 (*Hodonínka*), 747 (*Přerovská*) and 757 (*Flandria*). Recombination of the components took place in the subsequent three generations. The *Oslava* is early to mid-early variety with a good resistance to pathogens causing vascular wilt disease. It has got a spread out to partially erect tuft shape (height of 60–80 cm). The number of stems in the tuft is medium, the number of internodia is medium, too. The stem is medium to long. The terminal leave is elliptical, somewhat long (30–40 mm) and wide (15–20 mm). The number of inflorescence is mid-high; the color of flowers is light blue to blue and purple with a scarce mixture of colorful, cream, and yellow flowers. The number of pods in infructescence is medium to high, the number of seeds in a pod is medium. Pods are medium to strongly spiral-shaped. This variety was registered in 2003 and bred by Agrogen Ltd. Troubsko, a company who provided the seed. These varieties were grown on two locations (Žabčice and Troubsko), planted in 2008 and harvested in 2009 only two cuts (soil water logging) and in 2010 three cuts (Tab. I). Plants used in the experiments were pre-grown in a greenhouse, and at the age of about six weeks, they were planted. After planting plants were watered. During the vegetation in 2008 were done twice cuts and kicked. Were planted 52 plants in 4 rows in the plant spacing 50 × 50 cm and 84 plants in 7 rows in the plant spacing 25 × 25 cm, i. e. 4 and 16 plants per 1 m^2. The wider plant spacing were chosen to minimize errors in measuring RSS. Were evaluated 30 plants in each variant and each cut. The root system size was measured by ELC-131D LCR Meter at the measuring frequency of 1 kHz as described by Chloupek *et al.* (2006). The above ground phytomass of alfalfa was determined for the fresh matter.

The collected data were subjected to the multifactorial analysis of variance and post hoc L.S.D test at 0.05 and 0.01 levels of significance in STATISTICA 8.0 CZ program.

RESULTS

Root system size (RSS)

The higher RSS values were found on the Troubsko location (2.71 nF on average); in contrast, on the Žabčice location, RSS values of 2.27 nF were measured. The effect of location was highly statistically significant (Tab. II and III). This trend can be observed in Figures 1 and 3. The *Hodonínka* variety developed a bigger root system (2.59 nF on average); the *Oslava* variety, on the other hand, reached average RSS values of 2.39 nF. Likewise, the effects of variety and plant spacing on RSS were highly statistically decisive (Tab. II and III, Figures 1–3). Higher RSS value were achieved in wider spacing mean 2.99 nF; narrower spacing reached 1.99 nF on average. Additionally, the term of harvest also represented an important factor that influenced RSS. The highest values were found for

I: *Management of the experiment: Locations and data of planting and harvests (cuts)*

Locations	Plantings	Cuts				
		1st	2nd	3rd	4th	5th
Žabčice	April, 25th, 2008	May, 14th, 2009	August, 27th, 2009	May, 20th 2010	July, 1st 2010	September, 22nd, 2010
Troubsko	April, 28th, 2008	May, 26th, 2009	August, 28th, 2009	June, 1st, 2010	July, 20th, 2010	September, 15th, 2010

II: *Mean values of RSS and above ground phytomass of fresh matter with regard to location, variety, and plant spacing*

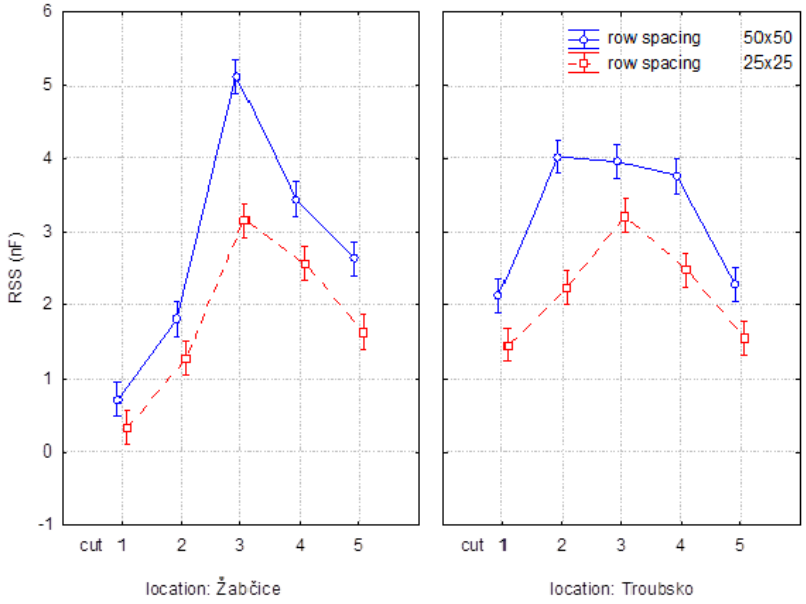
Factor	Mean values of RSS (nF)	Mean values of above ground phytomass (t.ha^{-1})	Factor	Mean values of RSS (nF)	Mean values of above ground phytomass (t.ha^{-1})
Žabčice	2.27	10.10	Cut 1st	1.16	10.98
Troubsko	2.71	19.57	Cut 2nd	2.34	15.63
Hodonínka	2.59	14.41	Cut 3rd	3.86	30.36
Oslava	2.39	15.24	Cut 4th	3.07	12.63
50 × 50 cm	2.99	11.04	Cut 5th	2.02	4.57
25 × 25 cm	1.99	18.64			

the third cut (the first cut in the second year) and this 3.86 nF on average; in contrast, the lowest RSS values were measured at the first cut (the first cut in the first year) and this 1.16 nF on average. The effect of the time of harvest on RSS was highly statistically significant, as displayed in Tab. II and III and Figures 1–3.

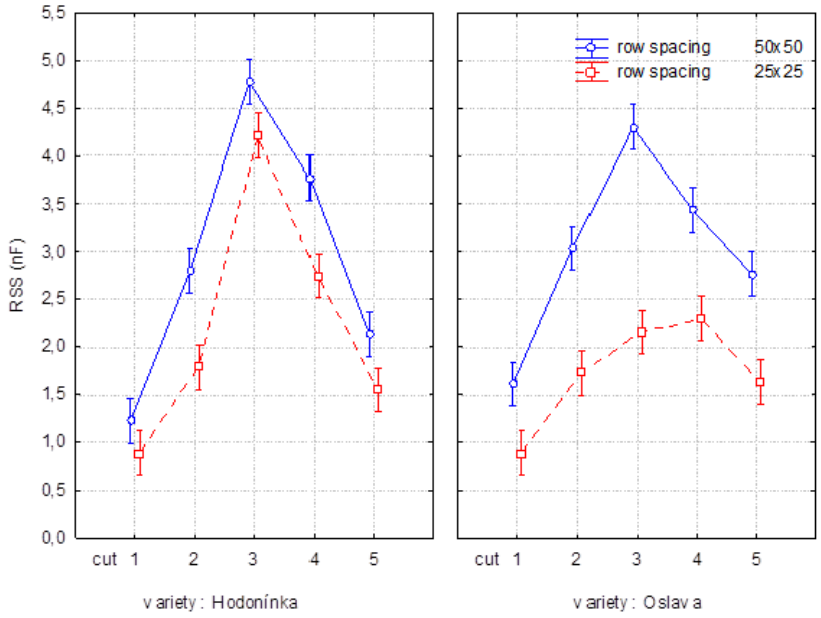
III: *Impact of cut, location, variety and plant spacing on alfalfa RSS and above ground phytomass of fresh matter*

Effect	d.f.	RSS MS	Sig.	Above ground phytomass MS	Sig.
Cut	4	42.41	**	3669.05	**
Location	1	9.68	**	4481.42	**
Variety	1	2.08	**	35.92	
Plant spacing	1	4.67	**	2887.66	**
Cut x location	4	8.81	**	234.90	**
Cut x variety	4	4.32	**	8.72	
Location x variety	1	6.67	**	40.29	*
Cut x plant spacing	4	1.01	**	129.49	**
Location x plant spacing	1	0.07		326.89	**
Variety x plant spacing	1	4.29	**	185.27	**
Error	177	6.56		414.23	
Total	199				

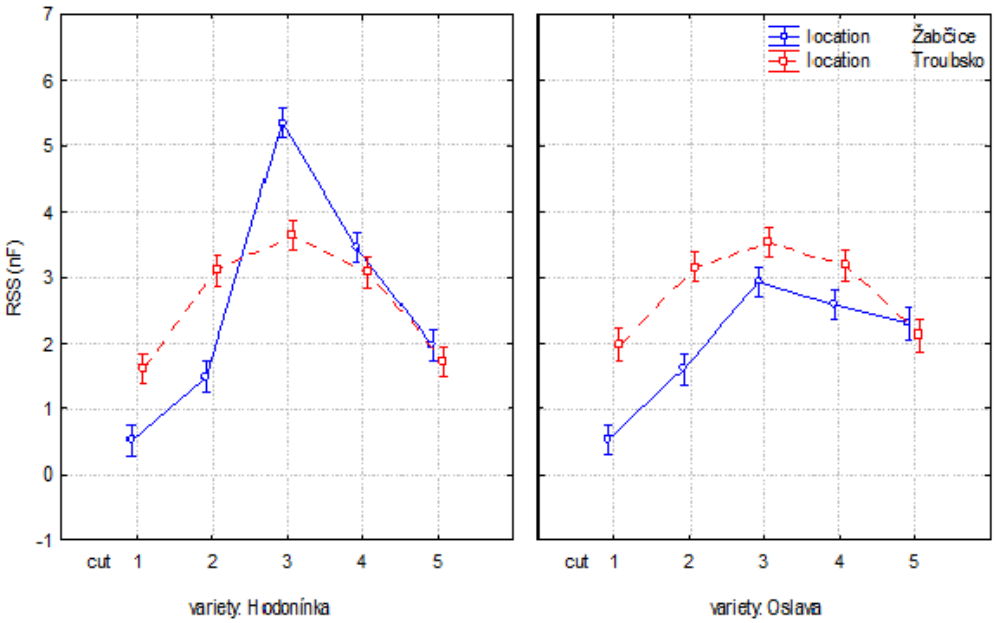
*$P < 0.05$, **$P < 0.01$



1: *Impact of location, cut and plant spacing on alfalfa RSS*
Note: Error bars represent confidence intervals, P = 0.05



2: *Interaction of variety, cut and plant spacing on alfalfa RSS*
Note: Error bars represent confidence intervals, P = 0.05



3: *Interaction of variety, cut and location on alfalfa RSS*
Note: Error bars represent confidence intervals, P = 0.05

The root system was not sufficiently developed during the first cut (Figure 1). Between first and second cut, RSS increased distinctly in all variants, and the maximum values were found for the wider spacing on the Troubsko location. On the Žabčice location at both types of plant spacing and on the Troubsko location at the plant spacing of 25 × 25 cm, the highest RSS was achieved in the third cut (the first cut in the second year of growing). RSS decreased during the subsequent cuts in all variants. The *Oslava* variety responded much better to less dense type of plant spacing. In case of the *Hodonínka* variety the wider spacing resulted in statistically significantly larger roots (Figure 2). The Žabčice location was more extreme in respect to RSS values of the *Hodonínka* that showed highly statistically significant diversities in the third cut, and in the fourth cut, statistically significant differences

were found, too (Figure 3). These extremes might have been caused by the year factor because in spring 2010, the Žabčice location had experienced worsened precipitation conditions due to which the plants had been stimulated to develop larger root systems. On the location Troubsko between evaluated varieties weren't in each cuts statistically significant differences at values RSS.

Above ground phytomass

The higher above ground phytomass values were reached on the Troubsko location – 19.57 t.ha^{-1} on average. On the Žabčice location, the average above ground phytomass was 10.10 t.ha^{-1} only. The impact of location was statistically highly significant (Table II and III). The *Oslava* variety reached the higher above ground phytomass per hectare – 15.24 t.ha^{-1}; the *Hodonínka* variety achieved an average above ground phytomass of 14.41 t.ha^{-1}. This small difference between *Oslava* and *Hodonínka* manifested itself by a statistically insignificant impact of variety on above ground phytomass (Table II and III). The plant spacing had a considerable effect on above ground phytomass. The best above ground phytomass was achieved at the plant spacing of 25 × 25 cm where the average above ground phytomass was 18.64 t.ha^{-1}; in contrast, at the plant spacing of 50 × 50 cm, the average above ground phytomass was only 11.04 t.ha^{-1}. The effect of plant spacing on above ground phytomass was highly statistically significant (Table II and III). The cut term was another factor influencing alfalfa's above ground phytomass per hectare. The best average above ground phytomass was achieved in the third cut – 30.36 t.ha^{-1}. The lowest average above ground phytomass was found in the fifth harvest term – only 4.57 t.ha^{-1}. The effect of the term of harvest on the above ground phytomass per hectare was highly statistically significant (Table II and III).

DISCUSSION

RSS was significantly ($P < 0.01$) influenced by location in the first, third, and the fifth cuts. RSS in Troubsko was larger than in Žabčice. The location impact was prevailing in the first two cuts. Dostál *et al.* (2009) measured the RSS of barley and established that it had been influenced by the location of the experiments (48–88%).

Stand density had a significant influence on RSS ($P < 0.01$) in all five cuts; this effect prevailed in the last three cuts. Plants in denser stands had smaller RSS than sparse stands. The effect of variety was significant in all cuts, with the exception of the second one. In the first two cuts, the effect of variety appeared smaller than the effects of location and density, but in the following three cuts (3rd–5th), it was stronger than the effect of location but still weaker than the impact of density. The *Oslava* variety had a larger RSS in comparison with the *Hodonínka*. In their winter wheat experiments, Acuña *et al.* (2007) confirmed that under dry conditions plants developed roots faster, the roots were reaching deeper and formed more robust root systems. In our experiment, this phenomenon was found only in the *Hodonínka* variety in the second and particularly the third cuts in dry location Žabčice where a rapid root mass growth could have been observed. The phenomenon was also confirmed by Hamblin *et al.* (1990) who found that plants developed larger root systems at times of drought.

This genetic controlled difference in RSS related to greater forage above ground phytomass could support selection for RSS as a selection criterion for forage above ground phytomass in alfalfa with respect to RSS, the interaction between varieties and stand densities was significant in four of five cuts with the exception of the fourth one. Similarly, this interaction was significant in the first four of the five cuts. Zhang *et al.* (1999) found that older varieties developed larger root systems at spring wheat in dry soils than the modern bred varieties did. In their publications, other authors have confirmed that stress factors (drought and waterlogging) promote root growth at wheat and barley (Morris *et al.*, 1991; Byerlee and Moya, 1993).

In a sparse stand, RSS was almost identical in both varieties; however, in a dense (25 x 25 cm) stand, RSS was smaller in the *Oslava* by more than 20%. Above ground phytomass higher by more than 20% (in comparison to *Hodonínka*) was found for sparse the *Oslava* stands; in case of the dense stands, the above ground phytomass was lower by nearly 10% in comparison to the *Hodonínka*. Moreover, in dense stands that are usually production standsand producing higher above ground phytomasss (22% in case of the *Hodonínka* and 9% in the *Oslava*). However, a larger RSS in 50 x 50 cm plant spacing did not respond similarly to forage above ground phytomass from the same area;. These plants could not probably eliminate the effect of a sparser stand by developing more stems. Chloupek *et al.* (1999) measured the size of the root system using same method for two alfalfa cultivars (*Zuzana, Pálava*). Progeny of plants with large RSSs inclined to having larger RSSs and higher forage above ground phytomass than progeny of plants with small RSSs. Lamb *et al.* (2003) compared ten alfalfa cultivars, two from each of the past five decades, four recently released cultivars, and two monitored cultivars for forage above ground phytomass and persistence at four locations. Year x location x cultivar-release date interactions demonstrated that the forage above ground phytomass and the final stand densities differed among the cultivars in each year of the experiment at each location.

SUMMARY

The goal of this study was to evaluate the size of the root system of alfalfa at various types of plant spacing (50 × 50 cm and 25 × 25 cm), on two different localities (Žabčice and Troubsko, CZ), and in two different varieties (*Hodonínka* reg. 1940) and (*Oslava* reg. 2003).

The root system size (RSS) was measured by electrical capacitance. The alfalfa above ground phytomass was determined in fresh matter. The collected experimental data were assessed by multifactorial analysis of variance and post hoc L.S.D test.

The results imply that the location factor was highly statistically significant in regard to the above ground phytomass as well as the RSS value. The effect of variety was highly statistically significant only in respect to RSS. The impact of variety on the above ground phytomass was not proven. The higher RSS values were found in the *Hodonínka* (2.59 nF). The effect of the type of plant spacing on both the above ground phytomass and the RSS was highly statistically significant. The higher average above ground phytomass was achieved at the narrower spacing. On the contrary, the higher average RSS values were found at the wider spacing (2.99 nF). The influence of the cut number on the above ground phytomass as well as the RSS was also statistically significant. The highest average above ground phytomass and the largest root system were found in the third cut. The above ground phytomass was 30.36 $t.ha^{-1}$ and RSS was 3.86 nF.

It was find out that at the time of the first cut, the root system was not fully developed which reflected in lower RSS values. At the time of the second cut, RSS distinctly increased in case of all variants and reached the maximum values at wider spacing in Troubsko.

The highest root capacity was found in the third cut (the first cut in the second year of growing) for both types of spacing on the Žabčice location and for narrower spacing in Troubsko. This was the maximum, and after that, the root capacity started decreasing. The *Oslava* variety responded better to sparse plant spacing. The *Hodonínka* variety had stronger correlations between the studied spacing types. Roots were statistically significantly larger when the 50 × 50 cm spacing was used. On the Troubsko location, both varieties performed in a similar way. The Žabčice location was more extreme in respect to RSS values in case of the *Hodonínka* variety that showed very high statistically significant differences in the third cut and significant differences in the fourth cut. These extremes might have been caused by the dry weather conditions of spring 2010 as a result of which plants were stimulated to develop larger root systems.

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REFERENCES

ACUÑA, T. L. B., PASINQUIN, E., WADE, L. J., 2007: Geotypic differences in root penetration ability of wheat trough thin wax layers in contrasting water regimes and in the field. Plant Soil vol. 301, p. 135–149.

BARNES, D. K., GOPLEN, B. P., BAYLOR, J. E., 1988: Highlights in the USA and Canada. In: HANSON, A. A., BARNES, D. K., HILL, R. R. (ed.): *Alfalfa and alfalfa improvement*. Agron. Monogr. 29, 1–24. ASA, CSSA, and SSSA, Madison, WI.

BYERLEE, D., MOYA, P., 1993: Impact of International Wheat Breeding in the Developing World, 1966–1990, Mexico, D. F.: CIMMYT.

DOSTÁL, V., STŘEDA, T., CHLOUPEK, O., 2009: Electric capacity as a measure of the intact root system size in the soil. In: *7th ISRR Symposium "Root Research and Applications"*. 1. vyd. Wien: BOKU Wien, 2009, 1–2.

HAMBLIN, A., TENNANT, D., PERRY, M. W., 1990: The cost of stress: dry matter partitioning changes with seasonal supplay of water and nitrogen to dryland wheat. Plant soil, Vol. 122, p. 47–58.

CHLOUPEK, O., 1972: *The relationship between electric capacitance and some other parameters of plant roots.* Biologia Plantarum, 14, 227–230.

CHLOUPEK, O., 1977: *Evaulation of size of a plants root system using its electrical capacitance*, Plant Soil, 48, 525–532.

CHLOUPEK, O., SKÁCEL, M., EHRENBERGEROVÁ, J., 1999: Effect of divergent selection for root size in field grown alfalfa. *Can J Plant Sci.*, 79: 93–95.

CHLOUPEK, O., 2000: *Genetická diverzita, šlechtění a semenářství.* 2. vyd. Praha: Academia, 193–197 s. ISBN 80-206-0434-2.

CHLOUPEK, O., EHRENBERGEROVÁ, J., OPTIZ VON BOBERFELD, W., ŘÍHA, P., 2003: *Selection of white clover (Trifolium repens L.) using root traits related to dinitrogen fixation.* Field Crops Res., 80, 57–62.

CHLOUPEK, O., FORSTER, B. P., THOMAS, W. T. B., 2006: The effect of semi-dwarf genes on root system size in field-grown barley. *Theor Appl Genet.* 112(5): 779–786.

LAMB, J. F. S., SHEAFFER, C. C., SAMAC, D. A., 2003: Population density and harvest maturity effects on leaf and stem yield in alfalfa. Agronomy journal, Vol. 95, issue 3, 635–641.

KENDALL, W., PEDERSON, G. A., HILL, R., 1982: Root size estimates of red clover and alfalfa based on electrical capacitance and root diameter measurement. Grass forage Sci., 37, 253–256.

MORRIS, N. L., BELAID, A., BYERLLEE, D., 1991: Wheat and barley production in rainfet mariginal environments of the developing World. In: CIMMYT World wheat facts and trends, Mexico, CIMMYT.

PELIKÁN, J., VYMYSLICKÝ, T., GOTTWALDOVÁ, P., 2007: The study of among–varieties variability within the assortment of alfalfa In: Proc. Aktuální poznatky v pěstování, šlechtění a ochraně rostlin, Brno, Czech Republic, 8–9 November 2007, 209–212.

STŘEDA, T., DOSTÁL, V., ULLMANNOVÁ, K., 2009: Root system as a factor of oil seed rape yield formation. In: *MendelNet'09 Agro – Proceedings of International Ph.D. Students Conference.* 1. vyd. Brno: Mendelu in Brno (in Czech).

ZHANG, R., ZHANG, D. Y., YUAN, B. Z., 1999: Study on the relationship between competitive ability and productive performance of spring wheat in semiarid regions of loess plateau. Acta Phythoecology, Vol. 23, p. 205–210.

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