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# VARIABILITY OF NITROGEN CONTENT IN THE NEEDLES OF DOUGLAS-FIR (*Pseudotsuga menziesii* Mir / Franco) PROVENANCES

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Nitrogen content in the needles of twenty Douglas-fir provenances, originating from different sites within the native range of the species in the USA, was studied in a Douglas-fir provenance test established at the montane beech site on acid brown soil. Based on the variability of nitrogen content in the needles, the intensity and dynamics of the physiological processes of Douglas-fir mineral nutrition were analyzed as the indicators of Douglas-fir adaptive potential to the sites in Serbia. All the trees of the study provenances were of the same age and grown under the same site and population conditions. The quantities of nitrogen absorbed in Douglas-fir needles were correlated with the geographical characteristics of the native sites of the observed provenances. The differences in nitrogen content in

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Douglas-fir needles point out the variability in the intensity of the physiological processes in the genotypes of the different provenances. Since the study Douglas-fir trees are cultivated on relatively small areas, in more or less equal general conditions, it can be concluded that the parameters of mineral nutrition depend on the genotypes constituting the gene pool of the study Douglas-fir provenances.

*Key words:* Douglas-fir, nitrogen,provenances, Serbia

## INTRODUCTION

Douglas-fir (*Pseudotsuga menziesii* Mir / France) is the most productive introduced coniferous species in Europe. Its native range extends from Canada in the north to New Mexico in the south of North America (HERMANN 1985, 1987). Geographical distribution of Douglas-fir is the largest of all conifers in western North America (CAMPBELL, 1992, CAMPBELL, SUGANO 1993). The introduction of species with such a wide natural range requires provenance testing before entering the new habitats of Serbia (ISAJEV AND LAVADINOVIĆ, 2008, RADONJA *et al.* 2003, TEŠEVIĆ *et al.* 2009) in the aim of the assessment of their genetic potential and adaptability to particular site conditions.

The intensity and dynamics of the physiological processes of Douglas-fir mineral nutrition are important indicators of the adaptation and effectiveness of Douglas-fir introduction to allochthonous sites in Serbia. The high production and quality of Douglas-fir biomass are determined by their genetic potential, favourable climate conditions, and by the high quantity of minerals in the soil where the trees grow (LAVADINOVIĆ *et al.* 1996, 2003, 2009).

Nutrient absorption from the soil by forest trees depends on site conditions, soil type and availability of plant nutrition elements. This is confirmed by high differences in the quantities of nutrients, primarily nitrogen concentrations in the foliage of forest trees growing on different soil types (LADANAI, 2008, BATOS *et al.* 2010, APONTE *et al.* 2011).

The quantity of nitrogen in the Douglas-fir tissues and organs depends on many factors, both internal - genotype, phase of ontogenesis, age, etc., and external, i.e. total environmental factors. The following ecological factors which affect the dynamics and effects of Douglas-fir mineral nutrition are regarded as the most important: precipitation, basic characteristics of the soil solution (particularly the soil solution concentration and pH reaction), main elements of soil solution, etc., the availability of the main nutritive elements in the soil, as well as humidity and temperature conditions.

Based on the variability of nitrogen content in the needles, the intensity and dynamics of the physiological processes of Douglas-fir mineral nutrition were analyzed as the indicators of Douglas-fir adaptive potential to the sites in Serbia. The results of the analyses are an important starting point for future selection of suitable genotypes – test trees in the seed stands of the provenances with above-average nitrogen content. The genotypes which will be selected in the future are those that, regardless of the lower N absorption, will yield higher quantities of biomass and

seeds, or will be tolerant to environmental conditions. These genotypes are very important for the future establishment of Douglas-fir cultural communities aiming at different targets in Serbia.

The results of the analysis of N concentration variability in the Douglas-fir needles, expressed as the percentage of dry matter, as well as the correlation with the estimation indicators of the trees of 28 provenances, are presented in Tables and Figures.

## MATERIALS AND METHODS

In the aim of the research of genetic variability and ecological adaptation of introduced species, Douglas-fir provenance tests were established in Serbia by the Institute of Forestry in Belgrade. The study Douglas-fir provenances originate from the whole Douglas-fir range in North America, Table 1.

*Table 1: General geographical characteristics of the provenances which are studied in the experiment in Mt. Juhor*

| Provenance | Our mark number | Latitude (°N) | Longitude °W | Altitude (m) | N (%) | Standardized deviation from the average (Z) |
|---|---|---|---|---|---|---|
| Oregon 205–15 | 1 | 43.7 | 123.0 | 750 | 2.14 | 1.0588 |
| Oregon 205–14 | 2 | 43.8 | 122.5 | 1,200 | 2.13 | 1.0350 |
| Oregon 202–27 | 3 | 45,0 | 122.4 | 450 | 1.97 | 0.6543 |
| Oregon 205–38 | 4 | 45.0 | 121.0 | 600 | 2.14 | 1.0588 |
| Washington 204–07 | 9 | 49,0 | 119.0 | 1,200 | 0.84 | -2.0343 |
| Oregon 205–13 | 10 | 43.8 | 122.5 | 1,050 | 1.94 | 0.5829 |
| Oregon 205–18 | 11 | 44.2 | 122.2 | 600 | 2.33 | 1.5108 |
| Oregon 202–22 | 12 | 42.5 | 122.5 | 1,200 | 0.84 | -2.0343 |
| Washington 202–17 | 15 | 47,6 | 121.7 | 600 | 1.15 | -1.2967 |
| Oregon 201–10 | 16 | 44.5 | 119.0 | 1,350 | 1.60 | -0.2260 |
| Washington 201–06 | 17 | 49.0 | 120.0 | 750 | 1.38 | -0.7495 |
| Oregon 202–19 | 18 | 45.3 | 123.8 | 300 | 1.82 | 0.2974 |
| Oregon 205–11 | 20 | 45.0 | 123.0 | 150 | 1.75 | 0.1309 |
| New Mexico 202–04 | 22 | 32.9 | 105.7 | 2,682 | 1.72 | 0.0595 |
| New Mexico 202–10 | 23 | 36.0 | 106.0 | 2,667 | 1.86 | 0.3926 |
| Oregon 202–31 | 24 | 44.3 | 118.8 | 1,500 | 1.66 | -0.0833 |
| Oregon 205–29 | 26 | 42.6 | 122.8 | 900 | 1.78 | 0.2022 |
| Oregon 205–08 | 27 | 42.7 | 122.5 | 1,050 | 2.05 | 0.8446 |
| Oregon 204–04 | 30 | 45.0 | 121.5 | 900 | 1.47 | -0.5353 |
| Washington 205–17 | 31 | 47.7 | 123.0 | 300 | 1.33 | -0.8684 |
| Average | | | | | 1.695 | |
| S | | | | | 0.42029 | |
| min | | 32,9 | 105,7 | 150 | 0.84 | -2.0343 |
| max | | 49,0 | 123,0 | 2682 | 2.33 | 1.5108 |

The test was established on Mt. Juhor - central Serbia, in the montane beech *Fagetum moesiacae montanum Rudski* site in 1982. The site altitude is 740 meters above the sea level, the slope of the terrain is 10 degrees, western aspect. The parent rock is gneiss, and the soil is dystric cambisol (acid brown soil). Based on the data obtained by the local weather station (Ćuprija) for the period 1961-2003, the characteristics of Mt. Juhor climate are: mean annual air temperature is 10.9°C; the warmest month of the year is July with 20.9°C, the coldest month is January with -0.4°C. Mean annual precipitation is 647.2 mm, with the highest rainfall in June (84.3 mm), and the lowest rainfall in February (41mm). Based on the general site conditions, it can be concluded that Douglas-fir trees grow under uniform general stand conditions. There are 2,083 trees per hectare, mean stand diameter ranges from 6.8 to 15.5 cm, and mean height ranges from 4.8 to 11 m.

The intensity and dynamics of the physiological processes of conifer mineral nutrition is one of major indicators of tree productivity, adaptation and successful introduction, WARREN, LIVINGSTON, TURPIN (2004), PÉREZ-SOBA, VISSER (2004), BALSTER, MARSHALL, CLAYTON (2009).

In the research of nitrogen variability in Douglas-fir needles, one-year-old needles were collected from the upper third of the crown during the vegetation dormancy. The quantity of nitrogen in the one-year-old Douglas-fir needles was determined using Kjeldah's method, and the statistical analysis was performed using Statistics software package.

## RESULTS AND DISCUSSION

The analyses of Douglas-fir nutrition macroelements in the needles showed the positive correlation between the tree estimation elements and the nitrogen content. The quantities of nitrogen in the needles of the observed provenances accounted for 0.83% to 2.32% (Figure 1). The highest quantities of nitrogen were determined in the needles of the following Douglas-fir provenances: 11, 1, 4, 2, and 27 (above 2% ), and the lowest quantities were measured in the provenances 9 and 12 (below 1.5%)

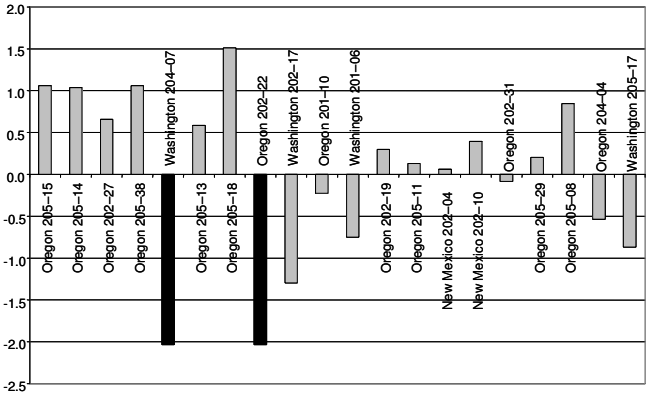


Figure 1. The variability of nitrogen content in Douglas fir needles

The research results point to a high variability of nitrogen content in Douglas-fir needles of different provenances, when they are grown under uniform site and stand conditions in Serbia. The highest quantity of this nutritive element was determined in the provenance Oregon 205-18, accounting for 2.33%. The lowest quantities of nitrogen in the needles were found in the provenances Washington 204–07 and Oregon 202–22.

The analysis of the multiple linear correlation shows that in Douglas-firs grown in Serbia, nitrogen uptake from the soil depended significantly on the geographical characteristics of the sites of the observed provenances in America (Table 2). The results of the experiments show that, under the same site conditions, lower quantities of nitrogen were absorbed from the soil by the provenances which originated from greater latitudes, and simultaneously from higher altitudes.

*Table 2: Regression Summary for Dependent Variable: N*

| | Beta | Std.Err. | B | Std.Err. | t(17) | p-level |
|---|---|---|---|---|---|---|
| Intercept | | | 6.7582 | 1.7607 | 3.8383 | 0.0013 |
| **GS** | -0.9420 | 0.328057 | -0.1039 | 0.0362 | -2.8714 | 0.0106 |
| **NV** | -0.7900 | 0.328057 | -0.0005 | 0.0002 | -2.4082 | 0.0277 |

R= 0.57247 $R^2$= 0.3277 Adjusted $R^2$= 0.2486
F(2,17)=4.1436 p<0.0342 Std.Error of estimate: 0.3643

The higher altitude and greater latitude certainly imply a colder and more humid climate, as well as a shorter growing season. At the same time, these conditions to a great extent slow down the decomposition of organic matter and also the mineralization of organic nitrogen and its occurrence in mineral forms and plant available forms. Since the observed provenances from different geographical areas of the USA developed under identical site and stand conditions in Serbia, the research results show that the provenances from colder and more humid areas adapted to the lower nitrogen requirements.

The results of the analysis of intra- and inter-provenance variability in the nitrogen content in the needles of the observed Douglas-fir provenances show that mean diameter (Figure 2), mean height (Figure 3), basal area (Figure 4), volume (Figure 5) and volume increment (Figure 6) of the trees did not depend on nitrogen content in Douglas-fir needles.

Starting from the fact that nitrogen plays a great role in different physiological processes in plant cells and tissues, numerous mutual growth processes requiring nitrogen are too complex to be presented only based on the content of this element in the needles. Still, the results of these analyses are important as they show the scope of variability in nitrogen content and the requirements of different Douglas-fir provenances at the sites in Serbia. Since all provenances were studied on

small sample plots with practically uniform site conditions, and as the supply of plant available nitrogen compounds in the soil was good, the variability in the content of nitrogen in the needles was primarily conditioned by the genetic specificities of the provenances.

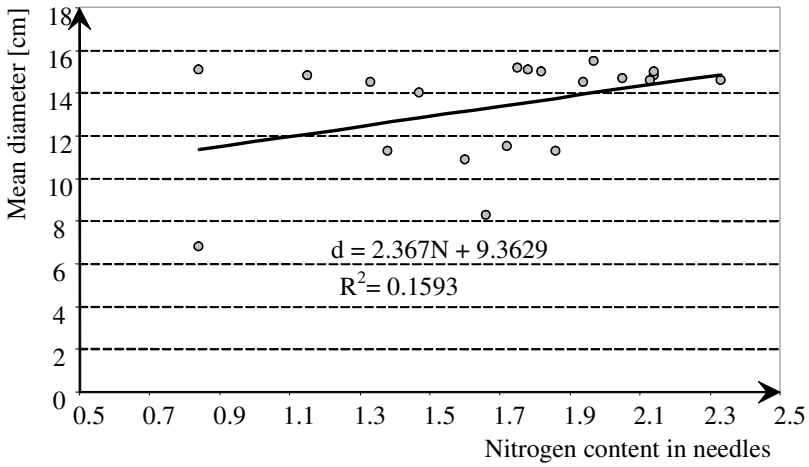


Figure 2. The dependence of the mean diameter of tree on the nitrogen content in Douglas fir needles

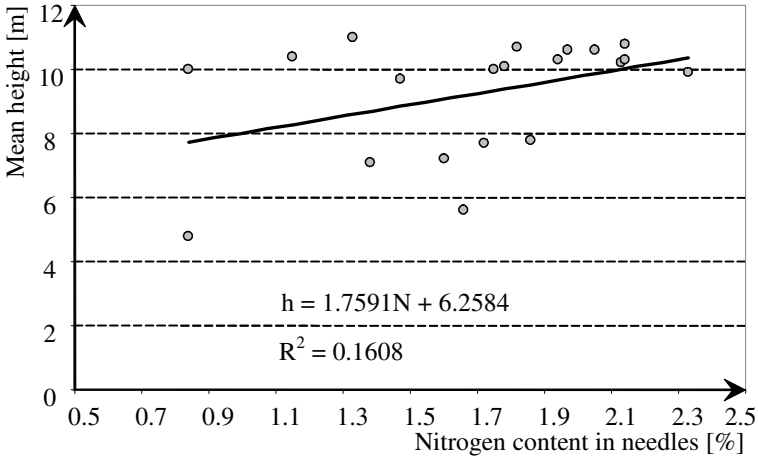


Figure 3. The dependence of mean tree height on the nitrogen content in Douglas fir needles

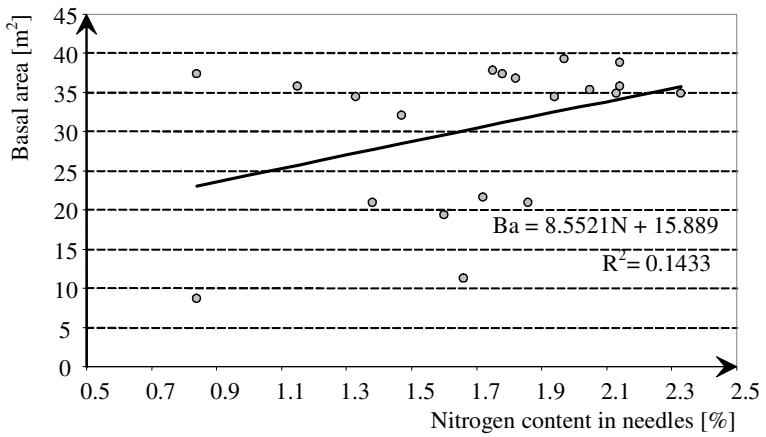


Figure 4. The dependence of basal area on nitrogen content in Douglas fir needles

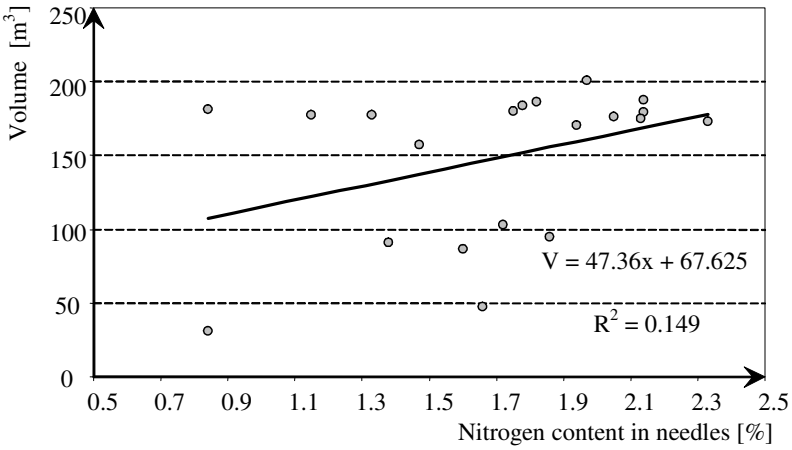


Figure 5. The depedence of tree volume on nitrogen content in Douglas fir needles

Based on the results of the analyses of variability of basic nutrient concentrations in Douglas-fir needles, it can be concluded that there were no major differences between the provenances. Taking into account the physico-chemical characteristics of the soil on which provenance tests were established, the relative uniformity of nutrient concentrations in Douglas-fir needles was mainly the result of uniform site characteristics of the sample plots, i.e. the good supply of plant

available nutrients in the soil. The inter-provenance variability of estimation elements was mainly conditioned by their genetic specificities, and not by the complex of ecological features of the sites in Serbia.

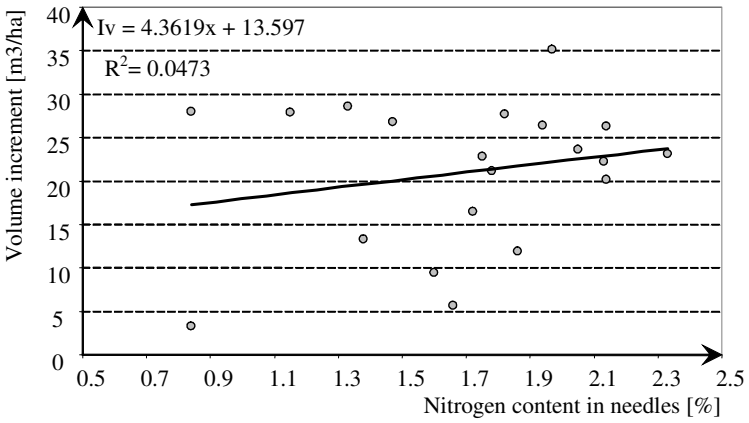


Figure 6. The dependence of volume increment on nitrogen content in Douglas fir needles

The differences in nitrogen concentrations in different Douglas-fir provenances point out the specificities in the absorption of this element. However, it is not a sufficient criterion for the evaluation of the photosynthetic production of the particular provenances, since they occur as the mixture of different genotypes, all of which, probably, differ in their ability to use certain elements in metabolic processes. Further researches on genetic-physiological traits of the selected provenances will reveal the genotypes characterized by the most desirable mineral nutrition demands and thereby the production of organic matter. According to PALLARDY (2008), it can be expected that different concentrations of mineral elements and production of nutrients can be obtained in different genotypes. In the economic sense, the best results will be obtained by using the genotypes which require lower quantities of mineral elements, with simultaneous high production of organic matter. The use of such genotypes for mass production of the reproductive material will provide the base for the establishment of economic plantations, which will be characterized by the high production of organic matter even at the poor sites in Serbia. The above researches of 20 Douglas-fir provenances showed the need of differentiated, i.e. specific nutrition of the trees from different geographical regions of this species in North America. However, sufficient attention has not been paid to this problem so far. The obtained results should be used in the nursery production, aiming at the increase in biomass production, by the input of small or no additional investments in fertilisation, which would reduce the production costs. The importance and relevance of the study results is in the fact that they can be used as the starting point for the future selection of suitable genotypes, as well as plus trees and test trees in seed stands of those provenances that were superior in the nitrogen content. Among the

genotypes which will be selected in future there will certainly be some which, by the absorption of the lower quantities of this element, will produce higher quantities of biomass and seeds, or will be tolerant to environmental conditions. These genotypes will be very important during the future establishment of the Douglas-fir plantations for different purposes in Serbia. The introduction of mycorrhizal fungi via the specially conditioned seedlings on poor soils would improve the uptake of water and minerals, as it is reported that, at autochthonous sites in North America, the effects of ecto- and ectendomycorrhiza of Douglas-fir with more than 2,000 species of mycorrhizal fungi are extremely favourable (Douglas-fir, *Pseudotsuga taxifolia*, 1970, U.S.D.A Forest Service).

## CONCLUSION

The differences in nitrogen content in Douglas-fir needles indicate the variability in the intensity of physiological processes in the genotypes of different provenances. Since Douglas-fir trees grow and develop in relatively small areas, with more or less uniform general conditions, it can be concluded that the parameters of mineral nutrition are determined by the different genotypes which constitute the gene pool of the observed Douglas-fir provenances. The study results point out the high nitrogen content variability in Douglas-fir needles of different provenances, when they are grown under uniform site and stand conditions in Serbia. The highest quantity of this nutritive element was reported in the provenance Oregon 205-18, accounting for 2.33%. The lowest quantity of nitrogen in the needles was found in provenances Washington 204–07 and Oregon 202–22. The determined range of variability can be implemented in the selection of less demanding provenances, which can be used also for the reforestation of the sites on different soil types, i.e. the soils characterized by a lower content of mineral nutrients.The statistically significant relations between nitrogen concentration in the needles and average tree heights indicate that the nourishment of Douglas-fir trees was uniform in all the study provenances. Further researches, with much more uniform material, preferably collected at the level of the selected test trees at the local autochthonous sites and within the half-sib and full-sib lines, will provide the results which can be used with more reliability in the establishment of Douglas-fir plantations in Serbia, which will be very important for the future selection of species and in the calculation of the necessary quantities of fertilisers.

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## VARIJABILNOST SADRŽAJA AZOTA U ČETINAMA PROVENIJENCIJA DUGLAZIJE (*Pseudotsuga menziesii* Mir / Franco)

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I z v o d

U provenijeničnom ogledu duglazije (*Pseudotsuga menziesii* Mir / Franco) osnovanom na staništu brdske bukve na kiselom smeđem zemljištu ispitivan je sadržaj azota u četinama dvadeset provenijencija duglazije poreklom sa različitih lokaliteta u okviru prirodnog areala vrste u SAD. Sva stabala ispitivanih provenijencija su iste starosti i odgajene pod jednakim staništnim i populacionim uslovima. Konstatovana je varijabilnost sadržaja azota u četinama duglazije.Razlike u sadržaju azota u četinama stabala duglazije ukazuju na postojanje varijabilnosti u intenzitetu fizioloških procesa kod genotipova različitih provenijencija. S obzirom na to da stabla duglazije rastu i razvijaju se na relativno malim površinama, sa manje-više ujednačenim opštim uslovima, može se zaključiti da su parametri mineralne ishrane determinisani različitim genotipovima koje čine genofond analiziranih provenijencija duglazije.Izražena varijabilnost za ukupni sadržaj mineralnih materija u četinama duglazije (gde je sadržaj pepela u dijapazonu od 3,30% kod provenijencije 22 do 9% kod provenijencije 17), kao i za promenljivost sadržaja azota, pokazatelj je specifičnosti genofonda različitih provenijencija prema mineralnoj ishrani. Utvrđeni opseg variranja je od aplikativnog značaja za selekciju manje zahtevnih provenijencija, koje se mogu koristiti i za pošumljavanja na lokalitetima sa različitim tipovima zemljišta, tj. onim koja se odlikuju skromnijim sadržajem mineralnih materija. Statistički signifikatne veze između koncentracije azota četinama i prosečnih visina stabala, ukazuje na to da je stanje ishrane stabala duglazije bilo ujednačeno kod svih ispitivanih provenijencija.Dobijene rezultate treba prihvatiti kao preliminarne. Oni nisu dovoljan kriterijum za vrednovanje uticaja parametara hranljivosti zemljišta na adaptabilnost duglazije na alohtonim staništima, zbog karakteristika uzorka koji je korišćen u analizama. Uzorak koji je korišćen za analize sastavljen je od velike mešavine različitih genotipova koji se verovatno razlikuju u sposobnostima za usvajanje i korišćenje hranljivih elemenata zemljišta i predstavljaju uopšteni pokazatelj na nivou vrste.

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