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GENETIC VARIABILITY OF WHEAT GERMPLASM REPRESENTED IN THE SOUTH PANNONIAN REGION

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In this study, genetic variability was investigated among 50 winter wheat varieties (*Triticum aestivum* L.) which are grown in parts of Croatia, Hungary, Serbia and Slovenia according to 22 morphological characteristics used for DUS (distinctness, uniformity and stability) testing. The average Dice similarity coefficient was 0.371. The determined similarity coefficient was in range 0.083 – 0.776. A significant variability of 6.21% in the breeding programs according to period was determined as well as significant variability of 3.10% between breeding programs. The UPGMA clustering divided investigated varieties into four main clusters. Based on data analysis, most distant varieties with best morphological characteristics were found which will provide valuable resource of new parent's combinations in future breeding programs. This paper also provided valuable assessment of morphological characteristics to define distinctness criteria in the DUS examination of wheat.

Key words: cultivars, DUS, similarity, wheat, variability

INTRODUCTION

Common wheat (*Triticum aestivum* L.) is the most widespread cultivated plant species for human consumption. In various forms wheat is used by more than one billion people in the world and it is grown on more than 220 million hectares, with a total annual production of about

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729 million tones (FAOSTAT, 2015). Continental Croatia is located in the southern part of the Pannonian region with an average wheat production of about 645 000 tones, which is grown on average at about 157 000 ha (CROATIAN BUREAE OF STATISTIC, 2015).

Genetic variability is one of the factors important for plant breeding. Gathering and collecting germplasm resources and genetic variability represent foundation of any breeding process (ALI *et al*., 2008; NEUMANN *et al*., 2011; NOVOSELSKAYA-DRAGOVICH *et al*., 2011). In breeding process, the choice of suitable parents is extremely important to ensure a wide genetic variability and thus allow selection of desirable genotypes from crossing (REIF *et al*., 2005; BEDE and PETROVIĆ, 2006).

Testing and evaluation of varieties morphological differences is of a great importance for breeding process because in classical breeding process determination of variability between varieties is based on a large number of morphological characteristics. In the last years, the numerous studies of genetic variation of morphological traits were conducted on a range of different species (SMYKAL *et al*., 2008; ČUPIĆ *et al*., 2009; TASUNOVA *et al*., 2010; TUCAK *et al.*, 2011; LI *et al*., 2012; RUKAVINA *et al*., 2013; DENČIĆ *et al*., 2015). For most species the first genetic maps were made using morphological markers and yet are still used today in many cultivars. The morphological characteristics are also used to describe new varieties when examining distinctness, uniformity and stability (DUS) in process of registration of new plant varieties as well as in process of granting plant breeder's rights (JONES *et al*., 2003; RUKAVINA *et al*, 2008). There are different opinions about the advantages and disadvantages of using morphological characteristics. The main disadvantages of morphological characteristics are the influence of environmental factors and developmental stage of the plant (WINTER and KAHL, 1995), while the SMYKAL *et al*. (2008) reports about main reasons and benefits of using morphological characteristics such as large number of characteristics and easier way of monitoring and evaluation.

Analysis of morphological characteristics gave a clear insight into the existing diversity in terms of breeding centers and a year of registration and directs towards the most diverse genotypes that can be used as a parent lines for a new selection cycle. The goal of the present study was to provide a clear assessment of the variability of wheat germplasm created in south Pannonian region. This paper also provides valuable assessment of morphological characteristics to define distinctness criteria in the DUS examination of wheat.

MATERIALS AND METHODS

Plant material and field trials

Research was conducted on 50 varieties of hexaploid wheat (*Triticum aestivum ssp. vulgare* L.) from five breeding centers but with a broad genetic origin. The breeding centers are located in continental Croatia. Varieties were selected based on market share and their significance in the production. The 17 varieties were from Agricultural Institute Osijek (PIO), 15 from BC Institute for Breeding and Production of Field Crops Zagreb (BC), 12 from Agrigenetics Ltd. (AG), 4 from Jost Seed-Research Ltd. Križevci (Jost) and 2 varieties from the Faculty of Agriculture, University of Zagreb (AFZG) (Table 1).

Trials have also included standard example varieties for studied morphological characteristics that are listed in the CPVO (Community Plant Variety Office) TP/003/4 Rev.2 (2011), protocol for wheat and their role was to clarify the expression of each of the observed characteristics.

Table 1. List of investigated winter wheat varieties

No.	Variety	Year of release	Origin	No.	Variety	Year of release	Origin
1.	FIESTA	1998	AG	26.	BC RENATA	2007	BC
2.	GABI	1999	AG	27.	BC LIDIJA	2009	BC
3.	MURA	2001	AG	28.	BC LIRA	2009	BC
4.	ATENA	2003	AG	29.	BC IRENA	2010	BC
5.	NIKA	2003	AG	30.	CERERA	1993	Jost
6.	HELIA	2005	AG	31.	DIVANA	1995	Jost
7.	KALISTA	2005	AG	32.	KOLEDA	1998	Jost
8.	MATEA	2005	AG	33.	TALIA	2004	Jost
9.	ANIKA	2006	AG	34.	ŽITARKA	1985	PIO
10.	DEA	2009	AG	35.	SRPANJKA	1989	PIO
11.	UNA	2009	AG	36.	BARBARA	1997	PIO
12.	EMA	2010	AG	37.	GOLUBICA	1997	PIO
13.	BANICA	1997	AFZG	38.	SUPER ŽITARKA	1997	PIO
14.	AFZG KARLA	2010	AFZG	39.	LUCIJA	2001	PIO
15.	SANA	1983	BC	40.	PANONKA	2001	PIO
16.	ADRIANA	1988	BC	41.	ALKA	2003	PIO
17.	MARIJA	1988	BC	42.	JANICA	2003	PIO
18.	MIHELCA	1996	BC	43.	SEKA	2006	PIO
19.	ZDENKA	1996	BC	44.	KATARINA	2006	PIO
20.	AURA	1997	BC	45.	AIDA	2006	PIO
21.	NINA	2000	BC	46.	FELIX	2007	PIO
22.	PRIMA	2001	BC	47.	ZLATA	2007	PIO
23.	BC ANTEA	2002	BC	48.	ILIRIJA	2008	PIO
24.	BC ELVIRA	2002	BC	49.	OLIMPIJA	2009	PIO
25.	BC MIRA	2007	BC	50.	NOVA ŽITARKA	2010	PIO

Field experiments were set up in field trials for DUS testing according to CPVO TP/003/4 Rev.2 (2011) on locations Osijek 45°32'N and 18°44'E (main location) and Klisa 45°46'N and 18°1'E (reserve location). At both locations trial was set up according to randomized block design with 2 replications and plot size was 6.25 m². Each plot included 200 plants / m², a total of 1250 plants per basic plot. For this study, the evaluation of morphological characteristics was conducted at both locations during the two growing seasons (2012/2013 and 2013/2014).

Additional trials for the characteristic - seasonal type were set up on location Klisa in two years according to CPVO TP/003/4 Rev.2 (2011). For this purpose all investigated varieties were sown in the spring time 2013 and 2014, out of frosts. Trials also included varieties Fidel and Slejpner, examples for the specified characteristic. The regular maintenance and protection measurements were conducted on trials.

Morphological characteristics

Variability of varieties was analyzed on the basis of 22 morphological characteristics: plant growth habit, frequency of plants with recurved flag leaves, time of era emergence, glaucosity of fleag leaf sheat, glaucosity of ear, glaucosity of neck, plant length, straw pith in cross section, shape of ear in profile, density of ear, presence of awns or scurs, length of awns or scurs at tip of ear, ear color, hairiness of convex surface of apical rachis segment, shoulder width of lower glume, shoulder shape of lower glume, beak length of lower glume, extent of internal hair of lower glume, grain color and seasonal type. Observations are done according to CPVO TP/003/4 Rev.2 (2011) protocol for DUS testing of wheat (Table 2).

Table 2. Morphological characteristics according to CPVO protocol for DUS tests of wheat

CPVO Nr.	Characteristics	Development phase[1]
2.	Plant: growth habit	25-29
3.	Plant: frequency of plants with recurved flag leaves	47-51
4.	Time of ear emergence (first spikelet visible on 50% of ears)	50-52
5.	Flag leaf: glaucosity of sheat	60-65
7.	Ear: glaucosity	60-69
8.	Culm: glaucosity of neck	60-69
9.	Plant: length (stem, ear, awns and scurs)	75-92
10.	Straw: pith in cross section (halfway between base of ear and stem node below)	80-92
11.	Ear: shape in profile	92
12.	Ear: density	80-92
13.	Ear: length (excluding awns and scurs)	80-92
14.	Awns or scurs: presence	80-92
15.	Awns or scurs at tip of ear: length	80-92
16.	Ear: color	90-92
17.	Apical rachis segment: hairiness of convex surface	80-92
18.	Lower glume: shoulder width (spikelet in mid- third of ear)	80-92
19.	Lower glume: shoulder shape (as for 18)	80-92
20.	Lower glume: beak length (as for 18)	80-92
21.	Lower glume: beak shape (as for 18)	80-92
22.	Lower glume: extent of internal hair (as for 18)	80-92
23.	Grain: color	92
25.	Seasonal type	-

[1] Decimal code of development phases according to EUCARPIA scale, Bulleti Nr.7.1974, pp. 49-52.

During each vegetation year seven morphological characteristics on 20 plants per plot were observed in field trials and the characteristic seasonal type. At the harvest time, sample of 120 ears per plot were taken in order to form samples of 20 ears for observation of 14 morphological characteristics: on the straw – pith in cross section; on the ear - shape in profile, density, presence of awns or scurs, length of awns or scurs at tip, color, hairiness of convex surface of apical rachis segment; on the lower glume: shoulder width, shoulder shape, beak length, extent of internal hair and on grain observation of color. In two growing seasons for each variety 1,120 plants were evaluated in the field and 2,240 ears in the laboratory, which was in total, for 50 investigated varieties, 56,000 observations in the field and 112,000 observations in the laboratory. Investigated varieties in the field trials were grouped according to the following characteristics recommended by CPVO (2011): straw – pith in cross section (halfway between base of ear and the stem node below), ear color, the presence of awns or scurs and seasonal type.

Statistical analysis

Statistical analysis included the results from 21 morphological characteristics. Due to the identical value for all varieties characteristic straw pith in cross section was excluded from the statistical analysis because it would have no impact on the determination of similarity. Based on the obtained notes the starting matrix was composed and used to calculate the Dice coefficient of genetic similarity (FERGUSON and CARSON, 2007). Similarities were calculated using the computer program NTSYS 2.2. (ROHLF, 2009). Similarity matrixes obtained from the morphological data were used to create a dendrogram using Unweighted Pair Group of Mathematics Average - UPGMA. The Mantel test was used for estimating the correlation between dendrogram and original matrixes (MANTEL, 1967). Similarity matrixes of morphological data were transformed to genetic distances for analysis of molecular variance - AMOVA (EXCOFFIER *et al*., 1992) to determine the genotypic variance between and within the assumed level structure of the tested varieties of winter wheat. Computer program Arlequin ver.3.5. (EXCOFFIER and LISCHER, 2010) was used for calculation of molecular variance.

RESULTS

Based on the analysis of wheat varieties morphological characteristics data, classification of investigated varieties was made according to the distribution of different expression states (Supplement 1). The average Dice genetic similarity coefficient (S_{ij}) was 0.371. Average similarity value between breeding centers was ranged from 0.29 to 0.39. The lowest similarity coefficient was between AG and AFZG (0.29), while even similarity was between AG and BC (0.37), AFZG and BC (0.38), AFZG and Jost (0.37) and PIO and Bc (0.39). The highest similarity within breeding center had Jost Seed-Research Ltd. (0.53) and lowest similarity had Agricultural Faculty Zagreb (0,24). The highest similarity coefficient was determined between varieties Cerera and Koleda (0.776), Aura and Zdenka (0.667), Bc Irena and Zdenka (0.655), Bc Mira and Zdenka (0.625) and varieties Prima and Nina (0.615). The lowest similarity value was 0.083 between varieties Ilirija and Panonka (Supplement 2).

The UPGMA clustering (Figure 1) divided investigated varieties into four main clusters. Cluster I was further divided into two sub clusters. First sub cluster consisted of AFZG Karla and in second sub cluster were grouped Ilirija, Talia, Helia, Mihelca, Kalista, Matea, Anika, Ema, Divana, Dea, Olimpija, Bc Lira, Cerera and Koleda. Cluster II comprised of Una, Barbara, Žitarka and Super Žitarka, while varieties Lucija, Seka, Gabi, Srpanjka and Panonka were grouped in cluster III. Cluster IV was further divided into four sub clusters. Variety Janica was allocated into first sub cluster, Nina and Prima were grouped into second sub cluster. Third sub cluster comprised of Adriana, Bc Elvira, Bc Mira, Alka, Banica, Bc Lidija, Nika, Zdenka and Aura, while fourth sub cluster comprised of Mura, Atena, Bc Renata, Fiesta, Marija, Golubica, Katarina, Aida, Felix, Zlata, Sana, Bc Irena, Bc Antea and Nova Žitarka. Correlation coefficient between similarity matrix and dendrogram (0.68) was highly significant ($P < 0.001$) after 1.000 permutations of Mantel test.

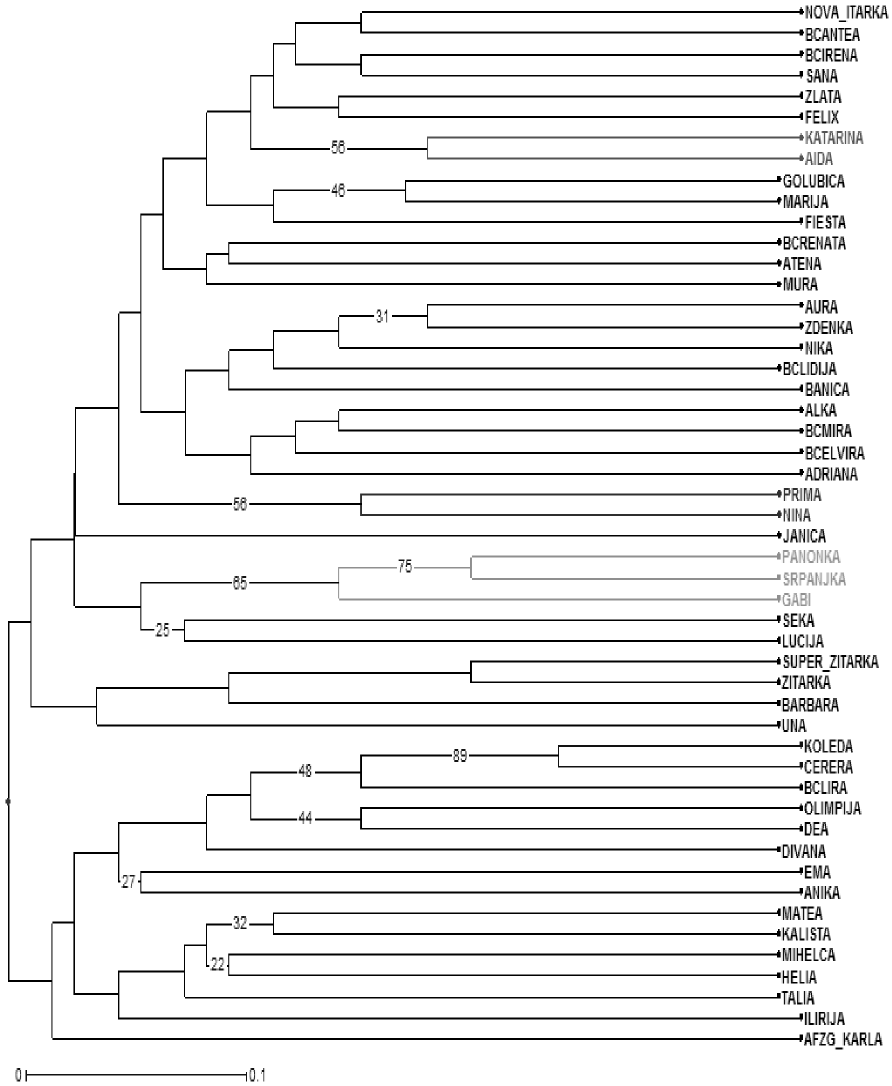

Figure 1. Dendrogram of the 50 wheat varieties based on morphological characteristics using Dice coefficient of genetic similarity

AMOVA analysis determined the distribution of the total variance based on the morphological characteristics data sets on levels of structure between breeding programs, within the breeding program by period, and between varieties at the period of the program. Periods are defined by year of release of varieties and the first period was until 2001 (including the year 2001), and the second period was after 2001. Largest share of variability (90.69%) was accounted for the differences between the varieties at the period of the program (Table 3). There was also a significant value of variability (6.21%) in the breeding programs according to period, and a significant value of variability (3.10%) between breeding programs.

Table 3. Results of AMOVA analysis based on morphological data

Source	**df**	**Sum of squares**	**Variance components**	**Share of variability %**	**φ**	**P(φ)**
Between programs	4	1.920	0.00993	3.10	0.03104	0.04
Within programs by period	5	1.902	0.1986	6.21	0.06408	0.003
Between varieties by period program	40	11.605	0.29012	90.69	0.09313	< 0.001
Total	49	15.426	0.31991			

DISCUSSION

Evaluation based on morphological differences between wheat varieties has a great importance for breeding because in the conventional breeding process determination of the variability between varieties is carried out with the assessment of a large number of morphological traits. Morphological characteristics are used when creating genetic maps, as well as to control the initial population and separating generation (ŠATOVIĆ, 1999). Today is an important application of morphological characters to describe new varieties when examining distinctness, uniformity and stability (DUS) in the process of variety registration as well as to plant variety protection (JONES *et al*., 2003; MARIĆ *et al*., 2004; RUKAVINA *et al*., 2008). Many researchers reported about the need to combine morphological traits with biochemical and molecular markers (ŠATOVIĆ, 1999; COOKE *et al*., 2003; COLLARD *et al*., 2005; BÖRNER, 2006).

This study determined relatively low average similarity value (0.371) among the investigated varieties which indicates the great morphological variability of wheat germplasm originally from continental Croatian as a south Pannonian region. Similar results were obtained by MARIĆ *et al*. (2004), ALI *et al*. (2008), SALEM *et al.* (2008) and PETROVIĆ (2011.) who found a relatively high distance between the tested varieties and UPGMA method showed the presence of significant genetic variability. Contrary to these results, MACCAFERRI *et al*. (2007) found the average genetic similarity value 0.73, and only a very distant line of durum wheat were distinguished on the basis of phenotype that included morphological characteristics recommended for DUS testing. The very low genetic similarity value (0.24) within breeding center Agricultural Faculty Zagreb was the result of the work of several breeding programs from which have been created investigated varieties.

Correlation coefficient of similarity matrix and dendrogram was 0.68 which indicate a good relationship between the results of similarity matrix and morphological cluster analysis. Genetic similarity of tested varieties based on morphological data showed clustering by the variety type and in some clusters by the origin. It should be noted that the characteristic no.10 (straw pith in cross-section) had the same state of expression for all investigated varieties and as such was excluded from the statistical analysis because it was determined that due to the expression there was no effect on estimating of distinctness, which is in accordance with research of JONES *et al*. (2003) and CABALLERO *et al*. (2010). Based on this knowledge appears that there is a need for proposal to Technical Working Party for Agricultural Crops of UPOV (International Union for the Protection of New Varieties of Plants) and Agricultural Expert Group of CPVO (Community Plant Variety Office) to delete this characteristic no.10 as a grouping characteristic and estimating distinctness in future revision of technical protocol for wheat DUS testing. According to obtained dendrogram, in the first cluster were grouped varieties which had present awns (Divana, Koleda, Cerera, Bc Lira, Olimpija, Dea, Ema, Anika, Ilirija, Talia, Matea, Kalista, Mihelca and Helia), and in sub cluster was separately allocated variety with awns AFZG Karla due to colored ear in stage of maturity. In this cluster sister lines Cerera and Koleda were pointed out due to result of same crossing NE 7060 76Y335 / VG-19 (Zlatna dolina X Kavkaz) and therefore it was expected for these varieties to have the highest similarity coefficient (0.776). Barbara, Super Žitarka, Žitarka and Una were aligned in second cluster and classification of the first three varieties can be associated with pedigree, because Žitarka is parental component of varieties Barbara and Super Žitarka. Reason of grouping Una in this group can be associated with the expression of the characteristics on the lower glume (shoulder width, shoulder shape, beak shape and extent of internal hair) and grain color. All of these are high yielding varieties belonging to the early to mid-early varieties, low to medium-high stem length, good quality and resistance to lodging (BEDE, 1994; PETROVIĆ, 2011; DREZNER, 2012). In particular sub cluster were grouped Seka, Lucija, Panonka, Srpanjka and Gabi belonging to the very early and early varieties, with assessed very narrow shoulder of lower glume. It can be concluded that the characteristics like date of ear emergence and shoulder width had an effect on the grouping of these varieties besides pedigree since Srpanjka is one of the parents is in the varieties Seka, Lucija and Gabi.

Fourth cluster included remaining varieties without awns, which were morphologically heterogeneous with respect to the characteristics of the ear and lower glume. Variety Janica allocated in a separate sub cluster, very close to the previous sub cluster, which can be associated with a pedigree because one of its parents is Srpanjka, but unlike the previous sub cluster it had strong expression of ear glaucosity as well as a different ear shape. Second sub cluster comprised of sister lines Prima and Nina which were the result of same crossing Sana / Gala and these varieties also had one of the highest similarity coefficient (0.615) which was expected. Grouping of varieties in third sub cluster (Alka, Bc Mira, Bc Elvira, Adriana, Banica, Bc Lidija, Aura, Zdenka and Nika), was under the influence of characteristic scurs length and medium-strong to very strong hairiness of apical rachis segment. Fourth sub cluster comprised of morphologically heterogeneous varieties, but it should be noted that variety Nova Žitarka was the only determined as alternative type after two years of seasonal testing in field trials.

According to AMOVA analysis high variation between varieties per period of breeding program was expected, since in the creation of varieties were used genetically different parents and different selection criteria. Significant, but much lower proportion of the variability between breeding programs can be linked to the fact that they relatively often use similar or partly shared parental components during the process of creating new varieties and in a very similar agro-climate conditions. Similar reasons for higher variability within, rather than between the assumed levels found ROUSSEL *et al*. (2004) for breeding centers in France, ROUSELL *et al.* (2005) for European countries, and PETROVIĆ (2011) for Croatian and foreign wheat.

CONCLUSION

Our data analysis based on morphological characteristics showed similarity coefficient ranging from 0.083 – 0.776 and it can be concluded that the use of morphological traits are of great importance for estimating the criteria of distinctness in DUS testing of wheat as well as for testing of genetic distances in wheat germplasm. On the basis of data analysis the farthest varieties of best morphological characteristics were found and that will provide the successful selection of new parent's combinations in future breeding programs.

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GENETSKA VARIJABILNOST GERMPLAZME PŠENICE ZASTUPLJENE U JUŽNO PANONSKOJ REGIJI

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Izvod

Gajenje kukuruza je nezamislivo bez primene herbicida, međutim zavisno od osetljivosti kukuruza moguća je pojava oštećenja od herbicida, što može negativno da utiče na prinos. To je najizraženije u proizvodnji semenskog kukuruza, zahvaljujući osetljivosti linija na razne stresne uslove uključujući i herbicide. Reakcije biljaka na herbicide je praćen velikim brojem biohemijskih reakcija koje uključuju i različite metabolite i antioksidante. Eksperiment je postavljen u cilju ispitivanja tri osetljive linije kukuruza (linije šećerca, kokičara i belog zrna) i variranja sadržaja rastvorljivih proteina, fitinskog i neorganskog fosfora kao značajnih metabolita na uticaj herbicida iz grupa triketona i sulfonilurea u periodu posle primene herbicida, kada su vizuelni simptomi najizraženiji i najviše koreliraju sa prinosom zrna. Variranja u sadržaju rastvorljivih proteina i naročito fitinskog i neorganskog fosfora ukazuju na osetljivost linija prema primenjenim herbicidima. Godina kao faktor je imala uticaja na ispoljavanje osetljivosti, kada su sadržaj rastvorljivih proteina i neorganskog fosfora u fazi 2-7 dana posle primene herbicida, kao i u fazi 21 dan posle primene bili praćeni smanjenjem prinosa zrna. Najveća osetljivost je zabeležena kod linije ZPT165b, u vidu najviših vrednosti ispitivanih metabolita u početnim fazama, naročito u nepovoljnoj godini za gajenje kukuruza. Od svih primenjenih herbicida, nikosulfuron je pokazao najmanju selektivnost utičući na smanjenje sadržaja ispitivanih metabolita.

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