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Technological quality traits phenotyping of *Camelina* across multienvironment trials

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ABSTRACT

Camelina (*Camelina sativa* [L.] Crtz.) is an oilseed crop of the *Brassicaceae* family. It represents revived crops, which was reintroduced to contribute in diversification of the used crops. Field trials were set in a total of five different locations across four years in which in Serbia (2015/2016, 2016/2017, 2017/2018, 2018/2019) and in Austria (2016/2017). In all samples, 12 different environments were processed. Analysis of the distributions of environments for oil content, which mirrored protein content, using the PCA analysis, shows a statistical significance of the first two main components, which participated in the phenotypic variation with a larger amount to additive component and with both axes having a statistically significant effect on the interaction. The first two main components jointly explained more than 80% of the variation in two traits. The statistical significance of the remainder is a consequence of an agronomically explicable variation. The genotypes NS Zlatka and NS Slatka, used in this study, come from elite breeding material that is engineered to provide a consistent return to the producer regardless of the amount of money invested in primary agricultural production. Incorporating these varieties into the crop rotation would broaden the range of species available in Balkan agriculture.

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Introduction

Camelina (*Camelina sativa* [L.] Crtz.) is an oilseed crop of the *Brassicaceae* family. It represents revived crops, which was reintroduced to contribute in diversification of the used crops, sideward its use as a fuel. *Green revolution* itself led to an intensification of agriculture and a fall in the number of crop species to less than 10 (FAO, 2019). Serbian agriculture has been dominated by a few high yield crops which deliver in enhanced vulnerability to diseases and do not go along with modern principles of agriculture and functional agro-biodiversification. *Camelina* could be necessary refreshment in crop rotation and also can be used as a cover crop (Gesch and Cermak, 2011; Zanetti et al., 2021). Usage of *Camelina* spring types as cover crops is a relatively recent innovation, which has been examined experimentally within areas of Minnesota (Johnson et al., 2017), thereby necessitating fine-tuning of agronomic practices on heterogeneous locations (Ott et al., 2019). But, before including it in intense agriculture a detailed agronomic analysis of all kinds should be done. Competitiveness in agronomic performance as compared to established oil crops is a

key factor for the economically successful establishment of a new crop. It has been listed among the most promising new crops for oil production in temperate regions due to its wide adaptability (Vollmann and Eynck, 2015; Kuzmanović et al., 2021). Different from other cultivated *Brassicaceae*, *Camelina* has a unique seed oil composition, with a low content of the undesirable fatty erucic acid, less than 4% (Zanetti et al., 2017). The improvement of oil quality has rarely been a target in *Camelina* breeding so far. However, higher concentrations of linolenic acid, which can only be supplied from linseed oil, would be required for different technical applications (Vollmann et al., 1997). Although some literature is available regarding the metabolic traits of this crop, reliable phenotyping of technological quality traits is lacking (Anderson et al., 2019). Multi Environment Trials (MET) makes it possible to identify genotypes that display a small temporal variability that is desired by and is beneficial to growers or cultivars that are consistent from location to location, which is desired by and is beneficial to seed companies and breeders. One of the main challenges in this type of field trials is to understand the genotype-environment

Table 1. List of environments and used codes.

| Code | | Location | Country | Year | Sowing season | Date of sowing | Date of harvest |
|------------|------|----------------|---------|------|---------------|----------------|-----------------|
| E1 | SO16 | Sombor | Serbia | 2016 | Spring | 01/04 | 01/07 |
| E2 | NS16 | Novi Sad | Serbia | 2016 | Spring | 29/03 | 01/07 |
| E3 | PA16 | Pančevo | Serbia | 2016 | Spring | 01/04 | 01/07 |
| E4 | SO17 | Sombor | Serbia | 2017 | Spring | 22/03 | 03/07 |
| E5 | NS17 | Novi Sad | Serbia | 2017 | Spring | 23/03 | 04/07 |
| E6 | PA17 | Pančevo | Serbia | 2017 | Spring | 28/03 | 03/07 |
| E7 | BP17 | Bački Petrovac | Serbia | 2017 | Spring | 22/03 | 04/07 |
| E8 | RŠ17 | Rimski Šančevi | Serbia | 2017 | Spring | 22/03 | 04/07 |
| E9 | TN17 | Tulln | Austria | 2017 | Spring | 25/03 | 01/07 |
| E10 | BP18 | Bački Petrovac | Serbia | 2018 | Autumn | 23/10 | 17/07 |
| E11 | RŠ18 | Rimski Šančevi | Serbia | 2018 | Autumn | 03/10 | 12/07 |
| E12 | RŠ19 | Rimski Šančevi | Serbia | 2019 | Spring | 03/04 | 05/07 |

interaction, seeking new ways of exploiting it and using it to benefit in the selection process (Olivoto et al., 2019).

The objectives of this study were to (1) determine seed yield, as an agronomic trait, and to determine oil and protein content as lead technological quality traits across 12 different environments to possibly select suitable genotype for each condition and to (2) identify the share of additive component and interaction in total phenotypic variability.

Material and methods

Field experiment

Field trials were seeded at five different locations across four years in which in Serbia (2015/2016, 2016/2017, 2017/2018, 2018/2019) and in Austria (2016/2017). In total samples 12 different environments will be processed (Table 1).

Trials lined at Sombor (45°77'N, 19°11'E and at 90 m elevation), Pančevo (44°56'N, 20°43'E and at 77 m elevation) and Novi Sad (45°23'N, 19°82'E and at 80 m elevation) [Serbia] were organised by the Department for Plant Variety Registration, Ministry of Agriculture. Trials lined at Rimski Šančevi (45°31'N, 19°82'E and at 82 m elevation) and Bački Petrovac (45°36'N, 19°59'E and at 86 m elevation) were conducted by the Institute for Field and Vegetable Crops, Novi Sad, Serbia. Austrian partner who obtained field trials in Tulln (48°19'N, 16°04'E and at 178 m elevation) and later results was the Department of Applied Plant Sciences and Plant Biotechnology, BOKU-University of Natural Resources and Applied Life Sciences. The soils on all five locations were quality chernozems followed with good humic horizons and a favourable air–water regime. Soils in Novi Sad, Rimski Šančevi, Pančevo and Bački Petrovac are classified as Calcic Chernozem, plots in Sombor as a Gleyic Chernozem (IUSS Working Group WRB, 2015). Whilst in Austria the soil at the trial site is classified as Calcaric Chernozem (Neugschwandter et al., 2019). Plots area varied from 2 m² in Tulln to 10 m² on

Serbian locations and on all plots seeding was accomplished by a plot drill. Trials in Serbia were arranged as a completely randomised block system in four replications, with an exception on E8 which was the same as in Austria – *alpha lattice* in three replications, from which these genotypes were singled out. Weeds were controlled entirely by hand. Harvest dates ranged from 1 July up to 17 July in Serbia, which was caused by dry conditions (Table 2).

Two different type camelina cultivars were tested in MET (Multi Environmental Trials), listed in Table 1. Both used genotypes NS Slatka and NS Zlatka are developed in the Institute for Field and Vegetable Crops (Novi Sad, Serbia) and recognised by amenable Ministry of Agriculture, Forestry and Water Management. Genotype NS Slatka was created by the process of self-fertilisation in a period of five years from Banat population of camelina, while NS Slatka was formed by the same process across five years from Ukrainian variety Stepski-1.

Oil and protein content

The total oil content of seeds was determined, as described in AOAC *Official Methods of Analysis 920.39* (1990). Protein content in Rimski Šančevi and Bački Petrovac was done *via* nitrogen content of seeds, which was determined using a *Modified Kjeldahl Procedure*, as described by AOAC *Official Method 988.05* (1990). Protein content in Sombor, Novi Sad and Pančevo was determined by the non-invasive and non-destructive technique of *Nuclear Magnetic Spectroscopy* in Agricultural Extension Service Sombor. Protein content in Tulln, Austria was determined non-destructively from whole seeds by *Near-Infrared Reflectance Spectroscopy* (NIRS).

Statistical analyses

Mean value was calculated for all analyses and presented as biplots. Also, the data were graphically analysed for interpreting GE interaction using the PCA biplot software, Statistica, Inc 2020.

Table 2. Weather conditions of temperatures and precipitation across five locations (Sombor, Novi Sad, Rimski Šančevi, Bački Petrovac and Tulln) in 2016, 2017, 2018 and 2019.

| 2016 | Temperature | | | | | | | | | | | | Average |
|-----------|---------------|----------|-------|-------|-------|-------|-------|--------|-----------|---------|----------|----------|---------|
| | January | February | March | April | May | June | July | August | September | October | November | December | |
| Sombor | 0.7 | 6.5 | 7.4 | 13.5 | 16.4 | 21.4 | 22.8 | 20.5 | 17.7 | 10.1 | 5.6 | -0.3 | 11.86 |
| Novi Sad | 1.3 | 7.5 | 7.8 | 14.2 | 16.9 | 21.7 | 22.8 | 21.1 | 18.5 | 10.2 | 6.3 | -0.3 | 12.33 |
| Pančevo | 2.3 | 9 | 9.1 | 15.5 | 17.5 | 22.5 | 24.4 | 22.3 | 19.7 | 11.1 | 7.7 | 0.9 | 13.50 |
| 2017 | | | | | | | | | | | | | |
| Sombor | -5.3 | 3.3 | 9.7 | 11.4 | 17.7 | 22.8 | 23.2 | 23.3 | 16.4 | 11.8 | 6.7 | 3.2 | 12.02 |
| Novi Sad | -4.9 | 4.2 | 9.9 | 11.4 | 17.6 | 23.2 | 24.3 | 24.8 | 16.9 | 12.5 | 7.1 | 3.8 | 12.57 |
| Pančevo | -3.3 | 5.4 | 11.5 | 12.7 | 18.4 | 24.3 | 25.9 | 26.1 | 18.4 | 13.9 | 8.4 | 5.1 | 13.90 |
| Bački P. | -5.1 | 4.3 | 10 | 11.9 | 18.7 | 23.8 | 24.8 | 25.1 | 17.4 | 12.5 | 7.1 | 3.7 | 12.85 |
| Rimski Š. | -4.9 | 4.2 | 9.9 | 11.4 | 17.6 | 23.2 | 24.3 | 24.8 | 16.9 | 12.5 | 7.1 | 3.8 | 12.57 |
| Tullin | -3.6 | 2.9 | 8.6 | 9.8 | 16.4 | 21.7 | 21.7 | 21.8 | 14.4 | 11.4 | 5.4 | 2.3 | 11.07 |
| 2018 | | | | | | | | | | | | | |
| Bački P. | 4.2 | 1.4 | 5.1 | 17.4 | 20.8 | 21.7 | 22.4 | 24.2 | 18.6 | 14.7 | 7.8 | 1.6 | 13.33 |
| Rimski Š. | 4.3 | 1.2 | 5 | 17.2 | 20.4 | 21.5 | 22 | 24 | 18.5 | 14.8 | 8 | 1.7 | 13.22 |
| 2019 | | | | | | | | | | | | | |
| Rimski Š. | -0.1 | 4.2 | 9.8 | 13.4 | 14.7 | 23.2 | 23.3 | 24.4 | 18.2 | 13.8 | 11.2 | 4.6 | 13.39 |
| | | | | | | | | | | | | | Sum |
| 2016 | Precipitation | | | | | | | | | | | | |
| Sombor | 48.3 | 82.9 | 54.8 | 22 | 62 | 101.2 | 115.2 | 81.5 | 68.7 | 77.7 | 44.9 | 2.4 | 761.6 |
| Novi Sad | 51.3 | 98.4 | 65.5 | 74.5 | 85 | 143.2 | 68.4 | 45.8 | 33.7 | 84.8 | 67.1 | 2.2 | 819.9 |
| Pančevo | 46.3 | 38.5 | 102.6 | 53.9 | 71.3 | 152.2 | 35 | 60.8 | 47.8 | 76.8 | 71.8 | 2.6 | 759.6 |
| 2017 | | | | | | | | | | | | | |
| Sombor | 21.8 | 45.5 | 44.8 | 46.2 | 55 | 51.8 | 55.6 | 24.3 | 69.4 | 48.7 | 33.5 | 46.1 | 542.7 |
| Novi Sad | 18.5 | 20.1 | 30.5 | 57 | 82.9 | 65.7 | 12 | 17.4 | 81.5 | 38.9 | 40.3 | 48.3 | 513.1 |
| Pančevo | 23.4 | 23.5 | 27 | 51.8 | 86.1 | 53 | 26.4 | 19.5 | 45.8 | 65.9 | 41.2 | 45.2 | 508.8 |
| Bački P. | 14.8 | 18.3 | 26.3 | 48.6 | 50.2 | 14.5 | 22.1 | 28.1 | 55.4 | 23.3 | 33 | 40.6 | 375.2 |
| Rimski Š. | 18.5 | 20.1 | 30.5 | 57 | 82.9 | 65.7 | 12 | 17.4 | 81.5 | 38.9 | 40.3 | 48.3 | 513.1 |
| Tullin | 21 | 25 | 29 | 76 | 69 | 50 | 106 | 57 | 79 | 61 | 26 | 23 | 622 |
| 2018 | | | | | | | | | | | | | |
| Bački P. | 40.8 | 77.1 | 52.1 | 42.3 | 63.1 | 109.8 | 89.4 | 21.1 | 42.7 | 5.1 | 24.1 | 40.6 | 608.2 |
| Rimski Š. | 47.5 | 81.9 | 60.6 | 49 | 64.2 | 163.2 | 81.2 | 51.2 | 27.1 | 7.4 | 24.6 | 59.2 | 717.1 |
| 2019 | | | | | | | | | | | | | |
| Rimski Š. | 45.8 | 17 | 15.9 | 54.1 | 147.6 | 63.7 | 21 | 79.1 | 53.1 | 20 | 53.7 | 61.1 | 632.1 |

Results

The mean values for all of the traits studied were estimated and shown as biplots (Figure 1). The contribution of each individual component can vary in different environmental conditions because the yield represents a complex of traits and its expression is determined by a large number of components.

When looking at the first trait that was tested, oil content, it is clear that the environments clustered in the upper right quadrant are significant, and that both tested genotypes achieved values above the average in all these environments. (Figure 1(a)). E1, E2, E3, E4, E5 and E6 were the conditions with the most visible performance. All these environments have one thing in common: spring sowing. In the second observed portion of Figure 1 (b), which reflects seed yield, it has been shown that the yield was above average in only four environments for both genotypes studied. E5, E6, E8 and E9 are the environments listed, with E9 being the only one that is in autumn sowing. Just three external conditions (E3, E8 and E12) obtained values higher than the average when both genotypes were analysed when it came to the percentage of proteins, as shown in Figure 1(c). All three environments were planted at the springtime.

On a PCA biplot for the percentage of oil, it can be seen that the IPCA1 graph's first axis represented 64.88% of the total phenotypic variability, which is made up of an additive portion (Figure 2(a)). IPCA2 graph's second axis accounted for 21.52% of the overall variance, showing that the experiment is highly multivariate. E9 stands out in comparison to other environments, which is not surprising given its location outside of Serbia's territory. The obtuse angles, formed by the values obtained by the examined values, suggest a smaller relation and similarity of that locality to the others. In terms of the stability of a genotypes achieved values across various environments, as measured by the distance from the coordinate origin, the NS Slatka achieved the most stable reaction on Rimski Šančevi in 2017 (E8). Surprisingly, the second genotype tested in the same environment, as well as NS Zlatka enviably stable reaction on E2 in Novi Sad in 2016, achieved a stable reaction. The PCA analysis grouped the environments with the most shared similarities in the upper right quadrant, which is consistent with the field trial setting, as the largest number of isolated environments is from 2017.

The first axis of the IPCA1 graph represented 39.09% of the overall phenotypic variability, as seen in the PCA

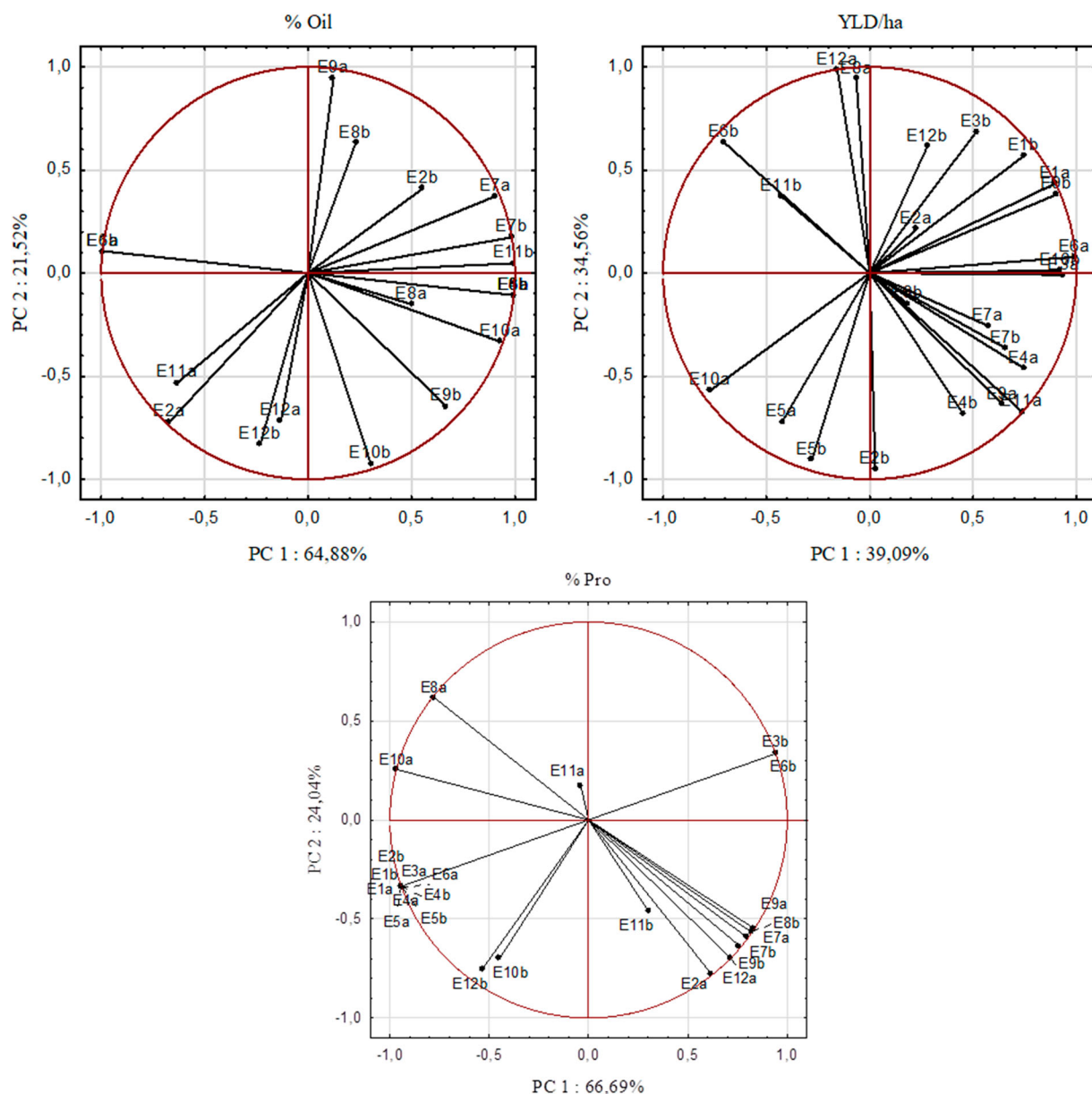


Figure 1. Scatterplot of mean values for analysed traits. Upper left (Figure 1(a)) for oil content (%), upper right (Figure 1(b)) for seed yield (t/ha) and bottom one (Figure 1(c)) for protein content (%). Red colour used for field exams in 2016, blue colour for 2017 and green colour for 2018.

biplot for seed yield (Figure 1(b)). The second axis of IPCA2 represented 34.56% of the total variance, indicating that the additive component share is equal to the multivariate component share for this trait. In terms of the stability of a genotypes achieved values in various environments, as measured by distance from the coordinate origin, it is clear that the genotype of NS Zlatka in Novi Sad in 2016 (E2) had the most stable reaction. On E8 and E11, NS Slatka achieved a desirable stable reaction. PCA analysis grouped the areas with the most similarities in the right lower quadrant.

When we look at the PCA biplot for protein percentage, it can be seen that the first axis of the IPCA1

graph represents 66.69% of the overall phenotypic variability, which is made up of an additive variable (Figure 1(c)). The second IPCA2 axis accounted for 24.04% of total variance, suggesting that the experiment is strongly multivariate. Stability-wise genotypes achieved values in multienvironments, as measured by distance from the coordinate origin, it is clear that the genotypes NS Slatka and NS Zlatka on Rimski Šančevi in 2018 (E11) achieved the most stable reaction. The distribution of environments is comparable in each of the three quadrants, indicating that more environments are optimal for the manifestation of this feature, which is grouped together based on its similarity.

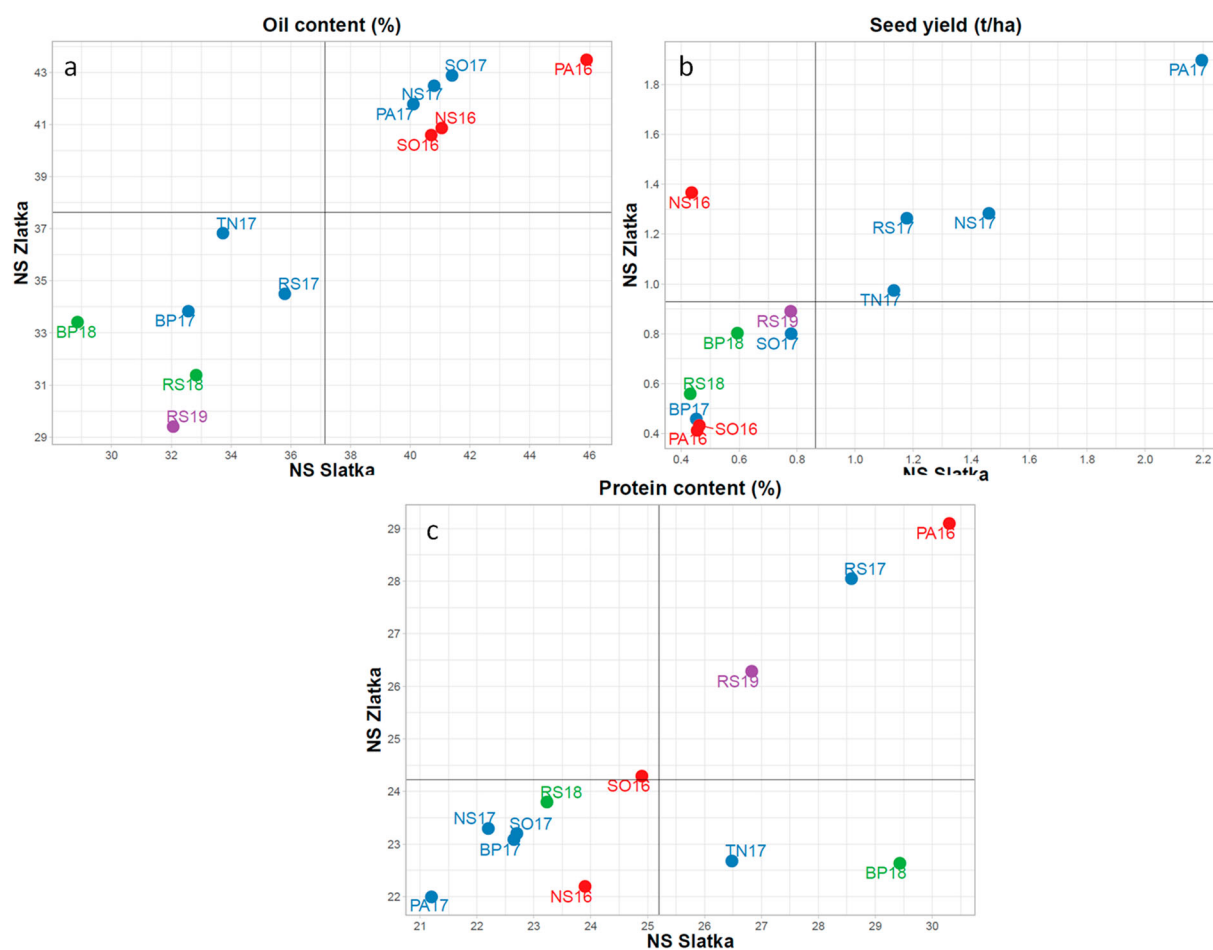


Figure 2. PCA biplot analysis for analysed traits. Upper left (Figure 1(a)) for oil content (%), upper right (Figure 1(b)) for seed yield (t/ha) and bottom one (Figure 1(c)) for protein content (%). In each figure besides code for environment stands letter (a) for genotype NS Slatka and (b) for genotype NS Zlatka.

Discussion

Conclusions about camelina adaptability and productive potential can be drawn from the data collected. Overall, camelina growing season was reported to be relatively short, supporting the stability of the investigated traits, which may make its inclusion into traditional crop rotations easier (Zanneti et al., 2017). Even though camelina is an old-world oilseed crop that has been grown for thousands of years (Berti et al., 2016), the main source of seed includes small seed repositories and private collections. Furthermore, according to Chao et al. (2019), some camelina seed accessions are possibly misclassified for summer- and winter-annual biotypes. All these are facts and state that studies like this, where all factors for manifestation of certain traits are broken into pieces, are necessary.

Analysis of the distributions of environments for oil content which mirrored protein content, using the PCA analysis shows a statistical significance of the first two main components, IPCA1 and IPCA2, which participated

in the phenotypic variation with a larger amount to additive component and with both axes having a statistically significant effect on the interaction variation, which is in agreement with Anderson et al. (2019). The first two main components jointly explained more than 80% of the variation in two traits. The statistical significance of the remainder is a consequence of an agronomically explicable variation. Given the additive component share and the interpretation that genotypes bear the total phenotypic variance, such findings are entirely expected. This means that the differences between the genotypes in the sample were the basis of the variation, which is in accordance with Cui et al. (2011). Analysis of the distributions of environments seed yield, using the PCA analysis, shows a statistical significance of the first two main components, IPCA1 and IPCA2, which participated equally in phenotypic variation with the same share of additive component and multivariation with both axes having a statistical significance. Nonetheless, genotype and environmental share along with interaction had almost the same impact on

variation. At this time, it is unclear if the differences in seed yield variation are due to genetic differences between summer- and winter-biotypes, the climate in which they were grown, the time of harvest, or a combination of these three factors. It can be imagined that the response is formed such that the climatologically element is behind it, despite the fact that it is in the realm of assumptions. Given that, with the exception of later months, the conditions in E2 are clearly favourable in terms of temperature and humidity, it is unquestionably one of the ways to approach the study of the remainder of the variance (Table 2). Based on the graphic representation (Figure 2), and in terms of stability values, it could be noticed that not all agro-ecological environments are at the level of the experimental overall average. As previously mentioned in the results, the most stable genotypes for the percentage of oil were NS Slatka in E8, NS Zlatka in E2, for seed yield and both varieties in E11 for the percentage of protein. From the standpoint of breeding, such a consistent reaction is understandable because all three environments are close to the origin of the raw breeding material. Naturally, the varieties designed on the same location will express the most stable reactions. The fact that the last month of camelina vegetation in E11 was relatively mild and agronomically favourable supports the high expression of these two genotypes across this environment. It's also worth noting that the genotype reaction in E2 is stable, but has slowed due to white rust and powdery mildew.

The genotypes NS Zlatka and NS Slatka used in this study come from elite breeding material that is engineered to provide a consistent return to the producer regardless of the amount of money invested in primary agricultural production. The foundation of agronomic production is the yield that can be counted on at any given time, and everything beyond that is beneficial. Incorporating these varieties into the crop rotation would broaden the range of species available in Balkan agriculture. The findings of this study could help future breeding programmes achieve higher-yielding genotypes with better-quality oil and protein content along with desirable stability.

Disclosure statement

We confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.

Notes on contributors

Dr Ana Marjanović Jeromela works at the Serbian Institute of Field and Vegetable Crops as a Principal Research Fellow.

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Dr Velimir Mladenov is university professor at Faculty of Agriculture, Serbia. He is dedicated in the field, laboratory and classroom to Plant Breeding.

M.Sc. Boris Kuzmanović is a PhD student of prof. Mladenov and dr Marjanović Jeromela. He works on his PhD on Camelina sativa.

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Danijela Stojanović is a person from the Ministry of Agriculture, Forestry and Water Management who leads the registration of oil crops in Serbia.

Dr Johann Vollmann is a university professor at BOKU, Vienna. He is a devoted soybean and camelina scientist and breeder who has passed on his love and experience to us.

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