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SOYBEAN SEED SCANNING FOR SIZE, GENOTYPE COLOR AND *Cercospora blight* DETECTION

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Abstract

Soybean cultivation in Europe has increased his importance due to the protein demand. Therefore, the scaling of different changes in cultivation technology and cultivar choosing is concerning to extend the areas of soybean crop zone by overcoming the biotic and abiotic stress. A number of 30 soybean genotypes had been studied by scanning a batch of seeds with GrainScan software in order to assess the seed size, color variation and mottling degree of grains. Further laboratory tests for the assessment of resistance of low temperature has been performed to check the relations between color code and degree of cold resistance of soybean genotypes. The work procedure is a fast option and low cost analysis for a primary seed health assessment.

***Key words**: soybean, GrainScan, seed scan, Cercospora blight, mottling, germination rate, low temperature, correlation.*

INTRODUCTION

In Europe the importance of soybean increased with the deficit of protein crops, estimated at 60-80% (Jerzak et al., 2020) and the increasing demand for the epoxidized soybean oil as a biobased and biodegradable polymer material. (Zych et al., 2021). 19 EU States signed the Europe Soya Declaration, a common agreement to reduce soybean imports (estimated at over 40 mil. tones) by cultivating non-GMO crops. (Dima, 2016; Bittner, 2019). Research works are following to extend the cultivated soybean area in colder climates like North of France and Poland (Jerzak et al., 2020; Boulch et al., 2021). Mature seeds of soybean cultivars vary in large colour groups of yellow type, green-type and black seed coat that reveals significant differences in composition of carotenoids components (Monma et al., 1994) and on common beans the microelements like Fe, Zn, Ca (Silva et al., 2012). Soybean seed mottling could have various causes from fungus and viruses pathogens. Often, purple colour of soybean grains is an indicator of purple seed stain (PSS) of soybean which is a major seed disease that cause grains value depreciation due to the purple discoloration and reduces vigor and quality. It is known that purple colour of soybean seeds is a light activated pigment named cercosporin and is produced by *Cercospora kikuchii* fungal pathogen.

Seed size is an important yield component, linked to the seedling vigour. On ideal moisture conditions larger size soybean seeds develops more vigorous seedlings with higher germination speeds (Pereira et al., 2013) although in the field conditions, planting methods and different conditions could advantage small seeds to obtain higher yields (Madanzi et al., 2010). In the research activity there is needed fast analysis methods for assessment and evaluation of different soybean cultivars. Seed dimensions also are correlated with vegetation period and therefore maturity group of the soybean cultivars (Greilhuber et al., 1997). Seed size measurement and description of colour and morphology is difficult to be performed by conventional methods due to the time and effort resources even for a small amount of samples. (Baek et al., 2020). To overcome this inconvenience and obtaining objective datasets about size, colour and seed shapes phenotypical analyses of grains using images was made for 30 soybean genotypes in order to find out the characters that could be used for soybean description and breeding process and finally emphasis and correlate with agronomical advantages of different genotypes.

MATERIALS AND METHODS

A total of 30 soybean genotypes were evaluated in this study, of which 10 are registered varieties and 20 are lines obtained in the soybean breeding program from NARDI Fundulea, Romania. (Table 1)

Field experiment was conducted at National Agricultural Research and Development Institute Fundulea, on a chambic chernozem soil in 2020 (44°26'39.07"N; 26°30'54.25"E). For all experiments, seeds were planted at a rate of 55 seeds/m^2. The field experiment was laid out in a Randomised Complete Block Design (RCBD). The plot size was 9 m^2. Planting date was at beginning of April (03 April). Applied crop technology was low input, without irrigation and additional nutrients. Two mechanical and two manual weeding works were performed after crop emerged.

Table 1. Breeding lines and registered varieties studied

No.	Soybean genotype	Maturity group
1	Safta F	0
2	F10-1443	0
3	F13-908	00
4	F13-993	00
5	Ilaria F	0
6	F13-1114	0
7	F13-1117	00
8	F13-1124	0
9	F13-1163	0
10	F13-1174	00
11	F14-878	00
12	F14-883	00
13	F14-892	0
14	F14-918	0
15	F14-924	0
16	F15-428	0
17	F15-749	0
18	F15-792	0
19	F15-828	0
20	F15-1026	0
21	Anduța F	0
22	Camelia F	0
23	Carla TD	000
24	Fabiana F	I
25	Flavia	00
26	Florina F	0
27	Larisa TD	0
28	Ovidiu F	0
29	Ricky	I
30	Teo TD	00

GrainScan software was used as low cost, and fast method for measurement of grain size and colour (Whan et al., 2014; Kinnikar et al., 2015). For capture the images of soybean genotypes studied a regular Canon MX390 PIXMA scanner was used. In order to avoid the seed shadows the equipment was covered by a black polyethylene sheet. A random number of soybean seeds from each genotype was scanned images files no bigger than 500 kilobytes. (Figure 1). After scanning and adjusting each file with the genotype name, the data has been generated with batch processing of files in separate .csv files. The class of values for size measures obtained are: area (mm^2), perimeter (mm), maximum length of the grain ellipse – noted with majellipse (mm) and the width – the minor axe of the best fit ellipse, noted as minellipse (mm). For the colour three values for red, green and blue codes was obtained.

Further, data editing was performed with Query instruments of MS Excel software (Figure 2).

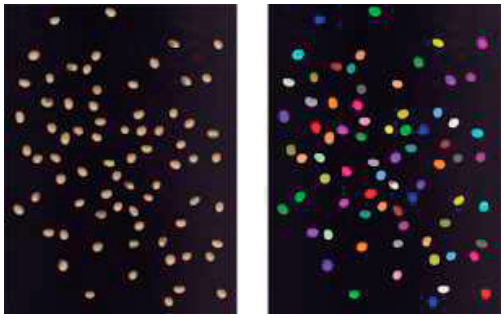

Figure 1. Scanned image of soybean seeds, variety Camelia F

Figure 2. Batch editing of .csv data at once from all images with Query instruments of MS Excel

A macro script was used to generate in a single cell each seed colour (Williams, 2015) (Figure 3).

```
Function myRGB(r, g, b)

    Dim clr As Long, src As Range, sht As String, f, v

    If IsEmpty(r) Or IsEmpty(g) Or IsEmpty(b) Then
        clr = vbWhite
    Else
        clr = RGB(r, g, b)
    End If

    Set src = Application.ThisCell
    sht = src.Parent.Name

    f = "Changeit(""" & sht & """," & _
                  src.Address(False, False) & """," & clr & ")"
    src.Parent.Evaluate f
    myRGB = ""
End Function

Sub ChangeIt(sht, c, clr As Long)
    ThisWorkbook.Sheets(sht).Range(c).Interior.Color = clr
End Sub
```

Figure 3. Macro script for combining three Red, Green, Blue values into a single cell colour, after Williams.

Number of spotted grains in red-brown and purple-black colours was counted from the scanned images and grains were analysed for purple seed stain (*Cercospora kukuchii*) and other fungal diseases by a classical method: seeds has been sterilized in a solution of 0.5% sodium hypochlorite (NaOCl) for 4 minutes and washed after with distilled water. Further was assured a potato dextrose agar environment and incubated at 25°C in the dark for 10 days. (Jordan et al., 1986). SPSS software was used for boxplots graphical representation of the seed dimensions results.

RESULTS AND DISCUSSIONS

The average daily maximum air temperature and total precipitation for each month during growing season from 2019 as compared with multiannual average are shown in Figure 4.

When plants were at the first growth stages in May, the amount of precipitation (124.4 mm) in 2019 was approximately 1.9 times that of multiannual average (62.5 mm). The average daily maximum air temperatures in May were 28.9˚C and 30.7˚C for MA, respectively.

When plants reached R5 or R6 growth stage in August, the total precipitation (12.6 mm) in 2019 was under normal of the zone (49.7 mm) and relative humidity was 60% in 2019 as compared cu 68% for MA. The average air temperatures in August were 35.2˚C and 35.3˚C for normal of the zone.

In conclusion, the year 2019 was characterized by abundant rainfall, high relative humidity and slightly lower temperatures in the first part of the soybean growing season, then from July was the drought until harvest.

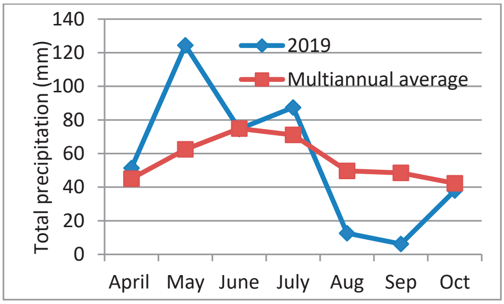

Figure 4.a. Total precipitation during soybean growing seasons

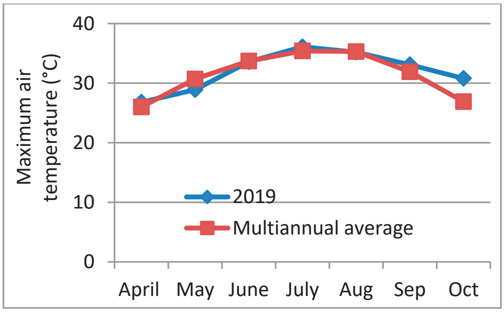

Figure 4.b. Maximum air temperature during soybean growing seasons

These conditions are favorable for *Cercospora* sp infection, which has been reported in the experimental field (Figure 5).

Figure 5. Aspect with soybean plants infested with *Cercospora sp*.

Soybean seed dimensions results and mottled grains

The seed dimensions resulted values from the image analysis: area (mm^2), perimeter (mm), length of seeds as majellipse (mm), and wide of seed as minellipse (mm) are presented in this paper in boxplots graphics. The outlier values, that differs from the lower bound and higher bound of each soybean genotype are explained partially as errors due to the different spots found on grains. Purple-black and brown-red spots (Figure 6) influenced the dimension results, due to the grain recognition area of Grainscan software. Spotted genotypes seems to have a wider range that characterize their dimensions and more values that are very far from the median and close range (outliers). For further soybean and other grain software development it will be necessary to integrate dimension analysis with characteristic of particular symptoms that could change the seed colour.

An initial manual counting of mottled seeds have been performed (Figure 6) also in order to

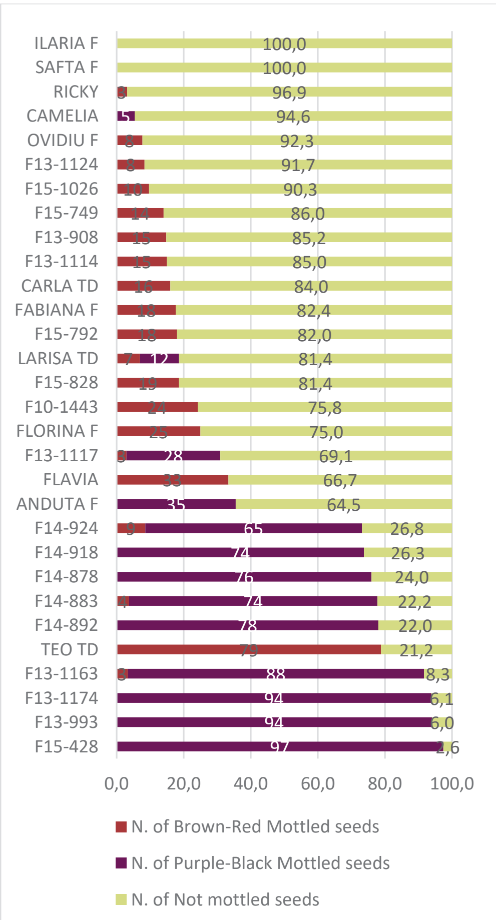

Figure 6. Percentage (%) of mottled soybean seeds of analysed soybean genotypes

Grains area

For grain area of soybean genotypes, analysed values range is spread from the lowest band of F15-792 (17.91 mm^2) to the highest band of upper limit of genotype Ilaria F (41.56 mm^2). Longer boxes of genotypes F13-1163, F13-1174, F13-993 although belongs to the susceptible group of very mottled seeds could also show that the breeding lines are heterogenous, as the seeds dimensions within the same genotypes differs. Scanned images from genotypes Ilaria F and Safta F didn't had mottled seeds. Therefore, their lower and higher bound limits (Ilaria F – between 37.11 and 41.56 and Safta F between 33.08 and 39.89) could be confidentially considered as reference values. Shorter boxes (Figure 7) of genotypes F15-749, F15-792 and their bottom position indicates that the grain area is smaller and seeds are uniform. Particularly, F15-426 bottom position in the graphic and larger large of values with many outliers is linked to the fact that the mottled seeds of this soybean breeding line was at 97.4%. (Figure 6).

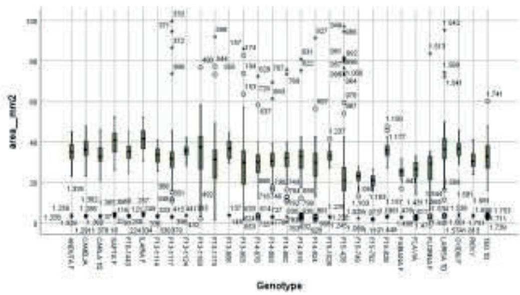

Figure 7. Area values of soybean grains (mm^2) of analysed genotypes

Grains perimeter

For grain perimeter of soybean genotypes, analysed values range is spread from the lowest bound of F14-883 (12.04 mm) to the highest bound of upper limit of genotype Anduta F (30.17 mm) (Figure 8). Large range of values for breeding line F13-993 outliers and spotted grains over 93.9% (Figure 6) place this genotype as the most heterogenous compared to others. Perimeter values for Anduta F, F13-114, F13-908, F15-792 and Fabiana F are closer to the median, that shows the uniformity of grains to this genotypes and a higher degree of heterogeneity for other studied lines.

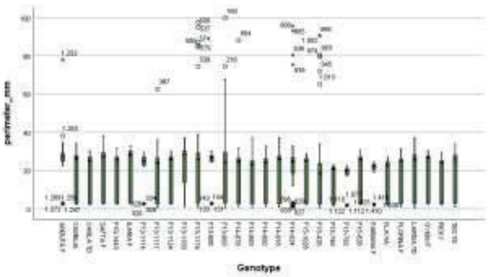

Figure 8. Perimeter values of soybean grains (mm) of analysed genotypes

Inner seed dimensions: Length and wideness of ellipse

Length and wideness values derived from the data of scanned images follow the same range pattern (Figure 9, Figure10), but as it was expected with lower values for the minimum of ellipse dimension (wideness). For both values genotypes F13-1174, F13-908, F13-993, F15-428, and Ovidiu F presented a high degree of heterogeneity, while other soybean grains values were less spanned.

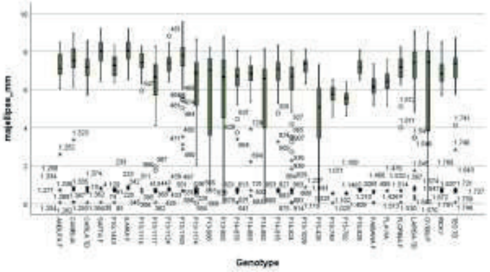

Figure 9. Length of seeds as majellipse (mm) values of soybean grains of analysed genotypes

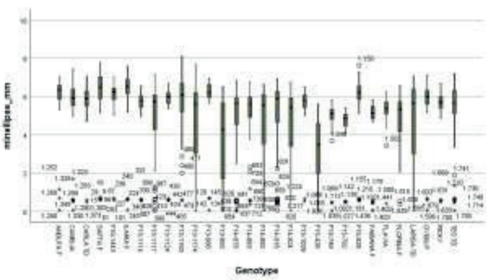

Figure 10. Wideness of seeds as minellipse (mm) values of soybean grains of analysed genotypes

In Table 2 are shown the mean RGB values and resulted color. As it was expected for genotypes Safta F and Ilaria F the color is close to natural because the scanned images did not show spots.

The color analysis also revealed purple color in case of the minimal mottled seeds of Ricky genotype but in genotypes Anduta F, Camelia, F13-114, F13-1124, F13-908, Florina F and Ovidiu F there are no perceptible color differentiations due to the frequency lower than 15% of mottled seeds.

Table 2. Mottled grains genotypes color generated by the mean of RGB codes

Genotype	grain ch1 code RED	grain ch2 code GREEN	grain ch3 code BLUE	Genotype color
ANDUTA F	185	156	146	
CAMELIA	183	156	133	
CARLA TD	148	136	774	
F10-1443	182	150	199	
F13-1114	185	153	141	
F13-1117	167	150	490	
F13-1124	189	159	128	
F13-1163	175	147	173	
F13-1174	174	154	187	
F13-908	191	161	128	
F13-993	164	161	366	
F14-878	172	142	354	
F14-883	172	158	394	
F14-892	172	160	412	
F14-918	174	152	274	
F14-924	181	197	294	
F15-1026	192	162	129	
F15-428	159	347	637	
F15-749	149	131	696	
F15-792	151	130	781	
F15-828	183	150	125	
FABIANA F	152	149	802	
FLAVIA	175	235	743	
FLORINA F	191	160	133	
ILARIA F	187	160	126	
LARISA TD	181	151	176	
OVIDIU F	192	158	126	
RICKY	159	129	604	
SAFTA F	185	158	124	
TEO TD	168	196	313	

Correlations between seeds color and germination, seedling weight and degree of soil cover

Seed germination is one of the important components of soybean seed quality.

In our study, there was a significant correlation between percent seed brown red colored and germination at low temperature, the correlation coefficient (r) was by 0.43 (significantly for P <0.1).

Some genotypes had high levels of coloration, but also had high seed germination (TEO TD, Flavia), while some genotypes had low levels of seed coloration, but had high percentages of seed germination (FD 15-792), (Figure 11).

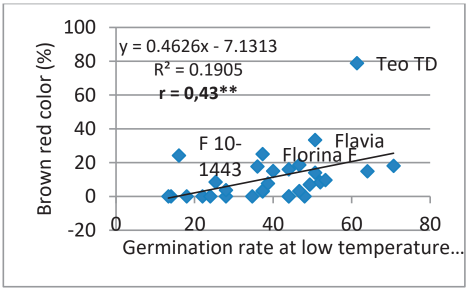

Figure 11. Correlation between germination rate and brown red color of seeds

There was a significant negative correlation between percent seed colored purple black (probably infected by Cercospora spp.) and germination (r = -0,46***), (Figure 12).

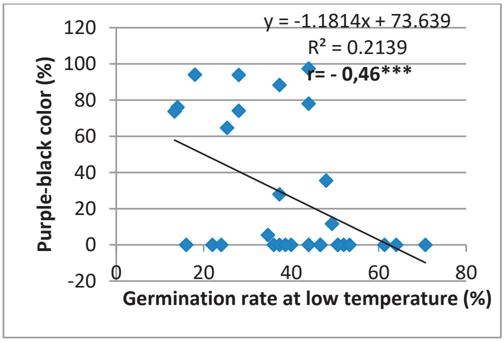

Figure 12. Correlation between germination rate and seeds purple black colored

A negative correlation was between seedling weight at low temperature and purple black color of seeds (Figure 13).

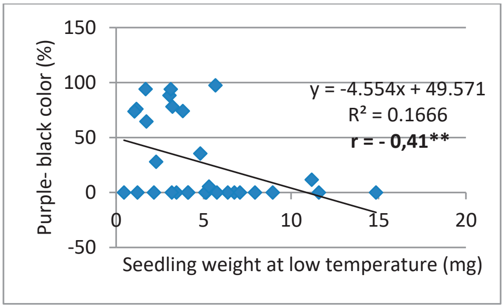

Figure 13. Correlation between seedling weight and purple black color of seeds

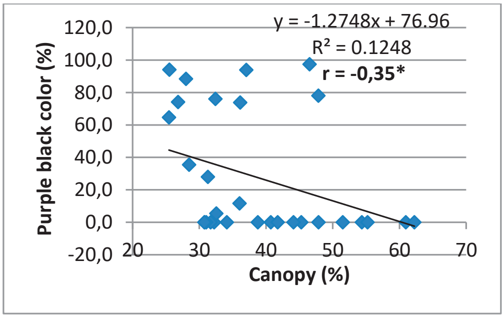

Figure 14. Correlation between degree of soil cover and purple black color of seeds

A negative correlation was between seedling weight at low temperature and purple black color of seeds (Figure 14).

CONCLUSIONS

There were differences in seed size and color among studied genotypes, that has been revealed by a simple scanner method and computer facilities.

Batch seed scanning for seed dimensions could be used as a tool for differentiate the uniformity and heterogeneity of different soybean breeding lines and as a fast method to detect the frequency above 15% of mottled seeds.

The correlations obtained shown that generally soybean genotypes more resistant to low temperature had low levels of coloration purple black which suggested probably low level of infection with *Cercosporea sp*.

Further research and algorithms are needed to fine tune the specific soybean grains color, obtain more precise dimensions of mottled seeds and diseases symptomatology.

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REFERENCES

Baek, J., Lee, E., Kim, N., Kim, S.L., Choi, I., Ji, H., Chung, Y.S., Choi, M.S. Moon, J.K. Kim, K.H. (2020). High throughput phenotyping for various traits on soybean seeds using image analysis. *Sensors (Switzerland)*, *20*. 1–9.

Bittner, U. (2019). Europe Soya Declaration, pp. 1–3.

Boulch, G., Elmerich, C., Djemel, A., Lange, B. (2021). Evaluation of soybean (Glycine max L.) adaptation to Northern European regions under different agro-climatic scenarios. *Oxford University Press, Annals of Botany*.

Dima, D.C. (2016). Soybean Demonstration Platforms: The Bond Between Breeding, Technology and Farming in Central and Eastern Europe. *Agriculture and Agricultural Science Procedia*., 10, 10–17.

Greilhuber, J.; Obermayer, R., 1997: Genome size and maturity group in Glycine max (soybean). *Heredity*., *78*, 547–551.

Jerzak, M.A., Śmiglak-Krajewska, M. (2020). Globalization of the market for vegetable protein feed and its impact on sustainable agricultural development and food security in EU countries illustrated by the example of Poland. *Sustainability (Switzerland)*, 12, 1–13.

Jordan, E. G., Manandhar, J.B., Thapliyal, P.N., Sinclair, J.B. (1986). Factors affecting soybean seed quality in Illinois. *Plant Disease*., 70, 246–248.

Kinnikar, A., Desai, P., Jahagirdar, S. (2015). Identification and Detection of Seed Borne Diseases of Soybean Using Image Processing-A Survey. *Int. J. Emerg. Technol. Comput. Sci. Electron*., *14*. 363–368.

Madanzi, T., Chiduza, C., Richardson-Kageler, S.J. (2010). Effects of planting method and seed size on stand establishment of soybean [*Glycine max* (L.) Merrill cv. Solitaire]. *Soil and Tillage Research*., *106.* 171–176.

Monma, M., Terao, J., Lto, M., Saito, M., Chikuni, K. (1994). Carotenoid Components in Soybean Seeds Varying with Seed Color and Maturation Stage. *Bioscience, Biotechnology, and Biochemistry*, *58.* 926–930.

Pereira, W.A., Maria, S., Pereira, A. (2013). Influence of seed size and water restriction on germination of soybean seeds and on early development of seedlings 1 Influência do tamanho de semente e da restrição hídrica na germinação de sementes de soja e no desenvolvimento inicial das plântulas. *Journal of Seed Science*., *35*. 316–322.

Silva, C.A., Abreu, Â. de F.B., Ramalho, M.A.P., Maia, L.G.S. (2012). Chemical composition as related to seed color of common bean. *Crop Breeding and Applied Biotechnology*., *12*. 5.

Whan, A. P., Smith, A.B., Cavanagh, C.R., Ral, J.P.F., Shaw, L.M., Howitt, C.A., Bischof, L. (2014). GrainScan: A low cost, fast method for grain size and colour measurements. *Plant Methods*., *10*. 1–10.

Zych, A., Tellers, J., Bertolacci, L., Ceseracciu, L., Marini, L., Mancini, G., Athanassiou, A. (2021). Biobased, Biodegradable, Self-Healing Boronic Ester Vitrimers from Epoxidized Soybean Oil Acrylate. *ACS Applied Polymer Materials*., 3. 1135–1144.