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ABSTRACT (FOR DISSEMINATION)	The work under package "SOYBEAN" in the framework of ECOBREED project (WP4) was focused on enhancing soybean genotypes for improved agronomic performance tailored to low-input and organic systems. Throughout project lifetime, WP4 partners focused on comprehensive phenotyping experiments to enhance stress tolerance against biotic (weeds, pests, diseases) and abiotic (drought and chilling stress) factors. Emphasis was also placed on evaluating crop and nutritive quality, as well as nitrogen fixation efficiency. A marker-assisted selection program was established to identify genes/QTL crucial for developing organic soybean varieties. Additionally, the project assessed the impact of cover crops and inoculation in soybean seed multiplication. The segregating material developed has entered the

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	breeding pipeline using the SSD method, and soybean lines were made available for variety testing and registration in 2021 and 2022. Finally, the variety NS ECOB, characterized by high protein content variety and belonging to the 00 maturity group, was registered in 2023. The outcomes of WP4 will significantly benefit the scientific community, offering valuable insights to early adopters of different methodologies. Moreover, farmers engaged in soybean variety testing directly benefit as end-users of the developed varieties and proposed technologies. The improved yield potential and better adaptation of soybean varieties contribute to the optimization of production processes, thereby enhancing the socioeconomic status of producers. Moreover, it has broader impacts on the entire organic food and feed sector.
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Table of contents

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Grant agreement No 771367

Executive summary

Soybean, crop with an annual production exceeding 370 million tons, has experienced significant developments in breeding and cultivation practices over the last 50 years. In line with global trends the work under package "SOYBEAN" in the framework of ECOBREED project (WP4) was focused on enhancing soybean genotypes for improved agronomic performance tailored to low-input and organic systems.

Throughout project lifetime, WP4 partners focused on comprehensive phenotyping experiments to enhance stress tolerance against biotic (weeds, pests, diseases) and abiotic (drought and chilling stress) factors. Emphasis was also placed on evaluating crop and nutritive quality, as well as nitrogen fixation efficiency. A marker-assisted selection program was established to identify genes/QTL crucial for developing organic soybean varieties. Additionally, the project assessed the impact of cover crops and inoculation in soybean seed multiplication.

The segregating material developed has entered the breeding pipeline using the SSD method, and soybean lines were made available for variety testing and registration in 2021 and 2022. Finally, the variety NS ECOB, characterized by high protein content variety and belonging to the 00 maturity group, was registered in 2023.

The outcomes of WP4 will significantly benefit the scientific community, offering valuable insights to early adopters of different methodologies. Moreover, farmers engaged in soybean variety testing directly benefit as end-users of the developed varieties and proposed technologies. The improved yield potential and better adaptation of soybean varieties contribute to the optimization of production processes, thereby enhancing the socioeconomic status of producers. Moreover, it has broader impacts on the entire organic food and feed sector.

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Introduction

Soybean represents one of the most important field crops in the world, with an annual production of over 370 million tons (FAOSTAT, 2021). Due to the progress that has been made and in the breeding of superior varieties and in cultivation practices, the global average yield of soybean increased by more than 150% over the last 50 years (1961-2021, FAOSTAT). Main goal of the WP4 was to develop soybean genotypes with improved agronomic performance and to offer know-how for seed cultivation practices tailor-made for low input and organic systems. During the project lifetime, the focus of activities was on phenotyping for biotic (weeds, pests, diseases) and abiotic (drought and chilling stress) stress tolerance screening, as well as crop and nutritive quality and N fixation efficiency. A marker-assisted selection programme was established to identify genes/QTL of particular importance for organic soybean varieties. In addition, an assessment of the effects of cover crops and inoculation in the process of soybean seed multiplication was performed. Segregating material that was developed is in breeding pipeline (SSD method) and soybean lines were available for variety testing and registration that were carried out in 2021 and in 2022. The chosen soybean lines went throughout the registration trials to evaluate their performance and adaptability. NS ECOB went through this process and it was registered in 2023. NS ECOB is the variety with high protein content (00 maturity group) specifically selected for organic and low input production requirements.

Material and methods

Material and methods on identification of useful traits

Phenotypic characterization of diverse soybean germplasm for identification of useful traits was conducted during 2020 and 2021 in three locations following a clearly defined protocol. In total, 206 accessions were divided into early and late, depending on the maturity group. The 117 genotypes from the 000 and 00 maturity groups were classified as early while 89 from 0, I, and II were classified as late. The selected material was tested in the trials that were sown at experimental fields of the Institute of Field and Vegetable Crops (IFVC) in Novi Sad, Serbia, at Saatzucht Gleisdorf GmbH (SZG) in Gleisdorf, Austria, and at the National Agricultural Research and Development Institute (NARDI) in Fundulea, Romania. The trials were set as augmented p-rep design with control and testing genotypes that were sown on 8m^2 plots at IFVC and SZG and 6 m^2 plots at NARDI. The data about phenology and crop development including emergence, flowering, and maturity date were assessed during the season. The evaluation was performed based on the predefined scale whose values ranged from 1 to 5. At maturity stage, five plants from each plot were sampled and used for morphological analysis

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which included traits such as plant height (PH), node number (NN), pod number (PN), number of branches (NB), first pod height (FPH) and thousand seed weight (TSW) data. Yield and yield components were recorded after the harvest was done. Post-harvest analysis of the soybean material also included protein and oil content analysis. This was done in Serbia (IFVC) on the samples from all three locations as SZG and NARDI provided the seeds from their trials to IFVC. The quality of the soybean genotypes was determined using near-infrared spectroscopy (NIRS) at Rimski šančevi in the laboratory at the Legumes department (IFVC, RS). At each location, the data about canopy cover (CC) was assessed two times in 2020 and 2021. First data collection was performed at the vegetative phase and second at the beginning of flowering (R1). At SZG and NARDI the CC was obtained using the mobile phone application Canopeo, while at the IFVC the unmanned aerial vehicle (UAV) equipped with a multispectral (MS) digital camera was used. The camera included five monochromatic sensors and one RGB camera. After photo acquisition, all images were stitched together in orthomosaic using Agisoft software. At IFVC, the images collected with the UAV were also used to extract values of vegetation indices (VIs) that can be used for phenotypic characterization of plant material. Calculation of CC and VIs for each plot within the trial was performed with the image analysis software called FIJI. Triangular green index (TGI), normalized difference vegetation index (NDVI), and normalized difference red edge index (NDRE) were used. The NDVI was calculated using NIR with R channel while NDRE was derived from NIR and RE. The TGI was based on the information obtained through the combination of R, G, and B channels. The VIs were extracted from the images collected in three-time points expressed through growing degree days (GDDs). In 2020 and 2021, the photo acquisition was performed at 230, 474, and 642 GDDs with a maximum difference between years of 22.2 GDDs.

Material and methods for weed screening

The field experiment regarding competitiveness against the weeds was conducted over a three-year period (2020, 2021, and 2022) at the IFVC in Novi Sad (RS). The trial design consisted of a split-split plot in 4 replications. The main plots were assigned to weed species (ABUTH, AMBEL and XANST). Subplots were assigned to soybean genotypes (NS Apolo, Fortuna and NS Zmaj). Sub-sub plots consisted of 5 density levels (0, 0.5, 1, 5, 10 plants per meter of soybean row). The competition design was additive, which means that the weed density varied while crop density was kept constant (Swanton et al., 2015). Soybean was planted at 450,000 seeds/ha at a row spacing of 50 cm. Weed-free plots were maintained with weekly hand weeding. Methods used for measuring were non-destructive, taken 6, 8, 10, and 12 weeks after emergence (WAE) of soybean. Plant height (cm) was measured with a wooden meter and the plant cover was assessed

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Horizon 2020
Grant agreement No 771367

using the application Canopeo. Soybean dry biomass was measured at 9 and 13 WAE each year. Biomass was dried in the green house until reaching constant weight and the weight was recorded. Statistical analysis was conducted using R 4.2.2. Results were reported separately for each year. The weed dry matter, leaf area and soybean grain yield were subjected to analysis of variance. Main effects and interactions were tested for significance using the Tukey test ($p < 0.05$).

Material and methods for disease screening

Screening under the field conditions

Disease severity index is a measure of the extent and severity of disease symptoms in plants. In this specific case, the disease severity index is measuring the natural severity of the *Macrophomina phaseolina* disease in soybean genotypes at four different environments in Serbia (year 2020 and 2021, drought location and regular). Ten random plants from each genotype were cut longitudinal through stem at maturity, and disease severity index were scored. In total, 206 genotypes were screen for natural infestation of *Macrophomina phaseolina*. The index is measured on a scale from 1 to 5, where 1 represents no symptoms of disease and 5 represents the most severe symptoms of disease. The higher the severity index, the greater the impact of the disease on the soybean crop. *Cut stem*: The V2 growth stage of soybean plants was reached before a sharp 40 mm cutting blade was utilized above a single leaf node. A 200 L pipette tip was used to extract mycelial plug from the growing margin of a 4-day-old *M. phaseolina* culture on PDA. Immediately after cutting the stem with a blade, the mycelial plug-tipped pipette is placed over it, making sure the agar is entrenched in the stem. Scores are determined by the length of the lesion. After the inoculation, lesion measures were taken on the third day and were then followed up on every third day for a total of five evaluations (3, 6, 9, 12 and 15 days). The surface below disease progression curves (AUDPC) for each entry was calculated to assess resistance to disease and chose superior lines. 25 plants per variety were screened. DPC trial: Toothpick method was implemented for soybean screening on DPC reaction. In brief, soybean plants were inoculated with mycelia grown toothpick at V4 phase of development between second and third node. Lesion length was measured every three days, in total 27 days experiment. Experiment was conducted at grown chambers (t=25^{o} C, 8/16 day).

Material and methods for *Nezara viridula*, the southern green stink bug (SGSB) screening

To assess the potential SGSB damage on soybean a set of trials was conducted in semi-controlled, field conditions at location Rimski šančevi (RS). Isolation cages, 1m in diameter and 1.8m in height, were mounted during 2018, 2020 and 2021. Testing was

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Grant agreement No 771367

done on five different soybean varieties that belong from 00 to III maturity groups (Fortuna, Romansa, Princeza, Sava and Senka). There were two types of cages, a control treatment without stink bugs, and a treatment with SGSB specimens. In each isolation cage with stink bugs, five specimens per plant were introduced at the end of July each year. Soybean plants were harvested at maturity stage and analysed in detail. Assessed parameters were height of the plant, number of lateral branches, number of seeds per plant, number of completely damaged seeds and thousand seeds weight.

Material and methods for drought tolerance trial

The initial testing of 206 soybean genotypes on abiotic stress tolerance was done in 2020 and 2021 at the experimental fields of the Institute of Field and Vegetable Crops (IFVC) at Rimski Šančevi (RS). The germplasm used within the research consisted of different varieties, divided into early and late based on the maturity group. The final number of genotypes in the early group was 117 and the rest, 89 were classified as late. The trials were set as augmented p-rep design with control and testing genotypes that were sown on 8m2 plots on the sandy soil with low fertility and poor water retention to simulate a drought environment. As a control, the same material was also sown on the chernozem soil, characterized by homogeneous texture and well-aggregated structure. The drought tolerance of the analysed soybean genotypes was evaluated by comparing their performance in a drought simulation environment and the control. The plant development data such as emergence, flowering, and maturity date were noted during the season. These data were timely assessed through periodic surveys over the trials. The moment when 50% of the plant emerged was noted as the emergence date for that plot. Flowering and maturity dates were recorded when 90% of the plants flowered/matured. In the soybean flowering phase (R1) the canopy cover (CC) data of all plots within the trials was assessed. The CC of each plot was calculated from the digital images collected with the multispectral (MS) camera and unmanned aerial vehicle (UAV). The camera included five monochromatic sensors and one RGB camera. The monochromatic sensors collect the reflectance data of the analysed soybean genotypes from the visible part of the light spectrum (R, G, B) as well as from the RE and NIR. The UAV flights were performed two times each year at 60m on the cloud-free sky, wind speed did not exceed 10 m/s. After photo acquisition, all images were stitched together in orthomosaic using Agisoft software. The data regarding spectral reflectance was used for the calculation of two vegetation indices (VIs), normalized difference vegetation index (NDVI) and normalized difference red edge index (NDRE). These two indices were used as stress indicators for the evaluation of analysed soybean genotypes. The NDVI was calculated using NIR with R channel while NDRE was derived from NIR and RE. In plants, values of both VIs range from 0-1, where higher values indicate healthy

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Horizon 2020
Grant agreement No 771367

vegetation. The VIs were extracted from the images collected in two time points expressed through growing degree days (GDDs). In 2020 and 2021, the photo acquisition was performed around 230 and 642 GDDs with a maximum difference between years of 22.2 GDDs. At maturity, five plants from each plot were sampled and used for morphological analysis which included traits such as plant height (PH), node number (NN), number of branches (NB), first pod height (FPH), and thousand seed weight (TSW) data. After the harvest, the yield of each plot was measured and calculated to 14% seed moisture. An online toolkit called iPASTIC was used for the calculation of nine stress indices based on the yield data of genotypes grown in drought simulation environments and in the control (Pour-Aboughadareh et al., 2019). The indices derived from iPASTIC software were tolerance index (TOL), mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HM), stress susceptibility index (SSI), stress tolerance index (STI), yield index (YI), yield stability index (YSI), relative stress index (RSI). The TOL index represents the difference between yield in control (Yp) and yield in stress conditions (Ys) and it's preferable to be as low as possible. The STI, MP, GMP, and HM are indices that can be used as an indicator of best-performing genotypes in both environments, the selection pattern is high index value. Finally, indices SSI, YI, YSI, and RSI are good for the evaluation of yield stability in stress and non-stress conditions. For SSI minimal values are desirable while for the other three indices tolerant genotypes are characterized by high values of YI, YSI, and RSI.

Material and methods for chilling tolerance trial

The trials in Germany observed chilling tolerance on germination and during flowering (10°C) in 2022 and 2023 (NATUR). Speed of growth during youth development was not observed. The trials took place in Dittlofsroda, central Germany, 100 km east of Frankfurt. 40 varieties were sown at three dates with 3 replications. The number of varieties had to be reduced from 50 to 40 due to bad seed quality. One variety (Strengs Weihenstephaner) was from own multiplication at the farm. The sowing dates were middle of April, beginning of May and middle of May. One plot was 50 seeds. A set of varieties (number 1-20) was sent by Saatzucht Gleisdorf from former ECOBREED trials.

Material and methods for N-fixing capacity screening

It the experiment 95 soybean genotypes from the maturity groups 00 and 000 were tested by BOKU in Austria (AT). The five different soybean subsets (genotype classes) were chosen so that a wide range of diversity in nitrogen metabolism and, seed protein content, could be observed: Subsets 1 and 2 breeding lines were obtained from a crossbreeding between a non-nodulating (rj1) and a nodulating (Rj1) parent, whereas lines from subset 1 displaying the non-nodulating phenotype and lines from subset 2

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Horizon 2020
Grant agreement No 771367

consistently nodulating. Subsets 3 and 4 included breeding lines previously selected for high seed protein content or high pod set, while subset 5 involved conventional soybean cultivars. In all three experimental environments subsets 3, 4 and 5 were planted in two replications. Due to the restricted seed supply subsets 1 and 2 were planted in 1-2 replications in the Tulln 2019, and in three replications in the Tulln 2020 and Gross Enzersdorf 2020 (AT).

Material and methods for genotyping

Genotyping was performed for screening of soybean genotypes from germplasm collections as well as breeding material for: (a) cadmium (Cd) accumulation, (b) supernodulation, and (c) disease tolerance. Total genomic DNA was extracted from fresh leaves using a modified CTAB method (Doyle and Doyle 1990) and from grounded dry seeds a modified SDS-based protocol (Cristina et al., 2017).

Screening for (a) cadmium accumulation

The main QTL responsible for controlling the accumulation of cadmium in seed was used to classify genotypes related to cadmium intake and the accumulation level to identify low cadmium uptake germplasm. Characterization of soybean lines for cadmium accumulation was performed using: SSR marker (SacK149) associated with Cda1 locus, derived cleaved amplified polymorphic sequence (dCAPS) marker in combination with BmrI restriction enzyme and KASP marker. In total, 28 soybean genotypes were screened for variability at the major QTL (Cda1) locus for Cd accumulation. Fifteen lines were selected based on yield results from trials in Serbia, Austria and Romania (Task 4.1), as genotypes with the highest yield. Characterization for seed Cd accumulation of all genotypes was performed using SSR marker SacK149. In addition, to check the reliability of obtained results, screening was also performed on 15 genotypes using dCAPS marker in P1B-ATPase gene and KASP marker.

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Horizon 2020
Grant agreement No 771367

Table 1 Protocols for PCR marker analysis for Cd accumulation.

SSR marker (SacK149)	
Concentration	PCR reagents
90-100 ng	genomic DNA template
1 x	buffer mix
0,3 µM	SSR primer mix
	Forward 5'-TGAACACATGCTCAACTTGTCA-3'
	Reverse 5'-CGTGTGGTTGCTATTAACTAAATGA-3'
1 U	Taq polymerase
PCR conditions: 95°C - 3 min, 35 cycles (95°C - 30 s, 57°C annealing temperature - 30 s, 72°C - 30 s) and 10 min final extension.	
Expected products: ~225bp = high Cd uptake, ~237bp = low Cd uptake. Gel electrophoresis: 3% "high resolution" agarose (CleverGEL-Clever Scientific), with ethidium bromide and visualized on UV light with Uvidoc HD6 system (Uvitec).	
dCAPS	
Concentration	PCR reagents
90-100 ng	genomic DNA template
1 x	buffer mix
0,3 µM	SSR primer mix
	forward primer (5'-TGACATCGGTATCTCACTGG-3')
	reverse primer (5'-ATGACATTCTCAATTAGCTTTC -3')
1 U	Taq polymerase
PCR conditions: 95°C - 3 min, 38 cycles (95°C - 30 s, 53°C annealing temperature - 30 s, 72°C - 30 s) and 10 min final extension. PCR product digestion: BmrI (NEB) restriction enzyme (3U), 37°C for 2,5 h.	
Expected products: ~145bp = high Cd uptake, ~25bp + 120bp = low Cd uptake. Gel electrophoresis for the separation of digested PCR products was carried out in a 2,5% "routine use" agarose gel (CleverGEL-Clever Scientific).	
KASP	
Concentration	PCR reagents
20-30 ng	genomic DNA template
2 x	PACE 2.0 Genotyping Master Mix (3CR BIOSCIENCE);
	primer mixture
	LowCd: GAAGGTGACCAAGTTCATGCTGACATCGGTATCTCAATGGG HighCd: GAAGGTCGGAGTCAACGGATTAGCTGACATCGGTATCTCAATGGA Common: GTTTCATTTGCAAGAGCTGAACCTGATAT *The KASP primers carry a standard FAM tail (5'-GAAGGTGACCAAGTTCATGCT-3') and HEX tail (5'-GAAGGTCGGAGTCAACGGATT-3') with different fluorescence signals.
PCR conditions: 94°C - 15min, 10x Touchdown: 94°C -20s, 65°C - 57°C (the annealing temp decreased by 0.8°C per cycle), 30 cycles (94°C -20s, 57°C -60s)	
The plate fluorescent readings were performed in a FLUOstar Omega Microplate Reader (BMG LABTECH). The genotyping data analysis and reporting was processed with KlusterCaller software (LGC, Biosearch Technologies).	

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Screening for (b) supernodulation

The screening of 13 soybean genotypes was performed by SNAP markers for gene GmNARK (Glycine max nodule autoregulation receptor kinase) associated with supernodulation (Kim et al. 2005). Molecular assay was screening A/T single nucleotide polymorphism of the GmNARK gene using A allele-specific SNAP primer (NARK-A) and T allele-specific SNAP primers (NARK-T) and specific PCR conditions (Table 2).

Table 2 Protocol for PCR marker analysis for supernodulation.

Primers specific to the A/T single nucleotide polymorphism of the GmNARK gene		
Allele	Primer sequence	PCR product size
NARK-A	F: CAACCTCACCGGCGTACTTCCGA	343
	R: CCTCAGCGTCTTCAACTTCGACAAACTC	
NARK-T	F: GAACAACCTCACCGGCGTACTTCCTT	346
	R: CCTCAGCGTCTTCAACTTCGACAAACTC	
	F: GTGAGGGCGGCGAGCTCCAA	374
	R: AATCAGAGAGACATGAGAAGCTGTGTGTGCTA	
PCR conditions		
Concentration	PCR reagents	
60-90 ng	genomic DNA	
2x	DreamTaq Green PCR Master Mix	
0,3 µM	forward primer	
0,3 µM	reverse primer	
	Sterile distilled water	
PCR conditions: Denaturing step: 95℃ – 3 min, Amplification step (35 cycles): 95℃-30s, 64℃-30s, 72℃-30s and Extension step: 72℃ -7 min.		
Expected Product Size: A allele (full-length GmNARK -normal phenotype) – 343bp, T allele (super-nodulation phenotype) – 346bp, A and T bands – heterozygous (normal phenotype)		

Screening for (c) disease tolerance

The genome-wide association study (GWAS) was performed to dissect the complex genetic architecture of soybean quantitative resistance to Sclerotinia sclerotiorum and to provide effective molecular markers that could be used. The diversity panel for S. sclerotiorum GWAS was selected based on molecular and phenotypic data availability, consisting of 84 genotypes of different origin. This panel was inoculated with S. sclerotiorum in greenhouse conditions, where the phenotypic response to infection was characterized. Under controlled conditions, the mean length of stem lesions was analysed in three repetitions. This genotype collection was genetically characterized using a genotyping by sequencing (GBS) approach, optimized for soybean (ApeKI enzyme). The TASSEL 5 GBSv2 pipeline was used to call SNPs under optimized parameters, using the Williams 82 assembly 2 (Wm82.a2). After imputing missing data and filtering, around 18,000 SNPs were obtained for association analyses by using the Fixed and Random Model Circulating Probability Unification (FarmCPU) method, using R/GAPIT 3.0 package. This approach accounted for population structure and genetic

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relatedness. The false positives in the GWAS study were corrected using a threshold of FDR-adjusted p-values <0.1.

GWAS approach was also performed to identify allelic variation associated with resistance against northern stem canker, caused by *Diaporthe caulivora*, and provide molecular markers useful in breeding programs. Phenotypic data were presented as a response to northern stem canker infection (% of dead plants) and was obtained from screening soybean germplasm in task 4.1. The same panel of 88 genotypes was genotyped using a GBS approach, optimized for soybean. The TASSEL 5 GBSv2 pipeline was used to call SNPs under optimized parameters, using the Williams 82 assembly 2 (Wm82.a2). After imputing missing data and filtering using an MAF of ≥0.05 as a cut-off, it was selected 32,836 polymorphic SNP markers used in the GWAS. The resulting SNPs were distributed over the whole genome. The GWAS was performed with the Fixed and Random Model Circulating Probability Unification (FarmCPU) method (R/GAPIT 3.0 package), which accounted for population structure (PCA). The false positives in the GWAS study were corrected using a threshold of FDR-adjusted p-values<0.1. In the study of Maldonado dos Santos et al. (2019), it was suggested that a single major gene located on Chromosome 14 was responsible for resistance to *Diaporthe aspalathi*, causing southern stem canker (SSC). In this study were identified a total of 19 SNPs on Chromosome 14 showing significant associations with SSC resistance. Furthermore, Maldonado dos Santos et al. performed haplotype analysis and identified four haplotypes containing the combination of three SNPs that were able to discriminate resistant and susceptible genotypes (Table 3).

Table 3 SSC Haplotypes obtained using SNPs from the GWAS in the study of Maldonado dos Santos et al. (2019).

Haplotype	**SNPs Positions on Chr14 of the soybean genome Wm82.a2**			**SSC Phenotype**
	1,744,370	1,768,793	1,744,518	
Hap1	C	C	C	R
Hap2	C	C	A	
Hap3	C	G	A	
Hap4	T	G	A	S
R: SSC-resistant accessions; S: SSC-susceptible accessions				

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Material and methods for cover crops evaluation

The experiments that simulate seed multiplication process were set up according to a complete block design with four replicates at two locations Čurug (organic production) and Rimski šančevi (low-input production) during 2020-2022, Serbia (RS). A pure rye crop (1) and mixture of peas and oats (2) were sown as a cover crop, while the control treatment (C) was an area without cover crop (period autumn – spring). During 2022 and 2023 a trial with winter vetch as cover crop was set up in Serbia where the same methodology was applied. After mulching of cover crop biomass and conservation tillage two soybean varieties were sown NS Mercury (00) and NS Altis (0). Soybean was harvested, moisture was measured and well as natural yield, followed with laboratory standard germination test (germination energy and germination). Based on reliable calibration models, the spectral characteristics of the examined sample are related to the content of the component of interest. Total protein and oil content of soybean were analysed by Antaris II Thermo Scientific FT-NIR, while OMNICTM software was used for data processing and calibration. Phenolic compounds were extracted from milled (IKA®, A11 basic, Germany) and sieved (using a 0.25 mm sieve) samples. Powdered material of the control samples (seed and herbal powder) was tested in a preliminary extraction procedure (100% and 80% acetone, ethanol and methanol solutions) to obtain the highest content of total phenolics and flavonoids and the most favourable extraction agent was used in further analyses. The extraction of phenolic compounds was performed in a cooled ultrasonic bath for 1 h, centrifuged (10 min at 33600 xg) and supernatants were analysed for total phenolics and total flavonoid content. Total phenolic content was determined by the Folin-Ciocalteu method slightly modified by Mikulič-Petkovšek et al. (2012). Soil parameters were analysed at the Laboratory for Soil and Agroecology of the Institute of Field and Vegetable Crops, Novi Sad. Yield t/ha was obtained in all treatments, at each location. Near-infrared spectroscopy was used for evaluation of nutritional quality. The determination of weed species was performed on $1m^2$ on 3 replicates on each cover crop. The green and dry mass of weeds t/ha was as well as weed determination was done in the cover crop, but also in the control plot. Seed health screening was done on soybean seed samples (25 grains) with a magnifying glass 5.0 magnification was examined. Seeds that are not infected with downy mildew were placed in sodium hypochlorite NaCIO-8% suspension for 5 minutes and then rinsed in sterile water twice. Seeds were dried on sterile paper and transferred to a PDA substrate paper and PDA substrate. Seeds were left in an incubator at a temperature of 23 degrees and after 7 to 10 days an examination of seed health was carried out (microscopy and morphological determination). The data were statistically processed (StatSoft Inc., Tulsa, USA) using the analysis of variance (ANOVA) statistical method, followed by mean separation according to the Fisher's LSD test ($P < 0.05$).

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Horizon 2020
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Material and methods for inoculant use evaluation

The effects of *Bradyrhizobium japonicum* strains on microbial soil properties, yield, yield-related traits, and grain quality were examined under low-input field conditions at the Rimski Šančevi experimental field (IFVCNS, RS) in three production seasons (2019-2021). In 2021, an additional treatment which included inoculation strains and micronutrients was tested. Two soybean varieties, NS Apolo ((I) and Rubin (II), were used in the 2019 and 2020 experiments, while only the variety NS Apolo (I) was tested in 2021. Liquid inoculum (109 CFUs/ml) was mixed with a carrier (sterile peat), and soybean seed inoculation was performed just before sowing. In 2021, the nutrient complex with the following composition was included, (% m/m): S – 5.2; Mg – 3; Mn – 1.5; Fe – 1; Zn – 1; Cu – 0.5; B – 0.3; Mo – 0.01. Soil samples for microbial analyses were collected randomly from the soybean rhizosphere at full bloom (R2) and full maturity (R8). Abundance of examined microbial groups (total number of bacteria, number of azotobacters, free-living N2 fixing bacteria, ammonifiers, fungi, actinomycetes) was assessed by the indirect dilution method followed by plating of soil suspension on selective nutritive media. Dehydrogenase activity (DHA) (EC 1.1.1.) was determined spectrophotometrically by measuring the extinction of coloured triphenylformazan (TPF) at 485 nm (Casida et al., 1964). The data were statistically processed (StatSoft Inc., Tulsa, USA) using analysis of variance (ANOVA), followed by mean separation according to the Fisher's LSD test ($P < 0.05$).

Material and methods for production of elite varieties and advanced breeding lines

Useful traits were identified and used as the basis of the breeding programs at IFVC (RS) and SZG (AT). The breeding results were also validated within this task. The first step in every breeding program is the selection of the parents that will be used for the crossing and development of the initial population. This step is crucial for the final result because a specific breeding goal can be achieved only if the starting population has adequate genetic variation. The pure line selection method does not involve the creation of a starting population by artificial hybridization. In this case, the breeder selects the best individuals from the bulk of the existing variety and then observes their progeny to find genotypes equipped with desirable traits (Miladinović et al., 2008). Production of new soybean lines suitable for organic production can be done through an ideotype survey. The ideotype in crops refers to a set of morphological, physiological, and other important traits that are important for optimizing crop performance (Martre et al., 2015). In this study, we established the protocol for the detection of soybean ideotypes (Table 4).

Table 4 Soybean variety ideotype survey.

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		SOYBEAN VARIETY IDEOTYPE SURVEY		Ecobreed trials results - organic germplasm collection		
	Units	Description	Trait	minimum	average	maximum
Plant development	days	Time from emergence to first flower	Time to flowering	27	40	50
	days		Time from flowering to maturity	55	87	108
	days	From emergence to maturity	Maturity days	89	123	160
	%		Canopy cover at V4	18	52	74
	%		Canopy cover at R1	45	83	100
Yield and yield supportive traits	cm		Plant height at maturity	40	90	153
	score	1- no lodging, 5-full lodging	Lodging at maturity	1	2,7	5
	score	1-no shattering, 5-full shattering	Pod Shattering	1	1,2	5
	kg/ha		Yield	454	2529	5825
Seed related traits	score	0-absent, 1-present	Mottling score	0		1
	score	0-absent, 1-present	Hilum color	0		1
	score	0-absent, 1-present	Seed coat color	0		1
	g		1000 seed weight	106	170	311
Plant architecture	number	Number of fertile nods	Nodes number at the main stem	7	14	22
	cm		First pod height	8,7	16	33
	score	1-determinant, 5-semi-determinant, 9-indeterminant	Stem determination	1	5	9
	number		Number of branches	0	1,8	4
Chemical composition	%	seed protein content at dry matter	Protein content	36,5	41,7	49
	%	seed oil content at dry matter	Oil content	16	20,7	22,7

Results and conclusions

Results on Screening of genetic resources and breeding material

The trials for screening of diverse soybean germplasm for useful traits showed differences between the analysed genotypes (Fig. 1).

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Fig 1 Variability of soybean genotypes in IFVC trial (RS).

The average number of days from sowing to flowering varied less between the locations compared to number of days from sowing to harvest (Table 5).

Table 5 Average number of days from sowing to flowering/harvest in 2020 and 2021 at IFVC, SZG and NARDI.

		Average number of days from sowing to flowering			Average number of days from sowing to harvest		
Year	**Trial**	IFVC	SZG	NARDI	IFVC	SZG	NARDI
2020	Early	52	51.7	67	134.4	149.6	132
	Late	59.8	58.7	74	145.3	157.3	142
2021	Early	52.2	53.2	53.7	140.9	146.9	129.3
	Late	56.4	60.1	62.6	144.9	158.1	152.1

In 2020 at IFVC (RS), SZG (AT) and NARDI (RO) the difference in average number of days from sowing to flowering was 0.3 - 15 days for early genotypes and 1.1 – 15.3 days for late, with longest period at NARDI. In 2021, discrepancies between all three locations (including NARDI) for the early group ranged from 0.5-1.5 days and 2.5-5.8 for the late group. Regarding the average number of days from sowing to harvest, genotypes sown at SZG demanded the longest period to mature in both years. In 2020, early and late trials were harvested after 150 and 157 days, respectively. It was around two weeks later compared to the IFVC. Also, in 2021, at SZG both early and late accessions matured later than those at IFVC and NARDI with differences of 6 to 17.6 days depending on the

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trial and location. The difference in the number of days from sowing to flowering/harvest within the trials at each location did not vary more than 6.5 days between the years. In the R1 phase of soybean development the highest CC was recorded at IFVC (Fig. 2). At the same time, the smallest variation between the obtained data was observed in Serbia compared to Austria and Romania, especially for early trial in 2021.

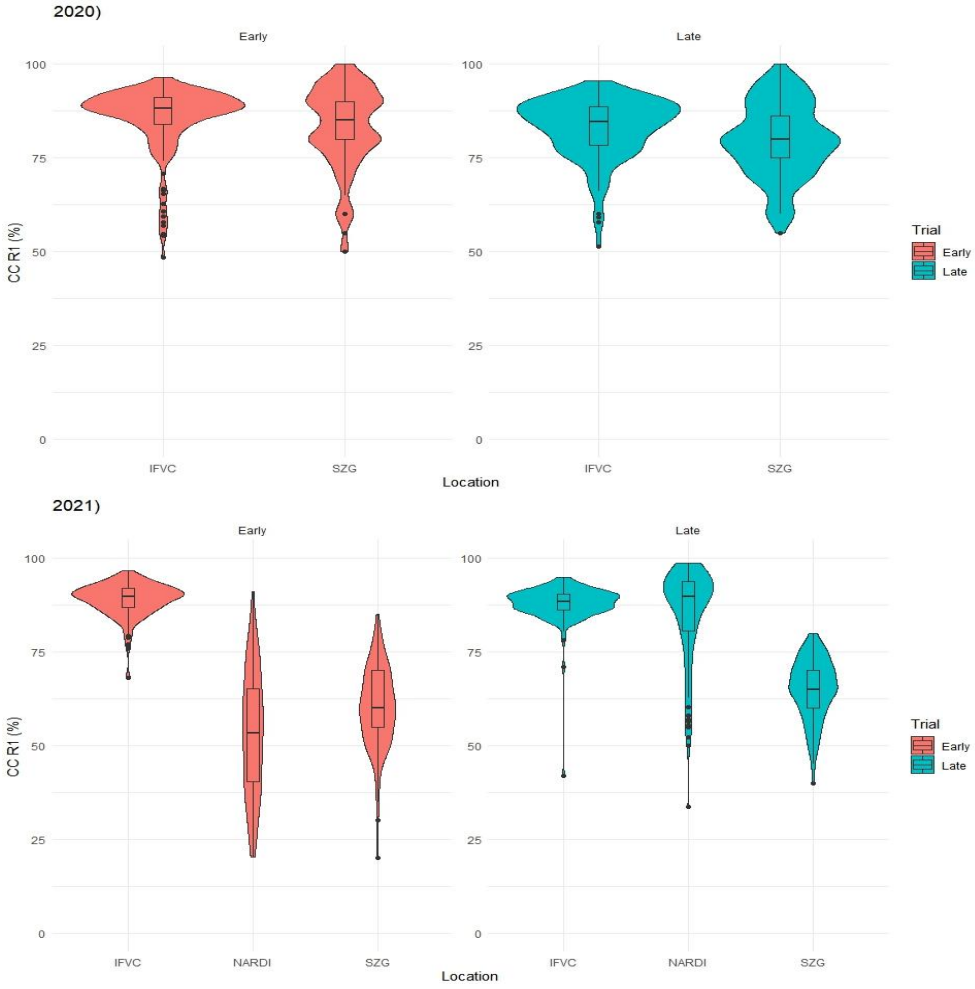

Fig 2 Canopy cover (%) of soybean genotypes grown at IFVC, NARDI and SZG in 2020 and 2021.

At each location, the CC of some plots was very low mainly as a consequence of plant loss during the growing season. Opposite to the IFVC site, the percentage of CC at SZG in 2021 was 28% (early) and 19% (late) lower than in 2020 with no plots having full canopy closure. Variety Pando stood out as the accession with the highest CC in both sites in 2020 while a year later the same result was repeated only at IFVC. The highest plant height was recorded at SZG in both years except for the 2021 late trial in NARDI, while the shortest plants were recorded at IFVC (Fig. 3). The height of the analysed genotypes varied depending on the maturity group. On average late accessions were higher compared to the early ones, especially in 2020.

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Grant agreement No 771367

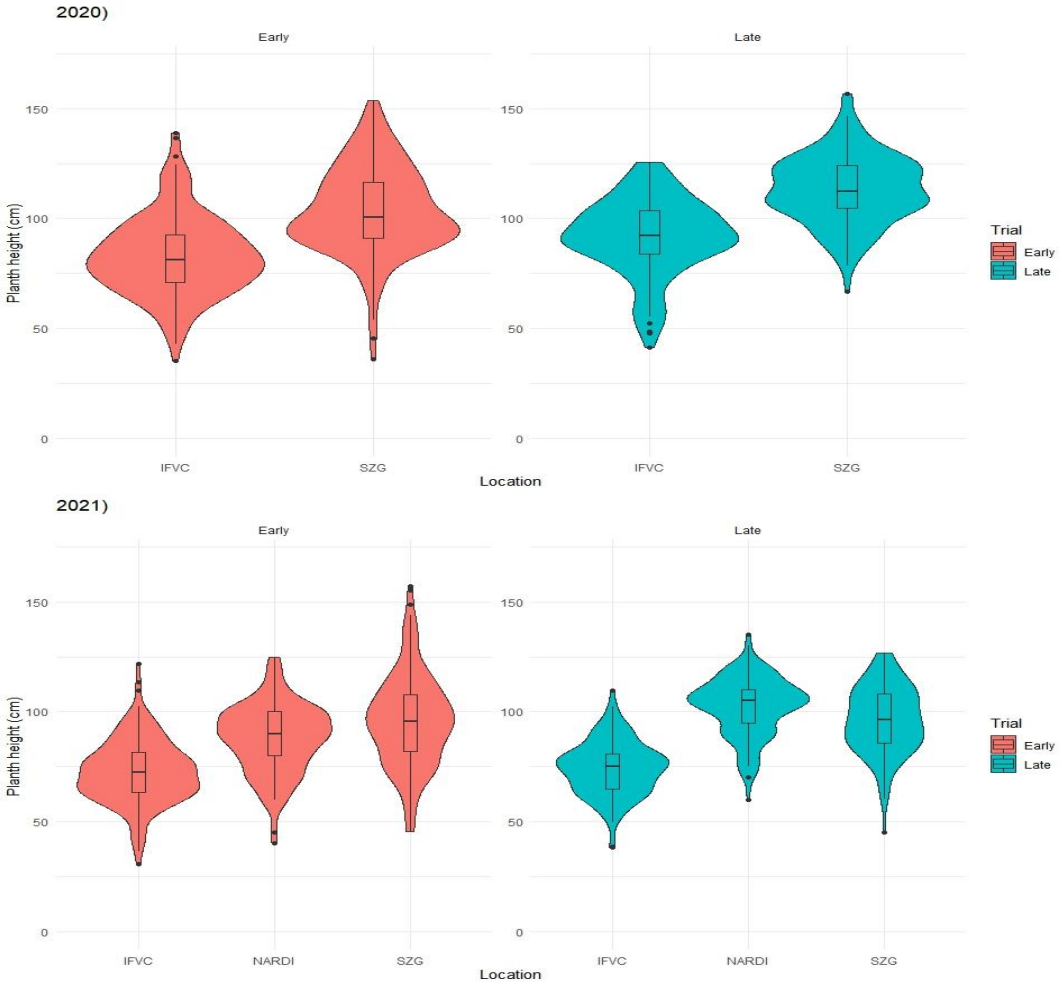

Fig 3 Plant height (cm) of soybean genotypes grown at IFVC, NARDI and SZG in 2020 and 2021.

In both years, higher yields were observed in the late trials compared to the early except in NARDI where genotypes with the shorter growing period outperformed the late ones (Fig. 4). IFVC was recognised as a highest yielding location for all trials except for the early trial that was set up at SZG in 2021. The lowest performance of soybean genotypes was recorded at NARDI when comparing the three locations. The yield of soybean genotypes at IFVC and SZG depending on year and trial ranged between 0.23 – 6.63 t/ha and 0.4 –5.22 t/ha, respectively. At NARDI, results were obtained only for 2021 where yield was in the range of 0.08 to 2.63 t/ha. A smaller data variation was observed at NARDI compared to IFVC and SZG.

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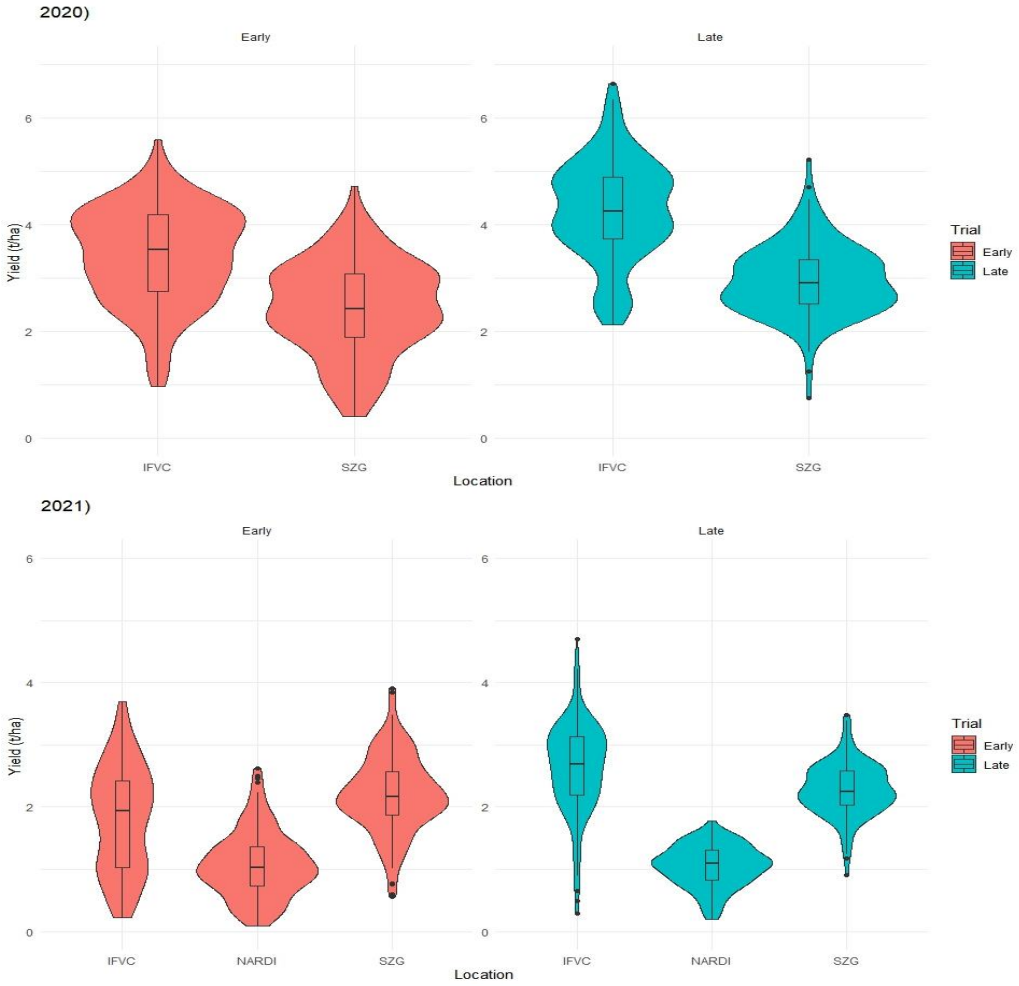

Fig 4 Yield (t/ha) of soybean genotypes grown at IFVC, NARDI, and SZG in 2020 and 2021.

As for yield component traits such as TSW, the data were different between locations in 2020 and 2021. The 1000 kernel mass ranged from 70 to 350g depending on year and trial site. Early genotypes on average had higher values of TSW compared to late in all trials (Table 6).

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Grant agreement No 771367

Table 6 Average thousand seed weight (g) of soybean genotypes grown at IFVC, NARDI and SZG in 2020 and 2021.

Year	Trial	IFVC	SZG	NARDI
2020	**Early**	209.9	192.5	–
	Late	180.6	170.2	–
2021	**Early**	171.6	201.8	145.4
	Late	156.2	165	113.6

In 2020, soybean genotypes grown at IFVC achieved higher values of TSW compared to those at SZG while in 2021 it was the opposite. The lowest 1000 kernel mass was achieved at NARDI for both early and late genotypes. Two genotypes, Toyokomachi and Toyomusume were identified as accessions with the largest seeds. Toyokomachi's TSW ranged from 194 to 316g and Toyomusume from 259 to 350g depending on year and location. After harvest, the analysis of protein and oil content were performed. The results showed that highest protein content was achieved at IFVC in all trials while the lowest values were recorded at SZG. Regarding oil content it was vice versa, especially in 2021 when more oil was accumulated in soybean genotypes that were grown at SZG then at NARDI and IFVC (Fig. 5).

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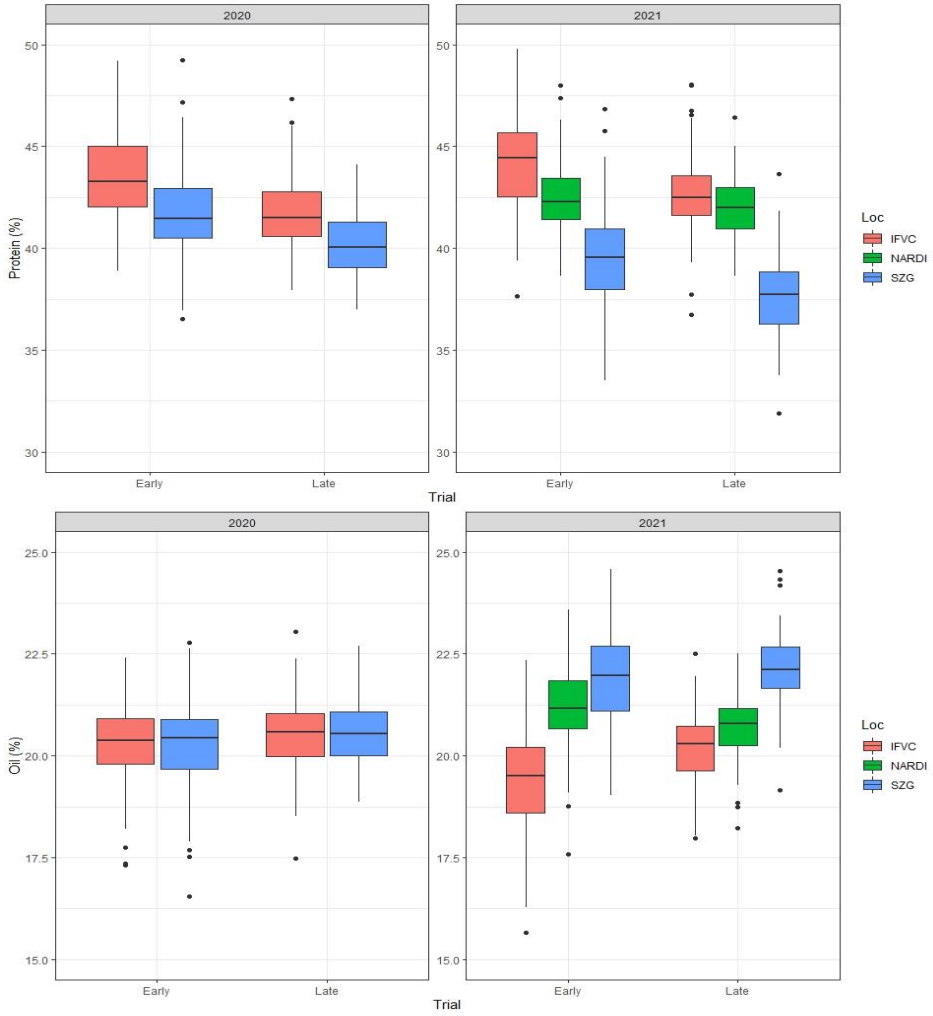

Fig 5 Protein (%) and Oil (%) content of soybean genotypes grown at IFVC, NARDI and SZG in 2020 and 2021.

According to the obtained results, the larger variation in quality parameters across the years was observed at SZG compared to IFVC. Namely, difference in protein content at IFVC between 2020 and 2021 was around 0.9% depending on the trial while at SZG it was 2.4%. At the same time oil percentage varied 0.63% in Serbia and 1.57% in Austria. The results of a one-year trial (2021) at NARDI showed that in terms of quality, this location for soybean production was positioned in the middle of the other two locations. Distribution of yield, protein and oil content and their relationship observed in 2020 and 2021 is shown in Fig. 6 and Fig. 7.

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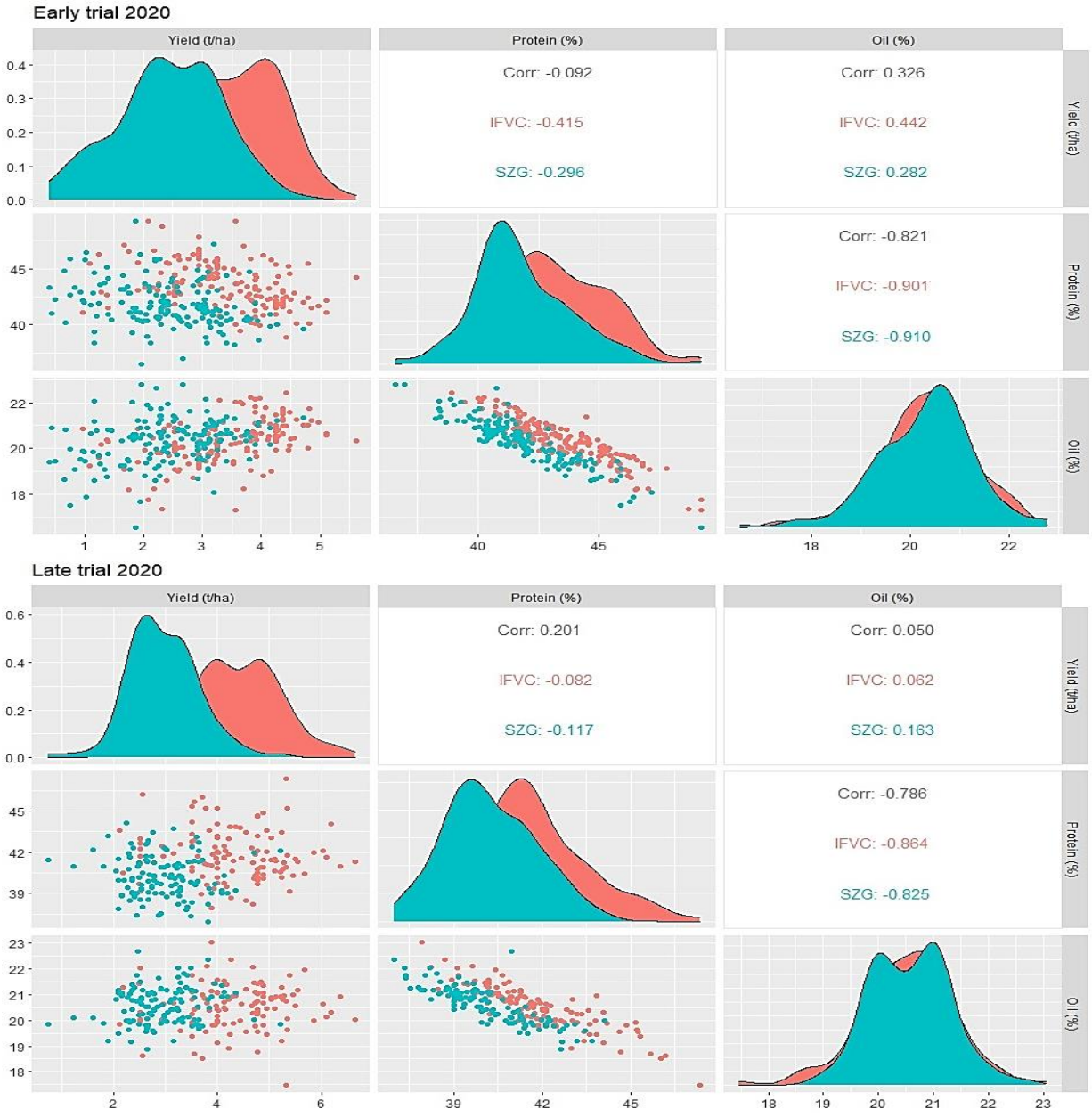

Fig 6 Distribution and correlation of yield, protein and oil content of soybean genotypes grown at IFVC, NARDI and SZG in 2020.

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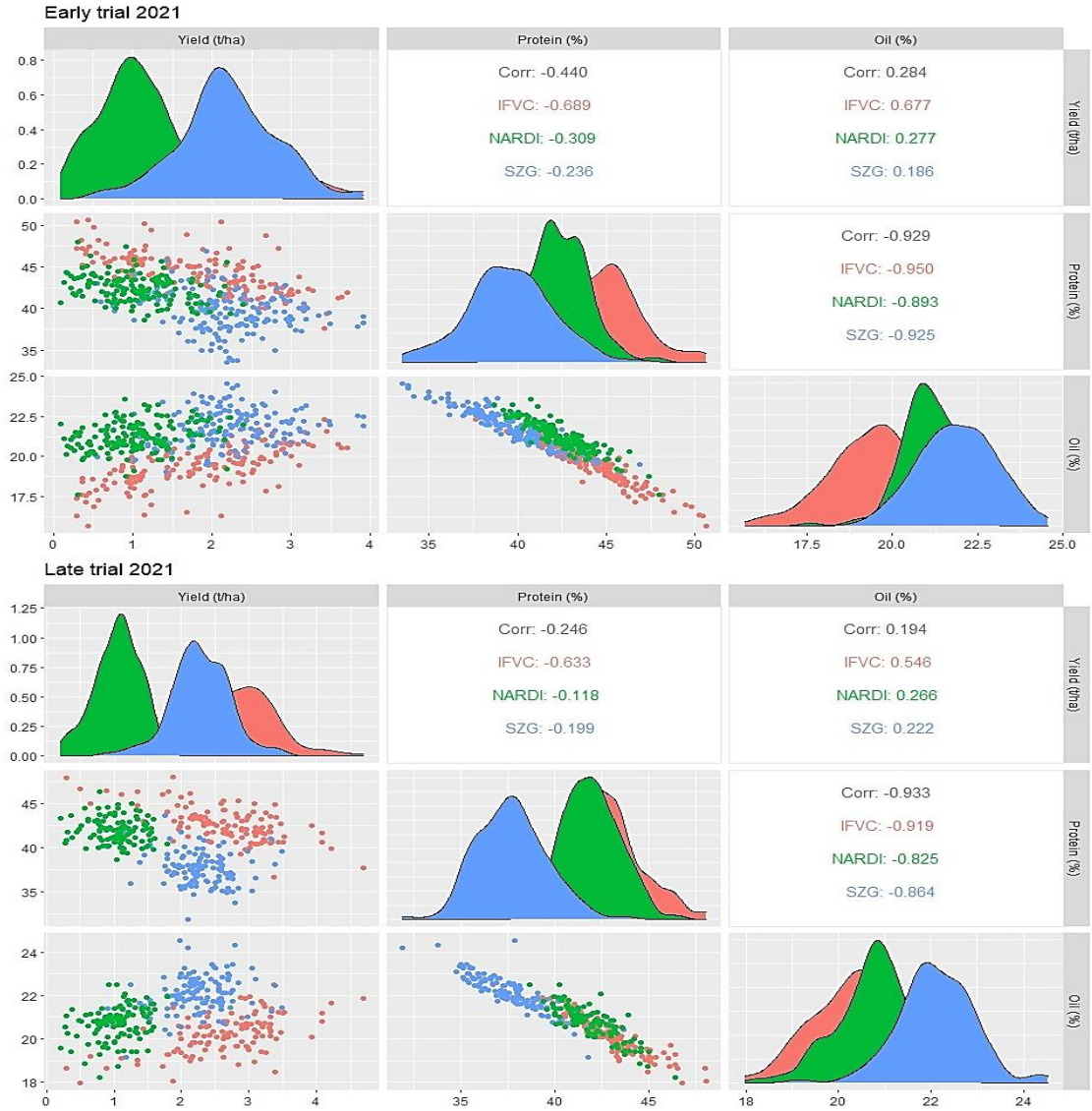

Fig 7 Distribution and correlation of yield, protein and oil content of soybean genotypes grown at IFVC, NARDI and SZG in 2021.

The results showed how yield and quality parameters are grouped depending on location. This grouping was especially pronounced for the late genotypes in both years. Distribution of all characteristics at NARDI was narrower compared to the IFVC and SZG, indicating smaller variability of the obtained results. The relationship between seed yield and protein and oil content was different. In all trials yield was negatively correlated with protein and positively correlated with oil content. Also, it was observed that the amount of accumulated protein and oil were inversely proportional i.e. increase of one led to decrease in the other. At IFVC soybean genotypes were also evaluated based on the three VIs, TGI, NDRE and NDVI. The image of each index suggests the presence of variability between genotypes based on the light reflectance (Fig. 8).

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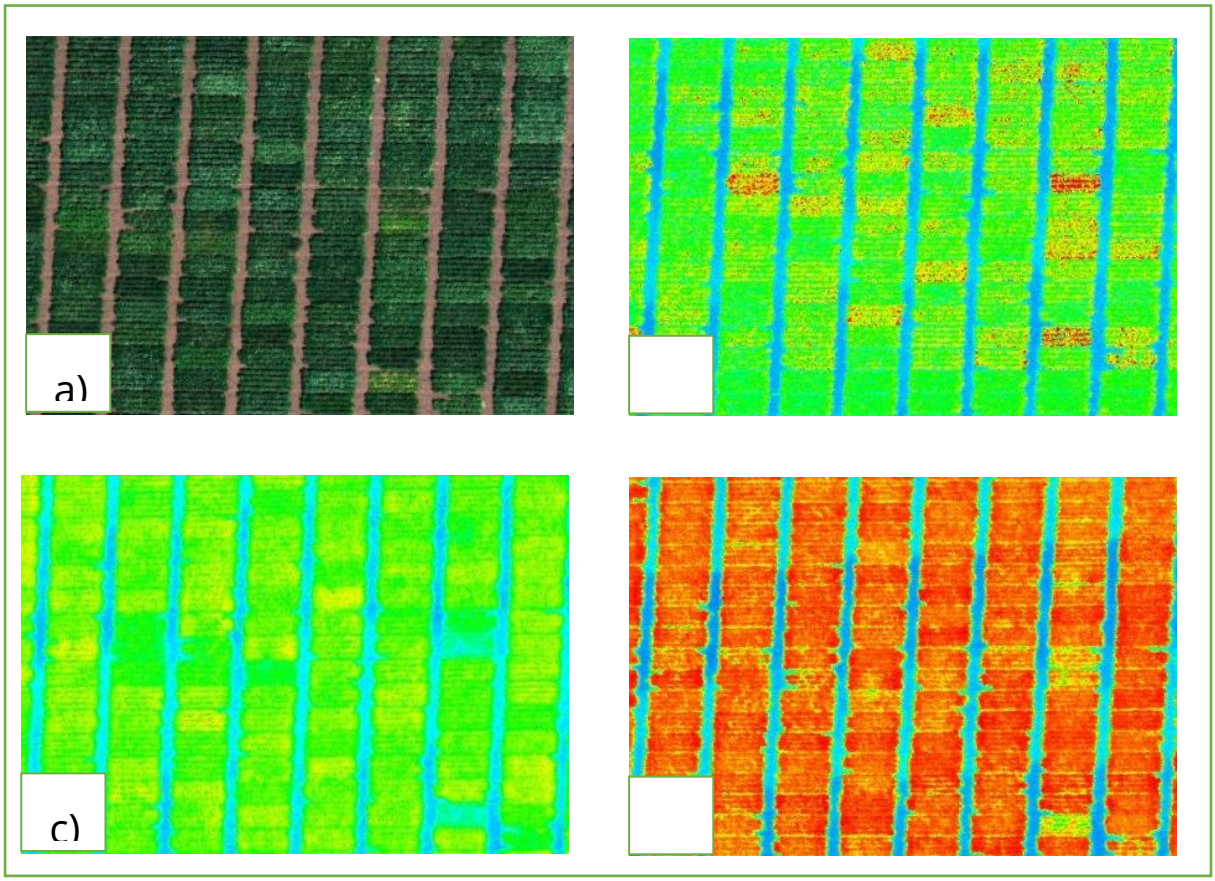

Fig 8 Images of soybean plots and vegetation indices (VIs) taken with UAV multispectral camera. a) raw image; b) triangular green index (TGI); c) normalised difference red edge index (NDRE); d) normalised difference vegetation index (NDVI).

Values of all three VIs varied depending on the time point in which photos were taken. In both years NDVI and NDRE increased as the season progressed while soybean genotypes showed different light reflection patterns, explained through the TGI (Table 7.)

Table 7 Variability of soybean vegetation indices depending on growing degree days (GDDs) at IFVC in 2020 and 2021.

Year	Vegetation index	230 GDD	474 GDD	642 GDD
2020	NDVI	0.72	0.88	0.91
	NDRE	0.10	0.19	0.26
	TGI	4.43	9.23	7.66
2021	NDVI	0.72	0.84	0.91
	NDRE	0.09	0.20	0.22
	TGI	7.52	5.60	8.25

In 2020, the highest value of TGI was recorded at 474 GDDs while in the same time point in 2021 it was the lowest compared to 230 and 642 GDDs. At 474 GDDs the TGI was highest in 2020 and lowest in 2021. This was opposite to the NDVI and NDRE whose values reached peak at 642 GDDs in both years. Also, these two indices were more

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stable, which was proven with the similar data obtained at the same time points within two seasons. The relationship between yield of all soybean genotypes (early and late) grown at IFVC and three VIs was analysed (Fig. 9).

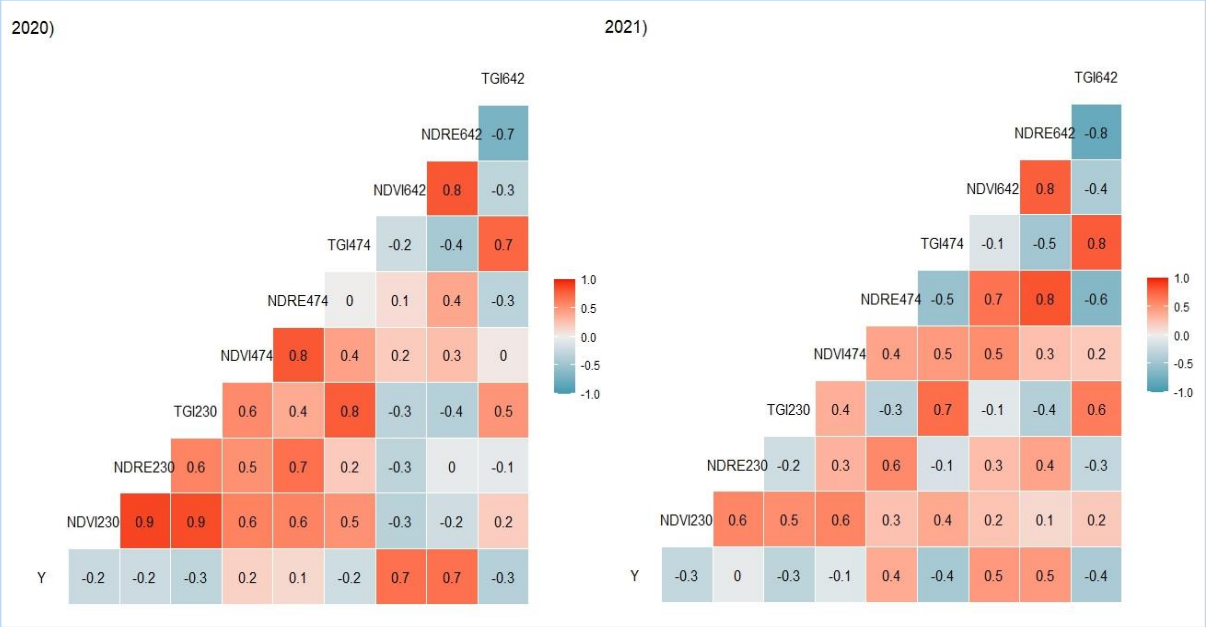

Fig 9 Correlation between combined yield (Y) of early and late soybean genotypes at IFVC and three different vegetation indices at 230, 474, and 642 growing degree days (GDDs). Normalised difference vegetation index (NDVI); normalised difference red edge index (NDRE) and triangular green index (TGI).

Highest value of correlation coefficient was observed with NDVI and NDRE at 642 GDDs with 0.7 and 0.5 in 2020 and 2021, respectively. The obtained results showed that significant amount of yield variability could be explained through the values of VIs calculated at later stages of plant development.

Results for weed screening

Weeds, especially *Abutilon theophrasti* (ABUTH) compete with crops for various environmental resources, including water, which is one of the major limiting factors for optimal crop production (Benjamin and Nielsen 2006). Since agronomic characteristics are influenced by the environment in which the crop grows and develops (Costa et al, 2019), different agroecological conditions in the three seasons may influence the competitive response of each variety. In 2020, growing conditions were more favourable for soybean growth. Mean values of leaf area (cm^2/plant) of soybean plants showed that the variety Fortuna had the biggest leaf area compared to NS Apolo and NS Zmaj. In 8 WAE, Fortuna showed a bigger leaf area than other varieties in treatment with 1 and 5 *Ambrosia Artemisiifolia* (AMBEL) per one meter of soybean row (Table 8.)

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Table 8 Mean values of leaf area (cm^2/plant) of soybean plants showed varieties differences within weeds by density combinations per year.

2020									
8 WAE									
Weeds	**ABUTH**			**AMBEL**			**XANST**		
Density m^{-1}	0	1	5	0	1	5	0	1	5
NS Apolo	797 a	689 a	600 a	705 a	720 a	661 ab	772 a	643 a	588 a
Fortuna	947 a	775 a	617 a	1291 b	998 b	908 b	840 a	817 a	514 a
NS Zmaj	738 a	773 a	776 a	854 a	767 ab	599 a	863 a	793 a	628 a
12 WAE									
NS Apolo	1343 a	1015 a	793 a	1200 a	976 a	691 a	1263 a	944 a	536 a
Fortuna	1729 ab	1897 b	1122 a	1813 b	1539 b	1105 a	1519 a	1211 a	642 a
NS Zmaj	2025 b	1520 b	850 a	1963 b	1886 b	792 a	1648 a	1166 a	601 a
2021									
8 WAE									
NS Apolo	880 a	734 a	463 a	724 a	707 a	571 a	654 a	583 a	402 a
Fortuna	738 a	820 a	636 a	901 a	739 a	710 a	800 a	622 a	470 a
NS Zmaj	760 a	746 a	585 a	812 a	870 a	583 a	729 a	653 a	390 a
12 WAE									
NS Apolo	930 a	856 a	600 a	1240 ab	1018 a	905 a	1143 a	780 a	504 a
Fortuna	1287 ab	1226 a	992 a	1173 a	1107 a	764 a	1266 a	1114 a	592 a
NS Zmaj	1648 b	1478 a	803 a	1602 b	1047 a	858 a	1473 a	1089 a	578 a
2022									
8 WAE									
NS Apolo	717 a	639 a	350 a	686 a	521 a	598 a	666 a	511 a	310 a
Fortuna	1449 b	1594 b	1125 b	1452 b	1198 b	1077 a	1831 b	1189 b	1016 b
NS Zmaj	760 a	977 a	495 a	665 a	900 ab	653 a	915 a	743 ab	337 a
12 WAE									
NS Apolo	824 a	812 a	1016 ab	883 a	552 a	479 a	657 a	542 a	516 a
Fortuna	1279 a	1715 b	1247 b	1373 a	1236 b	998 b	1290 b	782 a	688 a
NS Zmaj	1028 a	1093 ab	606 a	1080 a	1068 b	717 ab	977 ab	813 a	408 a

No significant differences were found in treatments with other weeds 8 WAE of soybean. Densities of 1 and 10 weeds per meter of soybean showed similar results as previous in Table 8. In 12 WAE, the varieties Fortuna and NS Zmaj had larger leaf area than NS Apolo. In treatments with 1 ABUTH and AMBEL there were significant differences between varieties Fortuna and NS Zmaj compared to NS Apolo. In 2021, which was dry and unfavourable for soybean production, the results showed that at 8 and 12 WAE there were no significant differences in all treatments with weeds between soybean varieties. In 2022, entire growing season was characterised by low

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precipitation. Here, the variety Fortuna showed a larger leaf area than other varieties in all treatments except with for *Xanthium strumarium* (XANST) at 12 WAE were no significant differences between the treatments were recorded (Table 8). In addition to the results of leaf area, the dry biomass of soybean plants showed similar results. The variety Fortuna stood out as the variety with the smallest reduction in dry biomass compared to the other varieties in the trial (data not shown). In 2020, the variety Fortuna had higher grain yield compared to NS Apolo and NS Zmaj, while in 2021 and 2022 there were no significant differences between varieties (Fig. 10).

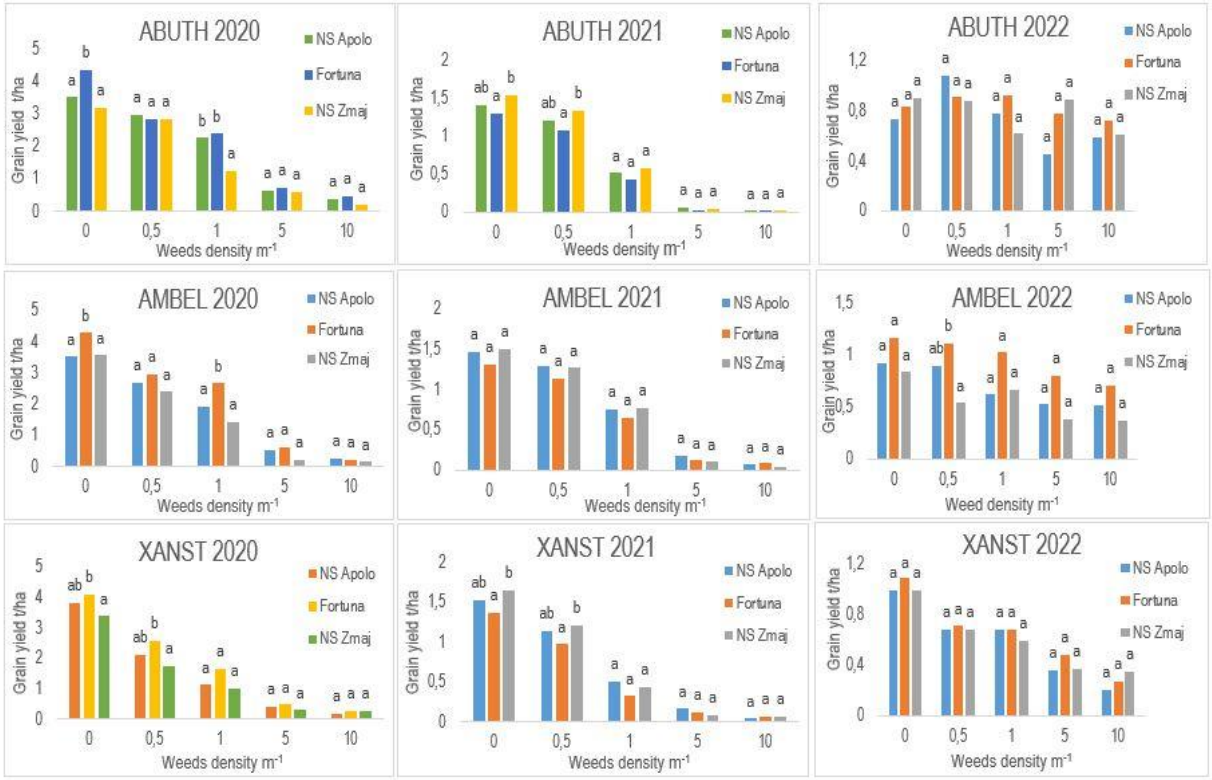

Fig 10 Effects of three weeds on grain yield (t/ha) of different soybean varieties.

Results on disease screening

The goal for the screening is to identify soybean germplasm that is resistant or tolerant to charcoal rot. The uniformity of disease pressure is an important factor to consider when conducting resistance screening for diseases. However, if the disease pressure is not uniform across screening conditions, the results may not accurately reflect the true resistance/tolerance. The Fig. 11 is displaying a disease severity index (DSI) across the four different environments.

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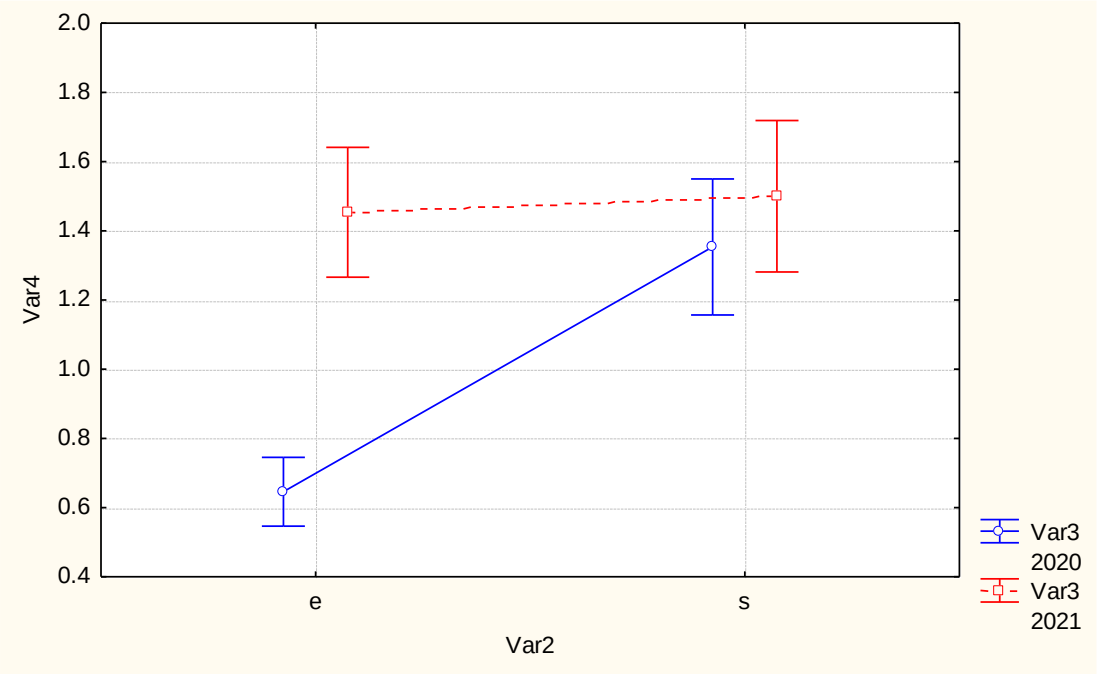

Fig 11 Disease severity index (DSI) across four different environments.

From the data collected over the four environments, it can be observed that the DSI is lowest in e2020 with a value of 0.65, while it is of similar value in other three environments (around 1.5). Uniform disease pressure means that the intensity of the disease is consistent across all environments, which allows for a fair and accurate comparison of the soybean varieties. If the disease pressure is not uniform, it may be difficult to differentiate between resistance and susceptibility due to other factors, such as differences in the environment or screening conditions. Due to uniformity criteria, the environment e2020 were excluded from assessment of soybean charcoal rot resistance.

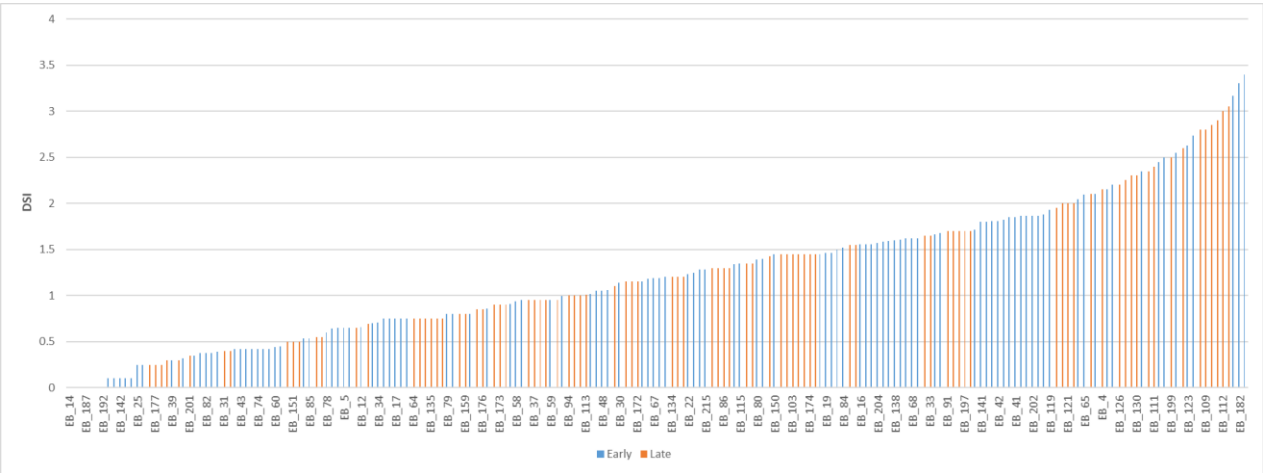

Fig 12 Disease severity index of 206 soybean genotypes.

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DSI ranged between 0 and 3.4, with an average value of 1.23. Seven genotypes had DSI equal to 0, which indicates identification of potentially tolerant/resistant genotypes. Seven potently resistant genotypes were screened by a more aggressive method (cut stem) to confirm the field trial results. Three genotypes were identified that are to a high extent resistant to charcoal rot.

Table 9 Potential resistant/tolerant soybean genotypes to charcoal rot.

EB code	Genotype	Origin	Pedigree	Maturity
EB_014	Maple arrow	Canada	Harosoy 63 X Holmberg 840-1-3	00
EB_047	Lada***	Russia Krasnodar	unknown	00
EB_049	Toyomusume	Japan	Tokei 463 x Toyosuzu	00
EB_187	Bettina***	Austria	unknown	00
EB_205	ES Commandor	France	unknown	000
EB_050	Trzic Rana***	Romania	unknown	0
EB_192	Backa	Serbia	(Fiskeby x Corsoy) x S-1346	0

***- confirmed resistance reaction

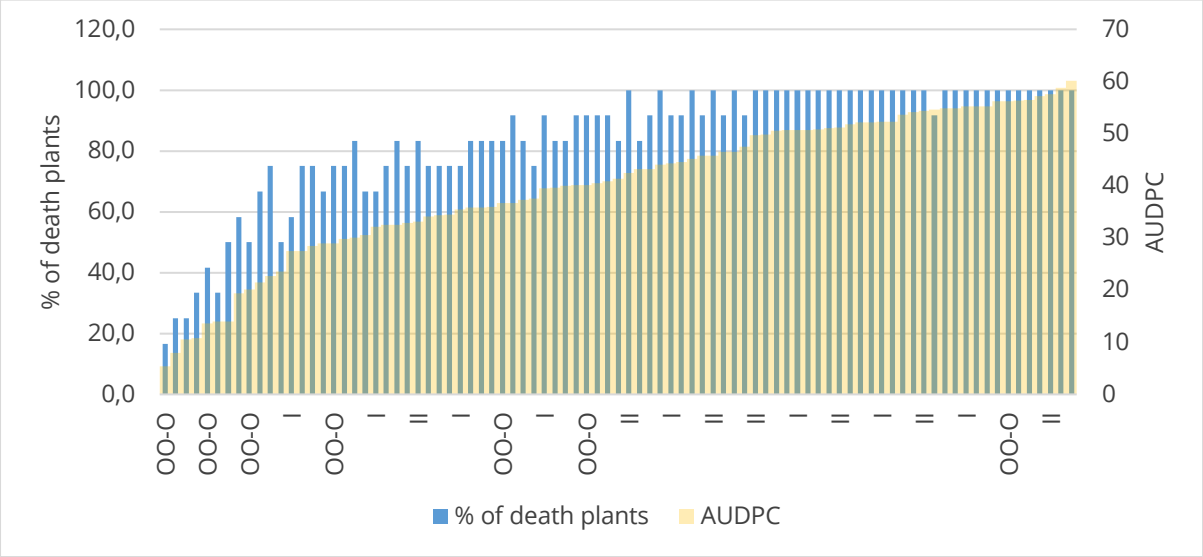

Fig 13 Reaction of soybean genotypes to *Diaporthe phaseolorum* var. *caulivora*.

Diaporthe phaseolorum var. *caulivora* (Dpc) causes soybean stem canker (SSC). The development and use of soybean varieties with resistance to SSC is an important component of an integrated disease management strategy. In addition to resistant varieties, other management strategies, such as crop rotation, tillage practices can also be used to help manage stem canker in soybean fields. Among tested soybean genotypes, there is no resistance to DPC while genotypes have different reactions. It is observed that early maturity genotypes are less susceptible to SSC (Fig. 13).

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Results on SGSB screening

One of the goals of this set of trials was to evaluate reactions of different soybean varieties to biotic stress caused by SGSB feeding since it has different influence on several aspects of the soybean plant. Overall, the level of SGSB damage on soybean depends on the variety and environmental conditions. SGSB did not influence soybean plant architecture, which is seen through a consistent number of lateral branches and plant height in the control and SGSB cages (Table 10).

Table 10 Plant height and number of lateral branches on five soybean varieties during 2018, 2020 and 2021.

Year	Variety	Fortuna (00)		Princeza (0)		Romansa (I)		Sava (I)		Senka (III)	
Trait		**Con.**	**Treat.**	**Con.**	**Treat.**	**Con.**	**Treat.**	**Con.**	**Treat.**	**Con.**	**Treat.**
2018	Plant height (cm)	61,2	59	97,3	85,3	74,3	66,8	72,7	79	100,7	96,57
	Number of lateral branches	2,9	1,6	2,4	5	2,1	2,22	0,5	0,44	0,71	1
2020	Plant height (cm)	87,1	82,5	65,2	72,4	75,5	73,9	85,5	89,78	108,1	98,5
	Number of lateral branches	0,4	0,7	4,6	2,4	2,8	4,5	0,6	1,33	3,7	2,5
2021	Plant height (cm)	66,32	64,9	64,8	71,5	57,2	64,38	68,2	72,89	95,56	90,44
	Number of lateral branches	1	1,1	1,5	2,5	2	2,12	0,8	0,56	2,33	1,67

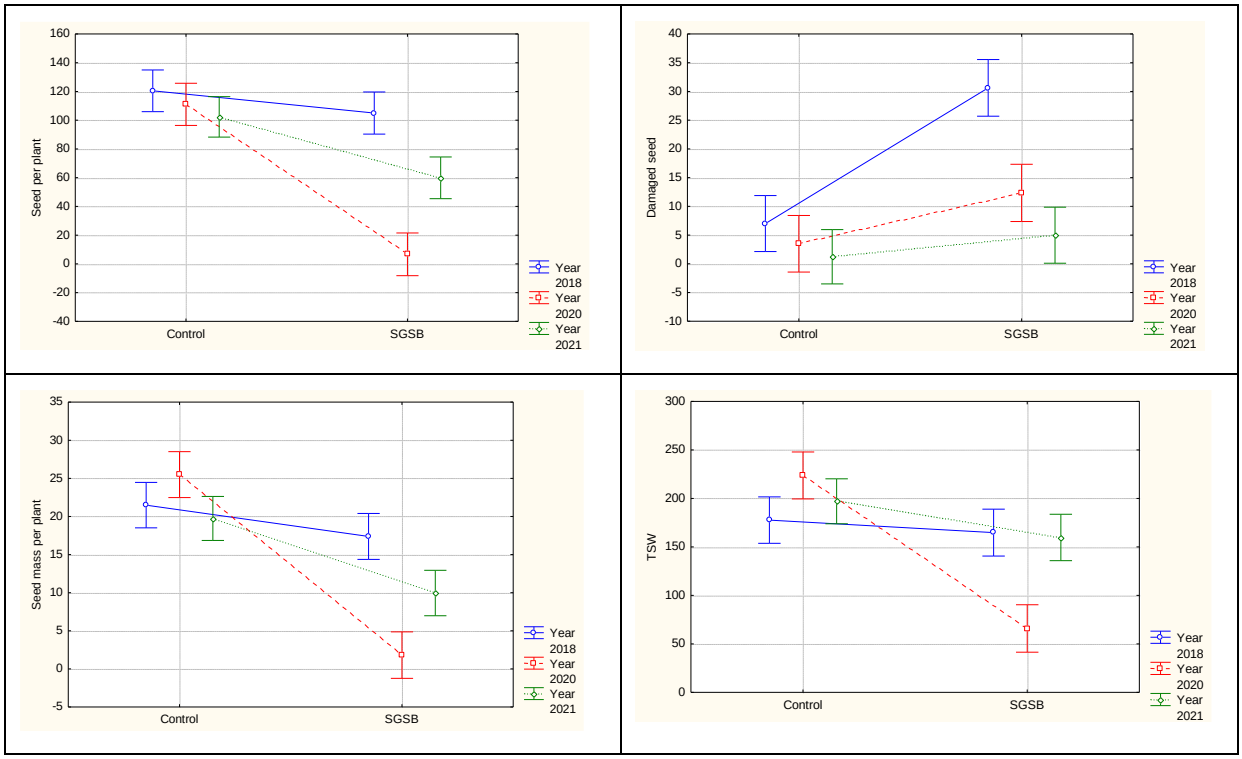

Fig 14 Differences between control and SGSB treatment expressed through number of seeds per plant, number of damaged seeds, seed weight per plant and thousand seed weight.

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Even though plant architecture was not influenced by SGSB, yield-related traits are more prone to insect feeding. The average number of seeds per plant in every experimental year was lower in the SGSB treatment. Seed number per plant on the variety Fortuna did not show great differences between control and SGSB treatment which can be partly explained by maturity group (00) since it is a very early variety. In contrast, all other varieties showed significant differences between the control and SGSB treatment, especially in 2020 where the level of damage was influenced by environmental conditions. Feeding of SGSB on soybean plants causes damaged seed, which is not suitable for planting or processing. Year 2018 was with the most evident differences with the highest number of damaged seeds which does not confirm data presented previously since in 2020 damage was so severe that seeds were not formed in most cases. Again, the variety Fortuna (00) did not show significant differences between the control and SGSB treatments. Seed weight was also influenced by SGSB feeding. In the year 2020, the most severe damage was observed. Moreover, SGSB caused direct damage on soybean seed, also insect feeding resulted in a physiological effect on soybean plants with a reduction in thousand seed weight (TSW). The varieties Fortuna and Sava seemed to be less influenced by SGSB for the most yield-related traits. The variety Fortuna avoided higher damages due to its maturity group (00). Maturity group of the soybean variety can be of key importance for avoiding higher damage from SGSB. Considering that use of pesticides is forbidden in organic production, selection of soybean varieties and timely application of cultivation practices are two options for overcoming crop failure due to SGSB damage. The third option is the use of traps and trap crops that need to be tested on a local basis.

Conclusions on screening of genetic resources and breeding material

In the conducted study for important phenotypic traits, divergent soybean genotypes were analysed at three locations. The germplasm consisted of accessions from different maturity groups (000-II) classified as early and late, originating from Japan to Canada. The southern location of IFVC and NARDI compared to SZG did not affect the required number of days from sowing to flowering as much as number of days to harvest which was significantly shorter in Serbia and Romania. Even though the trials were set up at different latitudes all genotypes matured properly and were harvested at the end of the season. This was the case in 2020 at two locations (NARDI trial was destroyed by pest) and in all three locations in 2021. Such information is of clear importance knowing that soybean is sensitive to photoperiod which limits the distribution of varieties from north to south (Zhang et al., 2007). Growth parameters varied depending on location in such way that soybean genotypes at IFVC achieved biggest CC in flowering while at the same

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time plants were shorter at the end of the season compared to the other two experimental sites in both years. Lack of height in soybean grown at IFVC did not affect seed production as Serbia was recognised as a highest yielding location for the most trials. At IFVC and SZG, longer growing period led to higher yields in the late genotypes as a consequence of the extended period of resource capture and utilisation (Miladinović, et al., 2008). This was not the case at NARDI in 2021, when early accessions slightly outperformed the late ones, which is not unusual in some cases. Namely, the varieties with the shorter growing period tend to go faster through plant development phases and thus have ability to avoid extreme temperatures and lack of precipitation during later stages of development. Unfavourable conditions can also affect TSW, this is proven by the data obtained from NARDI as this was the location where the smallest seeds were produced. Numerous soybean genotypes had TSW >250 g, some even >300 g which suggests that they could be used as an important source in selection of varieties suitable for human consumption. Such cultivars could be interesting in the production of edamame soybean, rich in health beneficial nutrients like isoflavones (Roland et al., 2011). It is well known that soybean seed can provide all essential amino acids (FAO/WHO/UN, 1985) and that the quality of protein is similar to those from an animal origin (Kudełka et al., 2021). Therefore, soybean is becoming more attractive as a food crop which is why breeders are focused on selection of varieties that will satisfy increasing demands. The data obtained through the field trials showed that early genotypes accumulated more protein and less oil than late. The IFVC location stood out as a site with the highest protein concentration compared to SZG and NARDI. At the same time, having high yield, total protein production per area was also biggest at Novi Sad. Still, quality and seed yield are negatively correlated and thus proper selection of varieties that satisfies both conditions is necessary. The screening of diverse soybean germplasm within the conducted study showed promising results as some accessions showed high protein content (>45%) while having good yields, over 3.5 t/ha. In the era of digital technologies, different tools, and techniques can be utilised to access important information about plant phenotypic characteristics that cannot be obtained with traditional methods. At IFVC soybean genotypes were analysed based on the values of three VIs (NDVI, NDRE and TGI) assessed at different GDDs. The NDVI for soil and plants ranges from 0-1, values close to the one represents dense, green healthy vegetation. A continuous increase in NDVI that was observed in both years at IFVC showed that soybean plots were not under any stress. As the season progressed, NDVI tends to saturate in dense vegetation (Gitelson, et al., 2004). In such case, the NDRE, index like NDVI can be more useful as it can penetrate deeper into the canopy and thus provide more precise information. The values of NDRE were similar to NDVI, confirming that soybean plants were in good condition. Unlike NDVI and NDRE which had the same pattern over the two years, the dynamics of TGI differed in 2020 and 2021. In 2020, the

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TGI reached its peak much earlier than in 2021. The obtained results can be interpreted differently as an increase of TGI is associated with a decrease of total chlorophyll (TCH) but at the same time is positively correlated with the leaf area index (LAI) (Hunt et al., 2013). Both traits are important for the photosynthesis and thus can have an impact on seed production. The yield of soybean genotypes in 2020 was almost twice as high as in 2021 while NDVI and NDRE had similar values in both years. This means that although they are associated with yield, such discrepancies in seed yield could not be detected with these two VIs. In that case, observed values of TGI suggest that yield variation between the years could be explained by differences in reflection of visible light.

Conclusions on weed competitiveness screening

The study on competitiveness between soybean and dominant weeds showed that the variety Fortuna performed better in terms of dry biomass accumulation and leaf area in the dry season compared to other varieties. In 2021 and 2022 the weed effect on soybean yield was masked due to the low precipitation. Parameters such as leaf area and dry biomass proved to be better indicators of the competitiveness than yield of soybean genotypes. The development of soybean cultivars with traits that increase competitive ability against dominant weeds in soybean would be beneficial. This approach would be helpful for organic producers as an additional weed management strategy.

Conclusions on disease screening

Among tested soybean genotypes the reaction to artificial inoculation with DPC was observed, that early maturity genotypes are less susceptible to SSC, while none of tested genotype were resistant.

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Results on drought tolerance

In the trials for abiotic tolerance, the difference between genotypes grown in drought simulation (Fig. 15a) and in control environments was observed (Fig. 15b).

Table 11 Average number of days from sowing to flowering/harvest in 2020 and 2021 at IFVC, SZG and NARDI.

		Average number of days from sowing to flowering		Average number of days from sowing to harvest	
Year	**Trial**	Early	Late	Early	Late
2020	Drought	35.7	41.7	115.8	125.6
	Control	52	59.8	134.4	145.3
2021	Drought	38.8	41.2	123.1	128.5
	Control	52.2	56.4	140.9	144.9

Fig 15 Difference between soybean genotypes in drought simulation (a) and in control environments (b).

The soybean genotypes exposed to drought, flowered and matured earlier compared to the control (Table 11). In the drought simulation environment the average number of days from sowing to flowering was 13-18 days shorter, depending on the maturity group and year. The soybean genotypes grown in the control also had a longer growing period of 17-20 days compared to genotypes exposed to abiotic stress. The research showed that unfavourable growing conditions affected the early and late group in a similar way as there were almost no differences in obtained results for the average number of days from sowing to flowering/maturity. Significantly lower values of CC in the flowering phase of soybean development were recorded for genotypes exposed to drought compared to the control in both years (Fig. 16).

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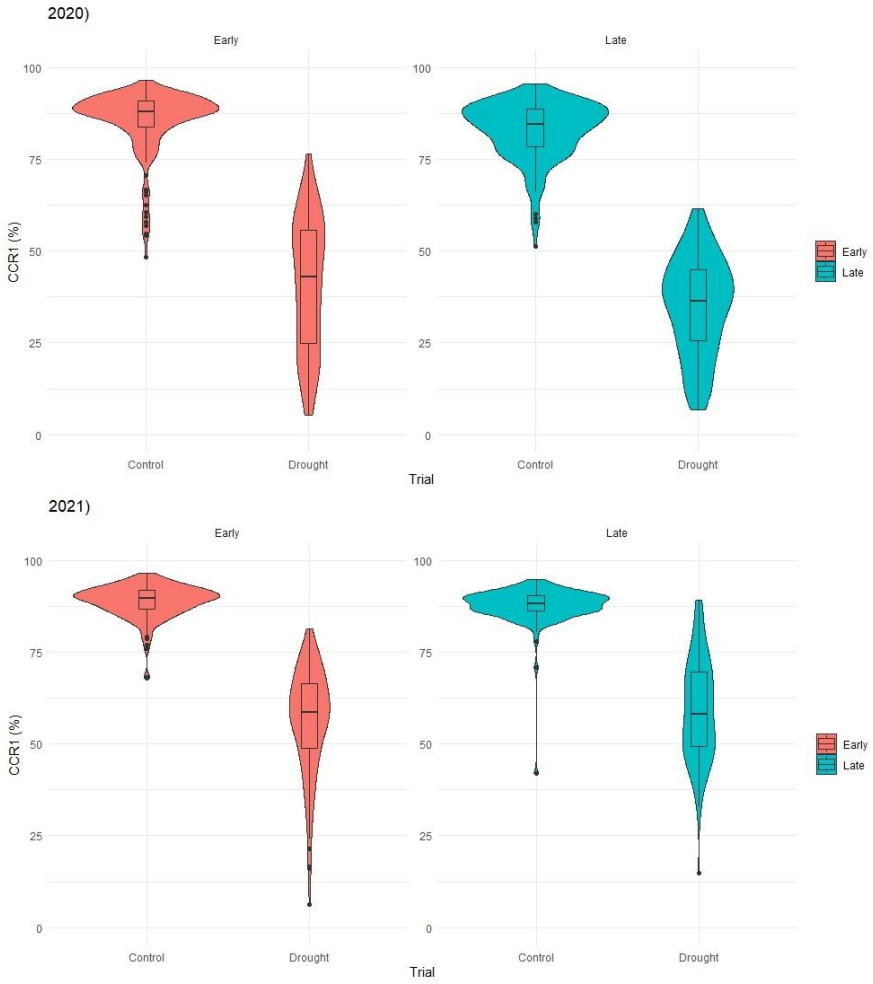

Fig 16 Canopy cover in flowering phase-CCR1 (%) of soybean genotypes grown in drought simulation and in control environments in 2020 and 2021.

Poor germination and plant loss during the season resulted in extremely low values of CC for some plots. The obtained results show that a bigger variation in CC data was observed for soybean genotypes grown in drought simulation compared to the control environment. This was especially pronounced in 2020 when the average CC for the early and late maturity group was 40.91 and 35.07 %, respectively. In 2021, the results of average CC were higher with 56.33 % for early and 59.31% for late genotypes. In the control experiment, the average CC of soybean genotypes ranged between 83-89 % depending on the maturity group and year. As for plant height the recorded data shows that taller plants were observed in 2020 compared to 2021 (Fig. 17).

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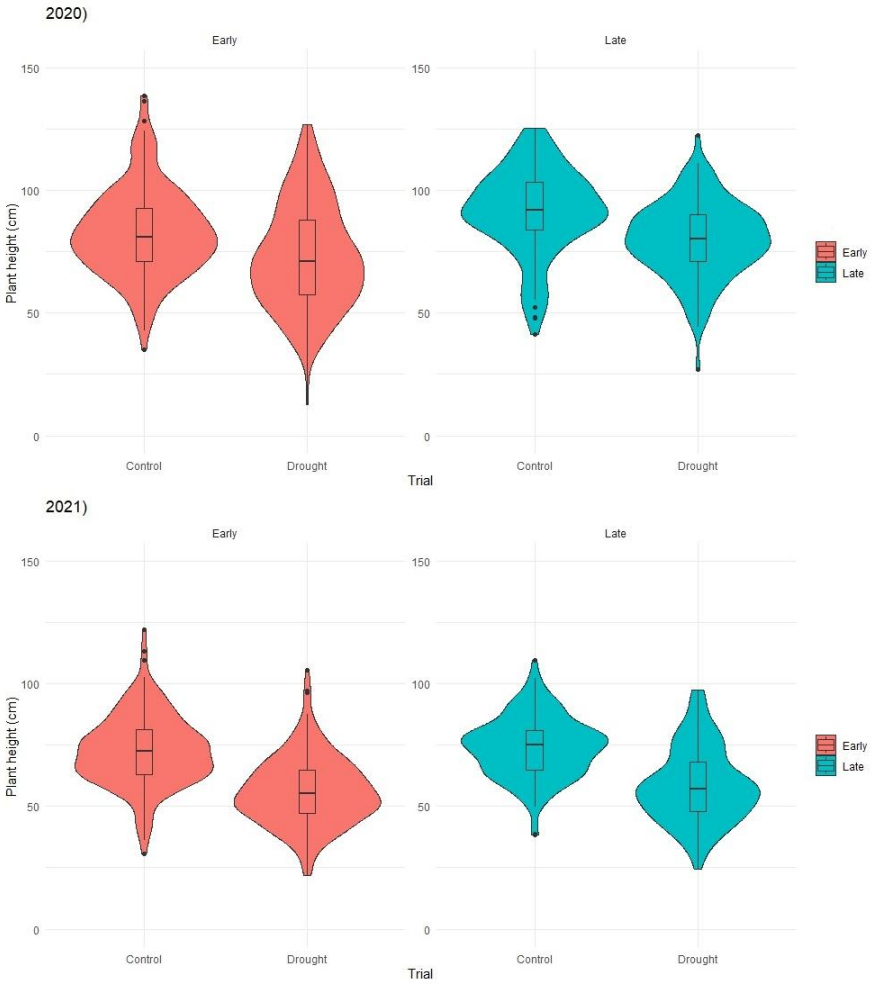

Fig 17 Plant height (cm) of soybean genotypes grown in drought simulation and control environments in 2020 and 2021.

The tallest plants were recorded in the 2020 late control group with an average of 91.59 cm while the shortest were in the 2021 early group with an average plant height of 56.22 cm. On average, the late genotypes were taller than the early ones. This was observed in the control and drought trials in both years. In general, the higher yields were recorded in 2020 compared to 2021. The soybean genotypes grown in the drought simulation environments had lower yields compared to the control (Fig. 18).

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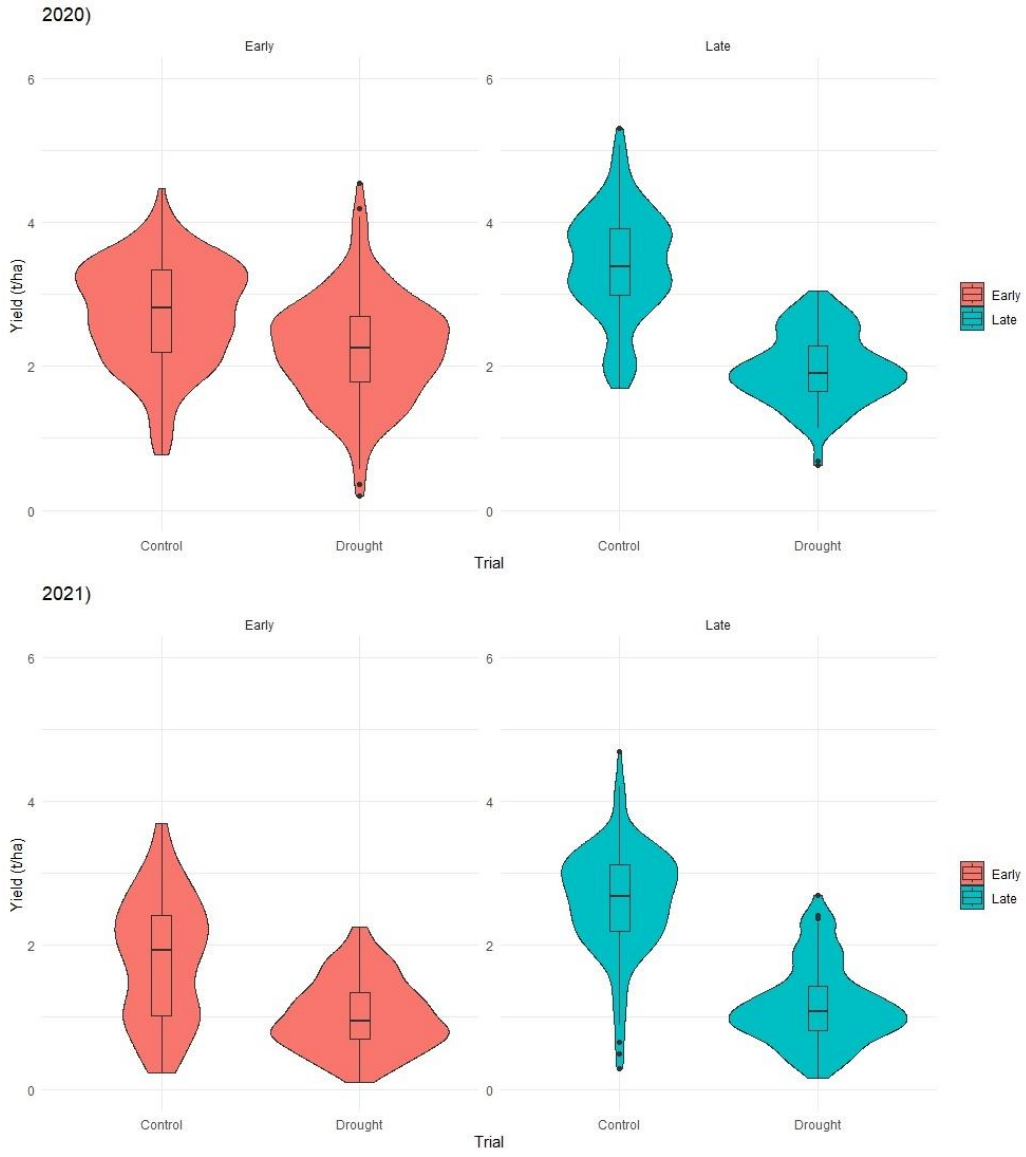

Fig 18 Yield (t/ha) of soybean genotypes grown in drought simulation and control environments in 2020 and 2021.

In 2020, the average yield of the drought trial was 2.25 t/ha and 1.96 t/ha for early and late soybean genotypes, respectively. In the control, the same germplasm achieved 2.73 t/ha and 3.42 t/ha which means that unfavourable growing conditions reduced the yield by 18 and 43 %. In 2021, the yield loss was even higher and ranged between 44 % for early to 56 % for the late group. The obtained data indicates that the soybean genotypes with shorter growing period had lower yield loss compared to the late germplasm.

The soybean genotypes under abiotic stress had lower values of TSW compared to the control in 2020 and 2021. This was observed for both the early and late maturity groups (Fig. 19).

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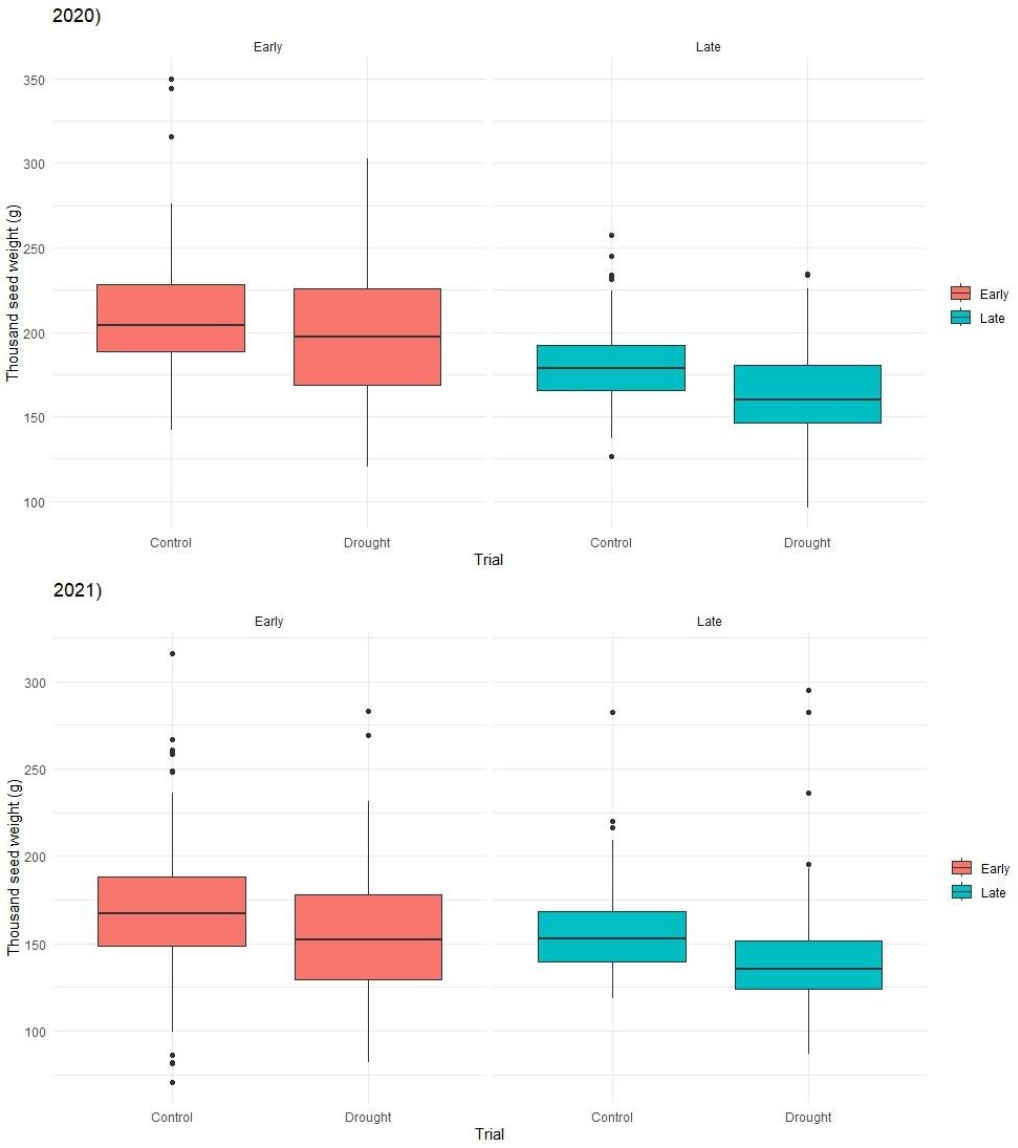

Fig 19 Thousand seed weight-TSW (g) of soybean genotypes grown in drought simulation and control environments in 2020 and 2021.

In 2020, the TSW of early genotypes in the drought trial ranged from 120 to 302 g while in control, values were from 141 up to 350 g. The TSW of the late group in the abiotic stress experiment was between 96-234 g and 126-257 g in the control. In 2021, seeds were generally smaller than the year before. The TSW of early genotypes under drought conditions varied from 82 to 283 g while in the control it ranged from 70-315g. The late group grown in an unfavourable environment achieved TSW of 86-295 g while in the control trial, it ranged between 118 and 282 g.

The effect of abiotic stress caused by drought on analysed soybean genotypes was also observed through the values of nine stress indices based on yield as the most important agronomic trait. The relationship between yield in control (Yp), yield in

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drought conditions (Ys), and all indices is shown through the values of Pearson's correlation coefficients between yields in control (Yp), yield in stress conditions (Ys) and nine stress indices for soybean genotypes grown in a drought simulation environment and control in 2020 and 2021. Tolerance index (TOL), mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HM), stress susceptibility index (SSI), stress tolerance index (STI), yield index (YI), yield stability index (YSI), relative stress index (RSI) (Fig. 20).

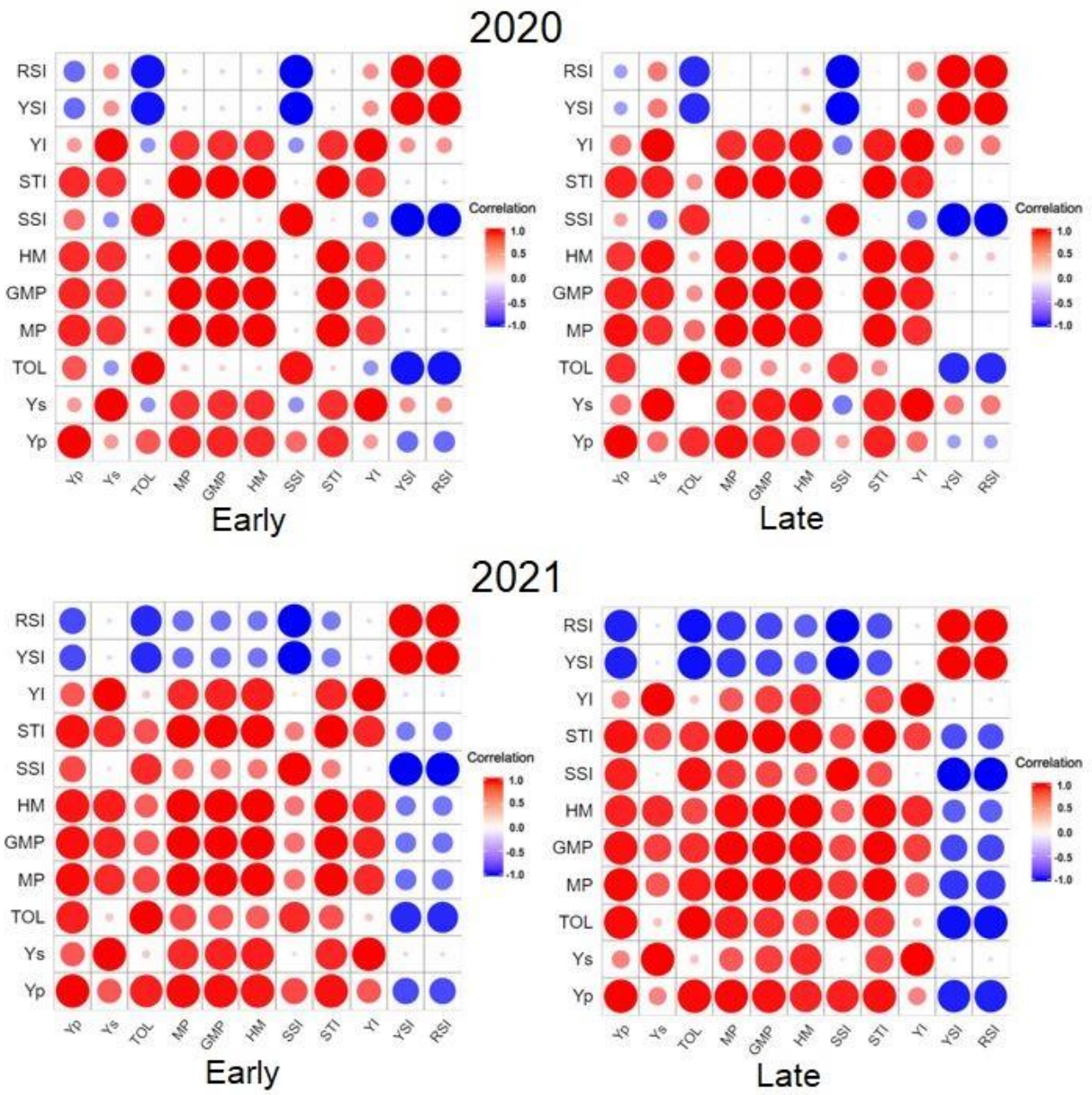

Fig 20 Pearson's correlation coefficients between yields in control (Yp), yield in stress conditions (Ys) and nine stress indices for soybean genotypes grown in a drought simulation environment and control in 2020 and 2021.

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In both years, a positive relationship was dominant for most variables while the higher values of Pearson's correlation coefficient were observed in 2021. The correlation between Yp and Ys for early soybean genotypes in 2020 and 2021 was 0.16 and 0.44, respectively. On the other side, the correlation between yields harvested in the control and drought trial for the late group was 0.33 in 2020 and 0.24 in 2021. Based on the TOL, SSI, YSI, and RSI indices, the best-ranking soybean genotype from the early maturity group in 2020 was Timirjazevskaja 144 while in 2021 it was Kamishunbetzu. The same indicators suggest that the smallest difference between Yp and Ys was recorded for the variety DH 4173 in 2020 and for the variety Trzic Rana in 2021. In both years, three soybean genotypes from the early and late group were ranked in the top 10 most stress-tolerant cultivars based on MP, GMP, and STI stress indices (Table 12).

Table 12 Ranking of soybean genotypes represented in the top 10 stress-tolerant cultivars based on MP, GMP, and STI stress indices in 2020 and 2021.

		Early			Late		
Year	**Stress index**	**Lissabon**	**Kitty**	**NS Virtus**	**NS Vasa**	**Hogar**	**Trijumf**
2020	**MP**	9	7	6	2	3	5
	GMP	8	6	7	1	3	9
	STI	8	6	7	1	3	9
2021	**MP**	9	7	2	1	4	5
	GMP	7	9	2	1	3	6
	STI	7	9	2	1	3	6

Spectral reflectance data from all trials was collected and used for the evaluation of analysed soybean genotypes. The values of individual channels from photos recorded with UAV were processed and NDVI and NDRE were calculated for every plot. The images of VIs suggest that there was a clear difference between soybean genotypes grown in drought stress compared to control environments (Figs. 21 and 22).

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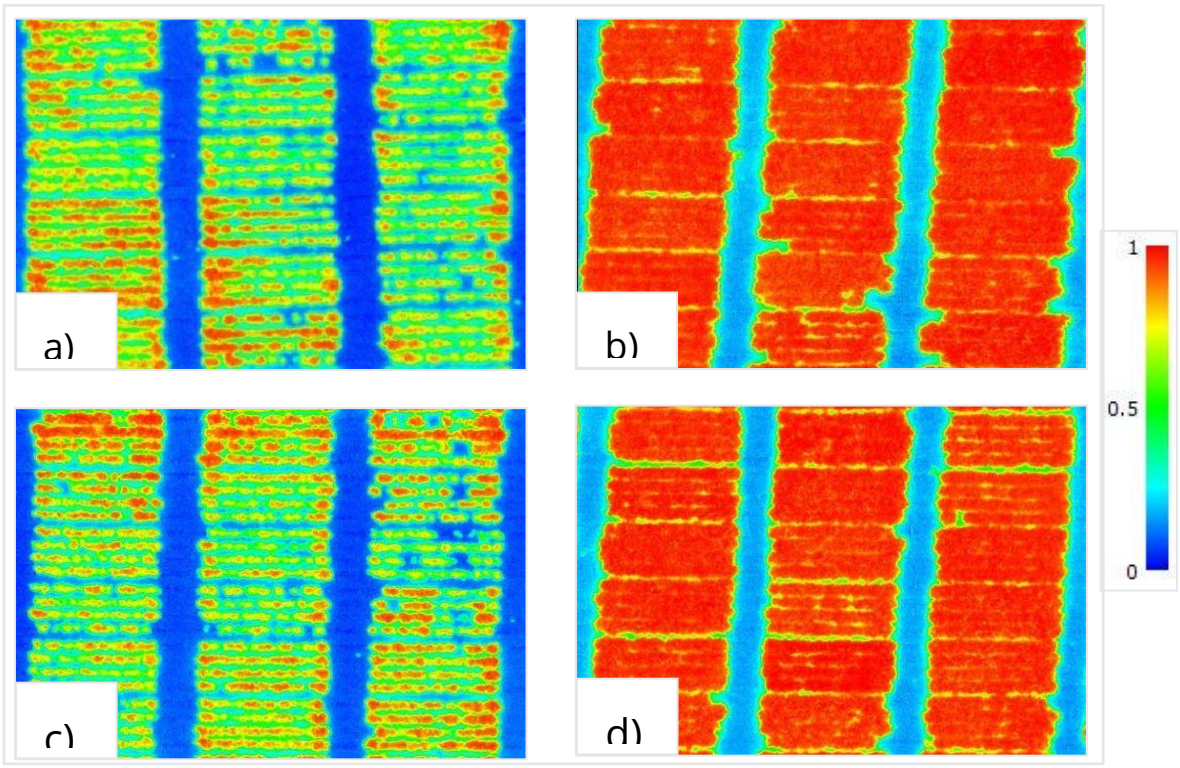

Fig 21 Example of normalised difference vegetation index (NDVI) of soybean genotypes grown under abiotic stress (drought) and control environment; a) early group grown in drought; b) early control; c) late group grown in drought; d) late control.

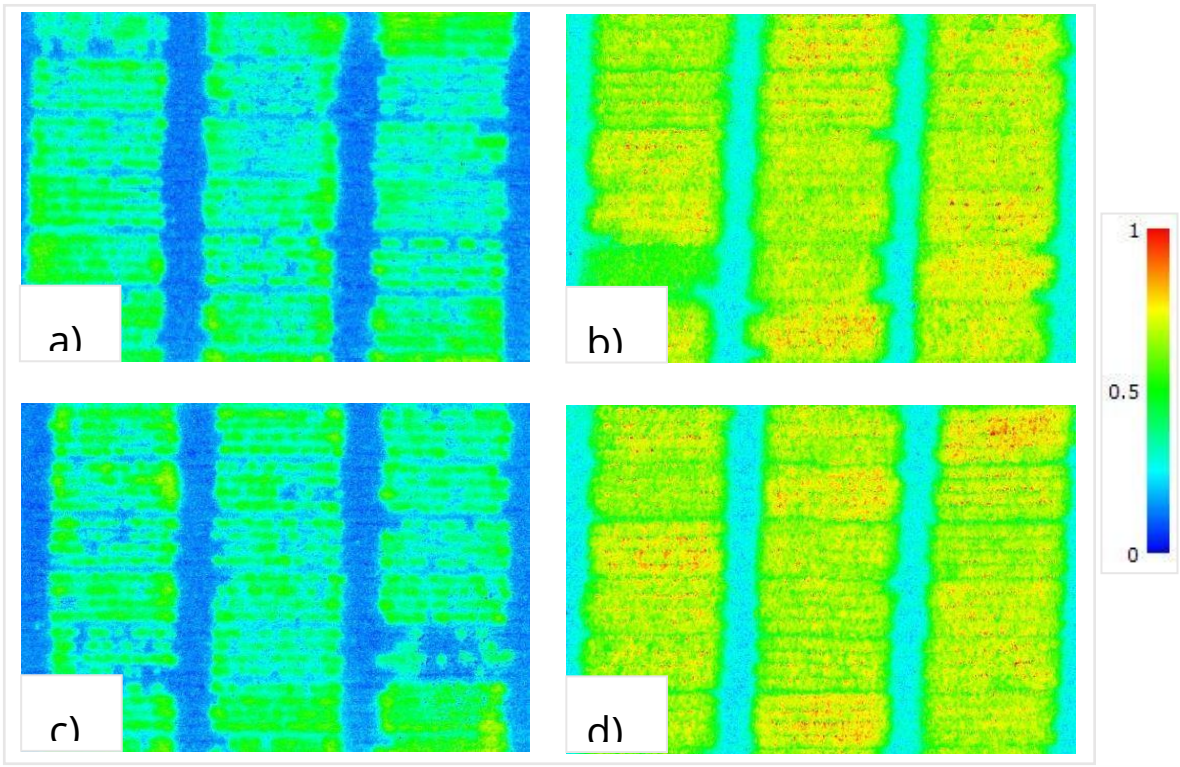

Fig 22 Example of normalized difference red edge (NDRE) index of soybean genotypes grown under abiotic stress (drought) and control environments; a) early group grown in drought; b) early control; c) late group grown in drought; d) late control.

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Clear difference between stress and control trials is noticeable based on the NDVI data at 642 GDDs (Table 13).

Table 13 The average values of normalised difference vegetation index-NDVI for soybean genotypes grown in drought simulation and in control environments at 642 GDDs in 2020 and 2021.

Year	Trial	Early	Late
2020	Drought	0.84	0.81
	Control	0.89	0.93
2021	Drought	0.85	0.83
	Control	0.91	0.91

Depending on the year, the early genotypes exposed to abiotic stress had lower NDVI values between 0.05-0.06 compared to the same plant material from the non-stress environment. For the late group, the drop in NDVI was even bigger and went from 0.12 in 2020 to 0.08 in 2021. The same pattern was observed for NDRE index (Table 14).

Table 14 The average values of normalised difference red edge-NDRE index for soybean genotypes grown in drought simulation and control environments at 642 GDDs in 2020 and 2021.

Year	Trial	Early	Late
2020	Drought	0.22	0.22
	Control	0.25	0.27
2021	Drought	0.18	0.16
	Control	0.22	0.22

The research showed that a greater decrease in NDRE was observed in late soybean genotypes compared to the early group. In 2020, the NDRE values for the late group dropped by 0.05 and further decreased to 0.06 in 2021. Meanwhile, the NDRE values for the early group decreased by 0.03 in 2020 and 0.04 in 2021.

Results on chilling tolerance

In Germany, during 2022 the weather conditions were very dry and hot especially from June to August so most varieties were mature by the beginning of September. For the first sowing date on 16th April there were differences in germination. Emergence was evaluated several times such that data about speed of germination could also be gathered. The best variety was Strengs Weihenstephaner with 41 plants on 23rd May followed by GL Susanna, Alicia with 26 plants, VM 18.184 with 25 plants and Salsa with 24 plants. Xonia, Altona, VM 11.965, Merlin and ES Commandor were the worst varieties with 3 to 5 plants. From the second and third sowing date a germination was lower because of dry weather conditions and there were no significant differences anymore.

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As plants were attacked by birds germination assessments had to be stopped in these 2 batches. Statistics between the different sowing dates are not possible because germination was more influenced by drought than by temperature. During flowering only 3 nights had relevant cold temperatures: 14th June (only some varieties in flower) and 21st June with minimum of 9°C at night and 2nd July with 10°C. Maturity of varieties was checked twice and distribution of pods was observed. Some plants of Xena, VM 17.155, Kristian, Caloria, GL Susanna, Salsa and Solena showed losses of one pod in the middle of the plant. For Xena observations from one of last years' farmers participatory field trials can be confirmed, for Solena from former trials. Some varieties lost pods at the top of the plants because of drought. Comparison of "official" maturity and "real" maturity did not show abnormalities and needs further investigation.

Fig 23 Chilling tolerance field trials in 2022,

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Table 15 Evaluation of varieties in 2022.

	Sowing date	16.04.2022	02.05.2022	18.05.2022	Missing pods
	Assessment	16.06.2022	03.06.2022	09.06.2022	04.09.2022
	soybean variety	Total No	Total No	Total No	% of plants
1	**GL Melanie**	21	4	4	
2	**GL Creme**	19	3	5	
3	**GL Theresa**	20	6	6	
4	**GL 1917012**	17	10	10	
5	**Paprika**	28	2	5	
6	**GL Susanna**	28	5	6	13
7	**Obelix**	14	6	6	
8	**Lenka**	14	2	3	
9	**Xonia**	4	3	4	
10	**Favorit**	19	7	7	
11	**Caloria**	20	5	5	17
12	**Solena**	20	2	4	7
13	**Korana**	22	4	9	
14	**Bei feng No. 3**	13	7	7	
15	**Sonali**	28	4	9	
16	**Angelica**	19	6	6	
17	**Atacama**	16	8	8	
18	**Kristian**	20	2	8	33
19	**Altona**	4	5	11	
20	**Salsa**	27	3	4	7
21	**Alvesta**	23	5	5	
22	**Merlin**	4	7	9	
23	**Strengs Weihenstephaner**	35	10	10	
24	**Tofina**	12	6	10	
25	**NS Mercury**	12	4	5	
26	**Es Mentor**	22	5	5	
27	**Abaca**	14	2	2	
28	**Achillea**	20	2	4	
29	**Alicia**	18	9	9	
30	**Adelfia**	14	2	3	
31	**Xena**	17	7	9	47
32	**VM 18.184**	21	2	3	
33	**Amadine**	12	2	4	
34	**EZG 22517**	8	3	4	
35	**EZG 22655**	14	6	7	
36	**VM11.965**	3	5	5	
37	**VM17.155**	16	4	5	33
38	**ES Commandor**	5	4	4	
39	**ES Compositor**	20	1	1	
40	**ES Collector**	13	3	3	

Test of the genotypes occurred in the cooling chamber of Bavarian State Research Institute. The method has already been used previously for genotypes from the Bavarian State breeding programme. 20 seeds each for control and treatment were surface sterilised and put in petri dishes with wet filter paper for 15 hours of swelling. The petri dishes were stored in a cooling chamber with 6°C whereas the petri dishes for control remained at room temperature. After 7 days the germination of control and treatments was measured. The petri dishes with treatment stayed in the room and were measured again after 24 hours and following five days (recovery) to evaluate the

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damage from cooling. Data shows germination of the genotypes in the control, after 24 hours and after 5 days. The tested genotypes did not show germination during cooling but after 24 hours of recovery germination was over 80% for some genotypes. This shows that some genotypes can tolerate cool temperature and are able to germinate afterwards without damage, e.g. Lenka, Caloria, Kristian and 22655. Other genotypes show a lower germination in recovery compared to the control treatment, e.g. Korana, ES Mentor und Abaca. The reason can be damage during cooling e.g. to the embryo.

Table 16 Results in cooling chamber**.**

No	Soybean variety	control	24 h	5 days
1	**GL Melanie**	45	30	75
2	**GL Creme**	100	70	95
3	**GL Theresa**	70	55	75
4	**GL 1917012**	100	85	95
5	**Paprika**	65	60	95
6	**GL Susanna**	100	85	95
7	**Obelix**	50	55	65
8	**Lenka**	70	85	95
9	**Xonia**	75	5	25
10	**Favorit**	90	65	85
11	**Caloria**	85	95	100
12	**Solena**	45	35	65
13	**Korana**	80	35	85
14	**Bei feng No. 3**	55	40	70
15	**Sonali**	95	70	100
16	**Angelica**	85	90	100
17	**Atacama**	65	80	90
18	**Kristian**	90	90	95
19	**Altona**	40	0	10
20	**Salsa**	80	50	90
21	**Alvesta**	80	25	80
22	**Merlin**	100	35	90
23	**Strengs Weihenstephaner**	65	50	90
24	**Tofina**	10	0	15
25	**NS Mercury**	0	0	5
26	**Es Mentor**	80	30	75
27	**Abaca**	75	15	75
28	**Achillea**	65	75	90
29	**Alicia**	70	5	35
30	**Adelfia**	90	55	85
31	**Xena**	40	0	20
32	**18.184**	35	5	60
33	**Amadine**	75	50	85
34	**22517**	90	50	95
35	**22655**	95	85	95
36	**11.965**	20	0	15
37	**17.155**	70	65	85
38	**ES Comandor**	35	10	20
39	**ES Compositor**	80	25	65
40	**ES Collector**	40	10	50

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Not all the genotypes showed similar results between the field and cooling chamber. Strengs Weihenstephaner, Alicia and EZG 18.184 were better in the field whereas GL 1917012, Lenka and Angelica were better in the cooling chamber. GL Susanna, Caloria and Kristian were very good in both field and cooling chamber.

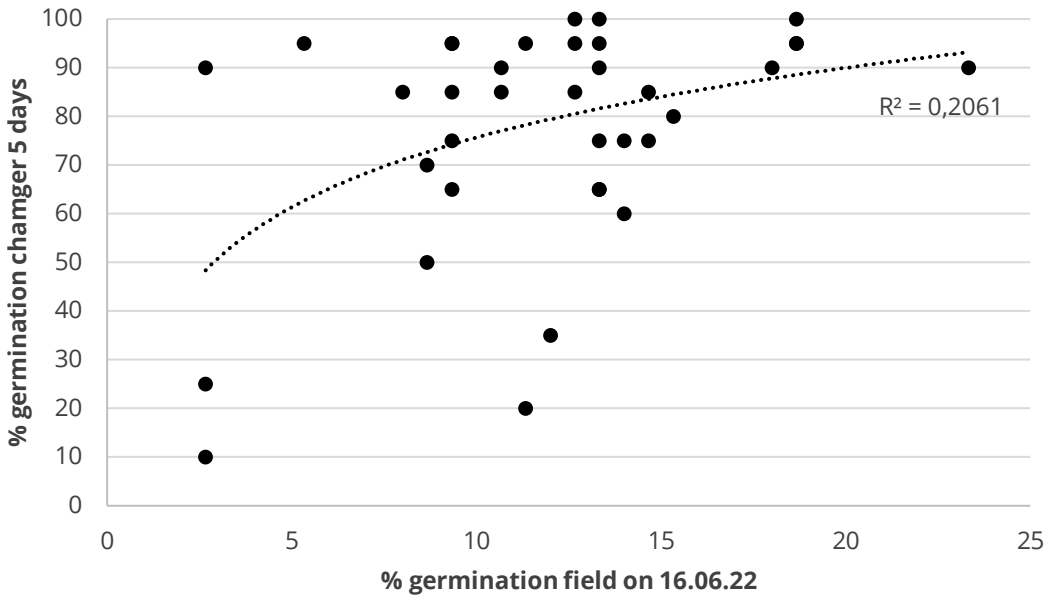

Fig 24 Comparison between germination in the field and cooling chamber in 2022.

During 2023, in Dittlofsroda, Germany 40 varieties were sown at three dates with 3 replications. The same varieties as 2022 were sown except for the worst ones in germination from the cooling chamber test. So Tofina, NS Mercury, 11.965 and ES Comandor were substituted by Tarock, Vineta PZO, Jenny and Simpol. The sowing dates were 22nd April, 4th May and 19th May. One plot was sown with 20 seeds. The weather conditions in March and April were very wet (200 mm rainfall) so that first sowing was not possible earlier. May and June were extremely dry then with only 16 and 18 mm of rainfall. The first sowing date germinated very well (more than double that in 2022) but at the second and third sowing dates germination was close to zero such that even weeds did not grow in the plots. Therefore data for the second and third sowing date are not shown. The best variety was again Strengs Weihenstephaner and second best was Sonali. There were only two nights with 10°C in June and July so there was no influence of cold temperature on flowering.

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Fig 25 Chilling tolerance field trials in 2023.

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Table 17 Evaluation of varieties in 2023.

	Sowing date	22.04.2023	16.04.2022
	Assessment	25.05.2023	16.06.2022
	Soybean variety	Total No (of 60)	Total No (of 150)
1	**GL Melanie**	8	21
2	**GL Creme**	23	19
3	**GL Theresa**	22	20
4	**GL 1917012**	24	17
5	**Paprika**	8	28
6	**GL Susanna**	23	28
7	**Obelix**	14	14
8	**Lenka**	13	14
9	**Xonia**	2	4
10	**Favorit**	19	19
11	**Caloria**	25	20
12	**Solena**	19	20
13	**Korana**	18	22
14	**Bei feng No. 3**	15	13
15	**Sonali**	28	28
16	**Angelica**	18	19
17	**Atacama**	19	16
18	**Kristian**	16	20
19	**Altona**	1	4
20	**Salsa**	14	27
21	**Alvesta**	21	23
22	**Merlin**	24	4
23	**Strengs Weihenstephaner**	33	35
24	**Tarock**	15	
25	**Vineta PZO**	14	
26	**ES Mentor**	16	22
27	**Abaca**	11	14
28	**Achillea**	16	20
29	**Alicia**	13	18
30	**Adelfia**	22	14
31	**Xena**	7	17
32	**VM 18.184**	15	21
33	**Amadine**	22	12
34	**EZG 22517**	17	8
35	**EZG 22655**	26	14
36	**Jenny**	19	
37	**VM17.155**	23	16
38	**Simpol**	23	
39	**ES Compositor**	12	20
40	**ES Collector**	10	13
	Average	17,2	16,9

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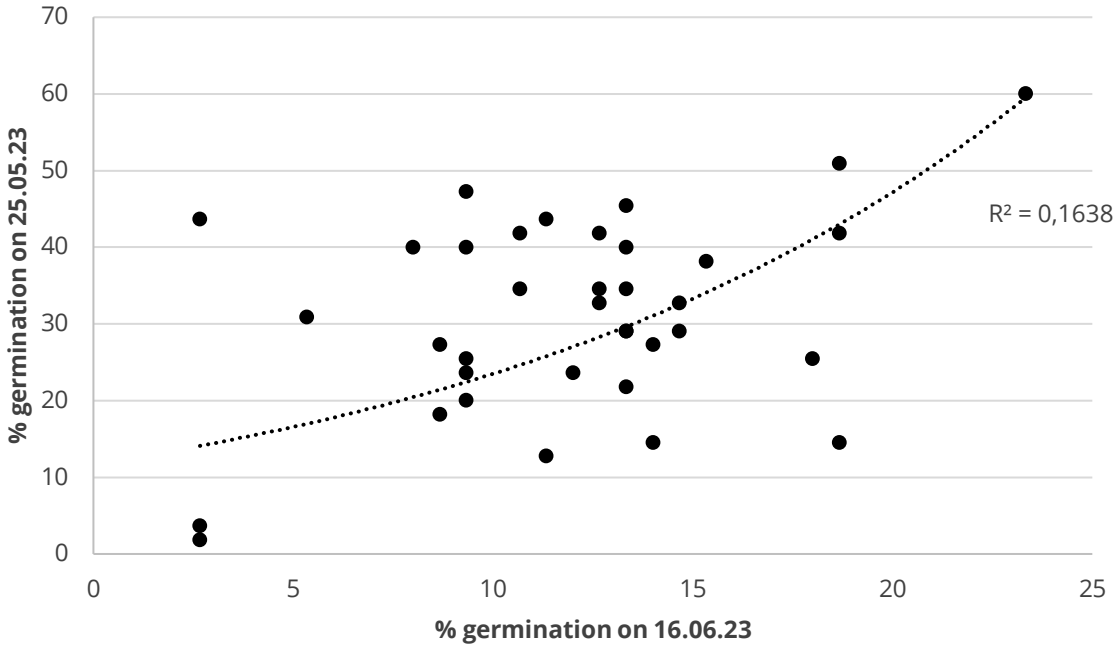

Fig 26 Germination in the field 2023.

Conclusions on soybean drought tolerance

A diverse soybean germplasm was tested for abiotic stress tolerance. Over 200 genotypes from maturity group 000-II were analysed for two years (2020 and 2021) in drought simulation environments and 40 for chilling tolerance. The results were compared with the control which consisted of the same plant material grown under non-stress conditions. Drought affected more or less all observed parameters within the trials. The soybean genotypes exposed to drought stress had significantly shorter growing period compared to the control. This was the case in 2020 and 2021 for both the early and late maturity groups. The negative effect of high temperatures and lack of moisture, reduced CC of most genotypes due to the poor emergence, plant loss and disturbed plant architecture. Still, some varieties were able to produce enough biomass and achieve high CC at flowering even under such harsh conditions which is already a positive indicator for detecting drought-tolerant material. Another important trait in plant development plant height was affected by unfavorable growing conditions in both years. This was observed for the early and late groups. The data from the two-year experiment indicates that the reduction in PH was more pronounced in 2021 than the previous year. This was the opposite to the observations for CC which can be the result of increased branching. The yield represents the most important agronomic trait and it is decisive in the selection of soybean genotypes tolerant to abiotic stress. In the drought simulation trials, early and late material was less productive compared to the control. The yield reduction was more pronounced in 2021 even though similar

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conclusions were obtained to the year before. The experiment on abiotic stress tolerance showed that genotypes from the early maturity group had a smaller yield loss compared to the late group. Depending on year, the genotypes with a shorter growing period were 18-43 % less productive than the control while for late varieties yield was reduced from 44-56 %. The early material passes crucial development stages before the driest period which may be one of the reasons for smaller yield loss. The unfavorable growing conditions also have negative effects on the yield components such as seed weight (Mwenye et al., 2016). This was observed in the soybean trial where the average TSW of soybean genotypes grown under abiotic stress conditions was 5-11% (depending on the maturity group and year) lower than in the control. The TSW data also confirmed higher drought susceptibility of late genotypes compared to the material with a shorter growing period. In general, early varieties made bigger seeds in both drought and control environments with a smaller reduction of TSW in stress conditions. Nevertheless, some cultivars such as Toyokomachi or Toyomusume were able to produce seeds with TSW over 250-300g even under drought which suggests how stable they are in different environments. On the other hand, the yield stability of analysed material was generally low. The highest observed value of the correlation coefficient between yield in stress and non-stress trials was for the early group in 2020 while the rest were even lower than that. The stress tolerance index provided deeper information on soybean drought tolerance at the genotype level. These indices could be divided into two groups depending on whether they measure simple yield penalty caused by the drought or they classify high-performance genotypes in both environments. Based on the values of TOL, SSI, YSI, and RSI, four varieties were selected as those with the lowest change between yields achieved in stress and non-stress conditions. Determination of drought tolerance using some of these indices was already confirmed in research on wheat (Sardouei-Nasab et al., 2019), barley (Khalili et al., 2016), and potato (Cabello et al., 2013). Still, the low yield penalty is not always an indicator for high tolerance genotypes. For example, a variety could be recognised as stable but with low yields in both environments thus it cannot be selected. On the otherhand, indices such as MP, GMP, or STI are good for the detection of best-performing genotypes in both drought and control environments. These varieties achieve high yields in good environments but also in stress conditions which represents the best combination. In the research on soybean, three cultivars from the early and three from the late group were ranked in the top 10 best-performing genotypes based on MP, GMP, and STI. Special attention should be paid to these genotypes as they were recognised as genotypes with high and stable yields across different environments and years. The great potential of digital technologies is exploited through remote assessment of different plant traits. The plant's spectral reflectance explained with values of VIs such as NDVI and NDRE provided important information about the reaction of analysed soybean material to

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abiotic stress. The results showed that values of both indices dropped in material exposed to drought conditions. This was expected knowing the fact that under stress conditions reflectance in the red part of the spectrum is increased while in the NIR regio it is reduced (Cure et al., 1989). This information can be significant for in-season monitoring of soybean genotypes exposed to unfavorable growing conditions.

Conclusions on soybean chilling tolerance trial

Research trials were conducted to assess the ability of soybean plants to tolerate cold temperatures during the germination and flowering stages, particularly at a low temperature of 10°C. Genotypes like Strengs Weihenstephaner and Sonali demonstrated their robustness and adaptability in the face of adverse conditions. This information is valuable for both soybean breeders and producers, helping them select suitable varieties for cultivation in regions with challenging weather conditions. Further research and experimentation may be required to understand the specific mechanisms underlying the success of these varieties and to optimise soybean cultivation practices in such conditions. Even though a great amount of data was collected during the experiments for soybean abiotic stress tolerance, the need for further analysis emerged. The study emphasised the need for robust, drought-tolerant soybean cultivars by highlighting the impact of meteorological conditions, particularly drought, on soybean germination, growth and pod formation.

Results on N-fixing capacity screening

Seed protein content and comparison of phenotyping devices

In harvest samples from various locations and genotype classes, a wide range of soybean seed protein content (290-490 g/kg) was found, suggesting variation in nitrogen metabolism between genotypes (Fig. 27). In both TU environments (but not in GE 2020), non-nodulating soybean (sub-set 1) had significantly lower seed protein content than their nodulating counterparts. As expected, the highest protein content was found in lines that had previously been selected for high seed protein content, while standard cultivars and genotypes chosen for high pod set had similar protein contents.

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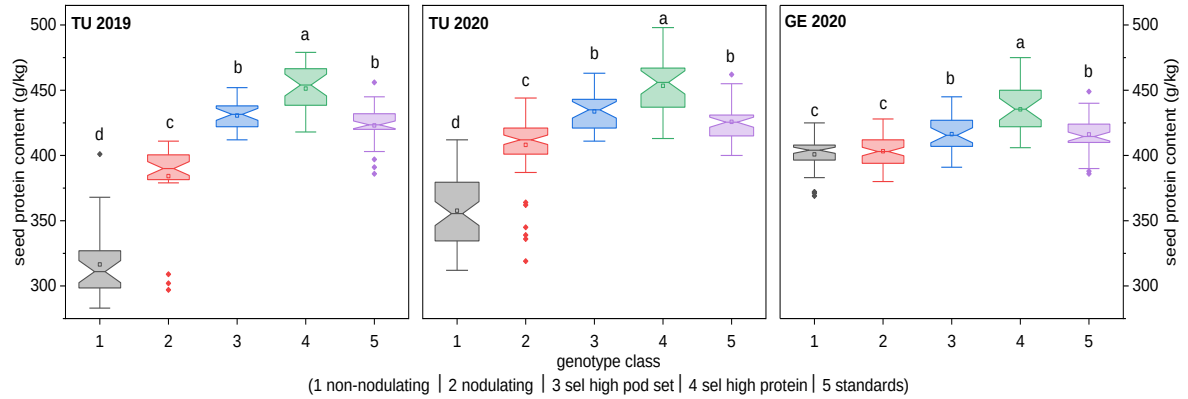

Fig 27 Variation of seed protein content (g/kg) for five genotype classes (sub-sets) across each of the three environments TU 2019, TU 2020, and GE 2020. Notched boxes with 25% - 75% range, bar marking 1.5 interquartile range, median line (notches indicating 95% lower and upper confidence intervals of median value), arithmetic mean (dot) and outliers (filled dots); letters above boxes indicating significant differences between genotype classes within environment (Tukey-Kramer multiple comparison at p=0.05 level). Source: doi.org/10.1016/j.compag.2022.107169

Differentiating between soybean genotypes may be possible using the determination of chlorophyll content by SPAD-meter (TU 2019 environment) as an indirect indication of crop N status. At later stages of development, SPAD measurements led to higher F-ratios and lower error mean squares (Table 18). Three SRIs derived from FieldSpechyperspectral data and associated with N uptake (NRI, PRI570, and MA1_R) displayed analytical power that were comparable or superior in terms of ANOVA parameters than SPAD values.

Table 18 Summary of comparative ANOVA results for SPAD meter measurements of chlorophyll content and spectral reflectance indices NRI, PRI570 and MA1_R from the same plots at three measuring dates during the TU 2019 season (for all data: standardisation through z-transformation) as well as their respective correlation to seed protein content. Source: doi.org/10.1016/j.compag.2022.107169

	R2-R3: Full bloom - Beginning pod (17/18 Jul 2019)				**R4: Full pod** (31 Jul/1 Aug 2019)				**R5-R6: Beginning - Full seed** (15/16 Aug 2019)			
ANOVA	**SPAD**	**NRI**	**PRI570**	**MA1_R**	**SPAD**	**NRI**	**PRI570**	**MA1_R**	**SPAD**	**NRI**	**PRI570**	**MA1_R**
Model F ratio	5.417	4.368	7.926	7.785	6.652	12.722	5.661	19.392	9.361	12.626	7.27	34.747
Genotype F ratio	5.488	4.388	8.039	7.941	6.696	12.987	5.764	19.803	9.553	12.777	7.274	35.13
Error mean sq.	0.274	0.331	0.194	0.197	0.228	0.124	0.263	0.083	0.166	0.125	0.21	0.047
Coefficient of correlation (r) to seed protein content (n=160)												
	0.404	-0.558	0.803	-0.715	0.503	-0.834	0.838	-0.872	0.799	-0.833	0.803	-0.880

The spectral index MA1_R demonstrated the best overall performance both in ANOVA and correlation analyses, and correlations between SPAD or SRIs and ultimate seed protein content likewise raised with developmental stage. In Fig. 28a, the correlation

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between SPAD measurement and seed protein content (r = 0.799) is shown; overlay distributions of the SPAD values reveal differences between the non-nodulating, high pod-set and the other genotype sub-sets. When compared to the SPAD parameter, the correlation between spectral index MA1_R and seed protein content (r = - 0.872) is higher, and the distinction across genotype classes is better (Fig. 28b, MA1_R overlay distributions).

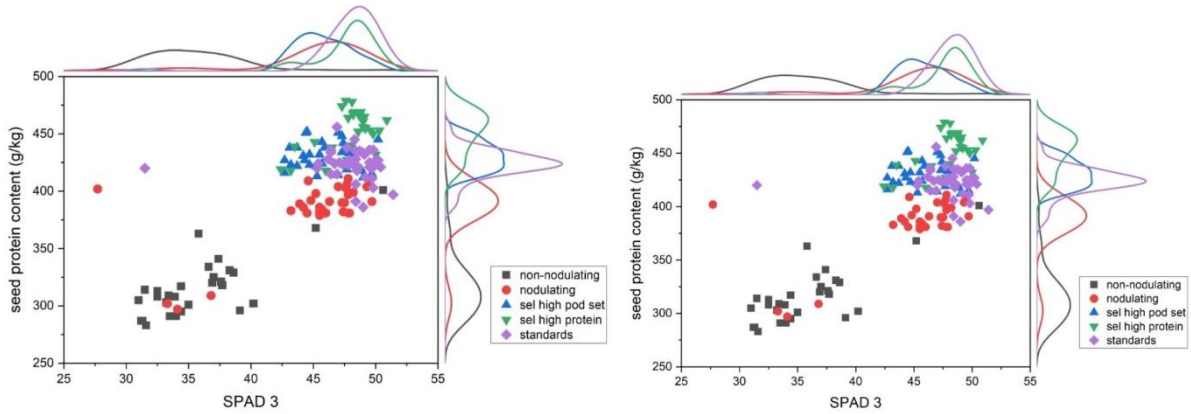

Fig 28 Seed protein content (TU 2019) as related to (a) SPAD measurement of chlorophyll content (r=0.799; n=160) and (b) spectral index MA1_R (r=-0.872; n=160) both determined at R5-R6 stage (15 Aug 2019) with overlay distribution of genotype classes (Kernel smooth density function). Source: doi.org/10.1016/j.compag.2022.107169

In the TU 2020 environment, an ASD hyperspectral device and a PolyPen RP410 were compared in addition to the SPAD meter. The PolyPen device's performance was comparable to the SPAD device both in terms of correlation to seed protein concentration (Fig. 29a) and in terms of the outcomes of the ANOVA (Table 18). Several SRIs developed from hyperspectral data acquired with the ASD devices outperformed the outcomes of PolyPen (index REIP in Fig. 29b).

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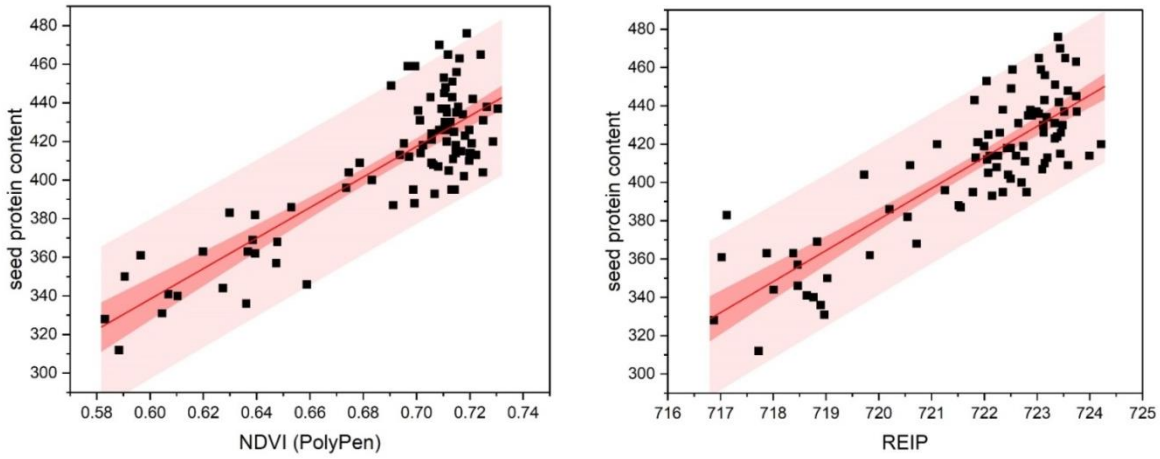

Fig 29 Scatter plots illustrating relationships between either NDVI index (Polypen RP410 NIR measurement; r=0.836; n=88) (a) or REIP index (ASD FieldSpec Handheld2 measurement; r=0.920; n=88) (b) taken at R3-4 stage (28 Jul 2020) and seed protein content (TU 2020). Source: doi.org/10.1016/j.compag.2022.107169

Hyperspectral measurement procedure

Instead of the usual single point measurements, the ASD hyperspectral device was moved over the canopy rows to capture as much spectral information as possible using 400 spectrum replications. As an example, the ranges of chlorophyll b content from individual spectral replications for four different genotypes are shown (Fig. 30). While the non-nodulating genotype has a noticeably lower chlorophyll b content, genotypes 2, 3, and 4 also demonstrate smaller differences.

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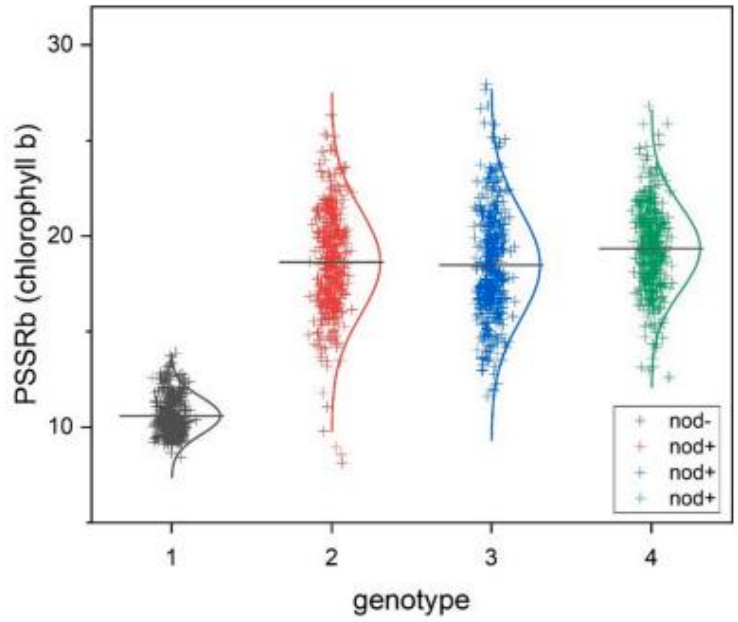

Fig 30 Index PSSRb indicating chlorophyll b content of 4 genotypes differing in nodulation status (1=non-nodulating, 2, 3, 4: nodulating) measured in a total of 400 replications each. Source: doi.org/10.1016/j.compag.2022.107169

Fig. 31 displays a stable ranking of genotypes after around 100 replications, and minor distinction between genotypes appear to be revealed, demonstrating the strength of the high number of spectral replications. This also relates to other indices, as shown, for instance, in Figs. 32 and 33 for carotenoid content (index PSSRc) and Fig. 34 for the double-peak canopy nitrogen index (DCNI) that is depicted here.

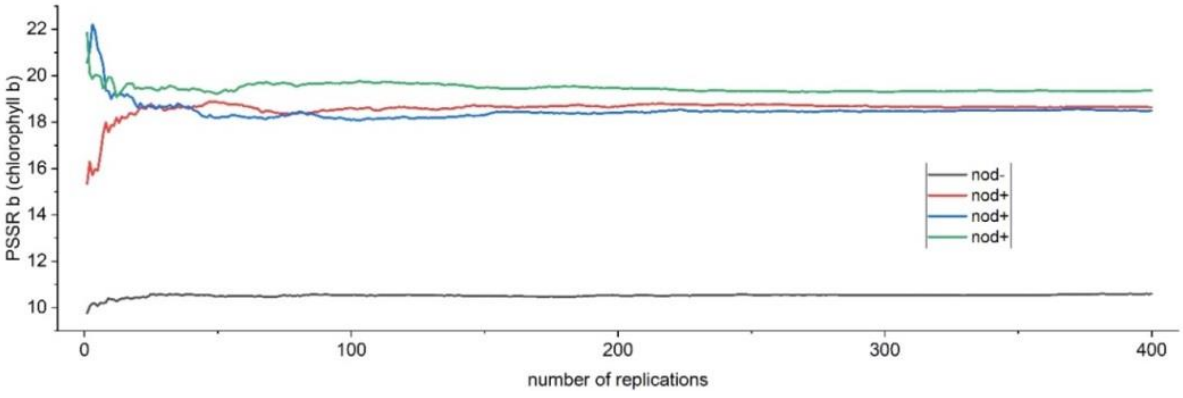

Fig 31 Effect of the number of replications for measuring chlorophyll b (PSSR b) in 4 genotypes differing in nodulation status. Source: doi.org/10.1016/j.compag.2022.107169

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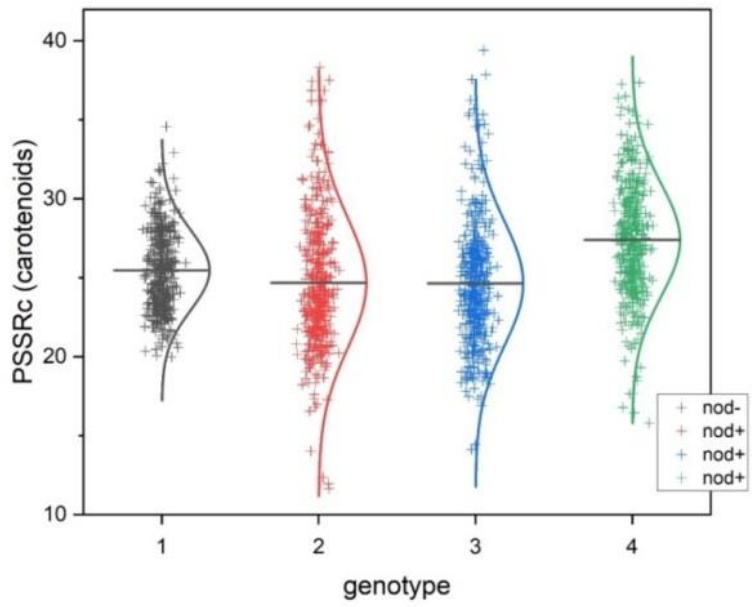

Fig 32 Index PSSRc indicating carotenoid content of 4 genotypes differing in nodulation status (1=non-nodulating, 2, 3, 4: nodulating) measured in a total of 400 replications. Source: doi.org/10.1016/j.compag.2022.107169

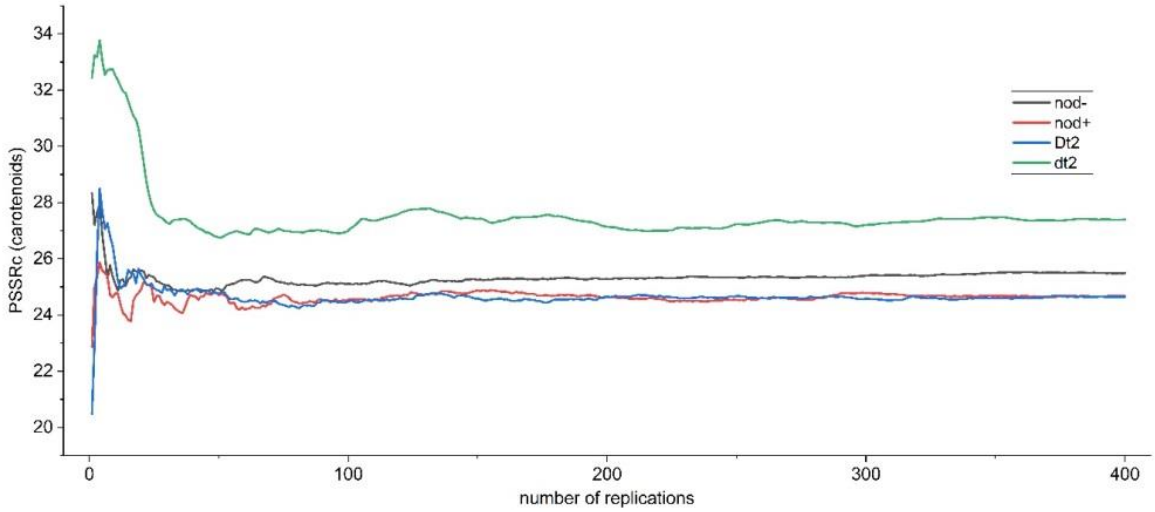

Fig 33 Effect of the number of replications for measuring carotenoids (PSSR c) in 4 genotypes differing in nodulation status. Source: doi.org/10.1016/j.compag.2022.107169

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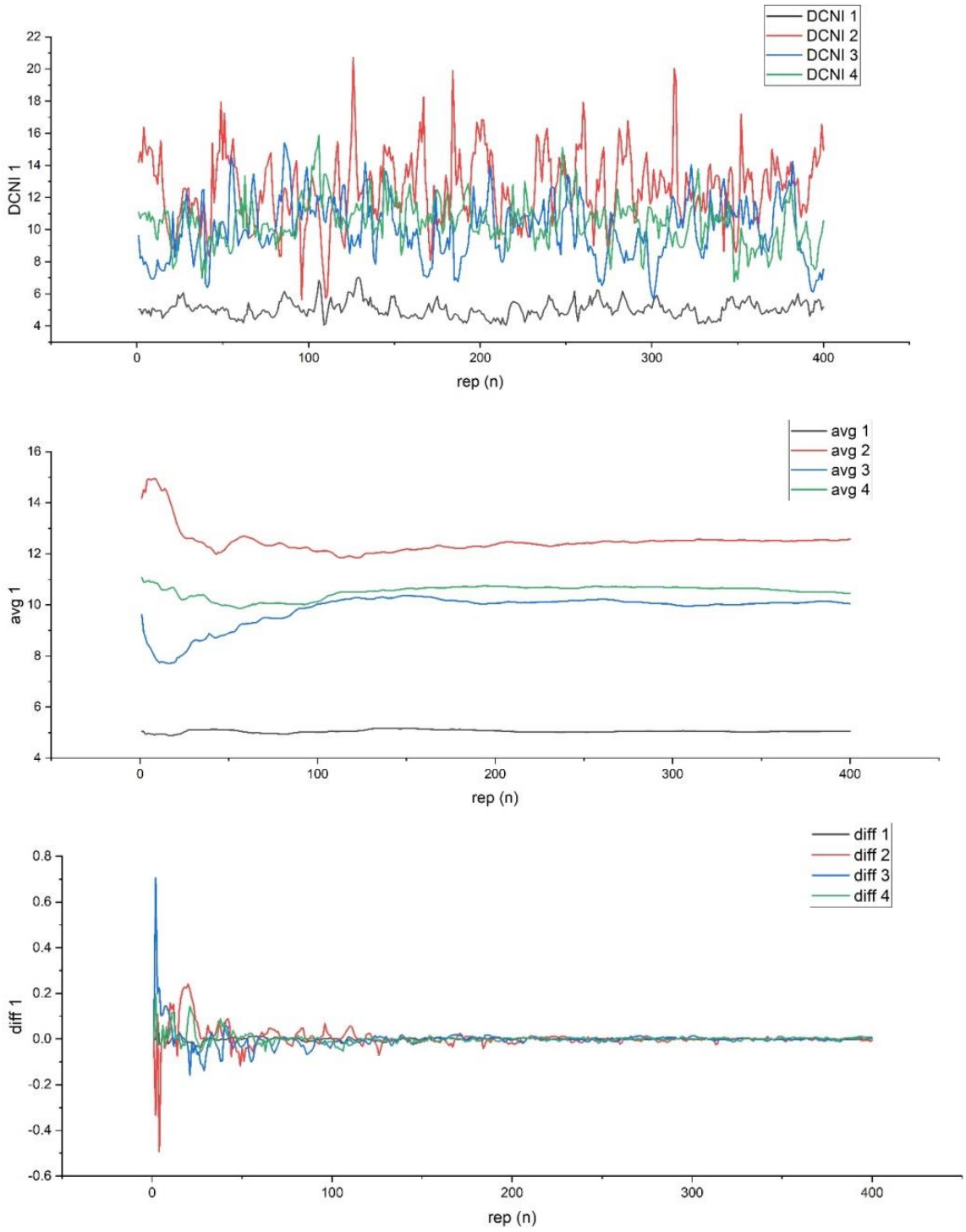

Fig 34 Double-peak canopy nitrogen index (DCNI) measured in 400 spectral replications for 4 different genotypes (1: non-nodulating; 2, 3, 4: nodulating). Individual DCNI values (a), DCNI averages across replications (b), differences to previous mean values from averaging across 1 to 400 replications (c). Source: doi.org/10.1016/j.compag.2022.107169

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Spectral correlations and spectral indices

For the nodulating and non-nodulating sub-sets of genotypes at various measurement times in the TU 2019 experiment, correlations between spectral reflectance at individual wavelengths and seed protein content followed clear patterns along the wavelength range from 325 to 1075 nm (Fig. 35). Patterns were less clear for the other sub-sets with less variation in protein content (Fig. 36), however the red edge correlation peak at about 700 nm is evident here as well. Using all genotype classes in one study, the correlations between spectral reflectance and either seed protein or sucrose content, across wavelengths, showed symmetrically diverging patterns (Fig. 37), which provides evidence of the negative correlation between protein and sucrose content.

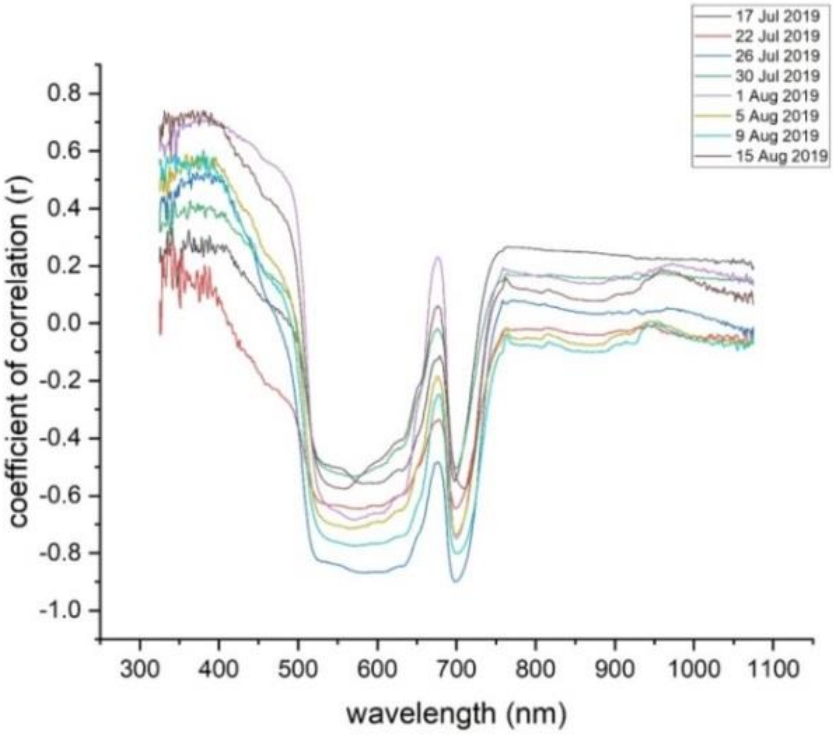

Fig 35 Relationship between hyperspectral reflection at eight different data collection dates during the soybean seed filling period and seed protein content of the harvest product: Correlograms describing correlations between reflectance at given wavelengths (1 nm increment) and seed protein content for the Tulln 2019 sub-sets 1 and 2 (nodulating and non-nodulating genotype classes). Source: doi.org/10.1016/j.compag.2022.107169

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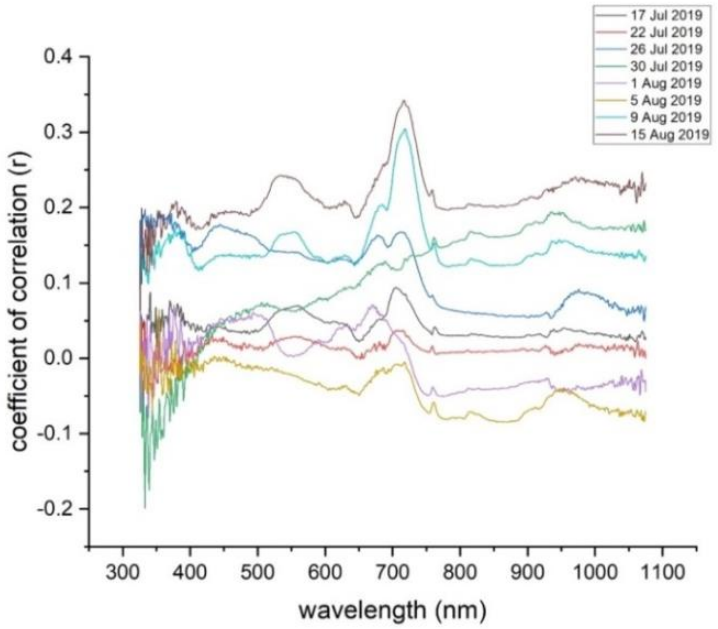

Fig 36 Correlation between hyperspectral reflection at eight different data collection dates during the soybean seed filling period and seed protein content of the harvested product: Correlograms describing correlations between reflectance at given wavelengths (1 nm increment) and seed protein content for the Tulln 2019 sub-sets c, d and e (high seed protein selections, high pod set selections, and standards).

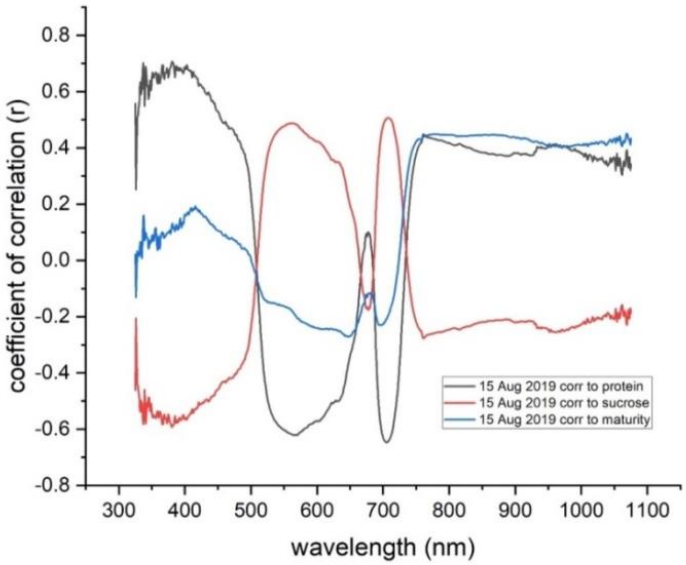

Fig 37 Relationship between hyperspectral reflection at the 15 Aug 2019 measurement date and different crop traits: Correlograms describing correlations between reflectance at given wavelengths (1 nm increment) and either seed protein content, seed sucrose content or time to maturity. The negative correlation between seed protein and seed sucrose content is evident from diverging correlations, whereas the correlogram for time to maturity is revealing a different pattern with highest correlations in the near-infrared region from 750 nm onwards. Source: doi.org/10.1016/j.compag.2022.107169

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In contrast, the correlogram for the time to maturity is distinct to those for protein and sucrose. The determination of dedicated spectral indices for a meaningful characterisation of genotype classes and individual genotypes was motivated by the correlations along wavelengths between spectral reflectance and individual attributes. Hence, the ANOVA findings showed highly significant differences between genotype classes for both phenotypic traits and SRIs. While genotypes were not significant for most of the water-related indices, differences between genotypes within genotype classes were significant for the majority of SRIs related to nitrogen metabolism.

Correlations between spectral predictors and seed protein content

A correlation between individual SRIs and phenotypic characteristics such as time to maturity, plant height, oil, protein, or sucrose content, and 1000-seed weight was found. In Fig. 38, eight SRIs with high correlations to seed protein content and their relationships to other seed characteristics and to pairwise SRI's are shown. The spectral index MA1_R demonstrated the highest correlation (r = - 0.75) to seed protein concentration across all settings, for the TU 2019 environment. While this correlation is based on individual plots (n = 630) across all environments, the high predictive power of MA1_R for seed protein content is evident from a correlation based on genotype means within environment of r = − 0.903 (Fig. 39a vs. 39b).

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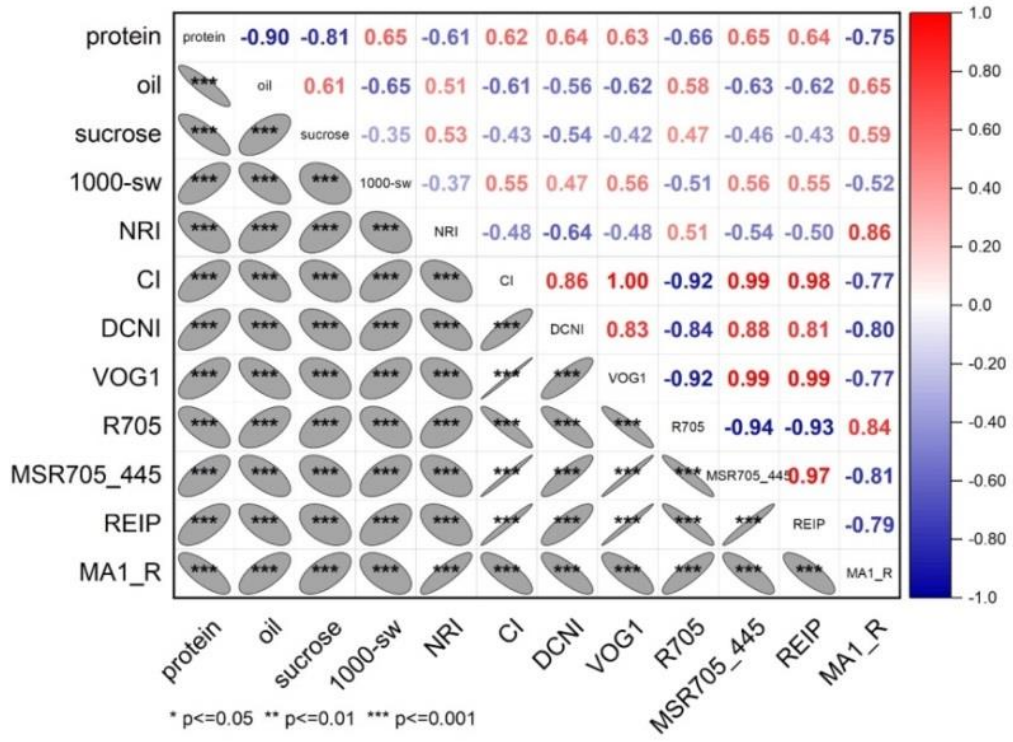

Fig 38 Pearson coefficient of correlation (r) between soybean seed traits and nitrogen/protein related SRIs (n=630, individual plots basis, 3 environments). Source: doi.org/10.1016/j.compag.2022.107169

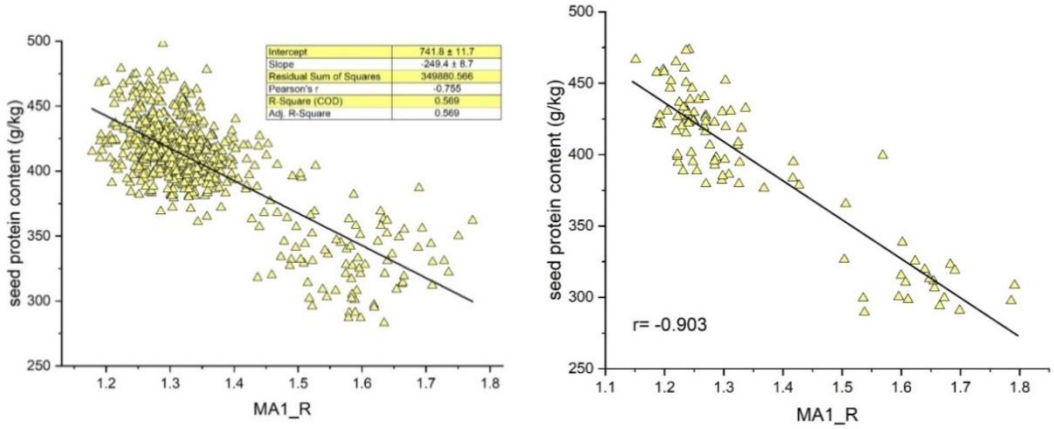

Fig 39 Correlation between index MA1_R and seed protein content; a: individual plot basis (all plots in all environments), b: genotype LSMEANS basis (means of TU 2019 experiment only). Source: doi.org/10.1016/j.compag.2022.107169

Instead of applying a single correlation/regression model to estimate the seed protein concentration from specific SRIs, PLSR-based models were created to use the full spectroscopic information available. R2 values of 0.844 and 0.805 for calibration and cross validation, respectively, were attained in models that included samples from all

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three environments (Fig. 40). Higher R2 values were observed in models for specific experimental sites (Fig. 41), and modelling outcomes are presented in Table 19 either separately for each specific environment or together across all environments.

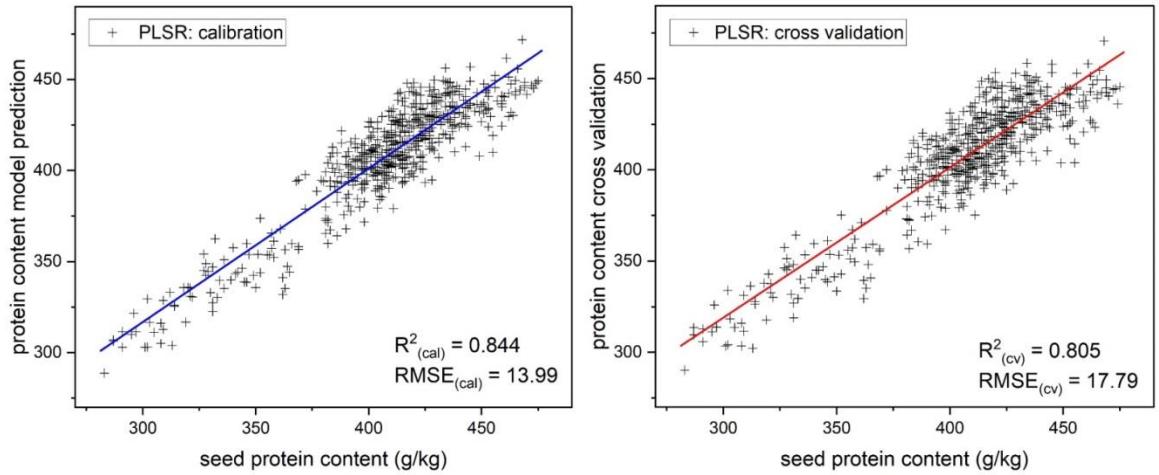

Fig 40 Relationship between seed protein content and PLSR calibration model (a) and cross validation (b) based on spectral data (n=589 plots) from all three environments. Source: doi.org/10.1016/j.compag.2022.107169

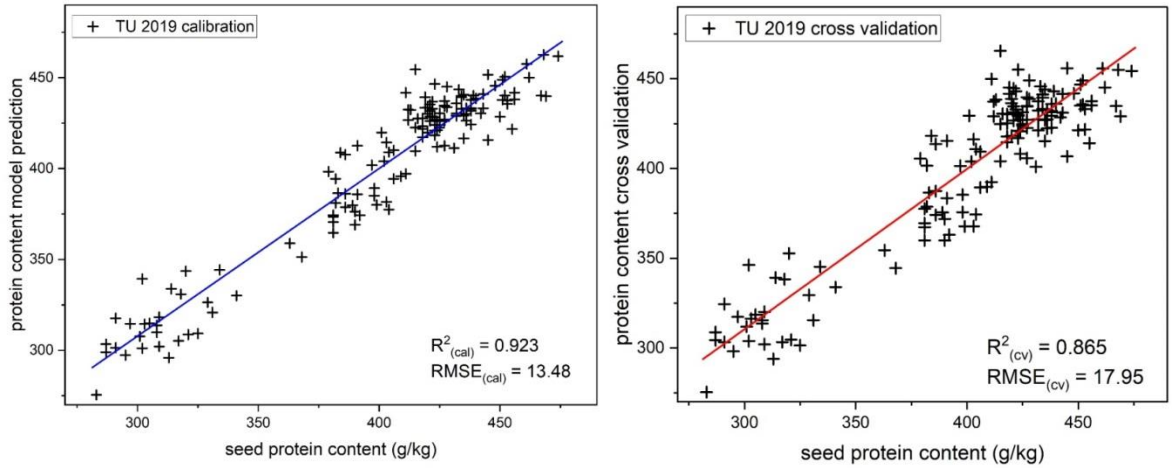

Fig 41 Relationship between seed protein content and PLSR calibration model (a) and cross validation (b) based on spectral data (n=149 plots) from TU 2019 environment only. Source: doi.org/10.1016/j.compag.2022.107169

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Table 19 Summary statistics for PLSR modelling results for prediction of seed protein content from hyperspectral reflectance data based on either individual environments or combined across all three environments. Source: doi.org/10.1016/j.compag.2022.107169

		TU 2019		TU 2020		GE 2020		all environments	
Parameter		all[a]	rem.outl.[b]	all	rem.outl.	all	rem.outl.	all	rem.outl.
No. of factors		12	12	12	12	12	12	15	15
RMSE[c]	- calibration	16.26	13.48	15.97	13.51	12.06	10.74	16.03	13.99
	- validation	20.68	17.95	19.32	16.39	14.26	12.63	17.79	15.64
R-square	- calibration	0.892	0.923	0.8269	0.8735	0.5073	0.6256	0.8008	0.8441
	- validation	0.8275	0.8653	0.7502	0.8158	0.3187	0.4878	0.7548	0.8052

[a] all plots/samples utilized in model; [b] outliers removed; [c] residual mean square error

For the GE 2020 environment, where seed protein content variation was lowest, the least accurate model was noted. The most significant wavelength regions contributing to the models are indicated in terms of their weighted regression coefficients for the four main regression factors (Fig. 42); green, red, red edge, and the infra-red shoulder at 770-900 nm are of particular importance in the first and second factor. PLSR models exhibit the highest precision in predicting seed protein content from spectral information.

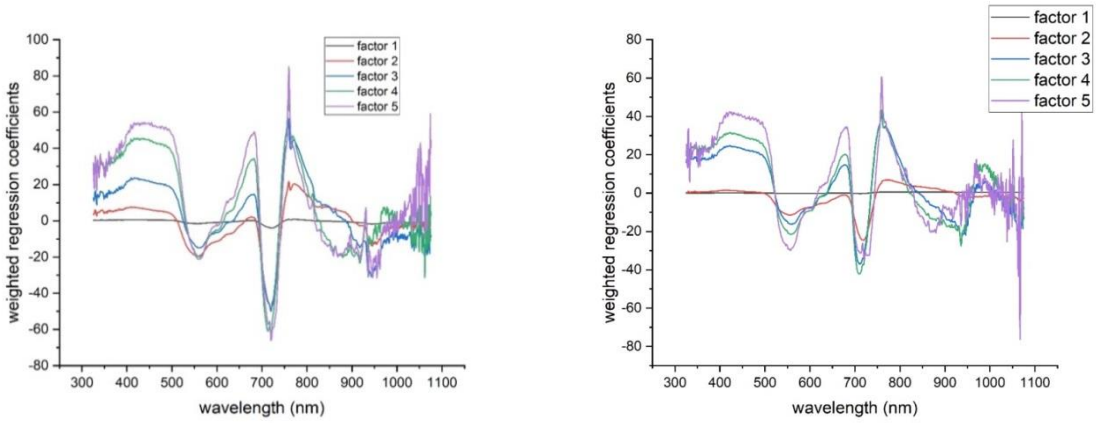

Fig 42 Weighted regression coefficients for most important PLS regression factors across the whole wavelength range; a: for the TU 2019 model, b: for the model across all environments. Source: doi.org/10.1016/j.compag.2022.107169

Both models that included all environments or certain environments solely had identical regression factor weights along wavelengths (Fig. 42). Additionally, as demonstrated above, the direct correlations between spectral reflectance and seed protein content are partially supported by regression factor weights (Fig. 43).

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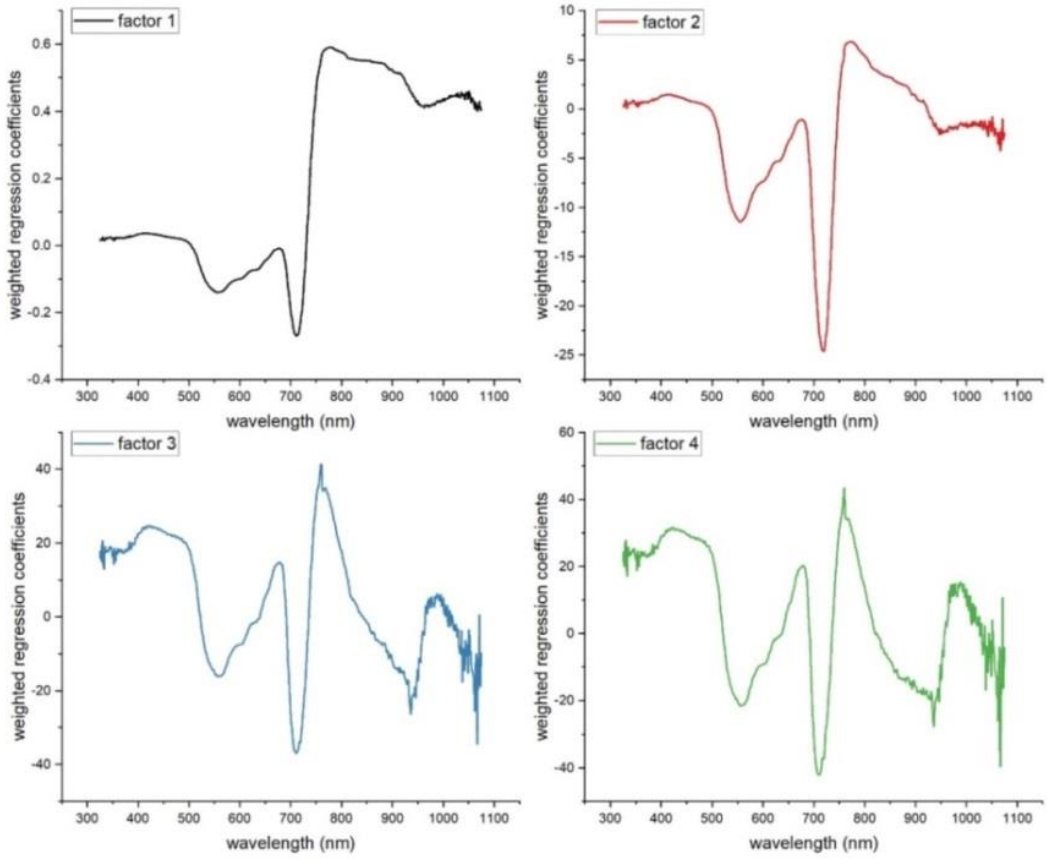

Fig 43 Weighted regression coefficients for four main PLS regression factors across the total wavelength range utilised (model across all three environments). Source: doi.org/10.1016/j.compag.2022.107169

Conclusions N-fixing capacity screening

The alternatives for estimating soybean seed protein content as a variable of interest using hyperspectral reflectance data acquired during the early phases of seed filling have been elaborated. Using PLSR models from hyperspectral data, coefficients of determination (R2) of 0.84 and 0.81 for calibration and validation, respectively, were identified for the prediction of seed protein content as an end-of-season attribute. These models appear to be more effective than SRIs with straightforward correlations to seed protein concentration based on a small number of spectral wavelength points. Strong high-throughput processes for screening numerous genotypes in soybean breeding programmes could more readily be developed using spectral index-based methodologies. As canopy N content results from both symbiotic di-nitrogen fixation and soil N uptake, this would indirectly quantify total N accumulation as well. Consequently, spectral indices related to N metabolism might be used to determine the amount of N that different genotypes absorb. The results showed that the chosen SRIs were successful in identifying significant quantitative differences across conventionally

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nodulating genotypes, demonstrating quantitative genetic differences in the N accumulation in addition to discriminating between non-nodulating and nodulating soybean. High symbiotic N fixation rates are particularly relevant for achieving a positive nitrogen balance as well as a beneficial crop rotation effect of soybean in organic production systems. Particularly under organic management practices, symbiotic di-nitrogen fixation of grain legumes has a significant impact on crop production, harvest product quality, and nitrogen (N) balance of crop rotations.

Results on genotyping

Genotyping for (a) cadmium accumulation

Three genotyping methods for detecting variability at the major QTL locus for Cd accumulation (Cda1) in soybean were performed using different marker systems: SSR (Sack149), dCAPS (Gm-dCAPS-HMA1) and KASP markers (Fig 44,-46).

All three protocols discriminated the homozygous and heterozygous material. Different assays showed the same results (the samples separated in the same way) (Table 20). Considering significantly faster analysis of the KASP technique compared to other two approaches, KASP could be the best option for further testing.

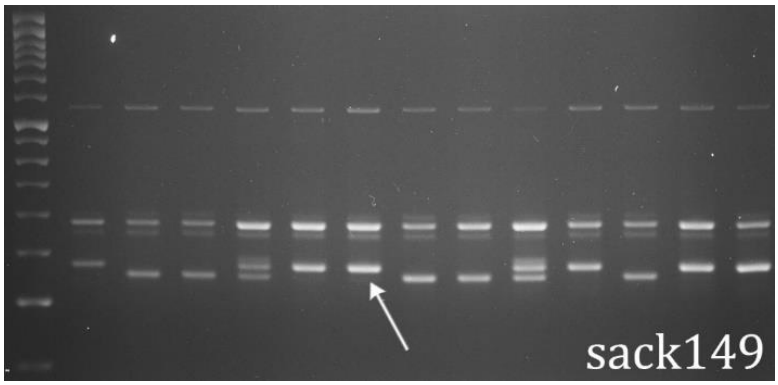

Fig 44 Electrophoretic profile obtained with Sack149 marker. The arrow denotes PCR product for the “low Cd accumulation” allele. M-50 bp DNA Ladder.

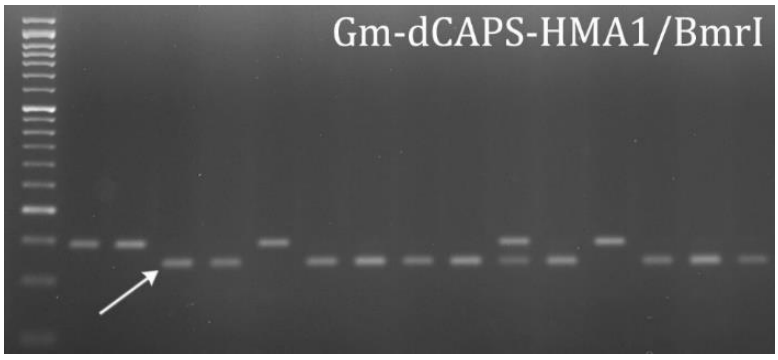

Fig 45 Electrophoretic profile obtained with Gm-dCAPS-HMA1 marker. The arrow denotes PCR product for allele “low Cd accumulation”. M-50 bp DNA Ladder.

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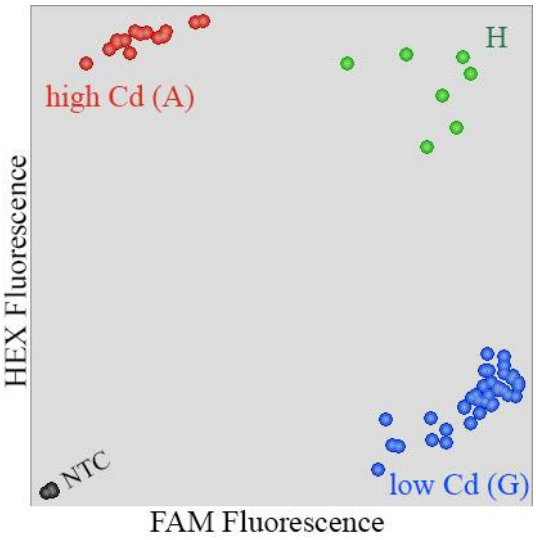

Fig 46 KASP assay for Cda1.

Of 28 tested genotypes, 13 soybean lines contained the low-cadmium accumulation allele in homozygous state and were desirable for including as parents into programmes for organic soybean breeding. Six of these lines (Kamianetz, Novosadska Rana, NS Albus, NS Kraljica, Tajfun, Xonia) were also selected as lines with high yield in field trials from Task 4.1. Eleven genotypes contained the high-cadmium accumulation allele in homozygous state, while four genotypes were heterozygous or heterogeneous, with issues of impurities.

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Table 20 Screening of soybean germplasm for Cd accumulation using different molecular markers.

No.	Accession name	Cadmium tolerance		
		SSR	dCAPS	KASP
1	Camelia F	high	high	high
2	Christine	low	low	low
3	Columna	low	low	low
4	Crina F	high	high	high
5	Daciana	H	H	H
6	Eider	high	high	high
7	Ezra	high	-	-
8	Fabiana F	H	H	H
9	GAZELA	high	-	-
10	GL Melanie	high	high	high
11	Kamianetz	low	-	-
12	Larisa	low	low	low
13	Lenka	high	high	high
14	Mercury	low	low	low
15	Novosadska Rana	low	-	-
16	NS Albus	low	-	-
17	NS Kraljica	low	-	-
18	NS Maximus	low	low	low
19	NS Vasa	high	-	-
20	NS Virtus	H	-	-
21	Oana F	high	high	high
22	Ovidiu F	low	low	low
23	Rubin	high	-	-
24	Sigalia	H	-	-
25	Steara	low	low	low
26	Tajfun	low	-	-
27	Valjevka	high	-	-
28	Xonia	low	-	-

Low-low cadmium accumulation; high-high cadmium accumulation; H-heterozygous/heterogenic

Genotyping for (b) supernodulation

Screening of the germplasm from ECOBREED project using molecular assay for *GmNARK* revealed the presence of both alleles (A and T) in all tested genotypes (Figs. 47 and 48, Table 21). This implicated that they have a normal phenotype, not supernodulating, as according to the literature, supernodulating genotypes showed only a band-specific to the T allele (Kim et al. 2005).

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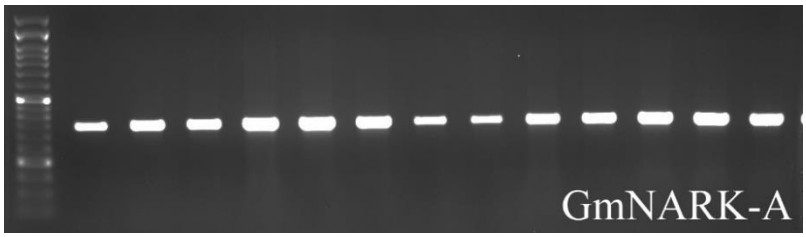

Fig 47 Electrophoresis pattern for A-allele.

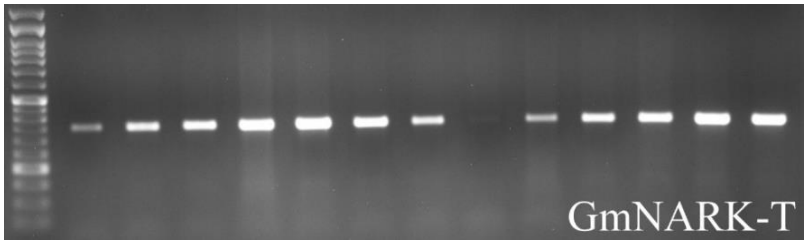

Fig 48 Electrophoresis pattern for T- allele

Table 21. Characterisation of soybean lines for supernodulation

No.	Accession name	NARK-A	NARK-T	Type (N/S)
1	NS Maximus	+	+	N
2	EM Neve	+	+	N
3	Daciana	+	+	N
4	Oana F	+	+	N
5	Columna	+	+	N
6	Crina F	+	+	N
7	Camelia F	+	+	N
8	Fabiana F	+	+	N
9	Ovidiu F	+	+	N
10	Miruna TD	+	+	N
11	Larisa	+	+	N
12	Eider	+	+	N
13	Steara	+	+	N
NARK-A- A allele-specific SNAP primer; NARK-T- T allele-specific SNAP primer; N-normal; S-supernodulating type				

Genotyping for (c) disease tolerance

GWAS approach identified 17 SNPs with significant association to disease response (Table 22), and all of the most highly resistant genotypes shared identical alleles at QTLs. In all SNPs, the most frequent allele was favourable as it was associated with shorter lesions. The strongest association was found on chromosome Gm18. The other significantly associated markers were found on chromosomes 1, 5, 7, and 9. As the size of this association panel was small, it is expected to capture only large effect QTLs, while some additional QTLs may have eluded detection. Based on haplotypes identified at the

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most highly resistant genotypes using significant SNP markers and available molecular data for ECOBREED soybean germplasm, the following soybean genotypes were determined to carry the highest number of SNP alleles that confer resistance (Table 22). Lines carrying the resistance allele for selected markers had lesions that were on average 42.1 mm (39.7-47.7) shorter than lines with the alternate allele. Furthermore, SNP markers from previously conducted genome-wide association studies (Iquira et al. 2015; Moellers et al. 2017; Wei et al. 2017) that were in strong association with the length of the lesions caused by *S. sclerotiorum* were compared to the significant SNP regions identified in ECOBREED (Table 23). The 94 markers important for disease resistance against *S. sclerotiorum* from other studies were located on all 20 soybean chromosomes. The shared significant regions among these studies and the GWAS from this project were on Chromosome 1 (2915019-3597388) (Moellers et al. 2017) and Chromosome 9 (31230369-32113409) (Moellers et al. 2017, Wei et al. 2017). None of the QTLs identified in the study of Inquira et al. (2015) proved to be the same as in this one, which might have been caused by the different genetic backgrounds of two association panels.

Table 22 Significant SNPs associated with the defence response to *Sclerotinia sclerotiorum*.

SNP	Chromosome	Position
S18_43348405	18	43348405
S5_40750374	5	40750374
S5_40752677	5	40752677
S5_40820956	5	40820956
S7_23283513	7	23283513
S18_43348430	18	43348430
S1_2765794	1	2765794
S1_3160446	1	3160446
S1_3161076	1	3161076
S1_2771348	1	2771348
S1_2829751	1	2829751
S1_3134270	1	3134270
S1_3137444	1	3137444
S5_40837630	5	40837630
S5_40924676	5	40924676
S1_3123217	1	3123217
S9_32640412	9	32640412

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Table 23 Identified ECOBREED soybean germplasm with haplotypes characteristic for genotypes resistant to *Sclerotinia sclerotiorum*.

No.	ECOBREED code	Accession name
1	EB_002	NS Kaca
2	EB_005	Fortuna
3	EB_006	NS Maximus
5	EB_077	Pando
6	EB_079	Gracia
7	EB_080	Favorit
8	EB_093	Astafor
10	EB_160	Zita
11	EB_162	NS-L-510016
12	EB_164	Venera
13	EB_169	Rubin
14	EB_173	Tajfun

In total, 15 SNPs associated with northern stem canker resistance were identified (Table 24). The most significant SNP association was observed on Chromosome 7, while other significantly associated markers were located on chromosomes 10, 11, 12, 13, 14, 16, 17, and 18. In all SNPs, the most frequent allele was associated with susceptible genotypes with a higher % of dead plants (DP%) assessed in task 4.1. The highest number of SNP alleles that confer resistance and could be used further as potential parents in organic soybean breeding programs were Favorit (EB_080) and Jelica (EB_081). Lines carrying the resistance allele for significant markers had DP% on average 48.1% higher than lines with the alternate allele.

Table 24 Significant SNPs associated with *Diaporthe complex* tolerance.

SNP	Chromosome	Position
S7_18783796	7	18783796
S10_33403518	10	33403518
S12_39599292	12	39599292
S16_32449620	16	32449620
S18_26503909	18	26503909
S12_20987398	12	20987398
S16_31603068	16	31603068
S12_20987408	12	20987408
S12_20987411	12	20987411
S12_20987417	12	20987417
S11_6339514	11	6339514
S13_7009929	13	7009929
S17_778539	17	778539
S16_32449710	16	32449710
S16_2430895	16	2430895

The genotype Jelica which was identified as resistant to northern stem canker using SNP markers from our study, was also confirmed as resistant to southern stem canker and

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had *Hap1* combination at Chromosome 14. Molecular marker data for SNPs S14_1744370, S14_1768793, and S14_1744518 were used to find other possible sources of resistance to *D. aspalathi* in soybean germplasm from the ECOBREED project. It was identified 13 soybean lines as resistant using defined haplotypes (Maldonado dos Santos et al. 2019) and are listed in Table 25. All of them had *Hap1* allele combinations at Chr 14.

Table 25 Identified ECOBREED soybean germplasm with haplotype *Hap1* characteristic for genotypes resistant to *Diaporthe aspalathi*.

No.	ECOBREED code	Accession name
1	EB_077	Pando
2	EB_081	Jelica
3	EB_093	Astafor
4	EB_094	Zlata
5	EB_121	Isidor
6	EB_151	Becejka
7	EB_154	NS Kraljica
8	EB_160	Zita
9	EB_162	NS-L-510016
10	EB_163	NS-L-510017
11	EB_166	NS-L-520019
12	EB_175	NS Sirius
13	EB_197	Belka

Conclusions on genotyping

Marker-assisted selection (MAS) is a valuable tool in organic crop breeding, allowing rapid screening at early developmental stages as well as more accurate and efficient identification and selection of plants with desired traits. Besides accelerating breeding, this approach reduces the need for extensive field trials and multiple generations of selection. Considering that organic breeding emphasises sustainable practices and minimal environmental impact, MAS can reduce the need for excessive land, water, and resources typically required for conventional breeding. In addition, it can help breeders to develop organic-compliant varieties by targeting specific traits and preserving organic standards, providing more efficient and sustainable strategies for organic production systems. In the conducted study for genotyping of important traits for organic production, divergent soybean genotypes from germplasm collection and breeding material were analysed. Obtained results provided the lists of genotypes with traits desirable for organic soybean breeding based on molecular markers. Cadmium (Cd) is taken up in plants by roots and can be translocated into aerial organs, where it can cause severe physiological and morphological damage to plants. Besides that, genotypes with low cadmium accumulation should be used in organic production to improve food safety. Low Cd accumulation in soybean seeds is under the control of a

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major gene (*Cda1*) with the allele for low accumulation being dominant. Three different protocols were used for germplasm screening (Jegadeesan et al., 2010; Benitez et al. 2012; Nissan et al. 2022) and all discriminated the homozygous and heterozygous material. The KASP marker assay is the fastest protocol for detection of the low cadmium accumulation allele. Among tested soybean genotypes for Cd accumulation, 13 cultivars showed the low-cadmium accumulation allele in homozygous state. Supernodulation in soybean could be an important trait showing potential for increased nitrogen fixation to subterranean ecological systems. The gene *GmNARK* controls autoregulation of nodulation. At the 959-bp position of the *GmNARK* sequence was identified SNP (A to T), where it was found that 'A-allele' encodes a full-length *GmNARK* protein and the 'T-allele' encodes a truncated *GmNARK* protein which is the mutant supernodulating allele. Based on *GmNARK* study all soybean cultivars showed genetically the haplotype for normal type of nodulation. Molecular screening of genotypes for disease resistance is especially important in organic farming where chemical interventions are limited, offering an efficient and sustainable strategy for organic production. Genotyping for resistance to *Sclerotinia sclerotiorum* is challenging in soybean, as no single gene provides strong resistance, but the resistance is quantitatively controlled by numerous genes or quantitative trait loci (QTLs). Thus, a genome-wide association study (GWAS) was performed to dissect the complex genetic architecture of soybean quantitative resistance to this pathogen. In total, 17 SNPs were identified to be significantly associated with the disease response, with the most frequent allele associated with shorter lesions. Based on molecular profiles, 14 genotypes were identified as resistant to *Sclerotinia sclerotiorum*, carrying the highest number of SNP alleles that confer resistance. 15 SNPs associated with northern stem canker resistance were identified, with the most frequent allele associated with susceptible genotypes. The highest number of SNP alleles that confer resistance were found in two genotypes, Favorit and Jelica that could be used as potential parents in organic soybean breeding programmes. Jelica was also confirmed as resistant to southern stem canker and had *Hap1* combination at Chromosome 14. Molecular marker data were used to find other possible sources of resistance to *D. aspalathi* in soybean germplasm from the ECOBREED project, identifying 13 soybean lines as resistant. Inconsistency was observed by comparing significant loci from association mapping for disease resistance (*Sclerotinia sclerotiorum* and *Diaporthe* complex) in the ECOBREED project with reported results from GWAS in previous research (Iquira et al. 2015; Moellers et al. 2017; Wei et al. 2017). One explanation for this might be the fact that a different set of accessions relative to previous studies was chosen for association panel. Thus, studied populations had a different genetic background and different QTLs are segregating in different mapping populations, causing also divergent QTL x genetic background interactions. Beside this, the fact that different inoculation method or

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treatment relative to previous work in this area was applied may also explain the lack of overlap. There is also possibility that different pathogen isolates, collected on different continents were used. All these facts can lead to high variability in phenotyping and consequently to different results. Other reasons explaining the inconsistency include genome coverage that was not equally similar, caused by exploiting different number of markers for association analysis, resulting in different linkage disequilibrium with the causal loci. Other possible reasons that might explain variable results in GWAS are QTL x environment interactions and different underlying mechanisms controlling resistance in different environments. Further research and experimentation may be required to understand the specific mechanisms underlying the disease resistance or tolerance.

Results on improving seed multiplication via the use of cover crops and seed inoculants

Results on cover crops

Soybean yield: During 2020 and 2022 soybean yield was in the range 1.9 – 3.3 t/ha (Fig. 49). Yield and yield parameters positively reacted to the mixture (P+O) as a pre crop. In both production systems (organic and low-input), the average yield for both types in 2022 was between 0.8 and 1.2 t/ha, which was affected by severe drought on both locations during July and August.

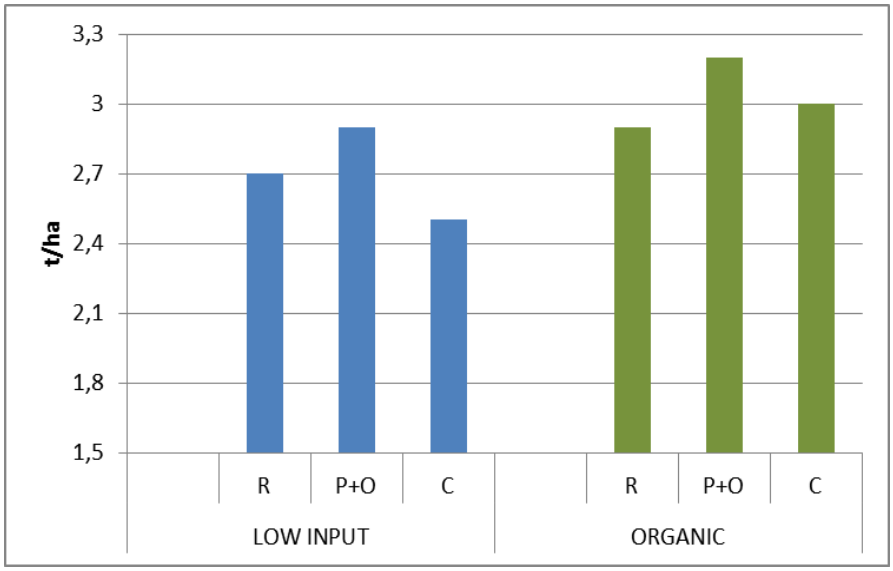

Fig 49 Soybean yield (t/ha) following rye and pea/oat cover crops compared to the control treatment.

Soybean seed quality: After harvest is each experimental year seed germination energy and germination (%) were determined. In 2020 (Fig. 50, 51) and 2021, the germination rates of the seeds under test reached as high as 98%. However, in 2022, germination

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dropped to 75%, primarily due to severe drought in the summer months and excessive rain in September during harvest.

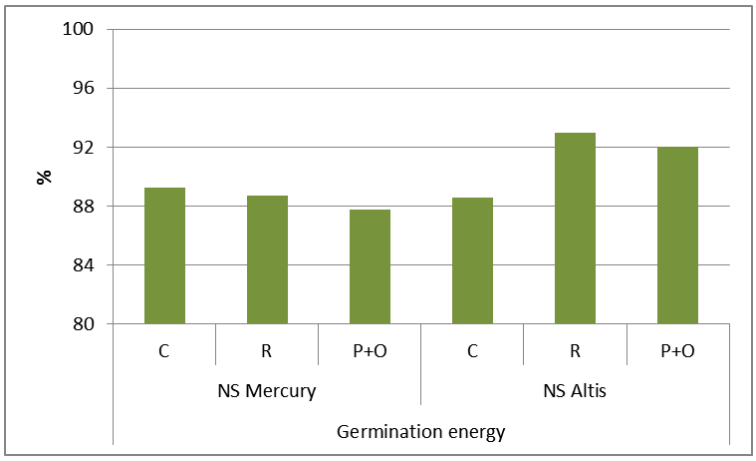

Fig 50 Organic production, soybean seed germination energy in 2020.

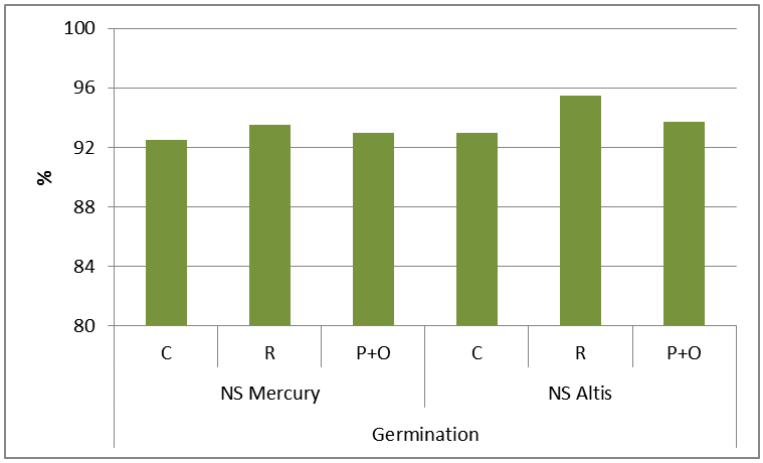

Fig 51 Soybean seed germination in 2020.

Soybean protein, oil phenols and flavonoids

Protein and oil content: The relation between protein and oil content and use of cover crops was seen through obtained results. There was no direct impact of cover crops on protein and oil content, with statistical differences identified only between selected varieties. In organic production the average protein content of different genotypes was in the range of 35.6 to 44.3 % for the two varieties (Fig. 52).

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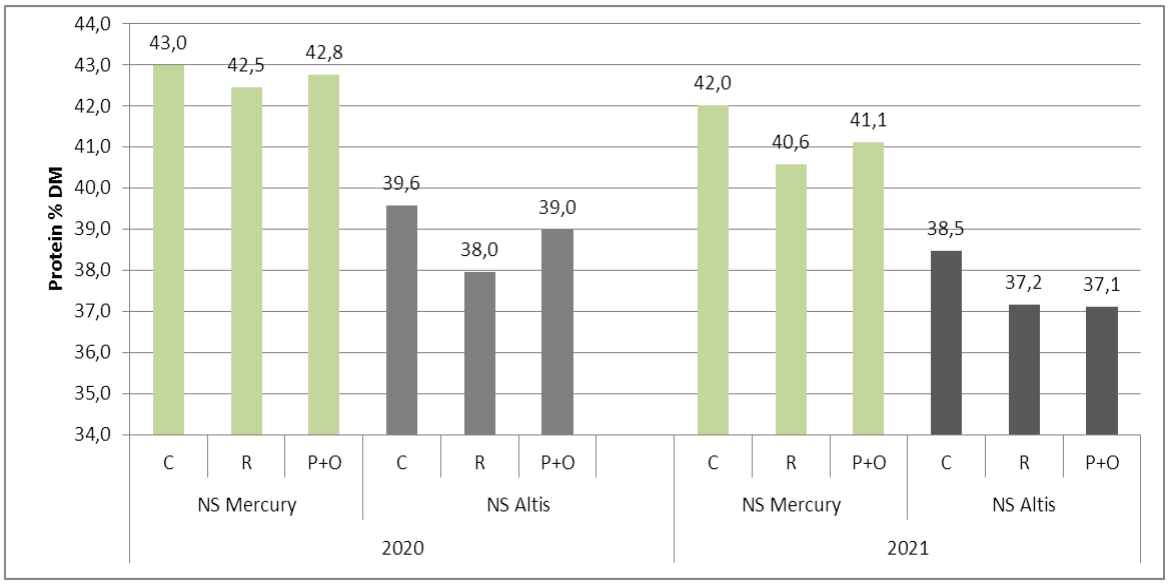

Fig 52 Protein (DM) in organic production in 2020 and 2021.

For the testing of seed multiplication two varieties were selected according to the end users, one variety that has high protein content (up to 45 %) NS Mercury and one that has higher oil content (22 % DM) NS Altis, which was confirmed in organic certified production conditions of this trial (Fig.53).

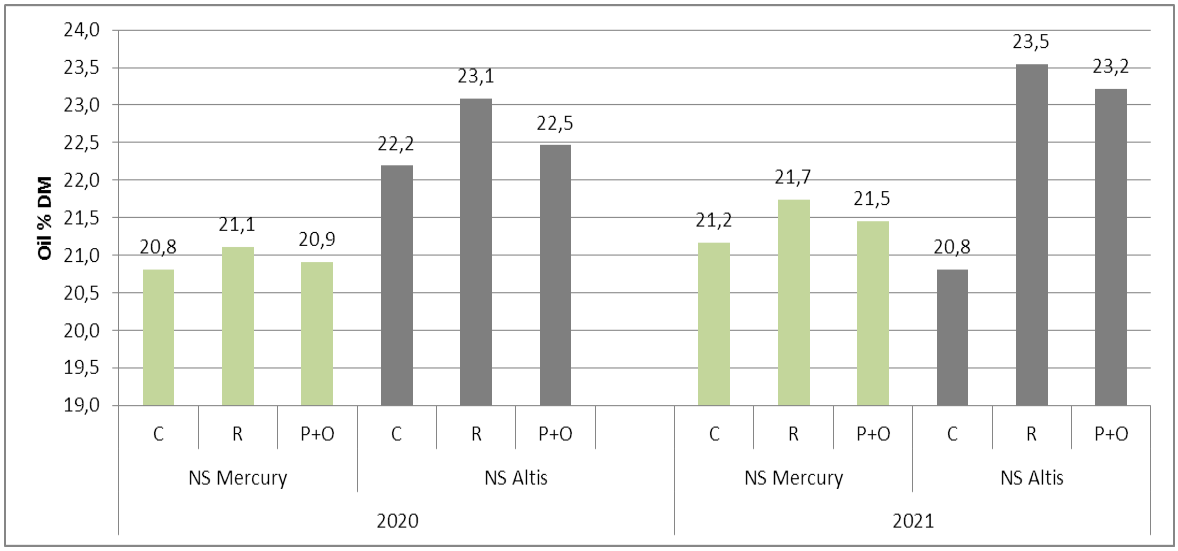

Fig 53 Oil (DM) in organic production in 2020 and 2021.

Total phenols and flavonoids: Phenols and flavonoids are secondary metabolites found in various plants, including soybean which have antioxidant properties. It is mostly unknown why particular flavonoids are produced and accumulate in various plant tissues after being stimulated either internally or externally (Shah and Smith, 2020). In some plant-microbe interactions in the rhizosphere, flavonoids are particularly effective at reducing biotic and abiotic stressors (Cetinkaya et al., 2017). Within trials (2020-2022)

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in RS total phenols and flavonoids were determined in soybean grains. According to gathered results, statistical differences were noted only for production year (Fig.54 & 55).

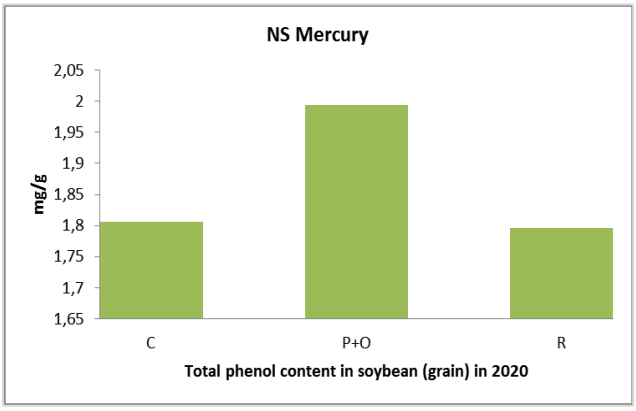

Fig 54 Total phenols in soybean seed in 2020 (NS Mercury).

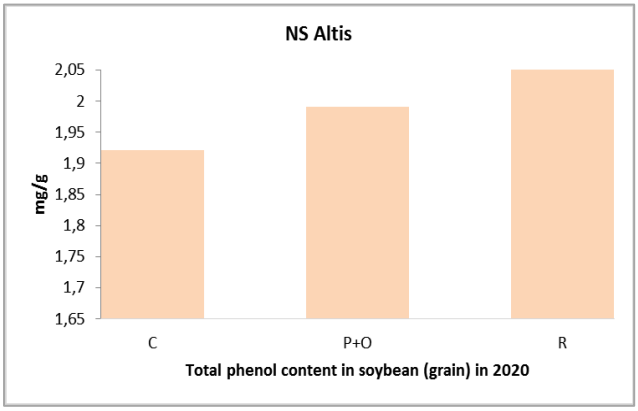

Fig 55 Total phenols in soybean seed in 2020 (NS Altis).

Soybean seed health and weed screening

Soybean seed health screening: The health of soybean plants was evaluated in the field following the use of cover crops. But also, screening of soybean seed health is particularly important during the seed multiplication process. The health status of seed is a key pre-requisite for gaining quality seed. The following fungal pathogens were identified: *Peronospora manshurica*, *Diaporthe phaseolorum, Alternaria* sp., *Fusarium* sp., *Cercospora* sp. and *Botrytis* sp. Statistical difference was not found during 2020 and 2021 on appearance of soybean seed causal agents (Fig. 56). In contrast, in 2022, a significant drought caused considerable challenges in the seed multiplication process, resulting in as much as a 50% infection rate among the screened seeds. The effect of cover crop, system of production and variety had no effect on seed health parameters, only production year. The correlation between adverse weather conditions, particularly high temperature appeared to be negatively influencing the quality of multiplied seeds.

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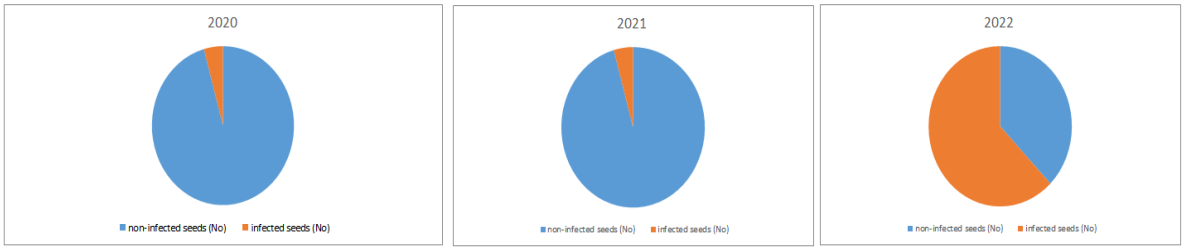

Fig 56 Number of soybean infected and non-infected seeds in 2020, 2021 and 2022.

Soybean weed screening in cover crops: Dense canopies of cover crops shade out weeds, reducing competition from subsequent soybean plants. Some cover crops (rye, oat, buckwheat) also release allelopathic chemicals that inhibit weed growth. The numbers of weed species that appeared at the site Rimski šančevi (RS) was up to 3, while in Čurug (RS), up to 5 were recorded. Weed assessment was carried out in both the cover crop and control plots.

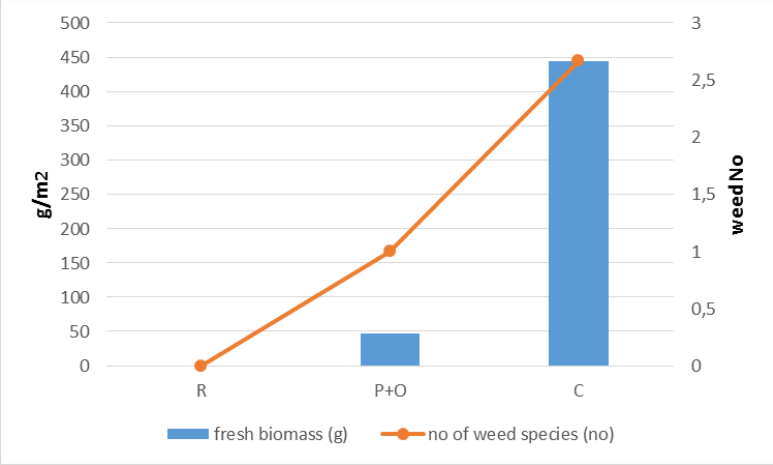

Fig 57 Weed screening in low-input in 2022.

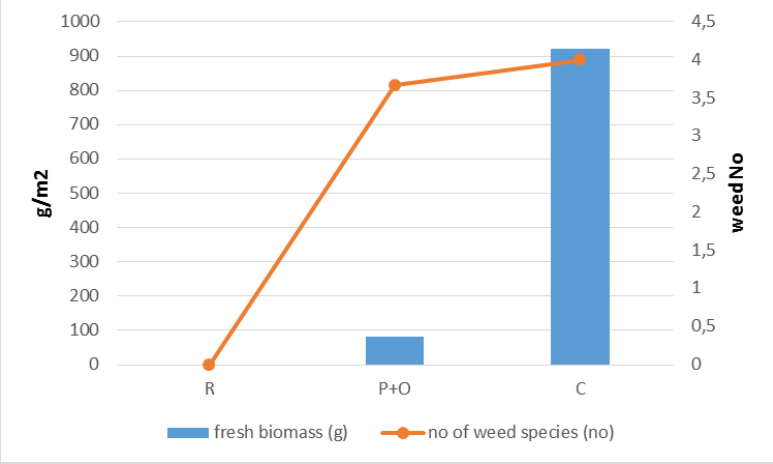

Fig 58 Weed screening in organic production in 2022.

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When it comes to the cover crop with rye (R), no weed species were identified, because rye is a strong competitor. When it comes to the cover crop, the P+O crop was moderately competitive with weed species, while the highest number of weed species was recorded in the control plot. Given that weeds use nutrients from the soil, the pressure of weed plants in organic and low-input production can be reduced by cover crop sowing. Weed pressure can be very high in low-input and organic production and with the introduction of cover crops the pressure can be decreased which is important during seed multiplication process, since one of the limiting factors in organic production are weed seed-banks.

Results on inoculant use

Whereas the mechanisms underlying plant-inoculant interactions are well studied, microbial inoculants effects on soil microbial communities, including their interactions, have received little attention. Introduction of microorganisms into the soil environment sometimes has undesirable ecological effects; therefore, it is important that their environmental effects are assessed. Rhizobia are reported to influence crop growth, yield, and nutrient uptake by different mechanisms which have an important role in seed multiplication. They fix nitrogen, increase the supply of other nutrients, produce plant hormones, help in promoting free-living nitrogen-fixing bacteria, enhance other beneficial bacteria or fungi, and control bacterial and fungal diseases (Trabelsi and Mhamdi, 2013). Although we understand little about the degree to which genetic and taxonomic microbial diversity affects functional ecosystem properties, it is accepted that higher-diversity ecosystems are frequently associated with soil fertility (Ambrosini et al., 2016). Impact of bacterial inoculation on agricultural systems is still unknown and varies according to the location, soil type, plant species and microorganisms introduced. Soil analyses performed during the flowering and maturity stages in 2019, indicate significant increase in abundance of *Azotobacter* spp. (71%), ammonifiers (98% and 216%), free N_2-fixing bacteria (152%) and actinobacteria (175% and 143%), following *B. japonicum* seed treatment. The significant beneficial effect of rhizobia inoculation on dehydrogenase activity (27-144%) was also recorded, unlike the total bacterial population and fungal abundance, which were not affected (Table 26).

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Table 26 Microbial abundance and activity in 2019.

Treatment	Variety	*Azotobacter* spp. × 10	Ammoni-fiers × 10^6	Total bacteria × 10^7	Free N_2-fixers × 10^6	Fungi × 10^4	Actino-mycetes × 10^4	Dehydrogenase activity
		CFU/g soil						(µg TPF/g soil)
Full bloom stage (R2)								
Control	NS Apolo (I)	118 b	55 b	221 a	140 a	14 a	8 b	15.83 bc
B. japonicum	NS Apolo (I)	202 a	109 a	323 a	160 a	12 a	22 a	33.91 a
Control	Rubin (II)	142 ab	31 b	197 a	135 a	12 a	7 b	13.76 c
B. japonicum	Rubin (II)	148 ab	98 a	311 a	173 a	9 a	17 ab	17.51 b
Maturity stage (R8)								
Control	NS Apolo (I)	99 b	99 a	213 a	187 bc	14 a	12 a	16.99 bc
B. japonicum	NS Apolo (I)	125 ab	112 a	189 a	257 ab	9 a	13 a	32.79 a
Control	Rubin (II)	144 a	59 a	151 a	114 c	11 a	17 a	14.71 c
B. japonicum	Rubin (II)	151 a	45 a	183 a	287 a	11 a	11 a	24.75 ab

The different letters within a maturity stage indicates a significant difference at $P < 0.05$. CFU – colony-forming unit

In the second year (2020), the only significant change in microbial community structure and activity due to inoculation was found in the abundance of *Azotobacter* spp. (56%) (Table 27).

Table 27 Microbial abundance and activity in 2020.

Treatment	Variety	*Azotobacter* spp. × 10	Ammoni-fiers × 10^6	Total bacteria × 10^7	Free N_2-fixers × 10^6	Fungi × 10^4	Actino-mycetes × 10^4	Dehydrogenase activity
		CFU/g soil						(µg TPF/g$^-$ soil)
Full bloom stage (R2)								
Control	NS Apolo (I)	136 ab	90 a	185 b	138 a	14 a	9 a	8.47 a
B. japonicum	NS Apolo (I)	142 ab	87 a	248 ab	190 a	14 a	11 a	6.27 a
Control	Rubin (II)	99 b	137 a	353 a	234 a	12 a	9 a	8.02 a
B. japonicum	Rubin (II)	155 a	128 a	283 ab	312 a	19 a	12 a	15.6 a
Maturity stage (R8)								
Control	NS Apolo (I)	126 a	206 a	366 a	251 a	52 a	31 a	9.20 a
B. japonicum	NS Apolo (I)	159 a	224 a	369 a	241 a	54 a	22 a	13.66 a
Control	Rubin (II)	65 b	215 a	403 a	304 a	37 a	24 a	11.48 a
B. japonicum	Rubin (II)	118 ab	179 a	393 a	276 a	37 a	18 a	10.33 a

The different letters within a maturity stage indicates a significant difference at $P < 0.05$. CFU – colony-forming unit

Bacterial inoculation efficiency is associated with the beneficial features of the inoculated bacterium, as well as with the complex network of interactions occurring in the soil (Ambrosini et al., 2016). The influence of bacterial inoculants on native microbial communities depends on numerous abiotic and biotic factors, considering that indigenous populations show higher resilience under specific environmental conditions.

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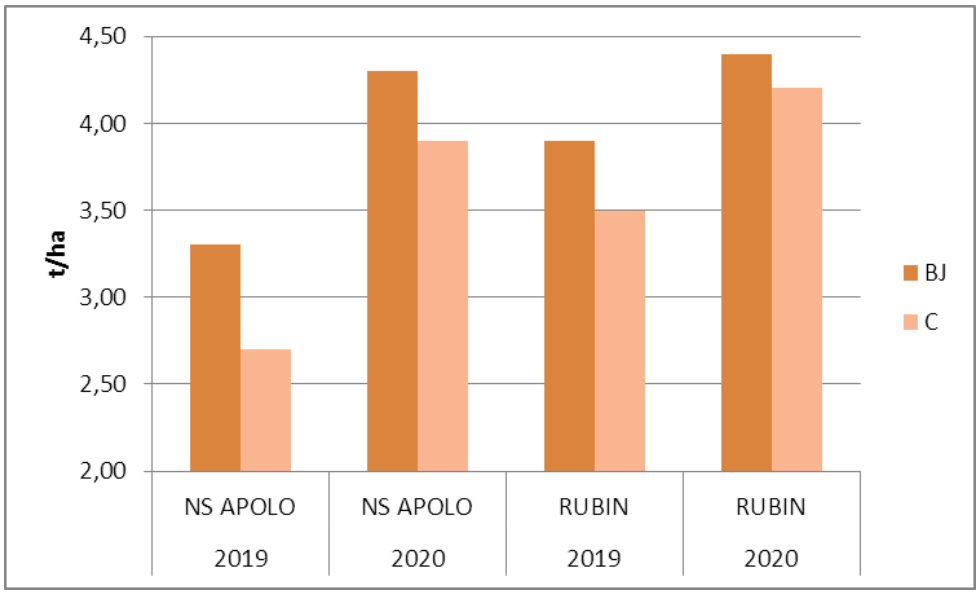

Fig 59 Yield of soybean (t/ha) during 2019 and 2020 as influenced by variety and following inoculation with *Bradyrhizobium japonicum* (BJ) in comparison with a control treatment (C)).

Table 28 Microbial abundance and activity in 2021.

Treatment	*Azoto bacter* spp. × 10	Ammoni-fiers × 10^6	Total bacter ia × 10^7	Free N_2-fixers × 10^6	Fungi × 10^4	Actino-mycetes × 10^4	Dehydrogenase activity
	CFU/g soil						(µg TPF/g soil)
Full bloom stage (R2)							
Control	12 b	53 b	41 a	37 b	15 a	3 a	4.31 a
B. japonicum	44 ab	131 a	87 a	137 a	18 a	7 a	6.88 a
***B. japonicum* + nutrients (% m/m): S – 5.2; Mg – 3; Mn – 1.5; Fe – 1; Zn – 1; Cu – 0.5; B – 0.3; Mo – 0.01.)**	86 a	153 a	75 a	141 a	12 a	8 a	8.71 a
Maturity stage (R8)							
Control	34 b	248 a	399 a	186 a	26 a	27 b	5.74 b
B. japonicum	33 b	200 a	481 a	217 a	26 a	39 ab	7.04 ab
***B. japonicum* + nutrients (% m/m): S – 5.2; Mg – 3; Mn – 1.5; Fe – 1; Zn – 1; Cu – 0.5; B – 0.3; Mo – 0.01.)**	91 a	206 a	592 a	249 a	74 a	47 a	11.04 a

The different letters within a maturity stage indicates a significant difference at $P < 0.05$. CFU – colony-forming unit. source doi10.18805/LRF-762

Both applied treatments in 2021, had a positive effect on the microbial soil properties, and the abundance of different bacterial communities were significantly enhanced compared to the control. More significant proliferation of azotobacter (617% and 167%), ammonifiers (189%), free N_2-fixers (281%), actinomycetes (74%) and dehydrogenase activity (92%) was obtained by applying inoculants with nutrient complex. However, total bacterial and fungal community abundance were similar independent of the treatments applied (Table 28). Results of the three-year study showed that over the

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course of a growing season, a more significant impact of inoculation was recorded at the full bloom phase. The results also indicate that bacterial communities were more sensitive to changes induced by inoculation compared to fungal communities, tending to significantly increase in abundance following inoculation. Fundamental divergences between bacterial and fungal ecology may have a part in this difference (Cornell et al., 2021). The analyses of key bacterial groups related to the N-cycle (azotobacter, ammonifiers, and free N_2-fixers) showed significant abundance increments due to inoculation. These outcomes can also facilitate understanding of how specific microbial functional groups are impacted by rhizobia introduction. More research is needed to understand interactions among inoculant establishment, and interactions with resident soil microbial communities. Long-term experiments could provide more details about the influence of rhizobia inoculation on soil microbiome structure and soil functionality. To maximise the positive effects of *Bradyrhizobium japonicum* on soybean yield (Fig. 60), it is essential for farmers to ensure that the right *Bradyrhizobium* strain is present in their soil, as the effectiveness of nitrogen fixation can vary depending on the specific strain and environmental conditions.

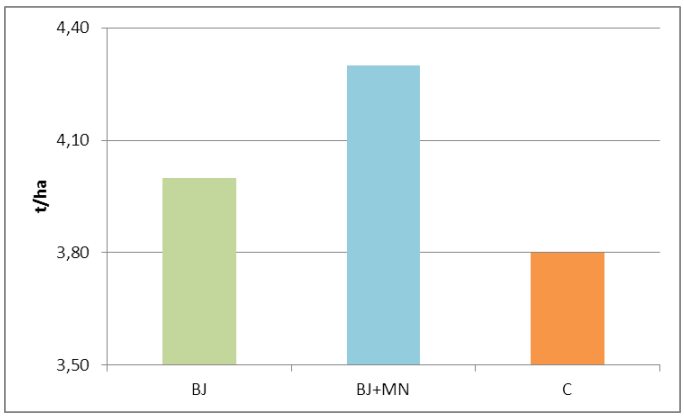

Fig 60 Yield of soybean (t/ha) during 2021 following inoculation with *B. japonicum* (BJ), *B. japonicum* (BJ) plus Micronutrients (MN) compared with a Control (C)).

Seed multiplication involves selecting and producing seeds from soybean plants with these desirable traits. Therefore, the quality and genetic potential of the seeds used for planting directly affect the yield of the resulting soybean crop. The integration of high-quality seeds from the seed multiplication process with the use of appropriate inoculants can enhance soybean yield. When high-quality seeds are used with effective inoculants, soybean plants are more likely to have vigorous growth, healthier root systems, and improved stress tolerance. This translates into increased yield potential. In summary, the relationship among seed multiplication, yield, and the use of inoculants is interconnected and crucial for successful soybean cultivation. High-quality seeds, produced through multiplication, provide the genetic foundation for optimal yield potential. When combined with effective inoculants, soybean plants are better

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equipped to utilise available nitrogen resources, leading to increased yields and potentially more sustainable and cost-effective farming practices.

Conclusions on cover crop testing and inoculant use

Inoculation and bio-priming are valuable tools for improving soybean seed multiplication. Both inoculation with *Bradyhrizobium* and cover cropping can create a synergistic effect on the multiplication of seed for low-input and organic production systems. The combination of improved nitrogen availability, reduced weed pressure, and enhanced soil health can lead to better overall soybean growth and, consequently, increased efficiency of seed multiplication. It is recommended to plan carefully crop rotation, cover crop selection, and to apply inoculation practices to optimise these benefits and to achieve sustainable and productive soybean seed multiplication in organic and low-input production systems. Additionally, local climate, soil conditions, and cultivation practices should be considered when implementing these practices.

Results on Production of elite varieties and advanced breeding lines

The identification of important traits through population and line selection of soybean was performed. Soybean lines were available for variety testing and registration that were carried out in 2021 and 2022. The chosen soybean lines went throughout registration trials in order to evaluate their performance and adaptability. Agronomic traits were examined, along with the soybean nutritional quality. NS ECOB (IFVC, RS) went through this process and it was registered in 2023. NS ECOB is a variety with high protein content (00 maturity group) specifically selected for organic and low-input production systems.

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Overall contribution to organic soybean breeding

The production of organic grain legumes with high-quality protein such as soybean for livestock and especially pig and poultry production requires development of soybean genotypes with increased agronomic performance, especially in organic systems was the main priority of work package “Soybean“. Work done under WP4 is valuable on first place to breeders and other scientists that are early adopters of different methodologies and also to farmers that were involved in testing of soybean varieties and that are direct users of soybean varieties and proposed technologies. Improved yield potential and better adaptation of soybean varieties will boost socio-economic status of producers through optimisation of production process and availability of varieties and seeds for organic and low-input systems, with impacts that will reflect on the entire organic food and feed sector. The WP4 outcomes indirectly benefit our citizens since soybean is main supplier of plant proteins as important component of organic supply chains.

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References

Ambrosini A, de Souza R, Passaglia LMP (2016) Ecological role of bacterial inoculants and their potential impact on soil microbial diversity. Plant and Soil, 400: 193–207.

Arao T, Ae, N, Sugiyama M, Takahashi M (2003) Genotypic differences in cadmium uptake and distribution in soybeans. Plant and Soil, 251(2): 247-253.

Araus JL, Kefauver SC, Zaman-Allah M, Olsen MS, and Cairns JE (2018) Translating high-throughput phenotyping into genetic gain. Trends in Plant Science, 23, 451–466. https://doi.org/10.1016/j.tplants.2018.02.001

Barnes ER, Jhala AJ, Knezevic SZ, Sikkema PH, Lindquist JL (2018) Common ragweed (*Ambrosia artemisiifolia* L.) interference with soybean in Nebraska. Agronomy Journal, 110(2), 646-653.

Benitez ER, Hajika M, Takahashi R (2012) Single-base substitution in *P1B-ATPase* gene is associated with a major QTL for seed cadmium concentration in soybean. Journal of Heredity, 103(2): 278-286.

Benjamin JG, Nielsen DC (2006) Water deficit effects on root distribution of soybean, field pea and chickpea. Field Crops Research, 97(2-3): 248-253.

Cabello R, Monneveux P, Mendiburu Fde, Bonierbale M (2013) Comparison of yield based drought tolerance indices in improved varieties, genetic stocks and landraces of potato (*Solanum tuberosum* L.). Euphytica 193(2): 147-156.

Callaway MB (1992) A compendium of crop varietal tolerance to weeds. American Journal of Alternative Agriculture 7(4), 169-180.

Cetinkaya H, Kulak M, Karaman M, Karaman HS, Kocer F (2017) Flavonoid accumulation behaviour in response to the abiotic stress: Can a uniform mechanism be illustrated for all plants. In Flavonoids - From Biosynthesis to Human Health; Intechopen: London UK

Cornell C, Kokkoris V, Richards A, Horst C, Rosa D, Bennett JA, Hart MM (2021) Do bioinoculants affect resident microbial communities? A Meta-Analysis. Frontiers in Agronomy 3: 753474.

Costa RF, Silva AG, Bessa OR, Dias MO (2019) Agronomic performance of transgenic soybean cultivars in Brazilian Cerrado. Acta Scientiarum. Agronomy, 41, e42713.

Cristina D, Ciuca M, Cornea CP (2017) Comparison of four genomic DNA isolation methods from single dry seed of wheat, barley and rye. AgroLife Scientific Journal, 6(1).

Cure WW, Flagler RB, Heagle AS (1989) Correlations between canopy reflectance and leaf temperature in irrigated and droughted soybeans. Remote Sensing of Environment 29(3): 273–280. https://doi.org/10.1016/0034-4257(89)90006-0

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13–15

Gitelson A (2004) Wide Dynamic Range Vegetation Index for Remote Quantification of Biophysical Characteristics of Vegetation. Journal of Plant Physiology 161(2): 165-173, ISSN 0176-1617.

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Horizon 2020
Grant agreement No 771367

Huff J, Lunn RM, Waalkes MP, Tomatis L, Infante PF. (2007) Cadmium-induced cancers in animals and in humans. International Journal of Occupational and Environmental Health 13(2): 202-212.

Hunt ER, Doraiswamy PC, McMurtrey JE, Daughtry CST, Perry EM, Akhmedov B (2013) A visible band index for remote sensing leaf chlorophyll content at the canopy scale. International Journal of Applied Earth Observation and Geoinformation 21(): 103–112. https://doi.org/10.1016/j.jag.2012.07.020

Iquira E, Humira S, François B (2015) Association mapping of QTLs for sclerotinia stem rot resistance in a collection of soybean plant introductions using a genotyping by sequencing (GBS) approach. BMC Plant Biology 15: 5. https://doi.org/10.1186/s12870-014-0408-y

Jegadeesan S, Ying Yu, R, Yu, K (2016) Genetic control of cadmium concentration in soybean seeds. Grain Legumes: 79.

Jegadeesan S, Yu K, Poysa V, Gawalko E, Morrison MJ, Shi C, Cober E (2010) Mapping and validation of simple sequence repeat markers linked to a major gene controlling seed cadmium accumulation in soybean [*Glycine max* (L.) Merr]. Theoretical and Applied Genetics 121(2): 283-294.

Khalili M, Pour Aboughadareh A, Naghavi MR (2016) Assessment of drought tolerance in barley: Integrated selection criterion and drought tolerance indices. Environmental and Experimental Biology 14: 33–41.

Kim MY, Van K, Lestari P, Moon J-K, Lee S-H (2005) SNP identification and SNAP marker development for a *GmNARK* gene controlling supernodulation in soybean. Theor Appl Genet 110: 1003–1010. https://doi.org/10.1007/s00122-004-1887-2

Kudełka W, Kowalska M, Popis M (2021) Quality of soybean products in terms of essential amino acids composition. Molecules 26: 5071.

Liebman M, Gallandt ER, Jackson LE (1997) Many little hammers: ecological management of crop-weed interactions. Ecology in Agriculture 1, 291-343.

Maldonado dos Santos JV, Ferreira EGC, Passianotto ALL, Brumer BB, Dos Santos AB, Soares RM, Torkamaneh D, Arias CAA, Belzile F, Abdelnoor RV, Marcelino-Guimarães FC (2019) Association mapping of a locus that confers southern stem canker resistance in soybean and SNP marker development. BMC Genomics 20: 798. https://doi.org/10.1186/s12864-019-6139-6

Romero Luna MP, Mueller D, Mengistu A, Singh AK, Hartman GL, Wise KA (2017) Advancing Our Understanding of Charcoal Rot in Soybeans, Journal of Integrated Pest Management 8(1): 8. https://doi.org/10.1093/jipm/pmw020

Martre P, Quilot-Turion B, Luquet D, Memmah M-MO-S, Chenu K, Debaeke P (2015) Chapter 14—Model-Assisted Phenotyping and Ideotype Design; Sadras, V.O., Calderini, D.F.B.T.-C.P., Eds.; Academic Press: San Diego, CA, USA; 349–373.

ecobreed IMPROVING CROPS

Funded by European Union
Horizon 2020
Grant agreement No 771367

Miladinović J, Hrustić M, Vidić M. (2008) Soja. Institut za ratarstvo i povrtarstvo, Novi Sad i Sojaprotein, Bečej, AMB Grafika, NoviSad.

Moellers TC, Singh A, Zhang J, Brungardt J, Kabbage M, Mueller DS, Grau CR, Ranjan A, Smith DL, Chowda-Reddy RV, Singh AK (2017) Main and epistatic loci studies in soybean for *Sclerotinia sclerotiorum* resistance reveal multiple modes of resistance in multi-environments. Scientific Reports 7: 3554. https://doi.org/10.1038/s41598-017-03695-9

Mohler CL, Liebman M, Staver CP (2001) Enhancing the competitive ability of crops. Ecological Management of Agricultural Weeds, 269-321.

Mwenye OJ, van Rensburg L, van Biljon A, van der Merwe R (2016) The role of proline and root traits on selection for drought-stress tolerance in soybeans: a review. South African Journal of Plant and Soil 33(4): 245–256.

Bellaloui N, Mengistu A, Zobiole LHS, Shier WT (2012) Resistance to toxin-mediated fungal infection: role of lignins, isoflavones, other seed phenolics, sugars, and boron in the mechanism of resistance to charcoal rot disease in soybean, Toxin Reviews 31: 1-2, 16-26. https://doi.org/10.3109/15569543.2012.691150

Nissan N, Hooker J, Pattang A, Charette M, Morrison M, Yu K, Hou A, Golshani A, Molnar SJ, Cober ER, Samanfar B (2022) Novel QTL for low seed cadmium accumulation in soybean. Plants 11(9): 1146.

Pour Aboughadareh, AM, Yousefian H, Moradkhani M, Moghaddam Vahed P, Poczai KHM Siddique (2019) iPASTIC: An online toolkit to estimate plant abiotic stress indices. Applications in Plant Sciences 7(7): e11278. https://doi.org/10.1002/aps3.11278

Roland WSU, Vincken J-P, Gouka RJ, Van Buren L, Gruppen H, and Smit G (2011) Soy isoflavones and other isoflavonoids activate the human bitter taste receptors hTAS2R14 and hTAS2R39. Journal of Agricultural and Food Chemistry, 59: 11764–11771.

Sardouei-Nasab S, Mohammadi-Nejad G, Nakhoda B (2019) Yield stability in bread wheat germplasm across drought stress and non-stress conditions. Agronomy Journa, 111(1): 175.

Sheirdil RA, Bashir K, Hayat R, Akhtar MS (2012) Effect of cadmium on soybean (*Glycine max* L) growth and nitrogen fixation. African Journal of Biotechnology 11(8): 1886-1891.

Swanton, CJ, Nkoa R, Blackshaw RE (2015) Experimental methods for crop–weed competition studies. Weed Science 63(SP1): 2-11.

Trabelsi D, Mhamdi R. (2013) Microbial inoculants and their impact on soil microbial communities: A Review. BioMed Research International 2013: 863240.

Vollmann J, Lošák T, Pachner M, Watanabe D, Musilová L, Hlušek J (2015) Soybean cadmium concentration: validation of a QTL affecting seed cadmium accumulation for improved food safety. Euphytica 203(1): 177-184.

Wei W, Mesquita ACO, Figueiró AA, Wu X, Manjunatha S, Wickland DP, Hudson ME, Juliatti FC, Clough SJ (2017) Genome-wide association mapping of resistance to a

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Brazilian isolate of *Sclerotinia sclerotiorum* in soybean genotypes mostly from Brazil. BMC Genomics 18:849

World Health Organization/Food and Agriculture Organization/United Nations University (1985) Energy and protein requirements Report of a Joint FAO/WHO/UNU Expert Consultation. In: WHO technical report series, no. 724. WHO, Geneva

Xu Y, Li P, Zou C, Lu Y, Xie C, Zhang X, Prasanna BM, Olsen M.S (2017) Enhancing genetic gain in the era of molecular breeding. Journal of Experimental Botany 68 (11): 2641–2666. https://doi.org/10.1093/jxb/erx135

Zhang LX, Kyei-Boahen S, Zhang J, Zhang MH, Freeland TB, Watson CE, Jr, Liu XM (2007) Modifications of optimum adaptation zones for soybean maturity groups in the USA. Online. Crop Management. https://doi.org/10.1094/CM-2007-0927-01-RS

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