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Horizon 2020
Grant agreement No 771367

D 3.8

Final publishable report on WP3

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SECURITY (DISSEMINATION LEVEL)	Public
CONTRACTUAL DATE OF DELIVERY	29 February 2024
ACTUAL DATE OF DELIVERY	26 January 2024
DELIVERABLE NUMBER	D 3.8
TYPE	Deliverable
STATUS AND VERSION	Final
NUMBER OF PAGES	68
WP CONTRIBUTING TO THE DELIVERABLE	WP3
LEAD BENEFICIARY	KIS
OTHER CONTRIBUTORS	UNEW, IHAR, MATE
AUTHOR(S)	Peter Dolničar, Uroš Žibrat, Jaka Razinger, Eva Praprotnik, Primož Žigon, Paul Bilsborrow, Enas Surfur, Ankush Prashar, Jaroslav Plich, Beata Tatarowska, Zsolt Polgar, Krisztián Frank
KEYWORDS	Potato, cultivars, advanced and conventional phenotyping, arbuscular mycorrhizal fungi (AMF), cover crops, seed quality, colorado potato beetle, wireworms, breeding, marker assisted selection, elite breeding lines
ABSTRACT (FOR DISSEMINATION)	The results of 6 tasks of WP3 of the ECOBREED project from 2018 to 2023 are presented. Conventional and advanced phenotyping were performed on 65 cultivars of the ECOBREED working collection to select the best cultivars for organic farmers and to evaluate traits useful for breeding programs. AMF compatibility studies were carried out on 20 selected varieties using a natural soil based AMF inoculant. To improve organic seed quality and vigour, the effect of cover crops was investigated on 6 varieties. New approaches using bio-insecticides against Colorado potato beetle and wireworms were evaluated. Molecular markers for late blight, viruses, nematodes and carotenoids were investigated. Marker-assisted selection for late blight and virus resistance was carried out, new resistant progenies were created and advanced potato clones were selected in the IHAR, KIS and MATE breeding programs. 3 new ECOBREED potato cultivars were developed.
DOCUMENT ID	D 3.8 Final publishable report on WP3

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Executive summary

Phenotypic characterisation of the ECOBREED potato working collection was carried out in Poland, Hungary, Slovenia and the UK during 2019-22 for cultivar suitability for organic production. The potato working collection comprised 65 varieties sourced from across Europe with potential for organic production systems. Phenotypic evaluation was carried out according to the agreed list of descriptors and included growth, foliar diseases, NDVI (Normalised Difference Vegetation Index), tuber yield, grading, tuber quality and cooking characteristics. In addition, Advanced phenotyping was carried out in the UK and Slovenia.

Tuber yield was highest in the UK, followed by Slovenia, Poland and Hungary, with clear differences between years. The top three varieties for tuber yield were different for each country. Tuber dry matter content was highest in the UK and lowest in Hungary (22.3 vs 15.6%) when averaged over all varieties in the 2020 and 2021 growing seasons. There were also clear differences between years, countries and varieties for tuber size, with a higher proportion of small tubers <45 mm in 2021 and large tubers >65 mm in 2020. Averaged over all varieties and the 2020 and 2021 growing seasons, Poland produced a higher proportion of small tubers and Slovenia a higher proportion of large tubers than other countries. Advanced phenotyping data collection in the UK and Slovenia was carried out using an unmanned aerial vehicle (UAV) equipped with a multispectral camera. The resulting correlation matrix shows that canopy characteristics and some indices have highly significant correlations with yield data, especially in 2020 and 2022.

AMF compatibility studies were carried out on 20 selected cultivars using a natural soil-based inoculant. The study showed low levels of natural mycorrhizal colonisation, although some cultivars were significantly better at forming mycorrhizal associations than others. Except for Salome, all cultivars showed significantly higher levels of mycorrhizal intensity than the two controls, Cara and Casablanca . Sarpo Mira stood out as having the highest mycorrhizal intensity and abundance of all cultivars. Ambo and Gatsby stood out for their respective maturity groups for mycorrhizal intensity and abundance, which again did not translate into greater plant growth responses compared to the other varieties in theirmaturity groups.

Cover crops are playing an increasingly important role in agricultural production, with the potential for much future adoption and use. The cover crop trials were conducted with individual cover crop species, i.e. black mustard (Brassica juncea), oil radish (Raphanus sativus), lucerne (Medicago sativa), black oat (Avena strigosa -) Common vetch (Vicia sativa and a mixture of all five species.. Four potato cultivars were used at both sites, i.e. Alouette, Carolus, Casablanca and Cara in the UK and Alouette, Carolus, KIS Kokra and KIS Tamar in Slovenia.

At both locations, yield and its characteristics were influenced by the choice of cultivar and cover crop in both years. The differences were significant in most cases, apart from the very dry year 2022 in Slovenia, when yields were very low. Most of the interactions werenot significant, which means that there was no relationship between cultivar and cover crop. Looking at the tuber quality traits observed in Slovenia, there were differences

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between cultivars in several traits, many of which are genetically determined (e.g. eye depth, resistances). It seems that under the stressful conditions of 2022, the choice of cover crop influenced (improved) tuber quality, at least in terms of shape regularity and silver scurf infection.

In 2021-2023, field trials were conducted to test four bioinsecticides i.e. Neemazal, Laser Plus, a conidial suspension of two isolates of the entomopathogenic fungus *Beauveria bassiana*, Novodor and RNA interference (RNAi) against Colorado potato beetle (CPB) larvae. The biological control agents were used individually and in combination against the larval population to evaluate their efficacy and potential synergistic interactions against CPB larvae. Spinosad was the most effective in suppressing the larvae, as its application at a 20-fold lower concentration resulted in over 90% larval mortality. Of the other treatments, only the use of azadirachtin showed a comparable effect in terms of a significant reduction in the number of larvae compared to the control. Combinations of different bioinsecticides did not show a better effect than their use alone. Some bioinsecticides were slow to act on larvae. The result of their use was not reflected in direct mortality, but in the reduction of larval feeding, which still provides adequate protection of potato leaves from damage.

For wireworm control, the results of testing different preparations based on entomopathogenic fungi (EPF) of the genus Metarhizium for wireworm control showed that EPF in granular form (especially ATTRACAP and the fungal formulation on rice) resulted in a reduction in the percentage of damaged tubers compared to planting potato tubers soaked in a fungal suspension. This was particularly evident at the site where wireworm pressure was highest. Therefore, introducing or enhancing EPF in the rhizosphere with the fungal formulation on rice (i.e. bioaugmentation) and using the attract-and-kill method with ATTRACAP can reduce the percentage of damaged tubers.

Molecular markers for late blight, viruses, nematodes and carotenoids were investigated in the ECOBREED working collection of 65 cultivars. Marker-assisted selection for late blight and virus resistance was carried out, new resistant progenies were created and advanced potato clones were selected in the IHAR, KIS and MATE breeding programmes during the project. 36 advanced breeding clones were offered to partners for exchange within MS 17.

Two new potato cultivars have been registered in KIS in the last three years: KIS Blegoš and KIS Tamar. At MATE, a new candidate cultivar called Balatoni sárga has been developed and will be registered in 2024. Four advanced KIS potato clones suitable for organic production, have been included in national VCU trials in the last three years: KIS 10-242/247-6, KIS 11-184/257-1, KIS 13-136/235-5 and KIS 14-136/256-26.

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1. Screening of genetic resources and breeding material

Many studies have shown that yields in organic production systems are lower than those in conventional. However, the yield gap varies between crop species, with tuber crops having a greater yield gap than cereals. For example, De Ponti et al. (2012) report that organic tuber crop production averages 70% of conventional in European studies, but with high variability (37–114%). The relatively large, variable yield gap between the two systems of potato production has been mainly attributed to poor control of foliar diseases in particular late blight (*Phytophthora infestans)* and limited fertilisation potential (Finckh et al., 2006; Van Delden, 2001, Haase et al., 2007). Breeding of widely adapted crop cultivars requires testing in multi-environment field trials accompanied by (G×E) interactions. Growing the potato working collection across 4 sites of contrasting pedo-climatic conditions under organic management allows the potential for selection of cultivars suited to a specific environment. However, the associated phenotyping through different generations has become a major limiting factor in the overall process due to extensive visual assessments, data recording etc. on millions of plants in the field. Developments in phenotyping have failed to keep up with those in breeding in particular molecular breeding. There is therefore an urgent need to develop and validate Advanced Phenotyping approaches using digital, thermal and hyperspectral imaging in terms of when, where and how these can be utilised. UNEW and KIS have developed high throughput phenotyping methods which enables the comparison with conventional phenotyping approaches. The evaluation using Advanced Phenotyping approaches in both the UK and Slovenia will enable an understanding of the potential that digital imaging will offer to the evaluation of crop breeding material in the future.

A key aim of the ECOBREED project is to increase the availability of seeds and cultivars for the organic and low-input sectors whereby multi-site testing across several years and pedo-climatic conditions is important.

1.1. Materials and methods

The ECOBREED potato working collection (65 cultivars) was planted for phenotyping at four locations (SI, HU, PL and UK) in the 2019-2022 seasons.

Phenotyping trials were planted at KIS, UNEW, MATE and UP in plots of 30 tubers (15 tubers per row with 2 rows) with 2 replicates of each cultivar in Poland; 3 replicates in the UK and Hungary and 2 replicates in 2019 with 3 in 2020 and 2021 in Slovenia. Organic farming management practices were used in all trials. Trials were harvested generally in September following which all potatoes were placed in store, weighed and graded manually followed by tuber quality and tuber disease assessments.

Advanced phenotyping data collection in the UK and Slovenia was carried out using an unmanned aerial vehicle (UAV) equipped with a multispectral camera from 2020 to 2022.

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1.2. Results

1.2.1. Yield and yield characteristics

Analysis was carried out for the common years i.e. 2020 and 2021 for all sites but using a limited number of traits i.e. tuber yield, tuber dry matter content and tuber grading. As different grading systems were used in the different countries the data for this was aggregated to form 3 tuber size categories i.e. small <45, medium 45-65 and large >65 mm to be able to carry out cross site statistical analysis. When averaged across all cultivars and countries tuber yield (Table 1) was higher in 2020 than in 2021 (24.1 vs 21.8 t./ha respectively). Yield was highest in the UK (22.3 t/ha) and lowest in Hungary (15.9 t/ha). The tuber dry matter content matched the tuber yield in that it was higher in 2020 than 2021 and it was also highest in the UK (22.3%) and lowest in Hungary (15.6%). Poland was characterised by having a large proportion of small tubers (0.49 in the <45mm category) compared with only 0.07 and 0.09 in Slovenia and the UK respectively. Slovenia had the highest % by weight of large tubers>65mm with 41% compared with only 12% in this category in Poland.

Table 1 Means (±se of the mean) for tuber yield, dry matter content and the proportion of tubers (<45, 45-65 and >65mm) across the 4 sites in the growing seasons 2020 and 2021.

	Yield t/ha	**DM %**	**Tuber grading**		
			<45	**45-65**	**>65**
Year					
2020	24.1±0.46	19.4±0.15	0.19±0.009	0.55±0.008	0.26±0.008
2021	21.8±0.54	18.3±0.16	0.40±0.010	0.42±0.009	0.18±0.013
Country					
UK	21.8±0.48 b	22.3±0.15 a	0.33±0.014 b	0.61±0.011 a	0.07±0.006 d
Poland	33.2±0.69 a	17.0±0.14 c	0.12±0.006 c	0.39±0.007 d	0.49±0.011 a
Slovenia	21.3±0.72 b	20.6±0.15 b	0.41±0.020 a	0.50±0.016 b	0.09±0.008 c
Hungary	15.9±0.40 c	15.6±0.16 d	0.33±0.015 b	0.45±0.010 c	0.22±0.011 b
Year (Y)	<0.001	<0.001	<0.001	<0.001	<0.001
Country (C)	<0.001	<0.001	<0.001	<0.001	<0.001
Genotype (G)	<0.001	<0.001	<0.001	<0.001	<0.001
Y x C	<0.001	<0.001	<0.001	<0.001	<0.001
Y x G	<0.001	<0.001	<0.001	<0.001	<0.001
C x G	<0.001	<0.001	<0.001	<0.001	<0.001
Y x C x G	<0.001	<0.001	<0.001	<0.001	<0.001

Means for each trait within a column followed by the same letter are not significantly different (p<0.05)

1.2.2. Resistance to late blight

From the detached leaflets test, the strongest infections of tested cultivars were observed in 2021. Mean values for this test were 2.0. In years 2019 and 2020 mean infection was weaker (4.7 and 6.8 respectively) (Fig. 1). In 2019 the isolate MP 324 was used, the virulence of this isolate was as follows: R1, R2, R3, R4, R6, R7, R11. In this year 11 cultivars were not infected i.e. Carolus, Levante, Twister, Otolia, Tinca, 12-LHI-6, Delila, Kelly, KIS Kokra, Sarpo Shona and Sarpo Mira. Mean values from 1.0 for cv Riviera to 8.9 for cv

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Twinner were obtained. In 2020 the isolate MP 324 was used. The virulence of this isolate was as follows: R1, R2, R3, R4, R6, R7, R11. In 2020 13 cultivars were not infected i.e. Carolus, Delila, Gatsby, KIS Kokra, Nofy, Omega, Sarpo Mira, Sarpo Shona, Tajfun, Tinca and Twister. For other cultivars mean values ranged from 1.2 for cv Riviera to 8.8 for Mayan gold, Gardena and Goldmarie NN. In 2021, the isolate 136/20 was used. The virulence of this isolate was as follows: R1, R2, R3, R4, R6, R7, R11. In 2021 the most resistant cultivars were Sarpo Mira (8.8), Alouette (8.7), Twister (8.5), Nofy (7.3), Carolus (6.2) and KIS Kokra (6.0). For other cultivars in the ECOBREED working collection the level of resistance ranged from 1.0 to 5.5.

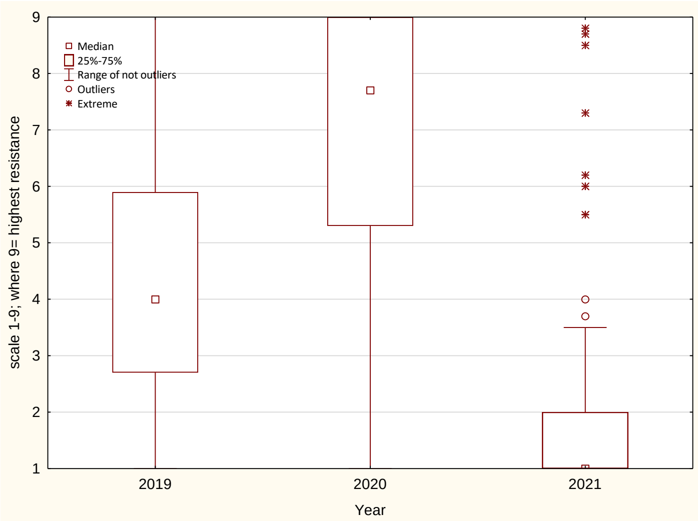

Fig 1 Results for detached leaflets test in individual years (POL 2019–21).

<u>IHAR Field tests (Poland)</u>

From the late blight field evaluation, the mean rAUDPC values for the most resistant cultivars evaluated in the 2020 and 2021 trials are presented (Table 2). The overall mean rAUDPC for the 65 cultivars evaluated in Boguchwała was 0.596 in 2020 and 0.259 in 2021. The highest mean rAUDPC for the two-year trial was recorded for Colomba (rAUDPC = 0.999) and the lowest for Carolus (rAUDPC = 0.002). For four cultivars, i.e. Kelly, Levante, Sarpo Mira, Twister and one clone 12-LHI-6, no late blight development was recorded in the two-year trial (Table 2). Late blight development was stronger in 2020 than in 2021 (Fig. 2).

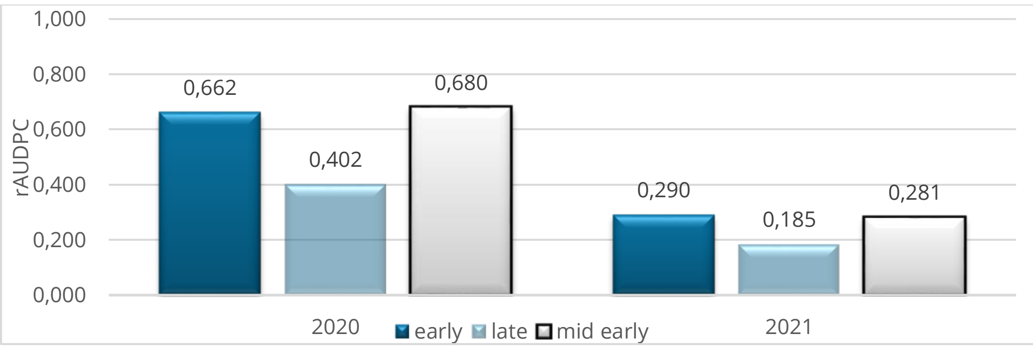

Fig 2 Mean values of rAUDPC for three maturity groups in Boguchwała (POL 2020–21).

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Table 2 Mean values of rAUDPC for the most resistant potato cultivars following a two-year field experiment (2020–21) in Boguchwała.

Cultivar	rAUDPC 2020	rAUDPC 2021	Mean rAUDPC 2020 and 2021
12-LHI-6	0.000	0.000	0.000
Kelly	0.000	0.000	0.000
Levante	0.000	0.000	0.000
Sarpo Mira	0.000	0.000	0.000
Twister	0.000	0.000	0.000
Carolus	0.004	0.000	0.002
Otolia	0.005	0.014	0.010
Tinca	0.005	0.000	0.003
Sarpo Shona	0.008	0.000	0.004
Tajfun	0.009	0.008	0.009
KIS Kokra	0.011	0.013	0.012
Nofy	0.014	0.000	0.007
Delila	0.015	-	0.015
Owacja	0.018	0.250	0.134
Bzura	0.019	0.002	0.011
Magnolia	0.026	0.300	0.163
Twinner	0.028	0.024	0.026
Valor	0.039	0.255	0.147
Alouette	0.085	0.000	0.043
Edony	0.110	-	0.110
Gardena	0.122	0.000	0.061

1.2.3. Advanced phenotyping

1.2.3.1 Slovenia

The F1 score obtained as the number of cultivars increased (Fig 3), showing a consistent decreasing trend. The highest performance, indicated by an F1 score of 0.971, was observed in distinguishing between two cultivars. Conversely, the lowest score of 0.476 was obtained when identifying among eight cultivars. Precision and Recall values closely aligned with the F1 score, suggesting that the model have not disproportionately favoured any specific cultivar, maintaining a balanced performance across different cultivars.

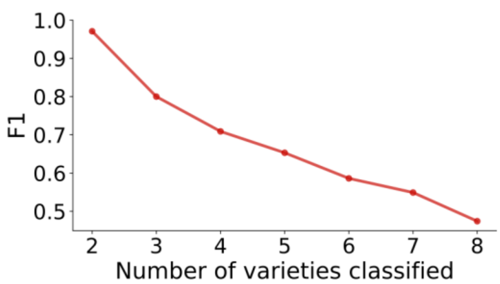

Fig 3 F1 score computed with respect to the number of cultivars using classified model.

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Regression metrics for various physiological targets are presented (Table 2). Each row corresponds to a distinct physiological value, with the "Target" column indicating the specific value under consideration. Metrics, computed in absolute values, show varying error magnitudes across physiological values. By comparing testing dataset RMSE with cross-validated training set RMSE – CV, it's observed the model avoids overfitting during optimization. Predictions consistently outperform a random base model, with R2 values exceeding 0.306. Notably, SPAD achieves the most accurate prediction with an R2 of 0.561 and RMSE of 0.403.

Table 3 Estimation of physiology values. The "Target" column specifies the specific physiology value for which the corresponding metrics are presented. The RMSE - CV column showcases the performance score attained through model optimization using cross-validation (CV).

Target	MAE	ME	R^2	RMSE	RMSE - CV
SPAD	0.315	1.381	0.561	0.403	0.443
GSW	0.044	0.247	0.306	0.062	0.067
E	0.978	4.529	0.366	1.284	1.366
PhiPS2	0.078	0.277	0.388	0.099	0.102
ETR	41.736	214.535	0.496	55.257	53.703

The metrics for predicting Alternaria fungi presence are presented (Table 4). Model performance was assessed individually for each cultivar and collectively for the combined set to observe adaptability. Pooled results yielded an F1 score of 0.825, indicating no overfitting towards any specific class (healthy or infected). Levante had the most favourable result (F1 score of 1.000), while Alouette showed the least favourable performance (F1 score of 0.620). Metrics may be skewed due to class distribution differences, like in the cultivar KIS Kokra, where relatively high values were noted.

Table 4 Disease detection. Binary classification scores for discerning between heathy and infected potato plants. The F 1 - CV column showcases the performance score attained through model optimisation using cross-validation (CV).

Cultivar	Accuracy	F1	Precision	Recall	F1 - CV
Alouette	0.620	0.630	0.660	0.620	
Carolus	0.880	0.880	0.910	0.880	
KIS Kokra	0.880	0.880	0.920	0.880	
KIS Tamar	0.750	0.750	0.750	0.750	
Levante	1.000	1.000	1.000	1.000	
Pooled	0.825	0.825	0.825	0.825	0.876

The significance of features, extracted from multispectral image channels, was assessed using SHAP values, with larger values indicating greater feature importance. Discriminative features for distinguishing cultivars (Fig. 4) manifest as combinations of spectral channels, with no singular raw spectral channel ranking among the top 10 features. Notably, three spectral indices—CIRE ((NIR / red edge) – 1), CVI ((NIR * red) / (green ** 2.0)), and BCC (blue / (red + green + blue))—provide the most significant information. Additionally, fundamental mathematical formulations, like the combination of NIR and red edge channels, contribute supplementary information. It is essential to

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note that the sequence of relevant features may exhibit bias due to sub-optimal classification when discerning all eight cultivars, resulting in a modest F1 score of 0.474. Regardless, the SHAP analysis demonstrated that more information is provided by the combination of features (derived from two or more spectral channels) compared to the utilisation of raw reflectance values.

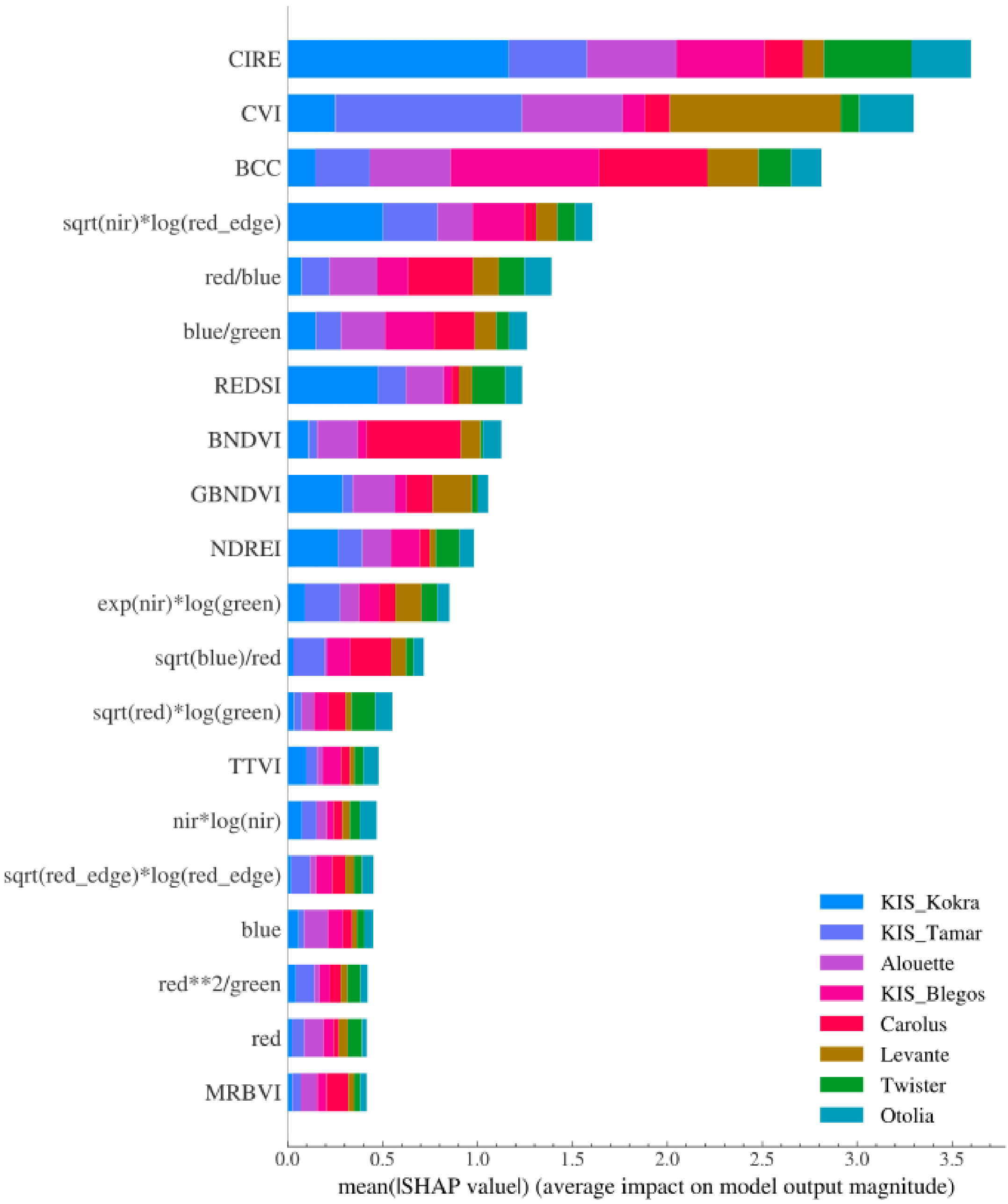

Fig 4 SHAP bar plot of the most relevant features for cultivar classification. Larger values indicate features that contribute more information and significance. Each bar's colour corresponds to the contribution of the respective cultivar.

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1.2.3.2 United Kingdom

Phenotypic data collection of potato field crops was carried out using Unmanned Aerial Vehicle (UAV) equipped with a Micasense Rededge MX multispectral camera designed to capture spectral information across the blue, green, red, red-edge and near-infrared wavelengths. Aerial images of the potato field were captured from a height of 30 meters with an 85% overlap between adjacent images. This strategic approach to image acquisition enhances the overall data quality and facilitates a more comprehensive analysis of the potato crops. Post-acquisition, a standardised set of processing procedures was implemented using the PIX4DMapper. This comprehensive process encompassed georeferencing, orthorectification, radiometric correction, conversion to reflectance units, and the creation of mosaic representations. These meticulous steps were undertaken to ensure the generation of precise, calibrated multispectral data essential for subsequent analytical tasks (e.g. evaluate plant count, canopy area, plant height etc).

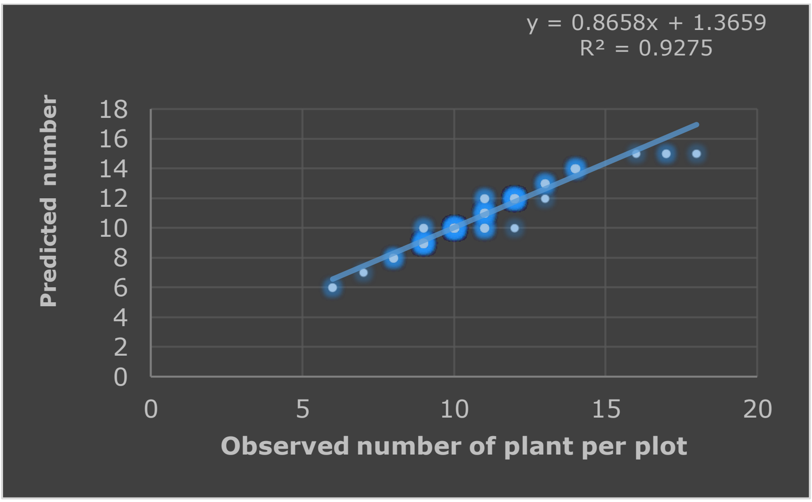

Fig 5 Comparison of plant count predicted using UAV flights with observed number of plants per plot.

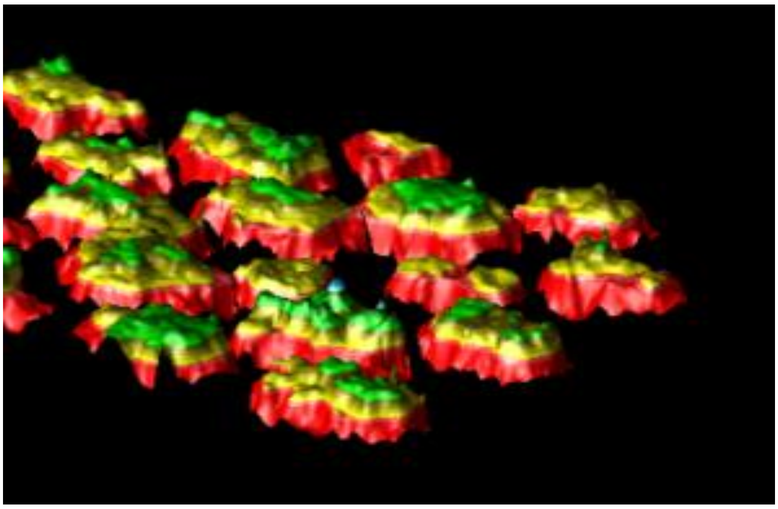

Fig 6 Example of a potato 3D model for extracting potato canopy characteristics (e.g height, canopy cover).

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Accurate plant count, vital for seed quality assessment was achieved using the fieldCount function in fieldimageR. Leveraging image segmentation from the EBImage package, the employment of distance and watershed transformations gave good accuracy in our data as shown (Fig. 5) Despite the relative simplicity, plant height and other morphological canopy characteristics can be labour- and time-consuming for large research datasets like ours. We employed an image analysis alternative to estimate plant height and other canopy parameters by creating Digital Surface Models during potato growth cycle (Fig. 6 and Fig. 7).

The data from the multispectral images was also used to calculate vegetation indices (VI) which are mathematical combinations of spectral bands and were used to highlight specific properties of vegetation. These indices help in monitoring plant heath, vigour, and stress as well as for estimating canopy area, biomass and chlorophyll content which are indictors of photosynthetic activity and in this project, we could extract data for each and every pixel to get a refined overview and hence these datasets were used in disease and yield modelling. The vegetation indices used for data analysis are summarised in Table 5 and an overview of field variability and extracts of index interaction with cultivar over the growth period (stolon initiation and tuber initiation) is presented in Figure 7 (only presenting mean datasets due to representation). The variability between cultivars increases as we go towards tuber filling stage and there is significant decrease in NDRE and NDVI values at the tuber filling stage most probably prompted by an increase in stress scenarios in the field as evaluated later.

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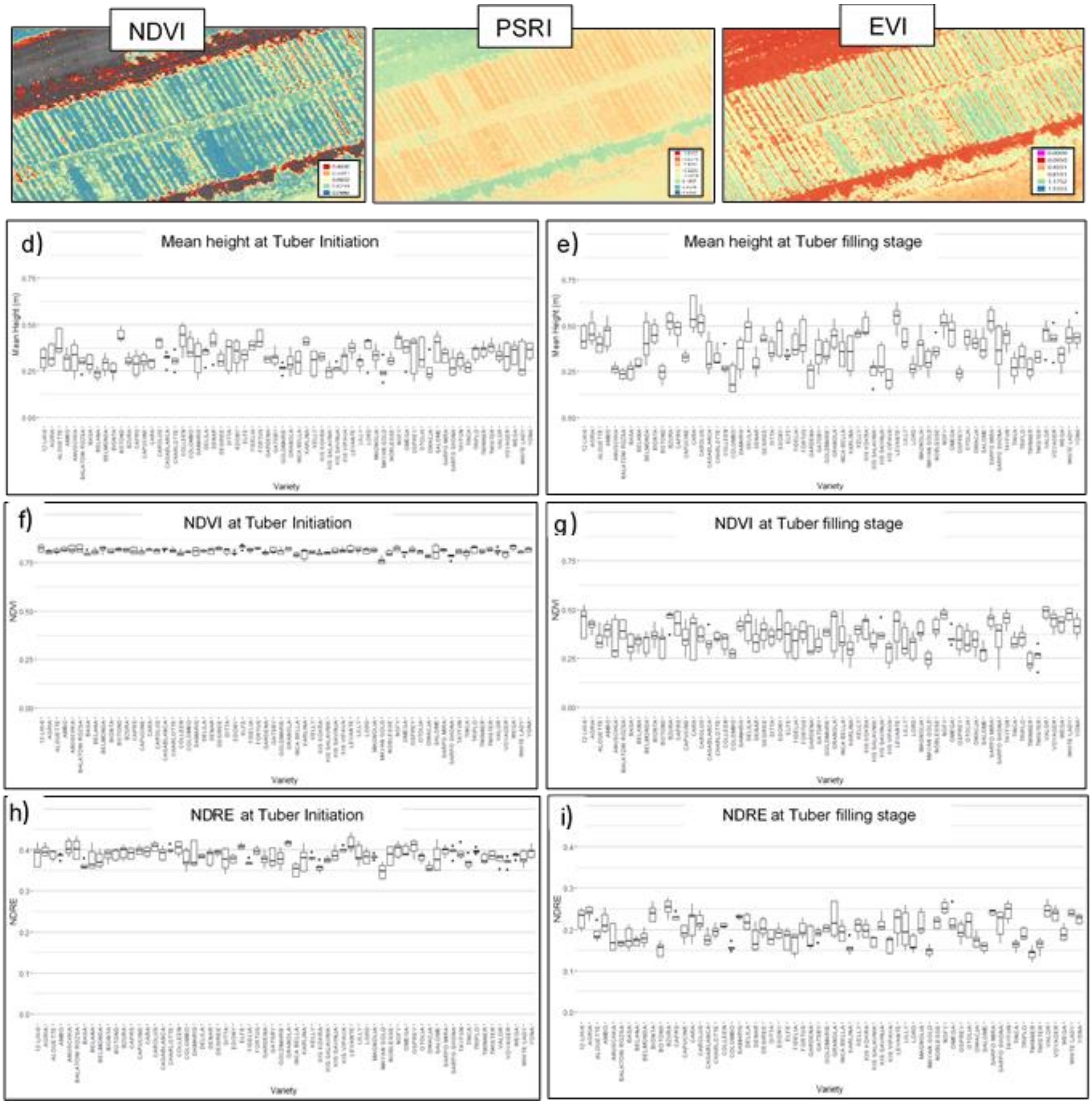

Fig 7 Field variability for different varities for 3 different indices from UAV multispectral data (a,b,c). UAV data from 2021 showing variability in morphological and spectral characteristics (a,b) NDVI at stolon initial and tuber initiation stage (c,d) NDRE at stolon initiation and tuber initiation growth stage and (e, f) Mean plant height at stolon initiation and tuber initiation growth stage.

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Table 5 Vegetation indices extracted from the multispectral images with formula and linked.

Vegetation index	Formulae	Related traits
NDVI (Normalised Difference vegetation index)	(NIR-R)/(NIR+R)	Chlorophyll, LAI, biomass
NDRE (Normalise Difference Red-Edge)	(NIR-RE)/(NIR+RE)	Chlorophyll
GNDVI (Green Normalised Difference vegetation index)	(NIR-G)/(NIR+G)	Chlorophyll, LAI, nitrogen content
BI (Brightness index)	sqrt((R^2+G^2+B^2)/3)	Vegetation coverage, water content
GLI (Green Leaf index)	(2*G-R-B)/(2*G+R+B)	Chlorophyll
VARI (Visible atmospherically resistant index)	(G-R)/(G+R-B)	Canopy, biomass
PSRI (Plant Senescence reflectance index	(R-G)/RE	Nitrogen, Chlorophyll, maturity
RVI (Ratio vegetation index)	NIR/R	Biomass, water content
TVI (Triangular vegetation index)	05*(120*(NIR-G)-200*(R-G))	Green Lai, canopy
CVI (Chlorophyll vegetation index)	(NIR*R)/(G^2)	Chlorophyll
EVI (Enhanced vegetation index)	2.5*(NIR-R)/(NIR+6*R-7.5*B+1)	Chlorophyll, biomass, nitrogen
CIG (Chlorophyll index – green)	(NIR/G)-1	Chlorophyll
CIRE (Chlorophyll index – Red edge)	(NIR/RE)-1	Chlorophyll
DVI (Difference vegetation index)	NIR-RE	Nitrogen, Chlorophyll

Yield prediction

The correlation matrix (Fig. 8) shows that the canopy characteristics and some indices show highly significant correlations with yield data especially in 2020 and 2022, whereas in 2021 although the relationship with yield is not significant the canopy characteristics are significantly correlated with some vegetation indices. To judge which parameters will be best suited to evaluate yield prediction, the random forest regression model was used with a test and train dataset split ratio of 80:20.

In 2020 using all the parameters the R-squared value was 0.412 with MSE = 0.951, RMSE = 0.975, MAE = 0.794. The model was improved when the top two best parameters including NDVI and canopy area was used giving R-squared value of 0.794 with MSE = 0.333, RMSE = 0.577 and MAE = 0.444. Hence the model indicates that NDVI is the most important variable in the model, followed by canopy area and average canopy height.

In 2021, using all the parameters the R-squared value was 0.541 with MSE = 3.501, RMSE = 1.871, MAE = 1.465. The model was improved when the top four best parameters including RVI, NDVI, EVI and GNDVI was used giving a R-squared value of 0.893 with MSE = 0.816, RMSE = 0.904 and MAE = 0.679. Hence the model indicates that NDVI is still one of the most important variables followed by other indices.

In 2022, from the correlation matrix average plant height from UAV flight 2 (Tuber initiation) showed the highest correlation followed by canopy area and maximum plant height with yield with correlation values of 0.739, 0.724, and 0.651, respectively. The Random Forest regression model highlighted that using all the parameters the R-squared value was 0.645 with MSE = 0.295, RMSE = 0.543, MAE = 1.465. The top parameters in this model were linked just to canopy area and canopy height excluding any indices.

Although the models created over three years gave a slightly different picture for the parameters linked to yield prediction but there is an overlap for canopy cover and height

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in 2020 and 2022 while NDVI exists in the top model for 2020 and 2021. Different factors might play a role in this and hence inclusion of proximal data, weather data and combining datasets for all years will help us refine this model.

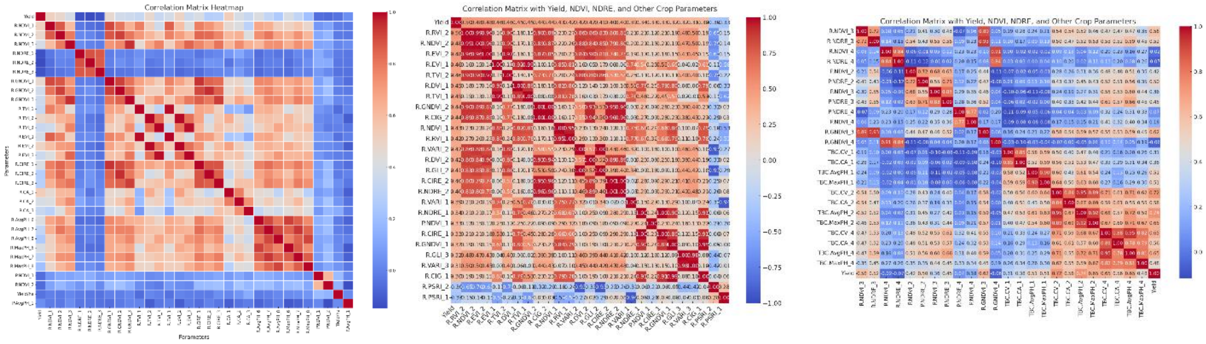

Fig 8 Correlation matrix highlighting correlations between canopy characteristics, spectral data and yield parameters for 2020, 2021 and 2022 respectively.

<u>Disease score classification in cultivars using UAV data</u>

Vegetation Indices (VI), canopy area (CA) and plant height (PH) extracted from multispectral UAV imagery were used to classify disease score gradings using random forest classification model for the 2020 and 2021 datasets incorporating 200 decision trees and a train test ratio of 70:30. Data collected at tuber bulking stage is presented using selected indices (NDVI, NDRE, GNDVI, TVI, EVI and CIRE) for 2020 and (NDVI, NDRE, GNDVI, BI, GLI, VARI, BGI, PSRI, RVI, TVI, CVI, CIG, CIRE and DVI) for 2021 classification. The reasoning being that the best accuracy to classify disease score was linked to tuber filling stage in 2020 and 2021 with an accuracy of 72% and 63% respectively. Slightly less accuracy for 2020 is probably due to the limitation in indices used in comparison to 2021 (Table 6).

Gini impurity, a measure of how frequently a randomly selected element is classified incorrectly was used in decision trees as a criterion for splitting nodes, where a node with a lower Gini impurity was regarded as purer. The average of these nodes or their improvement over all the forest's trees was then used to determine MeanDecreaseGini, suggesting that higher a variable's MeanDecreaseGini, the more significant that variable is in making accurate predictions.

Table 6 Accuracy, specificity and sensitivity of the random forest classification model.

Year	Accuracy	Precision	Sensitivity
2020	63 %	52 %	51 %
2021	72 %	60 %	75%

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Hence, it can be seen (Fig. 9) the most significant parameter for both 2020 and 2021 dataset is plant height (PH) followed by different indices which contributes to the random forest classification for disease scores.

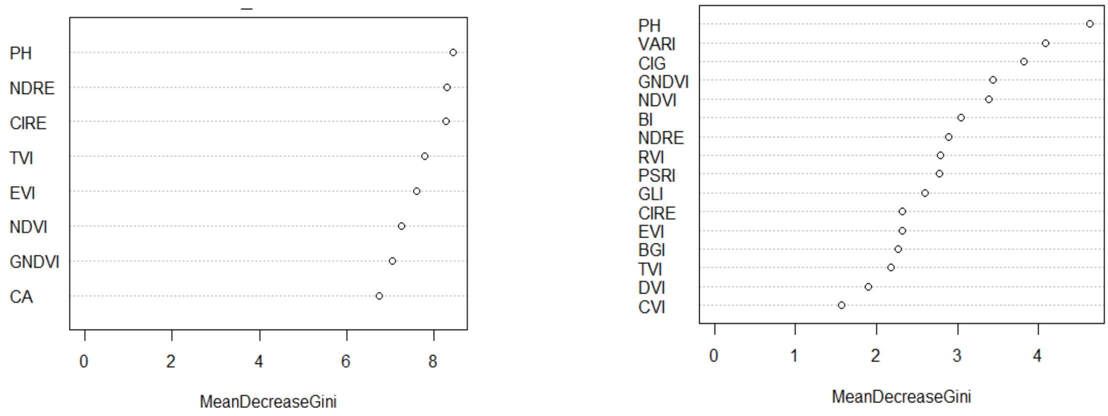

Fig 9 Variable ranking for classifying disease sore (left: 2020 dataset and right: 2021 dataset).

1.3. Conclusions

There were clear consistencies and differences in the results obtained between the four sites i.e. Levante was the highest yielding cultivar in all seasons in the UK but in Slovenia it was the 11th highest yielding when averaged across the 3 growing seasons. The top 3 performing cultivars in each country when averaged across the three growing seasons were all different. In contrast at the other end of the yield spectrum Mayan gold (diploid cultivar) had the lowest tuber yield in all seasons in the UK and Poland and the 2nd lowest yield when averaged across all seasons in Slovenia. However, in Hungary Mayan gold had an overall tuber yield of 15.33 t/ha compared with an overall mean of 16.48 t/ha across all 67 cultivars and growing seasons.

Palmer et al (2013) examining production constraints between different farming systems in a long-term field experiment at Nafferton Farm showed that organic potato yields were 56.5% of those in the 'conventional' system over a six-year study period (2004-09). Average tuber yields in this study varied from 22.3 t/ha in the UK to 15.9 t/ha in Hungary when averaged across all cultivars and growing seasons.

Including site as a factor in the analysis for a limited number of traits showed clear variation between sites in tuber yield, DM% and tuber grading. Poland produced the highest tuber yields and Hungary the lowest when averaged across all cultivars in the 2020 and 2021 seasons. The higher tuber yields in Poland were also supported by a higher proportion of large tubers in the >65 mm category.

Hagman et al (2009) evaluated 17 potato cultivars for suitability to organic production and observed that early maturing cultivars had greater chance to achieve significant tuber yield before the crop was infected by *Phytophthora infestans.* However, a significant interaction between maturation type and susceptibility to late blight has been identified (Visker 2005; Razukas et al. 2008) with early maturing cultivars often being more

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susceptible. This was observed in the present study, where early maturing cultivars were often the first to develop late blight, but the highest yields were achieved by both late and early maturing cultivars. Of the top 3 highest yielding cultivars in each country, it is only Levante in the UK and Michalina in Poland which were Early maturing all the others were either of Late or Intermediate maturity.

In advanced phenotyping top 3 performing cultivars in terms of tuber yield in each country were completely different but there were greater similarities at the lower end of the tuber yield spectrum.

Using AMMI modelling of the Poland dataset showed that genotype contributed 44.9 % of the observed variability with the environment 13.9% and the G x E interaction 31.2%.

Collection of Advanced Phenotyping data in the UK and Slovenia was carried out using Unmanned Aerial Vehicle (UAV) equipped with a multispectral camera. The resulting correlation matrix shows that the canopy characteristics and some indices show highly significant correlations with yield data especially in 2020 and 2022**.**

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2. AMF – compatibility screening

Some crop species more readily form mutualistic associations with mycorrhiza such as wheat and other cereals, whereas potato plants generally form weaker associations and are less important for plant growth (Davies et al., 2005). Differences in indigenous AM colonisation level among cultivars of potato have been reported before (Senes-Guerrero et al., 2014; Alaux et al., 2018), whereby selecting for this has the potential to reduce P fertiliser inputs and the environmental consequences of increased P supply to water systems.

2.1. Materials and methods

A 20-cultivar potato AMF screen was set up using soil taken from the field. The soil was taken from the 2020 ECOBREED potato phenotyping trial located at Nafferton Farm and sieved prior to transportation to the greenhouse. No external inoculum was used but the experiment is reliant on natural AMF in the soil. 1 tuber of the same 20 cultivars were planted on 1 March 2021. In addition, two cultivars (Cara and Casablanca) were used as controls with sterilised soil (48 hrs in the oven at 105 ^{o}C). The trial was harvested on the 18 May 2021 when soil was washed from the roots. Fresh and dry weight of root and shoot fractions were recorded and in addition a sample of fine roots from each pot was retained and transferred into 50 ml Falcon tubes with 50 % ethanol for AMF analysis. Root samples were stored at 4 °C in darkness prior to staining. The root staining process followed the protocol of the "Research institute of organic agriculture" (FiBL), Frick, Switzerland, modified from Vierheilig et al. (1998). The magnified intersection method by Mcgonigle et al. (1990) was used to assess mycorrhizal colonisation. Total colonisation is separated into the three structures, vesicles, hyphae and arbuscles.

2.2. Results

Except for the cultivar Salome, mycorrhizal intensity in all cultivars was significantly greater than the two control cultivars Cara c and Casablanca c (Table 7). Cara C recorded nominal values for most mycorrhizal parameters which indicates potential contamination during sterilisation of the soil. Sarpo Mira (late maturity) recorded the highest M% for all cultivars (1.4 % ± 0.4) and was significantly greater than all the other late cultivars. For medium maturity cultivars, Gatsby recorded the highest M% (0.9% ± 0.29) and Ambo was the highest for the early cultivars (0.6 ± 0.12) (Fig. 10). Frequency of mycorrhiza in the root system (F%) showed a similar pattern to mycorrhizal intensity Sarpo Mira, Gatsby and Ambo recorded the highest number for their respective maturity groups Late (51.3 % ± 4.78), Medium (40 % ± 2.36) and early (42 % ± 6.11). The Lowest value for mycorrhizal abundance barring the control cultivars (Cara and Casablanca) was again Salome (2 % ± 1.33), which was not significantly different to the Cara control (Table 7).

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Table 7 AMF colonisation of the potato cultivar screen.

Cultivar	F%	M%	m%	a%	A%	v%	V%	h%	H%	Ar%	Vr%	Hr%
Ambo	42±6.11	0.6±0.12	1.4±0.14	39.7±4.92	0.2±0.05	4.1±0.64	0±0.01	75.8±3.11	0.5±0.09	0.2±0.04	0±0.01	0.4±0.08
Casablanca	26.1±4.09	0.3±0.05	1.1±0.09	32.6±8.04	0.1±0.04	3.2±0.95	0±0	80.3±5.06	0.2±0.03	0.1±0.03	0±0	0.2±0.02
Casablanca c	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Colleen	16.8±2.96	0.2±0.08	1.4±0.23	29.7±8.65	0.1±0.04	0.3±0.33	0±0	82.9±5.67	0.2±0.07	0.1±0.03	0±0	0.2±0.06
Salome	2±1.33	0±0.01	0.5±0.29	5±2.89	0±0	0±0	0±0	47.4±27.39	0±0.02	0±0	0±0	0±0.01
Twister	25.3±5.01	0.4±0.12	1.5±0.33	29.6±3.59	0.1±0.03	2.7±0.44	0±0.01 fi	82.6±2.04	0.3±0.1	0.1±0.03	0±0	0.3±0.09
Agria	36.7±4.08	0.4±0.08	1.2±0.13	40±6.1	0.2±0.05	4±1.36	0±0.01	75.5±3.6	0.3±0.05	0.2±0.04	0±0.01	0.3±0.05
Cara	40.2±3.05	0.6±0.12	1.4±0.21	40.7±3.89	0.3±0.06	3.9±0.27	0±0.01	75.3±2.46	0.4±0.08	0.2±0.05	0±0.01	0.4±0.06
Cara c	2.7±1.25	0±0.01 a	0.8±0.25	17.5±11.09	0±0 a	0±0 a	0±0	65.1±22.44	0±0.01	0±0 a	0±0 a	0±0.01 a
Inca bella	26±7.7	0.3±0.08	1±0	35.1±7.06	0.1±0.02	3.6±0.44	0±0	78.6±4.5	0.2±0.07	0.1±0.02	0±0	0.2±0.06
Sarpo Mira	51.3±4.78	1.4±0.35	2.8±0.74	50.4±9.89	0.8±0.32	3.7±1.74	0.1±0.04	67.6±7.71	0.9±0.14	0.7±0.24	0.1±0.03	0.7±0.1
Sarpo Shona	20.7±3.86	0.3±0.07	1.2±0.23	21.9±5.85	0.1±0.03	1±0.47	0±0	87.7±3.52	0.2±0.05	0.1±0.03	0±0	0.2±0.04
Valor	18.7±2.91	0.2±0.05	1.3±0.17	51.8±4.33	0.1±0.04	3.3±0.8	0±0	68±3.39	0.2±0.03	0.1±0.03	0±0	0.1±0.02
Caprice	37.3±7.26	0.4±0.1	1.1±0.06	38.3±5.5	0.2±0.05	2.3±0.67	0±0	77.4±3.61	0.3±0.07	0.1±0.04	0±0	0.3±0.06
Charlotte	16±3.86	0.2±0.04	1±0	38.5±7.01	0.1±0.02	1.6±0.92	0±0	77.2±4.51	0.1±0.03	0.1±0.02	0±0	0.1±0.03
Gatsby	40±2.36	0.9±0.29	2.3±0.57	45.9±5.91	0.5±0.19	5.9±0.78	0.1±0.02	70.9±3.97	0.6±0.18	0.4±0.15	0±0.02	0.5±0.14
Lilly	18±2.49	0.2±0.03	1±0	35.1±8.96	0.1±0.02	0.7±0.67	0±0	79.5±5.63	0.1±0.02	0.1±0.02	0±0	0.1±0.02
Osprey	21.1±5.76	0.2±0.07	1.1±0.1	31.2±8.07	0.1±0.04	5.8±1.64	0±0.01	79.9±5.4	0.2±0.05	0.1±0.03	0±0	0.1±0.04
Tajfun	27.3±3.4	0.3±0.03	1±0	37.8±4.32	0.1±0.02	0.3±0.29	0±0	78.6±2.81	0.2±0.03	0.1±0.02	0±0	0.2±0.02
12-LHI-6	50.7±8.26	0.8±0.21	1.6±0.21	55.9±4.48	0.5±0.13	3.3±0.64	0±0.01	65±3.45	0.5±0.13	0.4±0.1	0±0.01	0.4±0.1
Levante	19.3±3.86	0.2±0.04	1±0	39.6±5.48	0.1±0.02	1.9±0.93	0±0	76.7±3.49	0.1±0.03	0.1±0.02	0±0	0.1±0.02
ANOVA												
p value	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.001	0.000

Means followed by the same letter are not significantly different p<0.05 (Tukey HSD)

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Within maturity groups, there was considerable homogeneity of values for F and M values. For F%, early cultivars, Casablanca, Ambo, Colleen, Twister were statistically different to Casablanca Control and Salome but not significantly different to each other. Gatsby recorded notably higher intensity levels than all medium cultivars, with Caprice Charlotte Lilly osprey Tajfun all recording similar means. The late cultivars Agria, Cara, Inca Bella, Sarpo Shona and Valor all recorded similar means (Table 8).

Ambo Gatsby and Sarpo Mira again recorded the highest arbuscule abundance in the root system for their respective maturity groupings. Early cultivars were all statistically similar with exception of Ambo (0.2 ± 0.05) (Table 8). Medium maturity cultivars were all statistically similar except for Gatsby (0.5 % ± 0.18) (Table 29) and late maturity cultivars were all statistically similar with the exception of Sarpo Mira (0.83 ± 0.32) (p value <0.05). Vesicles and hyphae both followed the same distribution as arbuscules.

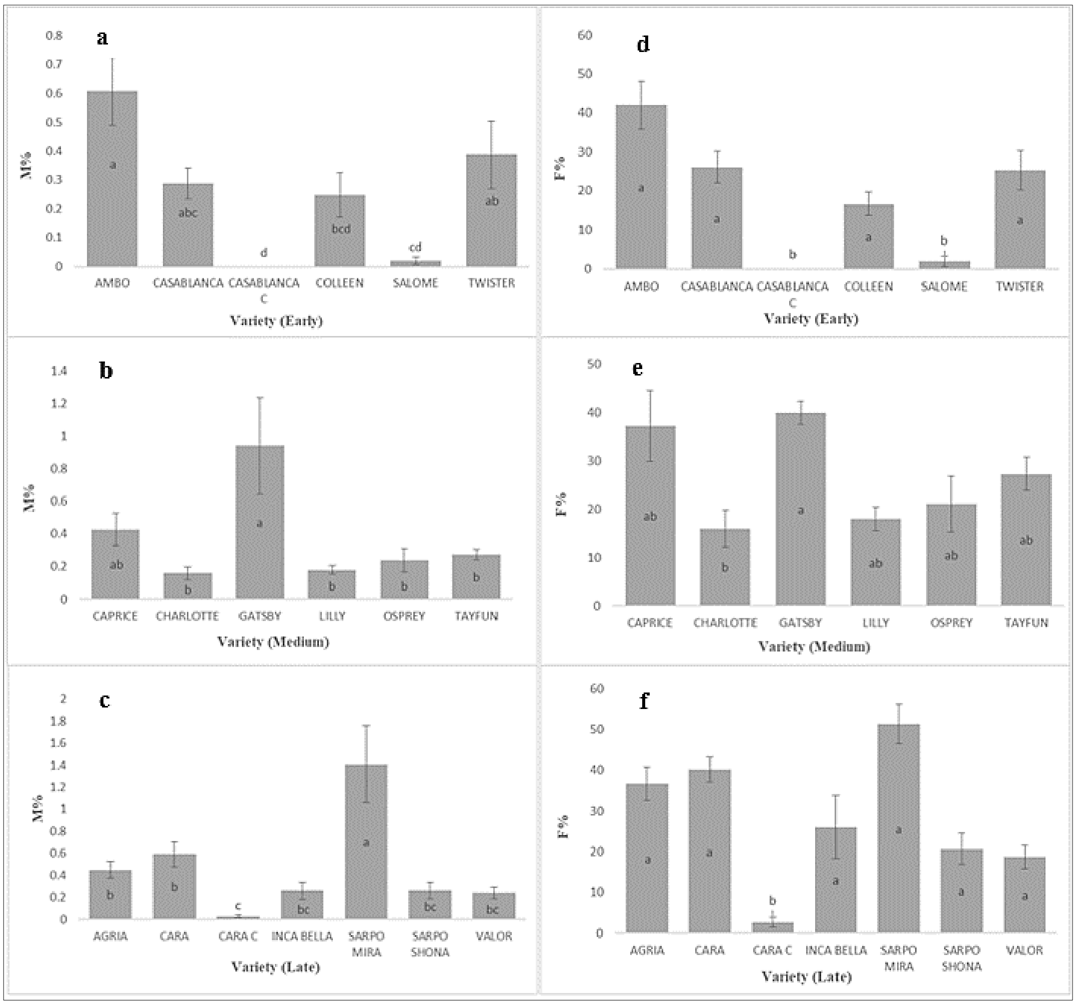

Fig 10 Frequency of mycorrhiza in the root system (M%) and intensity of mycorrhizal colonisation in the root system (F%) whereby; (a) M% for early cultivars, (b) M% for medium cultivars, (c) M% for late cultivars, (d) F% for early cultivars, (e) F% for medium cultivars and (f) F% for late cultivars. The same letter within each maturity group designates cultivars that are not significantly different according to the Tukey post hoc test ($P < 0.05$). Error bars represent ± standard error.

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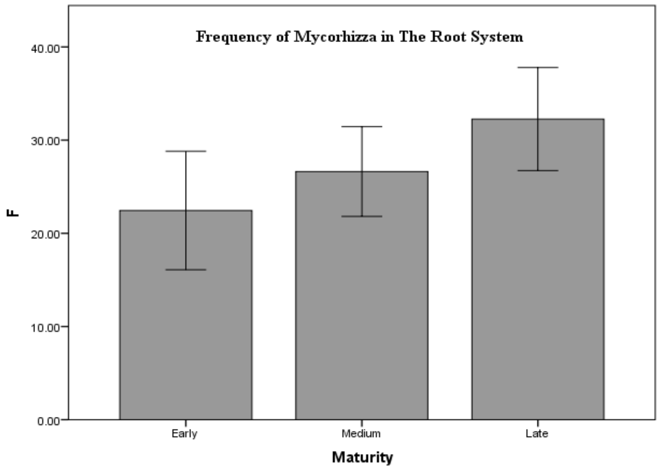

Fig 11 Frequency of mycorrhiza in the root system by maturity. Late cultivars were significantly different to early cultivars according to the Tukey post hoc test (P < 0.05). Error bars represent ± standard error.

Analysis of frequency of mycorrhiza in the root system by maturity group was also evaluated by the Tukey post hoc test which showed significant differences between the combined means of early and late cultivars (P - 0.042), though medium was not significantly different to either early or late cohorts (Fig. 11). Mycorrhizal intensity was also analysed to see if it varied between maturity groups, but no significant differences were found.

Mycorrhizal intensity and frequency in the root system were both negatively correlated with total dry weight (root + shoot) with respective p values (p<0.01. The correlations were relatively weak, as shown by the R2 linear values (0.068 and 0.161) and implies a negative effect of mycorrhizal association on plant growth (Figs. 27 & 28). Root and shoot biomass, root-shoot ratio and tuber number were also tested separately for correlation to M% and F% but were not significant.

2.3. Conclusions

The present study showed low levels of natural mycorrhizal colonisation across most cultivars, though some cultivars were significantly better than others at forming mycorrhizal associations. Sarpo Mira stood out as having the greatest mycorrhizal intensity and abundance across all cultivars. Sarpo Mira is a cultivar renowned for its high dry matter weight, owing to tall shoot growth and extensive root systems (Hagman et al., 2009). This combined with the cultivars' strong resistance to late blight make it a favourable cultivar for organic or low input systems (Rietman et al., 2012).

Ambo and Gatsby were standouts for their respective maturity groups for mycorrhizal intensity and abundance, which again did not materialise in greater plant growth responses compared to the other cultivars in their cohorts. Potato plants are not known for having high colonisation levels, however trace levels as low as 0.4% have been reported to improve growth (Niemera et al., 1995). Colonisation levels in this study were comparable to the afore-mentioned study, which again suggests an asymmetry of benefits for AMF and the plant host.

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3. Improving seed tuber quality and vigour via use of the cover crops

Cover crops are playing an increasingly important role in agricultural production with the potential for much greater future adoption and utilisation. In the past cover crops have generally been used to reduce soil erosion and nutrient loss and to provide a supply of nutrients particularly N to the following crop (Harramoto & Gallandt 2005). However, in recent years a move towards more regenerative production systems has seen an increased use of cover crops to provide living roots in the soil profile throughout the season with the potential to increase soil health. Many cover crops are grown in mixtures using brassica, cereal and legume species with differing benefits provided by the individual species.

For several years brassicas have been used as a cover crop due to their potential for soil biofumigation (Collins *et al*., 2006) which is particularly important in organic and low-input production systems where pesticide use is not allowed and/or restricted. There is also increasing evidence that cover crop species can be used to reduce pest and disease levels in potato. For biofumigation crops like Indian mustard *(Brassica juncea*) and oil radish (*Raphanus sativus*) with their rapid growth and ability to produce/release isothiocyanates have been well studied. Oilseed rape (*Brassica napus*) has also been shown to provide some control of early blight (*Alternaria solani*) (Runno-Paurson *et al*., 2019). Alfalfa (*Medicago sativa*) has been shown to reduce *Rhizoctania solani* severity by 50% in potato (Snapp *et al.,* 2005). This study looked at the potential for individual cover crop species to improve the growth and reduce the pest and disease levels in 4 cultivars of potato grown in Slovenia and the UK.

3.1. Materials and methods

Cover crop trials were set up at Nafferton Farm, Northumberland, University of Newcastle Upon Tyne, UK and in central Slovenia at Jablje, Kmetijski inštitut Slovenije in the 2020-21, 2021-23 and 2022-23 seasons.

Ccover crop trials was conducted with individual cover crop species, i.e. black mustard (*Brassica juncea* - 10 kg/ha), oil radish (*Raphanus sativus* - 15 kg/ha), lucerne (*Medicago sativa* - 25 kg/ha), black oat (*Avena strigosa* - 25 kg/ha) and common vetch (*Vicia sativa* - 25 kg/ha). A sixth treatment was included, which was a mixture of the 5 individual species drilled at a seed rate of 15 kg/ha. Treatment number 7 was the control with no cover crops (except for the 2021-22 season at UNEW).

In the UK the cover crop trial was ploughed in on 18th March 2022 with potatoes planted on the 14th of April. Tubers of 4 cultivars i.e. Carolus, Casablanca, Alouette and Cara were sourced from the UK with Carolus and Alouette also being grown in the corresponding trial in Slovenia. Four rows of each cultivar were planted with the inner two rows only being used for yield and tuber quality analyses. Pest and disesae assessments (early blight, late blight, Leaf roll and Virus Y) and in the field were carried out on 2nd, 11th and 18th of August 2022 with the crop being flailed on Tuesday the 23rd of August.

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The cover crop/potato trial was harvested between 8-12 September. Tuber yields were taken from the 2 middle rows . with , tuber number, dry matter content and tuber grading <25mm, 25-45mm, 45-65mm, 65-85mm and >85mm also determined. Disesae assessment in the field and of tubers was carried out according to the phenotypic descriptor list D3.1.

The second UK trial was planted on Wednesday 3rd August 2022 using the same design as in the previous season but with the inclusion of a control treatment (i.e. no cover crop). The remaining cover crop residues were ploughed into the ground on March 28 2023 prior to the land being cultivated on 17th April with potatoes being planted on Tueday 18th April. The potato trial was harvested on the 7th of Septemberwith foliar disease assessment (early blight only) was carried out on 10/07, 27/07 and 09/08 in 2023.

At KIS, the 6 treatments were drilled with a 1.25 m plot Wintersteiger drill (after the land had been ploughed and pressed about 4 weeks earlier) with dimensions of 6.25 x 50 m for each plot/species.

On 20 August 2020 the first cover crop trial was sown at KIS in IC Jablje. The establishment of cover crops in Slovenia was very good, so in 2021 the trial in Slovenia was ploughed in March and planted on 6th of May over the cover crops with 4 potato cultivars, i.e. Alouette, Carolus, KIS Tamar and KIS Kokra. Four rows of each cultivar were planted, with the inner two rows used only for yield and tuber quality analysis. Field disease assessments were carried out during the growing season. The trial was harvested on 26th of October 2021.

On 10th August 2021, a second cover crop trial was sown at KIS in IC Jablje. The establishment of cover crops in Slovenia was again very good, so in 2022 the trial in Slovenia was ploughed in March and planted on 12th April over the cover crops with 4 of the same potato cultivars as in the previous year, i.e. Alouette, Carolus, KIS Tamar and KIS Kokra. Four rows of each cultivar were planted, with the inner two rows used only for yield and tuber quality analysis. Field disease assessments were carried out during the growing season. The trial was harvested on 13th of October 2022.

Statistical analysis was performed in each year separately, as no control treatment was used in the 2021-22 trial.

3.2 Results

3.2.1. UNEW

3.2.1.1. Season 2021-22

Cover crop establishment was very good in the autumn of 2021 particularly the brassica species (black mustard and oil radish) but the black oat *Avena strigosa* showed low establishment. There was no symptoms of Virus Y at any of the assessment dates and for leaf roll virus only very low levels were detected and only in the cultivar Casablanca but with no effect of cover crop (data not presented). Following harvest tuber assessment showed no tuber irregularities with no tuber blight, scab or Rhizoctania evident on the tubers most likely due to the very dry conditions throught the growing season and at harvest in 2022.

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There were significant effects of potato cultivar (Table 8) on tuber yield (p<0.05), dry matter % (p<0.001), grading fraction 25-45 mm (p<0.01) and Rhizoctonia solani level (p<0.001). With respect to cover crop species there were significant effects non tuber yield (P<0.001), tuber DM% (p<0.001), grading fractions 25-45 mm (p<0.001) and 45-65mm (<0.05), eraly blight (p<0.001), late blight (p<0.001) and Rhizoctonia (p<0.05). There were no significant potatto cultivar × cover crop species interaction in 2021-22. When averaged across all cultivars and cover crop species Cara and the species mixtute produced the highest tuber yield. Conversely it was Cara and the species mixture which produced the lowest tuber dry matter %. Casablana showed the highest susceptability to early blight with Alouette and Carolus showing the highest susceptability to late blight.

For each poato cultivar it was different cover crop species that produced the highest tuber yield i.e. Lucerne in Alouette, the species mix in Cara, Oil radish in Carolus and Black oat in Casablana (Table 7). However Vetch resulted in the lowest tuber yield in Cara, Carolus and Casablanca with Black mustard producing the lowest yield in Alouette. Rhizoctonia levels were highest in Carolus and lowest in Casablana they were also highest following Vetch and lowest following Black mustard. There was much greater consisteny with respect to cover crop species on Rhizoctonia levels (Table 9) in that Black mustard produced the lowest Rhizoctonia levels in the avreties Cara, Carolus and Casablanca while the lowest levels in Aluette were followinhg Oil radish. The highest Rhizoctonia levels in Aluette, Cara and Casablanca were following Vetch while in Carolus it was following the cover crop species mixture (Table 10).

Table 8 Means of the effect of cover crop species and potato cultivar on the tuber yield, DM%, tuber grading, resistance to foliar disease and tuber disease levels at Nafferton Farm on 2021-22.

	Yield	DM		Tuber grading (mm)					EB*	LB*
	Rhizoc**									
	t/ha	%	<25	25-45	45-65	65-85	>85			
Cover crop (C)										
Lucerne	25.38	22.9	0	0.23	0.65	0.12	0	9.5	8.6	0.30
Black oat	24.31	22.8	0	0.23	0.72	0.04	0	9.4	8.7	0.46
Black mustard	23.00	23.5	0	0.25	0.70	0.04	0	9.6	8.5	0.14
Oil radish	24.11	23.1	0	0.24	0.70	0.06	0	8.5	8.5	0.35
Vetch	21.27	23.0	0	0.29	0.65	0.06	0	9.3	8.3	0.64
Species mix	26.02	21.6	0	0.20	0.72	0.08	0	9.3	8.6	0.45
Cultivar (V)										
Alouette	21.85	22.9	0	0.31	0.63	0.06	0	9.5	7.9	0.36
Casablanca	20.92	22.8	0	0.24	0.73	0.03	0	8.9	8.7	0.26
Cara	30.12	21.8	0	0.18	0.71	0.11	0	9.7	9.7	0.44
Carolus	23.16	23.8	0	0.24	0.70	0.06	0	9.9	7.9	0.48
Anova										
Cover crop	*	***	NS	**	NS	NS	NS	NS	NS	***
Cultivar	***	***	NS	***	*	NS	NS	***	***	*
C × V	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Resistance to Early blight (EB) and Late blight (LB) was assessed on a 1-10 scale where 1 = plants completely blighted (occasionally parts of stem not affected), 5= 50% of leaves infected and 10= occasional necrotic spots.

**Rhizoctania levels are presented as a proportion of total tuber weight

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Table 9 Tuber yields (t/ha) ± SE of the cover crop trial at Nafferton Farm in the UK in the 2021-22 season.

Cover crop species	Alouette	Cara	Carolus	Casablanca
Lucerne	24.01 ± 5.05	31.60 ± 2.81	24.85 ± 2.43	21.08 ± 1.25
Oil radish	21.26 ± 1.40	28.88 ± 0.88	24.89 ± 1.03	21.43 ± 1.89
Black mustard	20.23 ± 1.05	28.00 ± 2.08	24.27 ± 1.06	19.47 ± 1.33
Vetch	20.66 ± 0.13	27.00 ± 1.14	19.65 ± 1.31	17.78 ± 0.93
Black oat	23.75 ± 0.98	29.14 ± 0.63	21.21 ± 1.74	23.12 ± 1.60
Species mix	21.17 ± 4.04	36.12 ± 2.81	24.11 ± 2.86	22.67 ± 0.12

When averaged across all cover crop treatments the highest tuber yield was in Cara (30.12 t/ha) and the lowest in Casablanca (20.92 t/ha). When averaged across all four cultivars the tuber yield following the cover crop mixture was highest at 26.02 t/ha followed by Lucerne (25.38 t/ha) and the lowest following Vetch (21.27 t/ha). For the cultivars Cara, Carolus and Casablanca the lowest tuber yield was following Vetch but for the cultivar Alouette it was after Black mustard which was only 0.43 t/ha lower than Vetch. For Alouette the highest tuber yield occurred following Lucerne while for Cara it was after the mixture of cover crop species while for Carolus and Casablanca it was following Oil radish and Black oat respectively.

Table 10 Proportion of tubers infected with Rhizoctonia solani ± SE as influenced by potato cultivar and cover crop species at Nafferton Farm, UK in the 2021-22 season.

Cover crop species	Alouette	Cara	Carolus	Casablanca
Lucerne	0.46 ± 0.12	0.26 ± 0.15	0.39 ± 0.19	0.07 ± 0.04
Oil radish	0.16 ± 0.08	0.25 ± 0.20	0.61 ± 0.13	0.29 ± 0.17
Black mustard	0.06 ± 0.03	0.10 ± 0.01	0.28 ± 0.10	0.12 ± 0.02
Vetch	0.60 ± 0.06	0.88 ± 0.06	0.56 ± 0.15	0.52 ± 0.17
Black oat	0.53 ± 0.23	0.65 ± 0.06	0.35 ±0.03	0.30 ± 0.08
Species mix	0.38 ± 0.03	0.50 ± 0.13	0.67 ± 0.11	0.23 ± 0.10

3.2.1.2. Season 2022-23

In the following season despite being drilled in very dry conditions following a warm and dry summer at Nafferton cover crop establishment was good and with rain occuring in September followed by mild and good growing conditions a high level of crop biomass was achieved (Fig 12). Rapid growth in the autumn due to mild weather conditions meant that in December/January the oil radish, mustard and black oat species were at stem extension. Two very cold periods of weather for about 12 days in mid December 2022 and then again for about 10 days in mid January 2023 resulted in the sensence of the oil radish, mustard and black oat species together with a large amount of crop biomass in the species mixture treatment. This had not occured in the previous season and was klikely due to the early cover crop development into the more sensitive stem extension phase then combined with a prolonged period of freezing teamperate and snowfall resulting in senesence. This was not the case for the vetch and lucerne which were at a much earlier stage of their crop development cycle. The wet summer in the UK from the beginning of July resulted in highgh levels of tuber scab, *Rhizoctonia solani* and slug damage with 52 out of the 84 plots showing slug damage of harvested tubers (data not presented) .

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Tuber yields were lower in 2022-23 but again Cara produced the highest yield but this time it was Oil radish cover crop that produced the highest tuber yield being 12.52 t/ha higher than the control (Table 11). There were significant effects of poatto cultivar on tuber yield ($p<0.001$), tuber DM% ($p<0.001$), grading fractions 25-45 mm($P<0.001$), 65-85 mm ($p<0.001$), >85mm ($p<0.001$) and scab ($p<0.001$). With respect to cover crop species there were significant effects on tuber yield ($p<0.001$), grading fraction 25-45mm ($p<0.001$), 65-85 mm ($p<0.001$) and tuber scab ($p<0.05$). Although Rhizoctonia levels were higher in 2022-23 than the previous season there was no significant effect of potato cultivar or cover crop species. There were significant potato cultivar × cover crop species interactions on the grading fractions 25-45 mm ($P<0.001$), 45-65mm ($p<0.05$) and 65-85 mm ($P<0.05$).

No tuber scab had been reported following the very dry 2022 growing season but in 2023 scab levels were high with Carolus having 49% of tubers infected and Casablanca the lowest at 26%. The highest tuber scab levels occured following Vetch and the species mixture (45% of tubers infected) and the lowest following the control treatment (24% of tubers infected).

Fig 12 Cover crops established in the early autumn of 2022 at Nafferton Farm in the UK.

There was greater consistency in the effect of cover crop species in the 2022-23 season whereby Oil radish resulted in the highest tuber yield and Black oat the lowest tuber yield in all potato cultivars (Table 12). The highest proportion of tubers affected by Scab (Table 13) was following Vetch for Cara and Casablanca, following the species mixture for Alouette and following Black oat and the species mixture for Carolus. The lowest proportion of tubers affected by Scab occurred for Alouette following Vetch, for Cara following Oil radish and the control (no cover crop), for Carolus following Black mustard and for Casablanca following oil radish.

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Table 11 Means of the effect of cover crop species and potato cultivar on the tuber yield, DM%, tuber grading, resistance to foliar disease and tuber disease levels at Nafferton Farm in 2022-23.

	Yield	DM	Tuber grading (mm)					Scab**	Rhizoc***
	t/ha	%	<25	25-45	45-65	65-85	>85		
Cover crop (C)									
Lucerne	15.04	21.6	0	0.27	0.68	0.05	0	0.43	0.99
Black oat	10.57	21.9	0	0.38	0.58	0.03	0	0.37	0.92
Black mustard	23.79	21.9	0	0.19	0.68	0.13	0	0.37	0.96
Oil radish	29.23	21.8	0	0.16	0.70	0.13	0	0.27	0.99
Vetch	15.91	21.7	0	0.25	0.67	0.08	0	0.45	0.95
Species mix	23.32	22.2	0	0.18	0.61	0.19	0.01	0.45	0.99
Control	16.71	21.8	0	0.19	0.70	0.10	0	0.24	0.99
Cultivar (V)									
Alouette	16.34	22.0	0	0.33	0.65	0.02	0	0.41	0.96
Casablanca	19.70	21.5	0	0.26	0.69	0.05	0	0.26	0.99
Cara	23.82	22.0	0	0.12	0.61	0.25	0.02	0.31	0.97
Carolus	17.03	21.8	0	0.22	0.69	0.09	0	0.49	0.97
Anova									
Cover crop	***	NS	NS	***	NS	**	NS	*	NS
Cultivar	***	***	NS	***	NS	***	***	***	NS
C × V	NS	NS	NS	**	*	*	NS	NS	NS

* Early blight (EB) and Late blight (LB) were assessed on a 1-10 scale Scale of 1-10 where 1 = plants completely blighted (occasionally parts of stem not affected), 5= 50% of leaves infected and 10= no symptoms, occasional necrotic spots.
** Scab levels are presented as a proportion of tuber number
*** *Rhizoctonia solani* levels are presented as a proportion of tuber weight

Table 12 Tuber yields (t/ha) ±SE of the cover crop trial at Nafferton Farm in the UK in the 2022-23.

Cover crop species	Alouette	Cara	Carolus	Casablanca
Lucerne	13.23 ± 0.62	18.74 ± 1.62	14.41 ± 3.67	13.77 ± 0.68
Oil radish	22.76 ±0.66	34.76 ± 1.35	30.30 ± 1.40	29.10 ± 2.53
Mustard	18.75 ± 0.74	32.17 ± 1.87	21.55 ± 2.25	22.70 ± 0.97
Vetch	15.44 ± 1.48	17.54 ± 6.26	11.62 ± 4.78	19.05 ± 1.99
Black oat	9.62 ± 1.88	12.12 ± 4.48	7.28 ± 3.64	13.26 ± 1.42
Species mix	20.30 ± 0.86	31.6 ± 0.50	18.40 ± 4.05	22.99 ± 1.46
Control	14.30 ± 2.68	19.81 ± 3.73	15.67 ± 4.77	17.07 ± 1.88

Table 13 Proportion of tubers infected by scab in response to potato cultivar and cover crop species at Nafferton Farm, UK in the 2022-23 season.

Cover crop species	Alouette	Cara	Carolus	Casablanca
Lucerne	0.45 ± 0.14	0.50 ± 0.08	0.48 ± 0.14	0.29 ± 0.11
Oil radish	0.39 ± 0.12	0.11 ± 0.05	0.40 ± 0.06	0.17 ± 0.13
Mustard	0.41 ± 0.01	0.37 ± 0.11	0.19 ± 0.14	0.21 ± 0.11
Vetch	0.28 ± 0.01	0.55 ± 0.04	0.61 ± 0.13	0.37 ± 0.18
Black oat	0.36 ± 0.15	0.29 ± 0.11	0.59 ± 0.09	0.25 ± 0.09
Species mix	0.67 ± 0.15	0.24 ± 0.05	0.58 ± 0.04	0.32 ± 0.04
Control	0.34 ± 0.17	0.12 ± 0.05	0.27 ± 0.12	0.22 ± 0.12

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3.2.2. KIS

3.2.2.1. Season 2020-21

Very good cover crop emergence and development was achieved in the 2020 (Figure 2). The crops were clean, healthy and without weeds.

Fig 13 Cover crop trial established at KIS at Jablje farm taken on 29 September 2020.

Year 2021 was very wet in May, delaying emergence until the end of May, and very dry and hot in June. Soils compacted by the rain in May and high temperatures affected leaf growth, which was rather small at the end of June. The weather in July and August is crucial for late potato crops. In 2021 they were good without any stress, with enough rain and not too high temperatures, so the plants were able to produce good yields (Figure 13).

Yield

Tuber yields and yield characteristics of cultivars from the 2020-21 season in Slovenia are shown in Table 14. The yields are rather high due to good weather in 2021. The highest yields were achieved by the two Slovenian cvs. KIS Tamar (45.43 t/ha) and KIS Kokra (39.70 t/ha), which also had the largest tubers. They had significantly better yields than cvs. Alouette and Carolus. The differences in yields after cover crops were also statistically significant, with the highest average yields after lucerne (43.39 t/ha), followed by the mixture of species, and the lowest after oil radish and in the control without cover crops (Table 15). There were no significant differences in tuber number between cultivars and between different cover crops.

Table 14 Tuber yields (t/ha), number of tubers per plant and tuber sizes of cultivars at KIS in 2020-21 season.

Cultivar	Yield t/ha	N. of tubers	<25	25-45	45-65	65-85	>85
Alouette	32.38	16.7	0.01	0.62	0.37	0.00	0.00
Carolus	30.37	15.9	0.01	0.62	0.37	0.00	0.00
KIS Kokra	39.70	13.8	0.00	0.27	0.71	0.02	0.00
KIS Tamar	45.43	14.8	0.00	0.21	0.75	0.04	0.00

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Table 15 Tuber yields (t/ha), number of tubers per plant and tuber sizes of cover crops at KIS in 2020-21 season.

Cover crop	Yield t/ha	N. of tubers	<25	25-45	45-65	65-85	>85
Black mustard	33.99	14.4	0.01	0.45	0.54	0.01	0.00
Oil radish	29.31	12.3	0.00	0.33	0.41	0.01	0.00
Lucerne	43.19	16.5	0.00	0.35	0.60	0.04	0.00
Black oat	36.46	14.6	0.00	0.41	0.58	0.01	0.00
Common vetch	35.86	16.2	0.00	0.45	0.53	0.01	0.00
Species mix	38.50	15.4	0.01	0.44	0.54	0.01	0.00
Control	33.60	13.5	0.01	0.42	0.56	0.02	0.00

<u>Tuber quality assessment</u>

In December 2021, tubers from the 2020-21 season cover crop trial were analysed at KIS. A total of 14 quality parameters described in D3.1 (10 of which are listed in Tables 16 and 17) were evaluated. Hollow heart, black heart, internal heat necrosis and vascular discolouration were not observed, so these data are not included in the report. Traits were scored from 1 to 9, with 9 being the best, and diseases were scored as 1.

There were no significant differences between the cultivars (Table 17). Alouette had good shape regularity, the shallowest eyes, good uniformity, intermediate skin finish, but was more susceptible to secondary growth, with almost no rhizoctonia and common scab observed. It was the worst for silver scurf. Carolus had poor uniformity and skin finish but had the most secondary growth and severe common scab infection. KIS Tamar was among the best for almost all quality parameters, while KIS Kokra was the worst for uniformity of shape and depth of eyes, with additional problems of some cracks on tubers, most likely caused by rhizoctonia.

Table 16 Tuber quality characteristics of cultivars at KIS in the 2020-21 season.

	1-9	1-9	1-9	1-9	%	%	n.	1-6	1-5	1-5
Cultivar	**Regularitiy**	**Depth of**	**Uniformity**	**Skin finish**	**Mechanical**	**Secondary**	**Cracks**	**Rhizoctonia**	**Silver scurf**	**Common**
Alouette	7.7	8.5	7.5	5.1	0.0	0.4	0.0	0.1	4.5	0.0
Carolus	5.9	7.3	7.1	3.9	0.0	0.8	0.0	1.2	1.5	4.5
KIS Kokra	5.2	5.5	6.9	5.5	0.0	0.1	3.0	1.7	2.4	1.9
KIS Tamar	8.0	8.4	7.2	7.4	0.1	0.1	0.0	0.8	2.1	0.8

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There were also no significant differences between the cover crops, and in particular there was an improvement in quality compared to the control treatment. In the control there was more secondary growth, cracking and rhizoctonia and the tubers were less regular and uniform. Black mustard had the best shape regularity and uniformity, while lucerne and black mustard had the best skin quality. Black mustard also had the lowest number of cracks, followed by vetch. Infestation with common scab was lower after lucerne, oil radish and black mustard.

Table 17 Tuber quality characteristics of cover crops at KIS in the 2020-21 season.

	1-9	1-9	1-9	1-9	%	%	n.	1-6	1-5	1-5
Cover crop	**Regularitiy**	**Depth of eyes**	**Uniformity**	**Skin finish**	**Mechanical**	**Secondary**	**Cracks**	**Rhizoctonia**	**Silver scurf**	**Common scab**
Black mustard	7.1	7.6	7.6	5.8	0.0	0.3	0.5	1.0	3.1	1.5
Oil radish	6.7	7.3	7.1	5.7	0.0	0.3	0.9	1.0	2.5	1.5
Lucerne	6.8	7.2	7.1	6.1	0.0	0.3	0.9	0.8	2.8	1.4
Black oat	6.8	7.5	7.2	5.5	0.0	0.3	1.3	0.8	2.3	1.8
Common vetch	6.8	7.6	7.1	5.6	0.0	0.4	0.6	0.8	2.6	1.7
Species mix	6.5	7.1	7.3	5.2	0.1	0.2	1.1	1.1	2.7	1.9
Control	5.8	7.1	6.8	5.3	0.2	0.7	1.5	1.5	2.9	1.8

3.2.2.2. Season 2021-22

The year 2022 was very dry from winter onwards, with well below average rainfall for the first eight months of the year, with the exception of April (which was just in time for planting and emergence). In the trial area, we faced extremely dry conditions that affected potato yield. Heavy rain came in September, when it was too late for the potato crop.

Yield

The average tuber yields and other yield characteristics for each cultivar and cover crop from the 2021-22 season in Slovenia are presented in Tables 18 and 19.

Table 18 Tuber yields (t/ha), number of tubers per plant and tuber sizes of cultivars at KIS in 2021-22 season

Cultivar	Yield t/ha	N. of tubers	<25	25-45	45-65	65-85	>85	Dry matter %
Alouette	15.96	7.96	0.01	0.58	0.40	0.00	0.00	23.49
Carolus	16.25	10.30	0.02	0.74	0.24	0.00	0.00	23.47
KIS Kokra	14.77	8.18	0.01	0.54	0.44	0.00	0.00	25.54
KIS Tamar	14.79	7.11	0.02	0.45	0.51	0.02	0.00	24.35

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Table 19 Tuber yields (t/ha), number of tubers per plant and tuber sizes of cover crops at KIS in 2021-22 season

Cover crop	Yield t/ha	N. of tubers	<25	25-45	45-65	65-85	>85	Dry matter %
Black mustard	11.43	7.19	0.02	0.71	0.28	0.00	0.00	24.50
Oil radish	18.09	8.52	0.01	0.46	0.51	0.02	0.00	24.07
Lucerne	12.50	8.33	0.02	0.71	0.26	0.01	0.00	22.85
Black oat	18.35	8.72	0.01	0.46	0.53	0.01	0.00	24.43
Common vetch	18.01	9.28	0.01	0.54	0.44	0.01	0.00	24.48
Species mix	14.96	8.38	0.01	0.64	0.34	0.00	0.00	24.41
Control	14.94	7.87	0.01	0.50	0.48	0.01	0.00	25.26

There were no significant differences between the cultivars tested, while there were significant differences in yield between the cover crops. The significantly higher yields were achieved after black oats, oil radish and common vetch.

Dry matter content is highly dependent on cultivar and weather conditions. In 2022 the dry matter content was very high due to the dry growing conditions and low yields. There were significant differences in dry matter content between the cultivars tested and between the cover crops, but there was no interaction between them. The highest dry matter content was obtained with cv. KIS Kokra, followed by KIS Tamar. Cover crops also influenced the dry matter content, with the highest being achieved by the control, followed by black mustard and common vetch.

Tuber quality assessment

In December 2022, tubers from the 2021-22 season cover crop trial at KIS were analysed. A total of 14 quality parameters described in D3.1 and 10 of them listed in Table 19) were evaluated. Hollow heart, black heart, internal heat necrosis and vascular discolouration were not observed in 2022, so these data are not included in the report. Traits were scored from 1 to 9, with 9 being the best score, while for diseases a score of 1 was the best score. There were large differences between the cultivars, which was to be expected as resistance is genetically determined and depends on the genes in a particular genotype. Alouette had good shape regularity, the shallowest eyes, good uniformity, skin finish, but was more susceptible to secondary growth, with almost no rhizoctonia and common scab observed, but was the worst for silver scurf infection. Carolus had good uniformity and skin finish but had the most common scab. KIS Tamar was among the best for almost all quality parameters, while KIS Kokra was the worst for uniformity and eye depth, with additional problems of some cracks on tubers, most likely caused by Rhizoctonia (Table 20).

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Table 20 Tuber quality characteristics of cultivars at KIS in the 2021-22 season.

	1-9	1-9	1-9	1-9	%	%	n.	1-6	1-5	1-5
Cultivar	**Regularitiy shape**	**Depth of eyes**	**Uniformity**	**Skin finish**	**Mechanical damge**	**Secondary growth**	**Cracks**	**Rhizoctonia**	**Silver scurf**	**Common scab**
Alouette	7.9	8.0	7.5	7.0	0.2	0.2	0.0	0.0	3.3	0.0
Carolus	7.5	8.0	6.7	6.9	0.2	0.6	0.0	0.1	0.6	1.1
KIS Kokra	5.9	6.1	6.2	5.8	0.2	0.6	0.1	0.8	0.6	0.1
KIS Tamar	6.1	7.8	5.8	6.4	0.2	1.9	0.6	0.2	1.6	0.2

For the tuber quality of the crop following certain cover crops in 2022, there were differences in several tested traits. (Table 21). Eye depth is a highly heritable trait, so we did not expect large differences. Oil radish scored best for shape regularity, the control was the most uniform (at low yield) and the species mix gave the best skin finish. Oil radish and vetch had the least secondary growth. Species mix was most affected by rhizoctonia, control by silver scurf and trait black oat by common scab. Only some of the differences were significant and are shown in Tables 22 and 23.

Table 21 Tuber quality characteristics of cover crops at KIS in the 2021-22 season.

	1-9	1-9	1-9	1-9	%	%	n.	1-6	1-5	1-5
Cover crop	**Regularitiy shape**	**Depth of eyes**	**Uniformity**	**Skin finish**	**Mechanical damge**	**Secondary growth**	**Cracks**	**Rhizoctonia**	**Silver scurf**	**Common scab**
Black mustard	6.7	7.3	6.3	6.1	0.0	1.1	0.4	0.3	1.5	0.2
Oil radish	7.2	7.4	6.4	6.5	0.0	0.4	0.0	0.3	1.7	0.5
Lucerne	6.0	7.3	6.6	6.1	0.8	1.1	0.4	0.0	0.9	0.3
Black oat	7.1	7.5	6.7	6.6	0.0	1.3	0.3	0.3	2.0	0.6
Common vetch	6.7	7.3	6.3	6.7	0.0	0.4	0.0	0.3	1.2	0.2
Species mix	7.0	7.4	6.6	6.9	0.8	0.8	0.2	0.5	1.7	0.2
Control	7.0	7.4	6.8	6.3	0.0	0.8	0.0	0.0	2.3	0.3

Table 22 Multiple Range Tests for Regularity of shape by Cover crop.
Method: 95,0 percent LSD

Cover crop	***Count***	***LS Mean***	***LS Sigma***	***Homogeneous Groups***
Lucerne	12	6,0	0,166	X
Black mustard	12	6,66667	0,166	X
Common vetch	12	6,66667	0,166	X
Species mix	12	7,0	0,166	XX
Control	12	7,0	0,166	XX
Black oat	12	7,08333	0,166	XX
Oil radish	12	7,16667	0,166	X

Cv. Alouette had the best regularity of shape, followed by Carolus and KIS Tamar. The best regularity of shape was achieved after oil radish, followed by black oats, control and mixture. The worst regularity of shape was found after lucerne (Table 22), which could be caused by intensive growth due to more nitrogen in the soil.

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There were significant differences between cultivars for uniformity, rhisoctonia and common scab. Cv. Alouette was significantly better for uniformity than the Slovenian cultivars KIS Kokra and KIS Tamar, while Carolus had better uniformity only compared to KIS Tamar. In terms of rhisoctonia infection, KIS Kokra was much more infected than the others, which is in line with previous results. Cv. Carolus had the worst scab score, to the extent that the tubers were not marketable.

The significant differences in silver scurf infection in cover crops can be seen in Table 23. The lowest silver scurf infection was found after lucerne and the highest infection in the control plot. Besides lucerne, black mustard and vetch gave significantly less infected tubers compared to the control.

Table 23 Multiple Range Tests for Silver scurf by Cover crop.
Method: 95,0 percent LSD

Cover crop	*Count*	*LS Mean*	*LS Sigma*	*Homogeneous Groups*
Lucerne	12	0,916667	0,267	X
Common vetch	12	1,16667	0,267	X
Black mustard	12	1,5	0,267	XX
Oil radish	12	1,66667	0,267	XXX
Species mix	12	1,66667	0,267	XXX
Black oat	12	2,0	0,267	XX
Control	12	2,33333	0,267	X

At both locations, yield and its characteristics were influenced by the choice of cultivar and cover crop in both years. The differences were significant in most cases, with the exception of the very dry year 2022 in Slovenia, when yields were very low. Most of the interactions were NS, which means that there is no strong relationship between cultivar and cover crop. This means that a cover crop effect can be expected for most of the cultivars used by farmers.

For UNEW Rhizoctonia, scab and slug damage were more severe in the 2022-23 season in the UK, as Rhizoctonia symptoms tend to be more frequent and severe on cool, moist soils at temperatures of 16°C-23°C. Cultivar susceptibility to *R. solani* varies and was clearly evident in the 2021-22 season, but with much higher levels in 2022-23, no significant difference was observed between cultivars.

Looking at the tuber quality traits observed in Slovenia, there were differences between cultivars in a number of traits, many of which are genetically determined and were expected (e.g. eye depth, resistances). In 2021, when growing conditions were good, there were no differences in tuber quality between the different cover crop treatments. It seems that in the stressful conditions of 2022, the choice of cover crop (sown in the previous year) influenced (improved) tuber quality at least in terms of shape regularity and silver scurf infection.

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4. Colorado potato beetle and wireworm control strategies

4.1.Colorado potato beetle

The Colorado potato beetle (*Leptinotarsa decemlineata*; Coleoptera: Chrysomelidae) and wireworms (Coleoptera: Elateridae) are among the most important insect pests of potatoes worldwide. For Colorado potato beetle (CPB) damage is caused by larvae that feed on the leaves and stems of plants which can cause up to 80 % defoliation of the attacked plants. The above-ground destruction of potato plants can cause severe reduction in tuber size and overall yield (Alyokhin, 2009; Vincent et al., 2013). Colorado potato beetle can be controlled by classical 'chemical' insecticides which current EU agricultural policies are trying to reduce the use of (EU, 2022), or those based on plant extracts, entomopathogenic microorganisms and other substances that pose a lower risk for human and animal health and the environment. To evaluate several innovative CPB control strategies, we set up field experiments in 2020 and 2021 at the Agricultural Institute of Slovenia (KIS) and in 2021 and 2022 at the Hungarian University of Agricultural Sciences (MATE).

There are no commercial cultivars on the market that show a high level of resistance towards CPB, although some level of avoidance is seen in the cultivar Dakota Diamond (Thompson et al. 2008). Ghassemi-Kahrizeh (2009) showed that cvs. Delikat and Bridjet were less favourable for CPB compared to cvs. Agria and Stima, which can be used in plant breeding programs to produce resistant cultivars. To evaluate possible differences in genotype resistance 65 potato cultivars from the Ecobreed working collection were tested in 2021 and 2022 at Plant Breeding and Acclimatization Institute (IHAR).

4.1.1. Materials and methods

KIS - Bioinsecticides against Colorado potato beetle

In 2020 and 2021 two field experiments were carried out to test five bioinsecticides (Table 24) The biological control agents were applied against the larval population individually and in combination to explore the effectiveness and potential synergistic interactions against CPB larvae: spinosad, spinosad + *B. bassiana*, azadirachtin, azadirachtin + *B. bassiana*, *B. bassiana* and RNAi (Table 24).

Eight different treatments were applied on potato ('KIS Kokra' cultivar) plots infested with CPB larvae using a randomised complete block design with six replicates. In 2020 blocks were divided into seven plots (six treatments and untreated control), each measuring 3 x 2.5 m (7,5 m^2) and comprising 3 rows of potatoes. Applications of bioinsecticides were made with a backpack sprayer in the evening hours to take advantage of overnight favourable conditions of high humidity and the absence of solar radiation. Effectiveness of individual bioinsecticides was expressed as a reduction in number of larvae and differences in plant defoliation. Before the bioinsecticide application we selected and marked 5 CPB-infested plants per plot for further assessments. CPB larvae were counted before insecticidal spraying and on the 3^{rd} and 7^{th} day after (bio)insecticide treatment. Level of plant defoliation was assessed visually on the same plants immediately before spraying and on the 7^{th} day after treatment. Defoliation was assessed by visual inspection

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of 10 youngest compound potato leaves per plant using a rating scale that divided defoliation damage into 5 classes: 0 < 5 %, 1 5-10 %, 2 ≤ 50 %, 3 ≤ 75 %, 4 >75-95 % and 5 > 95 % of leaf surface eaten.

Table 24 List of all CPB control treatments tested in 2020 and 2021.

Treatment	Active substance	Concentration	Year applied
Neemazal - T/S	azadirachtin A (1 g/L)	0,5 %	2020, 2021
Laser plus	spinosad (480 g/L)	0,040 %	2020, 2021
Laser plus 0,2 dose	spinosad (480 g/L)	0,008 %	2021
B. bassiana	*Beauveria bassiana* (KIS isolates 2300 and 2121)	10^5 conidia/mL	2020, 2021
Novodor FC	*Bacillus thuringiensis* var. *tenebrionis* (20 g/L 10000 BTTU/g)	1 %	2021
RNAi	RNAi (dsMESH)	10 µg/mL	2020, 2021
***B. bassiana* + Laser plus 0,2 dose**	*Beauveria bassiana* (KIS isolates 2300 and 2121) + spinosad (480 g/L) 0,2 dose	10^5 conidia /mL 0,002 %	2021
***B. bassiana* + Neemazal – T/S**	*Beauveria bassiana* (KIS isolates 2300 and 2121) + azadirachtin (1 g/L)	10^5 conidia /mL 0,5 %	2020, 2021

For CPB data, statistical differences larval mortality according to Henderson-Tilton correction were calculated using ANOVA and Bonferroni's Multiple Comparison Test. Results of leaf damage increase were analysed using Dunnett's Multiple Comparison Test to assess whether the treatments significantly differ from the negative control ($p<0.05$). Results were analysed and visualised using Prism 8 software (GraphPad).

MATE

A small field experiment using a randomised complete block design with three replicates, was set up comparing the effectiveness of Laser Duplo, active ingredient 480 g/l spinosad (0,075 l/ha) and Biomit (5 l/ha) with proven or potential toxic effects against CPB by natural infection at the Potato Research Station, Keszthely. The cultivar Botond was used with 15 tubers per plot. Both protective chemicals were applied two times during the vegetation period in 2021 against the L2 CPB larvae, while due to the limited effect of Biomit in 2021 its application rate was increased five-fold in 2022 with the aim of covering all new developing leaves. Biomit as a dolomit based plant conditioner is enriched with several plant extracts and therefore has the potential to work as a repellent and an antinutritive substance for CPB according to its manufacturer (https://biomit-world.com/hu/biomit-novenykondicionalo/#pll_switcher).

The effect of the treatments was analysed in terms of the percentage of plant defoliation in the second week following application of the final treatment.

IHAR - Genotype differences and CPB

Tubers from 65 cultivars were planted in a cultivar trial in the field with 30 hill plots (two rows with 15 tubers in each) and with 2 replicates. Observations were conducted for two years in 2021, 2022 and 2023. The experimental field was located at IHAR in the central

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part of Poland in Młochów on a sandy loam soil. Colorado potato beetle damage of the potato foliage in the experiment was visually estimated as % of leaf surface destroyed.

4.1.2. Results

KIS - Bioinsecticides against Colorado potato beetle

Studies have been conducted on microbial insecticides summarising that different species or isolates of entomopathogenic fungi (EPF) against different host species show different pathogenicity (Wright and Ramos, 2005; Gödel et al., 2020). The present study indicated that the *B. bassiana* isolates KIS 2300 and KIS 2121 did not increase the mortality of CPB larvae. The highest efficiency of the tested bioinsecticides on CPB larvae was achieved by Laser plus applied at full and 20% of the full recommended dose (Table 25). In both experiments conducted in 2020 and 2021, the CPB mortality after application of Laser plus (full dose) was similar and amounted to 95.7 % or 90.7 %, respectively. Such high efficacy of Laser plus resulted in the fact that the addition of Laser plus at 20 % recommended dose did not differ significantly from efficacy of Lase plus at full recommended dose (assessed only in 2021). Also, despite showing a numerical increase in the efficacy of reduction of CPB larvae, the combination of Laser plus 20% dose and *B. bassiana* was not statistically different from the Laser plus full dose rate. Application of Neemazal – T/S caused a significant increase in mortality rate of CPB larvae i.e. from 31.8 % in 2020 to 61.7 % in 2021. However, the same mortality rates for Neemazal – T/S and the mixture *B. bassiana* + Neemazal – T/ indicates no obvious synergistic effect of these bioinsecticides. We observed no difference in larval mortality between the Novodor, RNAi and control treatments.

Table 25 Mean percent mortality (Henderson-Tillton corrected) of Colorado potato beetle larvae in in 2020 and 2021. Means followed by different letters within a year denote significant differences ($p<0.05$).

Treatment	Mortality (%)	
	2020	2021
Neemazal – T/S	31,8 bc	61,7 ab
Laser plus	95,7 a	90,7 ab
Laser plus 0,2 dose	nd	81,3 ab
B. bassiana	12,2 de	0 d
Novodor FC	nd	2,6 cd
RNAi	23,1 cde	25,2 bcd
***B. bassiana* + Laser plus 0,2 dose**	nd	98,9 a
***B. bassiana* + Neemazal – T/S**	43,8 b	25,4 bcd
negative control	0 e	0 cd

Assimilatory leaf area is a key factor which determines the productivity of arable crops and reduction of potato plant assimilation area due to CPB larvae feeding may lead to a reduction of tuber yield. The extent of losses depends on the degree of defoliation and development stage at which the defoliation occurs. In our experiments the leaf area consumed by CPB larvae was significantly lower in all treatments (except the *B. bassiana* and RNAi in 2021) compared to the untreated control in the first seven days after

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application (Fig. 14). The average level of plant defoliation ranged from 0.17 ± 0.17 (Laser plus in 2020) to 0.74 ± 0.19 (Novodor FC in 2021) which were significantly different from the control where the level of plant defoliation was 1.67 ± 0,08 (2020) and 1.89 ± 0.26 (2021) respectively.

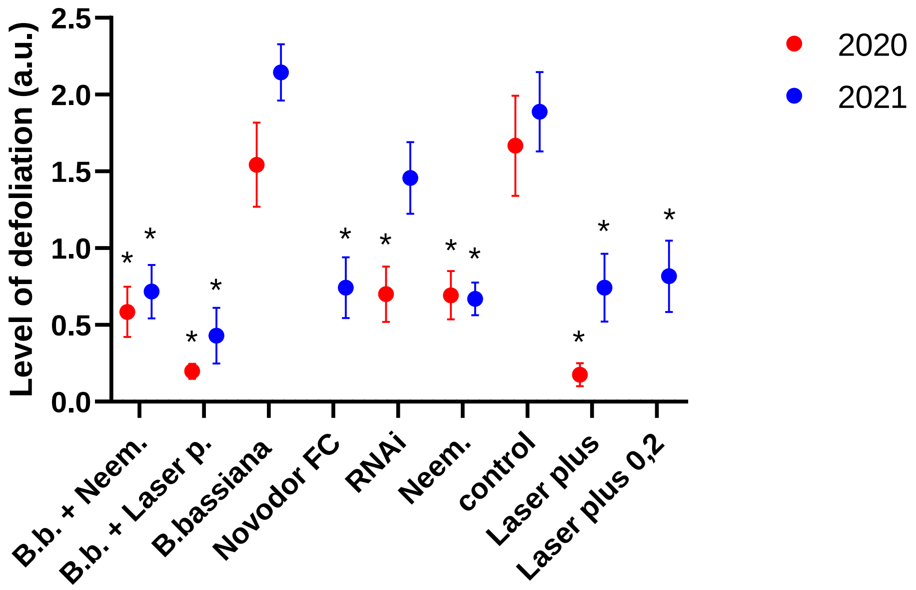

Fig 14 Potato leaf damage due to CPB herbivory expressed as leaf defoliation increase. Means marked with * are significantly different from the control treatment.

MATE

The insecticide Laser Duplo which is permitted for use in organic potato production was more effective in controlling CPB compared to the repellent Biomit. The application of Laser Duplo at two timings resulted in rather low 21 % level of defoliation of plants when averaged across the two growing seasons. The use of Biomit in 2021 (applied twice) resulted in high 70 % level of defoliation, while in 2022 (applied five times) resulted in a lower level of foliar defoliation at 52 %. The repellent effect of Biomit to prevent damage by CPB was not high enough for practical use to protect the plants and was not economically justifiable especially when applied five times (at 2-week intervals) during the season.

Table 26 Percentage of leaf damage by Colorado potato beetle on the cultivar Botond in experiments at MATE in 2021 and 2022.

	2021			Average	2022			Average
	I	II	III		I	II	III	
Spinozad*	27	15	18	20,0	17	28	22	22,3
Biomit**	80	75	56	70,3	57	48	52	52,3
*Spinosade was used two times in each season.								
**Biomit was applied two times in 2021 and five times in 2022.								

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IHAR - Genotype differences and CPB

In the season 2021 the level of damage caused by Colorado potato beetle ranged from 0% to 90%. In 2022 the damage caused by CPB was much lower than in 2022. The ANOVA revealed a highly significant effect for year (Y) on the % damage caused by CPB, while the effect of cultivar was not significant (Table 27).

Table 27 Sources of variation and ANOVA results for percentage damage caused by CPB (IHAR 2021 and 2022).

Sources of variation	ANOVA results					
	Sum of squares	Degrees of freedom	Mean square	F statistic	p-value	Significance
Cultivars (V)	15065.48	64	235.40	0.47491	0.998369	ns
Year (Y)	18696.01	1	18696.01	83.7089	0.000000	***
Maturity (M)	82.57	2	41.28	0.11107	0.894959	ns

ns=not significant
*significant at P < 0.05
**significant at P < 0.01
*** significant at P < 0.001

The highest percentage of damage averaged across 2021 and 2022 (Fig. 15) was recorded in cultivars: Twinner, Caprice, Belmonda, Agria, Gatsby, Coleen, Charlotte, Denar, Casablanca, Lilly and Ditta. For the cultivars Salome, Edony and Noblesse there were no damage caused by CPB in both years of the experiment.

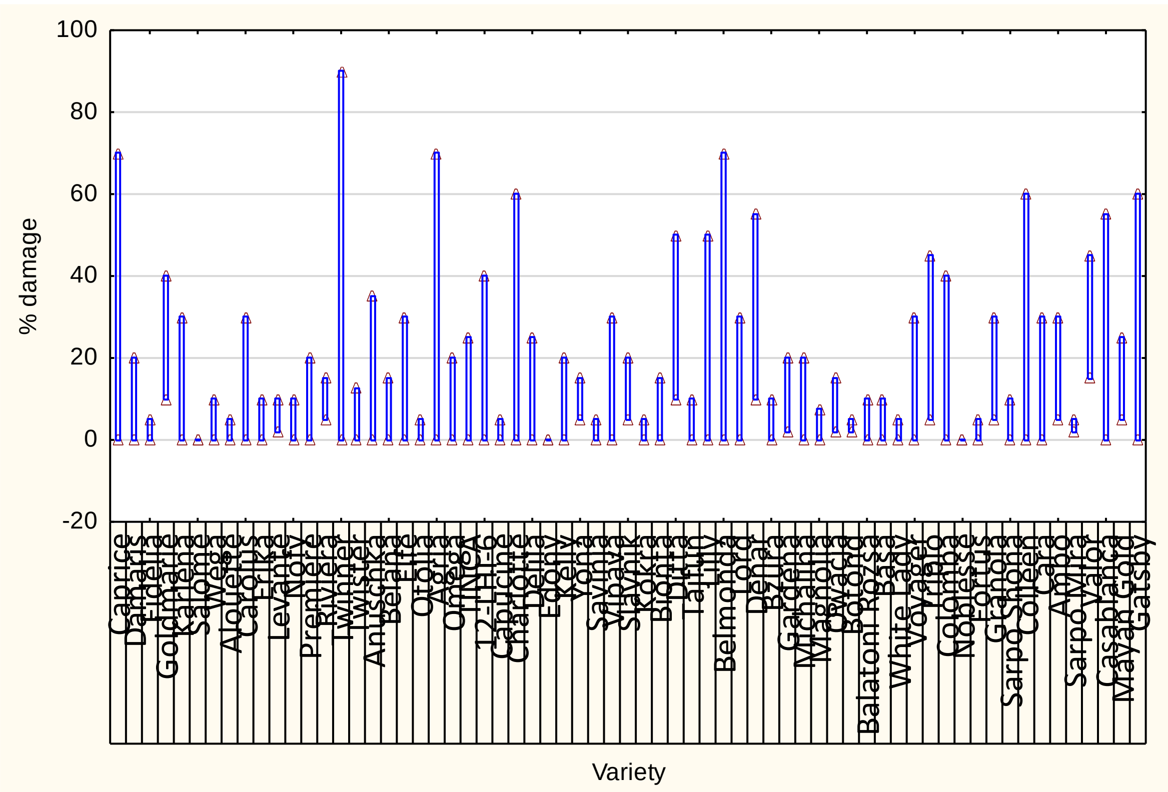

Fig 15 Mean % damage caused by CPB for 65 potato cultivars averaged across 2021 and 2022.

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4.1.3. Conclusions on CPB

Entomopathogenic fungi, especially from the genus *Beauveria* (Ascomycota: Hypocreales), have been shown to be a promising solution for the control of CPB in potato (Brandl et al. 2017; Wraight et al. 2008). *Beauveria* sp. naturally occurs in the soil and is therefore associated with soil-inhabiting insects like CPB larvae undergoing pupation. *Beauveria* sp. has also been effectively tested for CPB larvae control in the form of a foliar spray (Lacey et al. 1999).

Results of the study revealed that among the tested bioinsecticides all treatments with spinosad (Laser plus, Laser plus 0.2 dose and *B. bassiana*+Laser plus) provided significantly better control of larval population compared to all other insecticide treatments. The best results from the tested products showed Laser plus with significantly increased mortality rate and reduced feeding activity of CPB larvae when used at full and reduced dosage (0.2 of full dosage). Significant reduction in the number of CPB larvae was achieved also by the application of NeemAzal T/S and its combination with entomopathogenic fungi (*B. bassiana* + Neemazal – T/S). In addition to the direct impact and increase in mortality rate of different treatments, the indirect effect on the reduction of feeding is also important when evaluating their efficiency against CPB larvae. Results indicate that the bacterial based insecticide Novodor FC did not have a significant effect on CPB larvae mortality but could contribute to a successful strategy for CPB control by reducing plant defoliation caused by CPB larvae. However, insufficient efficacies were obtained with application of *B. bassiana* which showed low insecticidal activity in field conditions and was significantly less effective against CPB larvae which means that it can't be recommended as a ready-to-use bioinsecticide for CPB larvae control. Although in some studies RNAi has shown a positive effect against CPB larvae even when tested under field conditions (Petek et. Al. 2020) in our study the use of RNAi didn't show a significant effect on larval mortality and resulted in a significantly lower rate of plant defoliation only in 2020.

Based on the experimental results at MATE in Keszthely, the insecticide Laser Duplo prevented the defoliation of plants by CPB better than the natural repellent Biomit, which even following five times application could only reduce defoliation to 52%. Consequently, an even more frequent e.g. once a week application would be needed to prevent severe leaf damage, but that would make its application economically not viable.

The two-year research at IHAR revealed three cultivars (Salome, Edony, Noblesse) that might have a strong level of resistance towards CPB. Incorporation of host plant resistance into potato cultivars may be an effective tool in the management of CPB and is likely to be a valuable tool for integrated pest management approaches to CPB control.

4.2. Wireworms

Wireworms (Coleoptera: Elateridae) are the larvae of click beetles and they are also known as "elater larvae". They can be found in the soil where they feed on the underground parts of plants, such as the roots and tubers of potatoes. Damage from wireworms can include wilting, stunted growth and even plant death. Because wireworms can survive for several years in the soil, infestations can be difficult to control. Entomopathogenic fungi (EPF), particularly from the genus *Metarhizium* (Ascomycota: Hypocreales), have been shown to

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be a promising solution for controlling wireworms in potatoes (Reinbacher et al. 2021; Brandl et al. 2017; Kabaluk et al. 2005). With this in mind, we tested different formulations based on EPF *Metarhizium brunneum* and *Metarhizium robertsii* preparations, thus evaluating different modes of action such as plant growth stimulation, bioaugmentation and the »attract and kill« method.

4.2.1. Materials and methods

To evaluate several innovative strategies for wireworm control in potato fields, we set up two field experiments in 2020 and two in 2021. The Agricultural Institute of Slovenia's (KIS) mycological collection holds many isolates of entomopathogenic fungi (EPF) that have proven to be quite effective. Therefore, to test the bioaugmentation method, i.e. the introduction and potential multiplication of EPF in the rhizosphere, six of the most virulent KIS *Metarhizium brunneum* and *Metarhizium robertsii* isolates were formulated on rice and added to potato tubers at planting. To test the »attract and kill« method, the commercial granular bioinsecticide ATTRACAP (Biocare GmbH, active ingredient *M. brunneum* Cb15-III) was used. The ATTRACAP granules contain EPF (*M. brunneum* Cb15-III), starch and baker's yeast (*Saccharomyces cerevisiae*). When ATTRACAP is added to moist soil, yeasts begin to produce CO_2. Wireworms are attracted to the CO_2 source and consequently come into contact with the EPF present in the granules. To test plant growth stimulation method, we soaked potato tubers in spore suspension of six KIS EPF isolates for 1 h 30 min so that the spores would get adsorbed to the surface of the potato tubers and potentially colonise the external or internal tissues of the plants. Tubers were air dried and planted the next day (Fig. 16).

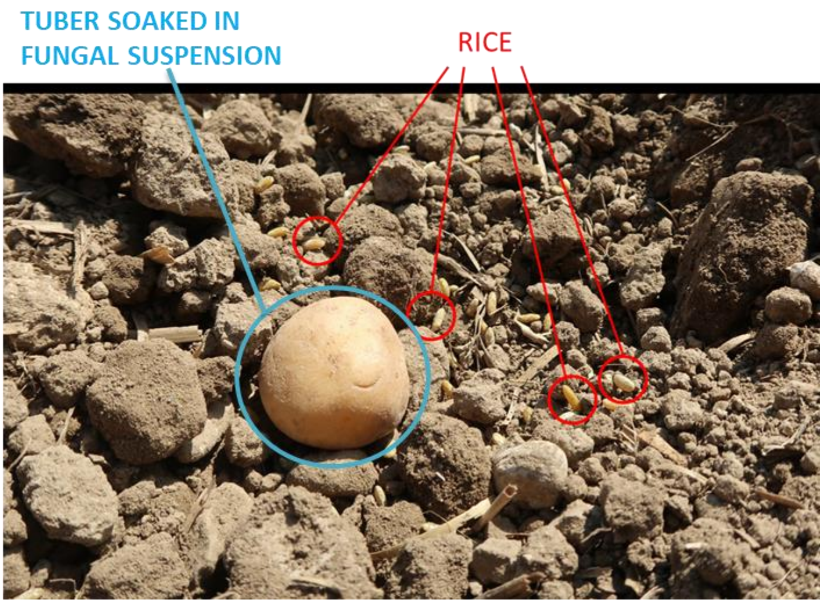

Fig 16 Potato seed soaked in fungal suspension with fungal formulation multiplied on rice.

We had seven treatments in the experiment: (a) potato tubers soaked in the fungal suspension of all six isolates (Potato_fungi), (b) fungi formulated on rice (Potato_rice) and (c) a combination of both treatments (Potato_fungi_rice). In addition, two treatments with the commercial bioinsecticide ATTRACAP were made, testing either full (30 kg/ha) or half

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(15 kg/ha) recommended dose and the commercial insecticide Force (Syngenta, active ingredient Tefluthrin, 15 g/kg) was used as a conventional (positive) control treatment alongside the untreated control (Table 28).

Table 28 List of wireworm control treatments tested in 2020 and 2021.

	2020	2021
Potato tubers soaked in the fungal suspension	✓	×
Fungi formulated on rice [51.3 kg/ha]	×	✓
Potato tubers soaked in the fungal suspension and fungi formulated on rice [51.3 kg/ha]	✓	✓
ATTRACAP full dose [30 kg/ha]	✓	✓
ATTRACAP half dose [15 kg/ha]	✓	✓
Negative control	✓	✓
Positive control – insecticide Force [5 kg/ha]	✓	✓

The experimental design was a complete randomised block design with six treatments and eight replicates in Field 1, Field 3 and Field 4, where we planted KIS-Savinja cultivar (N=576 tubers per treatment). Field 2 had six treatments and six replicates and we also planted the cultivar KIS-Kokra (N=252 tubers per treatment). At all locations, there were 2 rows of protection buffer zone on each side of the experiment. All fields were located at Agricultural Institute of Slovenia, Infrastructure Centre Jablje.

The effectiveness of tested methods was evaluated at potato harvest on 100 randomly selected tubers per plot as the amount of tuber yield and as the number of tubers where we counted more than one wireworm hole per tuber, since tubers with more holes are less attractive for consumers. Tubers from one row of each plot were additionally classified into four size classes (>65 mm; 65-45 mm; 45-25 mm; <25 mm) and the effect of the treatments on the number and mass of tubers in each size class was also tested.

For wireworm data, the effectiveness of treatments on the number and mass of tubers in each size class, on yield and on number of tubers with more than one hole was analysed using a one-way ANOVA, followed by Bonferroni-Holm multiple comparison test.

4.2.2. Results

The effectiveness of the different treatments was evaluated based on the number and mass of tubers in each size class, yield per row (in a single plot) and the number of tubers with more than one hole. Differences between treatments were not prominent (Fig. 17). Force appeared to be most effective in reducing the number of holes per tuber, especially on Field 1, where it reduced the number of tubers with more than one hole for 75.6%. The most effective EPF treatments for reducing the number of tubers with more than one hole were with potatoes soaked in fungal suspension at Field 1 (57.2% reduction) and potatoes soaked in fungal suspension together with fungi formulated on rice at Field 2 (43.9% reduction). In 2021, we made a change to one of the treatments, namely replacing potato tubers soaked in fungal suspension with fungi formulated on rice. In that year, the most effective EPF treatment for reducing the number of tubers with more than one hole was the half dose of ATTRACAP at Field 4 (49.5% reduction), while at Field 3 all EPF treatments resulted in higher number of tubers with more than one hole compared to the control.

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At all locations, tuber yield per row showed no significant differences between treatments, except a significant yield reduction from both the full dose of Attracap (21.3% reduction) and potatoes soaked in fungal suspension together with fungi formulated on rice (20.2% reduction) at Field 2. Nevertheless, full dose of Attracap increased yield by 16.7% at Field 3, but not significantly (Fig. 18). The treatments also had no significant effect on tuber number and mass in all four size classes (>65 mm; 65-45 mm; 45-25 mm; <25 mm).

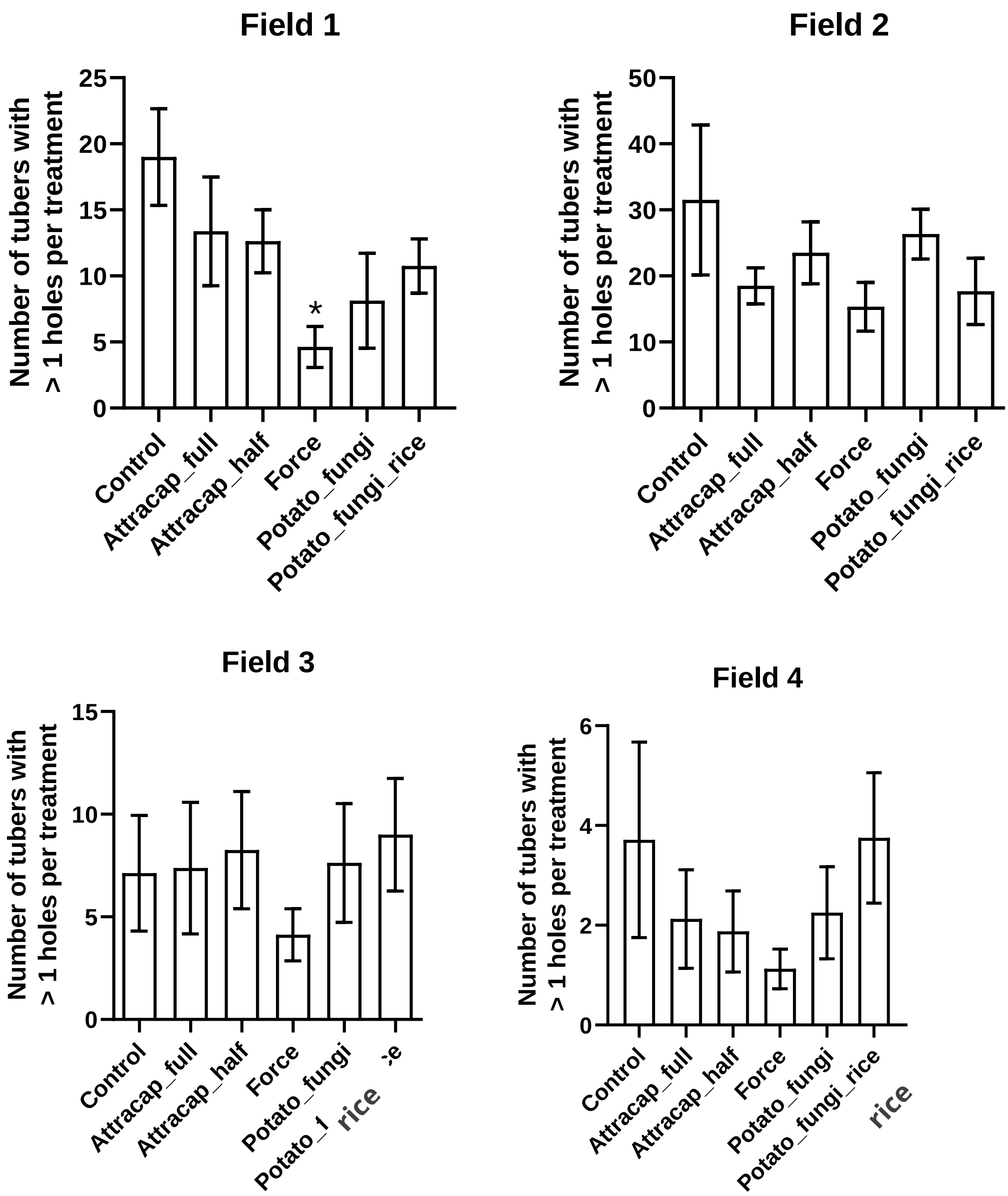

Fig 17 Number of tubers with more than one hole in different treatments in year 2020 (Field 1 and Field 2) and 2021 (Field 3 and Field 4). Means marked with * are significantly different from the control group mean (p<0.05).

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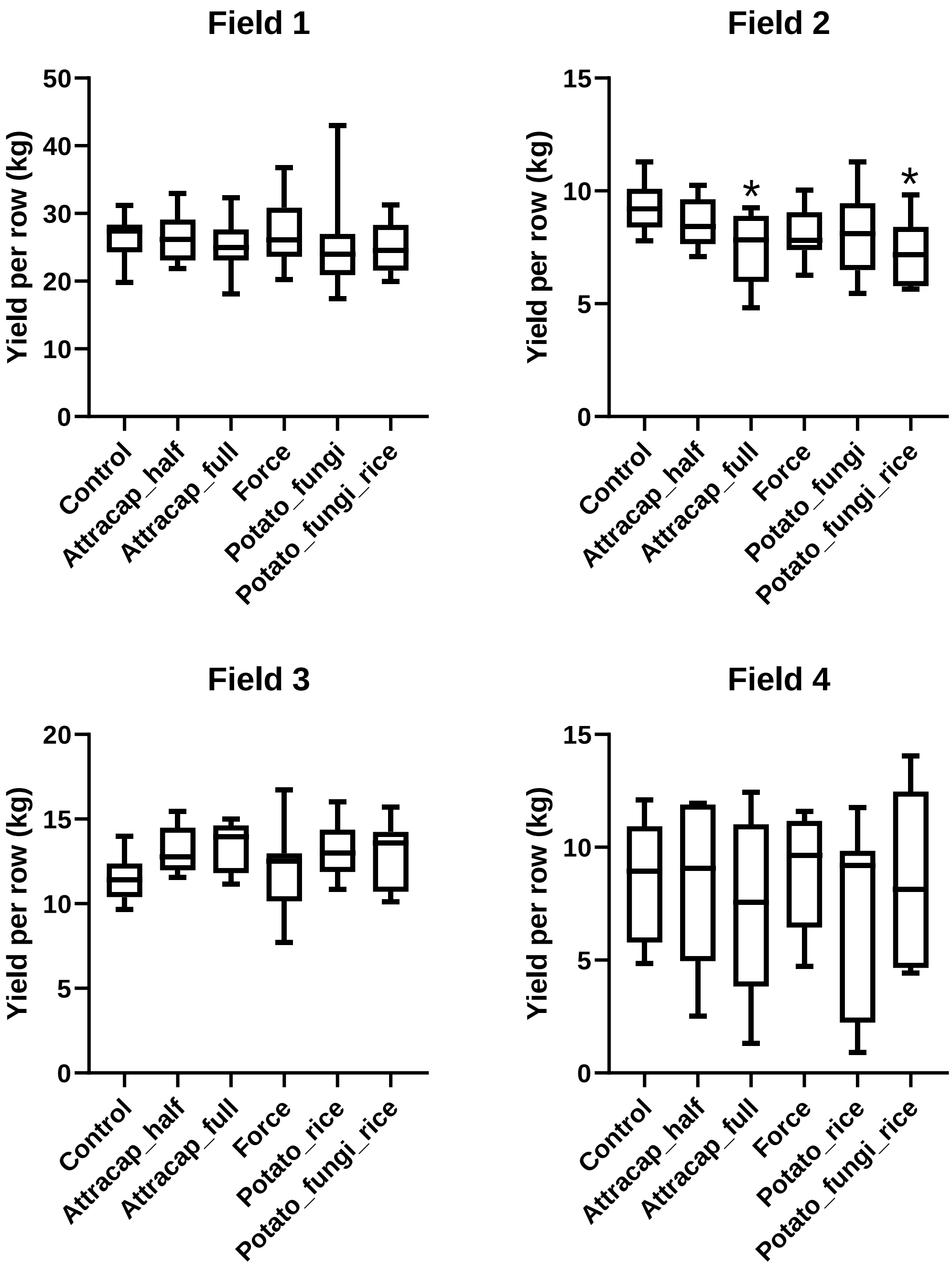

Fig 18 Potato yield (kg per row length 6.3 m) in different treatments per row in year 2020 (Field 1 and Field 2) and 2021 (Field 3 and Field 4). Means marked with * are significantly different from the control treatment (p<0.05).

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4.2.3. Conclusions on wireworms

Entomopathogenic fungi, especially from the genus *Metarhizium* (Ascomycota: Hypocreales), have been shown to be a promising solution for the control of wireworms in potato (Reinbacher et al. 2021; Brandl et al. 2017; Wraight et al. 2008). *Metarhizium* sp. naturally occurs in the soil and are therefore associated with soil-inhabiting insects like wireworms.

The results of testing various preparations containing *Metarhizium* on wireworms, especially potatoes soaked in fungal suspension and half dose of ATTRACAP resulted in lower number of tubers with more than one hole compared to the control. This was particularly evident at Field 1 and Field 4. Thus, the introduction of EPF in the rhizosphere as an attract-and-kill method, or as plant growth promotor by soaking tubers in fungal suspension, has the potential to reduce tuber damage.

Wireworms generally do not cause yield loss (Parker and Howard, 2001). This is consistent with our results, as wireworms did not affect the number or mass of the tubers and mostly did not cause yield losses at any location, apart from the full dose of Attracap and potatoes soaked in fungal suspension together with fungi formulated on rice at Field 2.

Overall, bioinsecticides based on entomopathogenic fungi were found to be quite effective in reducing potato damage caused by wireworms, as the effectiveness of the treatments, especially ATTRACAP half dose and potatoes soaked in fungal suspension, were comparable to that of a conventional insecticide based on tefluthrin (Force).

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5. Marker assisted selection

5.1. Screening a Potato Working Collection for the presence of resistance genes that are important for organic farming

The potato working collection established in WP1 consists of 65 cultivars potentially suitable for organic farming. However, these cultivars are also considered as a source of valuable resistance genes in new breeding programs. To confirm the presence of such genes in the cultivars of the working collection, the IHAR-PIB has screened all these cultivars with a set of molecular markers linked to resistance genes (R-genes) against late blight (*Phytophthora infestans* pathogen) and Potato Virus Y (PVY). These two potato pathogens are the most important for conventional and organic farming. In addition, all cultivars were tested for the presence of a marker linked to a gene conferring resistance to Potato Virus S (PVS).

5.1.1. Genes providing resistance against *P. infestans.*

Potato late blight, caused by the oomycete *Phytophthora infestans*, is generally regarded as the most important biotic factor limiting the yield of organic potatoes. There is no highly effective control measure against late blight in organic farming, and the development of resistant cultivars can play a key role in the sustainable control of the disease by organic farmers. The use of genetic resistance to develop resistant cultivars has long been one of the main objectives of potato breeding. The two main types of potato resistance to *P. infestans* are R-gene based (qualitative) and non-R-gene based (quantitative) resistance. Qualitative resistance is determined by key resistance genes (R-genes) that encode immunity through a hypersensitivity response (HR), which leads to cell death and rapid localization of the pathogen, preventing further colonization of the host tissue. Currently, the pyramiding of different broad-spectrum R genes in a cultivar is considered the best solution for breeding potato cultivars with a high level of durable resistance to late blight.

Examples of promising broad-spectrum resistance genes include *RB/Rpi-blb1* (Song et al. 2003), *Rpi-blb3* (Park et al. 2005), *Rpi-phu1* (Śliwka et al. 2006), which is identical to *Rpi-vnt1.1, Rpi-rzc1* (Śliwka et al. 2012), *R8 / Rpi-Smira2* (Rietman 2011), and others. The use of these R genes in the breeding of cultivars suitable for organic farming is extremely useful. However, the phenotypic evaluation of thousands of potato individuals in the selection process is very time-consuming and labour-intensive. In the case of pyramiding of R genes, the use of many different isolates with different (sometimes not yet existing) combinations of virulence/avirulence genes is required for adequate phenotyping. Marker-assisted selection (MAS) or marker-assisted gene pyramiding (MAGP) allows the selection of potato individuals with the desired R-gene composition without extensive phenotypic testing. There are many examples in the literature of the successful use of DNA markers for the selection of potato clones that have a specific R gene or a specific combination of R genes against late blight (Śliwka et al. 2010, Plich et al. 2017, Stefańczyk et al. 2020).

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5.1.2. Genes providing resistance against PVY and PVS

Potato virus Y (PVY) is an important pathogen affecting potato production worldwide. Three independent genes (*Ry-adg*, *Ry-sto* and *Ry-chc*) conferring genetic resistance to all known strains of the virus are currently being utilized in breeding programs to develop potato cultivars with extreme resistance to PVY infection. So far, only one R gene has been found in the potato gene pool that confers resistance to PVS, namely the Ns gene.

5.1.3. Screening of Working Collection

As part of the ECOBREED project, WP3, in the IHAR-PIB, all 65 potato cultivars from the working collection were analyzed for the presence of 8 DNA markers linked to R genes conferring resistance to late blight (*R1, R2*-family, *R3a, R3b, Rpi-Smira1, R8 / RpiSmira2, Rpi-chc1 and Rpi-vnt1 / Rpi-phu1*), two markers linked to an R gene conferring resistance to PVY (*Ry-sto* and *Ry-chc*), and additionally a marker linked to a resistance gene to PVS (Ns).

The selected genes and markers used for screening (with references) are listed in Table 29. All markers (except GP122 and SC811) are allele-specific and amplify the corresponding product directly after PCR. Only the markers GP122 and SC811 are CAPS (Cleaved Amplified Polymorphic Sequences) markers, and the PCR products should be digested with the endonucleases EcoRV and MboI. The PCR products were separated on 1.5% agarose gels stained with ethidium bromide and visualized under UV light.

Table 29 List of DNA markers used for the screening R genes in ECOBREED potato working collection.

Gene	Marker	Tm	Size of the product (bp)	References
		Resistance against *P. infestans*		
R1	R1-1205	60°	1205	Sokolova et al. 2011
***R2* (*R2* family)**	R2 / *Rpi-abpt*	57°	686	Kim et al. 2012
R3a	R3-1380	65°	1380	Sokolova et al. 2011
R3b	SHa	65°	982	Huang et al. 2005
Rpi-Smira1	45/XI	55°	~1000	Tomczyńska et al. 2014
R8 / Rpi-Smira2	R8-1276	62°	1276	Vossen et al. 2016
Rpi-chc1	Rpi-chc1	60°	~320	Monino-Lopez et al. 2021
Rpi-phu1 / Rpi-vnt1	Phu1_2069	67° - 62°	2069	Stefańczyk et al. 2020
		Resistance against potato viruses		
Ry-chc	Ry186	58°	587	Mori et al. 2011
Ry-sto	GP-122 (*EcoRV*)*	56°	564	Witek et al. 2006
Ns	SC811 (*MboI*)	56°	260	Witek et al. 2006

The results of the screening are summarized in the table above. Among the cultivars tested, the most frequently observed markers were those associated with genes: *R3b* (present in 47 cultivars), *R3a* (present in 36 cultivars) and R1 (present in 16 cultivars). Markers linked to genes from the *R2* family were found in 8 cultivars. We also found markers linked to genes: *Rpi-Smira1* (present in 14 cultivars), *R8/RpiSmira2* (present in 8 cultivars), *Rpi-chc1* (present in 3 cultivars) and *Rpi-vnt1 / Rpi-phu1* (present in 3 cultivars).

According to published data, genes *R1, R3a* and *R3b* provide resistance to a narrow spectrum of current races of *P. infestans* (almost 100% of isolates are virulent against these genes). Gene *R2* (and its orthologs/homologs) provides resistance to about 50% of the isolates collected. There are no data on the virulence of *P. infestans* against the locus *Rpi-*

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Smira1. The remaining 3 genes *R8, Rpi-chc1* and *Rpi-phu1/Rpi-vnt1* are considered broad spectrum resistance genes as less than 10% of isolates are virulent against each of these genes.

All cultivars were also analyzed for the presence of DNA markers associated with two R genes conferring resistance to PVY: *Ry-sto* and *Ry-chc*, as well as for the presence of the gene *Ns* conferring resistance to PVS. Based on these results (see Table 30), a marker linked to *Ry-sto* was found in 12 cultivars, while a marker linked to *Ry-chc* was found in 5 cultivars. A marker linked to the gene *Ns* was found in only one cultivar (Magnolia).

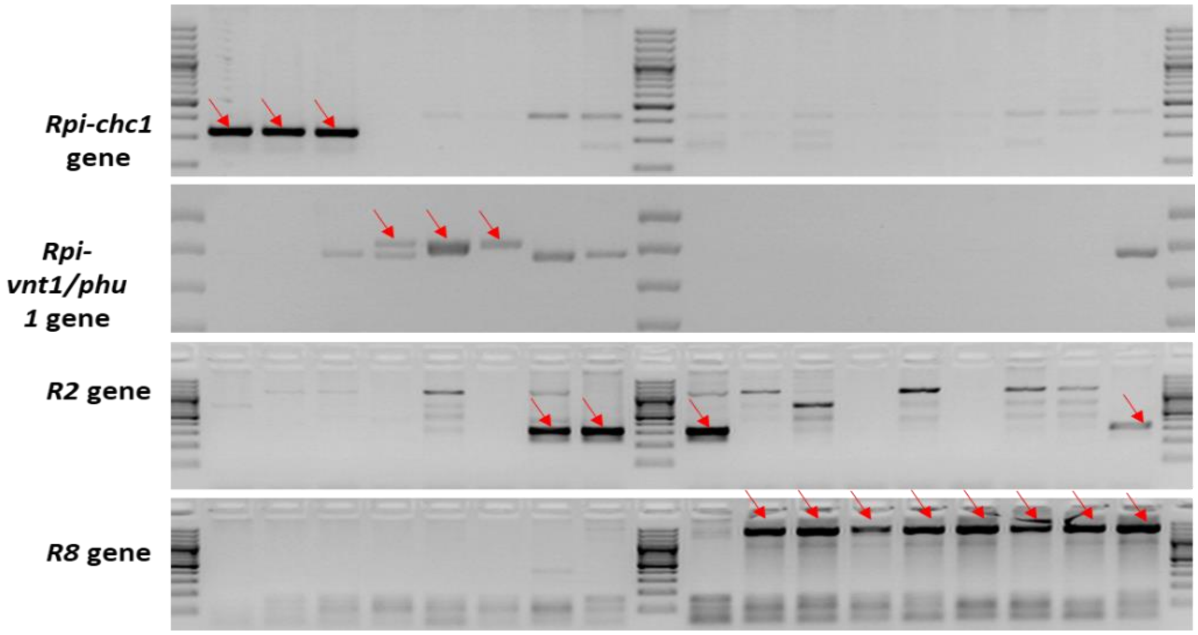

Fig 19 Example of band pattern of selected DNA markers after electrophoretic separation on agarose gel.

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Table 30 Results of the screening of the potato cultivar collection with DNA markers linked to the corresponding resistance genes: 1 – presence of the marker; 0 – absence of the marker.

	Cultivar	*R1*	*R2*	*R3a*	*R3b*	*Rpi-Smira1*	*R8 / Rpi-Smira2*	*Rpi-chc1*	*Rpi-phu1 / Rpi-vnt1*	*Ry-sto*	*Ry-chc*	*Ns*
1	Caprice	0	0	0	0	0	0	0	0	1	0	0
2	Damaris	0	0	0	0	0	0	0	0	1	0	0
3	Fidelia	0	0	1	1	0	0	0	0	1	0	0
4	Goldmarie	0	0	1	1	0	0	0	0	0	0	0
5	Karlena	0	0	1	1	0	0	0	0	0	0	0
6	Salome	0	0	1	1	0	0	0	0	0	0	0
7	Wega	0	0	1	1	0	0	0	0	0	0	0
8	Alouette	0	0	1	1	0	0	0	1	0	0	0
9	Carolus	0	0	1	1	0	0	1	0	0	0	0
10	Erika	1	0	1	1	0	0	0	0	1	0	0
11	Levante	0	0	0	0	0	1	0	0	0	0	0
12	Nofy	1	0	0	0	0	0	0	1	0	0	0
13	Premiere	0	0	0	1	1	0	0	0	0	0	0
14	Riviera	0	0	0	1	0	0	0	0	0	0	0
15	Twinner	0	0	1	1	0	0	1	0	0	0	0
16	Twister	0	0	1	1	1	0	1	0	0	0	0
17	Anuschka	0	0	1	1	0	0	0	0	0	0	0
18	Belana	0	0	1	1	1	0	0	0	0	0	0
19	Elfe	0	0	1	1	0	0	0	0	0	0	0
20	Otolia	0	0	1	1	0	1	0	0	0	0	0
21	Agria	0	0	1	0	0	0	0	0	0	0	0
22	Omega	0	0	1	1	0	0	0	0	0	0	0
23	Tinca	0	0	1	0	1	1	0	0	0	0	0
24	12-LHI-6	1	0	1	1	1	1	0	0	0	0	0
25	Capucine	1	0	1	1	0	0	0	0	0	0	0
26	Charlotte	1	0	1	1	0	0	0	0	0	0	0
27	Delila	1	0	1	1	0	1	0	0	0	0	0
28	Edony	1	0	0	1	1	0	0	0	0	0	0
29	Kelly	0	1	0	0	0	1	0	0	0	0	0
30	Yona	0	0	1	1	0	0	0	0	0	0	0
31	KIS Savinja	0	1	1	1	1	0	0	0	1	0	0
32	KIS Vipava	1	0	0	0	0	0	0	0	0	1	0

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33	KIS Slavnik	0	0	0	1	1	0	0	0	0	0	0
34	KIS Kokra	0	1	0	1	1	0	0	0	0	0	0
35	Bionta	1	0	0	0	0	0	0	0	1	0	0
36	Ditta	0	0	1	1	0	0	0	0	0	0	0
37	Tajfun	0	1	0	1	1	0	0	0	0	0	0
38	Lilly	0	0	1	1	0	0	0	0	0	0	0
39	Belmonda	0	0	1	1	0	0	0	0	0	0	0
40	Lord	0	0	0	1	0	0	0	0	0	0	0
41	Denar	0	0	0	1	0	0	0	0	0	0	0
42	Bzura	1	1	0	1	0	0	0	0	1	0	0
43	Gardena	0	0	1	1	0	0	0	1	0	0	0
44	Michalina	0	0	1	0	0	0	0	0	0	0	0
45	Magnolia	0	0	0	1	0	0	0	0	1	0	1
46	Owacja	1	1	0	1	1	0	0	0	1	0	0
47	Botond	1	0	0	0	1	1	0	0	0	1	0
48	Balatoni rozsa	1	0	0	1	0	0	0	0	1	0	0
49	Basa	0	1	1	1	0	0	0	0	1	0	0
50	White Lady	0	1	1	1	0	0	0	0	1	0	0
51	Voyager	0	0	1	1	0	0	0	0	0	0	0
52	Triplo	0	0	0	0	0	0	0	0	0	0	0
53	Colomba	0	0	0	1	0	0	0	0	0	0	0
54	Noblesse	0	0	1	1	0	0	0	0	0	0	0
55	Fortus	1	0	1	0	0	0	0	0	0	0	0
56	Granola	0	0	0	1	0	0	0	0	0	0	0
57	Sarpo Shona	0	0	0	0	1	1	0	0	0	1	0
58	Colleen	0	0	0	1	0	0	0	0	0	0	0
59	Cara	1	0	1	1	0	0	0	0	0	0	0
60	Ambo	1	0	1	1	0	0	0	0	0	0	0
61	Sarpo Mira	0	0	1	1	1	1	0	0	0	1	0
62	Valor	0	0	0	0	0	0	0	0	0	1	0
63	Casablanca	0	0	0	0	0	0	0	0	0	0	0
64	Mayan gold	0	0	0	0	0	0	0	0	0	0	0
65	Gatsby	0	0	0	0	0	0	0	0	0	0	0

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5.1.4. Genotyping of the CHY2 loci

Identification of the dominant allele 3 at the CHY2 locus was performed on the 65 potato cultivars from the working collection using a specific CAPS assay developed by Wolters et al. 2010. Genomic DNA was amplified with primers CHY2ex4F (5'-CCATAGACCAAGAGAAGGACC-3') and Beta-R822 (5'-GAAAGTAAGGCACGTTGGCAAT-3') to obtain a 308 bp fragment. Subsequent Alu digestion produces a diagnostic fragment of 163 bp in the presence of the dominant Chy2 allele 3, whereas all other (recessive) alleles at the same locus produce a specific fragment of 237 bp. PCR amplification was performed using PCR Mix Plus Green - High Specificity Ready Mix for PCR (A&A Biotechnology) and 300 nM of each primer in a final volume of 25 µl. Standard amplification conditions were as follows: initial denaturation of 4 min at 94°C followed by 30 cycles of 30 sec denaturation at 94°C, 30 sec annealing at 55°C and 1 min extension at 72°C. Reactions were terminated with an extension step of 7 min at 72°C. The CAPS markers of the CHY2 gene and the amplification products of the ZEP gene were separated on a 2% ethidium bromide-stained agarose gel.

The diagnostic fragment of the dominant Chy2 allele 3 was amplified in 50 out of 65 cultivars tested. Fig. 20 shows amplification of the CAPS marker linked to Chy2 gene allele 3. Clones with amplified diagnostic fragment were both yellow and white fleshed. Clones without this fragment were also both yellow and white fleshed. The correlation between the presence of Chy2 allele 3 and YI was r = 0.30*, while the correlation between the presence of Chy2 allele 3 and TC (mean) and TC 2020 was r = 0.26* and r = 0.31*, respectively (Table 31).

Table 31 Pearson's correlation coefficient of total carotenoids (TC) and yellow index (YI) with marker Chy2 gene allele 3.

Marker/Trait	YI	TC 2019	TC 2020	TC 2021	TC mean value from 3 years
Chy2	0.30*	0.18^{ns}	0.31*	0.032^{ns}	0.26*

ns = not significant; * significant at P < 0.05; ** significant at P < 0.01

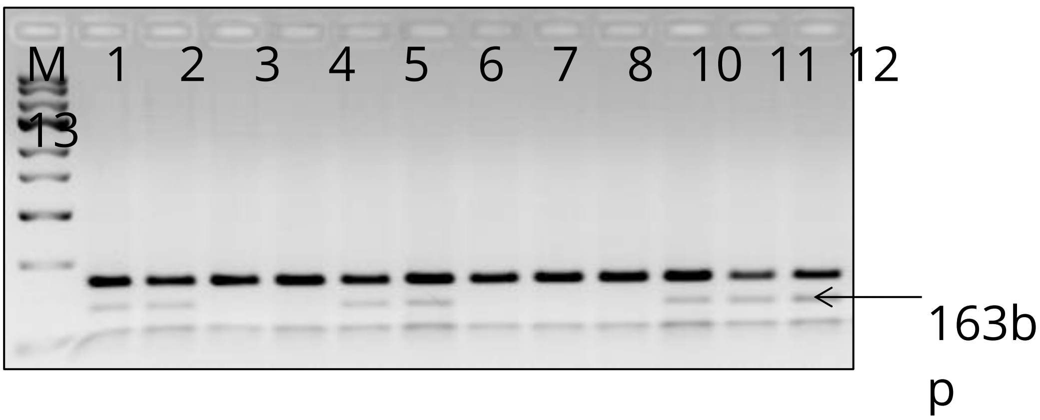

Fig 20 Amplification of CAPS marker linked to Chy2 gene allele 3. Cultivars having Chy2 gene allele 3 are shown in lanes 1,2,5,6,11,12 and 13, respectively. Lanes 3, 4, 7, 8 and 9 show cultivars lacks Chy2 gene allele 3. Lane M contains the 100bp DNA ladder.

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3.2. Marker-assisted selection (MAS) of genes conferring resistance to *P. infestans.*

3.2.1. IHAR-PIB

As part of Task 3.5 in IHAR-PIB, the usefulness of selected DNA markers for MAS was investigated. We screened 125 progeny clones from the EB-I population with DNA markers associated with resistance genes to *P. infestans*. EB-I originates from a cross between potato clone 15-V-271 and clone 15-V-54, which are donors of the resistance genes Rpi-phu1 and R8, respectively. Of these 125 progeny clones, 22 were susceptible (mean resistance value ≤ 6.0) to *P. infestans* and 103 clones were highly resistant (Fig. 21). The ratio of resistant to susceptible clones in this progeny was the expected 3:1 ($p<0.05$).

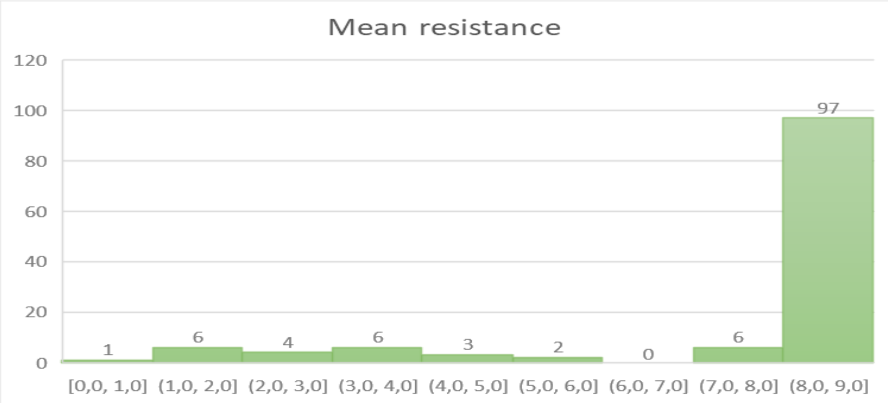

Fig 21 Histogram of mean resistance distribution in EB-I progeny.

To confirm the presence of the Rpi-phu1 gene in the progeny clones, the DNA markers phu6 and phu_2069 were used. To confirm the presence of the *R8* gene, we used the marker R8-1276. The presence of DNA markers is completely co-segregated with the phenotype and no specific product of the markers used was observed in any of the 22 susceptible clones. In 103 resistant clones, we confirmed the presence of the *Rpi-phu1* gene (36 individuals), the R8 gene (39 individuals) or both genes (28 individuals) (Fig. 22). This study is another excellent example of the utilization of DNA for MAS in potato breeding.

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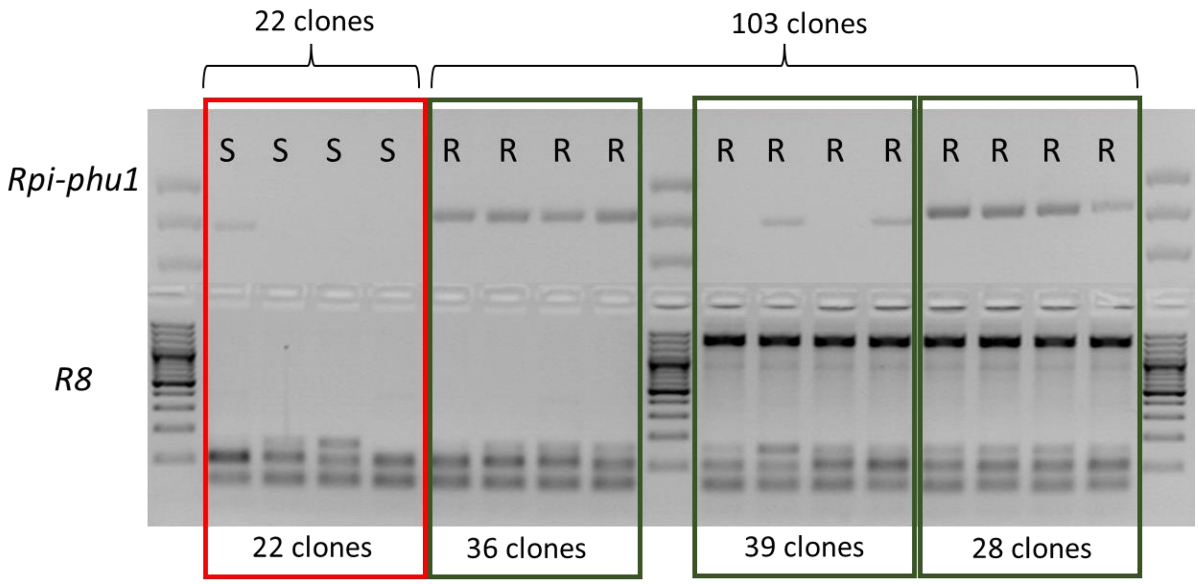

Fig 22 Picture of agarose gel after electrophoretic separation of PCR products of amplification of DNA markers linked with *Rpi-phu1* and *R8* gene in progeny EB-I.

5.2.2. KIS

Molecular markers for resistance genes against PVY and late blight are regularly used in the breeding programme of the Agricultural Institute of Slovenia. As part of the ECOBREED project, new resistant sources (genotypes tested for markers by IHAR from the working collection) were included in the program.

Over 2,100 seeds from 12 resistant families were sown from the 2017 crossing year and over 1,400 seedlings were produced in 2018. The molecular markers listed in Tables 64 and 65 were used at the seedling stage and 334 late blight resistant genotypes with at least two R genes (late blight or PVY) were selected, except for families with extreme PVY resistance due to the *Solanum stoloniferum* gene of the Dutch clone CIPC 2093, for which we do not yet have the marker. In this case, we selected PVY resistance based on infection with PVY during the first two years in the field. In 2019, multiplication of 334 genotypes took place in the greenhouse to obtain 4 normal-sized tubers for field planting in 2020 and 327 genotypes produced tubers. After the first field year in 2020, 95 clones were selected (Table 32).

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Table 32 Breeding scheme and number of selected clones of 12 resistant families from crossing year 2017 after first field year in 2020.

Carolus x Sarpo Mira			
R8	Rpi-Chc1	RyChc	A
+	+	+	6
+	+	-	5
+	-	+	6
-	+	+	11
Sum of selected clones			**28**

Crossings with Sarpo Mira				Toluca	KIS 13-268/256	Alouette	White Lady	Savinja
R8	Rpi-Blb2	RyChc	RySto	B	C	D	E	F
+	+	+	-	2				
+	-	+	+				2	
+	-	+	-		6	2		2
+	-	-	+				2	
Sum of selected clones		**16**		2	6	2	4	2

Crossings with Carolus		White Lady	Kokra	KIS 09-184/223-1	KIS 09-216/66-2	KIS 07-136/164-11	Savinja
Rpi-Chc1	RySto	G	H	I	J	K	L
+	+	10				4	3
+	n.d.		4	23	7		
Sum of selected clones	**51**	10	4	23	7	4	3

After a further two years of field selection, 36 advanced clones were selected and tested again with molecular markers. We were able to introduce several late blight and PVY resistance genes into the selected genotypes and detect them by molecular markers, such as:

R8, Rpi-chc and *RY-chc*

R8, Rpi-vnt1 and *RY-chc*

R8, Rpi-blb2 and *RY-chc*

R8, RY-chc and *RY-sto*

Rpi-chc and *RY-chc*

Rpi-chc and *RY-sto*

5.2.3. MATE

In the breeding programme of the Potato Research Station of the Hungarian University of Agriculture and Life Sciences (MATE), germplasms of different wild potato species (*S. stoloniferum, S. andigena, S. demissum, S. chacoense, S. hougasii*) have been used in the past to incorporate resistance genes against virus, nematode and late blight infection into

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breeding lines and cultivars. In the ECOBREED project, resistant potato cultivars were combined with high performing commercial cultivars to maintain the high level of resistance and improve the agronomic properties of the progeny. The progeny was genotyped with molecular markers for PVX, PVY and late blight resistance genes to select promising candidates for further breeding. Over 7000 seeds from 83 families from the cross year 2021 were sown and 6800 seedlings were produced. Molecular markers were applied to 500 genotypes at seedling stage in 2022 and 184 genotypes showing at least two resistance genes (late blight and PVY) were selected and planted in the field (Table 33). Of the planted tubers, 147 genotypes produced viable tubers, of which 14 clones were selected for further development in the breeding programme after phenotypic and qualitative evaluation (Table 34). Of these 14 clones, 11 produced new tubers with favourable phenotypic and qualitative characteristics. We succeeded in obtaining these clones with PVX, PVY and late blight resistance genes so that they can be used as breeding lines or further developed into candidate cultivars.

Table 33 The number and genotype of selected clones from crosses at the Potato Research Station.

Crossings with Bellarosa										
Rx1	Rx2	Ry_{adg}	Ry_{sto}	R1	R2	R3a	**94.405**	**02.222**		
+	+	-	+	-	-	+	38	27		
-	+	-	+	-	+	+	2			
+	-	-	+	-	+	+	2			
+	-	-	+	-	-	+		9		
Selected for planting							42	36		
Advanced clones							1	2		
Crossings with White Lady										
Rx1	Rx2	Ry_{adg}	Ry_{sto}	R1	R2	R3a	**98.7804.01 [2009]**	**S440**	**98.7804.01 [2015]**	**01.1395.01**
+	+	+	+	-	+	+	18	16	13	14
+	+	+	+	-	+	-		2		2
+	+	-	+	-	+	+	3			
+	-	+	+	+	-	+		6		
+	-	+	+	-	+	+	7		5	4
-	+	-	+	+	-	+		8		
-	+	-	+	-	-	+	4		1	1
-	-	-	+	+	+	+		1		1
Selected for planting							32	33	19	22
Advanced clones								3		8

Table 34 Advanced breeding clones of selected crossings at MATE.

Clone	Family	Rx1	Rx2	Ry_{adg}	Ry_{sto}	R1	R2	R3a
22.01	94.405 × Bellarosa		+		+		+	+
22.02	02.222 × Bellarosa	+	+		+			+
22.03	02.222 × Bellarosa	+	+		+			+
22.04	White Lady × 01.1395.01	+	+	+	+		+	+
22.05	White Lady × 01.1395.01	+	+	+	+		+	+
22.06	White Lady × 01.1395.01	+	+	+	+		+	+
22.07	White Lady × 01.1395.01	+	+	+	+		+	+
22.08	White Lady × 01.1395.01	+	+	+	+		+	+
22.09	White Lady × 01.1395.01	+	+	+	+		+	+
22.10	White Lady × 01.1395.01	+		+	+		+	+
22.11	White Lady × 01.1395.01	+	+	+	+		+	+
22.12	White Lady × S440	+	+	+	+		+	
22.13	White Lady × S440	+	+	+	+		+	+
22.14	White Lady × S440	+	+	+	+		+	+

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5.3. Conclusions

Within the program of evaluation of working collection with molecular markers the most frequently observed markers were those associated with genes: *R3b* (present in 47 cultivars), *R3a* (present in 36 cultivars) and R1 (present in 16 cultivars). Markers linked to genes from the *R2* family were found in 8 cultivars. We also found markers linked to genes: *Rpi-Smira1* (present in 14 cultivars), *R8/RpiSmira2* (present in 8 cultivars), *Rpi-chc1* (present in 3 cultivars) and *Rpi-vnt1 / Rpi-phu1* (present in 3 cultivars).

MAS was successfully applied in all three breeding programes with numerous progeins selected and advanced clones produced.

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6. Production of elite cultivars and advanced breeding lines

6.1. IHAR-PIB

Within the framework of Task 3.6 in the IHAR-PIB, four crossing programs were carried out (Table 35). These programs include 22 cross combinations between donors of different sources of potato resistance, mainly against *P. infestans*. During three growing seasons (2021, 2022 and 2023), about 4320 seeds from these combinations were sown and tubers from about 2130 seedlings were harvested. Almost 1240 newly bred potato clones (1st vegetative propagation of clones from the 2020 and 2021 crosses) were planted under field conditions and simultaneously subjected to marker-assisted selection and classical phenotypic selection. From this number of individuals, 150 clones combining two sources of resistance and a high level of agronomic traits were harvested and collected in cold storage.

Fig 23 Photo of field multiplication and phenotypic selection of potato clones.

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Table 35 Crossing programs performed at IHAR-PIB: numbers of seedlings and individuals selected with the use of MAS and classical phenotypic selections.

	No. of sown seeds / harvested seedlings	No. of 1st year clones in filed	No. of selected clones after MAS	No. of clones after first phenotypic selection	No. of clones planted for second phenotypic selection	No. of clones harvested in 2023
			Crossing program 2020			
2020	2021		2022		2023	
Otolia x Levante	300 / 120	115	82	44	20	12
Levante x Carolus	300 / 150	143	30	18	12	8
Alouette x Carolus	300 / 70	58	16	7	5	4
Bzura x Carolus	300 / 120	119	26	13	7	6
EB 19 – 20 x Carolus	300 / 160	150	33	26	20	8
EB 19 – 98 x Carolus	300 / 160	134	36	26	18	11
EB 19 – 20 x Levante	300 / 120	100	26	23	20	10
EB 19 – 98 x Levante	300 / 85	65	14	14	12	9
			Crossing program 2021			
2021	2022			2023		
Otolia x Gardena	300 / 130	109	78	32	-	32
Otolia x Sarpo Mira	300 / 180	147	115	27	-	27
Bzura x Sarpo Mira	300 / 140	112	28	8	-	8
Kuba x Sarpo Mira	300 / 220	135	30	15	-	15
			Crossing program 2022			
2022	2023					
Kelly x Carolus	120 / 95	-	-	-	-	95
Kelly x Nofy	120 / 80	-	-	-	-	80
Kelly x 21-IX-6	120 / 70	-	-	-	-	70
Kelly x 21-IX-13	120 / 70	-	-	-	-	70
21-IX-6 x Carolus	120 / 80	-	-	-	-	80
21-IX-6 x Nofy	120 / 80	-	-	-	-	80
			Crossing program 2023			
Ambo x Kelly	-	-	-	-	-	10 berries
EB 22-10 x Levante	-	-	-	-	-	7 berries
P-22-13 x Charlotta	-	-	-	-	-	6 berries
P-22-13 x Levante	-	-	-	-	-	6 berries

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6.2. KIS

At the Agricultural Institute of Slovenia (KIS), crossings of mother plants are carried out every year in a greenhouse. Every year, between 150 and 165 potato plants were grown on the brick in the greenhouse in Jablje for new crosses between resistant parents and high-quality cultivars.

Fig 24 Crossings were done in greenhouse raising mother plants on the brick.

In the years 2019 to 2023, numerous successful combinations were made between LB-resistant parents from the ECOBREED working collection (and some other sources of LB resistance) and high-quality commercial cultivars. there were 9 new combinations in 2022, 22 combinations in 2020, 31 combinations in 2021, 41 combinations in 2022 and 51 combinations in 2023.

The most frequently used sources of late blight resistance genes were the cultivars Sarpo Mira, Carolus, Alouette, Twister, Twinner and KIS Kokra. In the years 2021 to 2023, new late blight and PVY resistant KIS elite clones were introduced and frequently used for crosses in the breeding program, such as KIS 15-256/247-7, KIS 16-289/261-2, A 179.

6.2.1. Selection at seedling stage and on the organic fields

Around 5000 seedlings (genotypes) of new breeding populations are planted in the greenhouse every year in July. The seedlings of the resistant progeny are artificially inoculated (sprayed) with PVY and selection for PVY susceptibility (by visual inspection) is carried out during growth.

In the following year, one tuber per genotype is planted for selection on a conventional field as a single hill plot (approx. 3000 genotypes), and in the following years in 4 hill plots (approx. 300 genotypes) and 10 hill plots (approx. 150 genotypes).

Four tubers of each selected clone were collected from the 2017 and earlier generations to be planted in an organic field in 2021. In 2021, the tubers of 229 clones from the 2017 to 2011 crossing years were planted in an organic field for selection under organic conditions. Some of the advanced clones were resistant to late blight, as demonstrated by molecular markers. All were also extremely resistant to PVY. For all generations, 4 tubers per clone were planted together with the standard cultivars KIS Slavnik, Alouette, Carolus and KIS Kokra.

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In this way, from 2021, all clones of the 10 hill generations and later at KIS will be planted in both the conventional and organic system.

6.2.2. Use of molecular markers in potato breeding program at KIS

Molecular markers for resistance genes to PVY and late blight were applied in 2020 to all families with resistant parents from the crossing years 2013 to 2017, presenting the list of markers. From 2020 on, marker analyses are performed in selection process at KIS potato breeding program every year (Table 36).

Table 36 Number of clones selected in organic breeding and tested positive by molecular markers at KIS in 2020.

Crossing year	Number of advanced clones selected in organic breeding and tested positive by molecular markers*
2011	2 advanced clones (1 late blight resistant)
2013	2 advanced clones (1 late blight resistant)
2014	18 advanced clones (3 late blight resistant)
2015	17 advanced clones (1 late blight resistant)
2016	31 clones (4 late blight resistant)
2017	159 clones (37 late blight resistant)

All clones were proven before to be PVY resistant.

6.3. MATE

At the MATE Keszthely Potato Research Station, the entire breeding program is based on the pyramiding of important resistance genes against biotic and abiotic stress factors such as viruses, late blight, nematodes, potato wart, heat and drought at the varietal level. Our aim is to develop multi-resistant cultivars with high yield potential and culinary quality. The scope of MATE resistance breeding program as part of the ECOBREED project is shown in Tables 37 and 38.

Table 37 The number of crossings and planted genotypes in the ECOBREED project at MATE.

	Successful crossings made	Families sowed	F1 seedlings planted	Single hills planted	A clones planted	B clones planted	C clones planted	D clones, cultivar candidates planted
2020	-	32	9500	3698	467	42	23	18
2021	25	83	6800	7752	232	40	14	24
2022	17	74	6920	7566	232	14	7	12
2023	35	55	12000	4187	494	41	13	16

In the years 2021 to 2023, numerous successful combinations were made between resistant parents from our working collection and some other resistance sources and high-quality commercial cultivars. 25 new combinations were formed in 2021 (Table 79), 17 combinations in 2022 (Table 80) and 35 combinations in 2023 (Table 81). In each combination, one of the parents carried PVY resistance genes derived from either *S. stoloniferum* or *S. andigena*. The most frequently used sources of late blight resistance genes were the cultivars White Lady, Sarpo Mira, Sárvári Borostyán and Kastia.

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Table 38 The number of selected genotypes in the ECOBREED project at MATE.

	F1 seedlings harvested	Single hills harvested	A clones harvested	B clones harvested	C clones harvested	D clones, cultivar candidates selected
2020	9022	389	79	19	23	12
2021	6782	506	68	28	14	9
2022	6826	478	32	22	13	8
2023	11348	481	105	19	12	9

Every year, between 6,800 and 12,000 seedlings (genotypes) of new breeding populations were planted in the greenhouse. We did not select for agronomic traits in the seedlings of the resistant families, as this is not efficient at this stage. The following year, one tuber per genotype was planted in a conventional field as a single hill plot for selection. Between 3,698 and 7,752 genotypes were planted each year.

A total of 23,203 single hills with different types of resistance genes in their pedigree were planted and evaluated under field conditions in 2020 to 2023, respectively.

Fig 25 Evaluating the clones after harvest at the breeding field of MATE.

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6.4. Conclusions

The program of production of elite potato clones using MAS was successful at all three partner institutions. Numerous breeding populations and advanced breeding clones were developed. 36 of them were offered to the partners for exchange within MS 17.

Two new potato cultivars have been registered at KIS in the last three years:

KIS Blegoš – an early, yellow-fleshed, yellow-skinned, multipurpose potato cultivar of excellent quality for canning, baking, frying and chips. It produces a high early yield of numerous medium sized tubers. It is extremely resistant to PVY, has a long dormancy period and can be stored until late spring.

KIS Tamar – a late cultivar with light yellow flesh and yellow peel of excellent quality for canning and baking. It produces a high yield of large tubers in organic fields. It is extremely resistant to PVY, tolerant to stress conditions with long dormancy, can be stored until late spring.

At MATE new candidate cultivar called Balatoni Sárga has been developed and will be registered in 2024.

KIS advanced potato clones suitable for organic production that have been included in national VCU trials over the past three years:

KIS 10-242/247-6 – late, white flesh and skin, PVY extreme resistant clone, tolerant to drought stress

KIS 11-184/257-1 – late, yellow flesh, red skin, PVY extreme resistant clone, tolerant to drought stress

KIS 13-136/235-5 – late, yellow flesh, yellow skin, PVY extreme resistant clone, tolerant to drought stress

KIS 14-136/256-26 – intermediate, yellow flesh, yellow skin, PVY extreme resistant and late blight resistant clone

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