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Genetic diversity and proteomic analysis of vegetable soybean (*Glycine max* (L.) Merrill) accessions grown in mineral and BRIS soils

*Nor Hafizah Zakaria[1], Mohd Shukor Nordin[2], Maizatul Akma Ibrahim[2,3], Fadzilah Adibah Abdul Majid[1], Zarina Zainuddin[2,3]**

[1]*Institute of Marine Biotechnology, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, Malaysia*
[2]*Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia (IIUM), Kuantan, Pahang, Malaysia*
[3]*Plant Productivity and Sustainable Resource Unit, Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia (IIUM), Kuantan, Pahang, Malaysia*

**Corresponding author: zzarina@iium.edu.my*

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Abstract: Knowledge of the molecular mechanisms of response to environmental stress is fundamental for the development of genetically stress-tolerant crops. This study aims to find vegetable soybean accessions tolerant to cultivation in stressful tropical environments. Fourteen accessions of the vegetable soybean (*Glycine max* (L.) Merrill) were grown in mineral and beach ridges interspersed with swale (BRIS) soils. The genetic diversity, estimated using inter-simple sequence repeat (ISSR) markers, revealed 42.50% polymorphism and was regarded as moderate. The unweighted pair-group method arithmetic average (UPGMA) analysis allocated the tested accessions into five major clusters at a similarity coefficient level of 0.43. The lowest values of the genetic distance were between IIUMSOY11 and IIUMSOY13 & IIUMSOY13 and IIUMSOY14, indicating that these accessions were more genetically distant from the other accessions. Ten differentially expressed proteins were identified in the three selected accessions IIUMSOY1, IIUMSOY11 and IIUMSOY14 using mass spectrometry, revealing a unique expression of the proteins involved in the storage, flavonoid metabolism, protein modification, oxidative stress defence, carbohydrate metabolism and respiratory chain. The findings may be valuable for the selection of genetically diverse accessions, to enhance the breeding of vegetable soybean genotypes suitable for stressful tropical environments.

Keywords: environmental stress; ISSR; legume; polymorphism; protein expression; tolerant accession

The vegetable soybean (*Glycine max* (L.) Merrill) is one of the main legumes cultivated worldwide and consumed in the Asian diet due to its nutritional contents, such as protein, iron, vitamins, minerals, antioxidants and omega-3 fatty acids (Rizzo & Baroni 2018). This legume has great potential to be developed commercially as an animal feed, a food, and in industrial supplements (Yang et al. 2021). The global demand for the vegetable soybean is projected to continually increase in the coming years due to various factors, such as urbanisation, population growth and climate change (Henchion et al. 2017). To meet the increasing demand for this vegetable in the future, tolerant vegetable soybean varieties must be continually developed.

Assessment of the genetic relationships among the cultivated plants at molecular levels is a fundamen-

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tal component of crop improvement programmes. It provides information on the genetic variations among breeding materials, which is essential for the parental selection for future breeding (Begna 2021). According to Awoke (2022), a major challenge to vegetable soybean production is the difficulty in producing high-quality seeds. Hence, patterns of genetic diversity could assist the future breeding approach of the vegetable soybean.

Beach ridges interspersed with swales (BRIS) contain more than 90% of sand. They have not been well utilised for crop production due to several major constraints, such as their weak structure, low water retention, low nutrient content and high soil surface temperature (Tanaka et al. 2021). Abiotic stress arising from BRIS soils may adversely affect the plant growth, resulting in an insignificant decline in the crop productivity (Devasirvatham & Tan 2018). However, plants could develop cellular and metabolic adaptive mechanisms to recover from the damaging effects (Tandzi et al. 2019). To understand these mechanisms, a proteomic approach is useful for developing adapted accessions. It plays a role in identifying and characterising key proteins underlying the plant tolerance to a given stress factor, which can be used as a protein biomarker of the given stress (Liu et al. 2019).

The present study aims to evaluate the genetic diversity in 14 vegetable soybean accessions and analyse the seed proteome of the up-regulated and down-regulated proteins grown under mineral and BRIS soils to contribute to the knowledge of the genetic diversity and the proteomic analysis of the vegetable soybean for future crop improvement.

MATERIAL AND METHODS

Study site and plant materials. Seeds of 14 vegetable soybean accessions (Table 1) from the World Vegetable Centre, Taiwan, were cultivated at two locations: (1) a field at the Glasshouse and Nursery Complex (GNC), International Islamic University Malaysia, Kuantan, Pahang, Malaysia under a mineral soil, (2) a field at the Institute of Oceanography and Maritime Studies (INOCEM), Kuantan, Pahang, Malaysia under a BRIS soil. The mineral soil was considered less stressful than the BRIS soil based on the environmental conditions of the areas, as described by Naimah et al. (2014). The experiments were conducted from May until September 2015. A randomised Complete Block Design (RCBD) with three replications was applied for the experiment. Each replication had 14 rows corresponding to 14 accessions. Eight plants were grown per row. Nitrogen, phosphorus and potassium (NPK) Blue fertiliser was used during the crop cycle with an application rate of 2 t/ha (Naimah et al. 2014).

DNA extraction and ISSR amplification. Fresh leaves were obtained from the 30-day-old plants. According to the manufacturer's protocol, the genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Germany). The inter-simple sequence re-

Table 1. The pedigree of the 14 vegetable soybean accessions used in the study

Accession name	World vegetable centre line code	Pedigree
IIUMSOY1	AGS190	Veysoy #4
IIUMSOY2	AGS192	Taisho Shiroge
IIUMSOY3	AGS464	Dada Cha 2000 × (Taisho Shiroge × Neu Ta Pien 1)
IIUMSOY4	AGS346	[Ryokkoh × (Shih Shih × SRF 400)] × EMERALD
IIUMSOY5	AGS430	Ryokkoh 75 × KS 1
IIUMSOY6	AGS432	Ryokkoh 75 × KS 1
IIUMSOY7	AGS440	Ryokkoh 75 × Tanbaguro
IIUMSOY8	AGS465	Dada Cha 2000 × (Taisho Shiroge × Neu Ta Pien 1)
IIUMSOY9	AGS471	{Ryokkoh × [(PI 157424 × KS 8) × Neu Ta Pien 2]} × Dada Cha 2000
IIUMSOY10	AGS461	Dada Cha 2000 × KS 7
IIUMSOY11	AGS472	[(PI 157424 × KS 8) × (Ryokkoh × Tzuzunoko)] × Wuye-Edou
IIUMSOY12	AGS466	Ryokkoh///PI 157424/KS8//Neu Ta Pien 2////Wuyehedou
IIUMSOY13	AGS469	Dada Cha 2000/KS7
IIUMSOY14	AGS429	(PI157424 × KS8) × Ryokkoh 75

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peat (ISSR) polymerase chain reaction (PCR) was performed according to the method described by Gul and Alinca (2015). Thirty (30) ISSR primers (Table S1 in the Electronic Supplementary Material (ESM)) were purchased from First Base Laboratories, Malaysia. The primers were first screened, and the primers that gave clear and polymorphic patterns were used for the further analysis. The DNA amplification was performed in a reaction of 20 µL containing 30 ng of the DNA as a template, 10 µM of the primer and 10 µL of the PCR Master Mix (Vivantis) and distilled water to reach a total volume of 20 µL. The thermal cycler (Eppendorf Master Cycler, USA) was programmed into the following steps: 94 °C for 5 min, then 49 cycles of denaturing at 94 °C for 1 min, annealing at 41.0–56.0 °C (depending on the primers, see Table 1) for 1 min, extension at 72 °C for 2 min, and a final extension at 72 °C for 7 min. The PCR products were stained with ethidium bromide and visualised on a 1.5% agarose gel, and compared with the 100 bp and 1 kb ladders.

Data analysis for ISSR markers. Only the ISSR primers that gave clear and reproducible bands were chosen in the analyses (Bisen et al. 2015). The ISSR bands were manually scored as present (1) or absent (0). The genetic similarities were calculated according to the Jaccard similarities coefficient. Sequential agglomerative hierarchical non-overlapping (SAHN) clustering was applied using the unweighted pair group method with arithmetic averages (UPGMA). NTSYS-pc version 2.1 was used for the genetic similarity computing and dendrogram construction.

Protein extraction and quantification. Pods containing seeds were harvested in triplicate during the reproductive growth stage (R6). Following the instruction manual, proteins were extracted using a ReadyPrep Protein Extraction Kit (Bio-Rad, USA). The total protein contents were determined by the Bradford method employing a bovine serum albumin (BSA; Sigma-Aldrich, Germany) as the standard (Guo et al. 2015).

2D gel electrophoresis. The method was performed according to Guo et al. (2015). Initially, the protein samples were separated on pH 3–10 Immobilised pH Gradient (IPG) strips (Bio-Rad, USA). The samples were further separated using a linear immobiliser, pH 4–7 (Bio-Rad, USA) to improve the resolution. The proteins (80 µg) were first separated by isoelectric focusing (IEF). The strips were suspended in a rehydration buffer [8 M urea, 2% (w/v) 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 50 mM dithiothreitol (DTT), 1% (v/v) biolyte pH3/10 ampholyte, bromophenol blue and dH_2O]. The IEF was conducted using the PROTEAN IEF system (Bio-Rad, USA) at 20 °C and performed at 100 V for 20 min, 350 V for 1 h, 3500 V for 2 h, 10 000 V for 1 h and then held at 100 V. Before the second dimension, each gel strip was incubated and equilibrated in equilibration buffer 1 [50 mM tris/HCl pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, 0.002% (w/v) of bromophenol blue and 1% (w/v) DTT]. 2.5% (w/v) iodoacetamide was added to equilibration buffer 2 instead of DTT during the second step. The proteins were then separated in vertical 12% (v/v) polyacrylamide gels in an electrophoresis system (Bio-Rad, USA) at 150 V for 60 min.

Image acquisition and data analysis. The gels were visualised from three biological replicates using a high-resolution scanner (GS-800 Calibrated Imaging Densitometer; Bio-Rad, USA). The protein spots were analysed with the Bio-Rad PDQuest software (Ver. 7.2, Bio-Rad, USA). Manual editing was performed after the automated detection and matching process. Three replicated gels for each sample were analysed. Spots that showed differences in the relative protein expression levels between the mineral and BRIS soils were considered differentially expressed proteins.

Mass spectroscopy and data search. The differentially regulated protein spots in triplicate were excised using a sterilised blade, placed in Eppendorf tubes and sent to First Base Laboratories Sdn Bhd for in-gel digestion and electrospray ionisation mass spectrometry (ESI/MS). The protein identification was achieved by analysing the MS/MS spectra (Tandem mass spectrometry) using the MASCOT sequence matching software and the Swiss-Prot databases. Then, the proteins were analysed by matching the peptide fragmentation pattern to the theoretical sequences in National Center for Biotechnology Information database (NCBI). The selection of identified proteins was made based on the best ion score and the highest amount of the E-value (Risk et al. 2013).

RESULTS AND DISCUSSION

ISSR marker analysis. For the genetic diversity analysis, the ISSR marker has proven to be a low cost and high efficiency method and has successfully been applied in plant research, especially in soybeans (Nkongolo et al. 2020). In the present study, 30 ISSR primers were screened, and only 26 primers gave reproducible polymorphic bands in all 14 vegetable

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soybean accessions. An electrophoresis image of the ISSR amplification generated by primers UBC 807, UBC 811, UBC 836, UBC 843, UBC 857 and UBC 873 is shown in Figure 1. The size of the amplified products ranged from 150–2 500 bp. The scorable loci were 248, with 109 loci being polymorphic (42.50%). The number of bands varied from 6 to 13, with an average of 9.54 bands per primer (Table S1 in the ESM). The average number of bands using the ISSR primers previously reported were 11.92 (Arslan et al. 2020) and 6.5 (Aghaei et al. 2012). The polymorphism level, calculated as the number of polymorphic bands per primer, ranged from 9.0 to 83.0%. The moderate level of polymorphism indicates that the vegetable soybean accessions came from a narrow genetic base, potentially derived from a common ancestral gene (Shakoor et al. 2022). The level of the genetic diversity obtained in the current study agrees with the findings of a few authors, who reported an average level of polymorphism, with the random amplified polymorphic DNA (RAPD) primers among the soybean (*G. max*) accessions from different countries, of 35% with the highest value (43%) from Hungary (Nkongolo et al. 2020). However, higher polymorphic levels (78.4 and 70.0%) were reported by Bisen et al. (2015) and Kumar et al. (2022) in the study of soybean genotypes analysed with SSR markers, respectively. Conflicting reports on the polymorphism of soybeans in different studies could be due to the different primers and marker systems, and the different accessions used, making the results not easily comparable. This also emphasises the importance of parental selection to avoid any genetic relatedness and maintain the genetic diversity in breeding programmes (Nkongolo et al. 2020).

As shown in Table 2, the similarity coefficient values vary from 0.34 to 0.81, the highest similarity was observed between IIUMSOY5 and IIUMSOY6 (0.81) and the lowest were between IIUMSOY11 and IIUMSOY13 & IIUMSOY13 and IIUMSOY14, showing the value of 0.34. It indicates that these accessions are genetically diverse when compared to the other

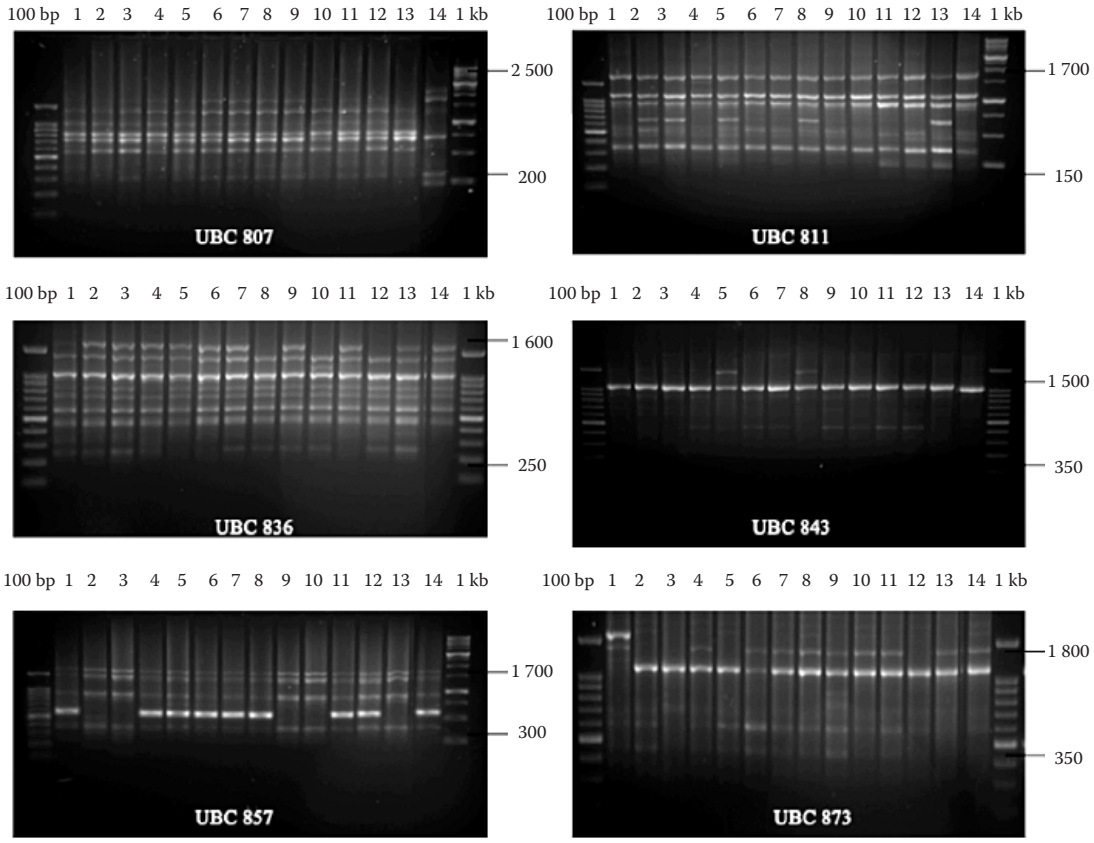

Figure 1. Banding profiles of primer UBC 807, 811, 836, 843, 857 and 873
1 – IIUMSOY1; 2 – IIUMSOY2; 3 – IIUMSOY3; 4 – IIUMSOY4; 5 – IIUMSOY5; 6 – IIUMSOY6; 7 – IIUMSOY7; 8 – IIUMSOY8; 9 – IIUMSOY9; 10 – IIUMSOY10; 11 – IIUMSOY11; 12 – IIUMSOY12; 13 – IIUMSOY13; 14 – IIUMSOY14

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Table 2. Jaccard's similarity coefficient of the 14 vegetable soybean accessions

Accession	IIUM-SOY1	IIUM-SOY2	IIUM-SOY3	IIUM-SOY4	IIUM-SOY5	IIUM-SOY6	IIUM-SOY7	IIUM-SOY8	IIUM-SOY9	IIUM-SOY10	IIUM-SOY11	IIUM-SOY12	IIUM-SOY13	IIUM-SOY14
IIUMSOY1	1													
IIUMSOY2	0.70	1												
IIUMSOY3	0.59	0.80	1											
IIUMSOY4	0.70	0.69	0.59	1										
IIUMSOY5	0.71	0.78	0.63	0.78	1									
IIUMSOY6	0.64	0.69	0.63	0.65	0.81	1								
IIUMSOY7	0.65	0.64	0.60	0.75	0.75	0.71	1							
IIUMSOY8	0.69	0.72	0.61	0.70	0.75	0.68	0.68	1						
IIUMSOY9	0.65	0.70	0.63	0.66	0.77	0.75	0.70	0.76	1					
IIUMSOY10	0.59	0.64	0.65	0.60	0.67	0.66	0.68	0.67	0.76	1				
IIUMSOY11	0.56	0.57	0.53	0.58	0.64	0.64	0.61	0.62	0.62	0.62	1			
IIUMSOY12	0.55	0.54	0.53	0.57	0.55	0.53	0.62	0.59	0.59	0.59	0.56	1		
IIUMSOY13	0.40	0.47	0.54	0.37	0.41	0.43	0.41	0.43	0.54	0.53	0.34	0.4	1	
IIUMSOY14	0.53	0.48	0.42	0.56	0.60	0.58	0.57	0.54	0.56	0.58	0.53	0.46	0.34	1

accessions, which could be the study's key finding. This germplasm derived from the IIUMSOY11 × IIUMSOY13 and IIUMSOY13 × IIUMSOY14 crossings could be rich sources of genetic diversity for future breeding purposes (Iannucci & Codianni 2019). The dendrogram constructed using the UPGMA clustering algorithm grouped the accessions into five clusters at a similarity coefficient level of 0.43 (Figure 2). Cluster 1 revealed the largest accessions, which were IIUMSOY1, IIUMSOY2, IIUMSOY3, IIUMSOY4, IIUMSOY5, IIUMSOY6, IIUMSOY7, IIUMSOY8, IIUMSOY9 and IIUMSOY10. In this cluster, IIUMSOY9 and IIUMSOY10 were closely related to IIUMSOY8, and these three accessions were grouped close to each other in a sub-cluster. IIUMSOY4 and IIUMSOY7 showed similarities with IIUMSOY5 and IIUMSOY6. IIUMSOY2 and IIUMSOY3 were grouped together in the same sub cluster. IIUMSOY1 showed some distant relationship and showed divergence compared with the other accessions present in this cluster. Cluster 2 was comprised of IIUMSOY11. IIUMSOY12 was grouped in cluster 3, and cluster 4 contained IIUMSOY14, while cluster 5 consisted of IIUMSOY13. IIUMSOY13 formed an out-group as it is not included in any of the main clusters and appeared as a separate branch at a similarity coefficient of 0.43 as the most genetically distant from the other accessions.

The dendrogram in Figure 2 showed that all the accessions were separated into five main clusters. It was suggested that the accessions within the same main cluster are closely related and could be derived from the same parental genes (Chen et al. 2020). Therefore, only one representative accession from each main cluster was chosen for the proteomic analysis to reduce the redundancy. In cluster 1, IIUMSOY1 was the most divergent compared to the other accessions. Therefore, it was selected for the analysis. Meanwhile, for the other clusters, IIUMSOY11, IIUMSOY12, IIUMSOY13 and IIUMSOY14 were further evaluated.

Proteomic analysis. The total proteins were extracted and separated by 2-DE gels. The initial separations were performed with the pH 3–10 strip. It showed that about 70% of the spots were concentrated in the region of pH 4–7. Therefore, an additional analysis with a pH 4–7 strip was undertaken to improve the spot resolution for the selected accessions of IIUMSOY1, IIUMSOY11 and IIUMSOY14. The selection was based on the abundance of protein spots in the pH 3–10 gels (Figure S1 in the ESM).

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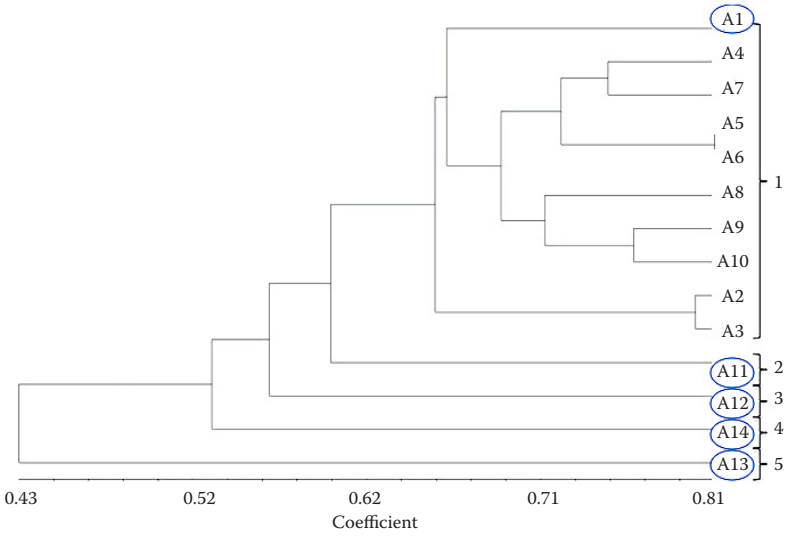

Figure 2. Dendrogram of the 14 vegetable soybean accessions
A1 – IIUMSOY1; A2 – IIUMSOY2; A3 – IIUMSOY3; A4 – IIUMSOY4; A5 – IIUMSOY5; A6 – IIUMSOY6; A7 – IIUMSOY7; A8 – IIUMSOY8; A9 – IIUMSOY9; A10 – IIUMSOY10; A11 – IIUMSOY11; A12 – IIUMSOY12; A13 – IIUMSOY13; A14 – IIUMSOY14; the circles indicate the selected accessions for the proteomic analysis

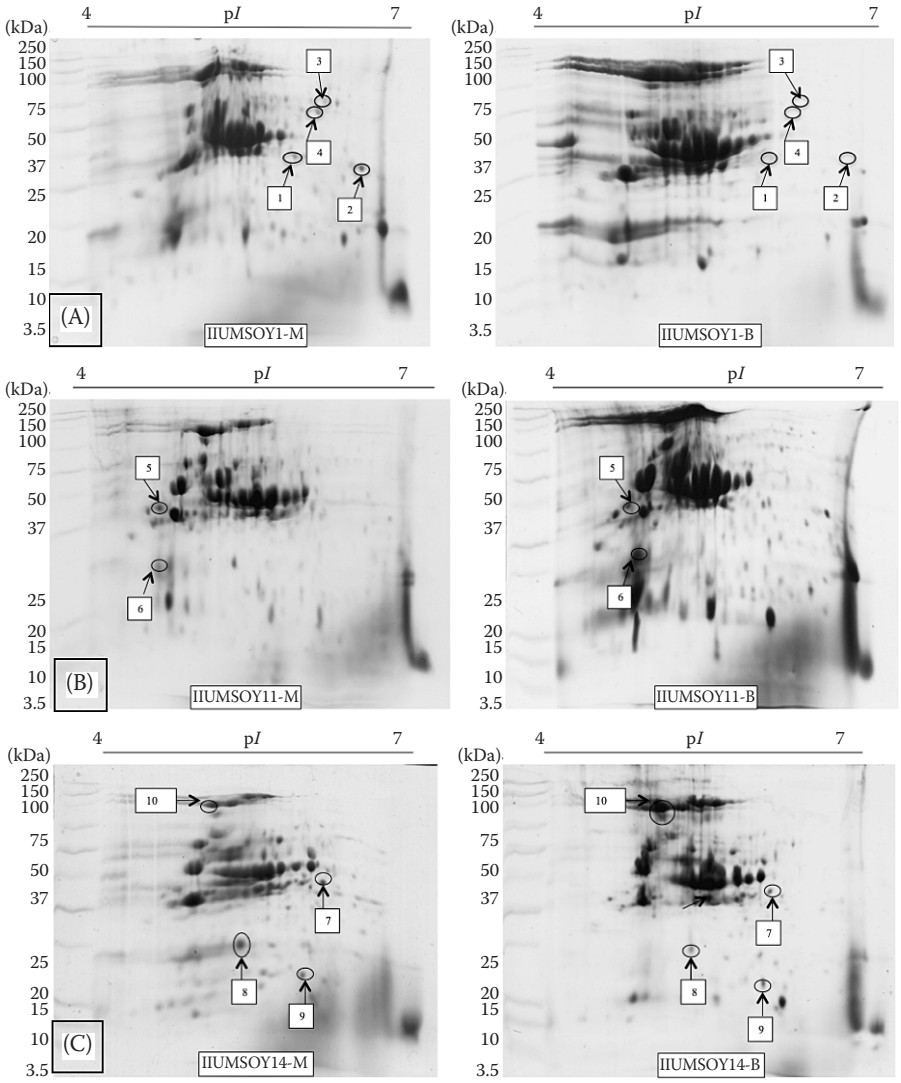

Figure 3. Differential protein expression in IIUMSOY1 (A), IIUMSOY11 (B) and IIUMSOY14 (C) using 2D-PAGE for pH 4–7
The black circles indicate the selected spots (1–10) for the protein identification; M – mineral; B – beach ridges interspersed with swale (BRIS); kDa – kilodalton (protein marker); p*I* – isoelectric point

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The PDQuest software analysis revealed a total of 239 spots for IIUMSOY1, 146 spots for IIUMSOY11 and 219 spots for IIUMSOY14 for both the mineral and BRIS soils. After being carefully viewed, four spots from IIUMSOY1, two spots from IIUMSOY11, and four spots from IIUMSOY14 were excised from the gels and selected for identification (Figure 3). The up-regulated and down-regulated proteins are summarised in Table S2 in the ESM. The identified proteins are highlighted in Table 3.

In IIUMSOY1, several protein spots (1–4) were identified, which were lectins, glucose and ribitol dehydrogenase (Glc/RibDH) and β-conglycinin, β-subunit (two spots). Lectin, a storage protein, showed low expression in the BRIS soil. Soy lectins are susceptible to heat stress. Therefore, the expression of this storage protein could be halted by the high temperature of the BRIS soil (Krager 2020). Glc/RibDH was expressed in a low amount under the BRIS soil. On the contrary, Witzel et al. (2010) reported the overexpression of Glc/RibDH in the tolerant lines of barley germinated under salinity stress.

Two spots of β-subunit of β-conglycinin were resolved from the analysis. These spots had different molecular weights, but the same isoelectric point. The variation in the protein spot distribution was most likely due to the post-translational modifications (Natarajan 2014). It was found that β-subunit of β-conglycinin accumulated in a low amount in the BRIS soil compared to the mineral soil, suggesting that this accession could not cope with the soil condition and consequently disrupted the expression of the storage proteins.

In IIUMSOY11, two protein spots were identified as probable thiol protease and Kunitz type trypsin inhibitor A. In the BRIS soil, probable thiol protease was down-regulated. A previous study on the drought stress of soybean nodules reported a similar result (Du Plessis et al. 2013). Meanwhile, the Kunitz trypsin inhibitors (KTIs) were expressed in a higher amount in the BRIS soil. This result is consistent with the Mafelo et al. (2020) study. They found that trypsin inhibitors played an important role in reducing the drought-induced oxidative stress in the *Arabidopsis* plant. In addition, evidence of proteases that lead to oxidative damage has previously been reported, indicating the involvement of this protein in abiotic stress susceptibility (Malefo et al. 2020).

In IIUMSOY14, isoflavone reductase (IFR) was identified. IFR is a crucial enzyme in the general flavonoid biosynthesis (Liu et al. 2021). In the cur-

Table 3. Differentially expressed proteins identified using ESI/MS/TOF-MS

Spot No.	M_w	NCBI accession No.	p*I*	Protein score/ ion score	E-value	Protein name	Function	Expression level	
								mineral	BRIS
1	30.90	gi|356499954	5.65	734/119	1.3e-12	lectin	storage protein	↑	↓
2	31.64	gi|1044537773	6.34	333/72	7.5e-05	glucose and ribitol dehydrogenase	carbohydrate metabolism	↑	↓
3	50.51	gi|121282	5.88	1 829/103	1.2e-10	β-conglycinin, β – subunit	storage protein	↑	↓
4	50.41	gi|1174098436	5.88	1 586/123	4e-10	β-conglycinin, β-subunit	storage protein	↑	↓
5	42.77	gi|129353	5.74	91/55	9.2e-06	probable thiol protease	protein modification	↑	↓
6	23.99	gi|125020	4.99	873/109	2e-11	Kunitz type trypsin inhibitor A	protein modification	↓	↑
7	33.94	gi|1198333535	5.75	92/37	0.22	isoflavone reductase	flavonoid metabolism	↑	↓
8	19.91	gi|951016693	5.20	80/66	0.00	ATP synthase subunit d, mitochondrial	respiratory chain	↑	↓
9	15.18	gi|351725359	5.27	80/38	0.00	superoxide dismutase	stress related protein	↓	↑
10	70.25	gi|121281	5.07	6 978/ 135	4.7e-14	β-conglycinin, α subunit	storage protein	↓	↑

M_w – molecular weight; p*I* – isoelectric point; BRIS – beach ridges interspersed with swale

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rent study, the low expression of IFR (BRIS soil) indicated that flavonoid compounds do not have a primary role in providing tolerance to environmental stress. A similar finding was reported in the study by Sobhanian et al. (2010), which found that IFR was down-regulated in the soybean seedlings under salt stress. Mitochondrial Adenosine triphosphate (ATP) synthase subunit d produces ATP from adenosine diphosphate (ADP) in the presence of a proton gradient across the membrane, which is generated by electron transport complexes of the respiratory chain (Neupane et al. 2019). This protein was expressed in a low concentration, suggesting a decrease in the ATP pool of the vegetable soybean cells in IIUMSOY14.

Next is superoxide dismutase (SOD), an antioxidant that protects cellular components from being oxidised by reactive oxygen species (ROS) (Ighodaro & Akinloye 2018). In this study, the SOD expression was up-regulated in the BRIS soil. SOD might respond to the high level of ROS accumulated when the plants were exposed to stress factors. BRIS soil has been described as a problematic soil which could induce oxidative stress in plants (Mustapha et al. 2017). A high accumulation of SOD in response to drought and salinity stress in soybeans was reported by Aleem et al. (2022). It is interesting to note that α subunit of β-conglycinin was highly induced in IIUMSOY14 grown under the BRIS soil. This finding is supported by Carrão-Panizzi et al. (2008) who studied the environmental effect on β-conglycinin of soybean cultivars in Brazil, citing that different protein fractions have a different thermal stability. They suggested that α subunit of β-conglycinin is thermally more stable in the heat condition than other subunits.

CONCLUSION

To our best knowledge, this is the first comprehensive study on the genetic diversity and proteomic analysis of the vegetable soybean under field conditions of mineral and BRIS soils. One major cluster consisting of a large number of accessions indicated a high degree of genetic resemblance and a narrow genetic base among the accessions used in the study. Due to the diverse similarity coefficients, the crossings of IIUMSOY11 × IIUMSOY13 and IIUMSOY13 × IIUMSOY14 could be used for future hybridisation programmes. Meanwhile, the proteomic analysis showed divergent stress responses among the accessions. The upregulations of KTI, SOD, and α subunit of β-conglycinin in IIUMSOY11 and IIUMSOY14 are the breakthrough information gleaned from the study which should be further explored in future works. Based on their diverse genetic relationships and proteomic responses, these two promising accessions may also contribute to the genetic enhancement of environmental stress tolerant varieties in the future.

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REFERENCES

Aghaei M., Darvishzadeh R., Hassani A. (2012): Molecular characterisation and similarity relationships among Iranian basil (*Ocimum basilicum* L.) accessions using inter simple sequence repeat markers. The Revista Ciência Agronômica, 43: 312–320.

Aleem M., Aleem S., Sharif I., Wu Z., Tahir A., Atif R.M., Cheema H.M.N., Shakeel A., Lei S., Yu D., Wang H., Kaushik P., Alyemeni M.N., Bhat J.A., Ahmad P. (2022): Characterisation of *SOD* and *GPX* gene families in the soybeans in response to drought and salinity stresses. Antioxidants, 11: 460.

Arslan E., Gülbahçe Mutlu E., Dursun Ö., Bağcı S.A. (2020): Comparative analysis of agronomic traits and ISSR method among some soybeans [*Glycine Max* (L.) Merr.] genotypes. KSU Journal of Agricultural Nature, 2: 687–696.

Awoke T. (2022): Performance evaluation of soybean (*Glycine max* L.) varieties for growth, yield and yield components under irrigation at lowland area of south Omo zone, Southern Ethiopia. Journal of Agriculture and Aqua-culture, 4: 1–6.

Begna T. (2021): Role and economic importance of crop genetic diversity in food security. Journal of Agricultural Science and Food Technology, 7: 164–169.

Bisen A., Khare D., Nair P. (2015): SSR analysis of 38 genotypes of soybean (*Glycine max* (L.) Merr.) genetic diversity in India. Physiology Molecular Biology Plants, 21: 109–115.

Carrão-Panizzi M.C, Kwanyuen P., Erhan S.Z., Lopes I.O.N. (2008): Genetic variation and environmental effects on beta-conglycinin and glycinin content in Brazilian soybean cultivars. Brazil Agricultural Research, 43: 1105–1114.

Chen H., Guo A., Wang J., Gao J., Zhang S., Zheng J., Huang X., Xi J., Yi K. (2020): Evaluation of genetic diversity within asparagus germplasm based on morphological traits and ISSR markers. Physiology and Molecular Biology of Plants, 26: 305–315.

https://doi.org/10.17221/38/2022-CJGPB

Devasirvatham V., Tan D. (2018): Impact of high temperature and drought stresses on chickpea production. Agronomy, 8: 145.

Du Plessis M., Vorster J., Kunert K. (2013): Cysteine protease expression and activity in soybean nodules during development and stress. South African Journal of Botany, 86: 164.

Gul I., Alinca S. (2015): Molecular characterisation and determination of morphological traits of the button medic (*Medicago orbicularis* L.). Pakistan Journal of Biological Sciences, 18: 141–145.

Guo C.G., Shang Z., Yan J., Li S., Li G.Q., Liu R.Z. (2015): A tunable isoelectric focusing via moving reaction boundary for two-dimensional gel electrophoresis and proteomics. Talanta, 137: 197–203.

Henchion M., Hayes M., Mullen A.M., Fenelon M., Tiwari B. (2017): Future protein supply and demand: Strategies and factors influencing a sustainable equilibrium. Foods, 6: 53.

Iannucci A., Codianni P. (2019): Phenotypic parent selection within a khorasan wheat collection and genetic variation in advanced breeding lines derived by hybridization with durum wheat. Frontiers in Plant Science, 20: 1460.

Ighodaro O.M., Akinloye O.A. (2018): First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine, 54: 287–293.

Krager J. (2020): Assessing the quantification of soy protein in incurred matrices using targeted LC-MS/MS. [Master Thesis.] Lincoln, University of Nebraska.

Kumar S.P.J., Susmita C., Sripathy K.V. (2022): Molecular characterisation and genetic diversity studies of Indian soybean (*Glycine max* (L.) Merr.) cultivars using SSR markers. Molecular Biology Report, 49: 2129–2140.

Liu W., Feng Y., Yu S., Fan Z., Li X., Li J., Yin H. (2021): The flavonoid biosynthesis network in plants. International Journal of Molecular Sciences, 22: 12824.

Liu Y., Lu S., Liu K. (2019): Proteomics: A powerful tool to study plant responses to biotic stress. Plant Methods, 15: 135.

Malefo M.B., Mathibela E.O., Crampton B.G., Makgopa M.E. (2020): Investigating the role of Bowman-Birk serine protease inhibitor in *Arabidopsis* plants under drought stress. Plant Physiology and Biochemistry, 149: 286–293.

Mustapha Z., Mat N., Othman R., Zakaria A.J. (2017): Quantification of BRIS soil bacteria at Tembila, Besut Terengganu. AGRIVITA Journal of Agricultural Science, 39: 252–256.

Naimah R., Nur Amirah Y., Adzemi M.A., Wan Zaliha W.S. (2014): Quality and growth development of roselle grown on bris soil in relation to regulated deficit irrigation. Journal of Tropical Plant Physiology, 6: 23–34.

Natarajan S.S. (2014): Analysis of soybean seed proteins using proteomics. Journal of Data Mining, Genomic & Proteomic, 5: 113.

Neupane P., Bhuju S., Thapa N., Bhattarai H. (2019): ATP synthase: Structure, function and inhibition. Biomolecular Concepts, 10: 1–10.

Nkongolo K., Alamri S., Michael P. (2020): Assessment of genetic variation in soybean (*Glycine max*) accessions from international gene pools using RAPD markers: Comparison with the ISSR system. American Journal of Plant Sciences, 11: 1414–1428.

Risk B.A., Edwards N.J., Giddings M.C. (2013): A peptide-spectrum scoring system based on ion alignment, intensity, and pair probabilities. Journal of Proteome Research, 12: 4240–4247.

Rizzo G., Baroni L. (2018): Soy, soy foods and their role in vegetarian diets. Nutrients, 10: 43.

Shakoor A., Zaib G., Zhao F., Li W., Lan X., Esfandani-Bozchaloyi S. (2022): ISSR markers and morphometry determine genetic diversity and population structure in *Hedera helix* L. Czech Journal Genetics and Plant Breeding, 58: 73–82.

Sobhanian H., Razavizadeh R., Nanjo Y., Ehsanpour A., Jazii F., Motamed N., Komatsu S. (2010): Proteome analysis of soybean leaves, hypocotyls and roots under salt stress. Proteomic Science, 8: 19.

Tanaka S., Saito H., Kajiwara N., Paing T.N., Mohd Yusoff K.H., Abe S.S., Nakao A., Yanai J. (2021): Long-term changes in paddy soil fertility in Peninsular Malaysia during 50 years after the Green Revolution with special reference to their physiographic environments. Soil Science and Plant Nutrition, 67: 80–88.

Tandzi L.N., Bradley G., Mutengwa C. (2019): Morphological responses of maise to drought, heat and combined stresses at seedling stage. Journal of Biological Sciences, 19: 7–16.

Witzel K., Weidner A., Surabhi G.K., Varshney R.K., Kunze G., Buck-Sorlin G.H., Börner A., Mock H.P. (2010): Comparative analysis of the grain proteome fraction in barley genotypes with contrasting salinity tolerance during germination. Plant, Cell & Environment, 33: 211–222.

Yang K., Qing Y., Yu Q., Tang X., Chen G., Fang R., Liu H. (2021): By-product feeds: Current understanding and future perspectives. Agriculture, 11: 207.

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