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Original Research

Selection of Tolerant and Susceptible Wild Soybean (*Glycine soja* Siebold & Zucc.) Accessions under Waterlogging Condition using Vegetation Indices

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Abstract

The waterlogging tolerance and susceptibility of 164 wild soybean accessions were evaluated. All plants were exposed to waterlogging conditions for 14 days, and visual score evaluation and detection of vegetation indices were performed at 14 and 21 days after waterlogging (DAW). According to our results, approximately 90% of the wild soybean accessions showed a visual score of 1.0-3.5 in both measurements. Among the 26 vegetation indices, only 17 showed statistically high correlation with visual score; however, the maximum *P*-value was less than −0.58. Therefore, correlation tests were re-performed using the selected wild soybean accessions (waterlogging-tolerant and waterlogging-susceptible accessions). As a result, significantly high *P*-values were detected for anthocyanin reflectance index (ARI1) (P = 0.98069 at 14 DAW; P = 0.86734 at 21 DAW), ARI2 (P = 0.98434 at 14 DAW; P = 0.87934 at 21 DAW), photochemical reflectance index (P = −0.9801 at 14 DAW; P = −0.9268 at 21 DAW), and simple ratio pigment index (P = −0.8841 at 14 DAW; P = −0.81292 at 21 DAW). Root morphological traits also showed significant differences between waterlogging-tolerant and waterlogging-susceptible accessions. In waterlogging-tolerant accessions, root length was 3.7–5.5-fold higher than that in waterlogging-susceptible accessions. Furthermore, waterlogging-tolerant accessions showed a 14.3%-56.3% increase in projected area compared with in waterlogging-susceptible accessions.

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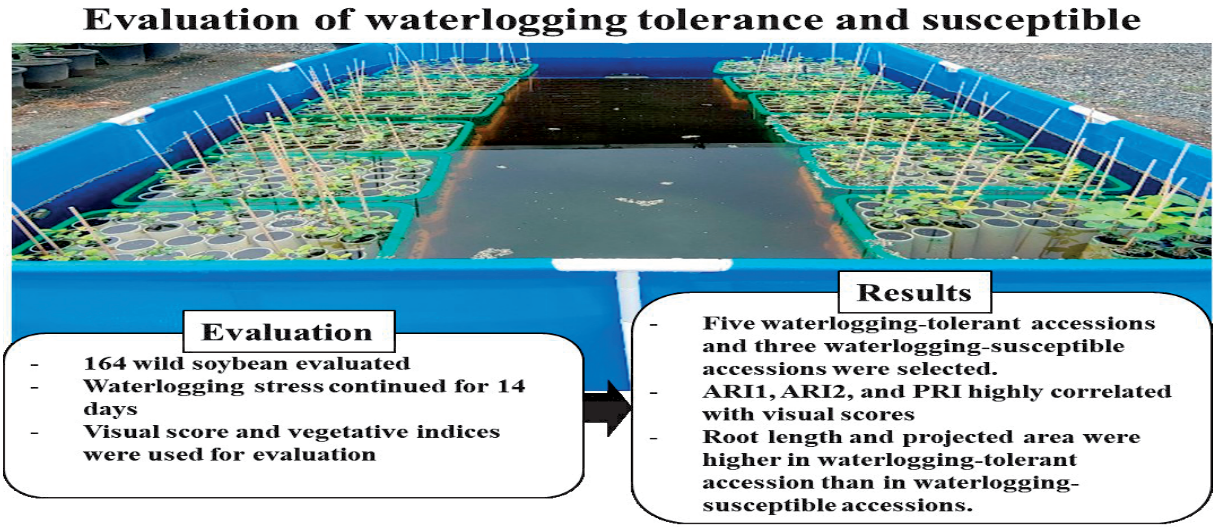
Graphical abstract



Keywords: root morphological traits, vegetation index, waterlogging, WinRHIZO

Introduction

Waterlogging stress is a major problem for the production of various crops, including cotton [1, 2], maize [2], oats [3], soybean [4], and sugarcane [5]. For soybean, waterlogging can result in heavy yield loss (17%–57%); therefore, several waterlogging-tolerant cultivars have been screened by soybean breeders [6, 7]. Furthermore, candidate quantitative trait loci also have been reported; however, the detailed physiological and genetic mechanisms of waterlogging in soybeans remain unknown [6, 7].

Waterlogging stress is caused by high water levels in the cultivated field during heavy precipitation, river flooding, and excessive irrigation [4]. Flash waterlogging in well-drained field conditions does not hinder the growth and development of soybean plants; however, waterlogging for an extended period of long restricts ordinary growth and development of the crop [8]. Waterlogging conditions in arable land result in the covering of soil pores with water; this leads to anoxia or hypoxia in the soil [4, 8]. Basically, plants need to absorb oxygen from the soil for various physiological processes, including mitochondrial respiration, which generates energy resources, such as nicotinamide adenine dinucleotide and adenosine triphophate. In the absence of oxygen, plants switch to an alternative physiological process called fermentation, which produces ethanol and lactate [9, 10]. Even with this alternative pathway, plants are unable to survive long-term waterlogging stress due to the lack of energy resources [11]. To cope with this, plants induce morphological changes in their shoot and root for capturing or transferring oxygen from the atmosphere to the plant body, particularly in the root zone [4, 5, 11]. The production of aerenchyma cells in the shoot and root is the most common response to waterlogging, as observed in rice, maize, and soybean [2, 11, 12]. Therefore, selecting soybean accessions that can easily produce aerenchyma cells in their body during waterlogging conditions will aid in the development of waterlogging-resistant cultivars.

Many plant breeders have evaluated waterlogging-tolerant and waterlogging-susceptible soybean (*Glycine max*) accessions generated via mapping population and reported several QTLs in chromosome 3, 5, 10, 11, 13, and 18, which are linked to waterlogging tolerance [7, 13-15]. However, the evaluation of waterlogging resistance and production of mapping population in wild soybeans (*Glycine soja*) remain unexplored to date. Wild soybeans are grown in a complex geography as well as in a wide range of climatic conditions; therefore, they often show improved stress tolerance than other cultivars [16]. Additionally, wild accessions confer to a wide range of genetic resources in major crops and are regarded as an important resource for plant breeding [17]. Wild soybean is used as food and feed with *Glycine max* and is also regarded as the progenitor of *Glycine max* [18]. Therefore, wild soybean has been considered a treasure of genetic resources, with high oil content, disease resistance, and environmental stress tolerance [18]. Despite wild soybean being an important genetic resource, adequate information on its resistance to waterlogging conditions is lacking. Therefore, this experiment was performed to evaluate waterlogging tolerance and sensitivity in wild soybean accessions. A total of 164 wild soybean accessions were evaluated using a state-of-the-art technology for wild soybean phenotyping.

Experimental Procedures

Plant Materials and Growth Conditions

Initially we planted 466 wild soybean seeds per replication; however, the data for only 164 seeds for three replications were obtained as the germination rate was low (Fig. 1). Accessions were donated by the Gene Bank of Korea, plant introduction (PI) was from USDA-ARS, Chung's wild germplasm collection (CW) was from Chonnam National University, Korea [19], and YWSs were from Yeungnam University (Prof. Eui-Ho Park) (Table S1). Seeds were scarified with a help of a nail clipper to enhance water uptake. The seeds were sown into polyvinyl chloride (PVC) pipes [6 cm (diameter) × 40 cm (height)] containing horticultural soil (Tobirang, Baekkwang Fertility, South Korea). When the seeds germinated, all pots were placed in a greenhouse located in the research center of Kyungpook National University, Daegu, South Korea. When the wild soybeans reached the V1 growth stage, all pots were placed in a pool of water for 2 weeks to ensure waterlogging conditions. Our experiment was conducted in three replicates per accession (n = 1). The 326, 339, and 335 wild soybean accessions were germinated in each replication. During the three replicates, only 164 wild soybean accessions were consistently germinated due to non-uniform seed germination (Fig. 1). The experiment began on June 3, 2019 and ended on September 5, 2019.

Analysis of Vegetation Index, Chlorophyll Content, and Visual Scores

To evaluate stress levels, we measured vegetation indices, chlorophyll content, and visual scores of soybean plants before and after waterlogging [(14 and 21 days after waterlogging (DAW)]. First, we measured various vegetation indices using PolyPen (RP410, Photon Systems Instruments, Czech Republic). To gather uniform data, we selected the second trifoliate leaf from every plant for measurement, and the average value of three different points was used for analysis. We used a chlorophyll meter (MC-100, Apogee Instruments Inc., USA) for determining the chlorophyll content and used the same leaf position for chlorophyll content measurement. All relevant data were collected from three replicates (n = 3). The equation of vegetation indices is shown in Table S2. In addition, we assessed the visual scores of soybean plants at 14 and 21 DAW and used a 1-5 scoring scale based on the extent of plant damage (Fig. 2). The wild soybean accessions were exposed to waterlogging for 14 days and scored 1 to 5 based on the damage symptom at 14 and 21 DAW. A visual score of 1 indicates no plant damage (healthy plant), score 3 indicates a 50% change in leaf color, and 5 indicates more than 80% of leaf color is yellow and red. Data were collected three times and are presented as the average±standard error (n = 3).

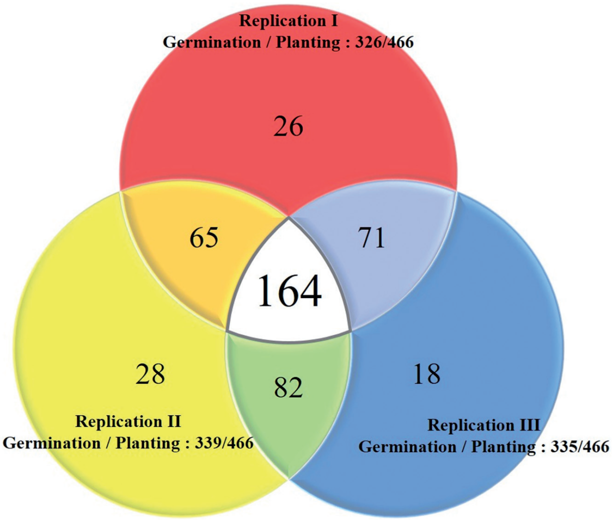


Fig. 1. Information on the number of germinated seeds for the three replicates. We used 164 seeds to evaluate waterlogging tolerance and susceptibility.

Determination of Shoot and Root Phenotype

Shoot and root samples were harvested at 21 DAW. We cut the shoot and root with a pair of scissors and immediately captured shoot images at a mini-studio to prevent drying of the leaves. The collected shoot images were analyzed using the WinRHIZO pro software (Regent Instruments Inc. Canada). For root collection, we poured soil from the pipes into a sieve and carefully removed the root from the soil. The collected roots were thoroughly washed with clean water to remove adhering soil particles and were stored in a plastic bag with distilled water to prevent them from drying. Root morphological traits were analyzed using the WinRHIZO pro software with captured images from a scanner (Expression 12000XL, Epson, Japan). The soil particles were further removed from the root samples and placed in a transparent tray (30 cm long × 20 cm wide) containing clean water for scanning.

Statistics Analysis

To determine the differences in data, we performed analysis of variance (ANOVA) (SAS release 9.4; SAS, Gary, NC, USA) for the visual scores and all phenotypic data. Mean value differences were determined using the Student's t-test at significance levels of P<0.05 and P<0.01. In addition, correlation analysis was conducted at a significance level of P<0.05. We performed statistical analysis with the data from the three replicates in order to obtain reliable results.

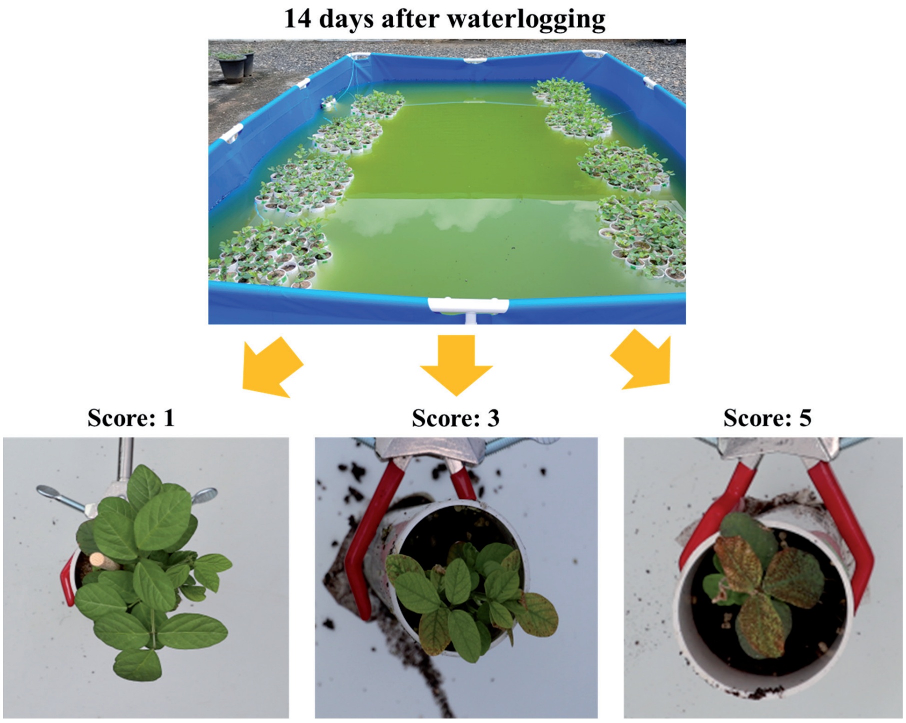


Fig. 2. Evaluation of waterlogging tolerance in wild soybeans. The wild soybean accession were exposed to waterlogging for 14 days and scored 1–5 based on the damage symptoms at 14 and 21 days after waterlogging. A visual score of 1 indicates no plant damage (healthy plant), score of 3 indicates a 50% change in leaf color was changed, score of 5 indicates over 80% of leaf color is yellow and red.

Results

Influence of Waterlogging Stress on Chlorophyll Content and Vegetation Index

According to ANOVA, all vegetation indices and chlorophyll contents showed significant differences in the 164 wild soybean accessions before waterlogging treatment (BW) (Table 1). At 14 and 21 DAW, all vegetation indices and chlorophyll contents showed significant differences at in 164 wild soybean accessions (P<0.0001) (Tables 2 and 3).

Selection of Waterlogging-Tolerant and Waterlogging-Susceptible Accessions

The condition of the plants was visually analyzed and rated on a scale of 1-5. Score 1 indicated that all plants showed no stress injury and score 5 denoted dead plants [20] (Fig. 2). The 466 wild soybeans were evaluated for waterlogging tolerance and susceptibility at an early growth stage. Based on leaf injury (yellow and red spots), score (1.0-2.0) was regarded as resistant to waterlogging and score (2.1-4.0) was regarded as moderately resistant to waterlogging. Likewise, score (4.1-5.0) was considered as susceptible to waterlogging. In this study, 22.0%, 75.6%, and 2.4% of wild soybean accessions were evaluated as resistant, moderately resistant, and susceptible to waterlogging, respectively at 14 DAW (Fig. 3). At 21 DAW, 34.8%, 64.0%, and 1.2% of wild soybean accessions were resistant, moderately resistant, and susceptible to waterlogging, respectively (Fig. 3). Most of the wild soybean accessions were resistant or moderately resistant when exposed to waterlogging for 14 days, whereas only 1.2% of soybean accessions were extremely susceptible to waterlogging stress. Based on the visual scores, five accessions that were highly tolerant to waterlogging stress (CW11598, CW14633, YWS 76, YWS 469, and YWS 602) and 3 accessions that were sensitive to waterlogging stress (CW11948, YWS 85, and YWS 545) were selected.

Correlation Test between the Visual Scores and Vegetation Indices in 164 Wild Soybean Accessions

A correlation analysis test between various vegetation indices and visual scores was performed to identify appropriate vegetation indices for stress resistance prediction. As shown in Table 4, many vegetation indices showed high correlation with visual scores. At 14 DAW, all vegetation indices, except chlorophyll content, (r = −0.06056, P<0.411) showed significant correlation with visual scores (Table 4). In particular, photochemical reflectance index (PRI) revealed the highest correlation (r = −0.57181, P<0.0001) with visual scores (Table 4). The same result was observed at 21 DAW; however, the correlation values of each vegetation index generally decreased compared with those at 14 DAW (Table 4). Similar to 14 DAW, the correlation between PRI and visual score showed a maximum value (r = −0.37995,

Table 1. Analysis of variance (ANOVA) results of the vegetation indices of the 164 wild soybean accessions. All data were collected before waterlogging treatment.

Vegetative index	Source of variation	*df*	Mean Square	*F*-Value	*P*-value	Vegetative index	Source of variation	*df*	Mean Square	*F*-Value	*P*-value	Vegetative index	Source of variation	*df*	Mean Square	*F*-Value	*P*-value
Chlorophyll	Var[1]	163	6407.633	7.62	<.0001	**ZMI**	Var	163	0.03432287	8.72	<.0001	**SIPI**	Var	163	0.00461885	7.62	<.0001
	Rep[2]	2	38806.351	46.14	<.0001		Rep	2	0.02367072	6.01	0.0152		Rep	2	0.00000116	0	0.9651
NDVI	Var	163	0.00758236	8.28	<.0001	**SPRI**	Var	163	0.00784379	4.51	<.0001	**GM1**	Var	163	0.22445043	7.75	<.0001
	Rep	2	0.00148598	1.62	0.2045		Rep	2	0.00908618	5.22	0.0236		Rep	2	0.06573764	2.27	0.1339
SR	Var	163	1.8398325	8.06	<.0001	**NPQI**	Var	163	0.00061671	3.72	<.0001	**GM2**	Var	163	0.20909681	8.03	<.0001
	Rep	2	0.1670393	0.73	0.3937		Rep	2	0.00000004	0	0.9872		Rep	2	0.08438909	3.24	0.0737
MCARI1	Var	163	0.00443364	3.09	<.0001	**PRI**	Var	163	0.00032002	7.6	<.0001	**ARI1**	Var	163	0.05932817	7.25	<.0001
	Rep	2	0.0011741	0.82	0.3669		Rep	2	0.00027923	6.63	0.0109		Rep	2	0.00498608	0.61	0.4362
OSAVI	Var	163	0.00306656	6.14	<.0001	**NPCI**	Var	163	0.00209454	4.44	<.0001	**ARI2**	Var	163	0.01525476	7.5	<.0001
	Rep	2	0.00002972	0.06	0.8076		Rep	2	0.00258569	5.48	0.0205		Rep	2	0.00106986	0.53	0.4693
G	Var	163	0.26293202	6.67	<.0001	**Ctr1**	Var	163	0.24416801	7.66	<.0001	**CRI1**	Var	163	4.2361635	8.69	<.0001
	Rep	2	0.09155693	2.32	0.1294		Rep	2	0.34213791	10.74	0.0013		Rep	2	0.1442406	0.3	0.5871
MCARI	Var	163	0.02483411	5.65	<.0001	**Ctr2**	Var	163	0.00696887	8.49	<.0001	**CRI2**	Var	163	4.6979871	8.73	<.0001
	Rep	2	0.03137675	7.14	0.0083		Rep	2	0.00361835	4.41	0.0373		Rep	2	0.2028623	0.38	0.5401
TCARI	Var	163	0.0166351	5.51	<.0001	**Lic1**	Var	163	0.00433383	6.97	<.0001	**RDVI**	Var	163	0.00213862	5.14	<.0001
	Rep	2	0.01965736	6.51	0.0117		Rep	2	0.00007006	0.11	0.7376		Rep	2	0.00001834	0.04	0.834
TVI	Var	163	6.0645512	3.15	<.0001	**Lic2**	Var	163	0.01015558	8.21	<.0001						
	Rep	2	0.8940429	0.46	0.4963		Rep	2	0.02274902	18.39	<.0001						

Var: variety, Rep: replication

Table 2. Analysis of variance (ANOVA) results of vegetation indices of the 164 wild soybean accessions. All data were collected 14 days after waterlogging treatment.

Vegetative index	Source of variation	*df*	Mean Square	*F*-Value	*P*-value	Vegetative index	Source of variation	*df*	Mean Square	*F*-Value	*P*-value	Vegetative index	Source of variation	*df*	Mean Square	*F*-Value	*P*-value
Chlorophyll	Var[1]	163	7371.372	2.63	<.0001	**ZMI**	Var	163	0.050567	2.49	<.0001	**SIPI**	Var	163	0.006089	2.52	<.0001
	Rep[2]	2	521328.2	186.11	<.0001		Rep	2	0.734959	36.14	<.0001		Rep	2	0.035141	14.53	<.0001
NDVI	Var	163	0.018603	2.63	<.0001	**SPRI**	Var	163	0.036505	2.84	<.0001	**GM1**	Var	163	0.257567	2.43	<.0001
	Rep	2	0.013898	1.96	0.1185		Rep	2	0.055608	4.33	0.005		Rep	2	0.28723	2.72	0.0443
SR	Var	163	2.336441	2.66	<.0001	**NPQI**	Var	163	0.000525	1.88	<.0001	**GM2**	Var	163	0.302928	2.56	<.0001
	Rep	2	3.031965	3.45	0.0165		Rep	2	0.001813	6.51	0.0003		Rep	2	0.982214	8.3	<.0001
MCARI1	Var	163	0.013407	2.21	<.0001	**PRI**	Var	163	0.001627	2.78	<.0001	**ARI1**	Var	163	0.268516	3.08	<.0001
	Rep	2	0.752067	123.91	<.0001		Rep	2	0.054324	92.96	<.0001		Rep	2	0.812543	9.32	<.0001
OSAVI	Var	163	0.007092	2.43	<.0001	**NPCI**	Var	163	0.01286	2.84	<.0001	**ARI2**	Var	163	0.05851	3.11	<.0001
	Rep	2	0.080559	27.63	<.0001		Rep	2	0.006908	1.52	0.2073		Rep	2	0.254077	13.49	<.0001
G	Var	163	0.572271	2.43	<.0001	**Ctr1**	Var	163	0.508289	2.44	<.0001	**CRI1**	Var	163	2.382385	2.64	<.0001
	Rep	2	37.80984	160.71	<.0001		Rep	2	31.22846	150.03	<.0001		Rep	2	97.40037	107.84	<.0001
MCARI	Var	163	0.040674	2	<.0001	**Ctr2**	Var	163	0.022111	2.68	<.0001	**CRI2**	Var	163	2.417497	2.6	<.0001
	Rep	2	5.326759	261.94	<.0001		Rep	2	0.117936	14.29	<.0001		Rep	2	115.8652	124.39	<.0001
TCARI	Var	163	0.031382	2.1	<.0001	**Lic1**	Var	163	0.008754	2.46	<.0001	**RDVI**	Var	163	0.00512	2.39	<.0001
	Rep	2	3.408751	227.96	<.0001		Rep	2	0.11077	31.09	<.0001		Rep	2	0.089973	42.03	<.0001
TVI	Var	163	18.96063	2.27	<.0001	**Lic2**	Var	163	0.023568	2.53	<.0001						
	Rep	2	775.9912	92.88	<.0001		Rep	2	0.300432	32.25	<.0001						

Var: variety, Rep: replication

Table 3. Analysis of variance (ANOVA) results of vegetation indices of the 164 wild soybean accessions. All data were collected 21 days after waterlogging treatment.

Vegetative index	Source of variation	*df*	Mean Square	*F*-Value	*P*-value	Vegetative index	Source of variation	*df*	Mean Square	*F*-Value	*P*-value	Vegetative index	Source of variation	*df*	Mean Square	*F*-Value	*P*-value
Chlorophyll	Var[1]	163	5388.391	3.23	<.0001	**ZMI**	Var	163	0.03737317	2.6	<.0001	**SIPI**	Var	163	0.002652	2.14	<.0001
	Rep[2]	2	112969.1	67.69	<.0001		Rep	2	0.41875887	29.19	<.0001		Rep	2	0.290153	233.73	<.0001
NDVI	Var	163	0.007959	2.32	<.0001	**SPRI**	Var	163	0.01636572	2.42	<.0001	**GM1**	Var	163	0.211143	2.64	<.0001
	Rep	2	0.335475	97.94	<.0001		Rep	2	0.40064847	59.33	<.0001		Rep	2	4.238823	52.93	<.0001
SR	Var	163	1.421391	2.42	<.0001	**NPQI**	Var	163	0.0010079	2.87	<.0001	**GM2**	Var	163	0.213828	2.58	<.0001
	Rep	2	87.57154	149.07	<.0001		Rep	2	0.00280985	8.01	<.0001		Rep	2	5.896187	71.22	<.0001
MCARI1	Var	163	0.006543	2.16	<.0001	**PRI**	Var	163	0.00078857	2.49	<.0001	**ARI1**	Var	163	0.181404	2.57	<.0001
	Rep	2	0.847959	279.8	<.0001		Rep	2	0.00009309	0.29	0.8294		Rep	2	0.715275	10.15	<.0001
OSAVI	Var	163	0.002315	2.06	<.0001	**NPCI**	Var	163	0.00523848	2.41	<.0001	**ARI2**	Var	163	0.037201	2.6	<.0001
	Rep	2	0.337327	299.77	<.0001		Rep	2	0.10284388	47.36	<.0001		Rep	2	0.130367	9.1	<.0001
G	Var	163	0.303484	2.41	<.0001	**Ctr1**	Var	163	0.26783727	2.78	<.0001	**CRI1**	Var	163	1.255471	2.15	<.0001
	Rep	2	36.31539	287.96	<.0001		Rep	2	3.0518425	31.73	<.0001		Rep	2	167.7606	287.85	<.0001
MCARI	Var	163	0.026468	2.87	<.0001	**Ctr2**	Var	163	0.00833364	2.42	<.0001	**CRI2**	Var	163	1.240795	2.11	<.0001
	Rep	2	1.179712	127.85	<.0001		Rep	2	0.27977524	81.36	<.0001		Rep	2	146.9214	250.36	<.0001
TCARI	Var	163	0.020522	2.77	<.0001	**Lic1**	Var	163	0.00289057	2.06	<.0001	**RDVI**	Var	163	0.001759	2.07	<.0001
	Rep	2	1.0988	148.15	<.0001		Rep	2	0.44281075	314.96	<.0001		Rep	2	0.309015	363.49	<.0001
TVI	Var	163	8.56143	2.13	<.0001	**Lic2**	Var	163	0.01444206	2.78	<.0001						
	Rep	2	1097.567	273.05	<.0001		Rep	2	0.02265641	4.36	0.0048						

Var: variety, Rep: replication

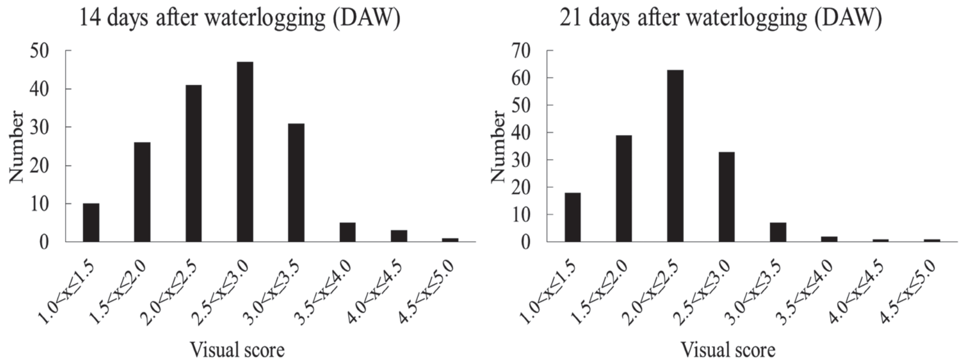


Fig. 3. Distribution of the visual scores of the 164 wild soybean accessions at 14 and 21 days after waterlogging.

P<0.0001) at 21 DAW but decreased when compared with that at 14 DAW (Table 4).

Correlation Test between the Visual Scores and Vegetation Indices Among Eight Selected Wild Soybean Accessions

Significant correlation was observed for the comparison of vegetation indices and visual score ratings in 164 wild soybean accessions, but the value was low due to moderate visual ratings. The vegetation indices of the eight selected wild soybeans were used for correlation testing. Interestingly, only four vegetation indices, namely simple ratio pigment index (SPRI), anthocyanin reflectance index 1 (ARI1), anthocyanin reflectance index 2 (ARI2), and PRI showed significant correlations with visual scores. At 14 DAW, SPRI (r = −0.8841, P<0.0082), ARI1 (r = 0.98069, P<0.0001), ARI2 (r = 0.98069, P<0.0001), and PRI (r = −0.9801, P<0.0001) showed significant correlations with visual scores (Table 5). At 21 DAW, similar results were observed; therefore, these four vegetation indices can better reflect stress injury than others.

Changes in Vegetation Indices during Waterlogging Stress in Selected Wild Soybean Accessions

The influence of various vegetation indices for waterlogging is presented in Fig. 4. For SPRI1, most of the waterlogging-tolerant accessions did not show significant difference between BW and after waterlogging. Moreover, compared with BW, the SPRI value significantly decreased in all of the waterlogging-susceptible accessions at 14 and 21 DAW (Fig. 4). On the other hand, the ARI1 and ARI2 values showed an opposite trend to SPRI1 in waterlogging-susceptible accessions. Similar to SPRI1, most of the tolerant accessions did not show any significant difference between BW and after waterlogging (Fig. 4). However, the values of ARI1 and ARI2 increased 2.0-8.0 fold when accessions were exposed to waterlogging (14 DAW) or were past the waterlogging stress threshold (21 DAW) (Fig. 4). PRI revealed distinguishing differences between waterlogging-tolerant and waterlogging-susceptible accessions. Overall, increased or similar PRI values were found in waterlogging-tolerant accessions when comparing before and after waterlogging (Fig. 4). However, in the waterlogging-susceptible accessions, all PRI values were positive BW but negative after waterlogging (Fig. 4). Finally, most of the waterlogging-tolerant accessions showed similar values when exposed to waterlogging stress; however, the values of susceptible accessions fluctuated. PRI showed a negative value in the waterlogging-susceptible accessions upon waterlogging.

Root Characteristics of Selected Wild Soybean Accessions

Waterlogging-tolerant and waterlogging-susceptible accessions showed different root morphological traits, as shown in (Fig. 5). Comparison between tolerant and susceptible accessions revealed that root length significantly increased in waterlogging-tolerant accessions, specifically in accession 884, which showed the highest root length (Fig. 5). Root length was not statistically different in susceptible accessions. In the case of the projected area, most of the tolerant accessions, except accession 659, showed higher projected area than susceptible accessions. Likewise, accession 884 and 1022 showed the maximum values in the tolerant accessions Fig. 5. Overall, the projected area was ranged from 7.7 to 15.1 cm^2 in waterlogging-tolerant accessions, whereas susceptible accessions ranged from 5.1 to 6.6 cm^2. Therefore, the projected area was increased by 14.3%-56.3% in waterlogging-tolerant accessions compared with in susceptible accessions, particularly accession 888 (Fig. 5). The link average length ranged from 0.23 to 0.261 cm, and accessions 659 and 1116 showed the maximum values, whereas others were not clearly distinguished (Fig. 5). In susceptible accessions, the link average length ranged from 0.185 to 0.247 cm, and accession 504 showed the lowest value compared with other susceptible accessions (Fig. 5). Compared between accessions 651 and 504, the link average length was increased by 29.1%.

Table 4. Correlation between the vegetation indices and visual scores of the 164 wild soybeans accessions after waterlogging.

Vegetative index	Pearson correlation coefficient *P*-value	
	14 days after waterlogging treatment	21 days after waterlogging treatment
Chlorophyll content	-0.06056	-0.0509
	0.4411	0.5175
SIPI	-0.4103	-0.31213
Structure Insensitive Pigment Index	<.0001	<.0001
NDVI	-0.42502	-0.30161
Normalized Difference Vegetation Index	<.0001	<.0001
SPRI	-0.53908	-0.26154
Simple Ratio Pigment Index	<.0001	0.0007
SR	-0.41052	-0.29212
Simple Ratio Index	<.0001	0.0001
ARI1	0.55379	0.31649
Anthocyanin Reflectance Index	<.0001	<.0001
ARI2	0.55609	0.32397
Anthocyanin Reflectance Index	<.0001	<.0001
GM2	-0.41348	-0.24526
Gitelson & Merzlyak Index	<.0001	0.0015
MCARI1	-0.47011	-0.19991
Modified Chlorophyll Absorption in Reflectance Index	<.0001	0.0103
PRI	-0.57181	-0.37995
Photochemical Reflectance Index	<.0001	<.0001
OSAVI	-0.48381	-0.3362
Optimized Soil-Adjusted in Reflectance Index	<.0001	<.0001
NPCI	0.52924	0.27952
Normalized Phaeophytinization Index	<.0001	0.0003
Ctr1	0.41741	0.17459
Carter Stress Index	<.0001	0.0254
Ctr2	0.45582	0.30313
Carter Stress Index	<.0001	<.0001
G	-0.48957	-0.24321
Greenness Index	<.0001	0.0017
Lic1	-0.47228	-0.33052
Lichtenthaler Index	<.0001	<.0001
Lic2	-0.44309	-0.25319
Lichtenthaler Index	<.0001	0.0011
RDVI	-0.49601	-0.31272
Renormalized Difference Vegetation Index	<.0001	<.0001

Table 5. Correlation between the vegetation indices and visual scores of the eight selected waterlogging-tolerant and waterlogging-susceptible wild soybean accessions.

Vegetative index	Pearson correlation coefficient *P*-value	
	14 days after waterlogging treatment	21 days after waterlogging treatment
Chlorophyll content	-0.68343	0.21608
	0.0905	0.6417
SIPI	-0.23709	-0.65002
Structure Insensitive Pigment Index	0.6087	0.114
NDVI	-0.5546	-0.76404
Normalized Difference Vegetation Index	0.1963	0.0455
SPRI	**-0.8841**	**-0.81292**
Simple Ratio Pigment Index	**0.0082**	**0.0262**
SR	-0.6716	-0.76532
Simple Ratio Index	0.0985	0.0449
ARI1	0.98069	0.86734
Anthocyanin Reflectance Index	**<.0001**	0.0047
ARI2	0.98434	0.87934
Anthocyanin Reflectance Index	**<.0001**	0.0032
GM2	-0.8254	-0.642
Gitelson & Merzlyak Index	0.0222	0.12
MCARI1	0.66774	-0.07336
Modified Chlorophyll Absorption in Reflectance Index	0.1012	0.8758
PRI	-0.9801	-0.9268
Photochemical Reflectance Index	**<.0001**	0.0027
OSAVI	-0.2792	-0.40402
Optimized Soil-Adjusted in Reflectance Index	0.5443	0.3687
NPCI	0.88266	0.80303
Normalized Phaeophytinization Index	0.0085	0.0296
Ctr1	0.12392	-0.7142
Carter Stress Index	0.7912	0.0714
Ctr2	0.51651	-0.3717
Carter Stress Index	0.2353	0.4117
G	-0.3645	-0.07522
Greenness Index	0.4215	0.8727
Lic1	-0.3097	-0.46056
Lichtenthaler Index	0.499	0.2983
Lic2	-0.94	-0.82187
Lichtenthaler Index	0.0016	0.0233
RDVI	-0.2474	-0.32749
Renormalized Difference Vegetation Index	0.5928	0.4734

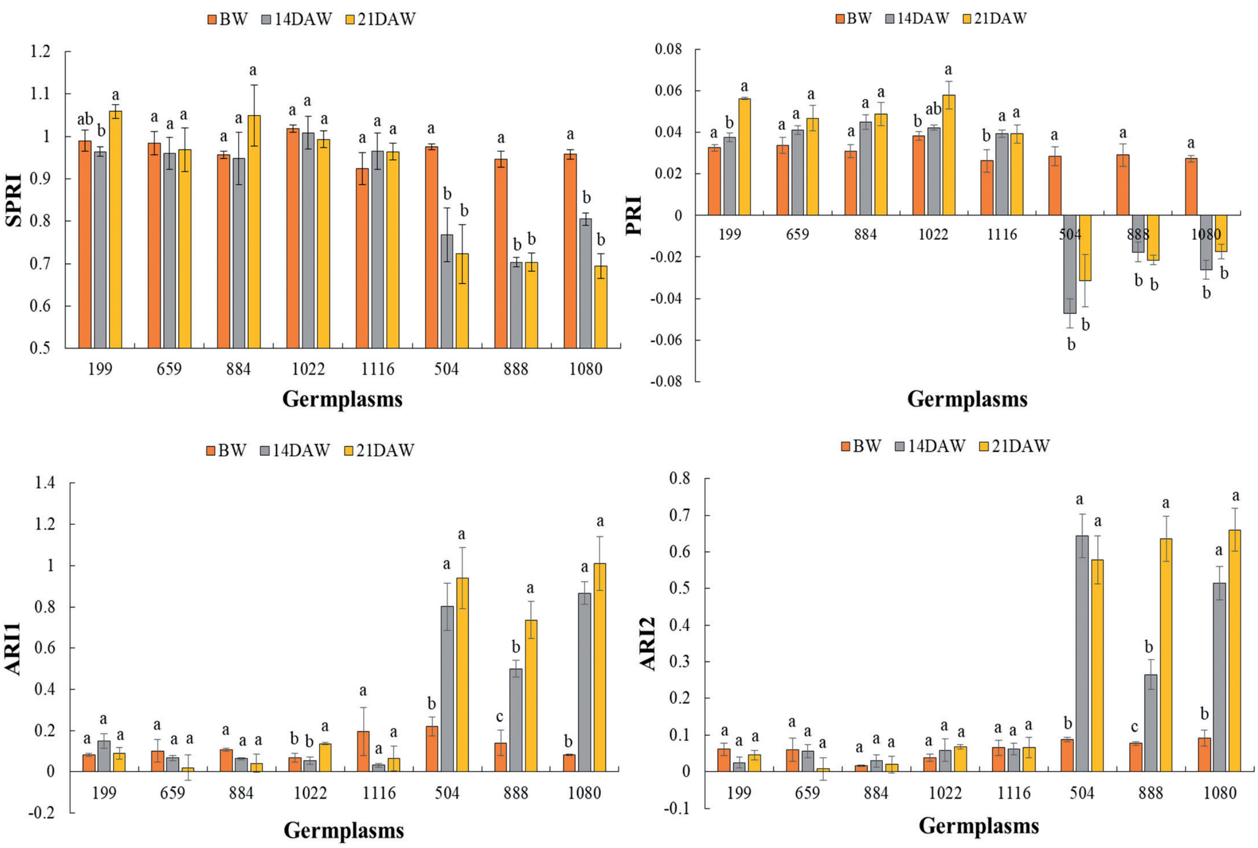


Fig. 4. Changed in the vegetation indices of the selected contrasting wild soybean accessions under waterlogging conditions. In the figure, different letters in error bars indicate significant different by Duncan's multiple range test (P<0.05).

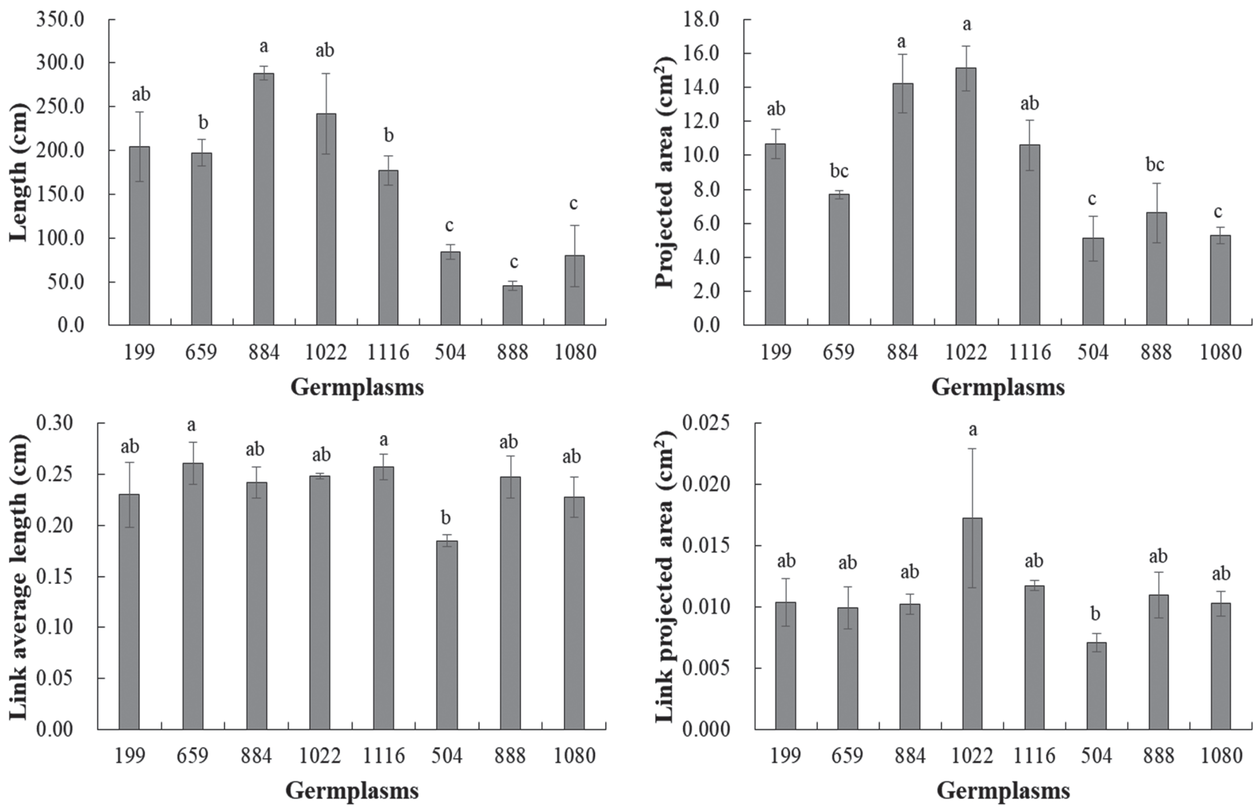


Fig. 5. Influence of root morphological traits in the selection of contrasting wild soybean accessions under waterlogging conditions. In the figure, different letters in the error bars indicate significant differences by the Duncan's multiple range test (P<0.05).

The link projected area was the highest in accession 1,022 and lowest in accession 504 (Fig. 5). Other accessions did not show a statistical difference for this trait (Fig. 5). The link projected area of accession 1,022 was 0.017 cm^2 and that of accession 504 was 0.007 cm^2; therefore, tolerant accession 1,022 was 58.8% higher than susceptible accession 504 (Fig. 5).

Discussion

Soybean is an economically important crop as it is a source of food, feed, and biofuel [19]. However, unexpected weather events, such as excess water levels in the field, negatively affect the soybean yield [4]. Therefore, the development of new soybean accessions is required for enhancing the productivity of soybean in various field conditions. To develop new varieties, finding variations in accessions is very important. *Glycine max* showed narrower genetic variations than *Glycine soja* due to genetic bottlenecks and manual selection [19, 20]. Therefore, it is essential to find out the novel variations found in wild soybean accessions [20, 21]. Both cultivated and wild soybeans belong to the genus *Glycine*, and wild soybeans are considered the ancestors of cultivated soybean; therefore, wild soybeans can be used for their genetic materials to improve various characteristics in cultivated soybean [19, 21, 22]. Despite of the importance of wild soybean for its genetic resources, the morphological traits of the shoot and root under waterlogging stress has not been characterized to date. Therefore, we used 164 wild soybean accessions and tested them to evaluate their stress responses for selecting contrasting wild soybean accessions. All potted plants were transferred to a pool of water to ensure a stress condition when overall wild soybean plants reached the V1 growth stage. For 2 weeks, a water level of 4–6 cm from the surface was maintained and then visual rating was performed on a 1–5 visual scoring scale at 14 and 21 DAW because visual rating is the most important method for selecting or evaluating stress resistance in certain conditions, such as drought stress [23], flooding stress [24], cyst nematode infestation [25] and two-spotted spider mite invasion [26].

In an experiment that was previously conducted, the waterlogging condition was for cultivated soybeans was maintained for 14 days and visual rating was performed on a 1-5 scale throughout the period [20]. In a similar experiment, waterlogging condition was maintained for 8-11 days and foliar damage or senescence was measured on a 1-9 visual scoring scale [27]. For waterlogging, the stress exposure period is an important factor because some genotypes may show similar responses when they are exposed to such stresses for too long or too short a period. In this experiment, the waterlogging condition was maintained for 14 days because the duration was relevant to the purpose of this experiment.

Various research groups have attempted to evaluate waterlogging tolerance and susceptibility in cultivated soybean since then; however, similar researches in wild soybeans are lacking [20, 27]. In another experiment, waterlogging tolerance and susceptibility was evaluated using 722 cultivated soybeans (maturity groups 4 and 5) for 5 years and reported that 52 soybeans showed tolerance and 57 soybeans demonstrated high sensitivity to waterlogging [27]. This shows that almost an equal number of cultivars were tolerant and sensitive to waterlogging. However, in this experiment, a greater number of wild soybean cultivars were waterlogging-tolerant (Fig. 3). Perhaps, these results are induced by the genetic diversity of wild soybean accessions. Studies have reported that wild soybeans have high adaptation to unfavorable environmental conditions [28, 29]. Therefore, only a small number of wild soybean accessions have been identified as waterlogging-susceptible.

The wilting status of plants was used for standard stress tolerance and susceptibility evaluation. Therefore, the wilting score has been used for selecting resistant genotypes in soybean [7, 20]. For this evaluation, breeders' experience is of utmost importance for accurate evaluation because plants can wilt even when exposed to various stress conditions. Furthermore, the range of the wilting score is not highly varying; therefore, breeders face this difficulty when the plants show moderate resistance. Subsequently, changes in leaf color, such as chlorophyll content and SPAD measurement, provide an alternative index for evaluating stress resistance [30, 31]. Recently, vegetation indices have been broadly used for predicting plant growth conditions [32]. The measurement of vegetation indices is highly preferred for high-throughput phenotyping due to its ease in obtaining data using a spectral camera and its large-scale coverage in the field [32]. In this experiment, chlorophyll content did not show any correlation with visual rating; however, several other vegetation indices showed high correlation with visual score (Table 4). Although there was a statistical correlation between visual scores and vegetation indices, the range of correlation value was low. The reason for this could be the similarity in visual scores for roughly 90% of the wild soybean accessions. Another reason could be the difference in the values of the visual scores and vegetation indices. As a result, correlation analysis was re-performed by selecting contrasting accessions. Therefore, only four vegetation indices with high correlation were confirmed, namely SPRI, ARI1, ARI2, and PRI. In particular, ARI1, ARI2, and PRI showed high correlational values for both measurements. ARI1 and ARI2 predict anthocyanin content via a non-destructive method and have been developed by the Gitelson [33]. Anthocyanins are known as water-soluble pigments derived from the flavonoids of higher plants and are responsible for the red coloration in plants [33]. Anthocyanins are accumulated in stress conditions, such as strong light, drought, fungal infection, nitrogen deficiency, and waterlogging [33-35]. In the present experiment, susceptible wild soybeans rapidly increased the development of red coloration in their leaves (Fig. 2). This led to a high correlation between visual scores and ARI1 and ARI2. PRI is based on the xanthophyll cycle pigment. Therefore, it reflects leaf fluorescence and photosynthesis [34]. For this reason, PRI has been used to detect water stress in crops [36, 37].

Root morphological traits are very important for water and nutrient uptake in plants and are widely studied for enhancing crop productivity [38]. Furthermore, various root morphological traits respond to osmotic stress conditions, such as drought and waterlogging [4, 38, 39]. Therefore, the root morphological traits between waterlogging-tolerant and waterlogging-susceptible accessions were analyzed and compared. The results indicated that waterlogging-tolerant accessions commonly show higher root length and root projected area than susceptible accessions. It has been reported that exogenously applied ethylene improves waterlogging resistance in *Glycine max* due to increased root surface area [4].

Conclusions

The waterlogging tolerance and susceptibility of 164 wild soybean genotypes were tested in three replicates. Our experiments confirmed that several wild soybean accessions are waterlogging-resistant. Furthermore, vegetation indices were observed to show a high correlation with visual score; therefore, they could be used as predictors of waterlogging resistance and susceptibility. In particular, ARI and PRI could be appropriate for precise accession screening. Therefore, those selected wild soybean accessions can be used for relevant researches.

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Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Material

Table S1. List of wild soybean accessions

Entry number	Genotype	IT number	Entry number	Genotype	IT number	Entry number	Genotype	IT number	Entry number	Genotype	IT number
11	CW 10050	-	666	CW 14643	IT 267444	911	YWS 152	-	1062	YWS 509	-
26	CW 10212	-	669	CW 14647	None	914	YWS 155	-	1066	YWS 513	-
30	CW 10245	-	673	CW 14651	IT 267445	928	YWS 176	-	1082	YWS 548	IT 247535
43	CW 10262	-	687	CW 14667	-	931	YWS 179	-	1097	YWS 571	-
82	CW 10461	-	692	CW 14673	-	935	YWS 188	-	1101	YWS 579	-
85	CW 10475	-	693	CW 14674	IT 267448	938	YWS 194	-	1105	YWS 585	-
148	CW 11260	-	694	CW 14675	-	943	YWS 261	-	1106	YWS 587	IT 247542
197	CW 11550	-	696	CW 14677	IT 267449	944	YWS 262	IT 247464	1108	YWS 589	-
199	CW 11598	-	703	CW 14686	-	947	YWS 273	-	1113	YWS 597	-
269	CW 12420	-	734	CW 14722	-	958	YWS 366	IT 247487	1116	YWS 602	-
285	PI 339731	-	750	CW 14748	-	961	YWS 370	IT 247490	1119	YWS 605	-
334	PI 597462A	-	751	CW 14749	-	967	YWS 399	-	1122	YWS 608	-
378	CW 11782	-	764	CW15259	-	968	YWS 402	-	1123	YWS 609	IT 247545
409	CW 13821	-	774	CW 14099	-	977	YWS 414	-	1138	YWS 639	-
421	CW 14125	-	776	CW 14104	-	980	YWS 419	-	1139	YWS 642	-
430	CW 14328	-	783	CW 15281	-	990	YWS 431	-	1153	YWS 678	IT 242679
447	PI 407299	-	786	CW 15284	-	991	YWS 432	-	1157	YWS 688	-
450	PI 424096	-	793	CW 15291	-	994	YWS 435	-	1160	YWS 692	IT 247560
455	PI 522180	-	801	CW 15302	-	998	YWS 440	IT 247508	1172	YWS 715	-
466	PI 464929A	-	812	CW 15313	-	1002	YWS 445	-	1173	YWS 716	-
489	CW 14858	-	826	YWS 1	-	1019	YWS 465	-	1174	YWS 717	-
504	CW 11948	-	827	YWS 4	IT 250557	1020	YWS 466	IT 247515	1178	YWS 722	IT 247568
507	CW 11955	-	832	YWS 10	-	1021	YWS 468	-	1182	YWS 726	IT 247569
551	CW 13176	-	833	YWS 11	-	1022	YWS 469	-	1198	YWS 767	-
554	CW 13201	IT 267421	836	YWS 18	IT 242674	1026	YWS 473	-	1200	YWS 777	-
575	CW 13274	-	837	YWS 19	-	1030	YWS 477	IT 247517	1209	YWS 860	-

Table S1. Continued.

579	CW 13303	-	840	YWS 23	IT 250514	1032	YWS 479	IT 247518	1219	YWS 913	-
581	CW 13313	IT 267426	848	YWS 34	-	1034	YWS 481	IT 247519	1242	YWS 1346	-
590	CW 13395	IT 267430	850	YWS 36	-	1035	YWS 482	-	1247	YWS 1353	-
594	CW 13432	-	853	YWS 39	-	1039	YWS 486	IT 247520	1253	YWS 1362	-
609	CW 14449	-	856	YWS 43	-	1040	YWS 487	-	1261	YWS 1374	-
624	CW 14580	IT 267433	862	YWS 53	-	1041	YWS 488	-	1265	YWS 1379	-
625	CW 14581	IT 267434	863	YWS 54	IT 250554	1042	YWS 489	-	1267	YWS 1381	-
626	CW 14582	IT 267435	864	YWS 55	IT 247444	1043	YWS 490	-	1274	YWS 1400	IT 242875
627	CW 14587	IT 267436	869	YWS 60	-	1048	YWS 495	-	1279	YWS 1405	-
641	CW 14604	IT 270014	874	YWS 65	-	1052	YWS 499	-	1282	YWS 1411	-
649	CW 14618	IT 267440	884	YWS 76	-	1055	YWS 502	-	1283	YWS 1412	-
657	CW 14631	IT 267441	889	YWS 86	-	1057	YWS 504	-	1299	YWS 1434	-
659	CW 14633	-	894	YWS 95	-	1058	YWS 505	-	1300	YWS 1436	-
	CW 14636	IT 267443	907	YWS 119	-	1059	YWS 506	IT 250444	1307	YWS 1449	-
665	CW 14642	IT 270016	908	YWS 120	-	1061	YWS 508	-	1330	YWS 1477	-

Table S2. Equations of the vegetation indices.

Index	Formula
Normalized Difference Vegetation Index (NDVI)	NDVI = $\frac{R_{NIR}-R_{RED}}{R_{NIR}+R_{RED}}$
Simple Ratio Index (SR)	SR = $\frac{R_{NIR}}{R_{RED}}$
Modified Chlorophyll Absorption in Reflectance Index (MCARI1)	MCARI1 = $1.2 \times [2.5 \times (R_{790} - R_{670}) - 1.3 \times (R_{790} - R_{550})]$
Optimized Soil-Adjusted in Reflectance Index (OSAVI)	OSAV I= $(1 + 0.16) \times \frac{(R_{790}-R_{670})}{(R_{790}+R_{670}+0.16)}$
Greenness Index (G)	G = $\frac{R_{554}}{R_{667}}$
Modified Chlorophyll Absorption in Reflectance Index (MCARI)	MCARI = $[(R_{700} - R_{670}) - 0.2 \times (R_{700} - R_{550})] \times (\frac{R_{700}}{R_{670}})$
Transformed CAR Index (TCARI)	TCARI = $3 \times [(R_{700} - R_{670}) - 0.2 \times (R_{700} - R_{550})] \times (\frac{R_{700}}{R_{670}})$
Traiangular Vegetation Index (TVI)	TVI = $0.5 \times [120 \times (R_{750} - R_{550}) - 200 \times (R_{670} - R_{550})]$
Zarco-Tejada & Miller (ZMI)	ZMI = $\frac{R_{750}}{R_{710}}$
Simple Ratio Pigment Index (SRPI)	SRPI = $\frac{R_{430}}{R_{680}}$
Normalized Phaeophytinization Index (NPQI)	NPQI = $\frac{R_{415} - R_{435}}{R_{415} + R_{435}}$
Photochemical Reflectance Index (PRI)	PRI = $\frac{R_{531} - R_{570}}{R_{531} + R_{570}}$
Normalized Pigment Chlorophyll Index (NPCI)	NPCI = $\frac{R_{680} - R_{430}}{R_{680} + R_{430}}$
Carter Stress Index (Ctr1)	Ctr1 = $\frac{R_{695}}{R_{420}}$
Carter Stress Index (Ctr2)	Ctr2 = $\frac{R_{695}}{R_{760}}$
Lichtenthaler Index (Lic1)	Lic1 = $\frac{R_{790} - R_{680}}{R_{790} + R_{680}}$
Lichtenthaler Index (Lic2)	Lic2 = $\frac{R_{440}}{R_{690}}$
Structure insensitive pigment index (SIPI)	SIPI = $\frac{R_{790} - R_{450}}{R_{790} - R_{650}}$
Gitelson & Merzlyak Index (GM1)	GM1 = $\frac{R_{750}}{R_{550}}$
Gitelson & Merzlyak Index (GM2)	GM2 = $\frac{R_{750}}{R_{700}}$
Anthocyanin Reflectance Index (ARI1)	ARI1 = $\frac{1}{R_{550}} - \frac{1}{R_{700}}$
Anthocyanin Reflectance Index (ARI2)	ARI2 = $R_{800} \times (\frac{1}{R_{550}} - \frac{1}{R_{700}})$
Carotenoid Reflectance Index 1 (CRI1)	CRI1 = $\frac{1}{R_{510}} - \frac{1}{R_{550}}$
Carotenoid Reflectance Index 1 (CRI2)	CRI2 = $\frac{1}{R_{510}} - \frac{1}{R_{700}}$
Renormalized Difference Vegetation Index (RDVI)	RDVI = $\frac{R_{NIR} - R_{RED}}{\sqrt{R_{NIR}+R_{RED}}}$