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Genotypic variations in phosphorus accumulation in wild cowpea relatives (*Vigna vexillata*) grown under phosphorus deficiency

Kanako Takada, Tomohiro Nishigaki , Yasuhiro Tsujimoto and Kohtaro Iseki 

Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan

ABSTRACT

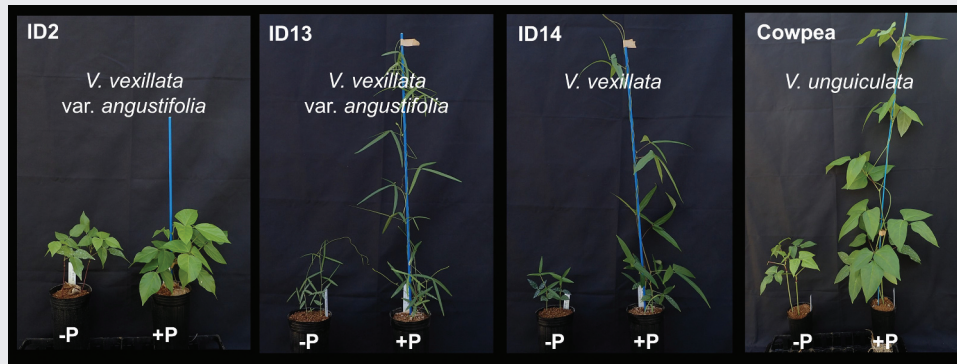
Vigna vexillata is a wild cowpea relative inhabiting tropical and subtropical regions. Because phosphorous (P) deficient soils are widely found in these regions, such wild relatives may have an ability to efficiently capture and utilize P in soils and thus can be potential materials of green manure to improve soil-plant nutrient cycles and subsequent crop production. Genotypic variations in shoot P content were evaluated in P-deficient (-P) and P-fertilized (+P) conditions of the pot experiment to clarify the ability of P accumulation in *V. vexillata*. Among the 14 accessions, five with a high shoot P content under P deficiency were identified. The high shoot P contents were attributed to large shoot biomass rather than high shoot P concentrations. The larger biomass of these accessions was consistent under the -P and +P conditions, indicating that potential growth characteristics without P restriction were a major driving factor of P accumulation even under P deficiency. In contrast, accessions with low shoot P content had small shoot biomass with high P concentrations. These accessions exhibited superior drought tolerance than those with large shoot biomass, presumably because of the small water consumption due to the small shoot biomass. The trade-offs between the shoot P accumulation and drought tolerance should be considered for genotype selection under the conditions where P deficiency and drought risks are often complex.

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Vigna vexillata; phosphorus deficiency; crop wild relative; genotypic variation



Introduction

Wild species inhabiting low fertile soils should have superior abilities to absorb or utilize limited amounts of nutrients for completing their life cycles. As plants accumulate soil nutrients in their body, such wild species are potential candidates for green manure for improving soil fertility of farmland suffering from nutrient deficiency. Tribouillois et al. (2015) evaluated 36 cover crops, including wild species, and revealed that the wilds had more diverse characteristics in nutrient accumulation and plant growth. Although some wild species, such as

white clover and wild subspecies of mustard, have been successfully adapted as cover crops to improve soil fertility (Alcántara et al., 2009; Hill et al., 2021), most cases targeted temperate climate conditions where rainfall amount is relatively high and where soil nutrient deficiency is not so severe. A number of studies in drier, warmer, and poor nutrient conditions such as the cases of tropical savannas and semi-arid climates are yet limited because available plant materials are not so abundant.

Vigna vexillata is a leguminous wild species genetically close to cultivated cowpea (*Vigna unguiculata*) and

CONTACT Kohtaro Iseki  iseki83@affrc.go.jp

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is widely distributed across tropical and subtropical Asia and Africa (Tomooka et al., 2011). In the tropics, soils are inherently low in the amounts of available phosphorous (P) and high in the amounts of P-fixing aluminum and iron oxides. Thus, P deficiency is one of the major crop production constraints (Nziguheba et al., 2015). Therefore, genetic resources of *V. vexillata* originating in low fertile soils would have various adaptation strategies to P deficiency, such as P solubilization by secreting organic acids into the rhizosphere and changes in root architecture (Krishnapriya & Pandey, 2016; Lynch & Brown, 2001). Lawn and Watkinson (2002) reported that some accessions of *V. vexillata* inhabiting alkaline soil may possess the ability to change the pH in their rhizosphere, which can also be effective in solubilizing the unavailable form of P.

It is known that plant nutrient uptake is inhibited by abiotic stresses (Bista et al., 2018; Brown et al., 2006). Some genetic resources of *V. vexillata* originated from dry regions are expected to have excellent adaptability to soil water scarcity. In our previous study, 69 accessions of *Vigna* species were tested for drought tolerance, and some accessions of *V. vexillata* from arid and semi-arid regions were identified to have tolerance to severe and extreme drought conditions (Iseki et al., 2018). *V. vexillata* accessions inhabiting coastal areas are also known to have salt stress tolerance (Iseki et al., 2016). As tolerances to salt stress and drought have common mechanisms in molecular level (Li et al., 2021; Wang et al., 2023), salt stress tolerance is also a promising characteristic that make this plant material applicable to tropical, low fertile soils with little rainfall.

According to the aforementioned characteristics, *V. vexillata* accessions with high P accumulation under P deficiency with drought stress are expected within the genetic resources, but such information is restricted for this species. The objective of this study is (1) to clarify the genotypic variation in P accumulation of *V. vexillata*

under P deficiency and (2) to know the relationship between P accumulation characteristics and drought tolerance. The possibility of utilization of *V. vexillata* as green manure plants under P deficiency with little soil water availability was discussed.

Materials and methods

1st pot experiment

Evaluation of P accumulation in *V. vexillata* accessions grown under P-deficient conditions

Plant materials and growth conditions

Fourteen accessions of *V. vexillata*, including two varieties (var. *angustifolia* and var. *macrosperma*), were used (Table 1). These accessions are single-seed descent lines in which most genomic regions are genetically fixed, and phenotypic segregation is rarely observed. All seeds were obtained from the GenBank of the National Agriculture and Food Research Organization, Japan. Accessions were selected from 108 accessions of *V. vexillata* collections derived from various environments in tropical and subtropical regions, showing different growth performances under P-deficient conditions in our preliminary experiment. A cultivated cowpea (*Vigna unguiculata* cv. IT98K-205-8) growing in low-fertility soil in West Africa was added as a reference accession to compare its response to P deficiency with that of *V. vexillata* accessions.

Pot experiments were conducted in a glasshouse at the Japan International Research Center for Agricultural Sciences (JIRCAS) in Tsukuba, Japan (36° 1' N, 140°1' E). P-deficient soil was collected from a subsoil layer (40–50 cm depth) in an experimental field at the JIRCAS Tropical Agricultural Research Front, Ishigaki, Okinawa, Japan (26°5' N, 135°7' E). The soil characteristics are listed in Table 2 (after Oo

Table 1. Plant materials used in this study.

ID	Accession number/Cultivar name	Scientific name	Origin	Status
ID1	JP256238	<i>Vigna vexillata</i>	Bhutan	Wild
ID2	JP256300	<i>Vigna vexillata</i> var. <i>angustifolia</i>	Cameroon	Wild
ID3	JP256309	<i>Vigna vexillata</i> var. <i>angustifolia</i>	Zimbabwe	Wild
ID4	JP256311	<i>Vigna vexillata</i> var. <i>angustifolia</i>	Tanzania	Wild
ID5	JP256315	<i>Vigna vexillata</i> var. <i>angustifolia</i>	South Africa	Wild
ID6	JP256326	<i>Vigna vexillata</i> var. <i>angustifolia</i>	Australia	Wild
ID7	JP256333	<i>Vigna vexillata</i> var. <i>macrosperma</i>	Costa Rica	Wild
ID8	JP256349	<i>Vigna vexillata</i>	Kenya	Wild
ID9	JP256353	<i>Vigna vexillata</i>	Congo	Wild
ID10	JP256364	<i>Vigna vexillata</i>	Zimbabwe	Wild
ID11	JP256376	<i>Vigna vexillata</i>	Togo	Wild
ID12	JP256407	<i>Vigna vexillata</i>	Australia	Wild
ID13	JP256412	<i>Vigna vexillata</i> var. <i>angustifolia</i>	Suriname	Wild
ID14	JP256417	<i>Vigna vexillata</i>	Senegal	Wild
COW	IT98K-205-8	<i>Vigna unguiculata</i>	Nigeria	Cultivated

Table 2. Physicochemical properties of the P-deficient soil used in this study.

Parameters	Unit	
pH (H ₂ O)		4.86
EC	mS m ⁻¹	4.63
Total nitrogen content	g kg ⁻¹	0.91
Total carbon content	g kg ⁻¹	4.82
P retention capacity	%	57.5
Available P content [†]	mg kg ⁻¹	17.5
P _{oxalate} content	mg kg ⁻¹	207.5
Al _{oxalate} content	g kg ⁻¹	2.70
Fe _{oxalate} content	g kg ⁻¹	2.58
Sand	%	44.3
Silt	%	12.0
Clay	%	43.7
Texture		Silty clay

The data are cited from Oo et al. (2021).

[†]Bray II method.

P_{oxalate}: oxalate-extractable P.

Al_{oxalate}: oxalate-extractable Al.

Fe_{oxalate}: oxalate-extractable Fe.

et al., 2021). The experimental soil was characterized by its low pH (H₂O) of 4.86, its low plant-available P (17.5 mg kg⁻¹), its high P retention capacity (57.5%), and its abundance of active aluminum and iron oxides. These chemical properties are similar to those of major tropical weathered soils such as Ultisol and Oxisol, widely distributed across southeast Asia, tropical Africa, and South America (Soil Map of the World, FAO/UNESCO, n.d). The experimental soil was mixed with granite sand at a mass ratio of 30% to adjust the soil permeability and then placed in a long pot measuring 9 cm in diameter and 20 cm in height. The drainage was improved by placing approximately 100 cc of pumice stones at the bottom of the pot.

Two levels of P treatment were established: -P and +P. P fertilizer was not applied in the -P treatment, whereas triple superphosphate (TSP) was applied at a rate of 1.2 g P kg⁻¹ soil in the +P treatment. The soil was pre-mixed with TSP for the +P condition. Before sowing, minute scratches were made on the seed surface of *V. vexillata* accessions to promote water absorption, and the seeds were pre-germinated in Petri dishes. Subsequently, well-germinated seeds were selected and sown in pots at a rate of three seeds per pot. The seeds were sown on 18 October 2021. The pots were arranged in a randomized block design with three replicates. The total number of pots was 90, comprising 15 accessions, two treatments, and three replicates. The pots were placed in trays filled with 2 cm of water for continuous watering throughout the experiment. The soil surface's volumetric soil water content (VSWC) (0–5 cm) was approximately constant at 0.33 cm³ cm⁻³. The average air temperature and relative humidity in the glasshouse during the experimental period were 28.9°C and 41.7%, respectively, with a mean solar radiation of 3.8 MJ m⁻²

day⁻¹. Insecticides free of organic P were used as required.

Chlorophyll fluorescence and SPAD evaluation

Chlorophyll fluorescence was measured three times, that is, at 28, 30, and 32 days after sowing, using a FluorPen FP110 (Photon Systems Instruments, Drásov, Czech Republic). The maximum quantum yield of photosystem II under light-adapted conditions (Fv'/Fm') was employed as a parameter of leaf physiological status concerning photosynthetic electron transport. The measurement was conducted from 10:00 AM to 12:00 PM. The chlorophyll content was also determined using the SPAD-502 Plus (KONICA MINOLTA Inc., Tokyo, Japan) on the same date as the chlorophyll fluorescence measurements. The light-adapted, fully opened, uppermost leaves were used to measure two plants in a pot.

P and N concentration

At five weeks after sowing, total shoots were sampled for each pot. Soils and roots were carefully removed from the pot, and then soils were gently washed away to sample the remaining roots. Shoot and root biomass were determined by drying them at 80°C for >72 h. The dried samples were ground to determine the P concentration. The ground sample (100 mg) was deposited in a ceramic crucible for dry ashing at 550°C for 2 h. Subsequently, 5 mL of 0.5 M HCl was added to each crucible for digestion. The P concentration was determined via the molybdate blue method using a spectrophotometer (UV-1900, Shimadzu, Kyoto, Japan). The P content of the shoots and roots (mg P plant⁻¹) was calculated by multiplying the dry weight and P concentration. Approximately 100 mg of the remaining ground sample of the shoot was analyzed with an NC analyzer (Sumigraph NC-220F; Sumika Chemical Analysis Service, Ltd., Osaka, Japan) to determine the N concentration. The analysis for ID3, ID5, ID10, and ID14 could not be performed because of an insufficient sample size. The N content (mg plant⁻¹) was calculated by multiplying the dry weight and concentration.

2nd pot experiment

Evaluation of drought tolerance in *V. vexillata* accessions with different shoot P concentrations

Plant materials and growth conditions

Six accessions of *V. vexillata* with varying shoot P concentration and shoot biomass were selected

based on the results of P accumulation in the previous experiment. Three accessions (ID3, ID10, and ID12) had a high shoot P concentration and a small shoot biomass, whereas the other three accessions (ID7, ID8, and ID13) had a low shoot P concentration and a large shoot biomass.

Pot experiments were conducted in a glasshouse at the JIRCAS, Tsukuba, Japan (36°1' N, 140°1' E). The P-deficient soil, identical to that used in the previous experiment, was placed in a long pot measuring 9 cm in diameter and 20 cm in height. The drainage was improved by placing approximately 100 cc of pumice stones at the bottom of the pot. Two levels of water treatment were established: control and drought conditions. Well-germinated seeds were selected and sown in pots at a rate of three seeds per pot. The seeds were sown on 12 December 2022.

The pots were arranged in a randomized block design with three replicates. The total number of pots was 36, comprising six accessions, two treatments, and three replicates. For both treatments, the pots were placed in trays filled with 2 cm of water for continuous watering until the start of the drought treatment. The average VSWC prior to the treatment was $0.38 \text{ cm}^3 \text{ cm}^{-3}$. Four weeks after sowing, the drought-treatment pots were removed from the watering tray and placed on a mesh tray for 30 days to promote soil drying. The control pots were kept watered. The average VSWC of the soil surface (0–5 cm) during the drought treatment was $0.06 \text{ cm}^3 \text{ cm}^{-3}$, while that of the control was $0.22 \text{ cm}^3 \text{ cm}^{-3}$ in the same period. The mean solar radiation was $4.5 \text{ MJ m}^{-2} \text{ day}^{-1}$ with an auxiliary halogen lamp from 6:00 AM to 6:00 PM. The average daily mean temperature and relative humidity in the glasshouse during the experimental period were 26.0°C and 28.6%, respectively.

Chlorophyll fluorescence, stomatal conductance, and shoot wilting

The F_v'/F_m' was measured using the FluorPen FP110 twice weekly from 20 to 30 days after drought onset. The measurement was conducted from 10:00 AM to 12:00 PM. The stomatal conductance was also evaluated using a leaf porometer (SC-1, METER Group, Inc., Pullman, WA, USA) on the same date as the chlorophyll fluorescence measurements. The light-adapted, fully opened, uppermost leaves were used to measure two plants in a pot.

The process of drought-induced shoot wilting was evaluated by capturing images using a digital camera (WG-7, RICOH, Tokyo, Japan) according to the method

described in Takahashi et al. (2015). A picture was taken just 60 cm above each pot. Images were captured twice a week from the onset of the drought to 30 days after the treatment. The vertically projected leaf area of the three plants in each pot was calculated using analysis software (ImageJ, version 1.53k, National Institutes of Health; <http://rsb.info.nih.gov/ij/>).

Statistical analysis

Statistical analyses were performed using R version 4.1.1. Tukey's multiple comparison tests were performed to detect significant differences in shoot P content, root P content, shoot N concentration, and shoot N content among the accessions under -P and +P conditions. Significant differences in the root – shoot ratios between the treatments were detected for each accession using Welch's t-test. The exact t-test was also used to detect significant differences in projected leaf area, F_v'/F_m' , and stomatal conductance between the accession groups of high and low shoot P concentrations in the second pot experiment.

Results

1st pot experiment

Genotypic variation of P content of *V. vexillata* under P-deficient conditions

The average shoot P content of 14 accessions was $0.34 \text{ mg plant}^{-1}$ under -P conditions, considerably lower than $2.49 \text{ mg plant}^{-1}$ under +P conditions (Table 3). The cultivated cowpea had higher P contents than *V. vexillata* accessions, irrespective of P conditions. Among the *V. vexillata* accessions, ID2, ID7, ID8, ID9, and ID13 showed high shoot P contents ($0.44\text{--}0.58 \text{ mg plant}^{-1}$) under -P conditions. The high P content of ID2, ID7, and ID13 was consistent under +P conditions. The shoot P contents did not significantly differ among the *V. vexillata* varieties (var. *angustifolia* and var. *macrosperma*).

Regarding root P content under -P conditions, ID2, ID7, ID8, ID11, and ID14 showed higher values similar to the cultivated cowpea, although the differences among the accessions were not statistically significant. Most of these accessions also had higher root P content under +P conditions.

The average -P/+P ratio for shoot P content (0.18) was lower than that in roots (0.47), indicating that the difference in P content between -P and +P conditions was higher in shoots than in roots. The -P/+P ratios did not

Table 3. Shoot and root P contents under -P and +P conditions.

ID	Shoot P content (mg plant ⁻¹)		Ratio (-P/+P)	Root P content (mg plant ⁻¹)		Ratio (-P/+P)
	-P	+P		-P	+P	
ID1	0.31 ± 0.04 cd	0.74 ± 0.27 e	0.42	0.09 ± 0.03 a	0.10 ± 0.12 c	0.90
ID2	0.48 ± 0.04 a	4.26 ± 0.84 ac	0.11	0.15 ± 0.06 a	0.48 ± 0.04 bc	0.31
ID3	0.20 ± 0.02 bd	0.65 ± 0.42 e	0.31	0.04 ± 0.03 a	0.04 ± 0.04 c	1.18
ID4	0.25 ± 0.06 bd	1.94 ± 0.44 ce	0.13	0.05 ± 0.03 a	0.12 ± 0.13 c	0.39
ID5	0.22 ± 0.03 bd	1.63 ± 0.49 de	0.14	0.07 ± 0.02 a	0.29 ± 0.04 bc	0.24
ID6	0.22 ± 0.05 bd	1.48 ± 0.5 de	0.15	0.08 ± 0.03 a	0.17 ± 0.08 c	0.48
ID7	0.44 ± 0.04 ac	4.97 ± 1.27 ab	0.09	0.14 ± 0.07 a	1.36 ± 0.56 a	0.10
ID8	0.58 ± 0.03 a	2.43 ± 1.16 ce	0.24	0.13 ± 0.1 a	0.19 ± 0.11 bc	0.70
ID9	0.50 ± 0.03 a	2.90 ± 1.13 bce	0.17	0.09 ± 0.03 a	0.12 ± 0.08 c	0.74
ID10	0.24 ± 0.01 bd	0.79 ± 0.37 e	0.31	0.04 ± 0.03 a	0.09 ± 0.11 c	0.46
ID11	0.32 ± 0.03 cd	3.25 ± 0.87 bcd	0.10	0.16 ± 0.05 a	0.83 ± 0.47 ab	0.20
ID12	0.30 ± 0.09 bd	1.60 ± 0.37 de	0.19	0.08 ± 0.05 a	0.21 ± 0.08 bc	0.38
ID13	0.47 ± 0.06 a	6.69 ± 1.55 a	0.07	0.11 ± 0.03 a	0.59 ± 0.24 bc	0.18
ID14	0.17 ± 0.06 b	1.53 ± 0.32 de	0.11	0.16 ± 0.04 a	0.53 ± 0.04 bc	0.30
Average	0.34	2.49	0.18	0.10	0.37	0.47
COW	0.77 ± 0.02	6.86 ± 0.74	0.11	0.14 ± 0.01	0.44 ± 0.04	0.32

Data are presented as mean ± standard deviation of three replications.

According to multiple comparison tests, means with the same letter are not significantly different at $p < 0.05$.

Table 4. Shoot N contents and concentrations under -P and +P conditions.

ID	Shoot N content (mg plant ⁻¹)		Ratio (-P/+P)	Shoot N concentration (%)		Ratio (-P/+P)
	-P	+P		-P	+P	
ID1	3.30 ± 0.60 c	4.71 ± 2.26 c	0.70	3.26 ± 0.27 ab	3.31 ± 0.17 a	0.98
ID2	6.18 ± 0.61 b	24.01 ± 5.74 ab	0.26	3.45 ± 0.31 ac	3.06 ± 0.20 ab	1.13
ID3						
ID4	2.69 ± 0.06 c	6.45 ± 1.31 bc	0.42	3.10 ± 0.01 ab	3.09 ± 0.05 ab	1.00
ID5						
ID6	2.46 ± 0.69 c	5.28 ± 1.65 c	0.47	3.47 ± 0.36 ac	3.53 ± 0.11 a	0.98
ID7	6.84 ± 0.89 ab	15.57 ± 1.41 ac	0.44	3.39 ± 0.09 ac	3.15 ± 0.20 ac	1.08
ID8	8.79 ± 0.92 a	19.50 ± 10.88 ac	0.45	2.64 ± 0.18 b	3.18 ± 0.31 ac	0.83
ID9	6.33 ± 0.79 b	9.12 ± 2.75 bc	0.69	2.95 ± 0.37 bc	2.67 ± 0.15 bc	1.10
ID10						
ID11	3.54 ± 0.44 c	12.52 ± 2.01 bc	0.28	2.99 ± 0.11 bc	2.51 ± 0.33 b	1.19
ID12	2.97 ± 0.52 c	6.55 ± 1.16 bc	0.45	3.37 ± 0.08 ab	3.65 ± 0.28 a	0.92
ID13	8.05 ± 1.58 ab	33.20 ± 14.22 a	0.24	3.81 ± 0.19 a	3.63 ± 0.16 a	1.05
ID14						
Average	5.11	13.69	0.44	3.24	3.18	1.03
COW	17.55 ± 0.98	43.27 ± 7.26	0.41	2.19 ± 0.13	2.88 ± 0.04	0.76

Data are presented as mean ± standard deviation of three replications.

Accessions without values were not evaluated due to insufficient sample sizes.

According to multiple comparison tests, means with the same letter are not significantly different at $p < 0.05$.

correlate with the absolute values of the shoot and root P contents. The ratios of the cultivated cowpea were below the averages for both shoot and root P content.

Shoot N contents and concentrations under P-deficient conditions

The shoot N content genotype averages were 5.11 mg plant⁻¹ and 13.69 mg plant⁻¹ under -P and +P conditions, respectively (Table 4). The *V. vexillata* accessions with high shoot P content (ID2, ID7, ID8, ID9, and ID13) also had high shoot N content (6.18–8.79 mg plant⁻¹). The cultivated cowpea had higher N contents than *V. vexillata* accessions, irrespective of P conditions. The accessions with higher shoot N content under -P conditions tended to have higher N shoot content under +P

conditions. The -P/+P ratios of shoot N content (average: 0.44) were larger than those of shoot P content in all tested accessions.

Minimal variation was observed in the shoot N concentration between the P conditions. The same values in the averages (3.24%) and -P/+P ratio (1.03) of shoot N concentration were obtained under both P conditions. Although significant differences were detected in shoot N concentration among the accessions, these differences were not significant.

According to the above results, shoot dry weight and leaf physiological status under P deficiency were analyzed by focusing on their relationship with shoot P concentration rather than shoot N concentration.

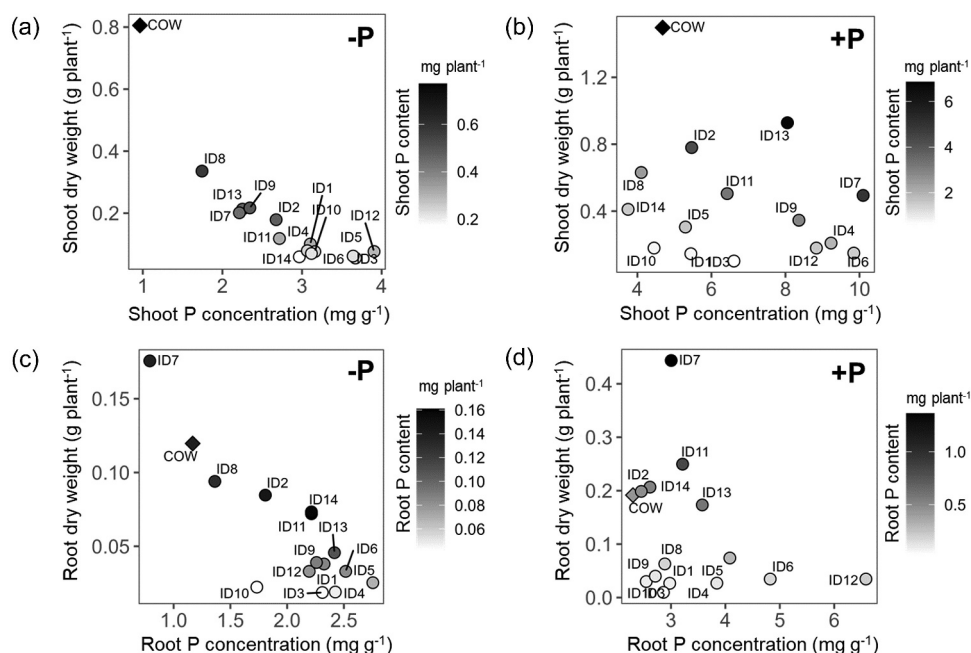


Figure 1. Relationships between P concentration and plant dry weight under -P and +P conditions. (a, b) shoots, (c, d) roots, (a, c) -P conditions, and (b, d) +P conditions. Each point represents the mean of three replications. Gray scales show the P content of each accession. Black and white colors indicate the accessions with higher and lower P contents. The rhombus represents the cultivated cowpea.

Phosphorus concentration and plant biomass

Under -P conditions, shoot P concentration was negatively correlated with shoot dry weight (Figure 1a). Accessions with higher shoot dry weight, including cultivated cowpea, had lower shoot P concentrations, whereas accessions with lower shoot dry weight had higher shoot P concentrations. Higher shoot P content was observed only in the former group.

Under +P conditions, there were still no accessions with higher shoot dry weight and P concentration, while

many had lower values for both traits (Figure 1b). Higher shoot P content was observed in accessions on the negative relationship's upper marginal line.

A similar relationship between P concentration and dry weight was also observed for roots (Figure 1c). The accessions with higher root dry weight showed higher root P content under -P conditions. The correlation was weaker under +P conditions (Figure 1d).

The root – shoot ratio of dry weight under -P and +P conditions is shown in Figure 2. The ratio tended

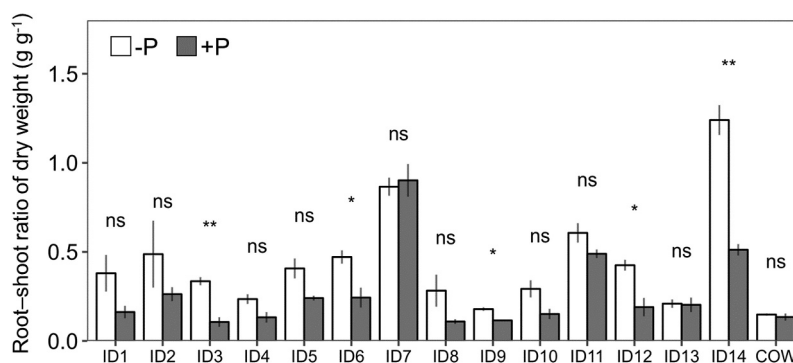


Figure 2. Root – shoot ratios under the -P and +P conditions. Data are presented as mean \pm standard error for three replications. Statistical significance between the different P treatments was indicated as ns: not significant, *: significant at $p < 0.05$, and **: significant at $p < 0.01$.

to be higher under -P conditions than under +P conditions, although significant differences were rare. The root – shoot ratio was unrelated to shoot or root P content or concentration. Among accessions with higher shoot P contents, similar root – shoot ratios were observed under -P and +P conditions for ID7 and ID13, while higher ratios were observed under -P conditions than under +P conditions for ID2, ID8, and ID9.

Phosphorus concentration and leaf physiological status

Although a significant decrease in F_v/F_m' was observed in the cultivated cowpea, chlorophyll fluorescence was minimally affected by P deficiency in *V. vexillata* accessions that the averages were 0.66 and 0.67 under -P and +P conditions, respectively (Figures 3a,b). There was no clear tendency between F_v/F_m' and shoot P concentration. Moreover, higher F_v/F_m' values were observed regardless of the shoot P content.

The SPAD value under -P conditions was higher (30.9) than that under +P conditions (27.8) (Figures 3c,d). There was no clear relationship between SPAD values and shoot P concentration or shoot P content under both -P and +P conditions. The cultivated cowpea showed the highest SPAD values under both P conditions.

2nd pot experiment

Drought tolerance under P deficiency

The larger projected leaf area at the drought onset was observed in the accessions with low shoot P concentrations than those with high shoot P concentrations (Figure 4a). As the soil drought progressed, the projected leaf area was reduced in all the accessions. The decreases in the former accessions were more drastic than in the latter. The significant difference between the accessions with high and low shoot P concentrations was diminished 30 days after treatment. The projected leaf areas under control conditions did not decrease during the experimental period. The areas were continuously higher in the accessions with low shoot P concentration.

The F_v/F_m' tended to be similar for the accessions with high and low shoot P concentrations at the drought onset (Figure 4b). As the soil drought progressed, larger decreases were observed in the latter accessions than in the former. Significant differences were observed from 10 to 30 days after treatment. The F_v/F_m' values below 0.3 indicated the leaves were close to wilting in the accessions with low shoot P concentration. Under control conditions, no decreases in F_v/F_m' were observed, and the values were similar for the accessions with high

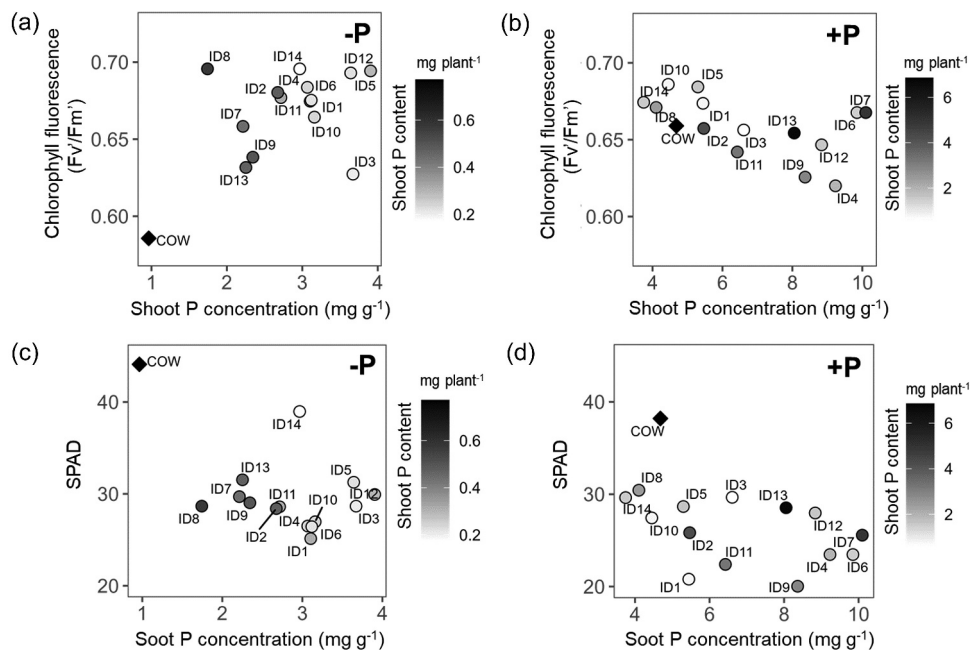


Figure 3. The relationship between shoot P concentration, chlorophyll fluorescence, and SPAD under -P and +P conditions. (a, b) chlorophyll fluorescence, (c, d) SPAD, (a, c) -P conditions, and (b, d) +P conditions. Each point represents the mean value of three replicates. Gray scales show the P content of each accession. The accessions with higher and lower P contents are indicated by black and white colors. The rhombus represents the cultivated cowpea.

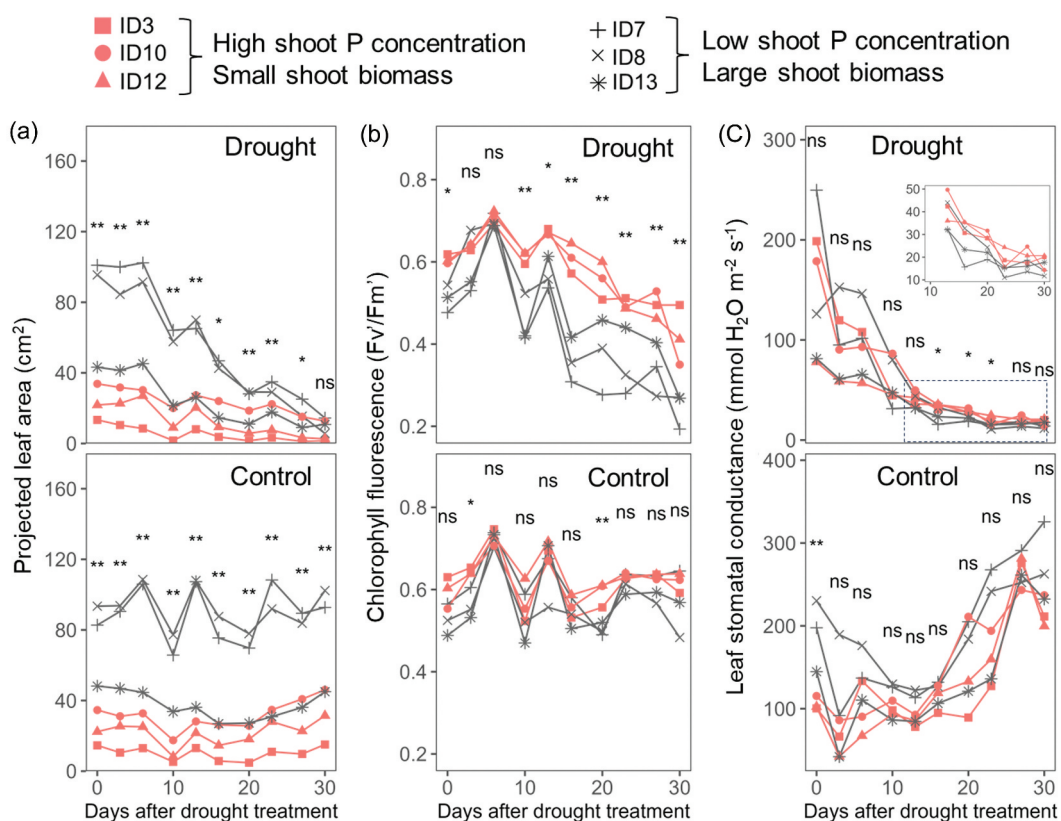


Figure 4. Responses of *V. vexillata* accessions with different shoot P concentrations to drought stress under P-deficient conditions. (a) projected leaf area, (b) chlorophyll fluorescence (F_v/F_m), and (c) leaf stomatal conductance. Symbols and lines with red and gray colors represent accessions with high shoot P concentration and small shoot biomass and accessions with low shoot P concentration and large shoot biomass, respectively. A significant difference in the values at each date between the accession groups with high and low shoot P concentration was indicated as follows: ns: not significant, *: significant at $p < 0.05$, and **: significant at $p < 0.01$.

and low shoot P concentrations throughout the experimental period.

The stomatal conductance under drought conditions started to decrease immediately after the drought onset and dropped below $50 \text{ mmol m}^{-2} \text{ s}^{-1}$ in all tested accessions after 13 days of treatment (Figure 4c). The accessions with high shoot P concentrations tended to have a higher stomatal conductance than those with low shoot P concentrations from 16 to 23 days after treatment, but the difference was diminished at the end of the experiment. Under control conditions, no specific trends in stomatal conductance changes were observed among accessions with different shoot P concentrations.

Discussion

This study revealed large genotypic variation in shoot P accumulation among the *V. vexillata* accessions, representing a range of adaptation strategies of each accession to their original environments. The accessions with

high shoot P content (ID2, ID7, ID8, ID9, and ID13) had relatively large shoot dry weights with low P concentrations. This result indicates that biomass production rather than P concentration contributed to P accumulation. The same accessions with high shoot P content were observed under +P conditions, indicating that the large shoot biomass was caused not by a trait specific to the P-deficient conditions. In wild species, fast leaf development conferring the large shoot biomass is thought to result from natural selection in nutrient-rich environments (Poorter & Remkes, 1990). Therefore, contrary to our expectations, genetic resources inhabiting relatively fertile soils will be favorable to explore to obtain accessions with high P accumulation.

Fast leaf development requires an efficient P acquisition strategy to meet plant growth and maintain the minimum level of tissue P concentration (Vance et al., 2003). Changes in root architecture are a mechanism to efficiently acquire soil P (Gilroy & Jones, 2000). However, in our study, changes in root – shoot ratios between -P and +P conditions

were inconsistent among the accessions with high shoot P content. The ratios were higher under -P conditions than those under +P conditions for ID2, ID8, and ID9. These accessions reduced shoot growth and maintained root growth by preferentially distributing carbon assimilates to the roots under P-deficient conditions. On the contrary, ID7 and ID13 did not show drastic changes in the root – shoot ratio between -P and +P conditions, similar to the cultivated cowpea, indicating that different P-acquiring mechanisms would function for the different *V. vexillata* accessions.

In contrast to the above accessions with high P contents and low P concentrations, *V. vexillata* accessions with low P contents and high P concentrations have small shoot biomass caused by slow leaf development. This phenotype may depend on nutrient conservation by modifying carbon metabolism to circumvent P-requiring steps (Plaxton & Carswell, 1999) or altering tissue lipid composition (Essigmann et al., 1998).

Under this study's advancing drought stress conditions, the higher photosynthetic activity became obvious in accessions with a higher shoot P concentration than those with a lower shoot P concentration, which indicated that the leaves in the former accessions were still alive. At the same time, those in the latter were close to wilt, although both had similar leaf areas and stomatal conductance. The accessions with high shoot P concentration would retain surplus P, which is more than the immediate need for plant growth, in tissues for adaptive or tolerance mechanisms against future environmental stress (Ågren, 2008). In addition, the small shoot biomass confers a lower water consumption than the large shoot biomass, which is also an excellent advantage for long-term survival under progressing drought conditions.

Significant genotypic variation of *V. vexillata* in shoot and root P content under P-deficient conditions, which was also associated with drought adaptability, indicated that these genetic resources were applicable in diverse environments of P deficiency with different soil water availability. Accessions with high P contents are generally preferred for P acquisition. However, the trade-offs between P content and P concentration under -P conditions could be interpreted as trade-offs between P capturing capacity and drought adaptability, which implies that accessions with low P content are suitable for drought-prone environments.

The stability of the shoot N concentration across different P conditions indicated that differences in shoot N content were primarily caused by differences in shoot biomass, the same as that observed in shoot P content. Güsewell and Koerselman (2002) also

reported that plant N concentration was less variable than P concentration, and N concentration varied more among species than among environments. Therefore, the stability of N concentration is not a specific trait of *V. vexillata* but a general phenomenon widely observed across species. Shoot N concentration was unsuitable as an indicator of plant nutritional response to different P conditions.

P deficiency disrupts the photosynthetic apparatus owing to photooxidative damage (Shi et al., 2020; Zhang et al., 2014). However, contrary to our expectations, Fv'/Fm' and SPAD were similar under -P and +P conditions. Furthermore, the genotypic differences in Fv'/Fm' and SPAD were unrelated to P content and concentration. This could be because the growth conditions in this study were insufficient to induce photooxidative damage and abiotic stresses other than P deficiency, such as drought, high irradiation, and high temperature. Accessions with higher P contents and lower P concentrations were preferable under conditions where photooxidative damage was negligible.

This study focuses on the P accumulation during the early vegetative periods of the plants because it is a critical growth stage to establish biomass under low soil fertility conditions (Iseki et al., 2021). However, further studies are needed to fully elucidate the performance of *V. vexillata* under P deficiency throughout the growth period, especially concerning symbiotic nitrogen fixation, which is a key trait for low P adaptation.

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No potential conflict of interest was reported by the author(s).

ORCID

Tomohiro Nishigaki  <http://orcid.org/0000-0002-6669-803X>
Kohtaro Iseki  <http://orcid.org/0000-0001-8721-1553>

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