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澳洲野生綠豆表現型與環境因子間的關聯

The association between environmental factors and phenotypic characteristics of Australian wild mungbean (*Vigna radiata* var. *sublobata*)

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The association between environmental factors and phenotypic characteristics of Australian wild mungbean (*Vigna radiata* var. *sublobata*)

本論文係林雋君（r09b42020）在國立臺灣大學植物科學研究所完成之碩士學位論文，於 2022 年 8 月 29 日承下列考試委員審查通過及口試及格，特此證明

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摘要

綠豆是一種重要的豆類作物。綠豆會與根瘤菌共生進行固氮作用，因此相當適合作為綠肥植物在輪作系統期間種植，恢復土壤肥力。種植綠豆目前所遇到的逆境有鹽害、高溫、乾旱、淹水等，在種植時往往會需要挑選得以應對特定逆境的品系。作物的育種時常會從作物的野生品系著手，尋找那些在野外歷經天擇、已具備抗逆境特性的個體。我們針對澳洲野生綠豆(*Vigna radiata* var. *sublobata*) 的族群結構進行分析，並藉由溫室實驗測量這些野生綠豆性狀、探討其性狀與氣候因子之間的關聯。研究結果顯示澳洲野生綠豆在基因上大致可以分為東、西澳兩大族群。這兩群的野生綠豆在性狀上具有顯著差異，其中包含：果莢大小、種子大小、葉片裂緣程度、生長習性、莖寬、節間數量及開花時間，同時這些性狀在與氣候因子的關聯性分析中表現出顯著相關。結果顯示乾旱較長的西澳地區，其綠豆的表現型具有較少裂緣的葉片、偏向直立型的生長習性、較細的主莖、較少節間數量、較早的開花時間、以及較大的果莢跟種子。從我們的結果可以了解澳洲野生綠豆性狀可能受到當地氣候長久下來的天擇，因而具有不同的表現型。

關鍵字：野生綠豆、表現型、多樣性、氣候、環境、關聯性分析

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Abstract

Mungbean is one of the most important legume crops in the world. The mungbean's symbiotic relationship with rhizobia could perform nitrogen fixation and maintain soil fertility in the crop rotation system. Due to the salinity, high temperature, drought, and waterlogging, the accession of mungbean used must be considered to overcome the stress. The breeding of crops often starts with the wild plants which have been selected by the habitat environments and have the resistance to stress. With wild mungbean accessions from Australia, we examined their population structure, measured several traits, and analyzed the correlation between traits and environmental factors. Our results showed that mungbeans in Australia could be genetically classified into two genetic groups, one in Western Australia and one in Eastern Australia. There were significant differences between the two groups' traits, which included pod size, seed size, lobed leaflets, growth habits, stem width, internode number, and flowering time. The wild mungbeans from Western Australia were with fewer lobed leaflets, erecting plant growth habits, thinner main stems, less internode number, early flowering, and higher yields. Also, these traits were significantly correlated with environmental factors. The different phenotypes of wild mungbean in Australia may result from the long-term selection in the environments. These findings may provide scientists with inspiration for breeding.

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Keywords:

Wild mungbean, phenotype, variation, climate, environment, correlation analysis

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Introduction

Mungbean (*Vigna radiata* (L.) R. Wilczek var. *radiata*) is one of the important legume crops widely planted in Asia, Africa, and Australia. Seeds of mungbean contain about 20.97–31.32% protein (1), which is higher than most seed crops, such as soybean (18–22%) and maize (7–10%) (2, 3). The high protein content makes mungbean an alternative protein source that ensures food security in food-deficient regions (4). Mungbean is often planted as a green manure crop in the crop rotation system because of its symbiotic relationship with rhizobia (5), which reduces the use of nitrogen fertilizers and achieve sustainable agriculture. However, the yields of mungbean may be reduced by abiotic stress such as salt, drought, heat, and waterlogging (6, 7). Nowadays, the strength of global warming and the frequency of extreme climates increase, such as extreme high (low) temperatures, the prolonged dry season, and high-intensity rainfall in a short period. Moreover, global warming causes the melt of glaciers and leads to the rising of sea level, which results in the loss of habitats and arable lands.

In harsh environments, plants have several strategies to overcome stress. Take water limitation as an example, which may force plants to evolve phenotypes withstanding droughts, such as drought-escape, drought-avoidance, and drought-tolerance characteristics (8-10). Plants with the drought-escape strategy develop a quick

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life cycle to reproduce before the dry season. The drought-escape traits include a short vegetative period, high metabolic rate, and early flowering (8, 9). Plants with a drought-avoidance strategy tend to maintain high water content during the dry season. The related traits include extensive root systems to search for underground water, less plant water loss, and frequent closure of stomata, which results in the reduction of transpiration (11, 12). The plants with a drought-tolerance strategy could endure low hydration in tissues (13), which needs several mechanisms to keep the cell function working, including osmotic adjustment and antioxidant metabolism (12). The activation of antioxidant metabolism is used to avoid the high concentration of reactive oxygen species (ROS) under water-limiting conditions. Temperature is another factor influencing a plant's growth and yield. If the temperatures are high, plants may evolve a high transpiration rate to cool down quickly (14). Another characteristic is the expression of heat shock proteins to prevent the incorrect folding of proteins (15).

Adaptation to environments is an important result of evolution, which involves mutation, migration, genetic drift, and natural selection. Migration provides the gene flow between populations, allowing some new alleles to be introduced into populations. Genetic drift represents the phenomenon that alleles' frequencies are influenced by random effects. Mutation and recombination causes the difference in fitness among individuals and provides the materials to be filtered by natural selection (16).

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Individuals with higher fitness may have advantages in survival and more opportunities to leave their genes to their offspring. Some alleles' frequencies may increase after generations of natural selection, which leads to the occurrence of more individuals with certain phenotypes in a population. Due to the genetic changes caused by generations of selection, even when the offspring of a species are transplanted into a stable environment, they will exhibit characteristics found in the original environment.

Unlike wild species under strong natural selection, most cultivated crops, such as maize, soybean, and mungbean, were domesticated by humans (17, 18). The domestication process has four stages. The first stage starts with a long period of cultivation and the selection of certain genotypes, which may take a long time. Following the first stage, the second stage is accompanied by the increase of certain alleles' frequencies, which may lead to an increase in yield. In the third stage, the domesticated crops spread out and are planted in environments different from their origins, which leads to the need for new cultivars that could overcome the new diversified environments or fit the flavor of locals. In the fourth stage, the cultivars with high yields, uniformity, and quality are bred to develop large-scale agriculture (18). After domestication, the genetic variation of cultivars is often less than the wild type due to the selection or the exclusion of certain traits according to human's will. Some traits that are necessary for the wild plants are removed in cultivars to result in better

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yields or stable quality, such as the pod-shattering in wild mungbean. The shattering of pods can ensure the spread of seeds, while this characteristic will lead to the loss of yields in agriculture. There is another example, calcium oxalate crystals are the defense against herbivores in wild spinach. After the domestication event, the content of calcium oxalate crystals in spinach is reduced to get a better taste (19). However, domestication may lead to the loss of gene diversity. The gene lost may include those with resistance to abiotic stress or biotic stress.

Most scientists may want to restore the gene of stress resistance in crops through molecular biotechnologies**,** such as CRISPR-cas9 and *Agrobacterium tumefaciens*-mediated plant transformation. However, there is another way of breeding new cultivars without the generation of genetically modified organisms (GMOs), which relies on the gene diversity in the wild species and candidate gene study. Wild species are rich in gene diversity, among which there may be genes with stress resistance. Candidate gene study often starts with the biological, physiological, and pathology of species so as to find the locus that is related to diseases or stress. Genome-wide association studies (GWAS) and quantitative trait locus (QTL) mapping are often used to detect disease-resistant genes or the abiotic stress resistance gene. After finding the genes we need, the process of conventional breeding will be accelerated, quickly obtaining new cultivars.

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The phenotype studies and the correlation analysis between climates and species phenotype are important to link how traits are shaped and how species adapt to environments. A local researcher in Australia, Dr. Robert J. Lawn, has investigated the phenotypes of *Vigna radiata* var. *sublobata*. In one of his studies (20), he crossed *Vigna radiata* var. *sublobata* with *Vigna radiata* ssp. *radiata* and obtained healthy and fertile offspring, exhibiting the close relationship between the cultivar and wild mungbeans. He measured phenology, leaf-related traits, biomass, and yields in other studies (21, 22). In the study about phenological traits (21), he performed common garden experiments in different seasons and found that those early flowering individuals will live with a longer flowering period. He also found there were two accessions from inland Northwestern Australia that showed early flowering but a short flowering period. In the study about leaf-related traits, biomass, and yields (22), Dr. Lawn compared these traits between the cultivar and wild mungbeans. He found that leaf-related traits were mostly associated with the mean daily temperature during his experiment. The leaf mass production is positively correlated to the temperature. For biomass, his results exhibited that it has a positive correlation to the duration of growth. The yields of wild mungbeans in his study were positively correlated with the duration of the reproduction stage, which suggested an indeterminate growth characteristic in the wild type. In another study about the traits of wild mungbean (23), he recorded the flowering time, lobed

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leaflets, seed size, yields, seed protein content, and seed hardness. In this study, he exhibited that there might be differences in phenotypes among Western Australia and Eastern Australia's wild mungbeans. In general, his mungbean samples from Eastern Australia had more lobed leaflets, longer flowering periods, higher yields, and a perennial lifestyle. The perennial habit of wild mungbeans was investigated in another study by Dr. Lawn (24). In this study, he collected the mungbeans with perennial lifestyles and measured shoot-related and root-related traits. These samples exhibited thickened lateral roots and the root biomass was positively correlated with the plant biomass and negatively correlated with the seed biomass. Dr. Lawn's studies included many aspects, while the samples he used only covered parts of Australia. There is a lack of phenotype studies with large amounts of accessions from both Western and Eastern Australia.

Our studies want to make up for the lack of phenotype studies with complete accessions in Australia and discover what shaped these phenotypes. With the germplasm from all over Australia, we measured a series of wild mungbean traits and examined their correlation to environmental factors. Our results may provide new opportunities for breeding new cultivars of mungbean which is able to live under stresses.

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Materials and Methods

Plant materials and common garden experiment

One hundred and twenty-nine accessions of wild mungbean (*Vigna radiata* var. *sublobata*) were selected from the Australian Grains Genebank (AGG) germplasms. The seeds were originally collected from Eastern Australia, Northern Territory, Western Australia, Papua New Guinea, and West Timor. Detailed geographic information was provided by AGG. The common garden experiment was performed in the greenhouse of the National Taiwan University from July 2020 to January 2021. The experiment consisted of five randomized blocks, with each block containing 129 accessions, each in one pot (12.6 cm in height and 14.7 cm in diameter). Two individuals of the same accession were initially planted within the same pot, and after 9 weeks, the pots were thinned to one individual each. The sacrificed individuals were used for trait measurements requiring destructive sampling (see below). The blocks were rotated around the greenhouse chamber every alternate day to reduce micro-environmental effects. The temperature was controlled at 26~28°C, and the illumination was controlled by covering the glass wall with hoods and masks to avoid sunburn. The plants were watered every two days. Hyponex No.2 fertilizer was diluted into the 1% solution with water, and 250 ml solution was applied for each pot every three weeks.

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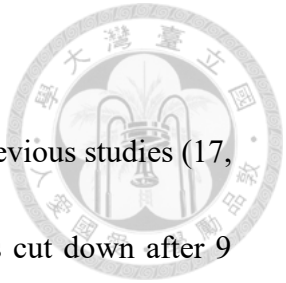

Trait measurement

The quantitative traits (Table 1) were measured according to previous studies (17, 21, 22). In each pot, one of the individual's aboveground part was cut down after 9 weeks from sowing to measure plant fresh weight, biomass, leaf area, leaf fresh weight, and leaf dry weight. Plants and leaves were dried in the oven EYELA WFO-600SD under 55°C for two days. Weight measurements were performed with the electronic scale. Leaf fresh weight was measured as the mean of two healthy, complete leaves without the petiole. We used the scanner ArtixScan F2 to scan fresh leaves. Leaf area was measured as the mean of two complete leaves with ImageJ. The unit used in ImageJ was set as centimeters. Based on the traits measured above, we calculated other traits. Leaf density was defined as the ratio of leaf (fresh or dry) weight to leaf area. Specific leaf area was defined as the ratio of leaf area to leaf dry weight. Leaf water content was defined as the difference between leaf fresh weight and leaf dry weight. Relative leaf water content was defined as the ratio of leaf water content to the leaf fresh weight. Plant water content was defined as the difference between plant fresh weight and biomass. Relative shoot water content was defined as the plant water content divided by plant fresh weight.

After thinning down the pots, the remaining individual of each pot was cultivated until 24 weeks, and the following traits were measured. Chlorophyll content was

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measured as the mean value of three terminal leaflets with the SPAD-502 meter after 13 weeks from sowing. Leaflet length, leaflet width, and the shortest width of the leaflet were directly measured as the mean of the three healthiest terminal leaflets per plant with a ruler after 18 weeks from sowing. The shortest width of the leaflet was defined as the sagging part of the terminal leaflets. Leaflet width-length ratio (w-l ratio) was defined as the ratio of leaflet width to leaflet length. Leaflet width-shortest width ratio (w-s ratio) was defined as the ratio of leaflet width to the shortest leaflet width. Growth habit was identified as erect (value = 1), semi-erect (value = 2), and trailing (value = 3) after 18 weeks from sowing. Leaf shape of mungbean was identified as hastate (value = 5), sub-hastate (value = 10), sub-globose (value = 15), and globose (value = 20) after 19 weeks from sowing while considering all of the leaves per plant.

During the experiment, we recorded the date of the first bud, the date of the first flower, the date of the first mature pod, and the date of the last mature pod. Pod-production period was defined as the difference between the date of the first mature pod and the last mature pod. Flower color was identified as white-yellow (value = 1), yellow (value = 2), creamy-yellow (value = 3), dark yellow (value = 4), and green-yellow (value = 5).

Stem length, the height to the first branch, reproductive length of the stem, internode number, side branch length, and stem width were measured with band tape

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and the digital caliper at 19 weeks after sowing when the growth stopped. The height to the first branch was defined as the length from the plant basal part to the first branching node. The reproductive length of the stem was defined as the length from the last node to the stem top, which is the region flowering occurs. Side branch-stem ratio was defined as the ratio of side branch length to the stem length. Mean internode length was calculated as the following equation, mean internode length = (stem length - reproductive length - first internode length) / internode number. Stem color was identified as five colors and transformed into two traits, stem green level and stem pink level. Stem green level ranges from one to five. Stem pink level ranges from one to four.

In the meantime, we collected and counted pods of each plant. Pods were dried to measure the pod-related traits. Seed number per pod was averaged from three pods. Pod-twists were measured as the mean of three pods' number of twists. Seed weight was measured as the mean of thirty complete seeds by an electronic scale. Expected seed number was estimated with pod number multiplied by seed number per pod. We used the scanner ArtixScan F2 to scan pods and seeds. Pod length was measured as the mean of three pods with ImageJ. Seed area was measured as the mean of ten seeds. The unit used in ImageJ was set as centimeters. Pod length of each roll was defined as pod length divided by pod-twists. Pod color was identified as light brown (value = 1), brown (value = 2), green-brown (value = 3), dark brown (value = 4), black (value = 5), and

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dark black (value = 6). Pod hair was identified as light (value = 1), medium (value = 2), high (value = 3), and very high (value = 4). Pod-shatter type was identified as no-shatter (value = 2), semi-shatter (value = 3), and fully-shatter (value = 4). Seed coat color was identified as green-black (value = 1), brown (value = 2), black-brown (value = 3), and black (value = 4). Seed pattern was identified as non-pattern (value = 1) and mottled (value = 2). All of the qualitative traits were turned into value before analyses, as shown above.

Environmental factors and niche modeling

The environmental data were obtained from the Worldclim database version 2.1 (during 1970-2000) (https://www.worldclim.org/), and the global aridity index and potential evapotranspiration climate database version 3.0 released by CGIAR CSI (https://cgiarcsi.community/). The data used in the analysis were from November to April, the growing season of wild mungbean (Lawn, personal communication). All of the environmental factors were downloaded with a resolution of 30 seconds. Twenty-four environmental factors, including BIO1-BIO19, aridity index, evapotranspiration, solar radiation, wind speed, and water vapor pressure (Table 2), were extracted with the R package, Raster (25). BIO12, the annual precipitation was replaced with the mean precipitation. The environmental data were transformed with rank-based inverse normal transformation to reduce effects from extreme outliers. The climate diagrams

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(Figure 6) were made with the R package, climatol (26), and the data from the Worldclim dataset from November to April during 1970-2000.

To investigate the potential distribution of wild mungbean, we performed the niche modeling for geographical areas 66.5 – 155.77 °E and 32.92 °N – 27.82 °S, which includes India, Southeast Asia, Indonesia, and Australia, the region with wild mungbean records. The niche modeling was performed with the software MAXENT 3.4.1 (27, 28). Pairwise correlation between environmental factors was calculated to exclude highly correlated factors (Pearson's correlation coefficient higher than 0.8). We chose BIO1 – mean annual temperature, BIO2 – mean diurnal range, BIO3 – isothermality, BIO12 – annual precipitation, BIO15 – precipitation seasonality, and BIO18 – precipitation of warmest quarter to be used in the niche modeling. The geography information we used contained records from AGG and the global biodiversity information facility (GBIF), including 22 occurrence points in India, 27 in Southeast Asia, and 39 in Australia. For niche modeling, we set 10000 background points, ten-fold cross-validation, and five thousand times maximum iterations. We used the downscaled CMIP5 data of the MIROC-ESM model from WorldClim. The time scale used included mid-Holocene, Last Glacial Maximum, and Last inter-glacial period. The ASCII files produced by MAXENT were used to estimate Schoener's D index (29). With this value, we could quantify the overlap between each niche simulation. Schoener's D ranges from 0 to 1,

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and the high value suggests a high overlap between modeled distributions. Another niche modeling was performed to investigate the distribution of different genetic groups within Australia (109.84 – 158.03 °E and 0.32 °N – 44.4 °S). The geography information used here was provided by AGG, including 28 dots in Western Australia and 31 dots in Eastern Australia. We used the present data from WorldClim. For niche modeling, we set 10000 background points, ten-fold cross-validation, and five thousand times maximum iterations.

Population genetic analysis

The read mapping, SNP calling, and population structure analyses were performed by members of the Lee lab. In brief, Illumina raw reads were trimmed with Trimmomatic v0.38 (30). Adaptor sequences were removed with Trimmomatic v0.38. The cleaned reads were mapped to the wild mungbean reference genome with BWA v0.7.15 (31). One hundred and eleven accessions' genome information was used to find the single nucleotide polymorphisms. The SNPs are called with GATK v4.2.2.0. Using vcftools v0.1.13 (32), we removed the indels and retained bi-allelic SNPs with the minimum allele = 2, the maximum = 2, and the max-missing value = 0.9. The quality score was set as 30.

The wild mungbean population structure in Australia had been clarified in the previous research from our laboratory with the software ADMIXTURE v1.3.0 (33).

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Our laboratory member estimated the cross-validation error (CV error) with different *K* values, which assumed the possible population number from two to five. We selected the result of the two groups (*K* = 2, CV error = 0.2805) as biologically meaningful clusters to proceed to the next analysis. The *Q* value larger than or equal to 0.7 was used to define whether the accession was pure or admixed.

Statistical analysis

After comprehending there were two genetic populations in Australia, our statistical analysis was focused on the difference between the two groups. All trait data were transformed with rank-based inverse normal transformation to reduce effects from extreme outliers. Our statistical analyses were performed with JMP, and R. With the traits of each accession, the principal component analysis (PCA) was performed to distinguish the two genetic groups' overall trait differences. We chose 34 traits and performed PCA with the R function prcomp (34). The traits measured on those 9-week plants and the shortest width of the leaflet were excluded in the PCA due to excess amounts of missing data.

To examine the difference between two genetic groups' individual traits, we used analysis of variance (ANOVA). In JMP13.0.0., the accession was set as a random effect nested within the fixed genetic group effect. The individual's trait data were taken as

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response variables. We took the *P* values less than 0.05 as significant and compared these results with the Q_{ST} value.

With JMP13.0.0, we calculated the least-square mean of each accession, where the accession was set as the random effect. With accession-level traits and climates data, the regression analysis was performed with R to identify the correlation between environments and accession's mean trait value. In the function y = ax+b, we used the traits of each accession as y and the environmental factors as x. The model was run independently for trait-environment combinations. *P* values less than 0.05 were considered significant.

Although we investigated whether genetic groups or local environments affect plant traits, genetic groups and environmental factors are highly correlated. To investigate their individual effects independently from each other, we constructed another model. With JMP13.0.0, individual trait data were used as the response variable. Accession was set as a random effect nested within the fixed genetic group effect. Environment (numeric) and its interaction with genetic groups were also set as fixed effects. The model was run independently for each trait-environment combination. *P* values less than 0.05 were considered significant.

The Q_{ST} and F_{ST} statistics (35) were performed to investigate the type of natural selection on traits between populations. F_{ST} represented the genetic variation among

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different populations, and Q_{ST} was estimated as the trait variation as a proportion of total variation among populations. With JMP13.0.0, we took accession and the genetic group as random effects; meanwhile, accession was nested within the genetic group. The result included three variance (σ^2) components belonging to the genetic group (GG), accession (AC), and residual, respectively. The formula of Q_{ST} was shown as follows: $Q_{ST} = \sigma^2_{GG}/(\sigma^2_{GG}+2\sigma^2_{AC})$. F_{ST} was calculated from bi-allelic SNPs with vcftools v0.1.13. A trait's Q_{ST} larger than the 95 % tail of F_{ST} distribution meant that this trait's difference between the two populations was larger than most of the genetic variations, showing a tendency for disruptive selection. In contrast, the Q_{ST} less than F_{ST} suggested the stabilizing selection of wild mungbeans.

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Results

Wild mungbean's population structure in Australia

With ADMIXTURE, we identified that the wild mungbeans in Australia could be classified into two groups, 45 accessions in Western Australia, 55 accessions in Eastern Australia, and eleven admixed accessions (K = 2, Figure 1). On the other hand, there were four Eastern Australia accessions located in Western Australia, which might be the error of the GPS records. We excluded these four accessions in any following analysis with location or environmental factors. We investigated the proportion of trait and climate data with PCA. With a total of 34 traits, PC1 explained 19.67% of trait variation, and PC2 explained 17.13%. The two genetic groups of wild mungbeans are separated by PC2 (one-way ANOVA, $F_{1, 80} = 40.3827$, $p < 0.0001$) (Figure 2a), while the PC1 ($F_{1, 80} = 0.8102$, $p = 0.3708$) showed without significance between the two groups. We also performed the PCA with 24 climate factors (Figure 2b), both of PC1 (one-way ANOVA, $F_{1, 74} = 12.3111$, $p = 0.0008$) and PC2 (one-way ANOVA, $F_{1, 74} = 57.7662$, $p < 0.0001$) exhibited significance between genetic groups.

The climate of the two groups' habitats was significantly different according to the ANOVA analyses (Table 2). Eastern Australia generally has more precipitation and a longer growing season compared to Western Australia (Figure 6). The main growing season in Eastern Australia is from November to April. As for Western Australia, the

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growing season is limited from December to March (Figure 6).One-way ANOVA identified several traits significantly differentiated between the two genetic groups (Table 1), including w-s ratio (the degree of lobed leaflets), stem width, internode number, seed weight, and pod length of each roll (pod length divided by the pod twist number). With the F_{ST}-Q_{ST} comparison, we investigated whether divergent selection occurred among the two groups. Among these traits, only w-s ratio's Q_{ST} was higher than 95 % of SNP F_{ST} values (0.3037) (Figure 3a). With the highest Q_{ST} value, w-s ratio was lower in Western Australia, which suggested the less expressively lobed leaflet, and there were more accessions with lobed leaflet in Eastern Australia (Figure 4). Despite most traits' Q_{ST} being lower than F_{ST}, we found a strong association between traits' Q_{ST} and *P*-value (Figure 3a). In general, accessions from eastern Australia tend to have more lobed leaflets, a thicker main stem, more internodes, lighter seeds, and a longer pod length of each roll (less rolling in dried pods); those from Western Australia showed the opposite patterns.

Based on the difference in climate and F_{ST}-Q_{ST} statistics, we assumed that the different phenotypes between the two genetic groups might result from the adaptation to different environments. In Western Australia, the lack of water would limit the possibility of further reproductive chances. While in Eastern Australia, water resources are sufficient and able to prolong the growing period. Here, we had several expectations

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of the mungbean's phenotype. The mungbeans in Eastern Australia might have larger plants, more pods, and more yields than those in Western Australia, which may result from the longer growing season. In our results, thicker main stem, more internode number, later first flower date, and later last pod date from the Eastern Australia accessions were consistent with our expectations that accessions live under the longer growing season may grow as larger plant size and reach the reproductive stage lately. On the other hand, the accessions from Western Australia produced higher yields than those from Eastern Australia, which is not reasonable with the shorter growing season in Western Australia.

The influence of climate on wild mungbean's traits

According to our correlation analysis, the traits could be classified into four main categories (Figure 7). The first category includes pod and seed-related traits. The second includes relative leaf water content, w-s ratio, and some other traits that seem without connection to each other. The third includes mostly the phenological traits and the stem-related traits. The fourth includes biomass and plant-water-related traits (nine weeks), leaf-related traits, and some pod-related traits.

Environmental factors were also grouped into four types (Figure 7). The first type includes most of the temperature variables, precipitation seasonality (BIO15), solar radiation, and evapotranspiration. The second includes wind speed and longitude. The

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third includes latitude and the temperature in the coldest month. The fourth includes most of the rainfall variables, aridity index, and water vapor pressure.

The first category of trait is positively correlated with the temperature variables (annual mean temperature, the maximum temperature of the warmest month, and mean temperature of the wettest quarter) and negatively correlated with the precipitation variables (mean precipitation and precipitation of the driest month) (Figure 7). The first category of traits contains seed area, seed weight, pod length, and pod length of each roll. With ANOVA analysis, these four traits exhibit significant differences among the two genetic groups. They are positively correlated with the temperature variables and solar radiation (Figure 8-11) and negatively correlated with precipitation of the driest month (BIO14). With higher temperatures and strong light intensity in Western Australia, mungbeans develop bigger seeds and larger pods compared to those from Eastern Australia.

The second category of trait is negatively correlated with latitude and the minimum temperature of the coldest month (BIO6) and is positively correlated with wind speed and longitude (Figure 7). The second category of traits contains some important traits which exhibit differences among the two genetic groups, including w-s ratio, relative leaf water content, seed coat color, and plant growth habit (Table 1). w-s ratio is an index to determine a sample's lobed leaflet level. This trait is negatively

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correlated with annual mean temperature and latitude. Accessions with more lobed leaflets occur in Eastern Australia, which is the region with higher rainfall and a longer growing season compared to Western Australia (Figure 6). The Q_{ST} and F_{ST} comparison also indicates the differentiation of this trait between the two groups (Figure 3a).

Relative leaf water content is negatively correlated with mean precipitation (BIO12), precipitation of wettest quarter (BIO16), and water vapor pressure (Figure 13). This trait also shows significant differences between the two groups, and the accessions of mungbean in Western Australia have a lower relative leaf water content than those in Eastern Australia.

Plant growth habit is another trait that exhibits significant differences between the two groups. Most wild mungbeans in Eastern Australia grow with a more-trailing growth habit, and those in Western Australia grow with a more-erect growth habit. This trait is negatively correlated with mean temperature of coldest month (BIO6) and latitude and positively correlated with longitude (Figure 14).

The third category of traits is negatively correlated with the temperature variables (mean diurnal range and temperature annual range) and positively correlated with the precipitation variables (annual precipitation and precipitation of the driest month) (Figure 7). The third category of traits contains phenological traits, including first flower days, bud seen days, pod mature days, last pod days, and some other stem-related

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traits. These phenological traits show negative correlations with mean diurnal range (BIO2), temperature annual range (BIO7), evapotranspiration, and positive correlations with mean precipitation (BIO12), and precipitation of driest month (BIO14).

Stem-related traits in the third category include side branch length, stem width, and internode number. Side branch length is positively correlated with precipitation of the wettest quarter (BIO16) (Figure 17), which is reasonable that those accessions with more rainfall will develop a large plant size. Stem width and internode number are negatively correlated with mean diurnal range (BIO2), temperature annual range (BIO7), and positively correlated with precipitation of the warmest quarter (BIO18) (Figure 18-19). Stem width shows a significant difference between the two genetic groups, Eastern Australia's accession stems are thicker than those of Western Australia. A thicker stem may be able to bear heavier plant weight resulting from the long growing season. The wild mungbeans in Eastern Australia have more internode numbers and longer side branches than in Western Australia, which is also consistent with our expectations. The accessions that live in Eastern Australia have a longer growing period (Figure 6b) than in Western Australia, which facilitates mungbeans to develop a longer lifecycle.

In the fourth category, most traits exhibit weak correlations with climate variables (Figure 7). Despite the lack of significant correlation, plant water content and leaf water

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content exhibit slightly positive correlations with temperature and precipitation factors. As factors are higher, plant (or leaf) water content gets higher, which may be traits for adaptation to harsh environments.

The influence of climates while considering the groups' difference

Although the results above have shown significant correlations with climate variables, there is another possibility to be considered. The differentiation of traits may decouple with the difference between the two regions. The traits may just slowly differentiate as time pass by and don't associate with the differences in climates. To control this confounding factor, we performed an ANOVA analysis considering the genetic group as one of the fixed effects. The result of the ANOVA exhibits whether the traits in each genetic group are influenced by climates.

For the first category of traits, the traits highly influenced by environmental factors include seed weight, seed area, pod length, and pod length of each roll. Only solar radiation (srad) and evapotranspiration (evap) significantly influenced seed area (Figure 20). Our analysis exhibits the decoupling between yields and environmental factors. There might be other reasons that influence yields.

For the second category, relative leaf water content and relative shoot water content are significantly influenced by mean precipitation (BIO12), precipitation of the warmest quarter (BIO18), and precipitation of the coldest quarter (BIO19) (Figure 21).

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The relative water content is higher in Eastern Australia's accessions than in Western Australia. Another trait, w-s ratio, is significantly influenced by precipitation of the coldest quarter (BIO19), aridity index, evapotranspiration, and solar radiation. Those accessions from Eastern Australia were with more lobed leaflets.

For the third category, side branch length, internode number, stem width, first flower days, and last pod days are influenced by mean diurnal range (BIO2), precipitation seasonality (BIO15), evapotranspiration (evap), and solar radiation (srad) (Figure 22). The phenological traits should be correlated with temperature and photoperiod. Our analysis is in line with this phenomenon. The two phenological stages occur earlier in Western Australia's accessions than those in Eastern Australia. For the three stem-related traits, accessions from Eastern Australia were with longer side branches, more internode numbers, and thicker main stems.

For the fourth category, including plant water content, biomass, leaf fresh weight, leaflet length, and leaflet width, these traits are not significantly influenced by any environmental factors.

Niche modeling of wild mungbean

Vigna radiata var. *sublobata* is distributed in Asia, Africa, and Australia, while their closely related species are located in Asia, which suggests the origin of wild mungbean may be in Asia. The wild mungbeans may spread via Southeast Asia or

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Oceania to Australia. Our lab members have found the differentiation time between Asian and Australian mungbeans was about 50,000 years ago. Here we used data from the Worldclim dataset during the last interglacial period (120,000 years ago), last glacial maximum (20,000 years ago), and present to examine whether the distribution of wild mungbeans in the past is consistent with the differentiation time we calculated before.

Our result (Figure 23) showed that the climate during the last interglacial period in Australia was not suitable for wild mungbeans. Only South Asia and Southeast Asia's climates were suitable for living. As for the modeling during the last glacial maximum, we can tell that the results of Southeast Asia, Oceania, and Australia share a high overlap, which exhibits that the climate of the three regions at that period is suitable for mungbeans. This result suggests that wild mungbeans have the opportunity for large-scale dispersal during the last glacial maximum.

The second model is performed with the collection area of our samples in Western Australia and in Eastern Australia. According to our results, the wild mungbeans had not differentiated during the last glacial maximum (Figure 23). While in the present, our samples showed differentiation in niches between Eastern Australia and Western Australia (Figure 24).

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Discussion

The wild mungbeans are distributed in Asia, Africa, and Australia. The closely related species of mungbeans are mainly in Asia. Thus, scientists mostly assume that Asia is the origin of the wild mungbean (36). In addition to Asian studies, Australia has many records of wild mungbeans. Local scholars in Australia have investigated the wild mungbean (21-23, 37), while the wild mungbean's expansion path and its adaption mechanism to different environments are still unclear. Here, we investigated the difference in wild mungbean traits between Western and Eastern Australia. The phenotype study is an effective method to investigate how species adapt to environments with specific traits. Our results exhibit that wild mungbean in Eastern Australia develops a phenotype with a trailing-type plant, a thicker main stem, shorter pods, smaller and lighter seeds, and a late pod production date.

The trailing plant and thicker main stems in Eastern Australia are consistent with our expectations. With a longer growing season, Eastern Australia's accessions may have greater growth potential and develop a strong main stem to support the heavier weight. The trailing habit of pea species is often associated with indeterminate growth and perennial lifestyle (38). According to previous studies (23), the perennial lifestyle of wild mungbean is defined as the continuous growth after removing the above-ground part or after flowering (39). In the field record, the above-ground part of wild

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mungbeans with the perennial lifestyle will die in the drought season while its underground roots are thickened and remain alive until the next growing season (Lawn, personal communication). Despite there being only a few accessions in Eastern Australia with the perennial lifestyle, our results suggested that accessions in Eastern Australia might develop the perennial lifestyle. Western Australia has longer drought season, which may hinder the development of wild mungbean's underground roots (Figure 6a). However, the wet season is longer in Eastern Australia (Figure 6b), which may provide wild mungbeans an opportunity of developing tuberous roots. The distribution of our perennial accessions was consistent with the records that Dr. Lawn described in his studies (23), which suggests an evolutionary event within small regions.

The growth habit (indeterminate & perennial, determinate & annual) is an important characteristic while breeding new lines. The accessions with indeterminate growth and perennial lifestyle produce higher yields than the determinate lifestyle (40). The genetic mechanism of determinate growth in mungbean cultivars has been discussed from different perspectives (41, 42). A certain gene, *VrDet1* has been found to encode a signal protein of shoot apical meristem, thus controlling the expression of indeterminate growth. Mungbeans would develop into determinate growth when *VrDet1* is reduced (43). Despite the accessions being widely planted in Asia are those

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with determinate growth and annual lifestyle, the phenotype with the indeterminate growth may be a choice for breeders in seeking new lines.

Phenological traits are consistent with our expectations that mungbeans may adapt to the drought environment with early flowering after the natural selection in Western Australia. According to previous studies (8-10), mechanisms to overcome drought include three strategies, the drought-escape, the drought-avoidance, and the drought-tolerance. Plants with drought-escape characteristics tend to develop a rapid life cycle to escape growing in the drought period. Plants with drought-avoidance characteristics tend to maintain high water content in tissues by reducing water loss or promoting water uptake. Plants with drought-tolerance characteristics tend to live with low tissue water content, maintain the cell turgor and increase protoplasmic resistance. With the drought-escape characteristic, the metabolic rate is highly reinforced, which results in early flowering.

In our results, first flower date represents when a plant transit to the reproductive stage, which is affected by the temperature, water source, photoperiod, and stress (21, 44, 45). Flower dates will get earlier when the precipitation is less (Figure 15), which corresponds to the drought escape mechanism. The genes and mechanisms of early flowering or rapid metabolism have been researched in several species (46-48). In comparison, the research about SNPs related to flowering between Eastern Australia

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and Western Australia is still unclear, which is another point worth further studying. In all of the phenological traits, only the last pod date is significantly different between the two genetic groups. The accessions in Eastern Australia (122 days, Table 1) have a later last pod date than those of Western Australia (117 days, Table 1), which suggests the difference in the life cycle between the two groups. The longer growing period and more rainfall in Eastern Australia may allow mungbeans to postpone their life stage and even prolong their reproductive stage. We also examined the results of phenological traits and latitude which represent the photoperiod of certain regions. However, the correlation between latitude and these traits is not significant. The main factor in selecting flowering time may not be the photoperiod for wild mungbean. Photoperiod may just affect the flowering time in terms of phenotypic plasticity.

The lobed leaf index (w-s ratio) is an outstanding trait that exhibits significant differences between the two regions and has a negative correlation to annual mean temperature (BIO1) (Figure 12). Wild mungbeans in Eastern Australia develop more lobed leaflets than in Western Australia. However, our ANOVA analysis with the group control exhibits that w-s ratio is significantly influenced by mean precipitation (BIO12), precipitation of the coldest quarter (BIO19), and aridity index rather than the temperature-related factors (Figure 21). Previous studies have shown that woody plants in the temperate zone develop lobed leaves or margins with sawtooth to enhance the

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efficiency of photosynthesis in the early development stage of plants (49). In contrast, our accession's phenotypes showed that the margin of the leaflet might not be linked with temperature. An interesting study (50) from India's local researchers showed that wildtypes from New Delhi (average temperature during summer ranging from 25°C to 45°C) developed lobed leaflets. In addition to this trait, that research (50) also exhibited three accessions with lobed leaves were late-flowering. Combining previous research and our results, the prominence of this trait in Eastern Australia may not result from the selection of the climates, which may originate from their ancestors in Asia.

On the other hand, some traits are inconsistent with our expectations, including the pod and seed size. We expect that wild mungbeans in Eastern Australia will produce larger pods and have more seeds since Eastern Australia has more rainfall and a longer growing period. However, the results were the opposite, and the pods and seeds from Western Australia were larger than those from Eastern Australia (seed number per pod and pod number are not significantly different between the two populations). After examining all of the traits we measured, there might be differences in the two populations' survival strategies.

According to the C-S-R triangle theory (51), there is a trade-off between growth, maintenance, and reproduction. The plant species could be classified into three kinds of strategy: competitor, stress tolerator, and ruderal. The competitor lives in the region

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under light stress and low-intensity disturbance. Competitors are with rapid growth rates, high productivity, and high phenotypic plasticity. These features allow competitors to gain resources efficiently and quickly. The stress tolerator lives in the region under strong stresses and low-intensity disturbance. The stress tolerator has slow growth rates, high nutrient retention rates, low phenotypic plasticity, and certain physiological reinforcement, which can overcome certain stresses. The ruderal lives in the region with high-intensity disturbance and low-intensity stress. Ruderals are with fast growth rates, a short life cycle, and large amounts of seeds.

Based on our results (Table 3), the shorter growing season in Western Australia (Figure 6a) may force wild mungbean development as a ruderal. The long drought in Western Australia may cause the soil to dry out, in which case the underground roots cannot survive. So most of the nutrients are used for reproducing quickly, resulting in a faster lifecycle, larger pods, and bigger seeds. Furthermore, the early flowering of Western Australia accessions was a solution to escape from the drought season. While those accessions in Eastern Australia may be closer to the competitor, they live in a stable environment with a longer growing season. The development period from germination to reproduction may need longer time and nutrients, which may be why some accessions have developed thickened roots and remain alive until the next growing season. Most of the nutrients may be stored at the thickened roots; only a few

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are spent for offspring. The avoidance of producing seeds as much as possible may reduce the risk of wasting nutrients, which may be why Eastern Australia's mungbeans produce small seeds. In the long term, the wild mungbeans from Eastern Australia may produce more seeds than those from Western Australia if the environments are suitable. Our results showed that wild mungbeans had developed different strategies under different environments (Table 3).

Due to the less rainfall in Western Australia, we expected that this area's mungbeans would have mechanisms to escape the drought. Therefore, the samples from Western Australia may have drought tolerance. Another experiment shows accessions in Western Australia are with drought tolerance compared to those from Eastern Australia (Ting, unpublished). The lack of drought-tolerance in Eastern Australia accessions may result from the dependence on abundant rainfall. In the same experiment (Ting, unpublished), accessions from Eastern Australia are more pest-resistant than those from Western Australia. Both drought tolerance and pest resistance may be opportunities for breeding new lines.

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Conclusions

According to our result, there might be a disruptive selection on the wild mungbean population in Australia. Because of the difference in length of growing season between Eastern and Western Australia, wild mungbeans developed two strategies for adapting to specific environments, the ruderal, and the competitor. In Western Australia, the growing period is short, wild mungbeans may have to live with drought-escape characteristics and a rapid lifecycle (early flowering and short reproduction period) as the ruderal in the C-S-R triangle theory. While in Eastern Australia, the growing season is longer, which provides wild mungbeans an opportunity to develop as the perennial type with a longer and later reproduction period. For the vegetative-related traits, accessions from Eastern Australia exhibited thicker stems, more internode numbers, longer side branches, and a more trailing type than those from Western Australia, which suggested the difference in the growth habit from the selection of environments. Our results provided a clear relationship between environmental factors and Australian wild mungbean phenotype and offered scientists a new trail for breeding new cultivars.

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Tables

Table 1. The Q_{ST}, heritability, and *P*-value from ANOVA of four categories of traits

The mean of each trait in Western Australia (WA) and Eastern Australia (EA) are listed in the table (calculated with raw data). Data in bold exhibit the traits with *P*-value less than 0.05. (Degree of freedom = 1) (The category is classified as Figure 7.)

Trait	WA raw data mean	EA raw data mean	Q_{ST}	Heritability	-$\log_{10}$(*P*-value)	Organ	Category
w-s ratio	**1.061**	**1.191**	**0.323**	**0.563**	**4**	**leaf**	**second**
Internode number	**6.522**	**7.322**	**0.274**	**0.248**	**2.42**	**shoot**	**third**
Stem width	**2.685**	**3.076**	**0.22**	**0.47**	**2.602**	**shoot**	**third**
Pod length of each roll	**2.471**	**2.220**	**0.202**	**0.468**	**2.337**	**pod**	**first**
Seed weight	**11.476**	**9.456**	**0.169**	**0.769**	**2.409**	**seed**	**first**
Relative shoot water content	**0.846**	**0.854**	**0.162**	**0.397**	**1.561**	**plant**	**second**
Last pod date	**117.466**	**122.376**	**0.158**	**0.224**	**1.572**	**pod**	**third**
Leaf shape	**13.106**	**11.063**	**0.152**	**0.534**	**2.071**	**leaf**	**forth**
Seed area	**6.237**	**5.477**	**0.15**	**0.853**	**2.244**	**seed**	**first**
Stem pink level	**1.554**	**1.397**	**0.146**	**0.595**	**1.438**	**shoot**	**first**
Plant growth habit	**1.888**	**2.086**	**0.145**	**0.296**	**1.664**	**plant**	**second**
Branch-stem ratio	**0.825**	**1.097**	**0.128**	**0.349**	**1.642**	**shoot**	**third**
Pod shatter type	**3.413**	**3.611**	**0.122**	**0.407**	**1.539**	**Pod**	**third**
Pod twist	1.974	2.104	0.107	0.192	1.11	pod	forth
Pod length	**4.601**	**4.350**	**0.1**	**0.628**	**1.532**	**pod**	**first**
Side branch length	46.038	54.569	0.089	0.376	1.268	shoot	third
Seed coat color	**2.237**	**2.550**	**0.088**	**0.556**	**1.366**	**seed**	**second**
Stem green level	2.549	2.678	0.036	0.191	0.749	shoot	forth
Pod color	5.294	5.054	0.033	0.508	0.828	pod	forth
First flower days	70.213	73.240	0.032	0.587	0.873	flower	third
Relative leaf water content	0.844	0.849	0.031	0.407	0.714	leaf	second
Fresh leaf density	0.019	0.020	0.011	0.434	0.576	leaf	forth
Pod mature days	97.612	100.608	0.01	0.524	0.604	pod	third

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Pod production period	19.854	21.769	0.007	0.189	0.54	pod	second
Flower color	2.847	3.067	0.004	0.489	0.538	flower	forth
Plant fresh weight	8.240	7.911	0	0.162	0.129	plant	forth
Biomass	1.292	1.177	0	0.223	0.313	plant	forth
Leaf fresh weight	1.050	1.035	0	0.172	0.075	leaf	forth
Leaf dry weight	0.165	0.157	0	0.208	0.296	leaf	forth
Leaf area	55.435	52.272	0	0.192	0.25	leaf	forth
Dry leaf density	0.003	0.003	0	0.238	0.014	leaf	third
Specific leaf area	346.254	341.878	0	0.238	0.014	leaf	second
Leaf water content	0.885	0.878	0	0.16	0.069	leaf	forth
Plant water content	6.948	6.734	0	0.157	0.09	plant	forth
w-l ratio	0.644	0.641	0	0.492	0.03	leaf	forth
Chlorophyll content	32.245	32.883	0	0.248	0.106	leaf	forth
Leaflet length	6.920	6.739	0	0.332	0.441	leaf	forth
Leaflet width	4.464	4.305	0	0.257	0.481	leaf	forth
Leaflet shortest width	3.175	3.161	0	0.289	0	leaf	forth
Stem length	66.227	63.121	0	0.279	0.337	shoot	first
Mean internode length	1.759	1.981	0	0.307	0.439	shoot	forth
Expected seed number	90.246	95.357	0	0.352	0.035	seed	forth
Pod hair	2.470	2.480	0	0.342	0.046	pod	second
Seed number per pod	11.497	11.749	0	0.597	0.303	seed	forth
Seed pattern	1.971	1.944	0	0.869	0.364	seed	first
Total pods number	8.467	8.771	0	0.358	0.002	pod	forth

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Table 2. ANOVA analysis of environmental factors

The mean of each environmental factor in Western Australia (WA) and Eastern Australia (EA) are listed in the table (calculated with raw data). The climate data used is from the period 1970 to 2000. Data in bold exhibit the environmental factors with *P*-value less than 0.05. Data in bold exhibit the traits with *P*-value less than 0.05. (Degree of freedom = 1)

Environmental factors	WA raw data mean	EA raw data mean	*P*-value	*F*-ratio
Bio1－annual mean temperature (°C)	**28.91**	**25.96**	**<.0001***	**341.20**
Bio2－mean diurnal range (°C)	**10.89**	**9.51**	**<.0001***	**41**
Bio3－isothermality	**0.75**	**0.77**	**<.0001***	**51.07**
Bio4－temperature seasonality	1085.87	1074.21	0.4568	0.55
Bio5－max temperature of warmest month (°C)	**36.20**	**31.63**	**<.0001***	**215.09**
Bio6－min temperature of coldest month (°C)	**21.43**	**19.21**	**<.0001***	**126.3**
Bio7－temperature annual range (°C)	**14.77**	**12.42**	**<.0001***	**44.81**
Bio8－mean temperature of wettest quarter (°C)	**28.79**	**26.42**	**<.0001***	**286.41**
Bio9－mean temperature of driest quarter (°C)	**28.30**	**26.25**	**<.0001***	**256.79**
Bio10－mean temperature of warmest quarter (°C)	**29.72**	**26.72**	**<.0001***	**268.41**
Bio11－mean temperature of coldest quarter (°C)	**28.10**	**25.46**	**<.0001***	**389.27**
Bio12－mean precipitation (mm)	**158.53**	**179.58**	**0.0009**	**11.11**
Bio13－precipitation of wettest month (mm)	249.10	269.69	0.0589	3.59
Bio14－precipitation of driest month (mm)	**49.49**	**72.31**	**<.0001***	**91.79**
Bio15－precipitation seasonality	**0.52**	**0.46**	**<.0001***	**31.76**
Bio16－precipitation of wettest quarter (mm)	**653.98**	**745.46**	**0.0042***	**8.29**
Bio17－precipitation of driest quarter (mm)	451.63	469.71	0.1163	2.48
Bio18－precipitation of warmest quarter (mm)	**491.98**	**647.00**	**<.0001***	**65.28**
Bio19－precipitation of coldest quarter (mm)	**486.00**	**602.20**	**0.0002***	**14.08**
Evapotranspiration (mm/day)	**1147.51**	**1058.74**	**<.0001***	**26.57**
Aridity index	**0.97**	**1.02**	**0.1546**	**2.04**
Solar radiation (srad) (kJ/m^2 day)	**21767.92**	**21422.90**	**<.0001***	**17.66**
Water vapor pressure (vapr) (kPa)	**2.55**	**2.44**	**0.0008***	**11.48**
Wind speed (wind) (m/s)	**2.04**	**3.45**	**<.0001***	**368.52**

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Table 3. C-S-R Triangle theory and wild mungbeans in Australia

The least square mean values of traits are listed in the table. The three strategies and the environmental status in the C-S-R Triangle theory were briefly described.

Type	C-type	S-type	R-type
Environments disturbance	Low	Low	High
Environment stress	Low	High	Low
Strategy	Competing with other species by reaching resources efficiently.	With certain physiological reinforcement to overcome the stress.	With fast growth rates, a short life cycle and often reproduce large amounts of seeds.
Wild mungbeans in Australia	Eastern Australia		Western Australia
First flower date	0.365 (later)		0.134 (earlier)
Last pod date	0.180 (later)		-0.099 (earlier)
Internode number	0.226 (more)		-0.161 (less)
Stem width (mm)	0.245 (thicker)		-0.251 (thinner)
Plant growth habit	0.124 (trailing)		-0.146 (erect)
Seed area (mm^2)	-0.388 (small)		0.175 (big)
Seed weight (mg)	-0.385 (light)		0.166 (heavy)
Pod length (cm)	-0.275 (short)		0.149 (long)
Expected seeds number	-0.083 (similar)		-0.099 (similar)

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Figures

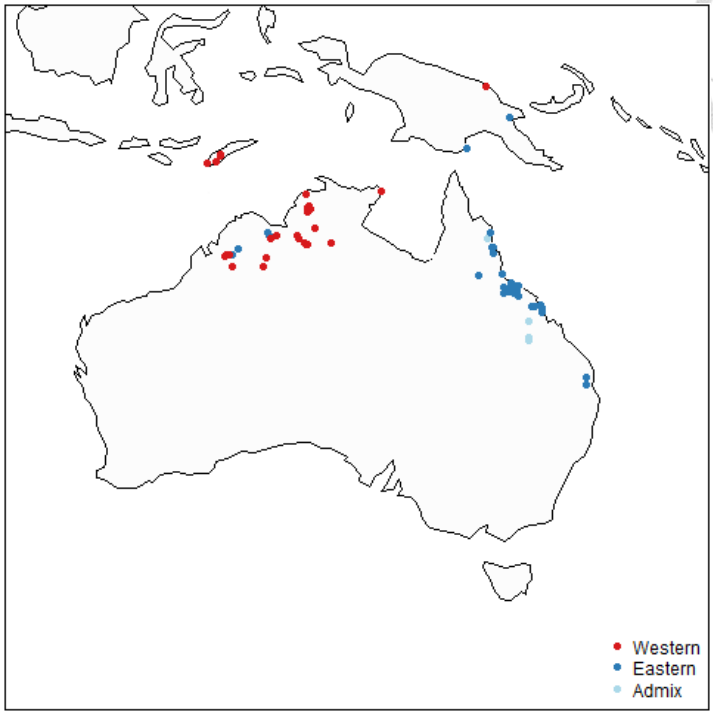

Figure 1. Sample collection sites

Different colors represent the classification of the genetic groups for each accession. Red indicates the Western Australian group, blue indicates the Eastern Australian group, and light blue indicates the accessions with admixed genetic composition.

doi:10.6342/NTU202203403

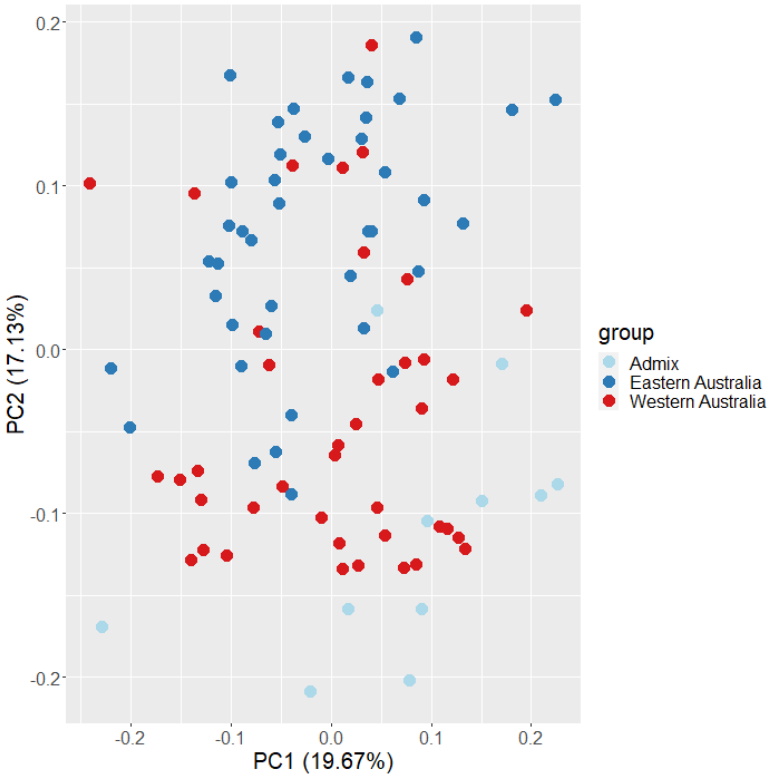

Figure 2a. The principal component analysis (PCA) of wild mungbean traits

Light blue represents the accessions with admixed genetic composition between Western and Eastern Australia, red represents the Western Australian group, and blue represents the Eastern Australian group.

doi:10.6342/NTU202203403

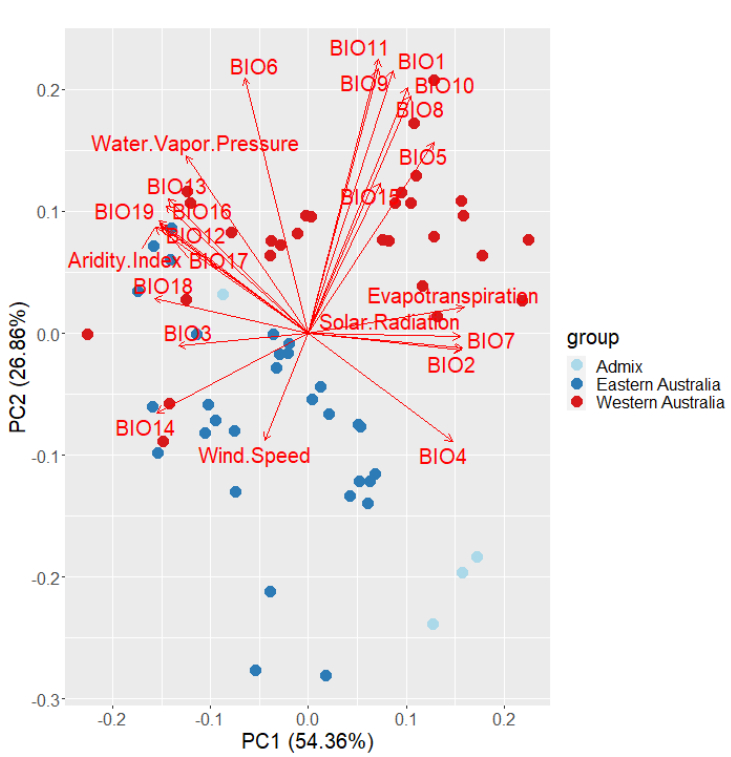

Figure 2b. The principal component analysis (PCA) of environmental factors of sample collection sites in Australia

Light blue represents the accessions with admixed genetic composition between Western and Eastern Australia, red represents the Western Australian group, and blue represents the Eastern Australian group.

doi:10.6342/NTU202203403

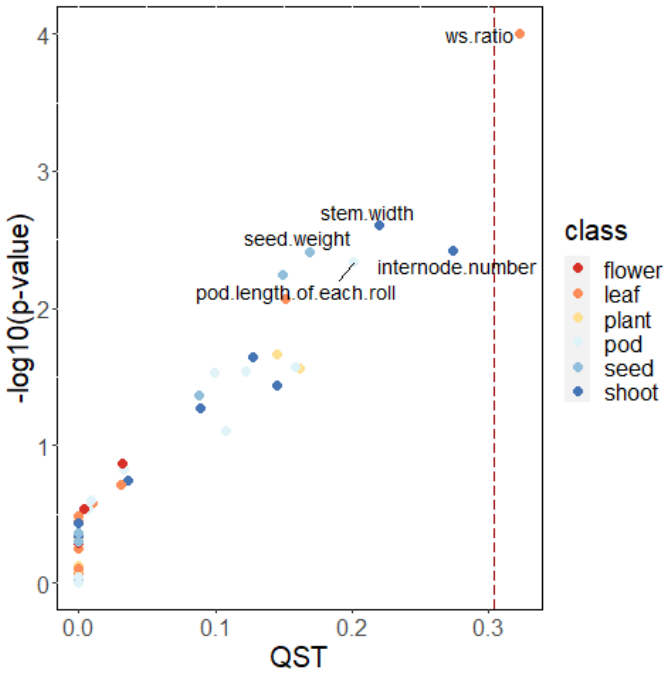

Figure 3a. The Q_{ST} statistics and the *P*-value from ANOVA analysis

The color of the dot represents the organ to that traits belong. The dots with the traits name listed are those with the top five lowest *P*-values. The dashed line represents the 95 % tail of the F_{ST} value.

doi:10.6342/NTU202203403

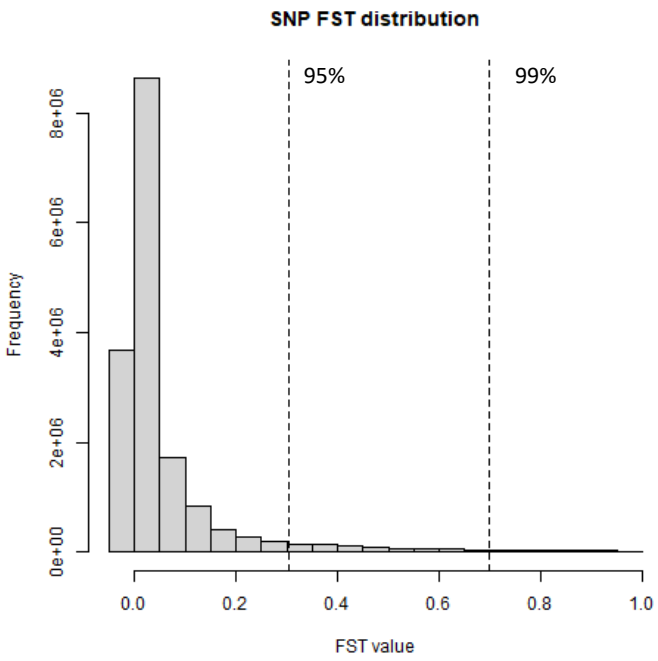

Figure 3b. The frequency distribution of SNP F_{ST} value

The dashed lines represent the 95 % tail and the 99 % tail of the F_{ST} value.

doi:10.6342/NTU202203403

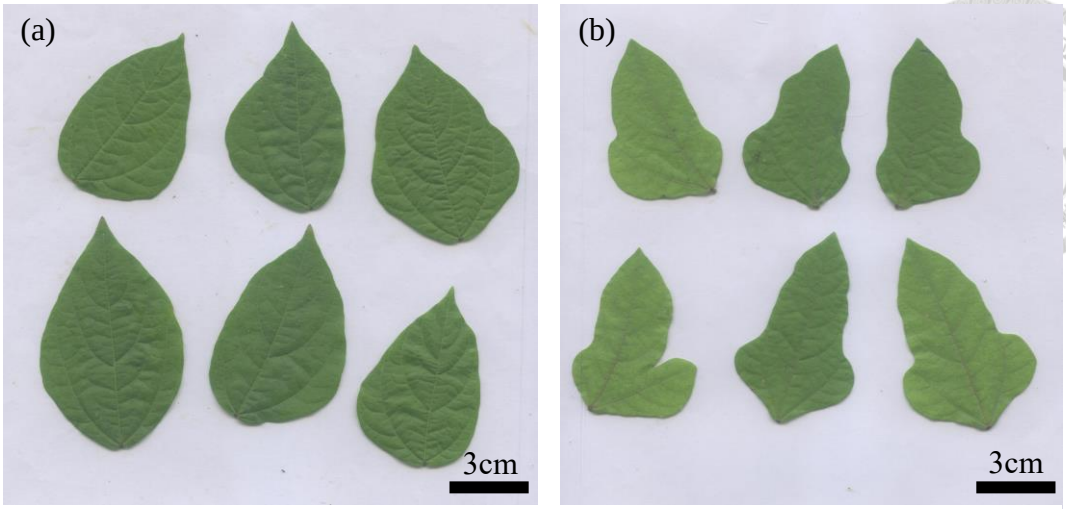

Figure 4. The leaf shape of accessions (a) CPI106932 and (b) CQ2235

The accession CPI106932 was collected from Western Australia. The accession CQ2235 was collected from Eastern Australia. Both the two pictures were taken nine weeks after sowing.

doi:10.6342/NTU202203403

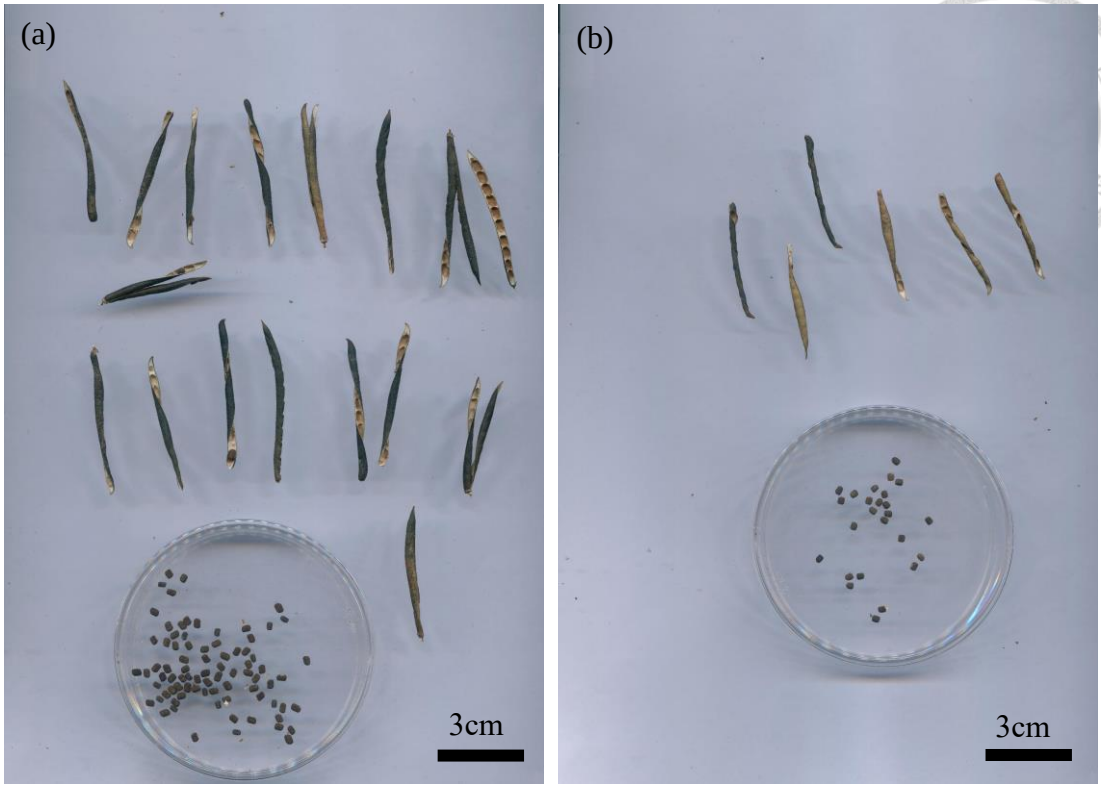

Figure 5. The pod and seed picture of accessions (a) CQ2240 and (b) CQ2226

The accession CQ2240 was collected from Western Australia. The accession CQ2226 was collected from Eastern Australia.

doi:10.6342/NTU202203403

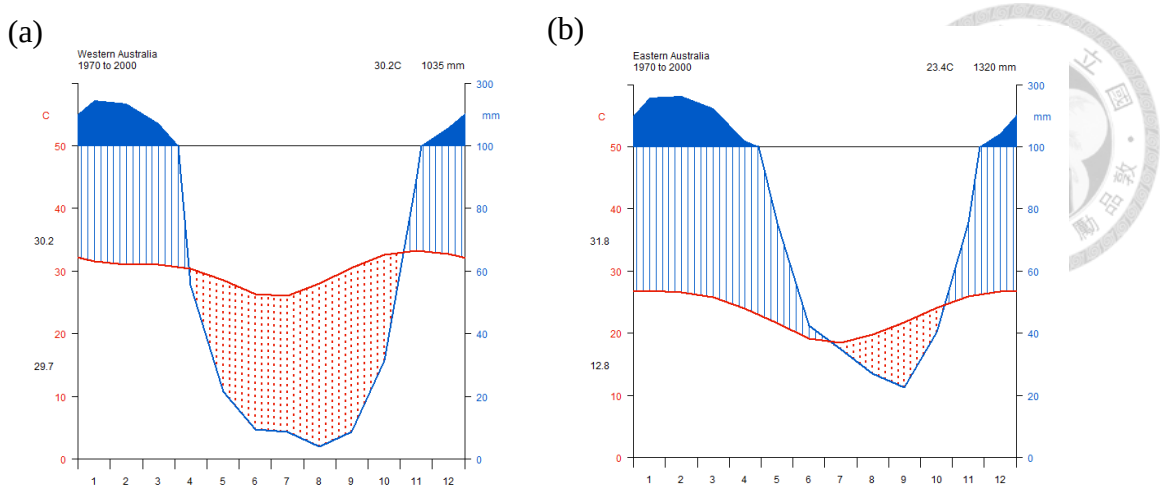

Figure 6. The climate of Western Australia and Eastern Australia during 1970-2000

The climate data are from Worldclim2.1. The x-axis is the month from January to December. The y-axis on the left represents the temperature. The y-axis on the right represents the precipitation. The blue line represents the precipitation of each month, and the red line represents the temperature of each month. The area filled with solid blue and blue lines represents the wet season, and the area filled with red dots represents the arid season. The mean annual temperature and annual precipitation are listed on the top right of the figure. These figures exhibited the growing season and drought season in Western and Eastern Australia.

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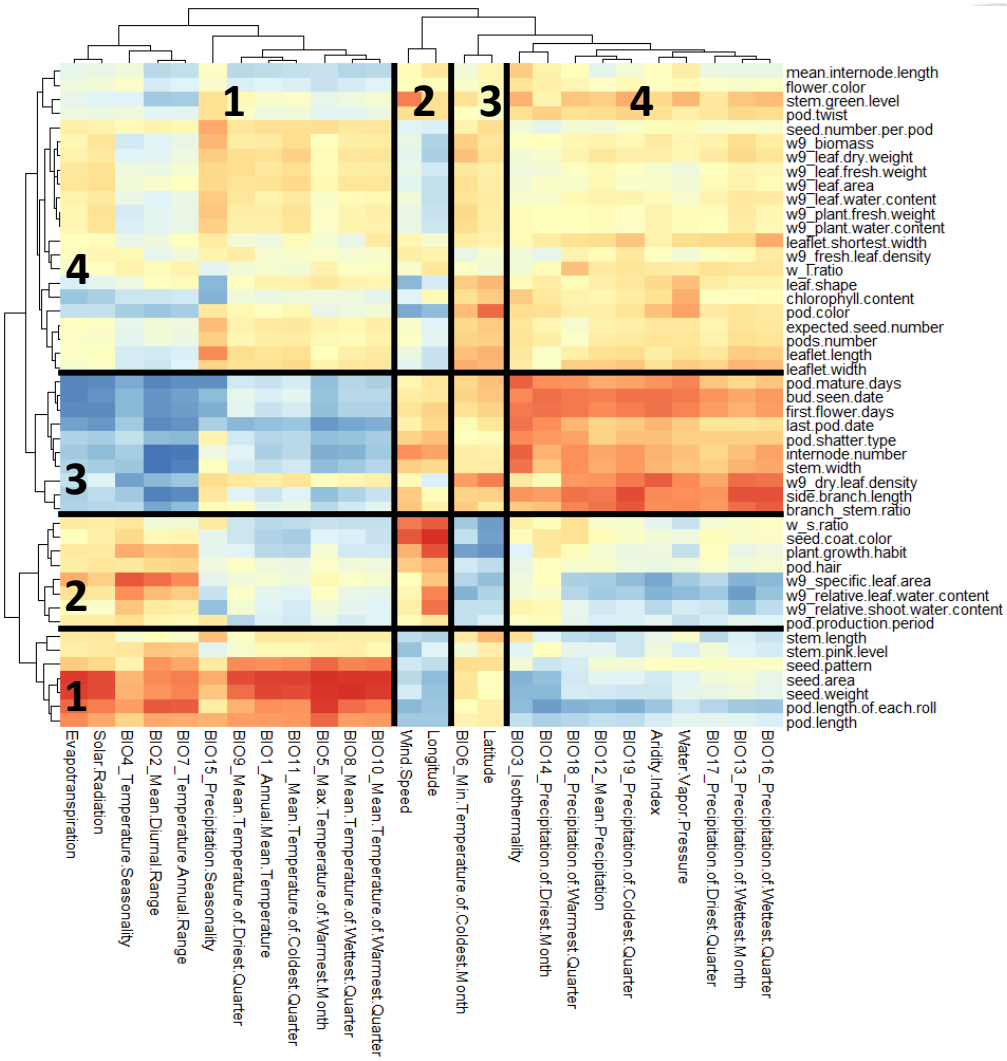


Figure 7. The heatmap of correlation between wild mungbean traits and environmental factors

Both traits and environmental factors could be classified into four groups. These groups were described in Results.

doi:10.6342/NTU202203403

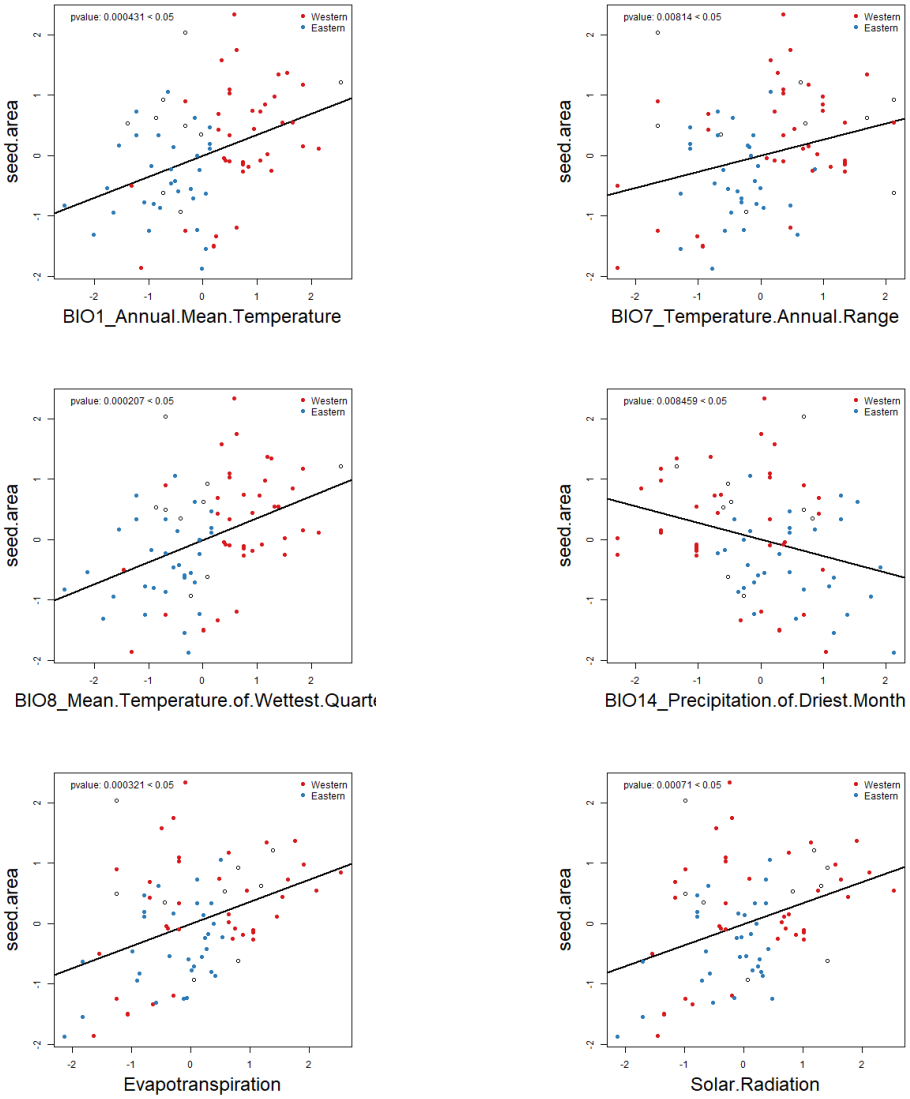


Figure 8. The correlation between seed area and environmental factors

All of the data were transformed with ranked-based inverse normal distribution.

doi:10.6342/NTU202203403

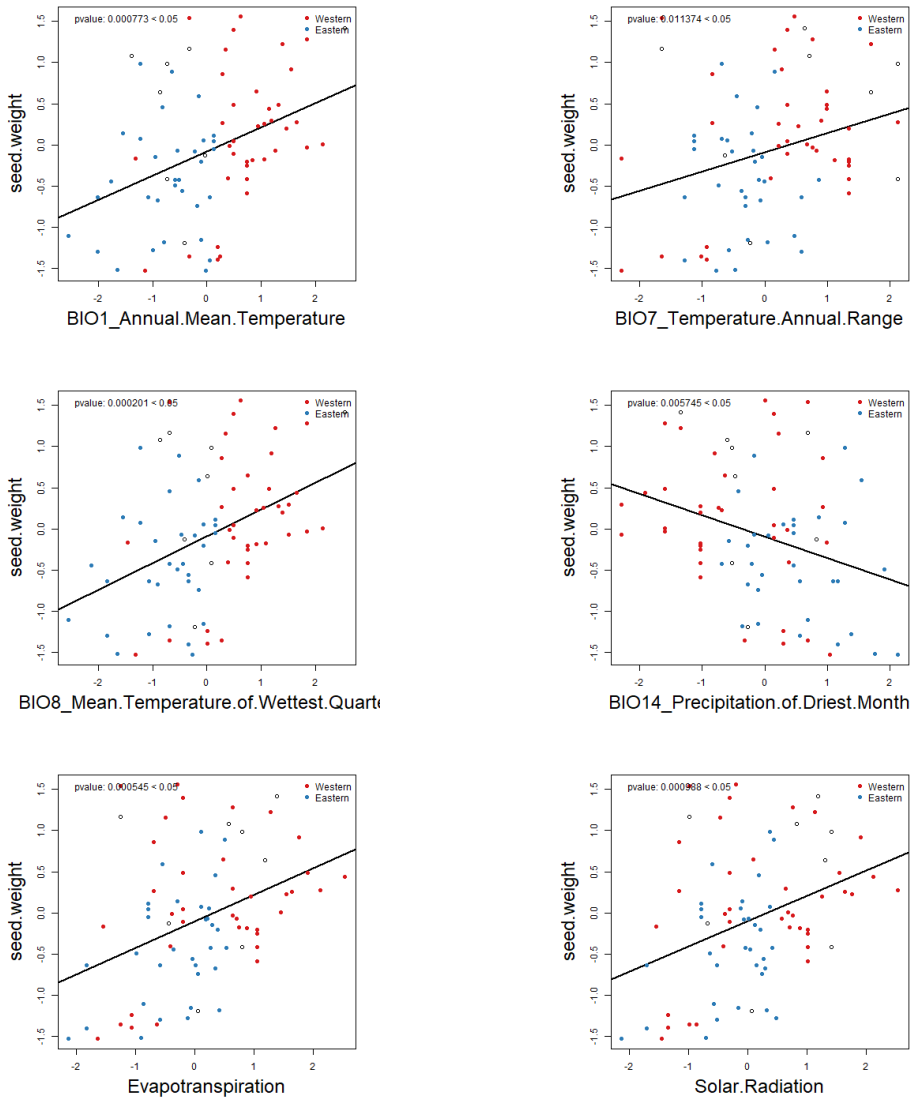


Figure 9. The correlation between seed weight and environmental factors

All of the data were transformed with ranked-based inverse normal distribution.

doi:10.6342/NTU202203403

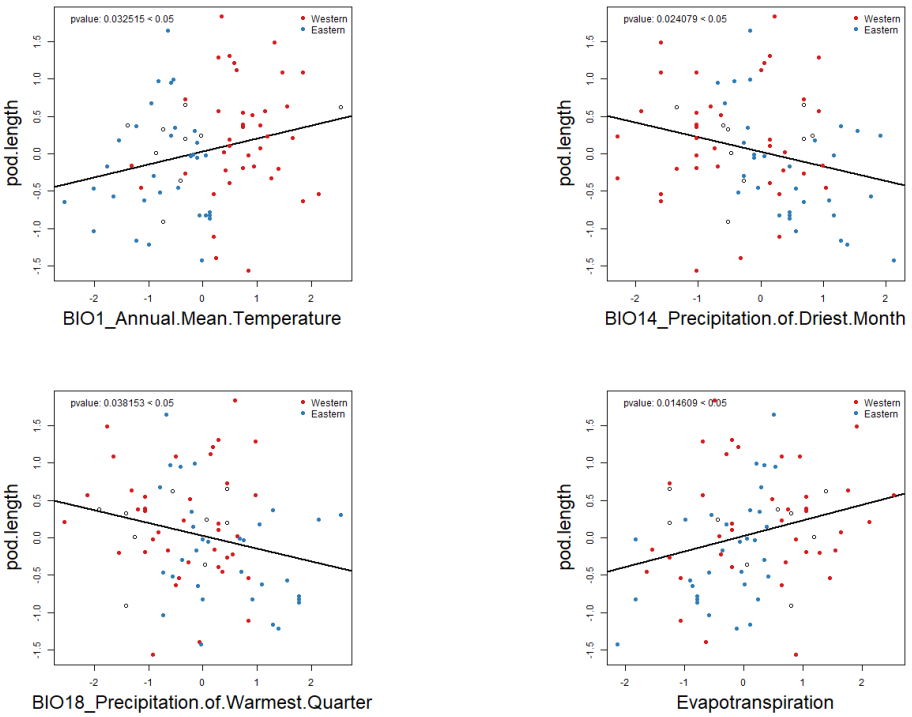


Figure 10. The correlation between pod length and environmental factors

All of the data were transformed with ranked-based inverse normal distribution.

doi:10.6342/NTU202203403

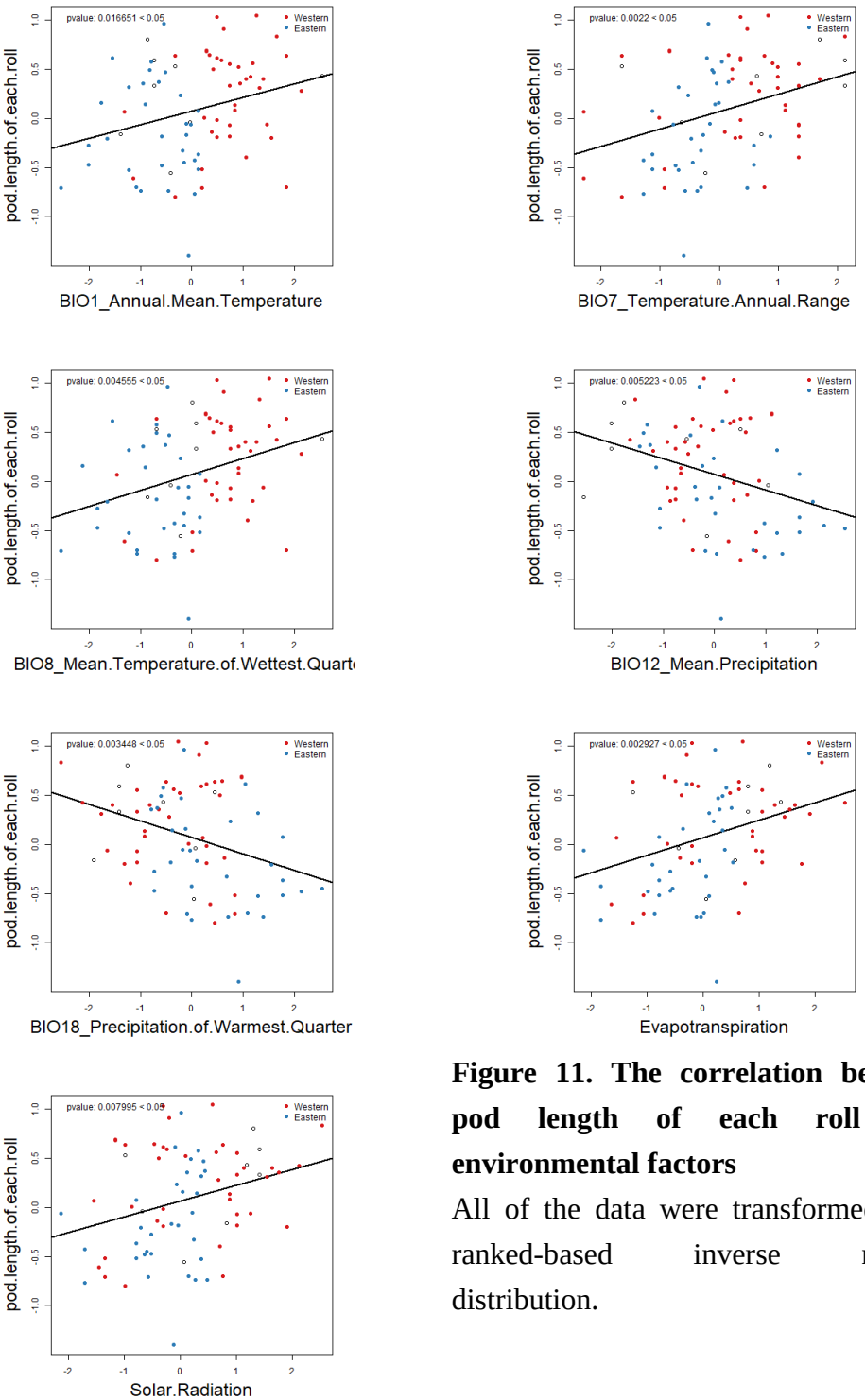
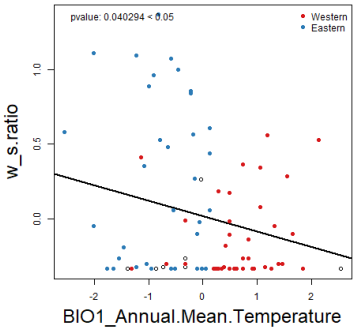
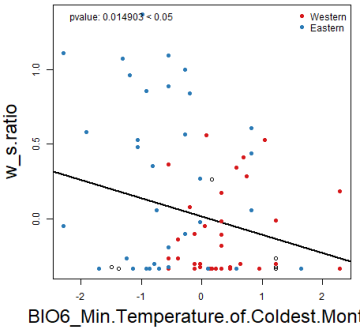


Figure 11. The correlation between pod length of each roll and environmental factors

All of the data were transformed with ranked-based inverse normal distribution.

doi:10.6342/NTU202203403





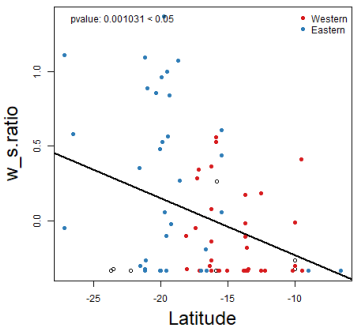


Figure 12. The correlation between w-s ratio (lobed-leaflets index) and environmental factors

All of the data were transformed with ranked-based inverse normal distribution.

doi:10.6342/NTU202203403

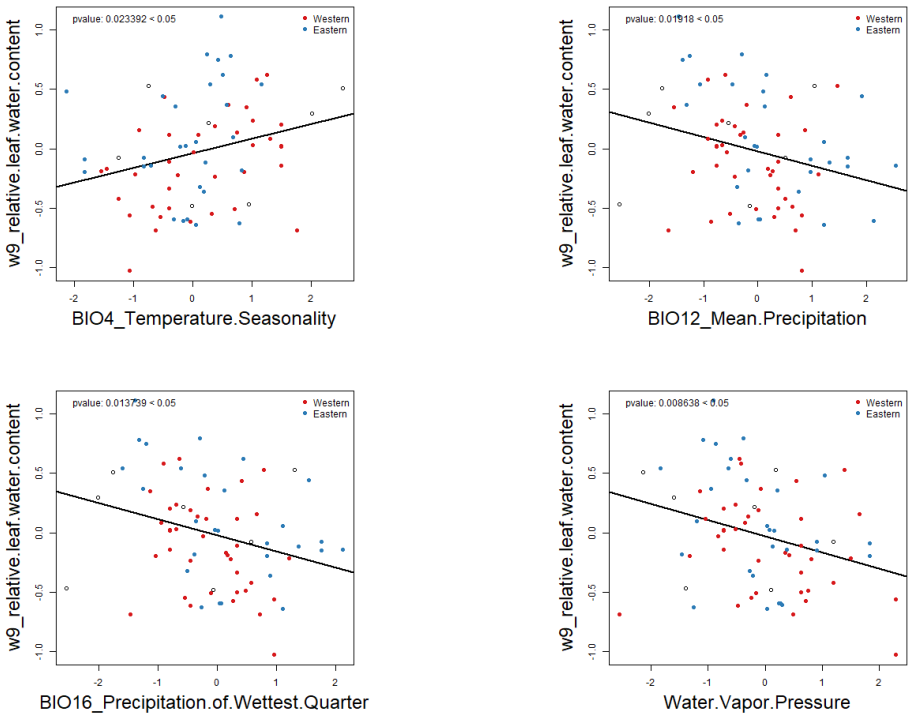
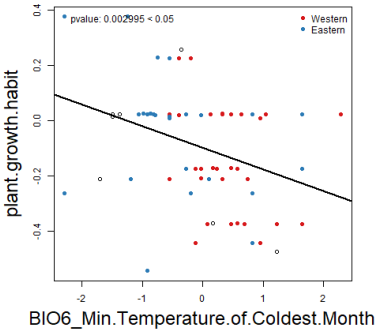
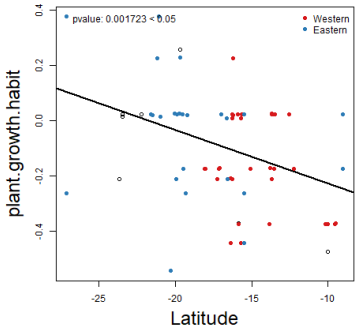


Figure 13. The correlation between relative leaf water content and environmental factors

All of the data were transformed with ranked-based inverse normal distribution.

doi:10.6342/NTU202203403





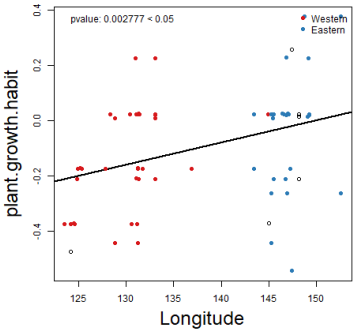


Figure 14. The correlation between plant growth habit and environmental factors

All of the data were transformed with ranked-based inverse normal distribution.

doi:10.6342/NTU202203403

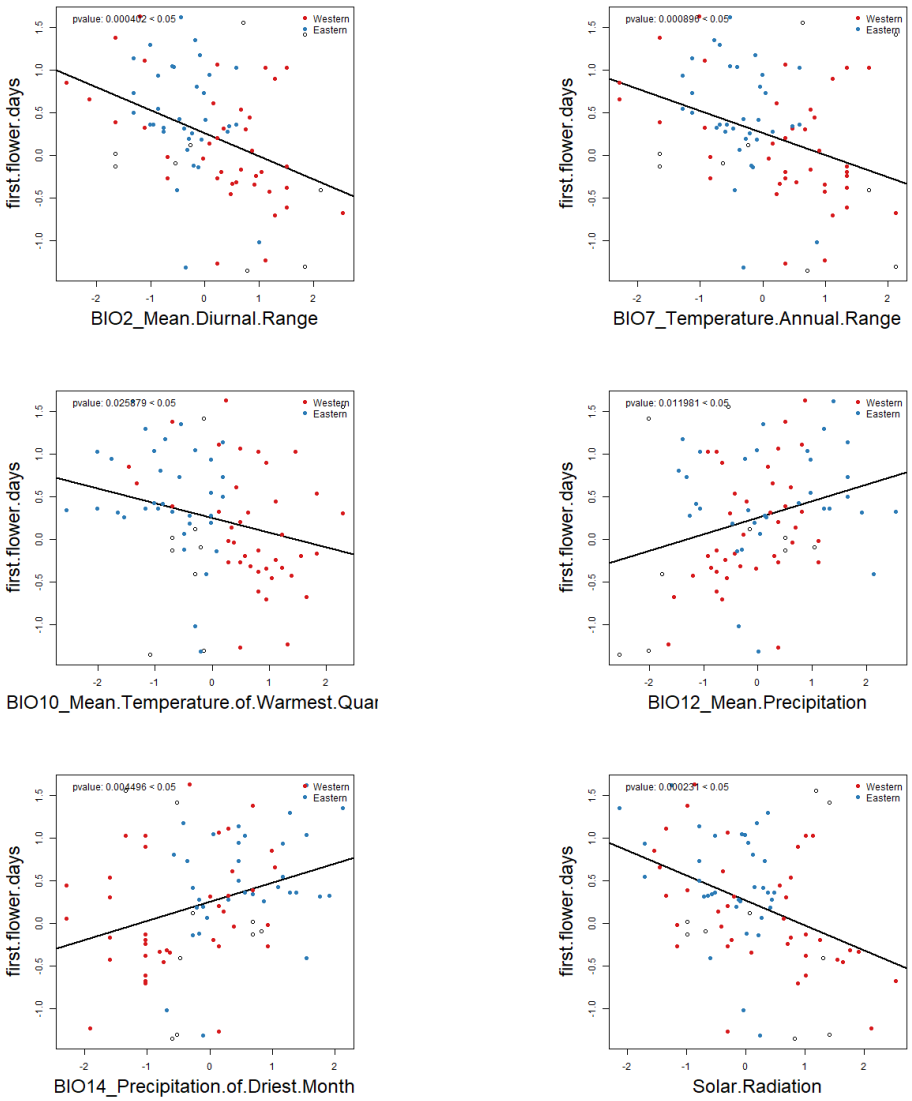


Figure 15. The correlation between first flower days and environmental factors

All of the data were transformed with ranked-based inverse normal distribution.

doi:10.6342/NTU202203403

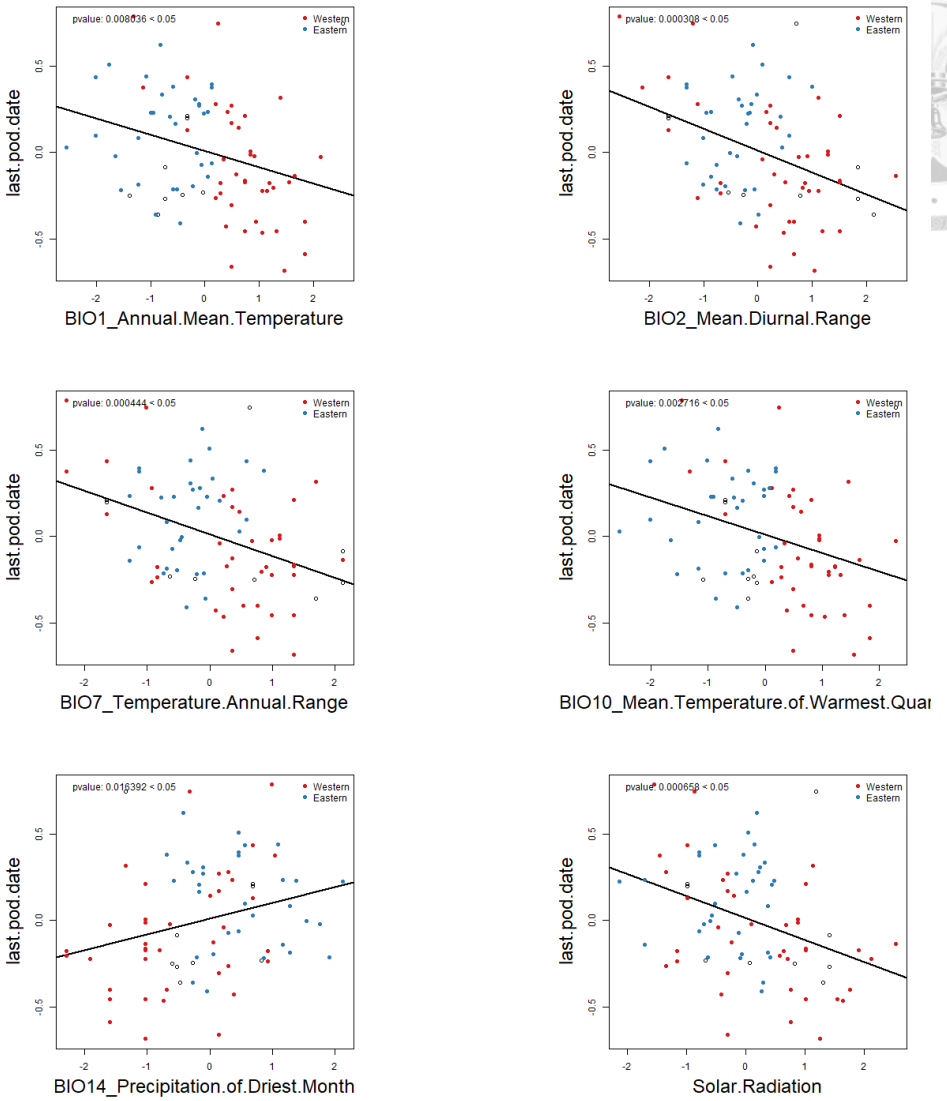


Figure 16. The correlation between last pod date and environmental factors

All of the data were transformed with ranked-based inverse normal distribution.

doi:10.6342/NTU202203403

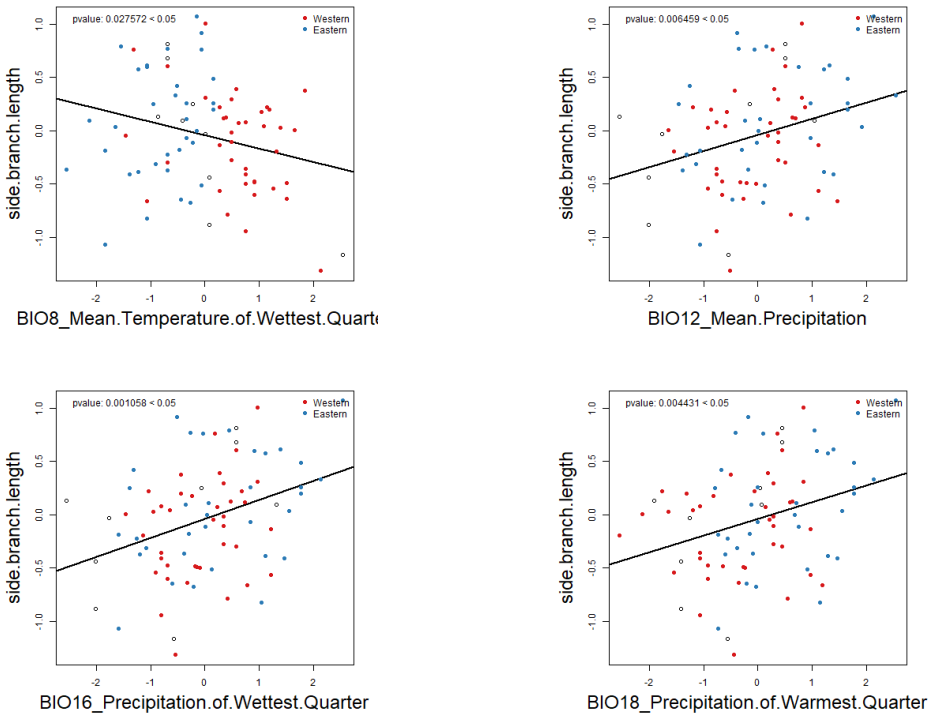


Figure 17. The correlation between side branch length and environmental factors

All of the data were transformed with ranked-based inverse normal distribution.

doi:10.6342/NTU202203403

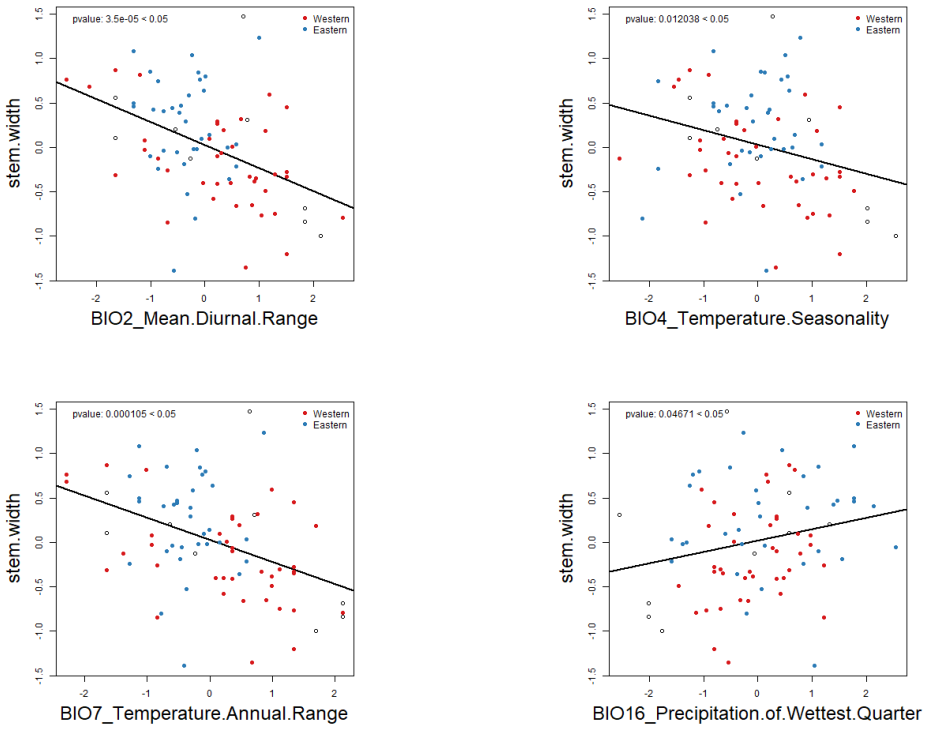


Figure 18. The correlation between stem width and environmental factors

All of the data were transformed with ranked-based inverse normal distribution.

doi:10.6342/NTU202203403

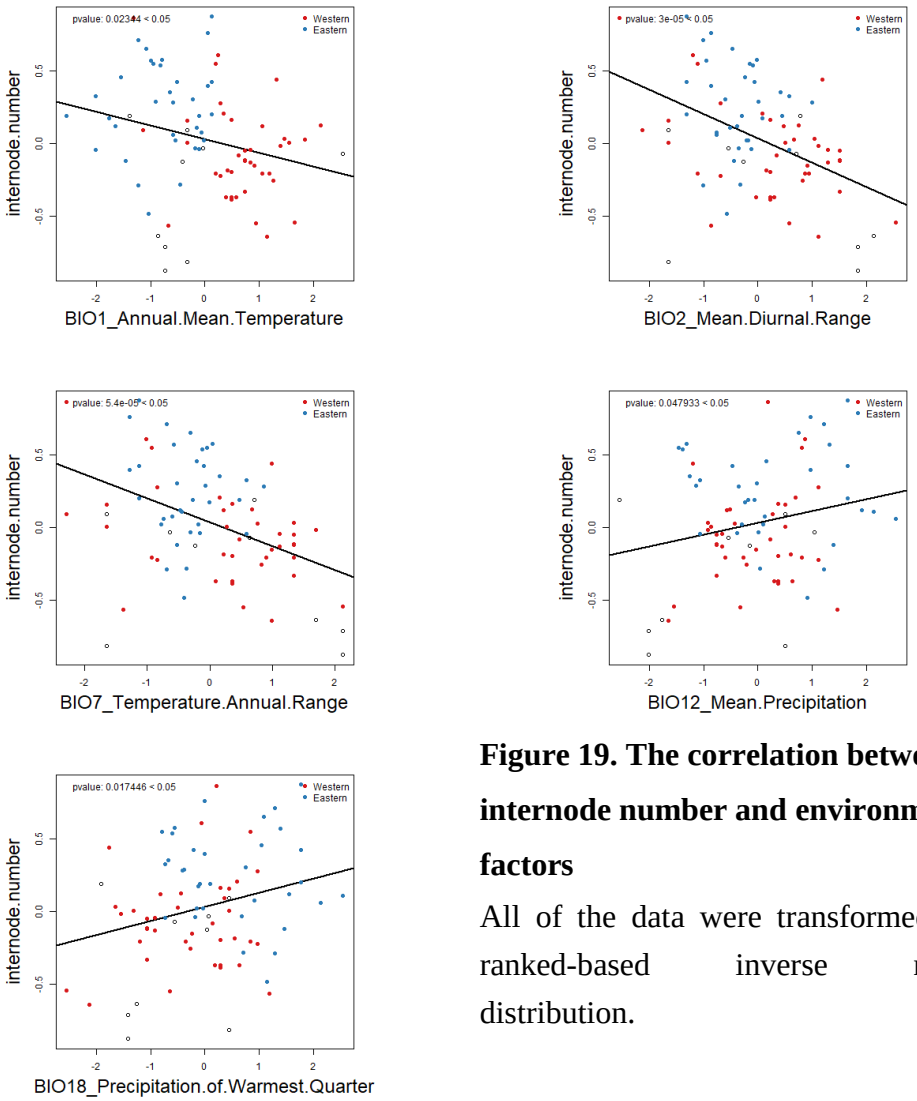


Figure 19. The correlation between internode number and environmental factors

All of the data were transformed with ranked-based inverse normal distribution.

doi:10.6342/NTU202203403

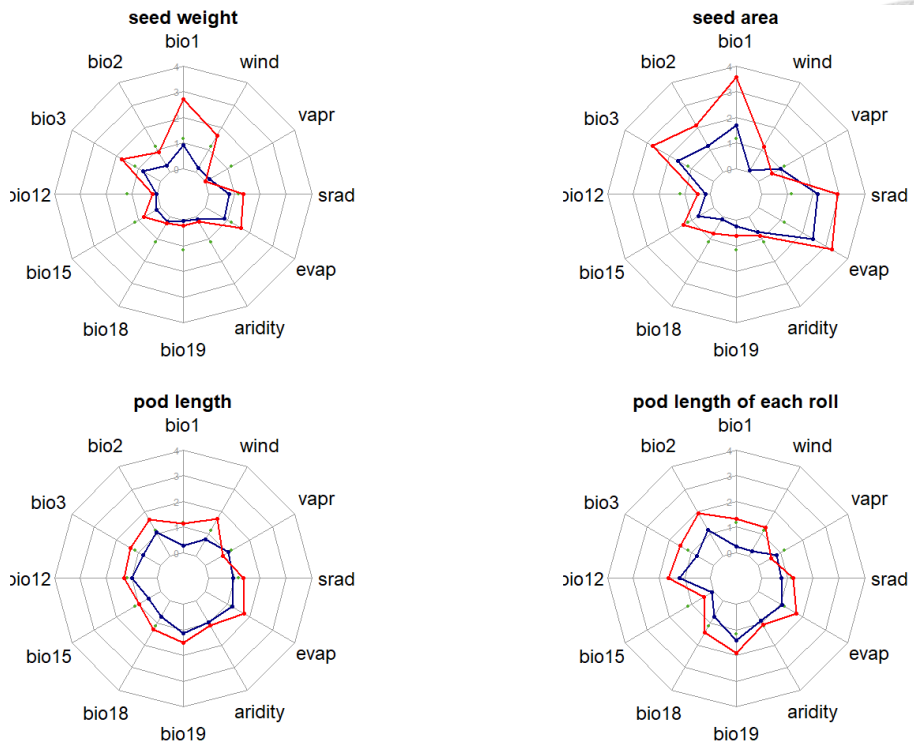


Figure 20. The influence of environmental factors on the first category of traits while controlling for accessions' genetic background

The factors represented here include annual mean temperature (BIO1), mean diurnal range (BIO2), isothermality (BIO3), mean precipitation (BIO12), precipitation seasonality (BIO15), precipitation of the warmest quarter (BIO18), precipitation of the coldest quarter (BIO19), aridity index (aridity), evapotranspiration (evap), solar radiation (srad), water vapor pressure (vapr), and wind speed (wind). The *P*-values of ANOVA were transformed with $-\log_{10}$. The red line represents the *P*-value of ANOVA without the control of genetic groups. The blue line represents the *P*-value of ANOVA with the control of genetic groups. The green dot represents the value, $-\log_{10}(0.05)$

doi:10.6342/NTU202203403

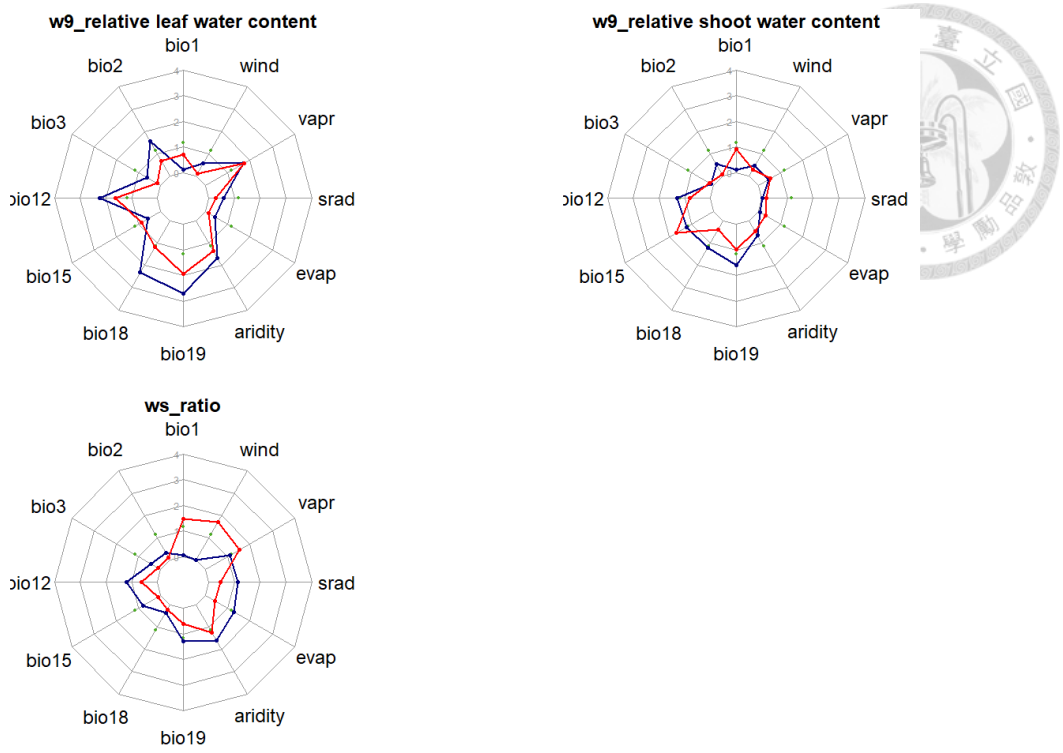


Figure 21. The influence of environmental factors on the second category of traits while controlling for accessions' genetic background

The factors represented here include annual mean temperature (BIO1), mean diurnal range (BIO2), isothermality (BIO3), mean precipitation (BIO12), precipitation seasonality (BIO15), precipitation of the warmest quarter (BIO18), precipitation of the coldest quarter (BIO19), aridity index (aridity), evapotranspiration (evap), solar radiation (srad), water vapor pressure (vapr), and wind speed (wind). The *P*-values of ANOVA were transformed with $-\log_{10}$. The red line represents the *P*-value of ANOVA without the control of genetic groups. The blue line represents the *P*-value of ANOVA with the control of genetic groups. The green dot represents the value, $-\log_{10}(0.05)$

doi:10.6342/NTU202203403

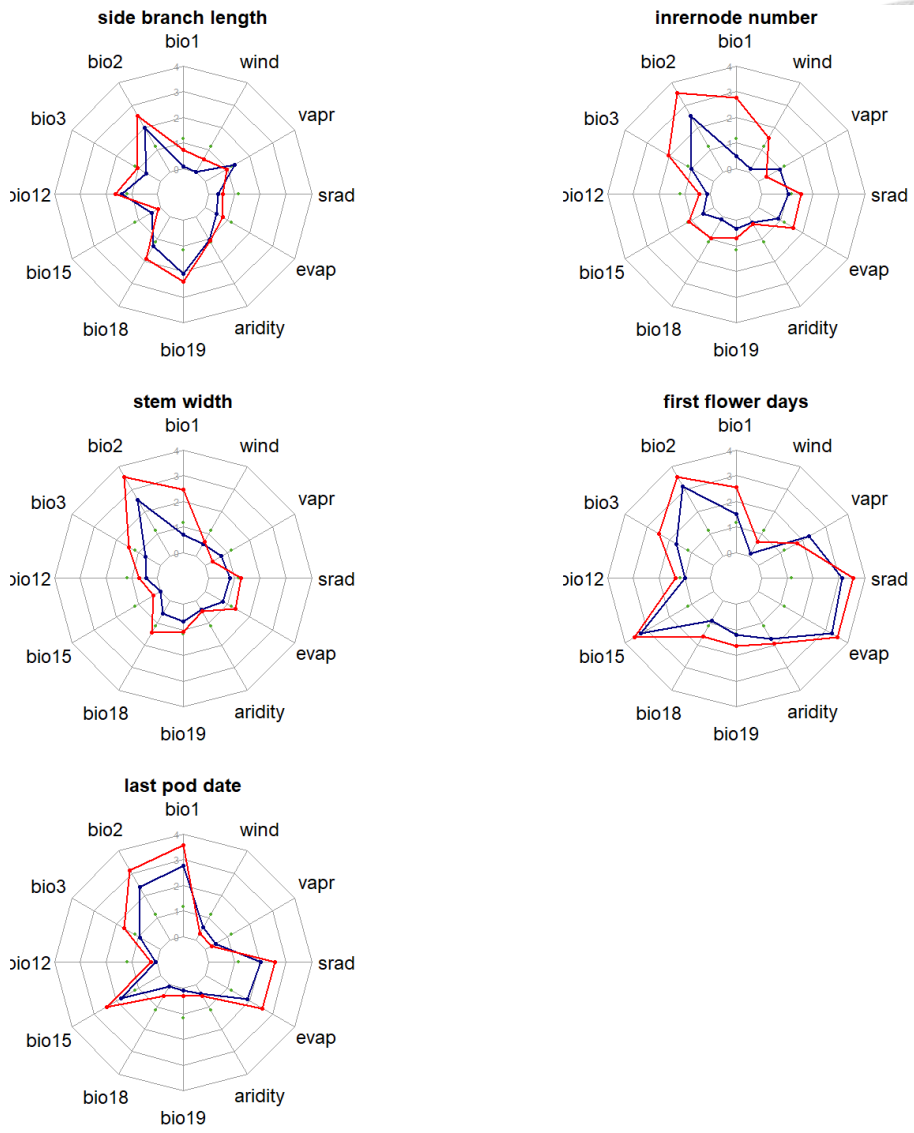


Figure 22. The influence of environmental factors on the second category of traits while controlling for accessions' genetic background

The factors represented here include annual mean temperature (BIO1), mean diurnal range (BIO2), isothermality (BIO3), mean precipitation (BIO12), precipitation seasonality (BIO15), precipitation of the warmest quarter (BIO18), precipitation of the coldest quarter (BIO19), aridity index (aridity), evapotranspiration (evap), solar radiation (srad), water vapor pressure (vapr), and wind speed (wind). The *P*-values of ANOVA were transformed with $-\log_{10}$. The red line represents the *P*-value of ANOVA without the control of genetic groups. The blue line represents the *P*-value of ANOVA with the control of genetic groups. The green dot represents the value, $-\log_{10}(0.05)$

doi:10.6342/NTU202203403

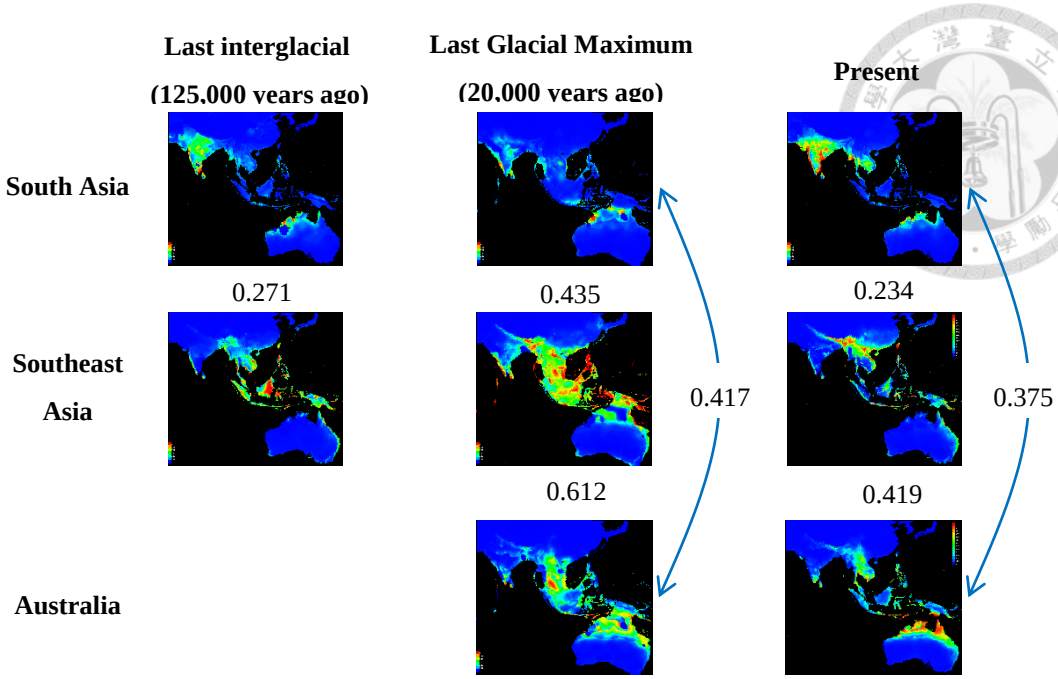


Figure 23. The niche modeling of wild mungbean during last interglacial, last glacial maximum, and present in South Asia, Southeast Asia, and Australia

Red represents the high density of wild mungbeans distribution, and blue represents the low density of distribution. Schoener's D values are noted between each set of results.

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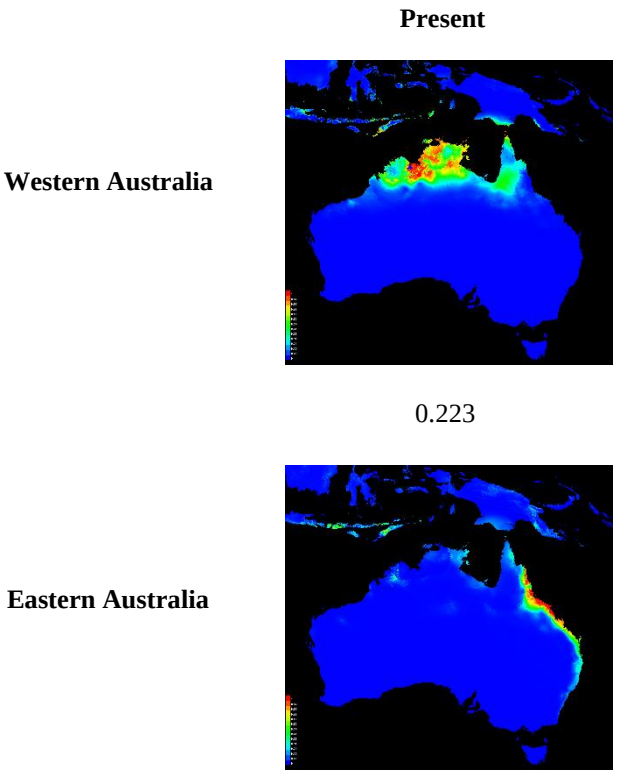

Figure 24. The niche modeling of wild mungbean in present in Western Australia and Eastern Australia

Red represents the high density of wild mungbeans distribution, and blue represents the low density of distribution. Schoener's D values are noted between each set of results.

doi:10.6342/NTU202203403