

Genetic analysis of root growth direction in soybean

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Chapter I

General introduction

The world is facing a serious challenge for food production and security in the near future. Despite the slowing of global population growth, Asia and Africa will still experience high population growth and expansion. The world population is estimated to reach 9.7 billion by 2050 (FAO, 2017), which correlates directly to the increase in demand for food, feed and biofuel production. The average annual growth rates for selected crop yields during the past 50 years showed in Fig. 1.1. It can be seen that in maize, rice, and wheat there have been average annual yield increases of slightly more than 1 percent since 1990s (FAO, 2017). However, these increases were substantially lower than they had been in the 1960s. Additionally, the data reported for soybeans and sugarcane showed less than 1 percent increase. With the increasing population, it is critical to increase the productivity/yield for these crops. Actually, according to FAO (2015), during 1961 to 2014, the world soybean harvest area and production increases annually by 3.16% and 5.11% respectively. In 2014, the world soybean harvest area covered 117.72 hectares or

increased by 5.45% from the previous year (Fig. 1.2). In spite of the increasing harvest areas and production, the productivity remains inadequate.

Among food crops, soybean holds an essential role in Asian countries. Soybean has been a staple source of protein in the Asian diet for thousands of years (Messina and Messina, 2010). Besides from being a source of protein ($\pm 40\%$), it also contains 35% of carbohydrate, 20% of fat, and 5% of mineral (Firdaus, 2015). Consumption of soybean in a sufficient amount (25g/day of whole soy protein) can improve the metabolism of the body, prevent cancer and cardiovascular diseases, reduce the effects of menopause and prevent the osteoporosis (Omoni *et al.*, 2005; Messina and Messina, 2010). In the food industry, soybean has been widely used in traditional non-fermented foods such as soy milk, tofu and oil, and in fermented foods such as soy sauce, natto, tauco, and tempeh.

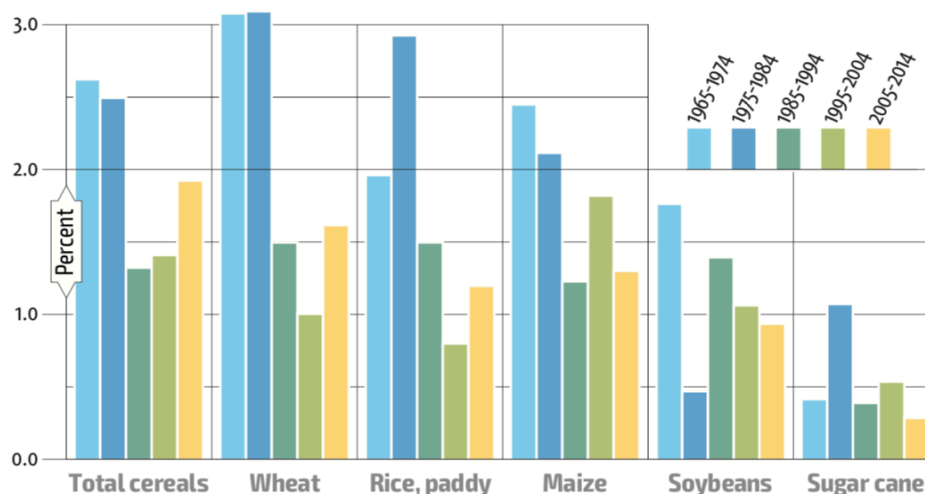


Fig. 1.1 Average annual growth rates for crop yields Source, FAO, 2016

In Indonesia, soybean is the third most essential food crop after rice and maize (Hasan *et al.*, 2015), thus making it the primary plant-based protein source. The consumption of soybean in 2015 was 6.12kg/capita/year and the productivity was 1.44 ton/ha, which makes Indonesia the

largest market for soybean in Asia. Ironically, in 2011-2015 the average value of Import Dependency Ratio (IDR) of soybean was 67.99% or 1.9 million ton. The domestic yield was not able to fulfil the demand of tempeh, tofu and the other products (Table 1.1). The low production rate of soybean crop in Indonesia is due to several factors. Firstly, white soybean, which is the main ingredient of tempeh and tofu, is not originally a tropical plant, resulting in a lower productivity compared to other countries (Fig. 1.3). Secondly, plant breeding and domestication have not fully succeeded to alter the photosensitive character of white soybean. On the other hand, black soybean, which is less photosensitive, receives less attention from plant breeders although it is more adaptable to Indonesia (Data Center And Agricultural Information System Ministry Of Agriculture Indonesia, 2016).

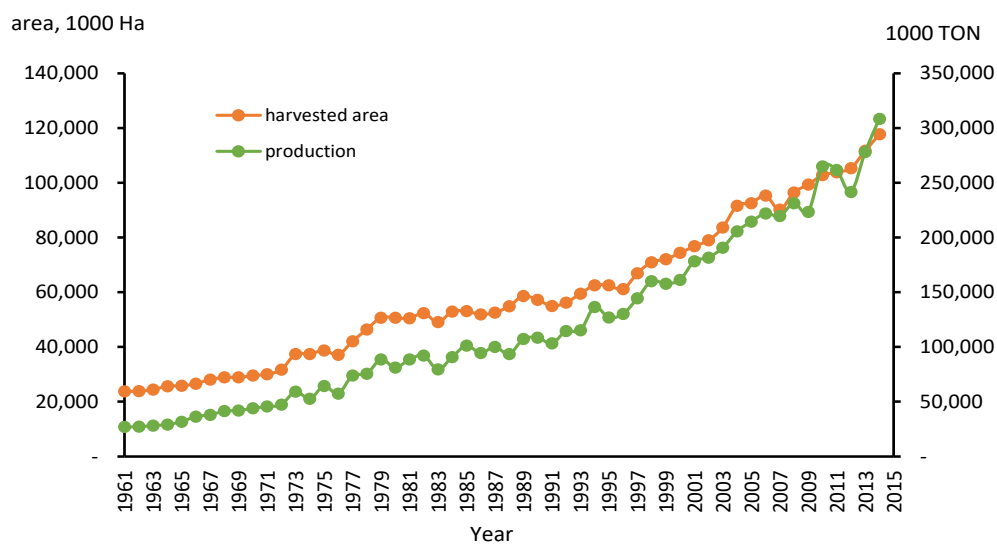


Fig. 1.2 World Soybean Harvest Area and Production, 1961-2014 Source: FAO, 2015

Table 1.1 Import Dependency Ratio (IDR) and Self Sufficiency Ratio (SSR) of Soybean in Indonesia 2011-2015

Variable	Year					Average
	2011	2012	2013	2014	2015	
Production (Ton)	851286.00	843153.00	779992.00	954997.00	963183.00	878522.20
Export (Ton)	8996.00	33950.00	11133.00	41304.00	14360.42	21948.68
Import (Ton)	2088616.00	1220120.00	1785384.00	1964080.90	2256931.68	1863026.52
IDR (%)	71.26	60.12	69.90	68.25	70.40	67.99
SSR (%)	29.05	41.55	30.54	33.19	30.05	32.87

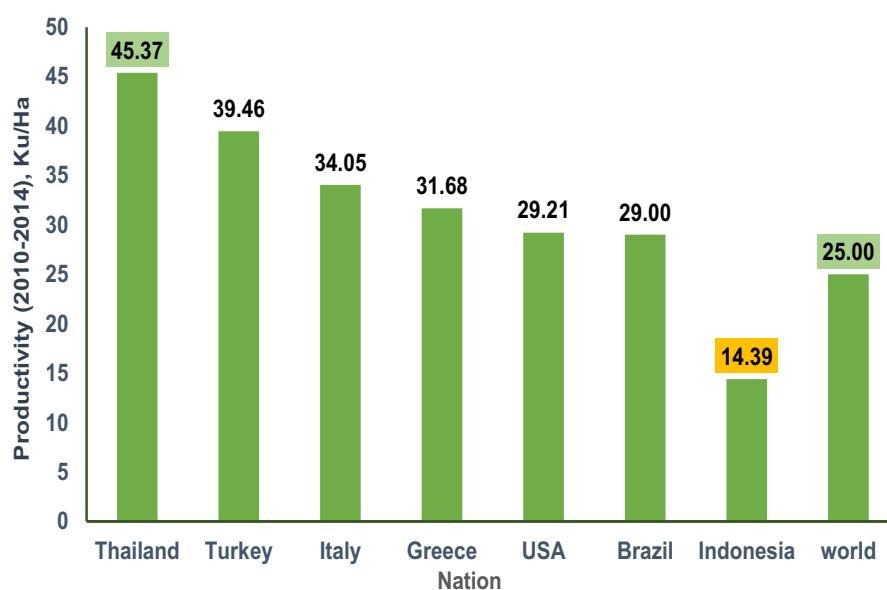


Fig. 1.3 World Soybean Crop Productivity 2010-2014

Aside from the factor above, climate condition also play a major rule in crop production. The global warming and El Nino which bring about drought condition in some area of Indonesia further created some significant problems in crop production including soybean crops. In drought condition, root is the first organ to respond to scarcity in the soil. The ability of the root to penetrate deeper area allow a better absorption of nutrients and water. Therefore, root architecture has a profound role in the growth and yield of plants. In drought condition the production of soybean

decrease until 50 % (Catuchi *et al.*, 2011). Thus, the introduction of specific varieties with improved drought tolerance is the most paramount goals in soybean breeding (Tuberosa and Salvi, 2006).

Root traits have the potential to be developed in crop breeding programs to optimize the ability to acquire soil resources. Root system architecture is recognized as having a relationship with the crop productivity, thus can be optimized to increase crop productivity, particularly for growth under low input condition involving unevenly distributed water and nutrient surrounding the rhizosphere.

In this study, the soybean (*Glycine max*) world mini-core collection (GmWMC) and recombinant inbred lines originated from the cross between Peking and Tamahomare (PT-RILs) were used for the experiment. Soybean GmWMC was used to evaluate the genetic variation of root traits (Chapter 2 and 4). Recombinant inbred lines were used for QTL analysis of root traits (Chapter 3 and 5).

Chapter II

Genetic variation of root traits among core collection

2.1. Introduction

Studying and observing the distribution of roots as ‘the hidden half’ (Waisel *et al.*, 1991) is very complicated because it requires an appropriate method and an understanding of the significant influences of the environment on root growth and direction. Root growth angle is defined by the direction of root elongation in the soil, which determines whether the plant develops shallow or deep roots. Root growth angle is controlled by several environmental factors, such as gravity, light, and water potential (Oyanagi, 1994). Studies on the root growth angle have been performed on wheat and rice. By using the basket method for wheat varieties, Hamada *et al.* (2012) defines the deep root ratio as above 45°, and found genetic variation (range 10 to 100%) in the deep root ratio among wheat core-collection. Several researchers established interval scales to define root growth direction. Uga *et al.* (2015) found genetic variation (range: 35°-80°) in the growth angle of the primary root. Kato *et al.* (2006) defines angles of more than 50° as deep root using the basket method. In another study, Hanzawa *et al.* (2013) divided the root growth angle into intervals of 0° to 30°, 30° to 60°, and 60° to 90°. Meanwhile, Tomita *et al.* (2017) evaluated the growth angle at the seedling stage in 10° intervals from 0° to 90. On the basis of former research,

it can be concluded that the root growth angle is one of the root traits that plays an essential role in determining the root system architecture. Identification of the genetic factors controlling the root growth angle is a key to evaluate root system architecture. The root system architecture is closely related to the crop productivity particularly under low amount of water and nutrients. The aim of this study is to evaluate the genetic diversity of root growth angle using soybean core collection.

2.2. Materials and methods

2.2.1 Plant Materials

Eighty lines of a soybean (*Glycine max*) world mini-core collection (GmWMC) (Table 2.1) provided by the Genetic Resources Center, National Agriculture and Food Research Organization, Japan, were used to evaluate the genetic variation in root growth angle.

2.2.2 Experimental procedures

Root traits were measured by the basket method (Oyanagi, 1994) with slight modification. A stainless mesh basket (10 cm diameter, 7 cm depth) was buried in the center of 1/5000 in a Wagner's pot filled with fine pumice originating from Kanuma, Japan (Fig. 2.1 (B)). A single seed was planted in the center of each basket at a depth of 1 cm. All pots were placed in a container in which the water level was adjust to 2 cm. After all the fine pumice become moist, seeds were placed on the soil so that the hypocotyl side was on the bottom. Twelve pots were put into a plastic

Table 2.1 Lines of GmWMC used in this study

WD/2014	Variety Name	Genebank ID, NARO in Japan	Status	Origin	
WD3	SEITA	GmWMC011	Landrace	Rep. Korea	√
WD4	MANSHUU	GmWMC012	Landrace	China	√
WD7	RIGAI SEITOU	GmWMC018	Landrace	China	√
WD8	CHOUSENSHU(CHA)	GmWMC019	Landrace	Korean Peninsula	√
WD9	POCHAL	GmWMC020	Landrace	Taiwan	√
WD10	NEZUMI META	GmWMC022	Landrace	Korean Peninsula	√
WD 11	CHIENEUM KONG	GmWMC024	Landrace	Rep. Korea	○ ²⁾
WD12	KONGNAMUL KONG	GmWMC027	Landrace	Rep. Korea	√
WD14	PEKIN DAI OUTOU	GmWMC035	Landrace	China	√
WD 15	MASSHOKUTOU	GmWMC042	Landrace	China	○
WD18	OKJO	GmWMC045	Landrace	Rep. Korea	√
WD20	HEAMNAM	GmWMC048	Landrace	Rep. Korea	√
WD21	HEUKDAELIP	GmWMC066	Landrace	Rep. Korea	√
WD23	PK 73-54	GmWMC071	Landrace	India	√
WD24	M 581	GmWMC072	Landrace	India	√
WD26	CHEONGYE MYONGTAE	GmWMC075	Landrace	Rep. Korea	√
WD28	PEKING	GmWMC084	Landrace	China	√
WD29	ANTO SHOUKOKUTOU	GmWMC086	Landrace	China	√
WD31	JEOKGAK	GmWMC094	Landrace	Rep. Korea	√
WD33	HAKKA ZASHI	GmWMC107	Landrace	China	√
WD34	KARASUMAME	GmWMC108	Landrace	China	√
WD36	WILLIAMS 82	GmWMC115	Breeders Line	USA	√
WD37	ODU	GmWMC118	Landrace	Rep. Korea	√
WD40	GAPSANJAE(LAE(I)	GmWMC122	Landrace	Rep. Korea	√
WD41	N 2295	GmWMC123	Landrace	Nepal	√
WD42	BHATMAS	GmWMC125	Landrace	Nepal	√
WD43	AOKI MAME	GmWMC129	Landrace	China	√
WD44	L 2A	GmWMC132	Landrace	Philippines	√
WD45	LOCAL VAR(SEPUTIH RAMAN)	GmWMC136	Landrace	Indonesia (Sumatra, Java, Borneo)	√
WD49	M 44	GmWMC143	Landrace	India	√
WD50	M 918	GmWMC144	Landrace	India	√
WD51	HM 39	GmWMC146	Landrace	India	√
WD52	COL/THAI/1986/THAI-78	GmWMC147	Landrace	Thailand	√
WD53	M 42	GmWMC148	Landrace	India	√
WD55	JAVA 7	GmWMC151	Landrace	Indonesia (Sumatra, Java, Borneo)	√
WD56	U 1290-1	GmWMC152	Landrace	Nepal	√
WD57	MANSHUU MASSHOKUTOU	GmWMC154	Landrace	China	√
WD58	U 8006-3	GmWMC156	Landrace	Nepal	√
WD61	COL/THAI/1986/THAI-80	GmWMC162	Landrace	Thailand	√
WD63	KARASUMAME(SHINCHIKU)	GmWMC165	Landrace	Taiwan	√
WD64	MERAPI	GmWMC166	Breeders Line	Indonesia (Sumatra, Java, Borneo)	√
WD65	L 317	GmWMC168	Landrace	India	√
WD 66	HAKUCHIKOU	GmWMC169	Landrace	China	○
WD67	M 652	GmWMC170	Landrace	India	√
WD68	U-1741-2-2 NO.3	GmWMC171	Landrace	Nepal	√
WD69	KARASUMAME(NAIHOU)	GmWMC173	Landrace	Taiwan	√
WD70	BISHUU DAIZU	GmWMC175	Landrace	China	√
WD71	SANDEK SIENG	GmWMC176	Landrace	Cambodia	√
WD72	CHIENGMAI PALMETTO	GmWMC181	Landrace	Thailand	√
WD73	LOCAL VAR.(TEGINENENG)	GmWMC182	Landrace	Indonesia (Sumatra, Java, Borneo)	√
WD74	KARASUMAME(HEITOU)	GmWMC183	Landrace	Taiwan	√
WD75	RINGGIT	GmWMC186	Breeders Line	Indonesia (Sumatra, Java, Borneo)	√
WD76	KADI BHATTO	GmWMC187	Landrace	Nepal	√
WD77	E C 112828	GmWMC188	Landrace	India	√
WD80	U 1155-4	GmWMC192	Landrace	Nepal	√

¹⁾https://www.gene.affrc.go.jp/databases-core_collections_wg_en.php

NIAS World Soybean Core Collection

²⁾ ○ : A line which cannot be used in Chapter II

container (W/D/H, 51 cm/81 cm/21 cm). Plants were grown in a greenhouse, with a temperature above 22°C at night and below 28°C during the daytime. Fourteen days after sowing, the baskets were carefully lifted. Penetrating roots were observed, and their number and the location of penetration were noted. Roots were classified into three categories according to growth angle (Fig. 2.1(B)). Roots with a growth angle of less than 30° were defined as shallow, those between 30° and 45° as intermediate, and those greater than 45° as deep. The experiment was conducted according to a completely randomized design with three replications (three pots per line).

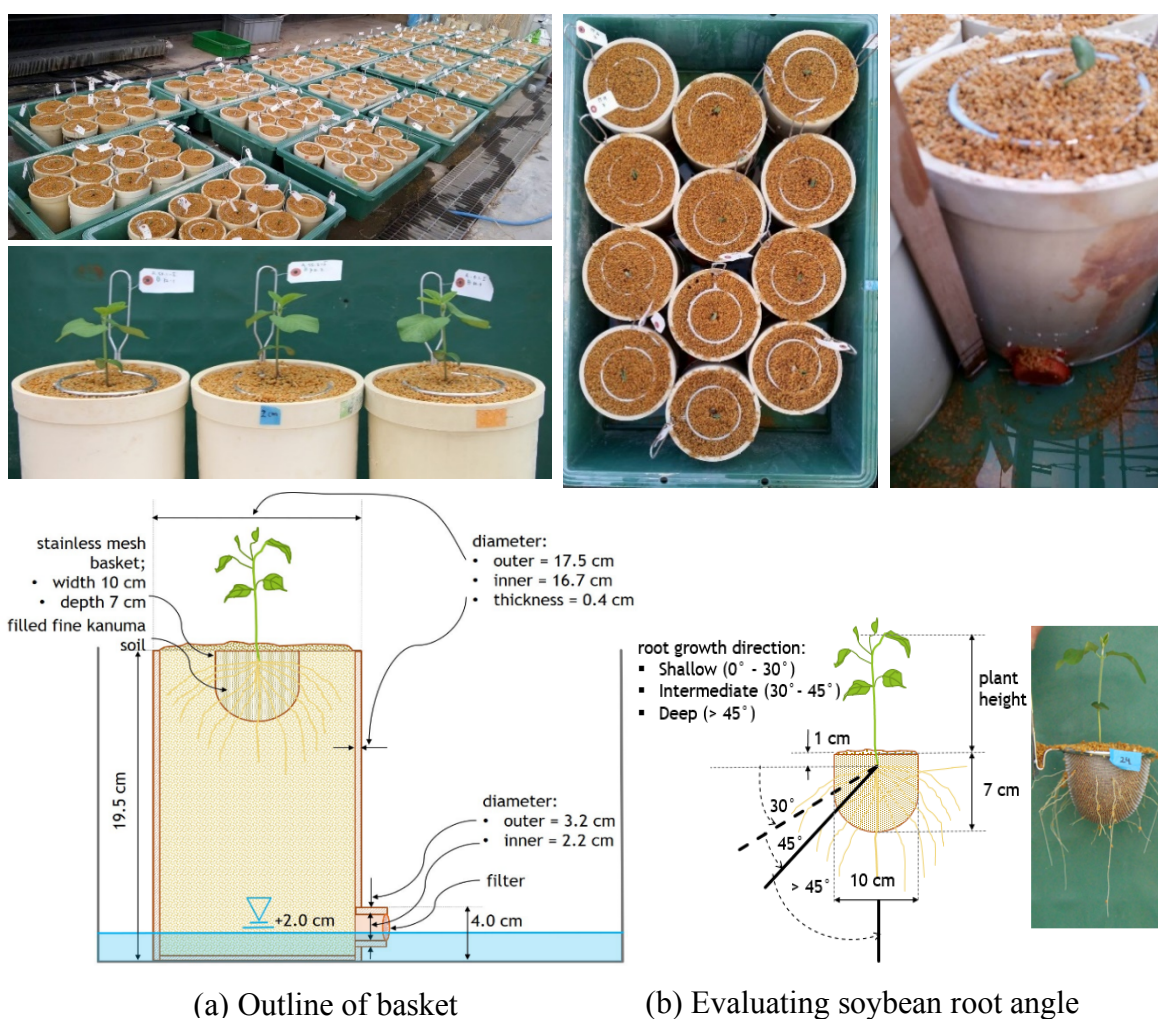


Fig. 2.1 Experimental system with basket method for soybean root

2.3 Results

To evaluate the genetic diversity of root traits, the root traits of the GmWMC were analyzed (Table 2.2, Fig. 2.3). Among 80 accessions of GmWMC, 18 lines were deleted because the seeds were not fully matured. Another 10 accessions were also eliminated from the data because of the accidental loss of plants during the experiment. The total root number ranged from 12.7 to 43.3, with an average of 25.9. In general, there were fewer deep roots than shallow or intermediate roots.

Table 2.2 Distribution of root traits in the GmWMC.

	Range	Mean	CV(%)
Shallow root number	1.7-19.3	10.5	35.0
Intermediate root number	2.0-31.3	8.1	57.3
Deep root number	2.0-12.7	7.3	29.3
Total root number	12.7-43.3	25.9	27.1
Shallow root ratio	4.8-55.6	41.1	23.2
Intermediate root ratio	12.9-89.5	30.1	38.0
Deep root ratio	5.7-50.0	28.9	24.7

Soybean is a species of legume that is native to East Asia, including China and Korea. Therefore, GmWMC accessions were divided into cultivars from China and Korea, and cultivars from other regions (Fig. 2.3). Some of the cultivars originating from China and Korea had fewer roots in all three categorized root depth types (comparing Fig. 2.3 (A) and (B)) and the ratios ((C) and (D)), indicating that the genetic diversity among cultivars from China and Korea is greater than those from other areas.

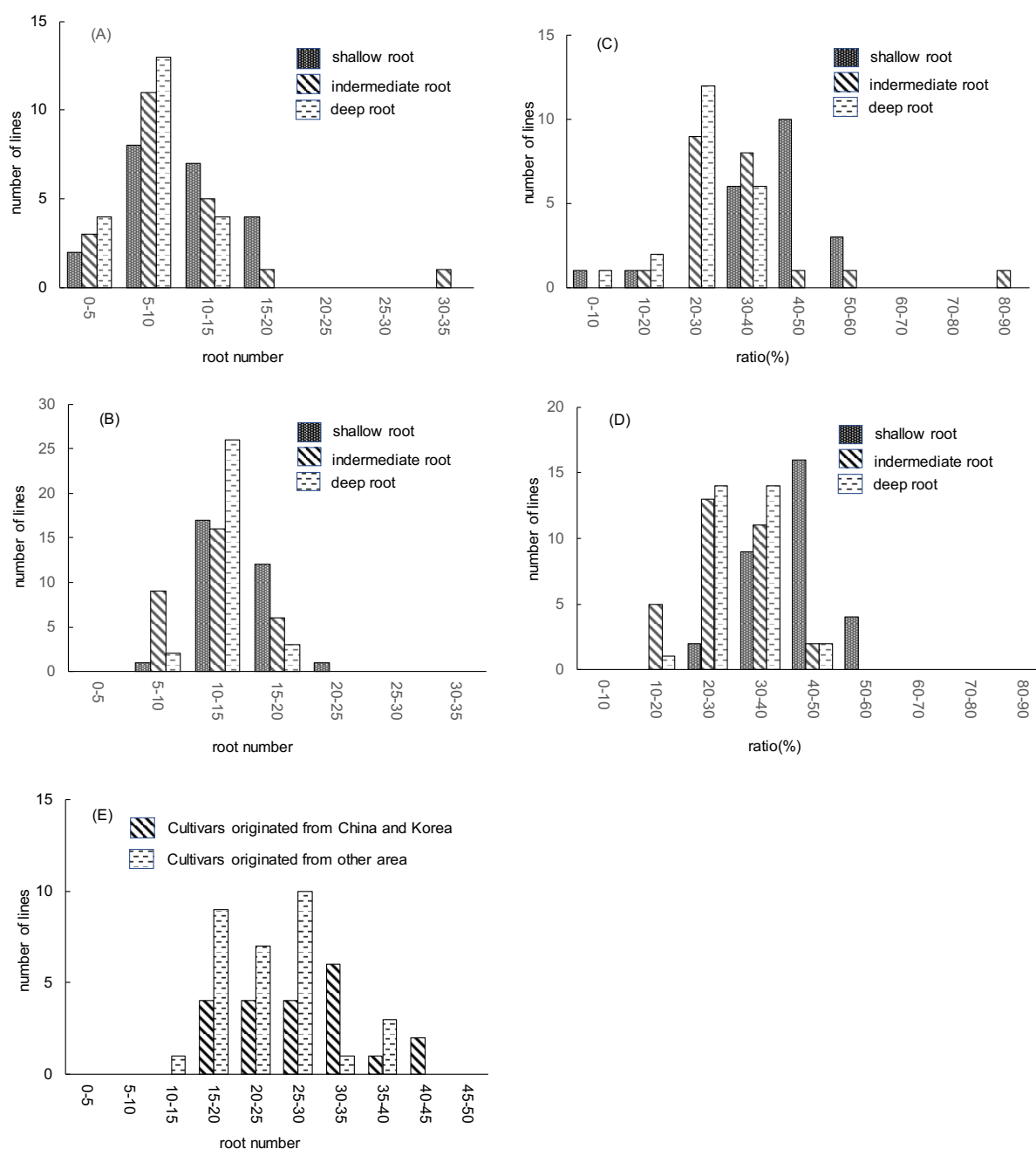


Fig. 2.3 Frequency distribution of GmWMC collection (A) Root number in cultivars originated from China and Korea (B) Root number in cultivars not originated from China and Korea (C) Root number ratio in cultivars originated from China and Korea (D) Root number ratio in cultivars not originated from China and Korea (E) Total root number

2.4 Discussion

In soybean, root traits have the potential to be developed to optimize the ability to acquire soil resources. Particular attention has been devoted to increasing crop yields without causing environmental damage (Gewin, 2010). Innovation in some methodologies has provided an opportunity to improve the root system to more effectively acquire soil resources and to better optimize agricultural land use. Root traits could be a challenging selection criteria in this regard.

Plants effectively respond to environmental factors such as drought conditions and nutrient deficiency. Previous research on soybean revealed that the effects of drought are related to phosphorus deficiency as it increases fibrous root density at the soil surface (Abdel-Haleem *et al.*, 2011). Treatment without nitrate promotes the elongation of the primary root (Saito *et al.*, 2014). The use of phosphorus in the legume group such as *Phaseolus vulgaris*, which have basal roots with a low phosphorous content, will result in branches growing at a shallower depth than roots with sufficient phosphorous. In cereal, low phosphorus availability also enhances the elongation of the primary root and the induction rate of adventitious roots (Hu *et al.*, 2011; Zhou *et al.*, 2008). The root architecture of soybean, in which shallow roots are dominant over deep roots, has the potential advantage of growing with a low phosphor content.

Deep rooting is considered to be an essential trait in the improvement of drought avoidance in crops (Manschadi *et al.*, 2006; Kirkegaard *et al.*, 2007). In waterlogged conditions, however, root systems ordinarily respond by remaining shallow (Sallam & Scott, 1987). For example, rice cultivars that are grown in upland areas tend to have deeper roots than lowland cultivars whereas rice in wetland areas genetically tends to grow at a shallower depth because the oxygen deprivation in the soil environment results in root damage (Yoshida *et al.*, 1982, O'Toole and Bland, 1987). In maize, shallower genotypes had significantly greater growth and phosphorus accumulation

compared to deeper genotypes with low phosphorus availability (Zhu *et al.*, 2005). Hence, in the case of phosphorus deficiency, a topsoil-foraging root system is considered to be better than a deep root system because phosphate often accumulates in the top soil layers. An intercropping system in maize and soybean revealed that the land equivalent ratio had a higher biomass and nitrogen content in maize intercropped with shallow-rooted soybean compared to deep-rooted soybean (Jinchi *et al.*, 2005).

It was revealed that features of the soybean root architecture are distinctly different from those of the Poaceae family, including rice and corn. The number of shallow roots in soybean was much higher than the number of deep roots, the opposite of what is seen in rice. Shallow roots have the advantage of supplying nitrogen gas from the atmosphere to nodule bacteria. Soybean root architecture is formed to fix nitrogen effectively.

Chapter III

QTL analysis of root traits using recombinant inbred lines

3.1. Introduction

QTL analyses have been introduced to clarify the genetic factors in root traits in some plants. QTL analysis is helpful, connecting the gap between genotype and phenotype. There are many loci/genes that control root trait. Uga *et al.* (2011) conducted research using the basket method to evaluate the deep rooting of rice in relation to the proportion of total roots that extended 50° to 90° along the bottom of the basket. They identified a QTL for deep rooting in rice, namely, *Dro1*.

Research on the genetic factors regulating the root architecture is mostly limited to the Poaceae family, including rice, maize, wheat, and is scarce in Dicotyledoneae. In the previous study (Chapter 2), the root growth angle using core GmWMC developed by Kaga *et al.* (2012) have been evaluated, and estimated the genetic diversity of the root architecture. Moreover, QTL analysis using recombinant inbred lines was conducted to identify the genetic factors underlying the root architecture.

3.2. Materials and methods

3.2.1 Plant Materials

Ninety-three recombinant inbred lines originating from a cross between Peking and Tamahomare (PT-RILs) were used for the QTL analysis of root traits. Peking is a landrace originating from China, and Tamahomare is an elite Japanese variety. PT-RILs were developed through successive inbreeding from F2 to F14. I reconstructed the linkage map for 93 RILs (F14) using 227 polymorphic simple sequence repeat (SSR) markers with MAPMAKER/EXE v. 3.0 software (Lander *et al.*, 1987) based on the linkage map for F8 plants consisting of 344 polymorphic SSR markers (Sayama *et al.*, 2009). QTL analysis was calculated using the R/QTL package (Broman *et al.*, 2003).

3.2.2 Experimental procedures

Root traits were measured by the basket method (Oyanagi, 1994) with slight modification. A stainless mesh basket (10 cm diameter, 7 cm depth) was buried in the center of 1/5000 in a Wagner's pot filled with fine pumice originating from Kanuma, Japan (Fig. 1(A)). A single seed was planted in the center of each basket at a depth of 1 cm. All pots were placed in a container in which the water level was adjusted to 2 cm. After all the fine pumice became moist, seeds were placed on the soil so that the hypocotyl side was on the bottom. Twelve pots were put into a plastic container (W/D/H, 51 cm/81 cm/21 cm). Plants were grown in a greenhouse, with a temperature above 22°C at night and below 28°C during the daytime. Fourteen days after sowing, the baskets were carefully lifted. Penetrating roots were observed, and their number and the location of penetration were noted. Roots were classified into three categories according to growth angle (Fig. 1(B)). Roots with a growth angle of less than 30° were defined as shallow, those between 30° and

45° as intermediate, and those greater than 45° as deep. The experiment was conducted according to a completely randomized design with three replications (three pots per line).

3.3 Results

To identify the genetic factors controlling root traits, QTL analysis was conducted. The range of the root traits of PT-RILs generally exceeded the parent values (Table 3.1, Fig. 3.1), indicating that the parents had alleles at different loci involved in root traits. In particular, the shallow root number of PT-RILs was greater than that of the GmWMC accession.

Table 3.1 Distribution of root traits in the parental cultivars and the RIL population.

	Parents		Range	RILs	
	Peking	Tamahomare		Mean	CV(%)
Shallow root number	13.00	20.33	5.67-33.67	16.02	34.04
Intermediate root number	12.00	17.00	1.33-17.67	9.20	33.74
Deep root number	8.67	13.33	1.00-11.67	5.89	31.93
Total root number	33.67	50.67	15.00-51.33	31.11	23.39
Shallow root ratio	38.77	40.47	29.42-80.14	51.57	20.28
Intermediate root ratio	35.35	33.18	5.33-48.34	29.19	28.42
Deep root ratio	25.87	26.35	5.26-32.07	19.24	28.26

A total of nine putative QTLs were identified for five of the seven tested traits (Table 3.2, Fig. 3.2). No significant QTLs for deep root number or deep root ratio were detected. Putative QTLs for shallow root number were located in Chr. 2, 7, and 19, named qSN1, qSN2, and qSN3, respectively. Peking-type alleles for qSN1 and qSN2 and Tamahomare-type alleles for qSN3 increased the shallow root number. The phenotypic variations explained by each QTL exceeded 20%. QTLs for intermediate root number were located in Chr. 2 and 19, named qIN1 and qIN2,

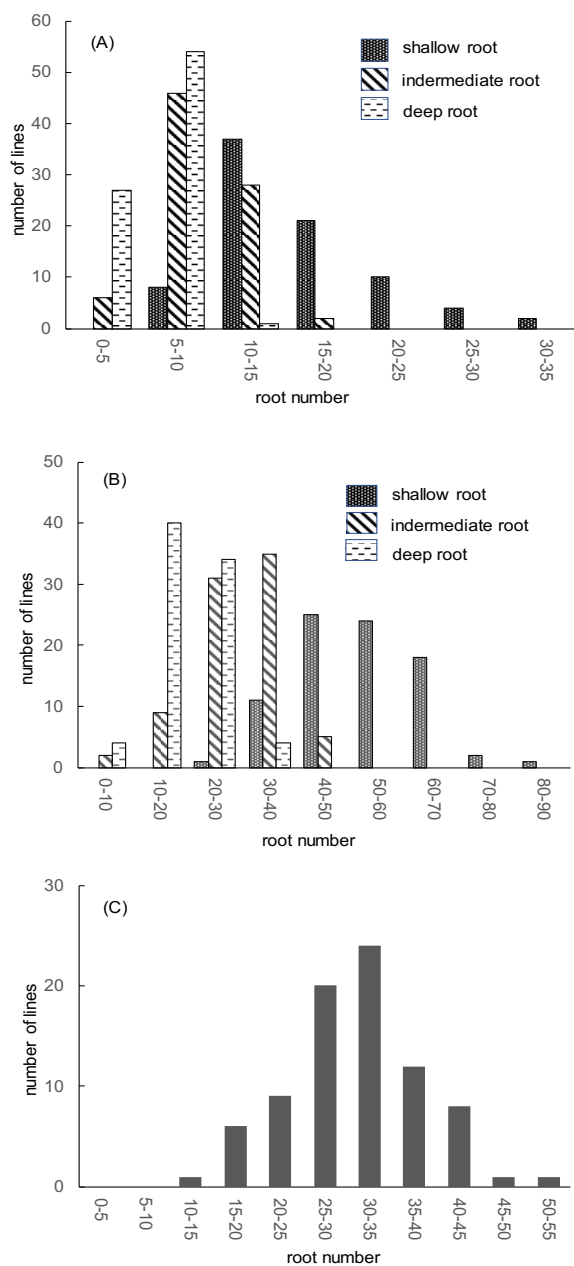


Fig. 3.1 Frequency distribution of PT-RILs (A) Root number (B) Root ratio (C) Total root number

Table 3.2 Putative QTLs detected for root traits with basket method

Trait	QTL	Marker Interval	Chromosome	Peak Position ^a	LOD	Additive	Contribution
	Nomenclature	flanking QTL peak	(linkage group)	(cM)		effect ^b	(%)
Shallow root number	qSN1	Satt558-Satt282	2 (D1b)	51	4.09	4.10	21.2
	qSN2	Satt551-Satt210	7 (M)	90	4.81	5.05	24.5
	qSN3	Satt284-Sat_113	19 (L)	73	4.29	-5.20	22.1
Intermediate root number	qIN1	Sat_139-Satt459	2 (D1b)	114	3.53	-2.25	18.6
	qIN2	Satt284-Sat_113	19 (L)	80	3.16	-2.65	16.8
Total root number	qTN1	Sat_162-Satt424	8 (A2)	54	3.92	7.20	20.4
	qTN2	Satt284-Sat_113	19 (L)	77	4.08	-9.40	21.2
Shallow root ratio	qSR1	Sct_067-Satt177	8 (A2)	23	3.25	3.15	17.3
Intermediate root ratio	qIR1	Satt459-Satt271	2 (D1b)	141	4.09	-2.50	21.2

^a QTL position was calculated from the first marker

^b A positive value indicates that the “Peking”-type allele increases phenotypic value and a native value indicates that the “Tamahomare”-type allele increases phenotypic value.

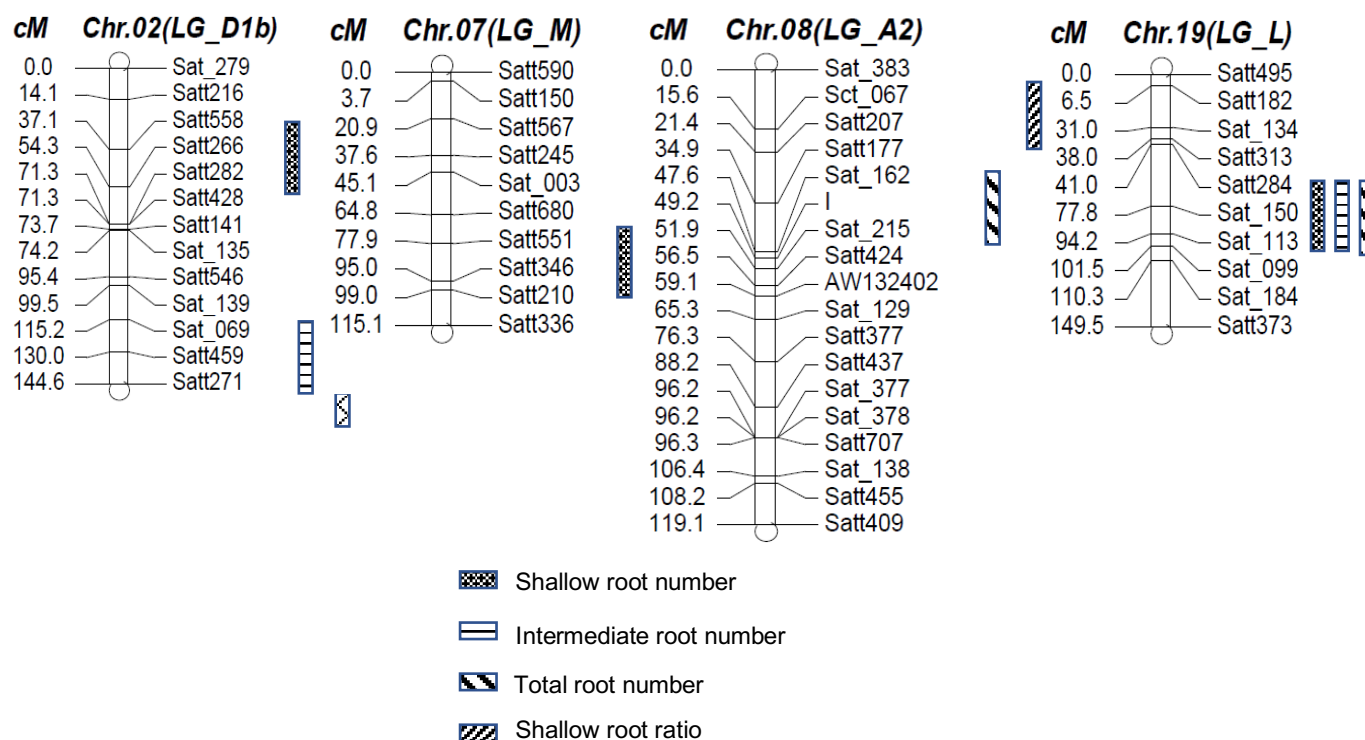


Fig. 3.2 QTL region of PT-RILs grown by basket method

respectively. QTLs for total root number were located in Chr. 8 and 19, named qTN1 and qTN2, respectively. Three QTLs, qSN3, qIN2, and qTN2, were located in the same region on Chr. 19, suggesting that pivotal genetic factors for increasing root number, especially shallow root number, are located in this region. A single QTL was detected for the shallow root ratio and the intermediate root ratio on Chr. 8 (qSR1) and Chr. 2 (qIR1), respectively. The chromosome regions of qSR1 and qIR1 were not overlapped with any QTLs for root number.

3.4 Discussion

In terms of root number, a major QTL was located on Chr. 19 (Satt284-Sat_113) for total root number as well as shallow root number and intermediate root number. Hwang *et al.* (2014) detected several QTLs of root nodule number and nodule weight. Among them, a QTL of root nodule weight was located at almost the same chromosome segment on Chr. 19. The low lateral root number may have caused the reduction in nodule number. Liang *et al.* (2014) reported several QTLs for maximum root length, lateral root number, and root weight. They detected four QTLs for lateral root number on Chr. 2, 5, 19, and 20. The QTL of intermediate root number on Chr. 2 (qIN1) in our study was located in the same chromosome region they detected. Brensha *et al.* (2012) reported the QTLs of several root traits in soybean. They reported the QTL of lateral root length and basal root thickness on Chr. 18, and the QTL of root fresh/dry weight on Chr. 3 and Chr. 8. Among them, we found the QTL of the shallow root ratio (qSR1) at the overlapping region of the QTL of root fresh weight on Chr. 8. We also found the QTL of total root number (qTN1) at the overlapping region of the QTL on root dry weight on Chr. 8. Further investigation and identification of the QTLs will clarify if a single QTL has pleiotropic effects on root traits or if contiguous QTLs have independent effects. In conclusion, the root distribution of soybean was

difficult to develop in deep regions. The shallow root architecture of soybean perhaps contributes to forming nodules because roots close to ground level likely provide atmospheric nitrogen to nodule bacteria easily. In the future, the identification of the corresponding genes through fine mapping should be conducted to establish efficient marker aided selection for root architecture.

Chapter IV

Root system architecture in the field

4.1. Introduction

Many researchers have suggested several approaches for the phenotyping of root traits. There are three different approaches for the phenotyping of root traits, field methods, greenhouse method and laboratory methods (Paez-Garcia *et al.*, 2015). In the field method, growth condition can be minimally controlled, which takes advantage of physiological and practical relevance but laborious. Furthermore, evaluation under field condition is challenging due to the wide diversity of soil condition. In laboratory method, the growth condition can be highly controlled and the root growth can be overserved in real time through non-destructive ways. Therefore, the investigation of root traits depends on the development of techniques and methods for root sampling and root trait measurement (Chen *et al.*, 2017).

The observation of root traits are currently in pursuit for varied reason. Non-destructive, quick analysis of root traits are complicated to accomplish as root part is hidden in the soil. Consequently, appropriate methods to measure root traits are required to improve root traits and to evaluate root growth responses to environmental variation (Le Marie *et al.*, 2014). The aim of

this study is to evaluate the root traits under the field condition and to clarify the relation with the results obtained under greenhouse condition (Chapter II)

4.2. Materials and methods

4.2.1 Plant Materials

Eighty lines of a soybean (*Glycine max*) world mini core-collection (Table 2.1) provided by the Genetic Resources Center, National Agriculture and Food Research Organization, Japan, were used to determine the proper method to evaluate the root traits. The researches were conducted in 2015 and 2016 in the experimental field of Kyoto University using the double basket method.

4.2.2 Experimental procedures

As shown in Fig. 4.1, Eighty lines of GmWMC and six typical lines were grown in the experimental field of Kyoto University. Two plastic mesh baskets were combined to grow soybean in the field. The inner basket (top diameter 18 cm, height 8.2 cm) was filled with field soil. The space between the inner basket and the outer basket (top diameter 22 cm, height 9.9 cm) was filled with fine pumice originated from Kanuma in Japan. This combined double basket unit was buried in the field and two seeds were sown at the center of the inner basket. Seven days after sowing, a seedling was thinned out to grow a single plant in each unit of double basket. In 2015, lines were grown in the field with the randomized block design with two replications (three plants per replication). After the flowering time, the inner basket was carefully lifted up and the penetrating root was observed, and their number and location of penetration were noted as shown in Fig. 4.1. Roots were classified into two categories according to their penetrating locations. Roots penetrated at P1 and P2 were designated as shallow root (S-root). Roots penetrated at P3 and P4 were designated as deep root (D-root). In 2016, six lines were selected based on the result of 2015 and

were grown in the field with the randomized block design with two replications (five plants per replication)

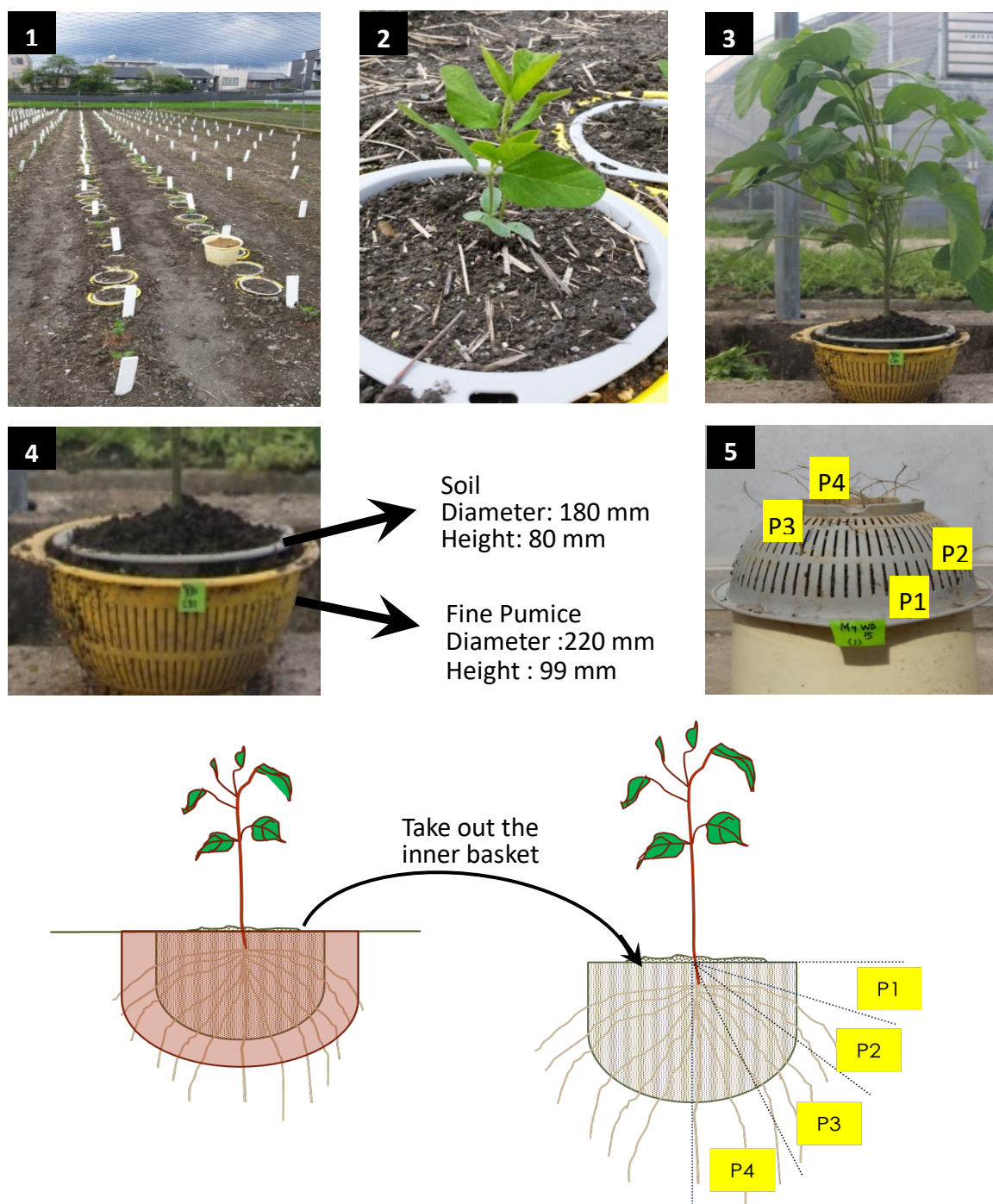


Fig. 4.1 Evaluating root traits in the field

4.3 Results

Among 80 accessions of GmWMC, 18 lines were deleted because the seeds were not fully matured. Another 7 accessions were also eliminated from the data because of the accidental loss of plants during the experiment. The frequency distribution of the ratio of S-root and D-root is shown in Fig. 4.2. Mean value of S-root ratio is higher than that of D-root ratio. This indicated that soybean tend to develop their root in the shallow part of the soil. The relationship between root number in S-root and D-root are shown in scatter plots in Fig. 4.3. Based on the number of S-root and D-root GmWMC accessions can be categorized into two Groups, namely S-root dominant type (Group II) and D-root dominant type (Group I). It indicated that most GmWMC accessions develop more S-root than D-root. As shown in previous chapter (Chapter 2), soybean elongate its roots into the shallow part of the soil even under the field condition.

The result of field experiment in 2015 and 2016 for the D-root and S-root ratios of six lines are shown in Table 4.1. Lines were classified into shallow-root dominant type (S-type), Intermediate-root dominant type (I-type) and deep-root dominant type (D-type) based on the S-root ratio in 2015. Lines classified as a S-type in 2015 were regarded as the I-type in 2016. Their S-root ratios were 71 and 73% in 2015, but they were only 48 and 41% in 2016. Moreover, their S-root number was significantly reduced in 2016 comparing with those in 2015 (Fig. 4.4). Lines classified as a D-type in 2015 also regarded as D-type in 2016. Their D-root number and ratios were not significantly different between 2015 and 2016. Lines classified as I-type in 2015, their D-root number and S-root number were reduced in 2016. The weather condition of 2015 and 2016 are shown in Fig. 4.5. It is quite obvious that amount of rainfall in 2015 was significantly lower than that in 2016. It might be possible that soil moisture content affect the S-root number. When there is enough amount of water in the surface area of the soil, S-root development was somehow

restricted. When there is a very few amount of water in the surface area of the soil, S-root development were intensively promoted.

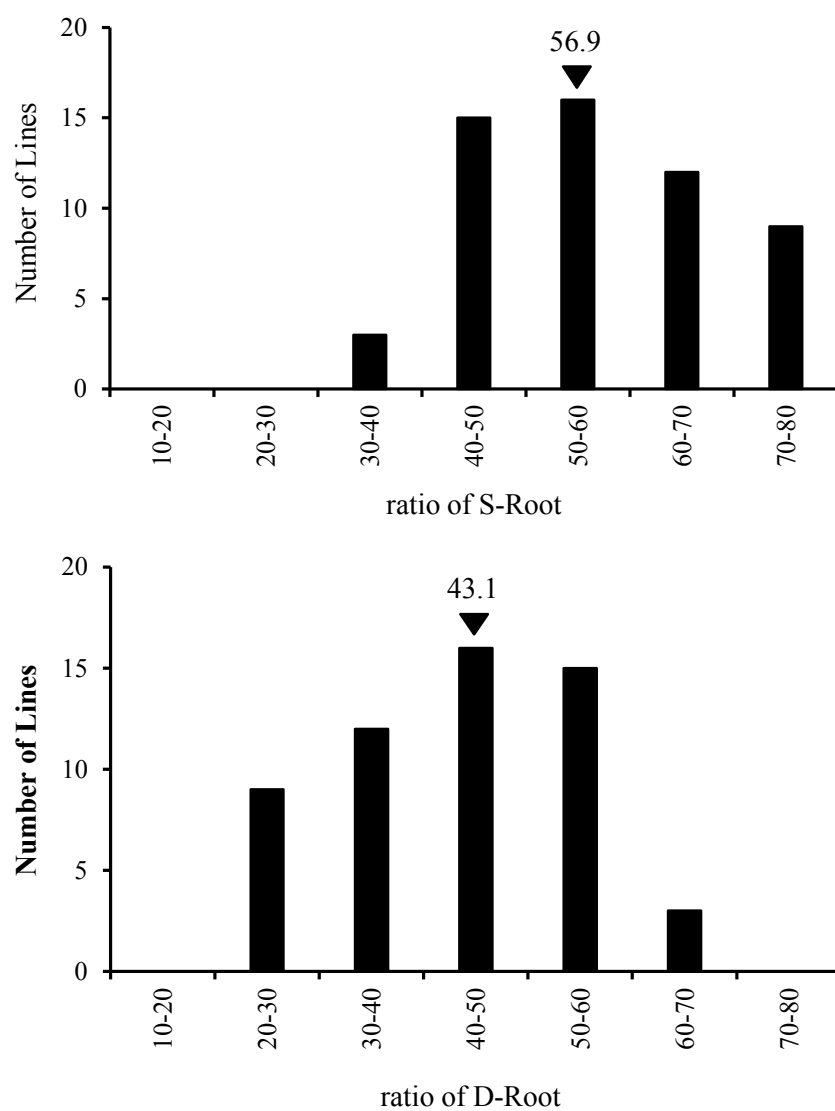


Fig. 4.2 Frequency distribution ratio of S-root and D-root in the field, 2015

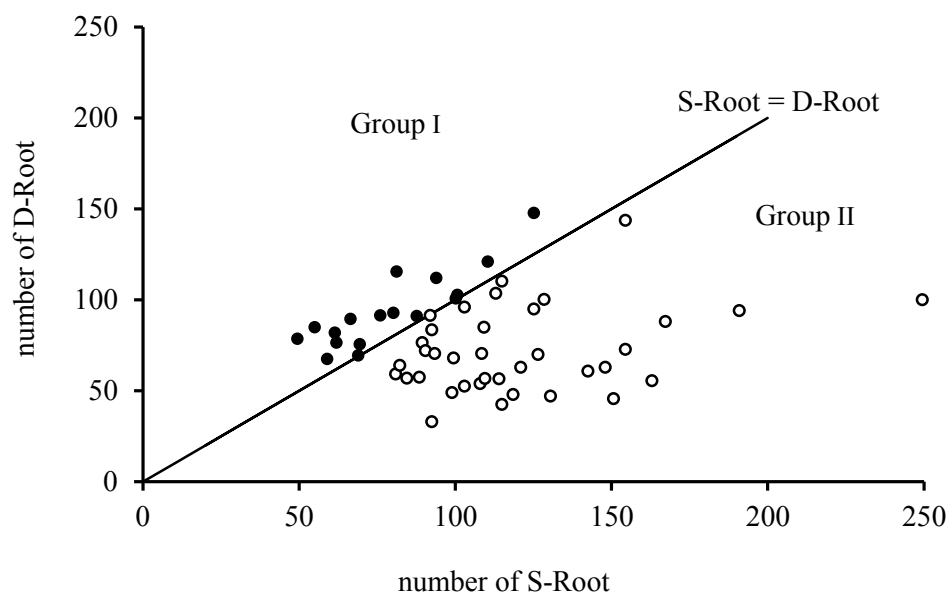


Fig. 4.3 Relationship number of S-Root and D-Root in the field

Table 4.1 Root percentage in typical line 2015 and 2016

LINE	2015 (%)		2016 (%)	
	S-root	D-root	S-root	D-root
D-28	42.86	57.14	33.3	66.7
D-36	39.29	60.71	25.2	74.8
S-43	71.39	28.61	48.5	51.5
S-67	73.52	26.48	41.4	58.6
I-11	51.76	48.24	49.1	50.9
I-66	56.17	43.83	63.9	36.1

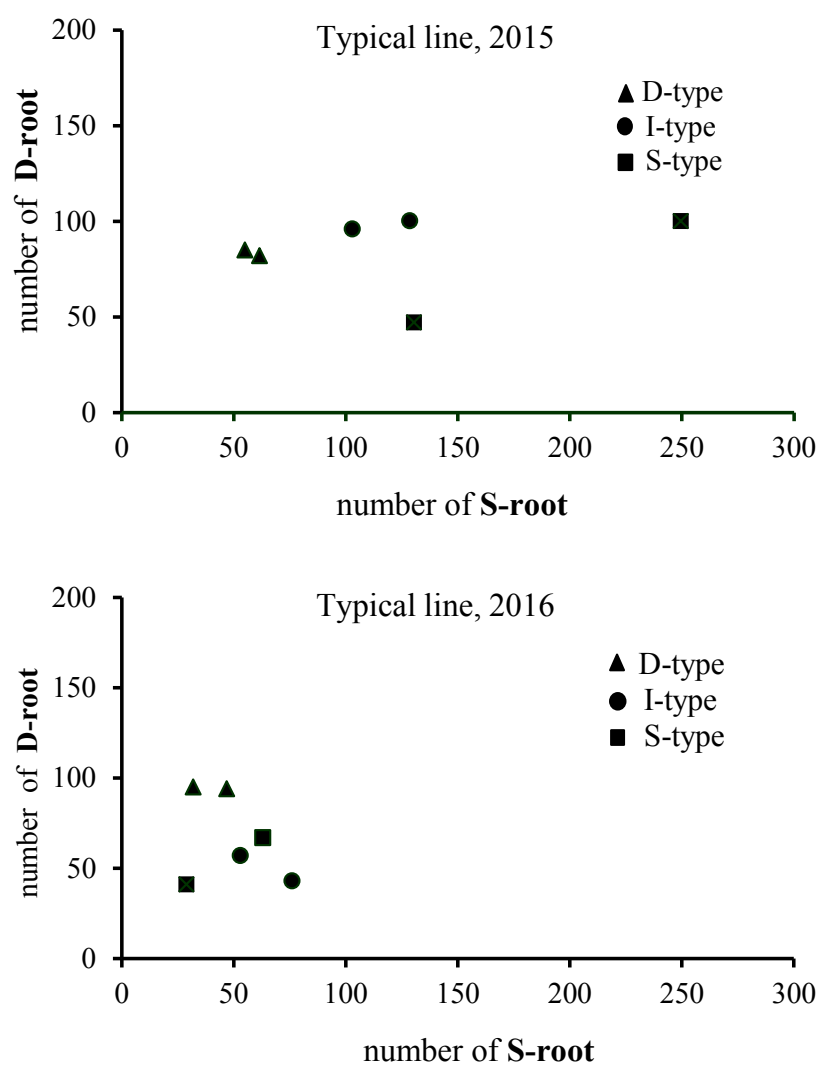


Fig. 4.4 Relationship number of S-root and D-root typical lines in 2015 and 2016

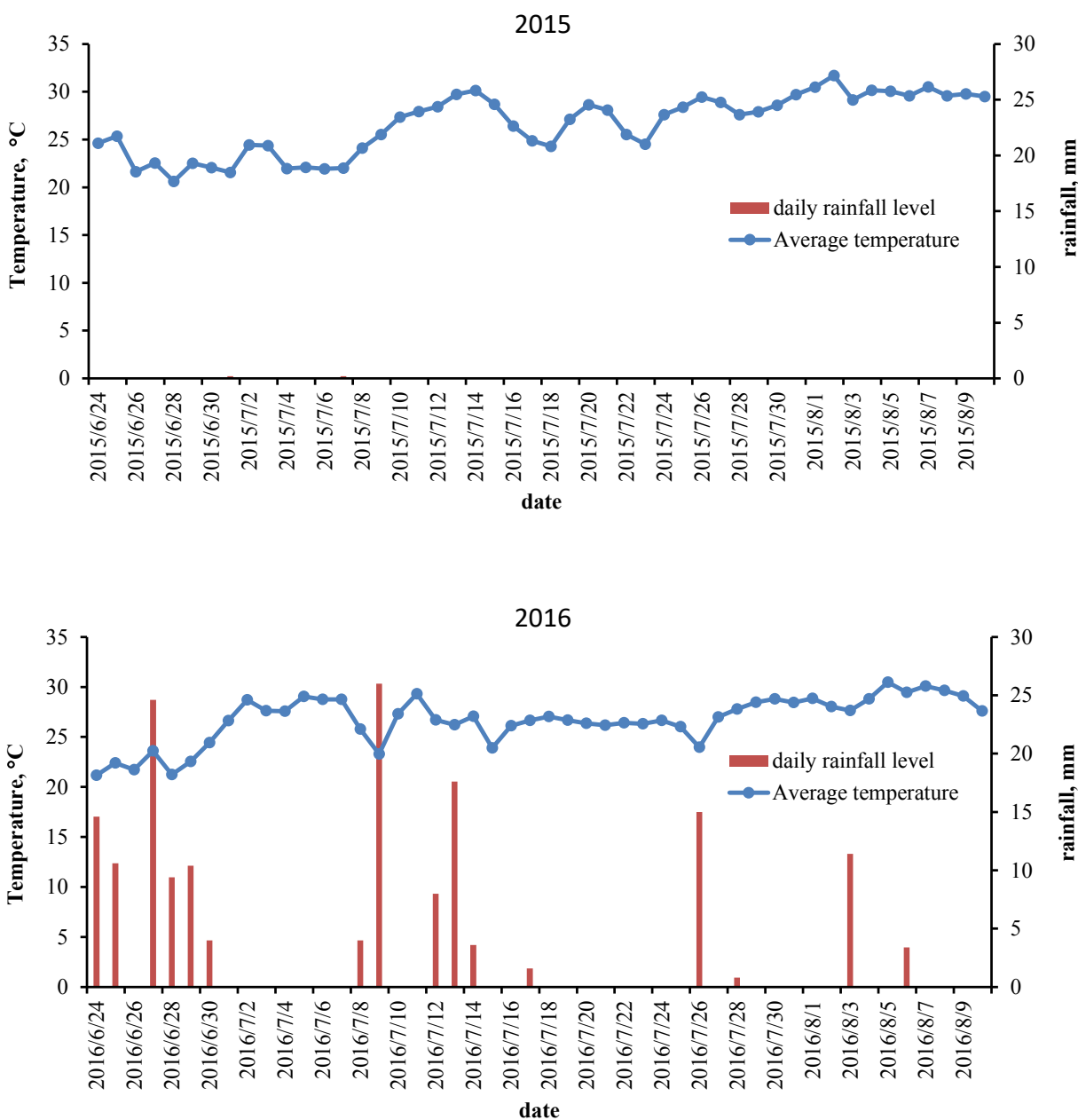


Fig. 4.5 The meteorological condition during the experiment in 2015 and 2016

Irrespective of the difference in the amount of rainfall between 2015 and 2016, D-root number of D-type was not largely affected. In contrast, S-type lines restrict their S-root number when there is enough water in the surface area of the soil. It can be concluded that the difference

in soil moisture in the field has a significant effect on the S-type lines in comparison to D-type lines.

To reveal the relationship between the field observation and the greenhouse observation (Chapter II) shallow root number and deep root number of Group I lines and Group II lines are shown in Fig. 4.6. Distribution of Group I lines and Group II lines were completely overlapped, and S-root dominant groups in the field experiment not always develop the larger number of shallow root in greenhouse.

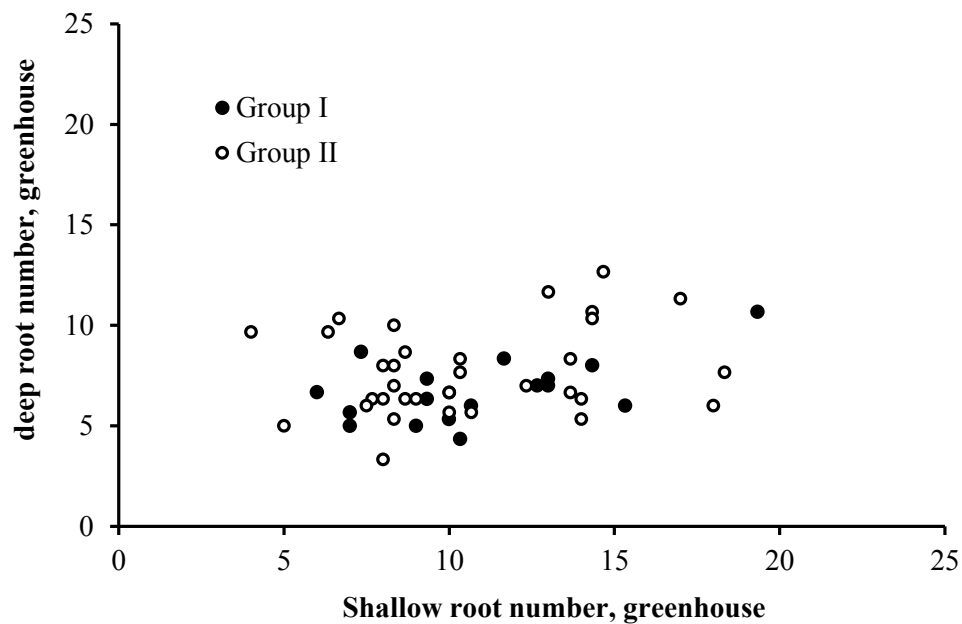


Fig. 4.6 Relationship root number in Group I and Group II in greenhouse

4.4 Discussion

Since D-root number was not largely affected by the water condition of the soil. The relationship between D-root number in the field and deep root number in the greenhouse was analyzed. The correlation between D-root number in the field observation and deep root number of the greenhouse experiment of Group I and Group II lines are separately shown in Fig. 4.7. They are positively correlated especially in Group I lines which can be restricted S-root development even under limited water content in the surface area of soil. Deep root number (D-root in Group 1 lines) in the field had a significant correlation with deep root number in the greenhouse ($r=0.52$). Meanwhile, deep root number (D-root in Group 2 lines) revealed a coefficient correlation with deep root number in the greenhouse ($r=0.23$). This indicates that lines belong to Group I are the lines with the poor ability to develop shallow root. Thus, Group I lines largely rely on the deep root development to access to the water resource.

Comparison of the result in the field condition using double basket method and greenhouse method using basket method for shallow root type showed that the root distribution reaches 70 percent in the shallow area using the basket method in the greenhouse and 56.9 percent using double basket method in the field. The number of roots counts that in the shallow region is larger than that in the deep areas.

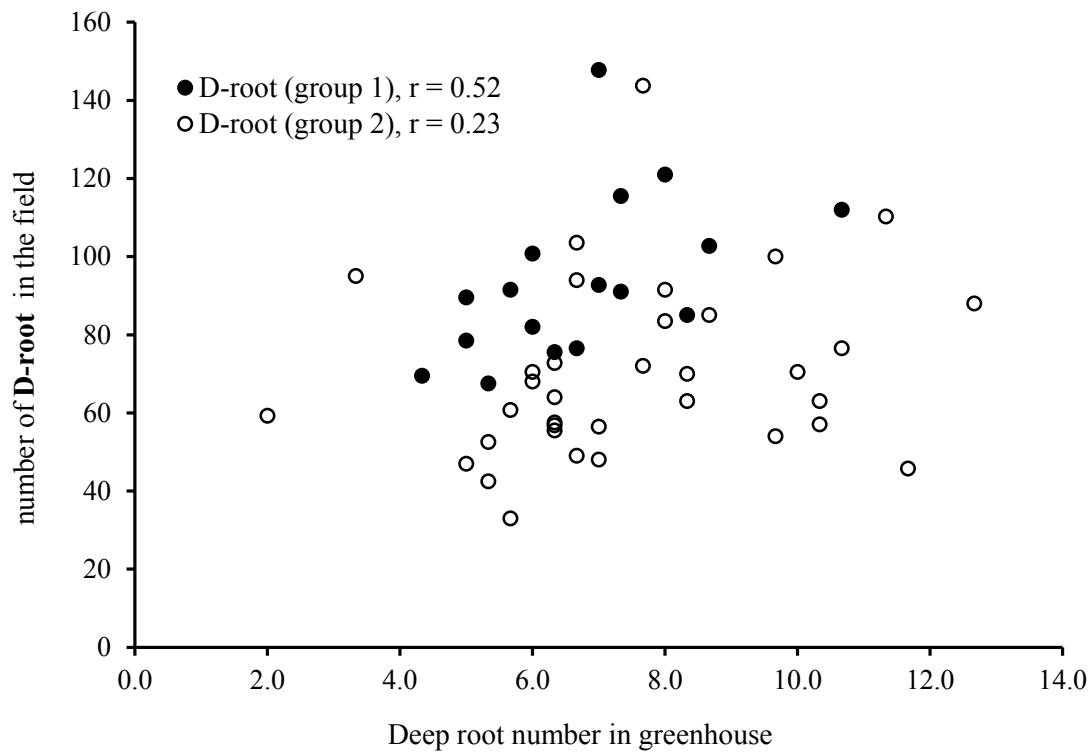


Fig. 4.7 Relationship between deep root number in greenhouse and number of D-root in the field.

Chapter V

Evaluation of root traits at the seeding stage using rhizobox system

5.1. Introduction

Research on root development was initiated in the field by interests in studying root growth under various soil conditions. The measurement of root traits and specific traits employs a wide range of equipments and techniques, with several are dependent on crop growth conditions, whether in the field or specific containers. Furthermore, each method has its own advantages and disadvantages, thus the best method is chosen based upon the following considerations. First, at what growth stage (seedling, vegetative, or generative stage) the plant is evaluated. Second, the seedling type of plant: whether hypogeal or epigeal. Third, the ease to observe the root growth and to reproduce the observation. Fourth, whether or not the method damages the root. Lastly, the ease to obtain high quality pictures of the root for documentation purposes.

The study of root traits in seedling using a germination paper was first developed for a study on *Phaseolus vulgaris* (Bonser *et al.*, 1996), with basal root as the focus of study. Later on, Liao *et al.* (2001) expanded the technique to allow measurement of the root length. In 2009, it was reported that this technique was successfully employed for a study on maize seedling (Hund *et al.*, 2009). QTL analysis based upon high density genetic maps has allowed a better understanding of

genetic control of root traits. The number of studies identifying QTLs in seedling root traits in wheat (Hamada *et al.*, 2012; Christopher *et al.*, 2013). The aim of the study is to apply rhizobox system for the QTL analysis of root traits.

5.2. Materials and methods

5.2.1 Plant Materials

Ninety-three recombinant inbred lines originated from a cross between Peking and Tamahomare (PT RILs) were used to identify for the QTL analysis.

5.2.2 Experimental procedures.

Seeds were surface sterilized by rinsing in 30 % hydrogen peroxide (H_2O_2) for two minutes and washing several times with sterile water. Sterilized seeds were placed in the middle of a moistened paper towel for 12 hours at room temperature 25 °C. Then, seeds were transferred to a moistened germination paper (Whatman) crease-side down in a rhizobox system to complete the germination. The plexiglass rhizobox system was based on the design developed for soybean. Each box measured length 34 cm x width 1.5 cm x height 27.5 cm. Chromatography paper (3 mm Chr) was folded in half and seeds were sandwiched in between. Papers were set in the rhizobox and the rhizoboxes were filled with vermiculite. The vermiculite was sterilized with oven at 200 °C for 8 hours and put in the rhizobox. Six hundred ml water was poured and kept for 2 hours, after paper was moistened the seed was placed inside the paper. The rhizobox was then covered with a black polythene film and placed inside the controlled environment room at 25 °C with 16-hours photoperiod. After six days treatment, seedlings were collected. All treatments were performed in two replicates. Root parameters, which consists of the number of

roots, root length, root growth angle, and root width and root depth were observed (Fig. 5.1). The root growth angle was measured from vertical axis by using software Image J.

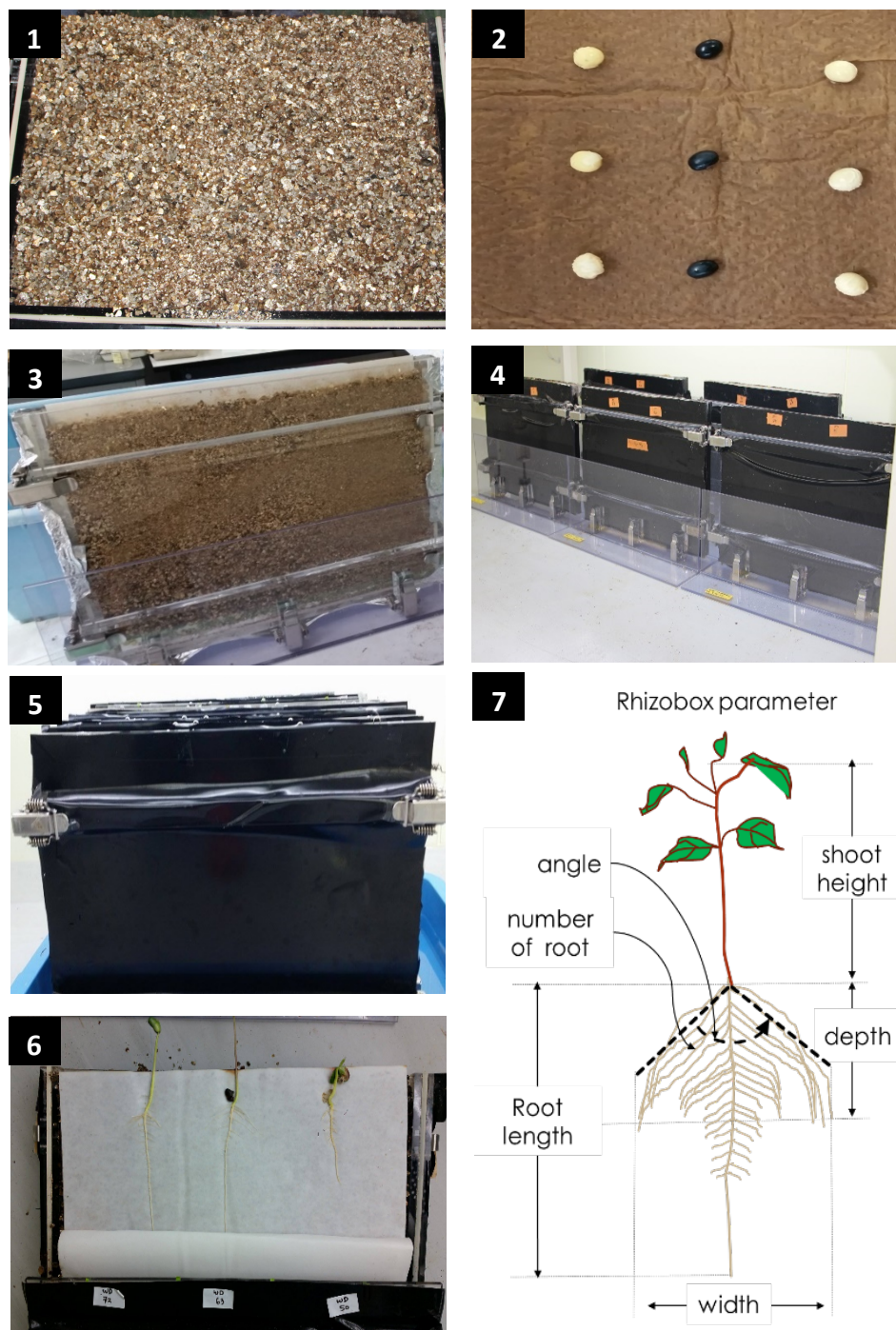


Fig. 5.1 Evaluating root traits in rhizobox system

5.3 Results

Values of the ranges, means and coefficient variations for five traits in PT-RILs are summarized in Table 5.1. The mean values of all traits PT-RILs population revealed a wide range of variation. The range of the root traits of PT-RILs generally exceed, those of the parents. The frequency distribution of the five traits in the PT-RILs are given in Fig. 5.2. For all traits, the PT-RILs population exhibited a broad and continuous distribution of values ranging between the values of the parental cultivar.

A total of nine putative QTLs were identified for the eight of the nine observed traits (Table 5.3). No significant QTLs for root growth angle was detected. Putative QTL for the root number was located on Chr.10 named qRN1. The phenotypic variation explained by each QTL range 14-21%. QTLs for root length was located on Chr. 8 and Chr.11, named qRL1 and qRL2, respectively. QTLs for the root width was located on Chr.9 and Chr.19 named qRW1 and qRW2 respectively. QTLs for root depth named qRD1, qRD2, qRD3 and qRD4 was located on Chr.9,10 and 19. The QTLs for root width (qRW1, qRW2) and Root depth (qRD1, qRD4) were located in the same position on Chr. 9 and Chr.19.

Table 5.1 Distribution of lateral root traits in the parental cultivars and the RIL population (Rhizobox)

traits	Parents		RILs		
	Peking	Tamahomare	Range	Mean	CV(%)
Number of root	15.50	27.00	7.50 – 37.50	21.38	28.95
Root length	17.25	17.25	7.75 – 23.00	16.49	20.25
Root growth angle	127.46	101.77	92.12 – 152.02	128.88	9.50
Root width	3.50	2.50	1.25 – 8.50	3.55	36.52
Root depth	1.50	2.00	0.80 – 4.75	2.15	39.27

Table 5.2 QTLs for root architecture traits of PT-RILs grown in Rhizobox

Trait	QTL	Interval	Chromosome (linkage group)	Peak Position ^a (cM)	LOD	Additive effect ^b	Variance (%)
No. of root	qRN1	BE801128-Satt592	10 (O)	69	2.95	-1.95	15.8%
Root length	qRL1	Satt215-Sat_129	8 (A2)	59	3.55	0.95	18.7%
	qRL2	Sat_272-Satt509	11 (B1)	27	2.61	0.85	14.1%
Root growth angle	Not detected						
Root width	qRW1	Sat_167-Sat_126	9 (K)	128	3.11	-0.95	16.6%
	qRW2	Sat_113-Sat_184	19 (L)	101	2.67	-0.35	14.4%
Root depth	qRD1	Sat_167-Sat_126	9 (K)	126	4.09	-0.57	21.2%
	qRD2	Satt358-Satt445	10 (O)	0	2.76	-0.54	14.8%
	qRD3	Satt592-Satt243	10 (O)	97	3.60	-0.24	18.9%
	qRD4	Sat_150-Sat_113	19 (L)	91	2.97	-0.28	15.9%

^a QTL position from the first marker.

^b A positive value indicates that “Peking”-type allele increases phenotypic value and a negative value indicates that the “Tamahomare”-type allele increases phenotypic value

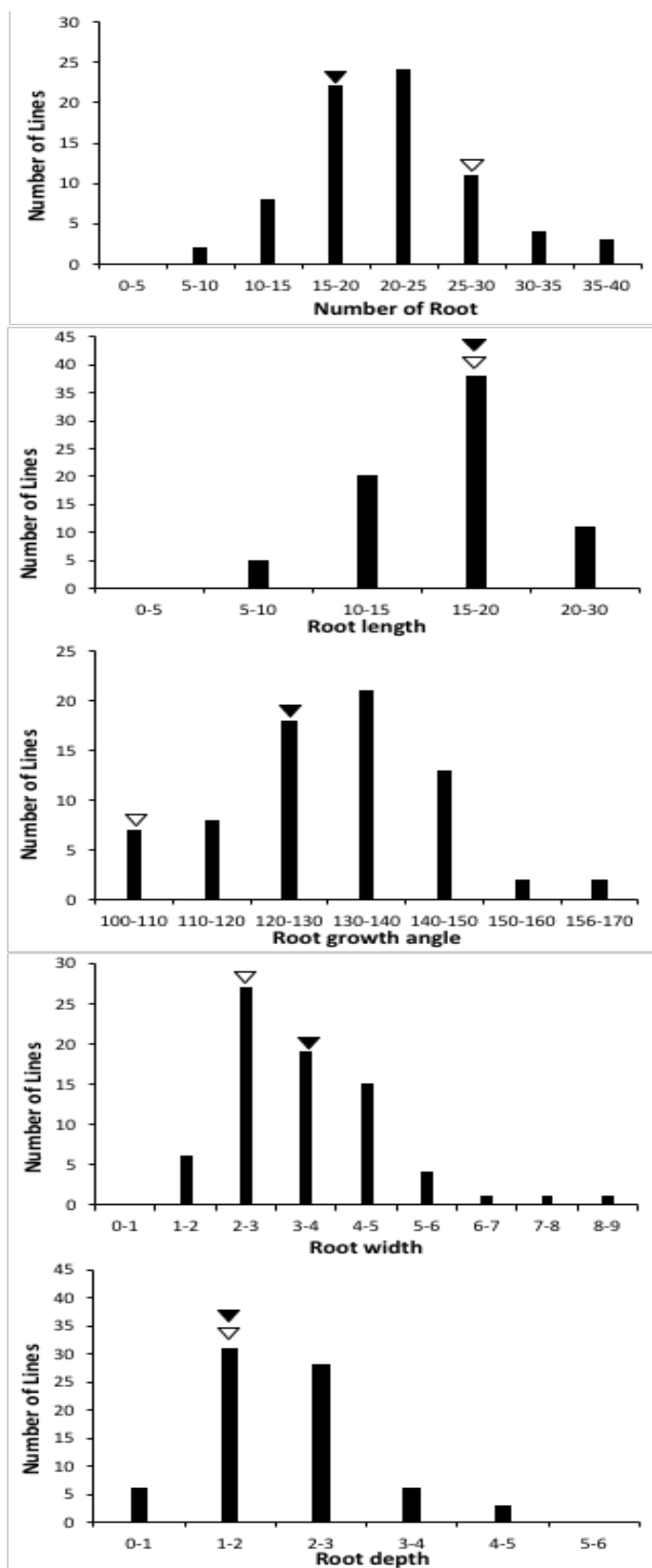


Fig. 5.2 Frequency distribution RILs Rhizobox (▼ Peking, ∇ Tamahomare)

5.4 Discussion

Many researchers who are working on root system architecture of crops exploited the seedling traits of the crops to save cost and time. In evaluating root traits at the seedling stage in this study, a rhizobox system combined with germination paper was used. Moistened vermiculite was used to supply water to the germination paper. Vermiculite can keep water, so as to supply moisture to the paper but water drained down in six days making the moisture content of upper part germination paper become lower than that of the lower part of the paper. This gradient of the moisture content very much affect the direction of roots elongation. When upper part of rhizobox was covered with polyethylene wrap, the growth angle of seedlings become large due to the high moisture content of the upper part of the germination paper (Fig. 5.3)

Mapping QTL is becoming increasingly essential in modern breeding programs through marker-assisted selection (MAS). In terms root surface area, two major QTL was located on Chr. 9 (Sat_167-Sat_126) and Chr.19 (Sat_113-Sat_184) for root width and root depth. Hwang *et al.* (2014) detected a QTL of root nodule weight was located on Chr.19. Meanwhile the QTL number of root and root depth on Chr.10. Liang *et al.* (2014) detected QTL for maximum root length and identified on Chr.11, Chr.19, Chr. 3, and Chr. 10. The QTL of root length was located on Chr.8. Brensha *et al.* (2012) detected several QTLs of root traits of soybean. Among them, a QTL of lateral root length and basal root thickness on Chr.18, and the QTL of root fresh/dry weight on Chr.3 and Chr.8. Among them I found the QTL of the root length at the overlapping region of the QTL of root fresh weight on Chr.8.

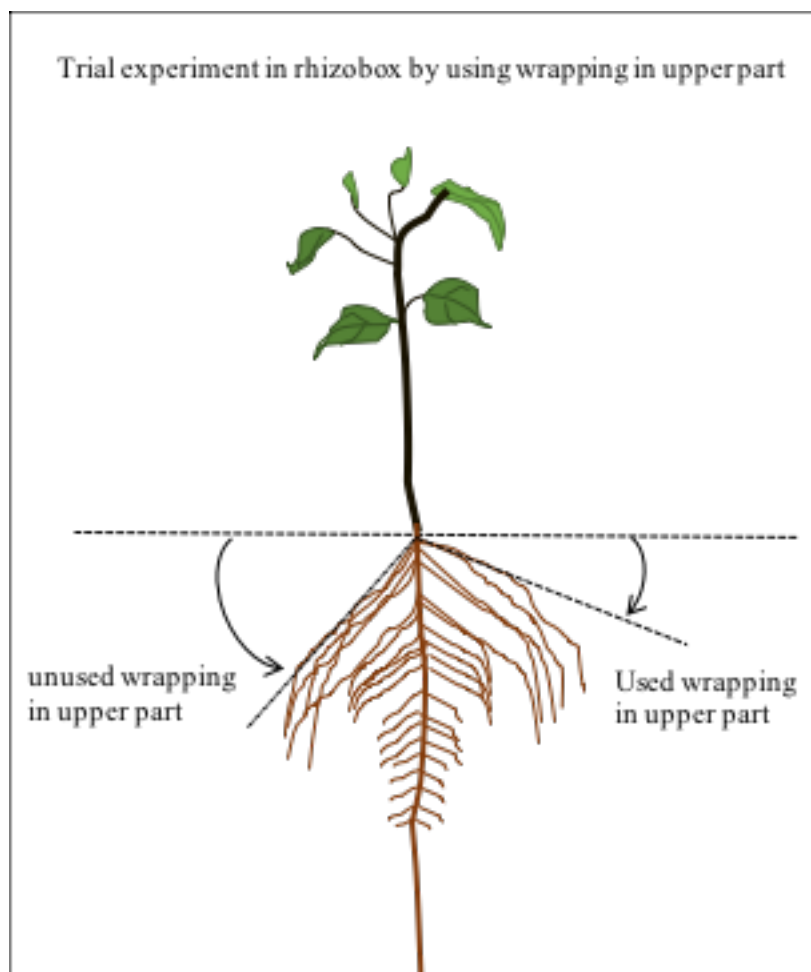


Fig. 5.3 The phenotyping of root growth angle due to the different moisture content in upper part

In Chapter 3, QTL of total root number (qTN1) identified on Chr.8 is over lapped with QTL of root length in rhizobox experiment. In conclusion, root distribution in rhizobox method was difficult to evaluate due to the un even moisture distribution of the germination paper.

Chapter VI

Conclusions

Soybean root traits are defined by the primary root, the lateral roots, their densities, and their distributions. The primary root is established as a framework to explore the soil for nutrients and water, and is formed at the basal pole of the embryo. Lateral roots, which are responsible for increasing water and nutrient intake. Roots can be modified by environmental factors, including temperature, water stress, and the availability of nutrients such as nitrogen and phosphorus. The selection of deep-rooted plants may thus be considered a characteristic of drought-resistant plant development that contributes to water absorption from deeper areas. In contrast, in waterlogged conditions, it is beneficial for plants to have shallow roots to absorb oxygen from the surface in order to prevent a lack of oxygen.

In this study, soybean (*Glycine max*) world mini collection (GmWMC) and recombinant inbred lines originated from a cross between Peking and Tamahomare (PT-RILs) were used for the experiment. GmWMC was used to evaluate the genetic variation of root traits (Chapter 2 and 4). Recombinant inbred lines were used for QTL analysis of root traits (Chapter 3 and 5).

The combination of basket method and Kanuma soil could make the water content in the soil as even as possible. Under this condition, the genetic diversity in the ability of the shallow

root development was found to be greater than the genetic diversity in the ability of the deep root development. QTL analysis also revealed the most of the QTLs related to the shallow/intermediate root development. Unlike rice and wheat, genetic factors regulating the gravitropism could not be found in soybean.

In the field experiment in 2015, lines classified as S-type (shallow root dominant type) was found to be D type in 2016. Due to the higher water content in the surface region of the soil in 2016, shallow root development was distinctly restricted in 2016. This indicated that S-type line in 2015 can promote shallow root development in response to the dry condition of the soil. On the other hand, D-type lines in 2015 can restrict shallow root development even under the dry condition of the soil. Instead, they tend to develop deep root in various water condition of the soil. Thus, deep root development in soybean might be regulated by the hydrotropism rather than gravitropism.

A positive correlation was found between the D-root number in the field and deep root number in the greenhouse. This indicates that lines belong to Group I (D-root dominant type) have a higher potential to develop deep root in the response to the water content of the soil. But this potential is not directly related to the gravitropism of soybean. In contrast, lines belong to Group II (S-root dominant type) have a comparatively lower potential to develop a deep root in response to the water content of the soil. Root growth direction was very sensitive to the gradient of water content of the soil.

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Deviona

June, 2018

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