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Genetic analysis of seed dormancy QTLs in yardlong bean [*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* Sesquipedalis Group]

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Abstract Seed dormancy is one of the key trait as the result of domestication in yardlong bean [*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* Sesquipedalis Group]. Seed dormancy is important trait related to seed viability and seed germination. Yardlong bean has higher seed coat permeability and better germination than its wild ancester. Currently, plant breeders prefer to use wild type to improve plant cultivars due to a lot of hidden useful genes such as disease and insect resistance, and resistant to inappropriate environment. The research finding reported the genome regions where QTLs for seed dormancy-related traits in yardlong bean including water absorption of seed, electrolyte leaching of seed and seed germination. Water absorption of seed was controlled by three QTLs with phenotypic variance explained (PVE) of 6.45-11.53% and alleles from yardlong bean increased water absorption. Electrolyte leaching of seeds was controlled by two QTLs with PVE of 8.71 and 9.83 % and alleles from yardlong bean increased the electrolyte leaching. QTL was not detected by composite interval mapping for seed germination, but fourteen markers were associated with seed germination which detected by single marker analysis. Thus, the result is useful for marker-assisted selection development and further *Vigna* breeding program.

Keywords: Yardlong bean, Seed dormancy, Quantitative trait loci

Introduction

Yardlong bean [*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* Sesquipedalis Group] was selected and developed from wild cowpea (*V. unguiculata* spp. *spontanea*). Yardlong bean and wild cowpea has been distinguishing the difference in morphological and physiological traits as the results of selection among domestication process, especially loss of seed dormancy is one of the most interesting character. Yardlong bean has higher seed coat permeability and better germination than wild cowpea. Seed

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dormancy is important trait related to seed viability and seed germination. Plant cultivar with high seed germination or high seed coat permeability has an impact on planting efficiency. High seed dormancy in *Vigna* crop need dormancy-break treatments before planting such as soaking in hot water, seed scarification, to allow water go through the seed coat and stimulate metabolism, therefore the cost for planting is increased and planting process is more delicate. However, seed dormancy has an advantage for seed sprout prevention while pod dehiscence under high humidity or wet condition in harvesting season. Genetic controlling seed dormancy have been reported in some studies. Seed coat of dormant seed in mungbean had higher lignin content, higher silica content and more fiber than normal seed (Rodringuez and Mendoza, 1990). Hard-seededness is one of seed dormancy character that controlled by one major gene (Imrie *et al.,* 1988; Inderjit *et al.,* 2005), or only minor genes (Humphry *et al.,* 2005). Unlike common bean, azuki bean and rice bean, hard-seededness was controlled by multiple genes (Koinange *et al.,* 1996; Isemura *et al.,* 2007, 2010). Currently, plant breeders prefer to use wild type to improve plant cultivars due to lot of hidden useful genes such as disease and insect resistance, resistant to inappropriate environment (Tanksley and McCough, 1997). Nevertheless, the problem of using wild type is undesirable traits such as climbing, pod dehiscence, seed dormancy might link with desirable traits (linkage drag). The objective of this study was to identify QTL controlling seed dormancy-related traits, which would be useful for marker-assisted selection development and further *Vigna* breeding program.

Materials and methods

950 BC_1F_2 plants which derived from a cross between yardlong bean (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* Sesquipedalis Group) accession JP81610 and wild cowpea (*Vigna unguiculata* subsp. *unguiculata* var. *spontanea*; Andersson and de Vicente 2010) accession JP89083 were used in this study. JP89083 was pollinated to JP81610 to produce F_1 hybrid plants. An F_1 plant was crossed as female parent with JP81610 to develop the BC_1F_1 population. Then, each of BC_1F_1 plant was self-pollinated to produce BC_1F_2 population. A total of 950 BC_1F_2 plants were evaluated three traits related to seed dormancy including seed germination, electrolyte leaching testing and water absorption of seed. Seed germination: 30 BC_1F_3 seeds of each 950 BC_1F_2 plants and parents were used. Seed germination was conducted using between paper methods. In brief, Total of 30 seeds were placed in rows on the wet towel, then placed a second wet towel to the first wet towel and rolled up the two towels with the seeds in-between and placed in a sealed container. The

counted seedlings were shoots and roots as viable seeds for the germination rate. Percentage of germination was calculated as follows: percentage of germination = [(Number of germinated seed*100)/Total number of tested seeds]. Electrolyte leaching testing: 30 BC_1F_3 seeds of each 950 BC_1F_2 plants and parents were soaked to 200 ml of deionized water for 24 hrs, then, stirred and poured the solution to beaker. The conductivity value of each plant was measured by using conductivity meter as procedure by Singh *et al.,* 2008. Water absorption of seeds: 30 BC_1F_3 seeds of each 950 BC_1F_2 plant and parents were soaked to the distilled water for 24 hrs. The weight before and after soaking were recorded. Ability of water absorption of seeds were done as seed weight to increase after soaking to the water (water absorption rate = weight increase (mg) per original seed weight). Mean, standard deviation and the frequency distribution of phenotypes in BC_1F_2 population were examined for each trait.

Genetic linkage map of yardlong bean and quantitative trait loci (QTL) analysis: The genetic linkage map of yardlong bean developed by Kongjaimun *et al.* (2012) was used for QTL analysis of seed dormancy-related traits. In brief, the map was constructed using 190 BC_1F_1 population from the cross between yardlong bean accession JP81610 and wild cowpea accession TVnu457 (or named JP89083 in this study). It consisted 226 simple sequence repeat (SSR) markers developed from cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*), azuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi), and mungbean (*Vigna radiata* (L.) Wilczek). QTL analysis was done by two methods, viz. single marker analysis (Kearsey and Pooni, 1956) and composite interval mapping (CIM; Zeng 1994). Single marker analysis was done by regression analysis at P = 0.001 using R-program 2.10.0 (R Development Core Team 2018). CIM was conducted to locate QTL positions onto linkage maps using software WinQTLCartographer version 2.5 (Wang *et al.,* 2007). Model 6 with control marker number and 'window size' of 5 and 10, respectively were used. Walk speed of 2 cM and forward regression were applied. Genome-wide significant LOD threshold for each trait was calculated by a 3,000-run of permutation test at P = 0.05. The experiment was carried out during year 2017-2018 at Laboratory of Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Thailand.

Results

Variation of seed dormancy-related traits in parents and BC_1F_2 population

Three of seed dormancy-related traits were investigated; seed germination, electrolyte leaching, and water absorption**.** The mean percentage of seed

germination of JP81610 and JP89083 were 100 and 0, respectively**.** Percentage of seed germination in BC_1F_2 population showed continuous variation with the mean, minimum and maximum were 22**.**4±16**.**2, 2**.**2, and 80**.**0, respectively (Figure 1). The mean electrolyte leaching of JP81610 and TVnu457 were 888**.**3 and 17**.**0 dS/m, respectively**.** Electrolyte leaching in BC_1F_2 population showed continuous variation with the mean, minimum and maximum were 717**.**8±162**.**4, 272**.**8, and 1070**.**7 dS/m, respectively (Figure 1). The mean water absorption of JP81610 and TVnu457 were 7**.**19 and 0**.**33 g, respectively**.** Water absorption in BC_1F_2 population showed continuous variation with the mean, minimum and maximum were 3**.**6±0**.**8, 1**.**6, and 5**.**9 g, respectively (Figure 1). As all of three traits showed continuous variation suggesting polygenic inheritance of the trait**.**

QTLs for seed dormancy – related traits

Seed germination: the result of single marker analysis for seed germination in the BC_1F_2 population was shown as Table 1. Fourteen markers were associated with seed germination located on LG02, LG03, and LG09 with explained 2**.**80 to 6**.**98% of the trait variation**.** While no QTL detected using composite interval mapping analysis**.**

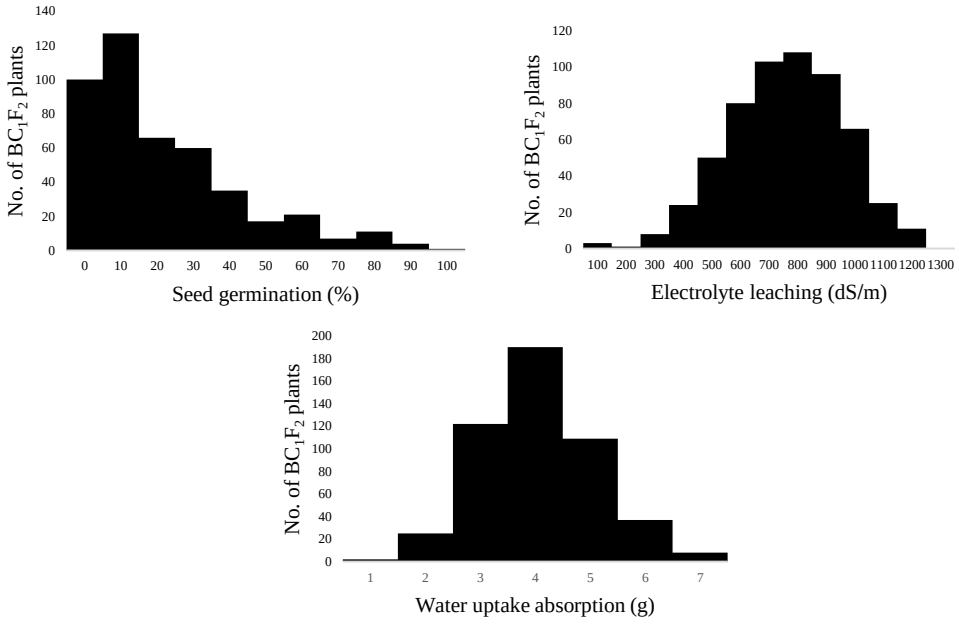

Figure 1. Frequency distribution of seed germination, electrolyte leaching and water absorbtion in BC_1F_2 population

Electrolyte leaching**:** the BC_1F_2 population, single maker analysis identified fourty-two markers associated with electrolyte leaching of seeds resulted to 3**.**55 to 8**.**68 % of the trait variation, located on LG01, LG02, LG03, LG06, LG07, and LG10 (Table 2). Composite interval mapping detected two QTLs, one each on LGs 1 and 3 is shown in Table 4**.** Phenotypic variance explained (PVE) by two QTLs were 9**.**83 and 8**.**71%, and the total variation was 18**.**54%. The additive effects of two QTLs were 102**.**91 and 95**.**95 which alleles of the yardlong bean increased electrolyte leaching.

Table 1. SSR markers associated with seed germination (%) in BC_1F_2 population as revealed by single marker analysis

LG[1/]	**Marker order**	**Marker name**	**P**	**R**[22/]	
02	2	cp01354	0**.**0170	4**.**49	*[3/]
02	3	VR0078	0**.**0248	3**.**84	*
02	5	cp08227	0**.**0047	6**.**98	**
02	6	cp11079	0**.**0454	3**.**36	*
03	17	cp03862	0**.**0444	2**.**80	*
03	18	cp03451	0**.**0205	4**.**34	*
09	6	cp06236	0**.**0204	3**.**86	*
09	7	CEDG304	0**.**0331	3**.**45	*
09	8	cp04908	0**.**0233	4**.**20	*
09	9	cp08239	0**.**0209	4**.**20	*
09	10	CEDG024	0**.**0180	4**.**30	*
09	11	cp01918	0**.**0080	5**.**31	**
09	12	cp02320	0**.**0090	5**.**32	**
09	13	cp02383	0**.**0112	5**.**52	*

[1/]Linkage group;
[2/]Coefficient of determination;
[3/]*, **, significant at P<0**.**05 and 0**.**01, respectively.

Water absorption: the result of single marker analysis for water absorption of seeds in the BC_1F_2 population was shown in Table 3. Fourty-six markers were associated with water absorption located on LG01, LG02, LG03, LG04 and LG07 with resulted to 1**.**78 to 14**.**40 % of the trait variation**.** Composite interval mapping detected three QTLs, one each on LGs 1, 3 and 4 (Table 4). Phenotypic variance explained (PVE) by the QTLs varied from 6**.**45 to 11**.**53%, and the total variation was 29**.**16%**.** The additive effects of these

QTLs varied between 0.42 and 0.56. All QTLs, alleles of the yardlong bean increased water absorption.

Table 2. SSR markers associated with electrolyte leaching of seed in BC_1F_2 population as revealed by single marker analysis

LG [1/]	Marker order	Marker name	P	R^2 [2/]		LG	Marker order	Marker name	P	R^2	
			0.00	6.6	***				0.00	6.5	**
01	8	cp08585	09	2	[3/]	02	16	cp04558	07	1	*
			0.00	7.0					0.00	7.1	**
01	9	cp07791	03	7	***	02	17	cp00500	07	9	*
			0.00	8.6					0.00	4.4	
01	10	cp02909	00	8	***	02	18	cp08299	68	4	**
			0.00	7.8					0.00	5.3	
01	11	cp08092	02	2	***	02	19	cp10889	52	6	**
			0.00	7.8					0.00	3.6	
01	12	cp02661	02	9	***	02	20	CEDG275	60	4	**
			0.00	3.5					0.00	5.2	
01	13	cp10384	23	5	**	02	21	CEDG207	94	9	**
			0.00	4.4				DMBSSR	0.00	6.4	
01	14	cp10941	22	6	**	03	1	228	11	4	**
			0.00	4.4					0.00	6.0	
01	15	cp05762	57	2	**	03	2	cp08179	12	7	**
			0.00	5.5					0.00	6.1	
01	16	cp06328	46	6	**	03	3	cp03846	22	5	**
			0.00	4.0					0.00	3.8	
01	17	cp07770	91	1	**	03	4	cp00361	70	2	**
			0.00	4.7					0.00	4.0	
01	18	CEDG288	91	4	**	03	5	CEDG205	55	9	**
			0.00	5.2					0.00	7.1	**
02	5	cp08227	26	6	**	03	6	CEDG208	04	1	*
			0.00	4.8					0.00	6.4	**
02	6	cp11079	34	4	**	03	7	CEDG186	06	7	*
			0.00	5.5					0.00	6.5	**
02	7	cp02612	14	3	**	03	8	CEDG159	06	0	*
			0.00	4.3					0.00	6.0	
02	8	cp08499	61	9	**	03	9	cp04508	15	5	**
			0.00	5.8					0.00	3.4	
02	9	cp01582	11	6	**	03	11	VR0086	86	6	**
			0.00	5.3					0.00	4.3	
02	10	cp03673	29	1	**	03	12	cp08786	78	9	**
			0.00	5.2					0.00	4.6	
02	11	cp03715	29	2	**	03	13	VR0140	28	4	**
			0.00	6.9					0.00	5.6	
02	13	cp00234	07	9	***	06	9	CEDG034	26	7	**
			0.00	6.6					0.01	4.9	
02	14	cp00801	07	7	***	07	3	cp10285	00	0	**
			0.00	6.0					0.00	4.4	
02	15	cp03494	07	3	***	10	18	CEDG150	71	2	**

[1/]Linkage group;
[2/]Coefficient of determination;
[3/]*,**,*** significant at P <0.05, 0.01, and 0.001, respectively.

Table 3. SSR markers associated with water absorbtion of seed in BC_1F_2 population as revealed by single marker analysis

LG [1/]	Marker order	Marker name	P	R^{2} [2/]		LG	Marker order	Marker name	P	R^2	
			0.00	6.1	**				0.00	4.9	
01	9	cp07791	54	2	[3/]	04	2	cp08304	16	5	**
			0.00	7.6	**				0.00	6.6	
01	10	cp02909	07	7	*	04	3	cp00455	16	2	**
			0.00	5.5					0.00	7.1	
01	11	cp08092	36	6	**	04	4	cp00972	14	1	**
			0.00	5.7					0.00	5.5	
01	12	cp02661	36	1	**	04	5	cp02569	14	6	**
			0.00	1.7					0.00	6.1	
01	13	cp10384	83	8	**	04	6	cp03981	14	7	**
			0.00	5.7					0.00	4.6	
01	16	cp06328	52	8	**	04	7	cp03876	21	8	**
			0.00	5.0					0.00	4.8	
01	17	cp07770	70	6	**	04	9	cp00106	67	8	**
			0.00	4.4					0.00	4.8	
01	18	CEDG288	70	0	**	04	10	cp10271	67	5	**
			0.00	4.7					0.00	6.1	
01	19	cp04570	52	2	**	04	11	CEDG107	12	8	**
			0.00	6.2					0.00	8.7	**
01	24	cp01826	27	2	**	04	12	cp00666	02	3	*
			0.00	5.1					0.00	11.	**
01	25	cp08288	52	3	**	04	13	cp01037	00	35	*
			0.00	4.6					0.00	11.	**
01	26	CEDG090	85	4	**	04	14	cp09102	00	51	*
			0.00	5.1					0.00	10.	**
02	1	CEDG293	89	1	**	04	15	cp08023	01	00	*
			0.00	5.2					0.00	9.8	**
02	2	cp01354	55	2	**	04	16	CEDG062	00	6	*
			0.00	3.8					0.00	11.	**
02	3	VR0078	79	0	**	04	17	cp04986	00	49	*
			0.00	4.8					0.00	7.6	**
02	5	cp08227	71	4	**	04	18	cp03825	02	3	*
		DMBSSR	0.00	13.	**				0.00	3.7	
03	1	228	00	10	*	04	19	CEDG127	95	4	**
			0.00	13.	**			DMBSSR	0.00	5.5	
03	2	cp08179	00	17	*	07	11	230	38	0	**
			0.00	14.	**				0.00	6.1	
03	3	cp03846	00	40	*	07	12	cp00806	14	0	**
			0.00	9.8	**				0.00	8.7	**
03	4	cp00361	01	0	*	07	13	CEDG111	01	4	*
			0.00	5.4					0.00	7.9	**
03	5	CEDG205	32	9	**	07	14	cp06427	03	8	*
			0.00	7.6	**				0.00	5.4	
03	6	CEDG208	02	0	*	07	15	cp05517	42	3	**
			0.00	6.2					0.00	5.6	
04	1	cp05679	16	2	**	07	16	cp07863	42	6	**

[1/]Linkage group;
[2/]Coefficient of determination;
[3/]*,**,***significant at P <0.05, 0.01, and 0.001, respectively.

Table 4. Quantitative trait loci detected for seed dormancy-related trait in the BC_1F_2 population by composite interval mapping

Trait	QTL name	LG [1/]	Marker interval	Position [2/]	LOD score	Additive effect	PVE (%) [3/]
Electrolyte leaching	*Ell1.1*+	01	cp02661-cp10384	63.55	20.04	102.91	9.83
	Ell3.1+	03	CEDG205-CEDG208	20.39	17.51	95.95	8.71
Water absorption	*Waa1.1* + *Waa3.1* + *Waa4.1* +	01	cp01826-cp08288	100.08	14.69	0.42	6.45
		03	DMBSSR228-cp08179	4.44	25.00	0.56	11.53
		04	cp01037-cp09102	23.46	25.47	0.56	11.18

[1/]Linkage group;
[2/]Position on the linkage group;
[3/]Percentage of phenotypic variance explained by the QTL.

Discussion

Seed dormancy is a major adaptive trait in plant, facilitates the survival of plant and provides the resistance to preharvest sprouting in Legume crops. The dormancy of seed could be evaluated from several characteristics such as seed germination, seed coat permeability, seed water absorption, and electrolyte leaching. Seed coat permeability is one of the first steps in breaking dormancy and beginning seed germination. Dormancy and viability can be maintained for long periods of seeds that showed water impermeability (Rolston, 1978). In soybean, many reports showed that there was very high degree of positive association of seed quality with slow rate of water absorption (Chachalis and Smith, 2000; Kuo, 1989). During water imbibition, seed leaches out some solutes, which included sugars, amino acids, most importantly electrolytes. Slow leaching of electrolytes is positively associated with seed vigor. Electrolyte leaching test has been carried out in soybean for testing the vigor of the seeds (Oliveira *et al.*, 1984; Yaklish *et al.*, 1979).

In this study, BC_1F_2 population was used for QTLs identification of seed dormancy-related traits including seed germination, electrolyte leaching and water absorption of seed. Electrolyte leaching and water absorption of seed were controlled by minor genes corresponded with the report of Humphry *et al.* (2005) that hard-seededness which is one of seed dormancy character controlled by only minor gene. Unlike common bean, azuki bean and rice bean,

hard- seededness were controlled by multiple genes (Koinange *et al.,* 1996; Isemura *et al.,* 2007, 2010) and by one major gene (Imrie *et al.,* 1988; Inderjit *et al.,* 2005).

Table 5. The QTLs for seed dormancy-related traits in *Vigna* species

Crop	Scientific name	Seed dormancy character	Number of QTLs	LG	PVE (%)	Reference
Azuki bean	*Vigna angularis*	Seed germination	5	1, 2, 4 and 9	*	Isemura *et al.* 2007
		Seed coat permeability	5	1, 2, 4 and 9	*	
Azuki bean	*Vigna angularis*	Seed germination	2	1	6.1-8.8	Kaga *et al.* 2008
		Seed coat permeability	2	1 and 6	9.9-25.9	
Rice bean	*Vigna umbellata*	Seed water absorption	5	2, 3, 4, 8 and 10	4.3 – 25.1	Isemura *et al.* 2012
Mungbean	*Vigna radiata*	Seed water absorption	4	1, 2, 3 and 4	4.6– 33.7%	Isemura *et al.* 2012
Cowpea	*Vigna unguiculata* ssp. *unguiculata*	Seed germination	1	1	12.9	Andargie *et al.* 2014
		Seed coat permeability	3	2 and 10	9.9-12.2	

* Explained in difference criteria.

Comparison between the locations of seed dormancy-related traits QTLs between yardlong bean and closely *Vigna* species based on common markers reported by Kongjaimun *et al.* (2012) as shown in Table 5. The QTLs of seed dormancy-related traits in this study, *Ell1.1*+ and *Waa1.1*+ were found in LG1 which was the same LG of seed germination QTLs and seed coat permeability QTLs of azuki bean and rice bean, as well as seed water absorption QTL in mungbean were existed. *Ell3.1*+ and *Waa3.1*+ were found in LG3, which was

the same LG of seed water absorption QTL in rice bean and seed water absorption QTL in mungbean were detected. *Waa4.1+* was found in LG4, which was the same LG of seed water absorption QTL in rice bean and seed water absorption QTL in mungbean were found. When compare the QTLs controlled seed dormancy-related trait of yardlong bean (in this study) and cowpea, the same species with yardlong bean, showed that three QTLs controlled this trait with only minor effect in cowpea corresponded to this study. In conclusion, this study is identified the genome regions where QTLs for seed dormancy-related traits in yardlong bean. Thus, the result would possible be useful for marker-assisted selection development and *Vigna* breeding program.

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