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## Research Paper

# Variability of arginine content and yield components in Valencia peanut germplasm

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Peanut seeds are rich in arginine, an amino acid that has several positive effects on human health. Establishing the genetic variability of arginine content in peanut will be useful for breeding programs that have high arginine as one of their goals. The objective of this study was to evaluate the variation of arginine content, pods/plant, seeds/pod, seed weight, and yield in Valencia peanut germplasm. One hundred and thirty peanut genotypes were grown under field condition for two years. A randomized complete block design with three replications was used for this study. Arginine content was analyzed in peanut seeds at harvest using spectrophotometry. Yield and yield components were recorded for each genotype. Significant differences in arginine content and yield components were found in the tested Valencia peanut germplasm. Arginine content ranged from 8.68–23.35 µg/g seed. Kremena was the best overall genotype of high arginine content, number of pods/plant, 100 seed weight and pod yield.

**Key Words:** groundnut, *Arachis hypogaea*, amino acids, diversity, agronomic traits.

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## Introduction

Peanut (*Arachis hypogaea* L.) is a legume crop grown mostly in the tropics and semi-arid tropics. The cultivated peanut is an allotetraploid species with a chromosome number of  $2n = 40$  (Seijo *et al.* 2007). Peanut seed is a rich source of protein, fatty acids, vitamins, fiber, and phytochemicals (Win *et al.* 2011). Arginine, an important amino acid with several health benefits, is found at higher concentrations in peanut seeds than in many other nut crops (Venkatachalam and Sathe 2006). Consumption of arginine at the level of 3–6 g/day has been found to improve the cardiovascular system, reduce intestinal permeability and activate the immune system (Field *et al.* 2000).

Defining the genetic variability in economically important traits is an important step in breeding programs. Information on the variation in arginine content in peanut germplasm is limited. To the best of our knowledge, only one

paper, arginine content in 108 accessions of the core collection of peanut germplasm has been published. In this study, arginine contents in peanut seeds ranged from 15–43 µg/g fresh weight (Dean *et al.* 2009). Amino acid profiles were studied in 17 peanut genotypes and the arginine content ranged from 8.98–11.2 µg/g (Anderson *et al.* 1998).

Amino acids in cotyledons of peanut genotypes were higher in Virginia and Valencia types than in other market types, with the arginine content of Valencia types ranging from 11.98–13.01% of total amino acid (Hovis *et al.* 1982). The range of protein content in peanut germplasm is also large, as seen in a 152 accession study of peanut genotypes in China, where total proteins ranged from 18.93% to 30.22% (Wang *et al.* 2011). From previous study, a large population which study about the variation of arginine content was reported only one (Dean *et al.* 2009). However, this study was not specific to the Valencia type of peanut. So the objective of the present study was to evaluate arginine content and yield components of a large, diverse group of Valencia peanut accessions for the purpose of identifying lines that would be useful in a breeding programs targeting high arginine content and pod yield.

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## Materials and Methods

### Plant materials and experimental design

One hundred and twenty eight accessions from the peanut “core” collection for Valencia, one Spanish (KKU 40) and one Virginia (KKU 60) peanut genotype were used for this study. These accessions were maintained in the genebanks of New Mexico State University, US, the International Crops Research Institute for the Semi-Arid-Tropics (ICRISAT) and Khon Kaen University, Thailand. Two varieties (KS 1 and KS 2) from Thailand were also used as Valencia peanut checks. The experiment was conducted at the Field Crop Research Station, Khon Kean University, Thailand during February–June 2012 and repeated during February–June 2013. A randomized completed block design (RCBD) with three replications was used. Plot size was 2 × 5 m and the spacing between row and plant was 50 and 20 cm, respectively.

### Crop management

The soil was plowed three times before planting. Sprinkler irrigation system was installed before planting. The seeds of each genotype were treated with Captan (3a, 4, 7, 7 a-tetrahydro-2-((trichloromethyl) thio)-1H-isoindole) at the rate of 5 g/kg seeds for controlling *Aspergillus niger*. At planting, three seeds per hill were sown and pre-emergent weed control herbicide (Alachor) was applied immediately after sowing. Rhizobium (mixture of strains THA 201 and THA 205; Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand) was applied in a liquid mixture on the day after planting. Seedlings were thinned to one plant per hill at 14 days after planting (DAP). Fertilizer (0, 56.25, 37.5 kg/ha of N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O, respectively) was applied 15 DAP. Gypsum (CaSO<sub>4</sub>) was applied at 312 kg/ha 40 DAP. Fields were scouted weekly for insects, weeds and diseases and treated as needed. Sprinkler irrigation was applied at two-day intervals to maintain soil moisture close to field capacity.

### Yield and yield components

At harvest time, total pods of each genotype were harvested from a 2 × 5 m<sup>2</sup>, and air-dried until reaching 8% moisture content and total pod yield was recorded. Twenty plants were randomly selected and the number of pods per

plant, the number of seeds per pod and 100 seed weight of each genotype were recorded.

### Arginine content analysis

Seeds from each plot were sub-divided to get 150 g. The 150 g samples of seeds were ground in a blender and a 10 g sub-sample was further ground using a mortar and a pestle. The sub-samples were then extracted using 30 ml of distilled water and filtered through No. 1 Wattman papers. To 5 ml of each sample, 6 M NaCl was added and heated at 90 ± 2°C for 90 minutes for protein precipitation. After heating, the samples were centrifuged for 10 minutes at 5,000 rpm and a 1 ml supernatant was transferred to a micro-tube and set aside at 4°C until analysis. Free arginine content was then analyzed using the Sakaguchi reaction as follows: to the sub samples, 0.1% α-naphthol (1 ml), 10% KOH (1 ml), 5% urea (1 ml) and 0.4% K-hypobromite (2 ml) were added (Basha *et al.* 1976), the reactions were incubated at room temperature for 20 min and then measured for absorbance at 520 nm using a spectrophotometer.

### Statistical analysis

Data were analyzed as a randomized complete block design using Statistix 8 (Statistix8 2003). The correlations between arginine content and yield and yield components were also analyzed using Statistix 8. A data matrix of the 130 accessions was constructed using the mean of yield and yield component characters and arginine content. The cluster analysis based on Ward’s method and squared Euclidian distance was performed and the dendrogram was constructed. All calculations were performed in SAS 6.12 software (SAS 2001).

## Results

### Combined analysis of variance

The year was significantly different ( $P \leq 0.05$ ) for number of pods per plant and pod yield, whereas genotypes were significantly different for all traits (**Table 1**). The interactions between year and genotype were significant for most traits except for number of pods per plant.

### Arginine, yield and yield components

The peanut genotypes with highest and lowest arginine content and agronomic traits are shown in **Table 2**. Arginine

**Table 1.** Mean squares from combined analysis of arginine content (Arg; µg/g), number of pods per plant (P/plant), number of seeds per pod (S/pod), 100 seed weight (100 SW; g) and pod yield (g/plant) of 130 peanut accessions across two years (2012 and 2013)

Source	df	Arg	P/plant	S/pod	100 SW	Pod yield
Year (Y)	1	0.2 ns	36550.2**	0.9 ns	17782.8 ns	42763.9**
Y*REP	4	32.3	216.0	0.1	53	101.1
Genotype (G)	129	50.4**	114.9**	0.6**	140.2**	51.2**
Y*G	129	25.1**	43.6ns	0.1**	28.9**	37.3**
Error	516	3.8	36.1	0.1	17.0	20.8
Total	779					
CV (%)		12.0	25.8	12.7	10.4	25.1

ns = not significant; \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ .

**Table 2.** Means for arginine content (Arg: µg/g seed), number of pods per plant (P/plant), number of seeds per pod (S/pod), 100 seed weight (100 SW: g) and pod yield (g/plant) of the ten highest and lowest genotypes based on arginine content in 2012 and 2013. Values with different letters within the same column in each group (high and low arginine groups) are significantly different at  $P \leq 0.05$  by DMRT

Entry	2012					2013							
	Accession	Arg	Pods/plant	Seeds/pod	100 SW	Yield	Entry	Accession	Arg	Pods/plant	Seeds/pod	100 SW	Yield
Ten highest varieties for arginine content													
82	New BG	25.90	33.41 B	2.18 BC	45.30 CD	24.78	78	DC2120 (S3873)	25.02	13.66 AB	2.35 ABC	38.60 BC	14.10 AB
83	S 3881	25.69	25.80 BC	2.06 C	53.08 BC	19.13	85	Kremena	24.02	17.20 AB	1.90 C	47.36A	15.43 A
80	S-3653	23.10	27.80 BC	2.56 AB	45.53 CD	25.31	103	ICG 5475	23.17	20.26 AB	2.11 BC	32.05 C	7.24 CD
85	Kremena	22.68	45.46 A	1.98 C	57.05 ABC	28.95	52	PI493405	22.86	14.80 AB	2.33 ABC	32.95 C	6.97 CD
18	PI536307	22.47	23.40 BC	2.95 A	52.58 BC	24.62	69	PI493630	22.60	12.86 B	2.15 BC	33.87 C	8.84 BCD
9	PI493381	22.46	25.93BC	2.93 A	47.28 CD	25.63	3	PI475921	22.35	12.86 B	1.95 C	36.86 BC	5.00 D
105	ICG 6022	22.28	19.26 C	3.00 A	63.66 AB	29.96	105	ICG 6022	22.24	11.13 B	2.73 A	44.60 AB	11.94 ABC
63	PI493565	22.07	28.26 BC	2.80 A	35.81 D	20.26	45	PI493325	22.07	22.86 A	2.45 ABC	35.36 C	8.87 BCD
129	KKU 60	21.84	31.26 B	1.80 C	65.81 A	33.76	44	PI468208	21.78	23.06 A	2.10 BC	38.26 BC	15.10 AB
53	PI493415	21.71	26.06 BC	2.63 AB	54.99 AB	22.18	4	PI475925	21.65	12.86 B	2.55 AB	34.71 C	10.41 ABCD
Ten lowest varieties for arginine content													
47	PI493340	10.70	27.53	2.60 B	41.83 A	27.33	106	ICG 6201	10.93	13.80	2.85 A	34.61 AB	7.50
41	PI576604	10.61	22.20	1.80 C	44.03 A	27.86	35	PI493518	10.66	15.46	2.70 AB	32.37 AB	10.84
2	PI475913	10.26	40.93	3.20 A	32.48 B	25.57	109	ICG 7181	10.59	12.13	2.70 AB	38.69 A	9.31
106	ICG 6201	10.23	23.73	2.60 B	43.37 A	25.62	114	ICG 10474	10.48	16.20	2.83 A	39.08 A	10.73
119	ICG 13856	10.14	34.00	2.40 B	41.62 A	27.34	72	PI493688	10.45	17.86	2.41 AB	28.64 B	7.38
98	ICG 2738	10.01	34.86	2.50 B	40.01 A	26.04	57	PI493458	10.23	11.40	2.83 A	28.54 B	6.86
60	PI493484	9.91	28.66	2.50 B	42.53 A	26.03	62	PI493523	10.02	16.20	2.56 AB	29.65 B	8.88
76	PI494019	9.87	31.93	2.50 B	45.89 A	27.72	73	PI493810	9.64	17.60	2.16 B	30.54B	11.74
26	PI338337	9.85	34.33	2.70 B	44.71 A	24.63	55	PI493446	8.51	18.33	2.45 AB	38.91 A	11.60
55	PI493446	9.35	30.46	2.20 BC	43.35 A	24.81	60	PI493484	7.44	17.60	2.90 A	34.07 AB	11.99
<b>Min</b>		<b>9.35</b>	<b>15</b>	<b>1.78</b>	<b>32.48</b>	<b>11.71</b>			<b>7.44</b>	<b>6.40</b>	<b>1.20</b>	<b>24.47</b>	<b>5.00</b>
<b>Max</b>		<b>25.90</b>	<b>51.13</b>	<b>3.23</b>	<b>65.80</b>	<b>37.74</b>			<b>25.02</b>	<b>30.26</b>	<b>3.45</b>	<b>52.16</b>	<b>19.26</b>
<b>Mean</b>		<b>16.13</b>	<b>30.17</b>	<b>2.51</b>	<b>44.51</b>	<b>25.57</b>			<b>16.10</b>	<b>16.15</b>	<b>2.58</b>	<b>34.96</b>	<b>10.77</b>

contents ranged from 9.35–25.90 µg/g seed in 2012 and 7.44–25.02 µg/g seed in 2013. The top five accessions for arginine content in 2012 were New BG, S3881, S3653, Kremena and PI 536307 with arginine contents being 25.90, 25.69, 23.10, 22.68 and 22.47 µg/g, respectively, whereas the five highest accessions in 2013 were DC 2120 (S3873), Kremena, ICG 5475, PI 493405 and PI 493630 with arginine contents being 25.02, 24.02, 23.17, 22.86 and 22.60 µg/g, respectively.

Numbers of pods per plant ranged from 15.0–51.13 pods/plant in 2012 and 6.4–30.26 in 2013, whereas the numbers of seeds per pod ranged from 1.78–3.23 to 1.20–3.45 seeds/pod in 2012 and 2013, respectively. One hundred seed weight ranged from 32.48–65.80 g in the first year and 24.47–52.16 g in the second year and pod yield (g/plant) ranged from 11.71–37.74 and 5.00–19.26 in the first and second year, respectively.

### Correlation between arginine content and agronomic traits

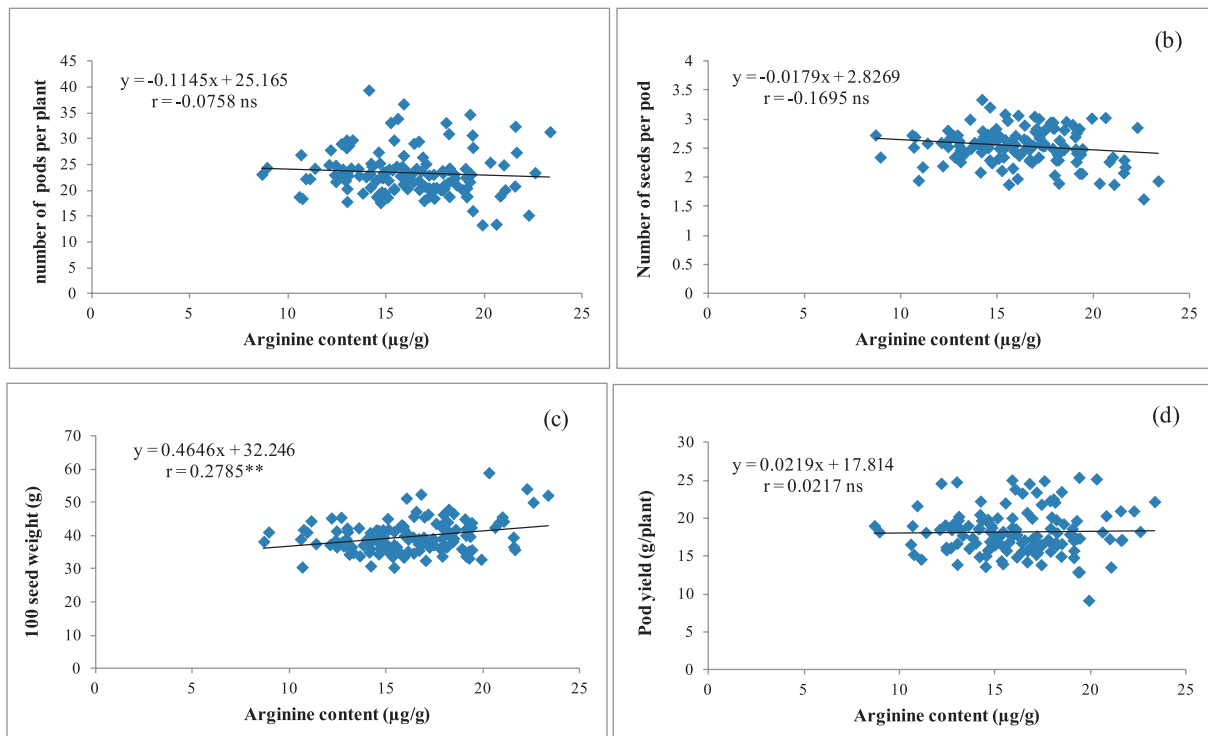
In this study, the arginine content was not correlated with any trait except 100 seed weight; however the correlation coefficient was low ( $r = 0.2758$ ) (Fig. 1). This indicated that a breeding program to develop a peanut line with a high arginine content and high pod yield is possible because the arginine content did not depend on the yield.

### Cluster analysis

One hundred and thirty peanut accessions were grouped into 10 clusters ( $R^2 = 0.70$ ) (Fig. 2) based on arginine con-

tent, number of pods per plant, number of seeds per pod, 100-seed weight and pod yield. The clusters should aid plant breeders in the development of new peanut lines with multiple desirable, yet unrelated, traits such as arginine content and yield.

Cluster one included 13 accessions originating from Bolivia, Argentina, Costa Rica, Kenya, Zimbabwe and USA. This cluster represented moderate arginine content (15.66 µg/g), high number of pods per plant (29 pods/plant), moderate number of seeds per pod (2.52 seeds/pod), low 100 seed weight (35.86 g) and moderate pod yield (18.95 g/plant). Cluster two consisted of 6 accessions originating from Argentina and Zaire and this cluster represented moderate arginine content (15.66 µg/g), high number of pods per plant (35 pods/plant), low number of seeds per pod (2.17 seeds/pod), low 100 seed weight (34.83 g) and moderate for pod yield (17.74 g/plant). Cluster three consisted of 23 accessions originating from Bolivia, Peru, Argentina, Venezuela, Zimbabwe, New Mexico State, Brazil, Zaire, Cameroon, Srilanka, Africa and one unknown accession. This cluster represented moderate arginine content, moderate number of pods per plant, moderate 100 seed weight and moderate pod yield, and high number of seeds per pod. Cluster four consisted of 14 accessions originating from Uruguay, Bolivia, Argentina, Benin, USA, Uganda and India. This cluster was characterized by low arginine content (12.51 µg/g), high number of pods per plant, high number of seeds per pod, high 100 seed weight and moderate pod yield.



**Fig. 1.** Relationship between arginine content and number of pods per plant (a), number of seeds per pod (b), 100 seeds weight (c) and pod yield (g/plant) (d) of 130 peanut accessions across two years. ns, \*\* = not significant and significant at  $** P \leq 0.01$ , respectively



parental lines in high arginine content breeding programs.

In this study, arginine content in Valencia peanut was not correlated with the number of pods per plant, number of seeds per pod, 100 seed weight and pod yield. The results indicated that arginine and yield traits segregate independently. Therefore, breeding for both high arginine and high yield should be successful.

The objective of this study was to evaluate the diversity of arginine content and identify lines for use in breeding programs that would aid the development of cultivars superior in yield and arginine by grouping the peanut accessions into 10 clusters based on arginine content, number of pods per plant, number of seeds per pod, 100 seed weight and pod yield. Breeders should be able to study the heritability of these traits and develop superior cultivars for them. For example, cluster five accessions were characterized as having low arginine content (12.78 µg/g) and pod yield. Cluster ten accessions were characterized as having high arginine content (19.75 µg/g) and pod yield. Cluster ten included Kremena, ICG 297, KKKU 40, KKKU 60 and ICG 6022. Kremena and ICG 6022 should be particularly interesting for breeding programs because they have high arginine content (23.35 and 22.26 µg/g), large seeds (52.2 and 54.13 g/100 seed) and high yield (22.19 and 20.95 g/plant). The origins of peanut plants in each cluster were different. For example, cluster ten with a high arginine content in the seed originated from New Mexico State, Mauritius, India and Thailand. Cluster four and five with low arginine contents also differed in origin. The result indicated that high or low arginine content in peanut seed was not related to their origin.

In conclusion, significant variation of arginine content was found within the Valencia germplasm core collection. Peanut genotypes Kremena and ICG 6022 had high arginine content, pod yield and a good agronomic trait. These two genotypes would be promising parents in breeding programs targeting improved arginine content and yield.

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