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Influence of seed moisture content on seed quality of Bambara groundnut (*Vigna subterranean* L. Verdc)

Wongwichaiwat, S.[1], Chotechung, S.[2], Kinnaret, P.[3], Kaewtaphan, P.[1*], Anothai, J.[4] and Phakamas, N.[1]

[1]Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand; [2]Suphunburi field crop research center, Suphunburi, 72160, Thailand; [3]Songkhla field crop research center, Songkhla, 90110, Thailand; [4]Agricultural Innovation and Management Division (Plant Science), Faculty of Natural Resources, Prince of Songkhla University, Songkhla, 90110, Thailand.

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Abstract Two varieties of bambara groundnut (*Vigna subterranean* L. Verdc) were evaluated for seed quality under different seed moisture contents between 8, 10 and 12%, and the seeds were stored under ambient condition for six months. Songkhla 1 maintained high germination percentage (91.11%) and high germination index (24.43) after storage for six months, whereas TVsu 1221 showed low germination percentage (54.67%) and germination index (14.83) in two months after storage. Differences among seed moisture contents were low and seed moisture content of 12% was better than seed moisture content of 8% for germination percentage and germination index. Variations between bambara groundnut varieties and seed moisture contents were low for shoot length and seedling dry weight. These parameters seemed to be not suitable for evaluation of seed quality of bambara groundnut.

Keywords: Bambara groundnut, Seed moisture content, Seed quality, Seed storage

Introduction

Bambara groundnut (*Vigna subterranean* L. Verdc) is leguminous crop indigenous to Africa and it is cultivated in the semi-arid tropics (Hillocks *et al*., 2012). Introduction of the crop into Thailand is still not clear. However, it is cultivated mainly in the South including Phatthalung, Songkhla, Yala, Narathiwat and Pattani (Siriwongpaisan, 2007). In Thailand, Bambara groundnut is grown in intercropping systems in rubber plantations, oil palm plantations and other perennial plants for the first 2-3 years of plantation establishment (Boonratsamee, 2023). Bambara groundnut can be planted in low fertility or sandy soil (Massawe *et al*., 2005). Bambara groundnut is a nutrient-rich plant containing 18-24% protein, 6-6.5% fat and 60-63%

* **Corresponding Author:** Kaewtaphan, P., **Email:** phissanu.ka@kmitl.ac.th

carbohydrate (Boonratsamee, 2023). It also contains antioxidants and phytochemicals useful for the human health (Ajibola *et al*., 2021).

Two recommended varieties including local variety and Songkhla 1 are available for bambara groundnut cultivation in Thailand (Suwanprasert, 2005). Local variety is characterized by its creamy yellow seed coat, small seeds and thick skin, and it has 150-180 days to harvest (Suwanprasert, 2005). Songkhla 1 was released in 1998 (Songkhla field crops research center, 1998). It is characterized by its purple red seed coat, larger seeds and 110-120 days to harvest (Songkhla field crops research center, 1998).

Bambara groundnut breeding program at Songkhla field crop research center has developed new breeding lines with high yield and good adaptation to growing environments in the South**.** TVsu 1221 is a promising line showing good yield in the South. It has creamy yellow seed coat, thin pods and 110-120 days to harvest (International Institute of Tropical Agriculture, 2023; Boonratsamee, 2023). Currently, it is under advanced evaluation and possible for release.

In Thailand, bambara groundnut is planted in the early rainy season to mid rainy season in May to August and harvest in the late rainy season in October to December, and the crop may encounter heavy rain during harvest. Farmers may collect seeds for planting in the next season. If they do not collected seeds, they have to buy fresh groundnut from the market for their seeds because no dried seeds are available in the local market (Suwanprasert, 2005).

Seed storage and seed maintenance is therefore important for farmers to use the seeds in the next growing season. It is also useful for bambara groundnut breeding in the preservation and collection of germplasm. Seed moisture content is an important seed component and an important factor affecting seed quality. It is also a main cause of seed degradation, which affects the shelf life of the seed.

Because seed moisture content plays an important role in all aspects of seed physiology. It is also an important cause of seed deterioration, which affects the shelf life of the seed. Water or seed moisture affects the metabolic activity within the seed. Seeds with low moisture content had a lower deterioration rate than those with high moisture content (Chanprasert, 1999).

In general, the seeds with moisture content lower than 10 percent can be stored for a long time, and seeds are less degraded than seeds at high moisture content (Anderson and Alcock, 1954). In a study in Thailand, the seed of a bambara groundnut variety, Songkhla 1, was oven-dry to obtain seed moisture content of lower than 11% and stored in woven plastic film tape sacks at room temperature for year without reduction in seed germination (Suwanprasert, 2005). Gerrano *et al.* (2021) reported that seed moisture content of freshly harvested bambara groundnut should be reduced to 12-14 percent. The seeds at this moisture content level are very dry and distorted,

and the seeds may not germinate. The seed with high moisture content, however, can be destroyed by fungi.

The information on the optimum moisture contents for seed storage is very rare. In Thailand, only Songkhla 1 was studied, and the information on the optimum seed moisture content for storage of TVsu 1221 is not available. The objective was to determine the optimum seed moisture content for seed storage of bambara groundnut seeds. The information obtained in this study will be used as a guideline for further seed storage of the bambara groundnut new varieties.

Materials and methods

Location and experimental design

The experiment was conducted at the laboratory of the Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang during October 2022 to April, 2023. A 2x3 factorial design was used. Two bambara groundnut genotypes including Songkhla 1 and TVsu1221 were assigned and factor A, and three moisture contents including 8%, 10% and 12% were assigned as factor B. Songkhla 1 is a released variety, and TVsu 1221 is a promising breeding line. The treatment combinations were arranged in a completely randomized design with three replications.

Seed preparation

Freshly harvested pods of the bambara groundnut genotypes were obtained from Songkhla in August 2022. The seeds were sun-dried for 3 days. The pods of TVsu 1221 dried faster than those of Songkhla 1 due to its thinner pods. The pods were threshed after 3 days of sun-drying.

The seeds were tested for moisture content and further oven-dried according to the method of the International Seed Testing Association (ISTA) with the following steps. Seed moisture content was measured using high constant temperature oven-dry method following the rules of ISTA (1996). The seeds were placed in the aluminum dish and dried in the oven at 103 ^{o}C for 24 hours until the weights were constant. Moisture content was calculated as follows;

$$\text{Moisture content (\%)} = \frac{\text{weight of fresh seeds} - \text{weight of dry seeds}}{\text{weight of fresh seeds}} \times 100$$

At the initial oven dry, Songkhla 1 had higher moisture content (48%) than TVsu 1221 (14%). After oven-dry, the seeds of both varieties had

moisture contents of 8, 10 and 12%, respectively. The seeds were sealed closed containers and stored at ambient temperature for 0, 2, 4 and 6 months.

Data collection

Data were recorded for germination percentage, germination index, shoot length and seedling dry weight. Germination percentage was determined according to ISTA (1996). Germination test was conducted using sand medium. The sand was sieved to discard unwanted particles. Germination trays were filled with the medium. Twenty-five seeds were placed in each plastic tray and then lightly covered with sand. The trays were watered at every day. The germination percentages were recorded at 4 to 14 days after seeding. Germination percentage was calculated as follows;

$$\text{Germination (\%)} = G/X \times 100,$$

Where;
G is number of normal germinated seedlings.
and X is number of seeds sown.

Germination index was determined by standard germination test. Normal seedlings were counted at 5 to 14 days after seeding. Germination index was calculated as described in the Association of Official Seed Analysts (2009) handbook on vigor as the following formula;

$$\text{Germination index} = \frac{\text{No. of normal seedlings}}{\text{Day to first count}} + \ldots + \frac{\text{No. of normal seedlings}}{\text{Day to final count}}$$

Shoot length was determined after 14 days of seeding. Ten plants were randomly chosen to measure length by measuring the length from the base to the tip of the seedlings (Association of Official Seed Analysts, 2009).

Seedling dry weight was determined after 14 days of seeding. The normal seedlings from each germination dish were collected, washed with running tap water and surface dried. The seedlings were oven-dried at 70 ^{o}C for 48 hours until the weights were constant.

Data analysis

Data were subjected to analysis of variance (ANOVA) using MSTAT-C software program from Michigan State University (Bricker, 1989). Duncan's Multiple Range Test (DMRT) was used to compare means at 0.05 probability level.

Results

Germination percentage and germination index

TVsu 1221 had significantly (P≤0.01) lower germination percentage and germination index than Songkhla 1 for most evaluation times at 2, 4 and 6 months after storage except at 0 month when these genotypes were not significantly different (Table 1). Songkhla 1 could maintain high germination percentage and high germination index until 6 months of storage, whereas TVsu 1221 had severe reduction in germination percentage and germination index.

At 0 month after storage, germination percentages of these genotypes were not different (93.33% for Songkhla 1 and 94.22 for TVsu 1221). Great reduction in germination percentage in TVsu 1221 was observed as early as 2 months after storage (54.67%). At 6 months after seeding, Songkhla 1 had germination percentage of 91.11%, and TVsu 1221 had germination percentage of 48.44%.

Table 1. Means for germination percentage and germination index of two bambara groundnut varieties as affected by seed moisture content evaluated at 0, 2, 4 and 6 months after storage

Factor		Germination (%) After storage (months)				Germination index After storage (months)			
		0	2	4	6	0	2	4	6
Varieties (A)									
Songkhla 1		93.33	92.00^{a}	88.89^{a}	91.11^{a}	23.00	24.13^{a}	20.24^{a}	24.43^{a}
TVsu 1221		94.22	54.67^{b}	15.11^{b}	48.44^{b}	23.72	14.83^{b}	3.25^{b}	11.92^{b}
F-test (A)		ns	**	**	**	ns	**	**	**
Moisture content (B)									
8 %		92.67	78.67	48.67	59.33^{b}	22.77	20.53	11.18	15.70^{b}
10 %		95.33	74.00	50.67	68.67^{ab}	23.60	19.92	11.29	17.82^{ab}
12 %		93.33	67.33	56.67	81.33^{a}	23.71	17.99	12.77	20.98^{a}
F-test (B)		ns	ns	ns	*	ns	ns	ns	*
Varieties (A)	Moisture content (B)								
Songkhla 1	8 %	93.33	89.33	86.67	94.67^{a}	23.00	22.85	20.17	25.32^{a}
	10 %	97.33	93.33	90.67	90.67^{a}	23.46	24.67	20.27	24.16^{a}
	12 %	89.33	93.33	89.33	88.00^{a}	22.53	24.86	20.27	23.80^{a}
TVsu 1221	8 %	92.00	68.00	10.67	24.00^{c}	22.53	18.21	2.18	6.09^{c}
	10 %	93.33	54.67	10.67	46.67^{bc}	23.73	15.17	2.31	11.49^{bc}
	12 %	97.33	41.33	24.00	74.67^{ab}	24.90	11.11	5.27	18.17^{ab}
F-test (A x B)		ns	ns	ns	**	ns	ns	ns	**
C.V. (%)		6.20	20.37	12.43	17.57	6.50	22.46	18.41	16.35

ns = non significant, * and **= significantly different at $P < 0.05$ and significantly different at $P < 0.01$, respectively. Means within the same column followed by the same letter are not significantly different by DMRT.

Germination index indicated speed of seed germination, and higher index indicated more rapid germination. Similar patterns of reductions of these genotypes were observed for germination index, and the data provided the similar information. Songkhla 1 had significantly higher germination indexes than TVsu 1221 at most evaluation times except at 0 month after seeding when these genotypes were similar.

Songkhla 1 and TVsu 1221 responded differently to seed moisture content for germination percentage and germination index. Seed moisture content did not have significant effect on germination percentage and germination index in Songkhla 1. However, it did have significant effects on germination percentage and germination index in TVsu 1221, and it seemed likely that low seed moisture content reduced germination percentage and germination index. Significant different between 8% and 12% were observed for germination percentage and germination index in TVsu 1221 at 6 months after storage.

Table 2. Means for shoot length and seedling dry weight of two bambara groundnut varieties as affected by seed moisture content evaluated at 0, 2, 4 and 6 months after storage

Factor		Shoot length (cm) After storage (months)				Seedling dry weight (g) After storage (months)			
		0	**2**	**4**	**6**	**0**	**2**	**4**	**6**
Varieties (A)									
Songkhla 1		23.49[b]	23.02[a]	21.95	21.83	3.49	3.13	3.01	2.81[a]
TVsu 1221		24.43[a]	21.44[b]	21.78	22.72	3.01	2.84	2.75	2.33[b]
F-test (A)		*	*	ns	ns	ns	ns	ns	**
Moisture content (B)									
8 %		23.19[b]	22.61	22.47	21.31	3.48	2.99	2.87	2.25[b]
10 %		23.78[ab]	22.14	21.66	23.30	3.13	2.95	3.05	2.75[a]
12 %		24.90[a]	21.95	21.47	22.20	3.14	3.01	2.71	2.71[a]
F-test (B)		**	ns	ns	ns	ns	ns	ns	*
Varieties (A)	Moisture content (B)								
Songkhla 1	8 %	22.67	22.48	22.27	21.27	3.54	3.01	2.94	2.66
	10 %	23.94	22.85	21.79	22.60	3.24	3.12	3.28	2.91
	12 %	23.84	23.74	21.78	21.61	3.68	3.25	2.80	2.87
TVsu 1221	8 %	23.70	22.74	22.67	21.36	3.41	2.96	2.79	1.85
	10 %	23.62	21.42	21.52	24.00	3.01	2.79	2.83	2.60
	12 %	25.96	20.17	21.15	22.80	2.59	2.77	2.63	2.55
F-test (A x B)		ns	ns	ns	ns	ns	ns	ns	ns
C.V. (%)		3.37	5.84	10.84	8.65	15.96	17.03	12.41	12.60

ns = non significant, * and **= significantly different at $P < 0.05$ and significantly different at $P < 0.01$, respectively. Means within the same column followed by the same letter are not significantly different by DMRT.

Shoot length and seedling dry weight

Significant different (P≤0.05 and 0.01) between bambara groundnut genotypes were found for shoot length at 0 and 2 months after storage and for seedling dry weight at 6 months after storage (Table 2). TVsu 1221 had longer shoot than Songkhla 1 at 0 month after storage. Songkhla 1 had longer shoots than TVsu 1221 at 2 months after storage, and Songkhla 1 also had heavier seedlings than TVsu 1221 at 6 months after storage. Significant different (P≤0.05 and 0.01) among three seed moisture contents were observed for shoot length at 0 month after storage and for seedling dry weight at 6 months after storage. At 0 month after storage, seed moisture content of 12% had significantly longer shoot than seed moisture content of 8%, whereas seed moisture content of 10% was not significantly different from seed moisture content of 8% for shoot length. Seed moisture contents of 10 and 12% also had significantly heavier shoots than seed moisture content of 8%.

Treatment combinations between bambara groundnut genotype and seed moisture content were not significantly different for shoot length and seedling dry weight at all evaluation times.

Discussion

Germination percentage and germination index

Bambara groundnut genotype had significant effects on germination percentage and germination index. It is clear that Songkhla 1 showed high germination percentage and germination index. The results indicated that Songkhla 1 could be stored at ambient condition for 6 months without severe reduction in seed quality. In contrast to Songkhla 1, TVsu 1221 had severe reduction in germination percentage and germination index as early as 2 months after storage, and at final storage for 6 germination percentage of this genotype was only 48.44% and germination index was only 11.92.

The results imply that TVsu 1221 was not suitable for long term storage, and its seed should be sowed soon after harvest to maintain high seed quality. According to Thai Seed Trade Association (2023), germination of commercial seed should be not lower than 80%. Physical factors and chemical factors of the seed might affect storability of the seed (Chanprasert, 1999). The Songkhla 1 has red seed coat, larger seeds and thin shell. The chemical compositions of the seeds consisted of 18% protein, 6% fat (Songkhla field crop research center, 1998) and 50-60% carbohydrate (Atoyebi *et al*., 2017). TVsu 1221 has creamy yellow seed coat, smaller seeds and thin shell. Chemical compositions of the seeds consisted of 21-23% protein and 4-5% fat (unpublished data).

Seed moisture contents in a range of 8 to 12% had low effects on germination percentage and germination index as significant differences

among seed moisture contents were found at 6 months of storage only. In faba bean (*Vicia faba* L.), seed moisture contents could be varied between 6 and 22% (Kooshki *et al*., 2018). In maize, moisture contents of commercial seed lots varied between 9.20 and 15.85% (Onwimol and Rongsangchaicharean, 2019). It is interesting to note here that the ranges of seed moisture content in previous studied were higher than in this study. However, the range of seed moisture contents in this study was rather narrow (4%) and expanding the range to higher moisture contents is still required to obtain conclusive results.

Bambara groundnut genotypes responded differently to seed moisture content. Seed moisture content did not have significant effects on germination percentage and germination index of Songkhla 1 as the differences among treatment combinations associated with Songkhla 1 at all evaluation times were not significant. However, small but significant effects were found for germination percentage and germination index of TVsu 1221 at 6 months after storage.

Expansion of storage times to 12 months and under controlled condition is still required to obtain more conclusive results as bambara groundnut is grown mainly in the rainy season although seed production can be carried out in the dry season with irrigation.

Shoot length and seedling dry weight

Seed moisture contents were significantly different for shoot length at 0 and 2 months after storage, and they were also significantly different for seedling dry weight at 6 months after storage. Identification of better genotypes for shoot length and seedling dry weight was difficult because the differences were not significant for most evaluation times. Although the differences were significant, the interaction between genotype and evaluation time could confound the results.

Seed moisture content of 12% seemed to be better than seed moisture content of 8% for shoot length at 0 month after storage and for seedling dry weight at 6 months after storage. However, the differences were not clear cut for these parameters because they were not significantly different for three evaluation times at 2, 4 and 6 months for shoot length and at 0, 2 and 4 months for shoot dry weight. According to Obilana and George (2021) seed storage is important for availability of the seed for planting in the next season and maintenance of seed quality. In this study, identification of the best seed moisture content was difficult because there were small differences among seed moisture contents for shoot length and shoot dry weight, and seed moisture contents were not significantly different at most evaluation times.

The interactions between genotype and seed moisture content were not significant for shoot length and seedling dry weight, and the treatment combinations of these factors were also not significantly different for shoot length and seedling dry weight at 0, 2, 4 and 6 months after storage. Because

identification of good genotype and seed moisture content with good storability is difficult using seedling length and seedling weight, these parameters are suitable for evaluation of seed quality of bambara groundnut, and the use of germination parentage and germination index is more suitable.

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