Effect of Seed Maturity and Invigoration on Seed Viability and Vigor, Plant Growth, and Yield of Bambara Groundnut (*Vigna subterranea* (L.) Verdcourt)

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Abstract

Bambara groundnut can be cultivated in marginal land, however, its productivity is still low. In order to increase productivity, high quality seeds must be used. Two consecutive experiments were conducted in the laboratory and field at Bogor Agricultural University from April to October 2010. Seeds of three maturity stages were harvested from a farmer's field in Sukabumi, April 2010. The seeds were dried down to 11.6% moisture content before being used. The objectives of the experiments were to evaluate the effects of seed invigoration applied to Bambara groundnut seeds of three maturity stages, on seed viability and vigor, plant growth, and yield. The laboratory experiment was arranged in a completely randomized design (CRD) while the field experiment was arranged in a randomized block (RBD) design. In both experiments, two factors were tested; seed maturity and invigoration. There were three stages of seed maturity i.e., seeds harvested at 119, 122, and 125 days after planting (dap). Seed invigoration consisted of untreated, *Rhizobium* sp., matriconditioning, matriconditioning plus *Rhizobium* sp. Matriconditioning was conducted using ratio of seeds to carrier (burned rice hull passed through 0.5 mm screen) to water of 5:3:3 (g) at 25°C for 3 days. *Rhizobium* sp. (0.48 g per 80 g seeds) was either applied on seeds just before planting or incorporated in matriconditioning. Results of the laboratory experiment showed that matriconditioning alone or matriconditioning plus *Rhizobium* sp. (biomatriconditioning) applied to seeds harvested at 122 or 125 dap improved seed viability (percent of germination) and vigor (speed of germination and index of vigor). However, there was no interaction between the two factors in the field experiment. Seeds treated with matriconditioning plus *Rhizobium* sp. or harvested at 125 dap showed the best improvement in plant growth (plant height) and yields (number and fresh weight of pods).

INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is mainly cultivated throughout the drier areas of tropical Africa, and the origin is most probably northern Nigeria and Cameroon. It has also spread to America, Australia, Central Asia, Indonesia, Malaysia and the Philippines (van der Maesen and Somaatmadja, 1992). The crop is cultivated from landraces, and farm yields are low and unpredictable. The problem of unpredictable yields has been attributed, at least in part, to variable and poor field establishment due to poor germination and/or seedling emergence (Linneman and Azam-Ali, 1993).

In its continent of origin, Bambara groundnut is harvested at 110-150 days after planting (dap), when plants start to turn yellow or 80% of pods mature (Department of Agriculture, Forestry, and Fisheries, Republic of South Africa, 2009). Early maturing

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genotypes mature ca. 90 days after sowing. Late maturing genotypes may require 150 days or more; mean temperature during the season influences the time taken to achieve physiological maturity (Linneman and Azam-Ali, 1993). In Indonesia, Bambara groundnut is harvested at 4-5 months (Redjeki, 2007) or 17-18 weeks after planting, depending on climate condition during harvest, 100% of the plant population are flowering at 70 dap and seeds can be harvested at 122 dap (Hamid, 2009). However, no study has been done on determination of physiological maturity in Bambara groundnut in order to obtain maximum seed quality.

Bambara groundnut can be cultivated in marginal land, however, its productivity is still low. In order to increase productivity, high quality seeds must be used. Seed invigoration, treatment on seed to improve its vigor, using matriconditioning has proved effective to improve seed quality, plant growth and/or yield in various crops (Khan et al., 1990; Ilyas, 2006). Matriconditioning was defined as preplant seed conditioning by mixing seeds with moist solid carriers devoid of osmotic solutes and with high water adsorptive capillary forces such as expanded vemiculite #5 and Micro-Cel E™ (Khan et al., 1990).

Little information on Bambara groundnut seed research is available, and no study has been done to improve seed quality through invigoration. Ilyas et al. (2003) studied improving seed quality, plant growth and yield of soybean through application of seed invigoration using matriconditioning plus *Bradyrhizobium japonicum* and *Azospirillum lipoferum* inoculants.

Therefore, the objective of this study was to evaluate the effect of invigoration treatments applied on different maturities of Bambara groundnut seeds on seed viability and vigor, plant growth and yield of Bambara groundnut.

MATERIALS AND METHODS

Seed Source

Seeds of Bambara groundnut were harvested from a farmer's field in Sukabumi-West Java, April 2010, at three maturity stages i.e., 119, 122, and 125 dap. The seeds were sun-dried down to ±11.6% moisture content and then stored in an air-conditioned room for 28 days before being used in laboratory and field experiments.

Experimental Design

There were two experiments conducted in this study: laboratory and field experiments. The laboratory experiment was arranged in a completely randomized design while the field experiment was arranged in a completely randomized block design. In both experiments, two factors were tested: seed maturity and invigoration. There were three stages of seed maturity i.e., seeds harvested at 119, 122, and 125 dap. Seed invigoration consisted of untreated, *Rhizobium* sp., matriconditioning, matriconditioning plus *Rhizobium* sp. (biomatriconditioning). Biomatriconditioning refers to matricondioning with the integration of biological agent(s). Each treatment was repeated three times. The effect of these factors on seed viability, vigor, plant growth and yield was investigated. The viability was reported as percent of germination while the vigor was measured as speed of germination and index of vigor. Plant growth was measured by plant height, number of leaves, and number of branches while yields as number and weight of fresh pods. The data were subjected to statistical analysis of variance (ANOVA), and Duncan's multiple range test (DMRT) was used to compare treatment means.

Invigoration Treatments

Seeds were matriconditioned in a 500 ml-covered transparent cup by mixing seeds with water and burned rice hull as the carrier at 25°C for 3 days. Burned rice hull was previously ground and sieved using a 0.5 mm screen size. The ratio of seeds to carrier to water was 5:3:3 (by weight in grams). *Rhizobium* sp. (0.48 g added in ± 10 ml water) was either applied to seeds (80 g) just before planting or incorporated into matriconditioning; in this case, 0.48 g *Rhizobium* sp. was added in 48 ml water and mixed with 80 g seeds and 48 g carrier. During conditioning the seeds were shaken for a minute once a day.

Laboratory Experiment

Conditioned seeds were sown in a transparent box of 25×20 cm filled two-thirds full with a mixture of equal portions of compost and burned rice hull. Twenty-five seeds were planted per box, and each treatment was replicated three times. The boxes were placed in a room at ambient temperature. Percentage germination was recorded as percent of total normal seedlings at first (7 dap) and final count (14 dap). The 'index of vigor' was taken as the percentage of germination at the first count. Speed of germination was calculated as the average number of seeds germinating each day and expressed as a percentage of the starting number.

Field Experiment

Conditioned seeds were planted in 36 plots of 2.5×6.0 m with a spacing of 50×60 cm, one seed per hole. At the same time, fertilizers were applied next to the planting hole: 100 kg Urea (45% N)/ha, 100 kg SP-36 (36% P_2O_5)/ha, and 75 kg KCl (60% K_2O)/ha. At 6 weeks after planting, when approximately 30% of the plants were flowering, soil was earthed up around the plant, covering ±2 cm of the plant as wide as the canopy diameter. Observation on plant growth was conducted at 5, 6, 7, 8 and 9 weeks after planting (wap) on plant height, number of leaves, and number of primary branches from 10 sample plants. Yields were measured at harvest (17 wap) on number of pods per plant as an average of 10 sample plants, and weight of fresh pods (kg) from all plants per plot.

RESULTS AND DISCUSSION

Table 1 indicates that application of invigoration treatment using matriconditioning alone (I2) or matriconditioning plus *Rhizobium* sp. (I3) on Bambara groundnut seeds harvested at different maturity i.e., 119 (M1), 122 (M2) and 125 dap (M3) as well as *Rhizobium* sp. alone (I1) on seeds with M3 maturity resulted on higher germination capacities compared to untreated on all seed maturity stages. A similar response was shown on the speed of germination. It was clear that even at M1 maturity stage, when the seed apparently had not reached its physiological maturity, matriconditioning or matriconditioning plus *Rhizobium* sp. improved both the percentage of germination and speed of germination.

These data indicated that seed harvested at 122 and 125 dap possibly had reached physiological maturity. Seed maturity is one of the factors that determine seed quality. Seeds harvested before or after physiological maturity will have lower seed quality than ones harvested at physiological maturity.

The highest index of vigor was obtained when seed harvested at 122 dap was matriconditioned. When matriconditioning plus *Rhizobium* sp. was applied on the three maturity stage seeds, there was no significant difference between the vigor of 122 and 125 dap-harvested seed, which were significantly higher than the vigor of 119 dap-harvested seeds. On the other hand, the untreated or *Rhizobium* sp. alone when applied to any stages of seed maturity, showed 0% index of vigor i.e., none of the seeds had germinated at the first time interval (7 dap). These data showed that matriconditioning or matriconditioning plus *Rhizobium* sp. applied on the three maturity stage seeds were able to accelerate seed germination (vigor) and improve viability.

In the field experiment, both factors did not significantly have an effect on vegetative parameters except that invigoration treatment significantly influenced plant height at 5-8 wap. Matriconditioning plus *Rhizobium* sp. provided the highest values among the other invigoration treatments especially compared to untreated seeds (Table 2).

Similarly, interaction between seed maturity and invigoration treatment did not significantly influenced the yield parameters. However, invigoration did influence both yield parameters i.e., fresh weight of pods per plot and number of pods per plant, while seed maturity significantly influenced only the number of pods per plant (Table 3). Previous work on Bambara groundnut suggested that the number of pods per plant was the most important trait (Redjeki, 2007; Makanda et al., 2009). Again, matriconditioning plus *Rhizobium* sp. provided the highest value for the number of pods per plant and was not significantly different with *Rhizobium* sp. alone. Matriconditioning plus *Rhizobium* sp. also resulted in the highest fresh weight of pods per plot even though it was not significantly different from other invigoration treatments. Seeds harvested at 125 dap indicated that the highest number of pods per plant was not significantly different from ones harvested at 122 dap but was if compared with the 119 dap-harvested seeds. On fresh weight of pods per plot, seed maturity did not show a significant difference, however, the 125 dap-harvested seeds showed higher values (Table 3). These findings confirmed the results in the laboratory experiment that the seeds harvested at 125 dap had reached the physiological maturity.

No study has been done on determination of physiological maturity in Bambara groundnut seed. Studies in peanut (*Arachis hypogaea* L.) showed that physiological maturity of the seeds was attained at different time depended on heat unit accumulation which were different among cultivars i.e., 'Landak' and 'Kidang' at 93 dap or 1497.5°Cd, 'Banteng' at 98 dap or 1598.7°Cd while 'Komodo' at 87 dap or 1397.6°Cd (Maria et al., 2000). Waemata and Ilyas (1989) reported that French bean (*Phaseolus vulgaris* L.) seeds 'Bandung local' reached physiological maturity 30 days after flowering (daf) indicated by maximum values on vigor and weight of dry seeds while the seeds harvested before and after physiological maturity (27 and 33 daf) showed a decrease in seed storability. In red bean (*Phaseolus vulgaris* L.) seed, physiological maturity was reached at 36 daf (Kartika and Ilyas, 1994).

In general, all invigoration treatments provided better results compared to untreated, either on vegetative (plant height) or yield parameters, and matriconditioning plus *Rhizobium* sp. showed the best treatment. The results generated in this study are generally in agreement with earlier findings in other crop species.

Khan et al. (1990) showed that large-size seed such as bean and soybean responded well to seed matriconditioning with Micro-Cel E. Matriconditioning improved germination, field emergence, plant growth and yield of various crops. Ilyas and Suartini (1998) proved that matriconditioning using sawdust upgraded low vigor seeds of yard-long bean into high vigor ones, and increased yield as well. Incorporation of *Bradyrhizobium japonicum* and *Azospirillum lipoferum* inoculants in matriconditioning using burned rice hull as the carriers improved seed quality, plant growth and yield of soybean better than matriconditioning or *Bradyrhizobium japonicum* and *Azospirillum lipoferum* alone or untreated. Furthermore, this invigoration technique also reduced N fertilizer use (only required 12.5 kg N/ha) (Ilyas et al., 2003). Some researchers reported that either *Bradyrhizobium* or *Rhizobium* strains were effective to form symbiostic relationships with Bambara groundnut (Linneman dan Azam-Ali, 1993).

Results of this study in the laboratory experiment showed that Bambara groundnut seed harvested at 122 or 125 dap and invigorated by matriconditioning or matriconditioning plus *Rhizobium* sp. showed better effects on seed viability (germination capacity) and vigor (speed and index of germination). In the field experiment, 125 dap-harvested seeds also gave better results in plant height and number of pods per plant. As on viability and vigor, matriconditioning plus *Rhizobium* sp. showed better effects as well on the number of pods per plant.

CONCLUSIONS

Invigoration treatment using matriconditioning or matriconditioning plus *Rhizobium* sp. applied to Bambara groundnut seeds harvested at 122 or 125 dap improved seed viability (percent of germination) and vigor (speed of germination and index of vigor) compared to untreated on all seed maturity stages. In the field experiment, seeds treated with matriconditioning plus *Rhizobium* sp. or harvested at 125 dap showed better improvement in plant growth (plant height) and yield (number and fresh weight of pods) compared to 119 and 122 dap. It is concluded that physiological seed maturity of Bambara groundnut was obtained at 125 dap, and matriconditioning plus *Rhizobium* sp. was the best seed invigoration treatment.

However, phenological study would be necessary to ascertain when Bambara groundnut seed reach its physiological maturity based on how many days after flowering.

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Tables

Table 1. Effect of seed maturity and invigoration treatment on seed viability and vigor parameters of Bambara groundnut.

Invigoration (I)	Seed maturity (M)		
	M1 (119 dap)[z]	M2 (122 dap)	M3 (125 dap)
	Germination capacity (%)		
I0[y]	12.0 Ab[1]	34.7 Ab	17.3 Ab
I1	33.3 Bab	50.7 ABab	68.0 Aa
I2	57.3 Aa	73.3 Aa	68.0 Aa
I3	54.7 Aa	65.3 Aa	70.7 Aa
	Speed of germination (%/d)		
I0	1.1 Ab	3.4 Ab	1.7 Ab
I1	3.4 Bab	5.2 Abab	6.5 Aa
I2	6.4 Aa	6.6 Aa	7.0 Aa
I3	6.5 Aa	7.5 Aa	7.7 Aa
	Index of vigor (%)		
I0	0.0 Ab	0.0 Ab	0.0 Ab
I1	0.0 Ab	0.0 Ab	0.0 Ab
I2	33.3 Ba	52.0 Aa	34.7 Bab
I3	16.0 Bb	46.7 Aa	41.3 Aa

[z] dap, days after planting.
[y] I0 (untreated), I1 (*Rhizobium* sp.), I2 (matriconditioning), I3 (matriconditioning plus *Rhizobium* sp.).
[1] Means separation at 5% level (DMRT); capital letters within seed maturity values, small letters within invigoration values.

Table 2. Effect of seed invigoration on the vegetative growth parameters of Bambara groundnut 5-9 weeks after planting.

Age of plant (wap)[z]	F test	Invigoration			
		I0[y]	I1	I2	I3
		Plant height (cm)[x]			
5	*	17.4 b[1]	17.4 b	17.6 b	18.4 a
6	*	17.9 c	18.6 b	18.8 b	19.7 a
7	*	18.4 b	19.0 ab	19.2 ab	19.5 a
8	*	19.6 b	20.3 ab	20.7 a	21.3 a
9	ns[w]	20.8	20.8	20.9	21.2
		No. of leaves[x]			
5	ns	9.0	8.8	9.4	10.2
6	ns	11.9	12.5	14.2	14.3
7	ns	15.6	15.9	16.9	17.7
8	ns	26.4	27.0	25.9	30.3
9	ns	39.2	41.8	38.7	42.5
		No. of primary branches[x]			
5	ns	4.3	4.3	4.4	4.5
6	ns	4.9	5.2	5.2	5.2
7	ns	5.6	5.7	5.8	6.0
8	ns	6.2	6.3	6.4	6.6

[z] wap, weeks after planting.
[y] I0 (untreated), I1 (*Rhizobium* sp.), I2 (matriconditioning), I3 (matriconditioning plus *Rhizobium* sp.).
[x] Values are average of 10 plants.
[w] ns, not significant (P>0.05).
[1] Means separation at 5% level (DMRT); letters within invigoration values for each plant age.

Table 3. Effect of seed invigoration on yield parameters of Bambara groundnut 17 weeks after planting.

Invigoration (I)	Seed maturity (M)			Invigoration means
	M1 (119 dap)[z]	M2 (122 dap)	M3 (125 dap)	
	No. of pods per plant[x]			
I0[y]	23.9	27.3	32.7	28.0 b[1]
I1	30.2	32.5	34.2	32.3 ab
I2	30.0	25.1	35.0	30.0 b
I3	33.3	38.3	37.3	36.3 a
Seed maturity means	29.4 B[1]	30.8 AB	34.8 A	
	Fresh wt. of pods (kg) per plot[w]			
I0	2.7	2.6	3.0	2.8 b[1]
I1	3.3	3.6	3.4	3.4 a
I2	3.7	2.7	3.3	3.2 ab
I3	3.7	3.9	3.0	3.5 a
Seed maturity means	3.2 A	3.2 A	3.3 A	

[z] dap, days after planting.
[y] I0 (untreated), I1 (*Rhizobium* sp.), I2 (matriconditioning), I3 (matriconditioning plus *Rhizobium* sp.).
[x] Values are average of 10 plants.
[w] Pods were harvested from all plants in plots of 2.5×6.0 m with spacing of 50×60 cm.
[1] Means separation at 5% level (DMRT); capital letters within seed maturity means, small letters within invigoration means.