

# Hydro-priming improved germination and vigour of kenaf (*Hibiscus cannabinus* L.) seeds

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#### Abstract

Kenaf is grown for fibre in many African countries and propagated by seed, thus biomass and economic yields depend on seed vigour and seedling stands establishment. However, kenaf seeds are reported to have poor physiological quality consequently limiting biomass productivity. In this study, seed invigoration treatment with various durations of hydro-priming including 0 hour (control), 24, 48 and 78 hours was investigated in 12 accessions of kenaf seeds. Data were collected on seed germination at 3 and 7 days after sowing (DAS), seedling length and weight upon emergence, while germination rate and a seed vigour index was calculated from the data. The data were subjected to the PROC GLM procedure of SAS statistical package. The results showed significant effects of hydro-priming and genotype on all the seed viability and vigour variables (P<0.05 and P<0.01). Mean viability of control seed lots at 7 DAS was 8.67% and significant differences were found between hydro-primed seeds and control seed lots for all the seed quality variables. Seeds hydro-primed for 24 hours had above 100% germination. Interactive effects of genotype and hydro-priming treatments showed that early germination and cumulative germination consistently increased from 6% in control seed lots to 31% in hydro-primed seed lots for 4 genotypes. Seed vigour of 9 genotypes (75% of experimented accessions) was also improved with seed hydro-priming for 24 hours in almost all the accessions. The results suggest that hydro-priming for 24 hours optimised seed germination and vigour and is therefore recommended for kenaf seed invigoration treatment before planting.

Key words: Hydro-priming, seed viability and vigour, germination rate, seed vigour index.

# Introduction

Kenaf (*Hibiscus cannabinus* L.) is a warm season annual fiber crop used as a cordage crop to produce twine, rope, and sackcloth derived from its stem. Kenaf is an alternative raw material for newsprint paper offering ecological advantage over paper production from trees. Moreover, kenaf is a candidate eco-friendly crop for phyto-remediation of marginal and polluted soils through it's capacity for sorption of heavy metals <sup>1,2</sup>. Kenaf is also a source of edible oil from its seeds.

Kenaf is propagated only by seeds, which is also the means of *ex-situ* conservation of kenaf germplasm resources. Because of high edible oil content of kenaf seeds, they rapidly lose germination capacity soon after harvest. Under conditioned storage at 20°C and 10% humidity, kenaf seeds remained viable for about 8 months<sup>6</sup>. However, under the humid tropical climates of Africa with average ambient temperature around 35°C and humidity above 60%, viability loss is faster. For example, Adeniyan (unpublished) reported that within a cropping season freshly harvested seeds of kenaf had lost viability below 10% in South western Nigeria. This constitutes a major limitation to the breeding and genetic resources conservation of kenaf in Nigeria.

Recent research on a range of crop species had shown that faster germination, early emergence and vigorous seedlings were achieved by controlled treatment of seeds by aerated hydration (hydro-priming) for certain number of hours followed by drying before sowing <sup>5</sup>. Several studies had supported the use of these methods for seed invigoration in various crops <sup>3, 10, 11, 13, 15</sup>. However, the secret to successful seed priming is ceasing the priming treatment at just the right time to allow re-drying, hence each species must be investigated for optimal priming treatments and treatment durations <sup>5</sup>. Therefore, the objective of this trial was to investigate the effects of various hydro-priming durations on kenaf seed germination and vigour.

#### **Materials and Methods**

The hydro-priming experiments were conducted at the Department of Plant Breeding and Seed Technology Laboratory University of Agriculture, Abeokuta, on seeds of 12 accessions of kenaf sourced from the IAR&T, Ibadan, Nigeria kenaf germplasm collection. The accessions were: Purple flower (Line 35), Tainung 2 21<sup>2</sup> (Line 26), Ex shika 24<sup>4</sup> (Line 19), Au-2452<sup>1</sup> (Line 29), Tainung 2 21<sup>1</sup> (Line 28), Ex giwa (Line 31) AC-313 29<sup>5</sup> (line44), G45 2<sup>3</sup> (line18), A-60-282 5<sup>1</sup> (Line10), S108/4 47<sup>3</sup> (Line 9), AU-75 41<sup>3</sup> (Line 16) and G-45<sup>2</sup> (Line 57) (Table 1).

Seed lots of each accession were divided into sub lots of 30

 
 Table 1. Germination and vigour of seed lots of 12 kenaf before hydro-priming experiments.

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Accession name	Accession	% Germination	Seed Vigour
	number	(Arc sine values)	Index (SVI))
1. Purple flower	Line 35	10.02	5.16
2. Tainung 2 $21^2$	Line 26	10.02	9.47
3. Ex-Shika 24 <sup>4</sup>	Line 19	10.02	3.21
4. Au-2452 <sup>1</sup>	Line 29	1.00	2.00
5. Tainung 2 21 <sup>1</sup>	Line 28	10.02	8.54
6. Ex-Giwa	Line 31	6.68	2.66
7. AC-313 29 <sup>5</sup>	Line 44	4.05	4.62
8. G-45 2 <sup>3</sup>	Line 18	6.71	7.68
9. A-60-282 5 <sup>1</sup>	Line 10	3.67	2.14
10. S108/4 47 <sup>3</sup>	Line 9	13.82	8.25
11. AU-75 41 <sup>3</sup>	Line 16	4.01	2.21
12. G-45 <sup>2</sup>	Line 57	3.67	4.48

seeds each which were packed in polyethylene net bags. The packed seeds were surface sterilised by soaking in sodium hypochlorite (NaOCl) solution for 10 min prior to priming treatment. The sterilised seeds were suspended in glass priming tanks filled with 1 litre of distilled water and fitted with an electric water pump (100 mbar capacity) to enhance air circulation and surface area for gas exchange. Priming duration of kenaf seeds in the aerated water were 0, 24, 48 and 72 hours and untreated seeds were the control lot. Seed packets were drawn from tanks and dried in room conditions for 24 hours before seed testing.

Seeds were tested for germination and vigour using the seedling growth tests. Germination tests were carried out on the 3 replicates of each priming treatment after drying. Seeds were germinated on blotter papers and germination count data were collected on daily basis for 7 days. Germination rate was estimated as reciprocal of time to cumulative seed germination according to Daniel <sup>8</sup> and Daniel *et al.* <sup>9</sup>:

 $GR = 1/t_{r} \cdot (\Sigma G)$ 

where t is time taken for seeds to reach maximum or cumulative germination G. Seedling length (SL) and dry weight (SW) data

were also taken at 3<sup>rd</sup> and 7<sup>th</sup> day of sowing (DAS) and used to calculate a seed vigour index as follows:

Seed Vigour Index (SVI) = % Germination x SL x SW

The data were subjected to the PROC GLM procedure of SAS statistical package <sup>15</sup>, to calculate analysis of variance (ANOVA). Germination data were arcsine square root transformed before testing for significance using ANOVA and *post-hoc* tests for mean separation were done with Least Significant Differences (LSD) at 5% probability level.

### Results

Germination and vigour was generally poor in the control kenaf seed lots before the priming treatments. Germination was  $\leq 10\%$  in seeds of 11 accessions of the 12 accessions and only one accession (S108/4 47<sup>3</sup>) had seed germination above 10% before priming (Table 1). SVI ranged from 2.0 to 9.27 among the accessions before the hydro-priming treatment.

Analysis of variance revealed high significance of hydro-priming duration treatments effect on all seed viability and vigour variables at  $P \le 0.01$  (Table 2). The effects of genotypes on almost all the viability and vigour traits were also highly significant ( $P \le 0.01$ ) except for seedling length that was significant only at  $P \leq 0.05$ . The post-hoc tests for mean comparison of seed responses to the hydro-priming duration treatments revealed the general trend of improved seed quality in all hydro-primed kenaf seed lots above the control seed lots (Table 3). Values of all the seed viability and vigour variables were lowest in control seed lots than hydroprimed seed lots (Table 3). Mean comparison among hydropriming duration treatments showed that hydro-priming of kenaf seeds for 24 hours resulted in significantly higher germination at 3DAS and 7DAS, GR, SL at 3 DAS and SVI than other hydropriming duration treatments. There is, however, the exception of SW at 3 DAS where hydro-priming for 48 hours significantly optimised seedling weight above all other hydro-priming duration and control treatments.

 Table 2. Means square values from ANOVA of the effects of various hydro-priming durations on seed viability and vigour of 12 accessions of kenaf.

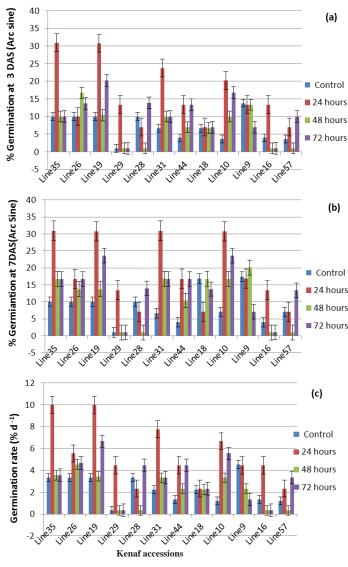
Sources of variation	Df	Arc sine Germination 3 DAS	Arc sine Germination 7 DAS	Germination rate $(d^{-1})$	Seedling Length at 3 DAS (cm)	Seedling weight at 3 DAS(g)	Seed Vigour Index (SVI)
Rep.	2	152.69	163.99	17.31*	8.02	0.026	908.97
Priming (P)	3	704.33**	648.55**	74.55**	41.83**	0.46**	1997.3**
Accession (A)	11	235.13**	387.78**	25.76**	7.32*	0.58**	594.93*
P*A	33	65.25	103.66	6.79	4.27	0.21**	255.14
Error	94	49.59	73.71	5.47	3.18	0.07	201.39

\*Significant F at P<0.05; \*\*Significant F at P<0.01.

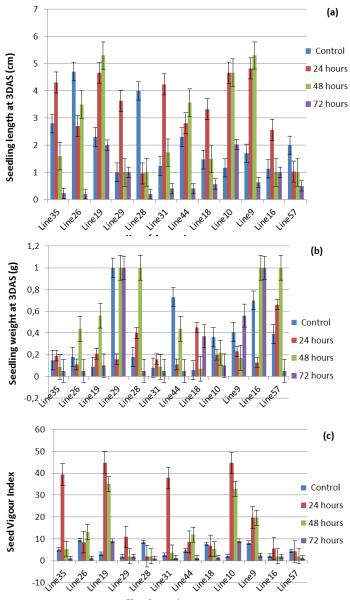
 Table 3. Comparison of various hydro-priming duration treatments on seed viability and vigour of 12 accessions of kenaf.

Hydro-primimg duration	Arc sine Germination	Arc sine Germination	Germination rate $(d^{-1})$	Seedling length at 3 DAS (cm)	Seedling weight	Seed Vigour Index (SVI)
(hours)	3 DAS	7 DAS	fute (u )	ut 5 Drib (em)	at 3 DAS(g)	
Control	6.97b	8.67c	2.31c	2.15b	0.36b	5.04bc
24	16.42a	18.47a	5.39a	3.32a	0.25b	19.44a
48	7.10b	10.76b	2.37bc	2.60ab	0.51a	11.25b
72	10.26b	13.65bc	3.44b	0.76c	0.28b	2.82c
LSD <sub>0.05</sub>	3.803	4.018	1.095	0.835	0.123	5.71

Plots of interactive effects of genotype and hydro-priming durations showed that generally, seeds of 9 of the 12 accessions tested (75%) showed positive germination and vigour responses to hydro-priming especially for 24 hours (Figs. 1 and 2). Seeds of lines 35 and 19 primed for 24 hours had 31% germination the highest value at 3DAS, while the control seed lots of lines 29, 44, 10, 16 and 57 had less than 5% germination (Fig. 1a). At 7DAS, seeds of lines 35, 19, 31 and 10 that were primed for 24 hours had 31% cumulative germination (Fig. 1b), and the highest values of germination rates ranging from 6.5 in line10 to 10 in lines 35 and 26 (Fig. 1c). Seedling length of seeds of lines 35, 19, 29, 31,10 and 9 had seedling length above 3cm at 3DAS when hydro-primed for 24 and 48 hours (Fig. 2a). Seedling weight was consistently above 0.5 g in seeds of 8 kenaf accessions that were primed for 48 hours (Fig. 2b), while seed vigour index was highest (above 35) in the 4 accessions that exhibited germination above 30% when hydroprimed for 24 hours (Fig. 2c).



*Figure 1.* Interactive effects of hydro-priming durations and variety on kenaf seed viability and vigour. a.) Early seed germination, b.) Cumulative seed germination and c.) Germination rate. Non overlapping standard error bars indicate significant differences between mean values.



Kenaf accessions

*Figure 2.* Interactive effects of hydro-priming durations and variety on kenaf seed vigour. a.) Seedling length, b.) Seedling weight and c.) Vigour index. Non overlapping standard error bars indicate significant differences between mean values.

#### Discussion

Hydro-priming improved seed quality of kenaf in terms of early and cumulative seed germination, rate of germination and seedling vigour traits. The improvement in early germination of all the hydroprimed kenaf seed lots at 3 DAS than control seed lots is attributable to increased metabolic activities in the hydro-primed seeds. The underlying physiological background of seed hydropriming treatments is the triggering of metabolic activities that result in germination <sup>3</sup>. Varier *et al.* <sup>16</sup> identified the proteins which appear specifically during seed hydro-priming of Arabidopsis as degradation products of the storage protein 12S-cruciferin *b*subunits. Other reserve mobilization enzymes such as those for carbohydrates and lipids mobilization are also activated during priming <sup>10,13</sup>. These indicate that enzymes involved in mobilization of nutrients are either synthesized or activated during seed priming, suggesting the most plausible explanation for the stimulated germination and germination rate performances found in hydro-primed kenaf seeds.

Besides seed germination capacity, germination rate and all the seedling vigour traits were significantly improved by hydropriming for 24 hours. Seedling vigour has been reported to significantly contribute to plant height if adult maize hybrid plant<sup>4</sup>. The performance of seedling vigour is important for productivity of kenaf, since its economic yield is mainly fibre from the stem. As shown in this study, there are potential biomass yield benefits for kenaf with hydro-priming especially for 24 hours. Plant biomass yield and eventual vigour is a product of enhanced respiratory processes since respiration results in the release of energy for protein synthesis and growth, which aerated hydration procedure of priming offers the hydro-primed seeds. Corbineau<sup>7</sup> observed that imbibition of tomato seeds results in sharp increases in energy products from respiratory process like adenosine triphosphate (ATP), energy charge (EC) and ATP/ADP (adenosine diphosphate) ratio which remain higher in primed seeds even after drying than in unprimed seeds. This was the basis for the enhanced vigour of primed tomato seeds above the unprimed seeds <sup>7</sup>. In essence, hydro-priming has a scientific basis for enhancing kenaf seedling vigour and ultimate plant productivity.

However, the maximum cumulative percentage seed germination observed in this study was 31% which was recorded for 4 kenaf genotypes in response to 24 hours of seed hydro-priming (Fig. 2b). This suggests that there is room for further exploration of more priming treatments and priming duration to maximize the productivity of kenaf through seed treatment. Priming methods can be divided into two groups depending on whether water uptake is uncontrolled (hydro-priming) or controlled (osmotic and solid matrix priming)<sup>12</sup>. The performance of each priming procedure is crop specific, indicating need for intensified research for optimal priming techniques for kenaf seeds.

#### Conclusions

This study established the potential application of seed hydropriming for the improvement of seed quality traits in kenaf. Besides mitigating the effect of poor seed germination capacity in the tropics, the study demonstrated that hydro-priming improved seed vigour which in turn holds promise for significant improvement in crop productivity of kenaf in Nigeria. Within the limits of the treatments imposed in this study, hydro-priming of kenaf seeds for 24 hours optimised seed germination and vigour. However, investigations of seed hydro-priming treatment durations below 24 hours will be desirable for further optimization of hydro-priming treatments of kenaf seeds.

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## References

- <sup>1</sup>Banuelos, G. S., Mackey, B., Cook, C., Akohoue, S., Zambrzuski, S. and Samra, P. 1996. Response of cotton and kenaf to boron-amended water and soil. Crop Science **36**(1):158-164.
- <sup>2</sup>Banuelos, G.S., Ajwa, H. A., Mackey, B., Wu, L., Cook, C., Akohoue, S. and Zambruzuski, S. 1997. Evaluation of different plant species used for phyto-remediation of high soil selenium. Journal of Environmental Quality 26(3):639-646.

<sup>3</sup>Basra, S. M. A., Zia, M. N., Mehmood, T., Afzal, I. and Khaliq, A. 2002. Comparison of different invigoration techniques in wheat (*Triticum aestivum* L.) seeds. Pakistan Journal of Arid Agriculture 5: 11-16.

- <sup>4</sup>Berzi, T., Janda, T., Hegyi, Z. and Pintér, J. 2010. Effect of drought stress at flowering on the water potential and photochemical reactions of reciprocal maize hybrids. Acta Agronomica Hungarica 58(3):219– 226.
- <sup>5</sup>Bradford, K. J. 1986. Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. HortScience 21:1105-1112.
- <sup>6</sup>Carberry, P. S. and Abrecht, D. G., 1990. Germination and elongation of the hypocotyls and radicle of kenaf (*Hibiscus cannabinus*) in response to temperature. Field Crops Research **24**(3–4):227–240.
- <sup>7</sup>Corbineau, F., Ozbingol, N., Vineland, D. and Come, D., 2000. Improvement of tomato seed germination by osmopriming as related to energy metabolism. In Black, M., Bradford, K. J. and Vasquez-Ramos, J. (eds). Seed Biology Advances and Applications: Proceedings of the Sixth International Workshop on Seeds. Merida, Mexico, CABI, Cambridge, pp. 467–474.
- <sup>8</sup>Daniel, I. O. 1997. Conservation of West African Yam (*Dioscorea* spp.) Germplasm: Physiology of Seed and Pollen Storage. Ph.D.dissertation, University of Ibadan, Nigeria.
- <sup>9</sup>Daniel, I. O., Oyekale, K. O., Ajala, M. O., Sanni, L. O. and Okelana, M. A. O. 2009. Physiological quality of hybrid maize seeds during containerized dry storage with silica gel. African Journal of Biotechnology 8(2):181-186.
- <sup>10</sup>Ella, E. S., Dionisio-Sese, M. L. and Ismail, A. M. 2011. Seed pretreatment in rice reduces damage, enhances carbohydrate mobilization and improves emergence and seedling establishment under flooded conditions. AoB PLANTS 2011 plr007 doi:10.1093/aobpla/plr007.
- <sup>11</sup>Farooq, M., Basra, S.M.A., Tabassum, R. and Afzal, I. 2006. Enhancing the performance of direct-seeded fine rice by seed priming. Plant Production Science **9**:446-456.
- <sup>12</sup>Heydekker, W., Higgins, J. and Gulliver, R. L. 1973. Accelerated germination by osmotic seed treatment. Nature **246**:42-44.
- <sup>13</sup>Mir-Mahmoodi, T., Ghassemi-Golezani, K., Habibi, D., Paknezhad F. and Ardekani, M. R. 2011. Effects of priming techniques on seed germination and seedling emergence of maize (*Zea mays* L.). Journal of Food, Agric. & Env. 9(2):200-202.
- <sup>14</sup>Sung, F. J. M. and Chang, Y. H. 1993. Biochemical activities associated with priming of sweet corn seeds to improve vigor. Seed Sci. & Tech., 2:97–105.
- <sup>15</sup>SAS Institute 1997. SAS/STAT Software: Changes and Enhancement through release 6.12. Cary, NC.
- <sup>16</sup>Varier, A., Vari, A. K. and Dadlani, M. 2010. The subcellular basis of seed priming. Current Science 99(4):450-456.

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