Effect of Kinetin, Thiourea and Thiourea dioxide, Light and Heat on Seed Germination and Seedling Growth of kola (*Cola nitida* (Ventenant) Schott and Endlicher)[1]

Gbolahan A. Ashiru
Cocoa Research Institute of Nigeria, Ibadan

Abstract. The effects of kinetin—(6-furfuryl-aminopurine), thiourea, thiourea dioxide, light and heat on germination of kola seeds were studied. The effect of chemicals on the germination of kola seeds was found to be highly significant. High temperatures and light seem to have no direct effect on germination. Seedlings from both fresh and stored kola seeds were normal at growth but the subsequent growth was enhanced in seedlings from stored kola seeds. None of the chemicals appear to have completely substituted for the postharvest requirement of fresh kola seeds. Germination of kola seeds by this method would considerably reduce the time required for producing seedlings for planting and grafting and would provide a higher percentage of uniform seedlings.

Introduction

Kola seeds,[2] *Cola nitida*,[3] Schott and Endlicher, require a period of dormancy or after-ripening treatment for effective germination and good seedling growth. The exact period required has not been fully investigated. Freshly harvested kola seeds when sown take between 3 and 9 months to germinate while seeds stored for about 7 months usually germinate within 3 to 4 months after sowing (4).

Clay (2, 3) and Dublin (4) reported the uneven and slow rate of germination in kola seeds. This was attributed to the stages of seed maturity and their origin. Both authors suggested that seeds should be pregerminated to obtain seedlings of the same age.

Several chemicals have been reported effective in breaking dormancy in some seeds. Thiourea was found effective for breaking the dormancy in seeds of lettuce by Thompson (6) and peach by Tukey (7).

The cytokinins have been found to induce growth and germination (5). Badizadegan (1) found that N^6-benzyladenine (BA) significantly stimulated and increased the germination of mature 'McIntosh' embryos. Stored and fresh kola seeds were used to study the methods of inducing quick germination within a 30 days period to obtain uniform seedlings.

19. ————, Herman Amberg, and Donald Crowe. 1966. Response of Concord grapes to light, exposure, and Geneva Double Curtain training. *Proc. Amer. Soc. Hort. Sci.* 89:268–280.
20. ————, E. S. Shepardson, and T. D. Jordan. 1967. The Geneva Double Curtain for vigorous grapevines. *N. Y. S. Agr. Exp. Sta. Bul.* 811:1–21.
21. Shepard, Paul H. 1946. Concord Moore and Campbell grown on vigorous rootstocks in Missouri. *Mo. State Fruit Exp. Sta. Bul.* 32.
22. Taschenberg, E. F. 1965. Phylloxera, does it pose a threat to New York vineyards? *Farm Research* 30(4):2–3.
23. Vaile, J. E. 1937. The influence of rootstocks on the yield and vigor of American grapes. *Proc. Amer. Soc. Hort. Sci.* 35:471–474.
24. Winkler, A. J. 1962. General viticulture. Univ. of Calif. Press. Berkeley, Calif. 633 p.

Materials and Methods

Kola seeds were obtained from open pollinated pods of *Cola nitida* trees. The post harvest treatments given to the seeds include the removal of the fleshy seed coats by soaking in water for about one day and drying in the sun for another day. When stored, the seeds were wrapped in banana leaves and kept in baskets. Banana leaves were changed on the average of once in 2 weeks. The temperature of seeds in storage ranged between 27° and 29°C. Seeds stored for 7 and 11 months were used in the experiments and compared with fresh seeds which were stored for not more than 6 days from harvest. A randomized design was used for each experiment.

In the first experiment fresh and stored seeds were soaked in kinetin 25, 50, and 100 ppm, thiourea 1000, 2000 and 5000 ppm, thiourea dioxide 1000, 2000 and 5000 ppm and in distilled water as control for 24 and 48 hr. The seeds were washed in running water for 2 min and sown at a depth of 2 inches in a moist mixture of 1:1 sand and sawdust in germination boxes covered with clear polythene sheets to conserve moisture. Twelve kola seeds were used for each treatment. The temperature within the germination boxes in the propagation room was maintained at 37° and 39°C and the illumination at the surface of the medium was about 1000 ft-c. The experiment was replicated 4 times.

In the second experiment 18 fresh and stored kola seeds were soaked for 24 hr in kinetin 50 and 100 ppm, thiourea 1000, 2000 and 5000 ppm, thiourea dioxide 1000 and 2000 ppm and distilled water. After treatment the seeds were sown in the same way as in the first experiment in plastic trays covered with black polythene sheet. The trays were kept in a dark incubator with a preset temperature of 38°C for 20 days after which they were removed to an out-door shed for another 10 days.

Records of germination were taken 30 days after sowing. Not all the seeds which germinated after 30 days were included in the data for analysis. The seeds were taken as germinated when their roots attained a length of at least 3 inches. In both experiments, the seedlings were transplanted into the nursery for further observations on their growth pattern.

Results

There was a definite difference in the germination pattern of stored and fresh kola seeds. In the stored seeds, the radicle emerged within 10 days after sowing and continued growth while the plumule started growth about 12 days later. In the fresh seeds the emergence of the radicle was not noticed until after 18 days and the

[1]Received for publication October 25, 1968.
[2]The reproductive organ of kola, *Cola nitida* and *Cola acuminata* commonly called a 'nut' is botanically a seed. In *Cola nitida*, the seed is made up of a fleshy seedcoat, 2, rarely 3 fleshy cotyledons and an embryo, while in *Cola acuminata*, the seed is composed of a fleshy seedcoat, 2 to about 8 fleshy cotyledons and an embryo.
[3]*Cola nitida* (Ventenant) was re-classified in 1832 by Schott and Endlicher; Schott and Endlicher, *Melostemata 33*.

plumule did not show any sign of growth until about 15 days later. As a result the seedlings from the stored seeds tended to grow bigger than those from fresh seeds.

In the stored seeds, the cotyledons were forced open by the activities of the enlarging cotyledonary stalks within 22 days of sowing while in fresh seeds splitting of the cotyledons did not take place until after about 35 days. Many seedlings obtained from the fresh seeds continued growth without splitting the cotyledons (Fig. 1).

Both seed types produced normal seedlings after germination although the seedlings from stored seeds were more vigorous than those from fresh seeds. There were no visible differences in the root systems of the seedlings obtained from stored and fresh seeds (Fig. 2).

Thiourea dioxide at 5000 ppm was toxic to both seed types with more pronounced effect on the fresh seeds. Thiourea at 5000 ppm was effective in breaking dormancy and inducing germination in stored seeds but slightly toxic to fresh seeds.

In experiment 1, the high temperature of 38°C and light intensity in the propagation room did not give any significant increase in the percentage germination over open air sowing. Observations showed however that the rate of initial germination was higher in the propagation room than in the open air 20 days after sowing but this advantage was lost by the thirtieth day. Seedlings obtained from the propagation room showed greater vegetative growth as compared to those from open air sowing.

Soaking the seeds for 24 hr gave a highly significant result over soaking for 48 hr (Table 1). There was an interaction between soaking and seeds which was significant at 1% level. The adverse effect of the prolonged soaking in chemicals on seed germination was noticed more on the stored seeds than the fresh seeds. This was probably due to the fact that stored seeds absorbed more of the solutions than the fresh seeds. The average weight increase per stored seed at 24 and 48 hr soaking was 0.31g and 0.58g while for fresh seeds the increase was 0.20g and 0.42g.

Table 1. Effect of 24 and 48 hr chemical soaking on germination of kola seeds. 12 kola seeds were used for each treatment.

		Average number of germinated seeds		
		24 hr soaking	48 hr soaking	Average
Seeds		7.77**	7.35	7.56
Chemicals	(ppm)			
Kinetin	25	7.81	8.06	7.93
	50	8.75	7.93	8.34
	100	8.62	7.56	8.09
Thiourea	1000	8.37	8.00	8.18
	2000	8.87	8.50	8.68
	5000	7.75	6.75	7.25
Thiourea dioxide	1000	8.75	8.00	8.37
	2000	7.31	7.56	7.43
	5000	5.37	4.50	4.93
Distilled water	0	6.12	6.68	6.40

Significant difference at 1% level = 0.579 (Tukey's test).
**Significant at 1% level.

The analysis of results for chemicals showed a high level of significance (Table 2), while the interaction between chemicals and seeds was significant at the 5% level (Fig. 3A). Thiourea was found to be the most effective chemical in inducing germination and in giving the highest germination percentage (85.1%) in stored seeds while in the fresh seeds, kinetin was best (56.3%). All the chemicals gave better germination percentages compared with controls (Fig. 3A).

In the second experiment, both fresh and stored seeds showed active germination within 15 days of sowing. The analysis of data for chemicals gave highly significant results (Table 3) and was comparable to results obtained

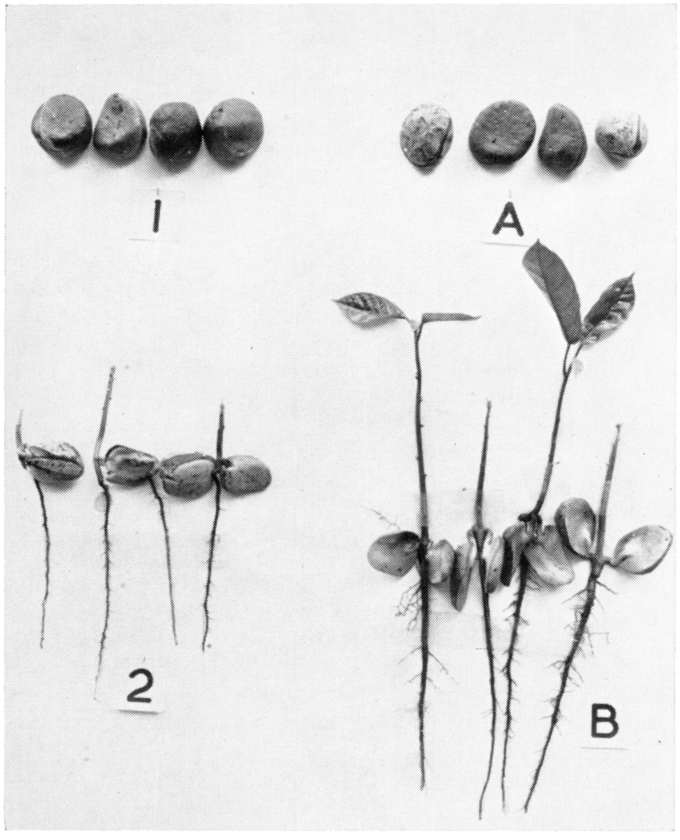

Fig. 1. Germination of kola seeds. *Top:* (1) Fresh seeds and (A) Stored seeds before sowing. *Bottom:* Seedlings obtained from (2) fresh seeds and (B) seeds stored 7 months, 6 weeks from sowing showing shoot development and degrees of cotyledon split.

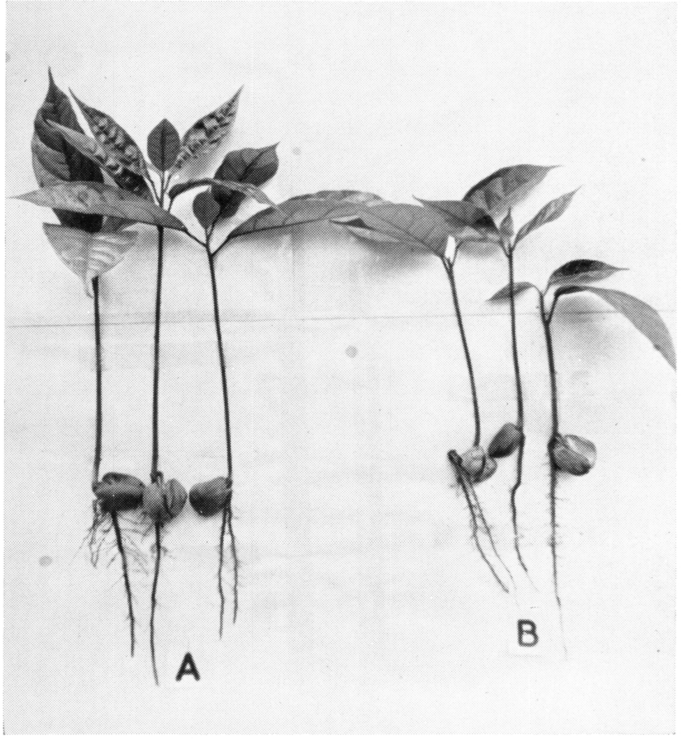

Fig. 2. Vegetative growth of kola seedlings, 10 weeks from sowing. (A) Seedlings from seeds stored 7 months, and (B) fresh seeds.

Table 2. Total number of germinated stored and fresh kola seeds: effect of chemical treatments on breaking dormancy. 12 kola seeds were used for each treatment.

		Average number of seeds germinated		
		Stored seeds	Fresh seeds	Average
Chemicals		9.21**	5.91	7.56
Chemicals	(ppm)			
Kinetin	25	9.25	6.62	7.93
	50	9.68	7.00	8.34
	100	9.50	6.68	8.09
Thiourea	1000	9.62	6.75	8.18
	2000	10.75	6.62	8.68
	5000	10.12	4.37	7.25
Thiourea dioxide	1000	9.62	7.12	8.37
	2000	9.25	5.62	7.43
	5000	6.50	3.37	4.93
Distilled water	0	7.87	4.93	6.40

Significant difference at 1% level = 0.258 (Tukey's test).
**Significant at 1% level.

Table 3. Total number of kola seeds germinated when incubated in the dark at 38°C for 20 days. Chemicals were applied to break dormancy. 18 kola seeds were used for each treatment.

		Average number of germinated seeds		
		Stored seeds	Fresh seeds	Average
Chemicals		13.81**	6.28	10.04
Chemicals	(ppm)			
Thiourea	1000	12.50	6.25	9.37
	2000	11.50	8.50	13.00
	5000	16.00	4.75	10.37
Thiourea Dioxide	1000	12.50	5.25	8.87
	2000	13.00	5.50	9.25
Kinetin	50	13.25	7.25	10.25
	100	16.50	7.75	12.12
Distilled water	0	9.25	5.00	7.12

Significant difference at 1% = 3.06 (Tukey's test).
**Significant at 1% level.

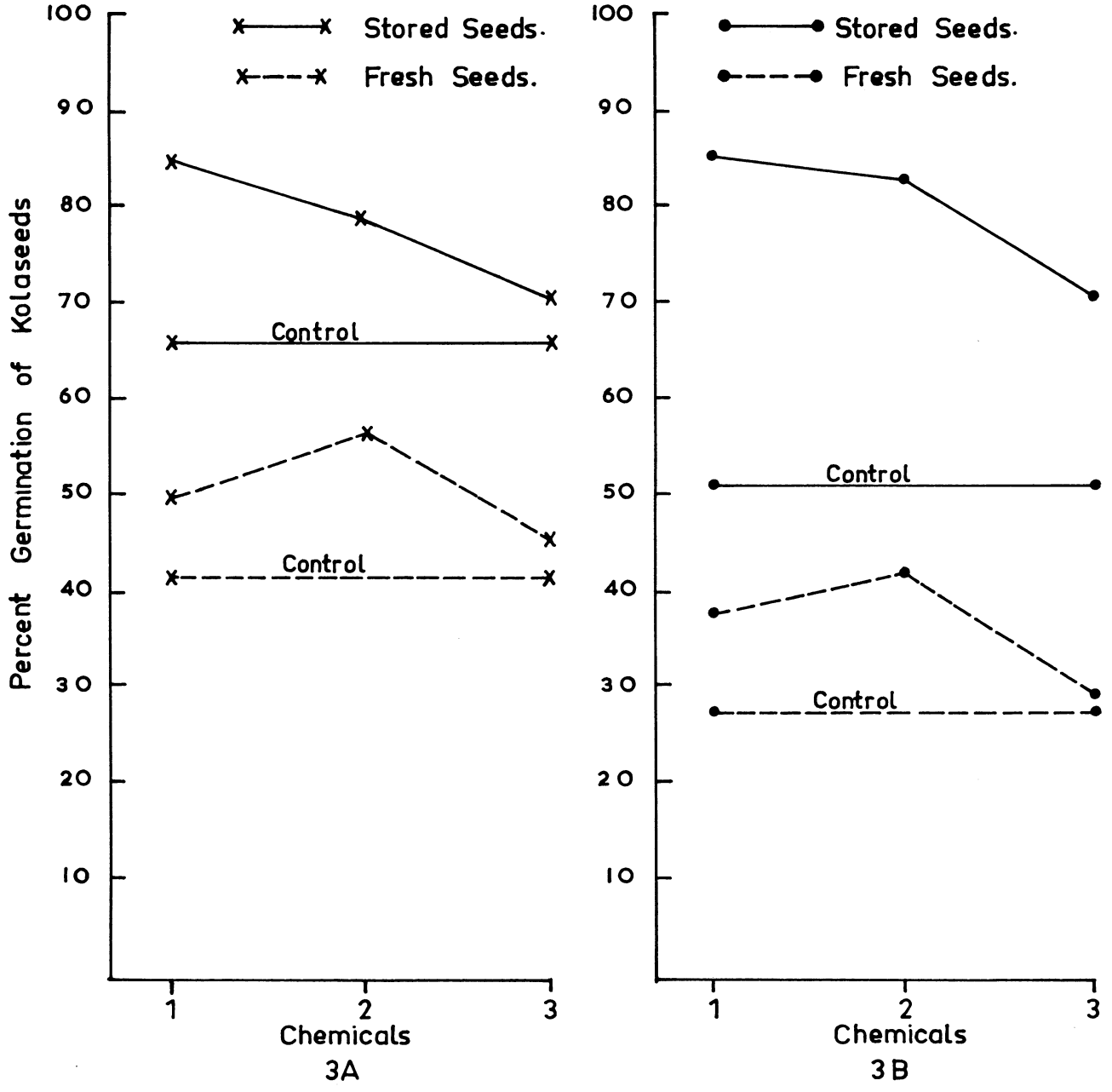

Fig. 3. The effect of thiourea (1), kinetin (2) and thiourea dioxide (3) on the cumulative percent germination of stored and fresh kola seeds in experiments 1 (3A) and 2 (3B). 432 and 504 kola seeds, stored and fresh were used in each chemical treatment in 3A and 3B respectively.

in the first experiment. Kinetin at 100 ppm and thiourea at 5000 ppm gave the best germination results in the stored seeds while thiourea at 2000 ppm and kinetin at 100 ppm gave the best result in the fresh seeds under the conditions of the experiment.

There was significant interaction between chemical treatments and seeds (Fig. 3B). The interaction effect of chemical and seeds in this experiment was very similar to what was obtained in experiment 1 (Fig. 3A).

Discussion

Kinetin, thiourea and thiourea dioxide were found to be very effective in inducing uniform germination in kola seeds. Except for thiourea dioxide the results were in close agreement with results obtained with same chemicals using other seeds Badizadegan (1), Thompson (6) and Tukey (7).

The exact physiological effect these chemicals have on the seed germination has not been determined. Since there was no visible change on the appearance of the large fleshy cotyledons, it seems very probable that the embryo which started enlarging shortly after treatment set up a chain of biochemical and anatomical changes in and around it, resulting in early germination.

Seedlings obtained from both stored and fresh seeds were normal contrary to some reports of anomalous growth of seedlings from seeds similarly treated with the same chemicals Badizadegan (1) and Tukey (7). Seedlings obtained from stored seeds were found to be more vigorous. This suggested that the chemicals either did not completely substitute for the postharvest requirements of the fresh seeds or that they gave additional stimulations to the seedlings obtained from stored seeds. Subsequent records on growth of both seedlings may throw more light on this aspect of their development.

Abnormally high temperatures and light intensities are not essential for germination though they may influence the initial rate of germination and subsequent seedling growth. Thiourea dioxide, though effective in inducing germination in kola seeds, is toxic to the seeds at higher concentrations. The use of thiourea to induce uniform germination in kola seeds is thus recommended.

The results of these experiments can readily be applied to large scale production of kola seedlings.

Literature Cited

1. Badizadegan, M., and Carlson, R. F. 1967. Effect of N^6 Benzyladenine on germination and seedling growth of apple *(Malus sylvestris). Proc. Amer. Soc. Hort. Sci.* 91:1–8.
2. Clay, D. W. T. 1960. Notes on nursery practises. *Min. Agr. Northern Nigeria Tech. Rept.* 13.
3. ————. 1963. Germination of the kolanut *(Cola nitida), Trop. Agr. Trinidad* 41:55–66.
4. Dublin, P. 1965. Le colatier *(C. nitida)* en Republique Centrafracaine, culture et amelioration. *Cafe, Cacao, Thé* 9:97–115.
5. Miller, C. O., F. Skoog, M. H. Von Sattza, and F. M. Strange. 1957. Kinetin, a cell division factor from deoxyribonucleic acid. *J. Amer. Chem. Soc.* 77:1932.
6. Thompson, R. C., and N. L. Horn. 1944. Germination of lettuce seed at high temperature (25 to 35°C) stimulated by thiourea. *Proc. Amer. Soc. Hort. Sci.* 45:431–439.
7. Tukey, H. B., and R. F. Carlson. 1945. Breaking the dormancy of peach seeds by treatment with thiourea. *Plant Physiol.* 20:505–516.