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# Study of germination conditions of cassava (*Manihot esculenta* Crantz) seeds obtained by genetic selection

**Adjata K. D.[1]*, Tchaniley L.[2], E. Banla[1], K. K. Tchansi[1] and Y. M. D. Gumedzoe[1]**

[1]Laboratory of Plant Virology and Biotechnology, High School of Agronomy / University of Lomé, P.O. Box 1515 Lomé, Togo.
[2]Département de production Végétale, Ecole Supérieure d'Agronomie / Université de Lomé, B. P. 1515 Lomé Togo.

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**Cassava (*Manihot esculenta* Crantz) is the third most important source of calories for human and animal in Africa. Its production is adversely affected by Cassava Mosaic Disease (CMD) which is the most important viral disease in cassava fields. To fight these virus diseases, it is significant to seek strategies of effective control while answering the taste of consumers. One of these strategies is to create new resistant/tolerant clones by genetic selection. However, in order to hasten the selection of these resistant/tolerant clones, it is important to know the germination conditions of these cassava seeds in order to have healthy planting materials for screening purposes in the field. With this purpose, a Split-Split-plot design was adopted to evaluate effect of various factors (sunning, germination medium and treatment by sulfuric acid, heat or cold) on the germination of seeds. The results were subjected to statistical analysis using GenStat and means were separated by Duncan's Multiple Range Test at 5% level of significance. These evaluation results revealed that germination of cassava seeds is influenced by degree of sunning. Amongst the treatments carried out, the treatment of the seeds by cold increased the germination percentage from 30 to 70%. The nature of the germination media used for seed germination in this study did not have any significant effect on the rate and speed of germination. The length of the germination can be reduced from 2 weeks-several months to 8-30 days under favourable conditions.**

**Key words:** Cassava seeds, clones, conditions, germination, selection.

## INTRODUCTION

Cassava (*Manihot esculenta* Crantz), of the family of *Euphorbiaceae*, is among the main starchy plants of Africa (Legg and Fauquet, 2004) and Asia (Hong et al., 1993; Saunders et al., 2002). It derives its importance from its storage root which is rich in starch and its leaves which is rich in protein. In Central Africa (Burundi, Congo, Central African Republic and Zaire) and, to a lesser extent, in West Africa, the leaf is used for human consumption, thus constituting an important source of protein.

While in some countries, cassava is behind foodstuffs such as rice, sorghum and yam, in many others (Congo, Central African Republic and Zaire) however, it is the staple food of the great majority of the population (Tsegai and Kormawa, 2002). Cassava is one of the leading food crops in tropical Africa and a tremendous asset for a region with food crisis. It also derives its importance from its great facility of cultivation and its various calorific products. The ethanol produced by fermentation of starch could totally replace petrol fuel. This is a major advantage in order to fight against pollution; all eyes are turning to biofuels (Ploypatarapinyo and Klinsukont, 1987; Nguyen et al., 2006).

World production of about 97 million tons in 1999 has

*Corresponding author. E-mail: dadjata@yahoo.fr

increased to 226 million tons in 2007. With 65.66% of the area planted of cassava, Africa accounts for about 94% of world production (FAO, 2008). The major producers in Africa are: Nigeria, Ghana, Benin and Togo.

In Togo, the yield in recent years is between 7 and 10 tons per hectare (for a crop with a theoretical potential of more than 80 tons per hectare) (DSID, 2008). This low efficiency is due to many biotic factors involving pests and disease-causing agents including viruses which occupy an important place (Legg and Thresh, 2003). Cassava mosaic disease (CMD) is the most devastating cassava disease causing a reduction of the production ranging from 5 to 95% (Fargette, 1985). The consequence of this results in the loss or abandonment of certain varieties and reduction of planted superficies (Legg and Fouquet, 2004). Since the input supply is scarce, the only way to raise the level of production is undoubtedly improved genetic potential of cassava in the development of new varieties that meet current growing conditions (Nassar and Ortiz, 2007). With this in mind, researchers have used the method of genetic improvement by hybridization to create a fairly large variability in cassava cultivars (Jennings, 1957; Hahn, 1980).

Thus, in the search for a strategy of effective control of CMD in Togo, the Laboratory of Plant Virology and Biotechnology (LPVB) initiated a program of resistant or tolerant cassava clone selection with initial results showing that it is possible to manage the disease through genetic creation of new clones (Adjata et al., 2011). However, the success of this program depends on the knowledge of the germination conditions of the hybrid seeds obtained after crossing. The present work which is part of the search for a strategy to fight against the CMD, aims at management of germination conditions of cassava seeds obtained by genetic selection.

## MATERIALS AND METHODS

### Plant materials

The plant materials used for the germination test are cassava seeds from the third generation of a back cross population harvested from female parents Kanigbeli Hybrid (KH). The male parents were Gbazekoutey Hybrid 1 (GH1), Gbazekoutey Hybrid 2 (GH2), Hybrid Bassar (HB) and Kanigbeli Hybrid (KH).

### Germination media

The seed germination media used are sand (S1); manure of *Cassia siamea* leaves (S2); manure of *Leucena leucocephala* leaves (S3); manure of cassava leaves (S4): and vermiculite S5).

### Effect of light on germination

The sown media were placed under sunlight (E1) and under screen house (E2) where the rate of light was almost zero.

### Preparation of seed germination media and treatment of seeds

All the germination media were sterilized with hot water before use. The treatment of seeds was done using sulfuric acid (T3), heat (T2) or cold (T1). For treatment with sulfuric acid (T3) or hot water (T2), the seeds were soaked either in concentrated sulfuric acid or water at 100°C for 5 s and then rinsed with water before sowing. For treatment with cold (T1), the seeds were placed in a refrigerator at 4°C for 24 h before sowing.

### Experimental layout

Seeds were sown in five prepared substrates of germination media using a split plot in a randomized complete block design.

### The studied factors

The studied factors were sunlight, the germination medium, seed treatment and the interaction between the different studied factors.

### The studied parameters

The studied parameters were ratio and speed of germination. The rate of germination was determined using the emergence of seedlings data. Number of germinated seeds was counted each day for 8 weeks. Speed of germination was based on the shortest period of time over which the maximum number of seeds germinated.

### Statistical analysis

The results were subjected to statistical analysis using GenStat and means were separated by Duncan's Multiple Range Test at 5% level of significance. Excel 2003 software was used for data processing requiring a graphic illustration.

## RESULTS AND DISCUSSION

### Germination rate and the effect of sunlight on the germination of seeds

The counting of germinated seeds from the emergence on the eighth day until the 60th day after sowing showed that germination was faster and at higher rate under sunlight (E1) than under screen house where the rate was almost zero. Statistical analysis of the variance revealed a significant difference between the two levels of the light factor ($P < 0.01$). The separation of the averages by Duncan's test showed that the germination rate "under the sunlight" (E1) was significantly higher than "under screen house" (E2) which is almost zero until the end of the experiment. The results of the evolution of the speed of germination of cassava seeds over time (Figure 1) showed that the germination started eight days after sowing and continued until the 30th day in the plots under sunlight (E1). In the plots under screen house (E2), germination started 13 days after sowing and continued until 20 days.

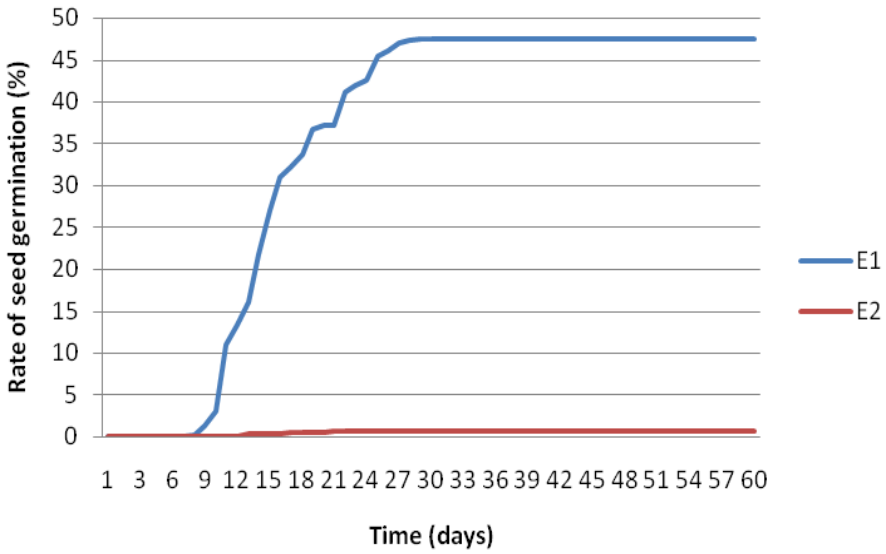


**Figure 1.** Effect of sunlight on the speed of germination of cassava seeds.

**Table 1.** Average number of germinated seeds for different seed treatment methods.

| Treatments | Average number of germinated seeds | Standard error |
|---|---|---|
| Cold (T1) | 219.67 | 1.25 |
| Heat (T2) | 125.0 | 2.45 |
| Sulfuric acid (T3) | 86.33 | 1.25 |

The result of the effect of sunlight on the speed of germination of cassava seeds (Figure 1) also shows that most of the seeds have germinated in the plots under screen house (E2) between the 10 and 20 days after sowing. It should be noted that the germination rate was virtually zero in the plots under the screen house (E2) where there was not enough light.

## Effect of treatment factors on the rate and the speed of germination

### *Effect of seed treatment on germination*

The results of seed treatments on germination rate (Table 1) show that the average number of germinated seeds is higher with the treatment by cold (T1). Similarly, higher rate of germination was obtained with the treatment by heat (T2) than treatment by sulfuric acid (T3). The results of the analysis of the variance of the data concerning the effect of seed treatment on germination rate showed a highly significant difference between treatments ($P < 0.05$). Separation of averages by Duncan's test showed that the germination rate is significantly higher with the treatment of seeds by cold compared to the other two treatments which are statistically equivalent.

### *Effect of seed treatment on the germination rate*

The results of the evaluation of germination over time in relation to the different treatments as illustrated in Figure 2 show that seed germination began 8 days after sowing with cold treatment and continued until the 28$^{th}$ day with a maximum rate of germination of 74.67% 28 days after sowing. Germination started 10 days after sowing with seeds treated with heat, and continued until the 30th day with a maximum rate of germination of 41.67%. Seeds treated with acid, started germinating 10 days after sowing and continued for 15 days with a maximum rate of 28%. The observation of the three curves shows that most of the seeds sprouted between the 8 and 30 days.

## Evaluation of the rate and speed of germination relative to the germination medium

### *Effect of germination medium on the germination rate*

The results of the effect of germination medium on the germination rate (Table 2) showed that the highest average number of sprouted seedlings was recorded with the manure of *Leuceana* leaves while the lowest germination rate was obtained with the manure of *Cassia*

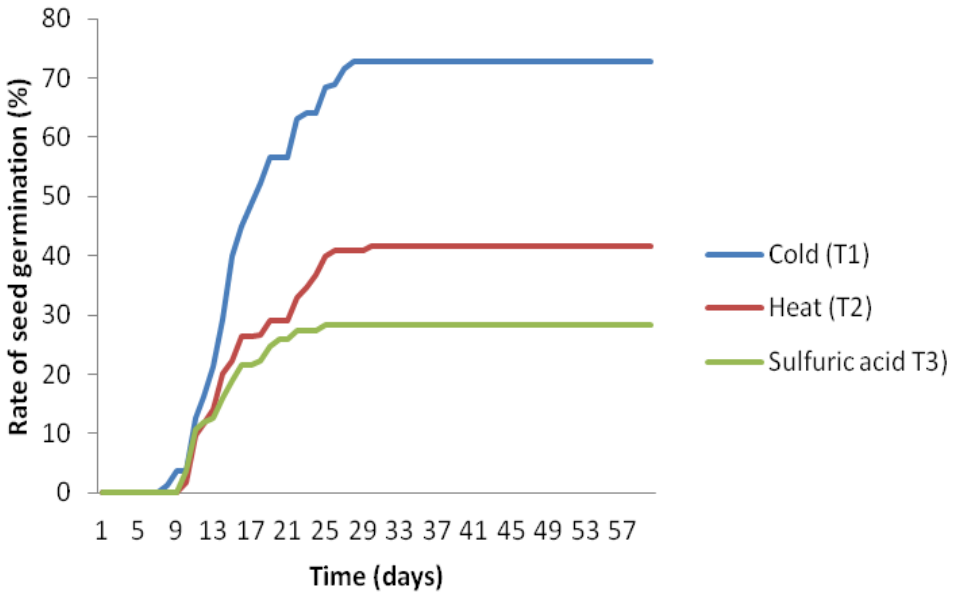


**Figure 2.** Effects of different seed treatment methods on germination rate of cassava seeds.

**Table 2.** Effect of germination media on seed germination rate.

| Germination medium | Average number of germinated seeds | Standard error |
|---|---|---|
| Sand | 80.33 | 1.25 |
| Cassia leaves | 72.33 | 2.49 |
| Leuceana leaves | 102.33 | 2.05 |
| Cassava leaves | 96.67 | 1.25 |
| Vermiculite | 77.33 | 2.05 |

leaves. The analysis of the variance ($P < 0.05$) revealed that there was no significant difference among the different germination media.

### *Effect of germination media on the speed of germination*

The results obtained from the effect of germination media on the rate of germination (Figure 3) showed that the germination began eight days after sowing on germination medium S1 (sand) and S2 (manure of *Cassia* leaves) whereas on manure of *Leuceana* leaves medium (S3) and Cassava leaves medium (S4), it began two days later. Germination lasted about 20 days regardless of the germination medium type. It was also noted that the germination rate after 30 days was greater than 30% regardless of the germination medium.

### Interaction between the different studied factors

There was significant interaction between seed treatment and sunlight factors in this study.

## DISCUSSION

### Effects of sunlight on the germination of seeds

The study carried out on germination conditions of cassava (*M. esculenta* Crantz) seeds obtained by genetic selection showed that sunlight has an undeniable effect on the germination of cassava seeds and a high rate of germination was obtained when the sowed seeds were placed in sunlight. The germination rate was virtually nil when the seeds were placed under screen house in the shade and received only a weak solar radiation. In addition, at the end of the experiment, the seeds that were in the shade, when placed in an open area, started sprouting after 8 days. All these observations show that germination is influenced by the degree of sunlight. It was noted that germination began earlier in sunlight 8 days after sowing and lasted for about 30 days whereas germination started 13 days after sowing in the screen house. The light rays thus have a beneficial effect on the germination of seeds because the germination rate obtained is much higher than the 30% usually encountered (Lefèvre, 1989). In addition, the fact that the germination rate is practically zero in the screen house

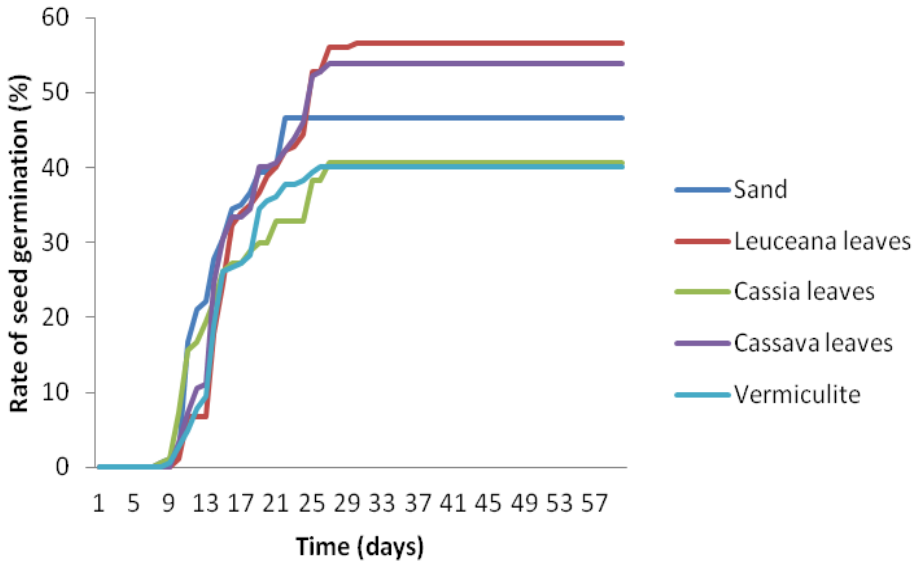


**Figure 3.** Effects of different germination media on germination rate of cassava seeds.

suggests that the absence of light ray is a limiting factor for the germination of cassava seeds.

### Effect of seed treatment on the germination

The highest germination rate of 73% obtained with the seeds treated with cold at 4°C for 24 h indicates that cassava seeds germination could be enhanced better with lower temperatures compared to 15 to 20°C for 8 to 16 h proposed by (Nassar and Teixeira, 1983). It has been observed during our experience that treatment by cold coupled with an optimum sunlight increases the rate germination of the seeds. This could explain the effect of light rays reaching the ground that heats it and creates the heat necessary for seed germination. Low germination rate recorded in seeds treated with acid indicates that sulfuric acid does not have a positive effect on the germination of cassava seeds. This result is inconsistent with the one reported by Lefèvre (1989).

### Effect of germination medium on the rate of germination of cassava seeds

The germination rate obtained at different germination media under the light rays is greater than 30%. However, statistical analysis revealed that there was no significant difference in the effects of these different media. This shows that the nature of the medium has no influence on the germination of cassava seeds. The only factor that has influenced cassava seeds germination during this study was the accessibility of sunlight. When the results obtained on equivalent germination media is taken into account, we note that percentages of germinated seeds depend essentially on the availability of light. For instance, under sunlight (E1), the percentage of germination was 47.5%, whereas under screen house (E2) this percentage is less than 1%. This is a real improvement since germination usually lasts two weeks to several months (Lefèvre, 1989). Given that the nature of the germination medium has no significant effect on the rate and speed of germination, the improvement could result from the interaction of the effects of light and seed treatment. It is important to notice that the lower and upper temperature thresholds for germination were not encountered in this study, however, effect of low and high temperatures among seeds in germination sensitivity were observed as it was reported by Orozco-Segovia et al. (1996); Kebreab and Murdoch (2000) and; Grundy et al. (2000) in the case of *Melastoma malabathricum* (*L.*) in Malaysia.

### Conclusion

It appears from this study on the conditions of germination of cassava seeds that the degree of sunlight has a positive influence on the ratio and the speed of seed germination. A high degree of sunlight could increase the speed and improve the germination rate.

The treatment of seeds by cold at a temperature of 4°C for 24 h can also increase the germination rate of cassava seeds to 70%. The nature of the medium has no effect on the ratio and speed of germination of cassava seeds. It was also discovered that germination could be extended on 30 days if conditions are favorable and most cassava seeds germinate between the 10th and 20th days if all requirements are met. Through this study, it could be said that the major problem which is the

uncertainty of handling cassava seed germination conditions is overcome and that now it will be possible to hasten cassava improvement programs in Togo.

## ACKNOWLEDGEMENTS

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