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THE INFLUENCE OF MOISTURE AND TEMPERATURE OF THE GERMINABILITY AND LONGEVITY OF TEA (*CAMELLIA SINENSIS* L.) SEEDS

A. R. Sebastiampillai & T. I. Anandappa

(*Tea Research Institute of Sri Lanka, Talawakele, Sri Lanka*)

The dry matter content of seeds of six tea clones was different and is probably determined genetically. The moisture content of fresh seeds expressed on a dry weight basis varied between 70-85%. The critical moisture level for the maintenance of high viability was around 28% with germination over 80%. Below this moisture level, the embryos were permanently damaged, so that subsequent resorption of water by the seed to the original moisture level did not improve its germinability. Fresh seeds were successfully stored at 100% RH and at a temperature of 5-7°C for 9 months. Tea seeds with a high moisture content could not be frozen at -2°C without injury to the embryo.

INTRODUCTION

Germinability and longevity of seeds are prime considerations in programmes that involve the development, multiplication and distribution of improved varieties of crop plants propagated from seed. The use of commercial tea seed has now assumed less importance in Sri Lanka since the successful development of vegetative propagation techniques. During the development phase of plant improvement, storage and improved germination of seeds play an important role, even in a vegetatively-propagated crop such as tea. All of the seeds necessary for experimentation may not become available at the same time in a perennial plant which has a long generation time. It has often become necessary to store the early batches of seed obtained from hybridization projects. The successful storage of seed is, therefore, an important and integral part of a tea breeding programme.

Tea seeds taken from freshly collected mature fruits germinate readily without any indication of dormancy. Germination commences at about the second week from sowing and a l viable seeds complete germination by about the eighth week. The earliness and the rate of germination is predominantly determined by the hard seed coat surrounding the cotyledons and the embryo (Gadd 1928; Leach 1936; Visser & Tillekeratne 1958). This association has been attributed to the impermeability of the seed coat to water (Tubbs 1932). The moisture content of seed has been shown to play an important role in the storage and viability of tea seeds (Leach 1936). These findings draw attention to the possible significance of the moisture status of the seed in germination. Any attempt at storage of tea seed should therefore take into account the high moisture requirement of the seed for the maintenance of viability.

Bulk storage of seed for about six months has been demonstrated by Leach (1936) in sand pits while Hume (1955) successfully stored seed in wooden containers at 20 – 40°F. The germination of experimental samples stored at O°C and 100% RH for a period of seven months has been given as above 70% (Visser & Tillekeratne (1958), while Gomez (1966) reported the successful storage of tea seeds in sealed polythene bags at 7°C for a period of more than three months.

In attempting to extend these studies further and to modify the conditions for prolonged storage of tea seeds some interesting results were obtained (Sebastiampillai; 1972). This paper reports these findings and attempts to evaluate the role of moisture on the germinability and the viability of stored seeds.

MATERIALS AND METHODS

Fruits were collected from seed bearers at St Coombs (1200 m amsl) and the seeds were extracted soon after collection. All cracked and immature seeds were discarded and so also were the floaters. Fresh weight determination of samples was made without delay. Dry weight was determined after drying the seeds initially for 24 h at 110° C and thereafter to a constant weight. The moisture content of seed was expressed as a proportion of the dry weight in all experiments. Germination of seeds was carried out in sand beds in an outdoor nursery. Seeds with emerging radicles were counted as having germinated.

EXPERIMENTS & RESULTS

Experiment 1— The variation in the dry matter content of seeds of six clones

Six replicated samples of 20 seeds each, from each of six clones were weighed soon after collection. The dry matter content of the seeds was determined by the method described earlier. Additional samples of 20 seeds each in triplicate from each clone were sown in sand beds and observations on germination were made at weekly intervals.

The results indicate that the dry matter content expressed as a percentage of the fresh weight was different for the six clones, with a corresponding variation in the moisture content of the seeds (Table 1). The differences in the dry weight of seeds were small, but were statistically significant.

TABLE 1 — *The dry matter content, moisture percentage and germination of seeds from six clones*

Clones	Dry weight of seed (%)	Moisture content (% of dry weight)	Germination (%)
TRI 2025	58.83	69.9	92
TRI 2024	57.80	73.0	90
TRI 2023	56.52	76.9	98
TRI 777	55.66	79.7	94
TRI 2026	54.62	83.1	96
TRI 2043	53.97	85.2	90
LSD ($P < 0.05$)	0.64	1.4	

The final germination percentage of the seeds at the end of eight weeks was over 90% in all six clones.

Experiment 2— The rate of germination of seeds with intact seed coat *vs.* cracked seed coat

Fresh seeds were soaked overnight in water. A quantity of this seed adequate for the experiment was wiped dry of surface moisture and put out in the sun for 30 minutes The cracked seeds were periodically removed into the shade and kept in water until a sufficient number of seeds were gathered for the experiment. Samples of 60 cracked seeds each, and replicated three times were sown along with similar samples with intact seed coats. Examination of seeds for germination commenced on the 10th day from sowing. The samples were examined again on the 14th day and thereafter at weekly intervals until the 9th week.

The cumulative germination percentages for the two treatments are presented in Fig 1. At the time of the first examination for germination (ten days from sowing), about 25% of the seeds with cracked seed coats had germinated. Gemination of seeds with intact seed coats, however, commenced on or about the 14'h day but 25% germination was attained only on the 22nd day. The seeds with intact seed coats lagged behind by about two weeks to attain the same germination percentage as that of seeds with cracked seed coats. This difference was maintained throughout until a 90% germination was attained by both treatments. This occurred during the 6th week in the treatment with cracked seed coats while the seeds with intact seed coats took eight weeks to attain the same germination percentage. The final germination percentages observed at the end of nine weeks in the two treatments were 97% and 98% respectively.

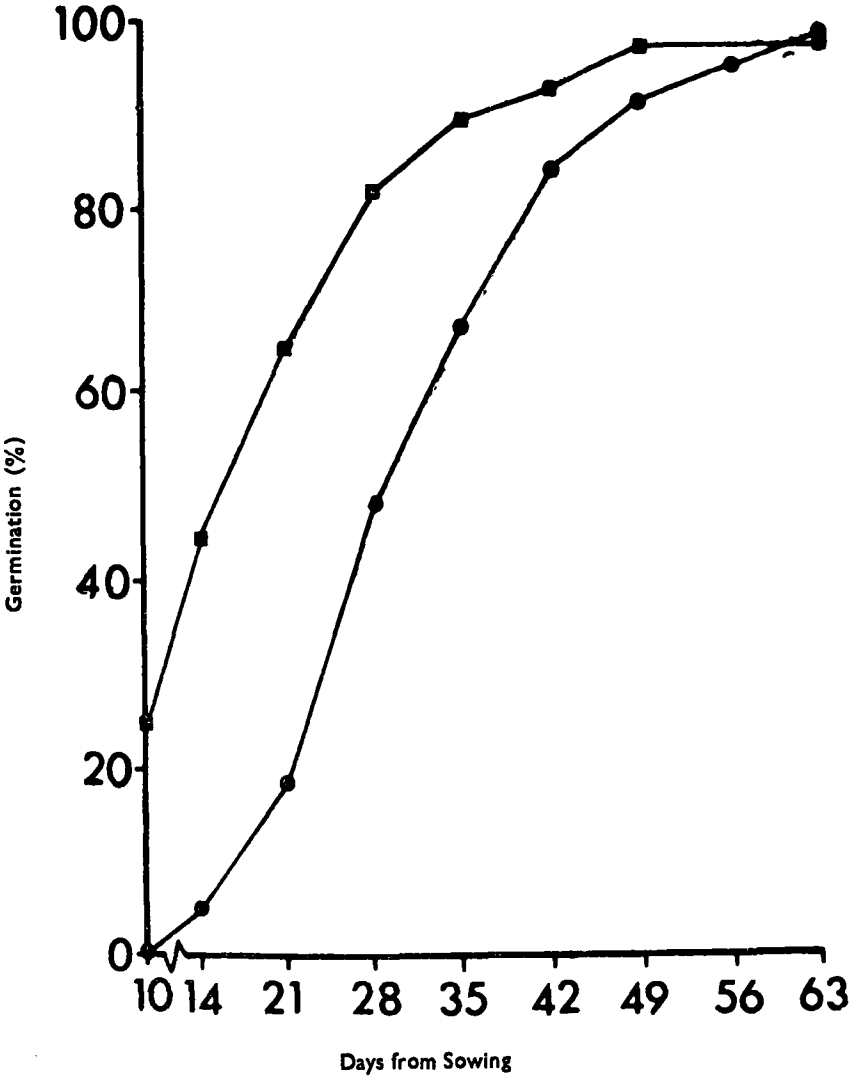

FIG. 1—*Germination of seeds with cracked and intact seed coat*

(■——■ = Cracked seeds; ●——● = intact seeds)

Experiment 3— **The effect of pre-soaking of seeds in water on germination**

Triplicate samples of 20 fresh seeds each, with an initial moisture content of 83% were immersed in water for different periods. Three samples were taken out at daily intervals for seven days, wiped dry of surface moisture and rapidly weighed to determine the increase in moisture. The weighed samples were immediately sown in sand beds. A record of germination was maintained from the second week onwards, at weekly intervals, after having made allowance for the number of days the seeds were in the soaking medium.

The moisture content of seeds soaked in water for different periods and the corresponding total germination percentage at the end of nine weeks for each of the treatments is given in Fig. 2. The increase in moisture content with time is gradual and small, the gain being about 14% over the control at the end of seven days. The moisture content of seeds pre-soaked for four days was 94% and thereafter there was no increase upto seven days of soaking. The total germination percentage at the end of nine weeks was unaffected by the immersion treatments, except for a small decrease in the treatment pre-soaked for seven days. This lowering of germination is perhaps due to microbial infection setting in with prolonged soaking. Infection of the seed occurred despite a daily change of the soaking medium.

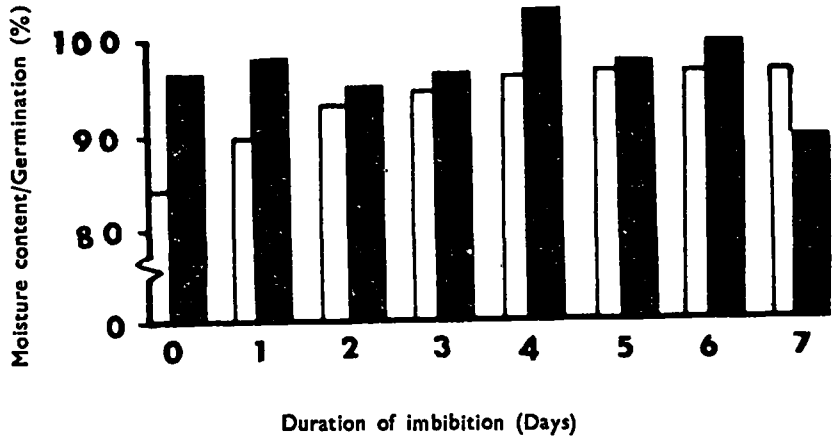

FIG. 2—*The relationship between moisture content* (□) *and germination* (■) *of pre-soaked seeds.*

The cumulative weekly germination percentages for the treatments pre-soaked in water for 0, 4 and 7 days are shown in Fig. 3. Soaking of seeds in water prior to germination enhances the rate of germination. The increased rate of germination of pre-soaked seeds is probably associated with the early cracking of the seed coat, observed in the treatments presoaked for more than four days. The difference in the rate of germination of seeds pre-soaked for four days to seven days was small and therefore there is no advantage in pre-soaking seeds for more than four days prior to sowing.

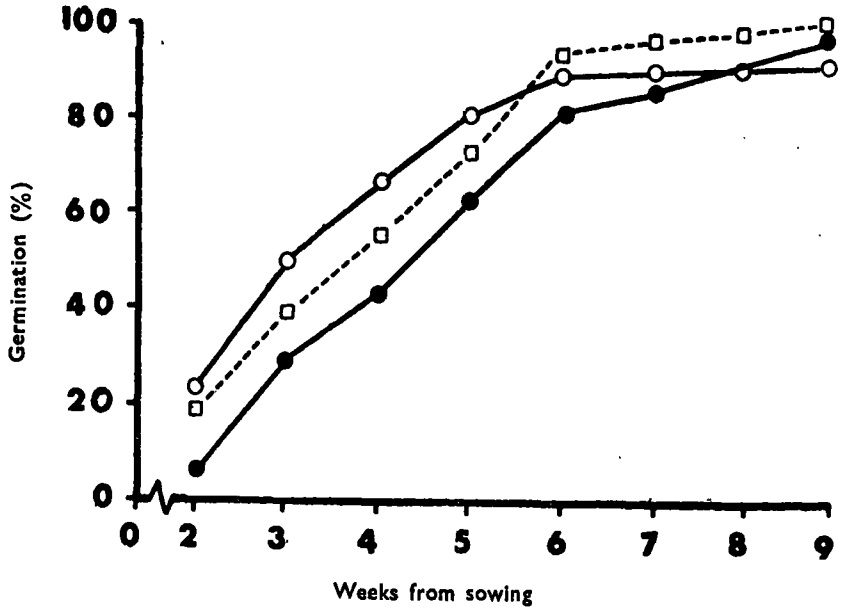

FIG. 3—*Rate of germination of seeds pre-soaked in water for different periods.*
(●—● = Control; □———□ = 4 days; O—O = 7 days)

Experiment 4— **Moisture loss and germination of fresh seeds at room temperature**

Several samples, each consisting of 20 seeds with an initial moisture content of 73% were weighed rapidly. One batch of samples was exposed to room temperature (23–27°C) in open Petri dishes. A second set of samples was enclosed in polythene bags with holes punched all round and similarly exposed to the conditions of the laboratory. Each sample was weighed daily and the samples, each with known moisture content, were sown at frequent intervals. The number of germinating seeds was recorded at weekly intervals, commencing from the 3rd week from sowing.

The moisture content of the seeds in the two treatments and the final germination percentages at the end of 8 weeks for the different moisture regimes have been plotted against time in Fig. 4. The moisture content of seeds stored in ventilated polythene bags decreased gradually and attained a moisture content of 20% in 30 days. Seeds stored in open petri dishes lost moisture very rapidly and reached a moisture level of 20% in six days. An equilibrium moisture content of 13% in this treatment was attained on the 10th day.

The corresponding germination percentages for each of the different moisture regimes in the seed indicates that the loss in germinability was only small until the moisture content dropped to below 28-30% in both treatments. The germination percentage of seeds with a moisture content greater than 28% was more than 80%. Thereafter, there was a significant decline in the total germination and the seeds were inviable when the moisture level dropped to below 13%.

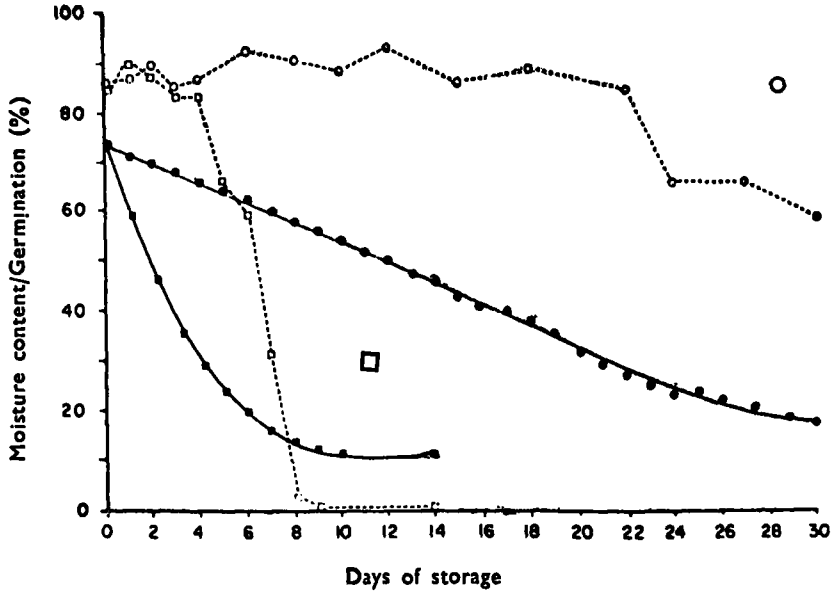

FIG. 4—*Moisture loss* (■—■ = open petri dishes; ●—● = punched polythene bags) and germination (□—□ = open petri dishes; O—O = punched polythene bags) of seeds stored at room temperature

Experiment 5— **The rate of resorption of moisture by seed dried to different moisture levels and their germination**

Samples of 20 seeds each with an initial moisture content of 73% were dried in an oven at 35–38°C. Samples were withdrawn from the oven at frequent intervals and weighed rapidly. By repeating this procedure, the moisture in the samples were reduced to 64, 49, 31, 21 and 12% respectively. Samples in triplicate for each of the different moisture regimes were put out for germination. Another set of samples whose moisture content was reduced to the same levels were soaked in water for varying periods until the seeds regained their original moisture content. Thereafter the samples were sown in germination beds.

It was observed that seeds initially having a higher moisture content were the first to reach maximum saturation (Fig. 5). Seeds with an initial moisture content of 73% reached a saturation moisture level of 85% in three days while seeds dried to less than 20% moisture failed to achieve the same level of saturation even after seven days. However, imbibition of water by seeds with an initial moisture content of less than 20% was quite considerable, and attained 72% moisture in seven days.

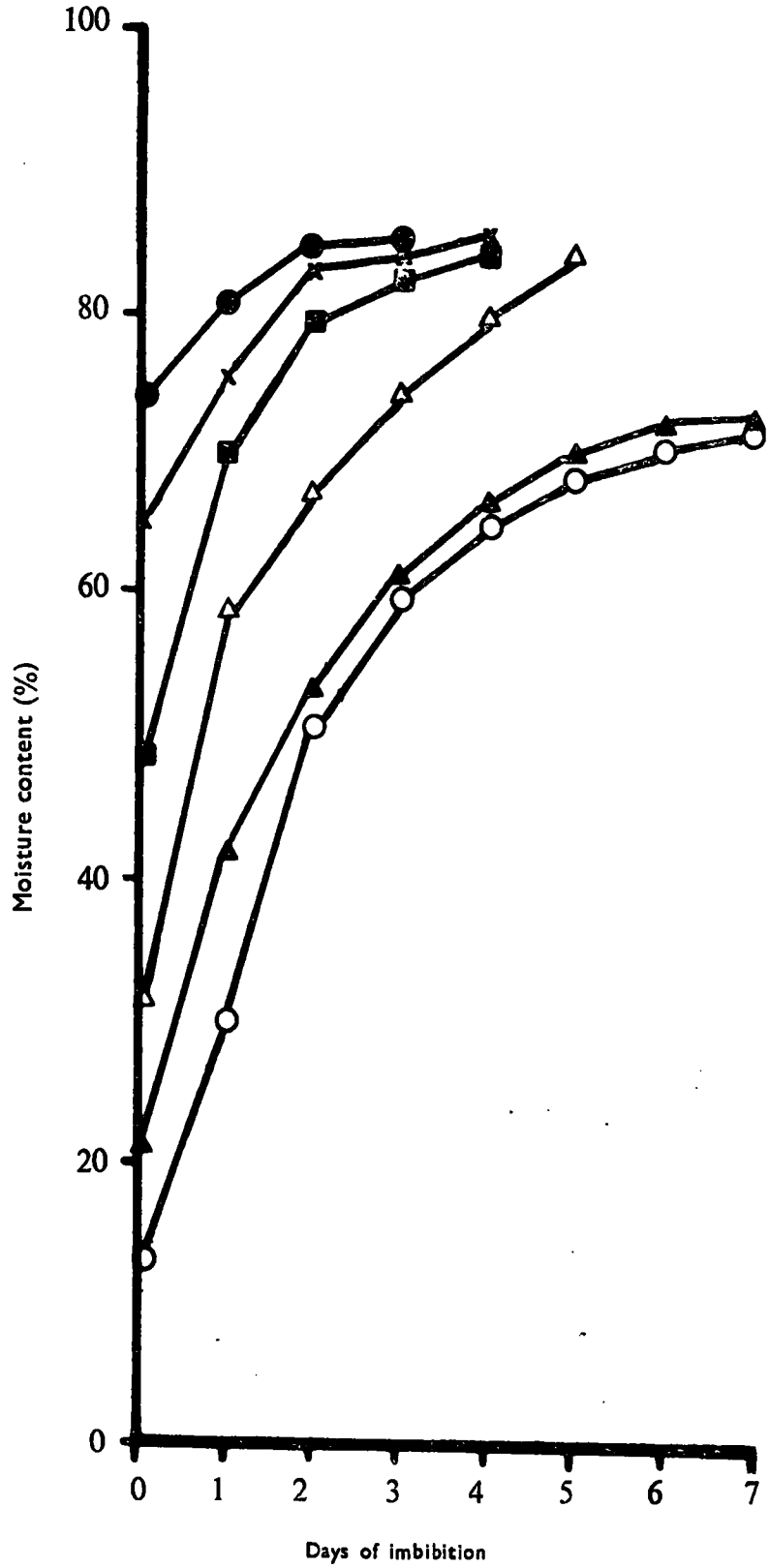

FIG. 5—*The rate of resorption of moisture by seeds with different initial moisture contents (12, 21, 31, 49, 64 and 73%)*

The final germination percentages for each of the treatments before and after resorption of water is shown in Table 2.

TABLE 2 — *Moisture content of seeds before and after water imbibition and their germination percentages*

Initial moisture content (%)	Germination (%)	Moisture content after imbibition %	Germination (%)
73	93	85	96
64	90	85	92
49	81	83	80
31	80	83	79
21	61	72	57
12	0	72	0

Imbibition of water by seeds did not significantly alter the final germination percentage of seeds. Seeds with an initial high germination percentage continued to maintain this high germination after imbibition of water. Seeds with a lower initial moisture content and a lower germination percentage imbibed a considerable amount of water, but this increase did not improve their germination capacity.

Experiment 6— **Viability of seeds stored at different temperatures for four weeks**

Several samples of 30 seeds each were subjected to the following storage treatments:

(a) Surface moisture on the seeds were wiped off and stored in open petri dishes in the laboratory, (22-27°C).

(b) Wet seeds, sealed in polythene bags and stored under laboratory conditions, (22-27°C.)

(c) Wet seeds sealed in polythene bags and stored in the refrigerator at 5-7°C.

(d) Wet seeds sealed in polythene bags and stored at -2°C.

Three samples of 30 seeds each sown at the beginning of the experiment served as control. Triplicate samples of the four treatments were drawn at weekly intervals and sown in sand beds. The number of germinated seeds were counted at the end of eight weeks from sowing.

The total germination recorded for each of the different treatments at the end of eight weeks is given in Fig. 6. Wet seeds stored in sealed polythene bags at 5-7°C (treatment b) continued to maintain its initial high germination of 90% even after four weeks. Seeds stored in open petri dishes (treatment a) and at -2°C (treatment d) became non-viable within two weeks. The loss in viability of seeds stored in polythene bags at room temperature (treatment, b) was gradual and 60% germination was recorded at the end of four weeks of storage. A small proportion of seeds in treatment (b) were also found to have cracked seed coats and there were visible signs of bacterial and fungal infection from about the second week. This suggests that the conditions of storage in treatment (b) are conducive to germination and that seeds cannot be stored for prolonged periods at high humidity and high temperature.

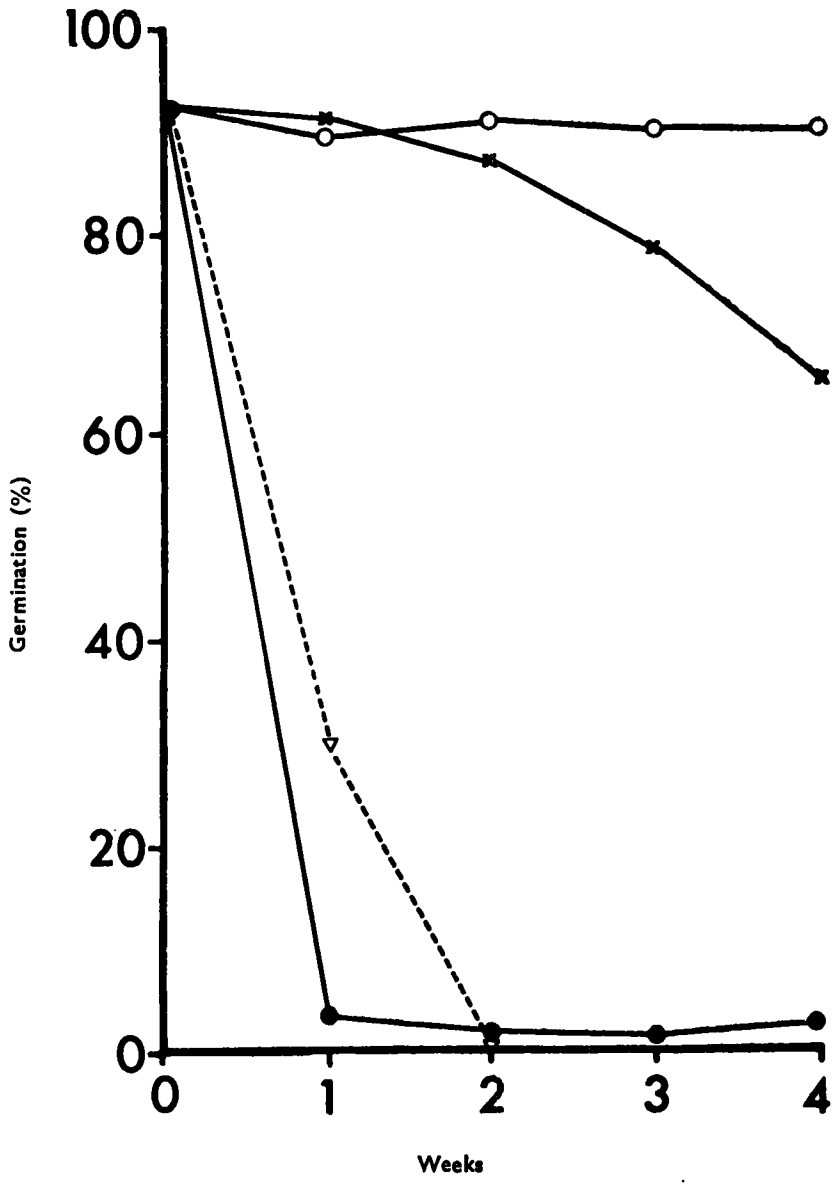

FIG 6—*Effect of Temperature of Storage on germination of seeds*
(●—● = *sealed bags at -2°C;* O—O = *Wet seeds in bags at 5-7°C ;* ∇—∇ = *Open dish at 22-27°C and* X—X = *Wet seeds in bags at 22-27°C*)

Experiment 7— **Long-term storage of seeds**

Fresh seeds were thoroughly washed in running water and the wet seeds were sealed in clean polythene bags. The bags were stored in the refrigerator maintained at 5-7°C. Triplicate samples of 30 seeds each were drawn at monthly intervals and germinated in sand beds.

The total germination percentage recorded at the end of nine weeks is presented in Fig. 7. The final germination percentages remained unaffected by this special storage conditions over a nine-month period. The combined cumulative germination percentages for all the stored samples, the unstored seed (control) and the samples stored for three months are given in Fig. 8. The stored seeds germinated earlier than fresh unstored seed, the rate being most rapid in seeds stored for three months. The rate of germination was variable for seeds stored for different periods. This difference in germination rates of stored samples is probably due to the different environmental conditions under which the stored samples were germinated.

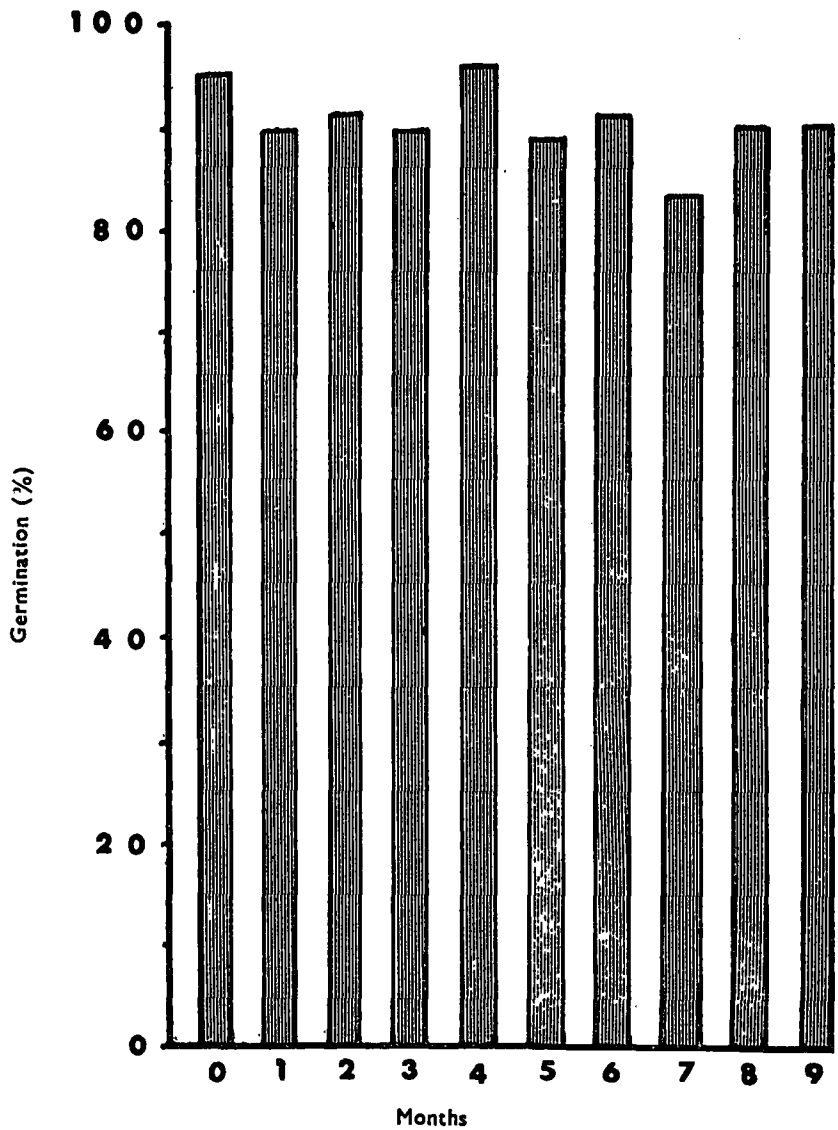

FIG. 7—*Effect of duration of storage in sealed bags at 5-7 °C on germination of seeds*

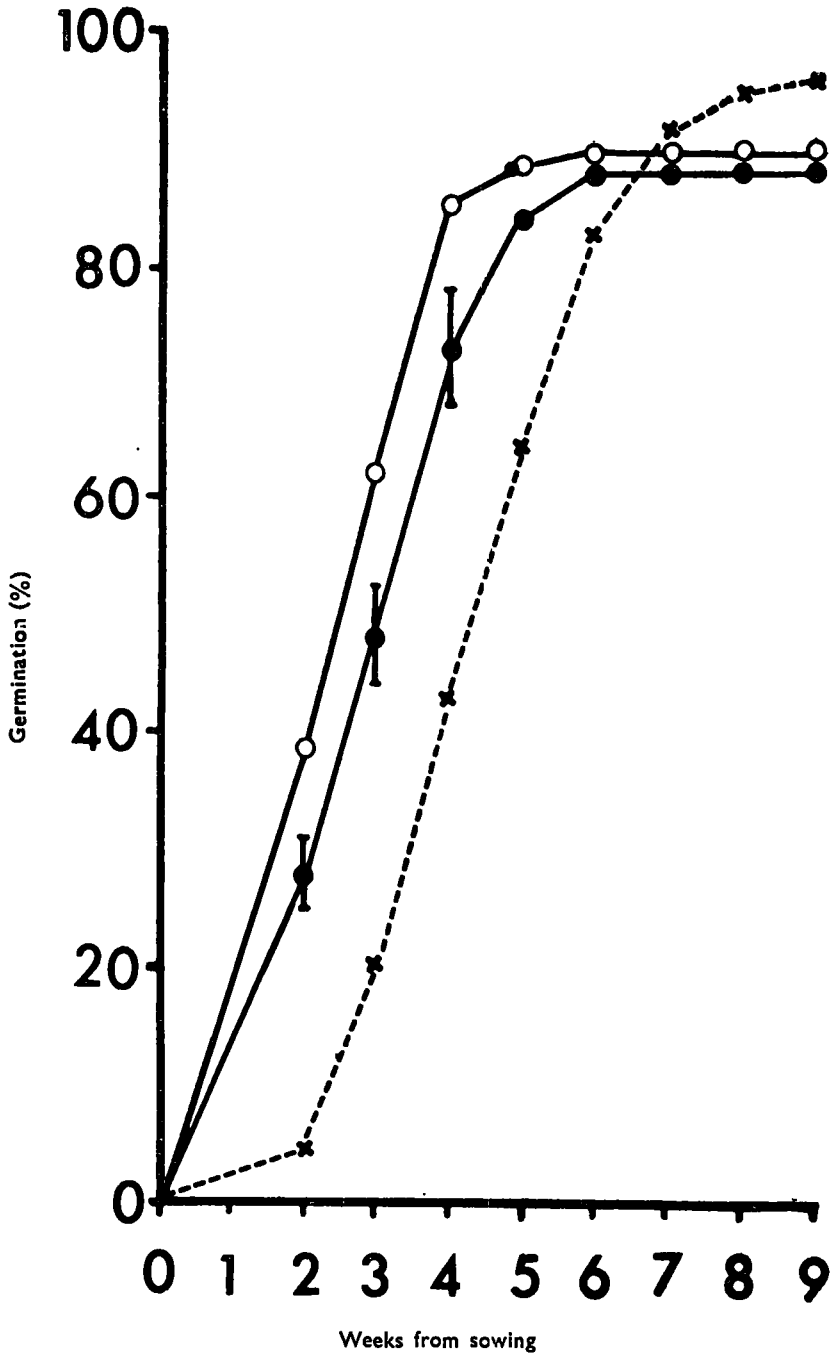

FIG. 8—*Rate of germination of stored and unstored seeds*

(x—x = Stored for 3 months; ●—● = Mean of 9 months storage and O—O = Fresh seeds)

DISCUSSION AND CONCLUSIONS

The moisture content of any seed is dependent on the growth conditions and the prevailing environment under which seed is collected. These same factors will influence the dry matter content of the seeds, since moisture and dry matter are inter-related products. The collection of seeds from one locality and it being done on the same day ensured that the seeds of the different clones developed under similar conditions. It was assumed that the seeds had reached physiological maturity since they were capable of germination soon after collection. The rapid determination of the fresh weights of seeds eliminated any experimental errors that could have occurred due to moisture loss or moisture absorption by the seeds. Seed size is known to be influenced genetically by the maternal parent. Since there are differences in seed sizes between clones, it seemed possible that the proportion of dry weight to the moisture content of seed may also be different. That there are differences in the dry weight of seeds between clones is confirmed in this investigation.

The cracking of the seed coat (Experiment 2) hastens germination suggesting that the seed coat forms a serious barrier to the early germination of seeds. It would be expected that the permeability of the seed coat would be greatest near the micropylar end. Imbibition of water by tea seed is very rapid reaching saturation moisture content in about three days (Experiment 3). Thus the conditions required for germination is satisfied very rapidly, but a delay in the emergence of the radicle of seeds with intact seed coats is probably due to a slow build-up of internal perssures required to crack the seed coat. It may therefore be concluded that the seed coat acts as a mechanical barrier to the early germination of seeds and not as an impervious layer to the imbibition of water as implied by Tubbs (1932) Cracking of the seed coat prior to sowing could therefore be adopted as an useful practice for hastening the germination of tea seeds.

Soaking the seeds in water for up to four days prior to sowing confers some advantage to the germination of seeds. The earliness of germination of pre-soaked seeds is probably due to the rapid elevation of seed moisture than that in the sand medium. In other words, seeds reach favourable germination conditions in water more rapidly than in the sand medium. Both pre-soaking and cracking of the seed coat will therefore realise the conditions required for the initiation of germination sooner than untreated seeds.

Experiment 4 clearly demonstrates that fresh tea seeds do not withstand desiccation and air drying without seriously affecting their germination. The short viability of tea seeds is due to the rapid loss in moisture from the seeds under normal atmospheric conditions. An equilibrium moisture content of about 13% by dry weight is rapidly attained by seeds exposed to the normal conditions in the laboratory. At this moisture level all seeds become inviable. Critical moisture levels in the seed for the maintenance of viability as indicated in this experiment is above 30%. A high initial moisture content and the prevention of moisture loss from the seed will therefore ensure maximum viability.

Imbibition of water (Experiment 5) is not a criterion of the viability of seeds. High initial seed moisture is an important factor which ensures high germination. Seeds when dried to different moisture contents lose their viability very rapidly. North (1948) has reported that the air temperatures for the safe drying of seeds varied from 32-60°C in different plant species. It is unlikely that the drying temperature of 35-38°C used in this experiment was injurious to the embryo. The

loss in viability is irreversible since there is no improvement in the germinability even after imbibition of water to their original moisture level. Therefore desiccation of seed after harvest is injurious to the embryo and should be avoided if storage is anticipated.

Optimal storage conditions differ with different species, but common factors of importance are humidity and temperature of the storage environment. The proper manipulation of these two factors will ensure the longevity of the seeds. The most satisfactory conditions for the long-term storage of tea seeds were found to be 100% RH and temperatures around 5–7°C. The storage of tea seeds for long periods at 5°C was reported by Katsuo *et al.* (1970), but they have not published any supporting evidence. Temperatures below freezing kill tea seeds due to the high moisture content in and around the seeds. A higher storage temperature and high relative humidity on the other hand provides the conditions necessary for germination resulting in the cracking of seed coat and promotes the activity of micro organisms resulting in a rapid deterioration of the seeds.

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