

# POSSIBLE CAUSES OF POOR GERMINATION OF LARGE CARDAMOM SEEDS

P. P. Khatiwada<sup>1</sup> and A. J. Murdoch

Department of Agriculture, The University of Reading  
Early Gate, Reading, RG6 2AT, Berkshire, United Kingdom  
(<sup>1</sup>Presently: Agriculture Research Station, Pakhribas, Dhankuta)

## ABSTRACT

*This paper reports on studies which were conducted to find out the reason(s) for the poor and irregular germination of large cardamom seeds including possible hardseededness, poor oxygen exchange, the presence of inhibitors, embryo immaturity or of a combination of these reasons. Poor (< 5 percent) germination was found in seeds even after a six month germination test at 20/30<sup>0</sup> C (16 h/ 8 h). Viability also dropped to 50.7 percent from 85.3 percent during this test. Similarly no seeds germinated over a shorter period (10 weeks) even after the application of dormancy-relieving treatments to seeds over 80 percent of which were viable. The seed coat did not greatly impede imbibitions of water. The moisture level in seeds increased from 16.1 percent to 37.8 percent in unrubbed seeds and 39.9 percent in individually rubbed seeds in 24 hours. Mechanical scarification (rubbing) treatment was found to be detrimental to the seeds. No germination and 100 percent decay were found in individually rubbed seeds during an 18week germination test compared to 32 percent in the unrubbed control. Elongation of the linear embryo during warm stratification suggested that embryo immaturity is one possible reason for dormancy in large cardamom. The embryo grew at a rate of 0.08 mm per week at 25<sup>0</sup>C but was 16.2 percent slower at 35<sup>0</sup>C. The warm stratified seeds did not, however, germinate in a 10 week test and indeed some loss of viability occurred. Further work on warm stratification is recommended but at a temperature lower than 25<sup>0</sup> C to avoid loss of viability. Similarly, temperature regimes other than 20/30<sup>0</sup> C and 5/25<sup>0</sup> C (8h/16h) are advised for germination tests.*

**Key words:** *Ammomum subulatum* Roxb, dormancy, embryo culture

## INTRODUCTION

Large cardamom (*Amomum subulatum* Roxb.) of Zingiberaceae family is a spice crop native to the eastern Himalayas (Purseglove *et al.*, 1981). In Nepal and India, large cardamom is propagated vegetatively by separating suckers as well as by seed (Mukherji, 1973; Sangraula, 1989; Karibasappa, 1990; Ojha, 1992). Gautam *et al.*, (1992) found that the former method is used to plant small areas and the latter for larger plantations. In Nepal, Splitting rhizome suckers is the main method of propagation (Hartkamp, 1993) but this can spread virus diseases like *Chhirkey* and *Foorkey*. Seed propagation is, therefore, attractive as the seedlings are free from these viral diseases (Purseglove *et al.*, 1981). Due to protracted germination periods - use of seed however, been confined to a few specialised nursery growers in Nepal (Hartkamp, 1993) despite efforts to use virus free material since 1976 (Ojha, 1992) *In vitro* propagation of virus-tested saplings may exploit the advantages of asexual propagation. It is, however, expensive and a poor transportation infrastructure in large cardamom growing areas limits the use of the planting material in Nepal. In order to maintain large cardamom cultivation, successful seedling production is, therefore, important.

Propagation of large cardamom by seeds is difficult and time consuming (Sangraula, 1989). Bhowmick and Chattopadhyay (1960) and Gautam *et al.* (1992) found that seeds take 6-8 months to germinate in the field. Low and irregular germination has also been reported (Ellis *et al.*, 1985). Additionally, a study conducted at Pakhribas Agricultural Centre, Dhankuta revealed that only 14 percent of seeds emerged in field conditions (PAC, 1994). In another study, 30-50 percent germination was reported (Cardamom Board, undated).

Low and irregular germination of large cardamom is sometimes thought to be due to dormancy in the seeds. Large cardamom seeds remain dormant for 7-8 months after harvest and this dormancy breaks naturally after completion of the period (Bhowmick and Chattopadhyay, 1960). However, Paudyal *et al.* (1993) reported 58 percent germination within three months of harvesting and did not find significant difference between control and dormancy-breaking treatments. Bhowmick and Chattopadhyay (1960) mentioned that irregular germination is due to variation in seed size and improper development of seeds in different parts of a capsule. Hardseededness is generally considered to be a problem in large cardamom. Chemical scarification has been proposed as a method of improving germination (Cardamom Board, undated; Gupta, 1982; John *et al.*, 1982). However, a preliminary trial carried out in the National Cardamom Development Programme, Fikkal revealed that acid scarification at different concentrations and durations did not affect the field emergence of large cardamom (Sangraula, 1989). In the same way, no significant difference between scarification (mechanical and chemical) and control was reported by Paudyal *et al.* (1993). Soil temperature has been associated with slow emergence. John *et al.* (1982) reported that emergence duration was halved with covering the nursery bed by polythene sheets. By way of contrast, no effect of a polytunnel on emergence was reported by Sangraula (1989). The literature on large cardamom clearly demonstrates an ambiguous and confusing picture of the germination problem with conflicting results, which do not provide practical solution or clear guidance for further experiments. This study, therefore, sought to find out the cause of poor and irregular germination of the crop.

## MATERIALS AND METHODS

### Seeds

Capsules of three cultivars (*Ramsahi*, *Dambarsahi* and *Kupringe*) were picked in October 1995 from Pakhribas Agricultural Centre, Dhankuta. Seeds from freshly harvested capsules in the bottom two thirds of inflorescence were extracted and washed with sand to remove the mucilage around the seeds. After three days drying in the shade, the cleaned seeds were packed in polythene bags and stored at room temperature until despatched on 17 December 1995. After arrival at Reading, United Kingdom on 8 January, the seeds were stored at 3-5°C in hermetically sealed packets. Seed moisture content on receipt was 16.0 to 16.3 percent (wet weight basis) by the low constant temperature methods (ISTA, 1993).

### Germination Test

Germination tests were carried out on top of two circles of Whatman Grade 181 seed testing paper in 9 cm diameter polystyrene petri dishes. Each dish was moistened with 4.5 ml of deionised water or aqueous chemical solution as per treatment. Where necessary, seeds were disinfected by dipping in 1 percent sodium hypochlorite solution for 10 minutes. Dipped seeds were washed in running deionised water for five minutes. Petri dishes were placed in transparent polythene bags to minimise moisture loss during tests. Incubators were maintained at alternating temperatures 5°/25°C (8h/16h) or 20°/30°C (8h/16h). Additional lighting was not

provided. Petri dishes were observed weekly and additional water was provided where necessary at the rate of 2 ml per dish. The germination criterion was 2mm radicle emergence. Four replications of 25 or 50 seeds were used in most experiments.

### **Viability Test**

The topographical tetrazolium test was used to check viability. Seeds were pre-imbibed overnight on germination paper moistened by deionised water. Imbibed seeds were then bisected by scalpel and dipped in 0.1 percent solution of 2,3,5-triphenyltetrazolium chloride. Test tubes containing the tetrazolium solution were wrapped with aluminium foil and placed at 30<sup>0</sup>C for about 48 hrs in a dark incubator. After incubation, seeds were washed twice by deionised water before counting the number of stained embryos. Two replicates of 25 seeds were used throughout the viability test. All analysis was based on proportions of stained embryos.

### **Mechanical Scarification**

For the individually rubbed treatment, individual seeds were rubbed on sand paper until the cotyledon become visible. For one and five minute rubbing treatments, batches of 60 seeds were rubbed between two sheets of sand paper. Two replicates of 50 seeds of each cultivar for each treatment were then placed in an incubator at 20<sup>0</sup>/30<sup>0</sup>C (8h/16h). The substrate was moistened by deionised water.

### **Soaking Treatments and After-ripening**

To test assumption that low oxygen permeability limits germination, seeds were soaked in 0.5M H<sub>2</sub>O<sub>2</sub> for 24 hrs at 20<sup>0</sup>C. Subsequent germination tests were carried out with 100 ppm GA<sub>3</sub> and 0.01M KNO<sub>3</sub>. To remove inhibitors, seeds were soaked on 3, 7 or 14 days in deionized water at 20<sup>0</sup>C. Water was changed daily during the course of soaking and germination tests were carried out in KNO<sub>3</sub> (0.01 M). For dry-after ripening treatment, seeds were hermetically sealed and placed at 60<sup>0</sup>C. Four days after-ripening period was selected after a preliminary study, which showed that this did not cause a significant loss of viability. A factorial experiment then included two levels of dry-after ripening (with and without) and two levels H<sub>2</sub>O<sub>2</sub> (soaked and unsoaked) followed by germination 100 ppm GA<sub>3</sub> and 0.01M KNO<sub>3</sub>.

### **Embryo Culture and Length**

Two experiments were designed to test the hypothesis that poor germination was related to embryo immaturity. *Ramsahi* - the most common cultivar in Nepal - was used in these experiments. The first, a factorial experiment comprised warm stratification treatments and included three levels of gibberellin (0, 100 and 500 ppm) at 25<sup>0</sup>C and 35<sup>0</sup>C. To ensure seeds were of similar size, seeds were sieved with 2.35 and 3.36 mm sieves and those retained by the 2.35 mm were used. Warm stratification took place in petri dishes as described for germination tests. Four replicates of 20 seeds were kept per treatment, but embryo lengths were only measured ten of these seeds. To take the sample of seeds for measurement, seeds in a petri dish were divided into four parts and two parts were randomly selected. Seeds were longitudinally bisected by scalpel and embryos were separated from cotyledons. Embryo length was measured with a microscope and a resolution of 1/22 mm. Some seeds did not contain embryos and these were omitted from analysis. Measurements were taken every two weeks for a period of eight weeks. Viability of measured embryos was assessed after six and eight weeks. Secondly, excised embryos without cotyledons were cultured in a growth medium. For disinfection, seeds

of *Ramsahi* cultivar were placed in a vial containing 100percent ethanol for 10 seconds and then dipped in 10percent sodium hypochlorite solution for 10 minutes. After rinsing three times in purified water, seeds were kept overnight in room temperature to imbibe in purified water. Embryos were then excised in sterile conditions under a laminar flow hood. An undamaged embryo was placed in each test tube containing Murashige and Skoog medium (Murashige and Skoog, 1962) and sealed by paper tape. Ten replicate embryos were cultured in the following treatments; (1) chilling at 3-5<sup>0</sup>C for a week, (2) in darkness for a week in a growth room and (3) in light in the growth room (the control). All test-tubes were transferred to the growth room after pre-treatment. The growth room was maintained at 22.5<sup>0</sup>C throughout the period with 18 hours light (white fluorescent) and 8 hours dark.

### Factorial Trial

A further factorial experiment tested whether dormancy might be due to more than one reason. Treatments comprised soaking (unsoaked and soaking in water or 0.5M H<sub>2</sub>O<sub>2</sub>), warm stratification (none and warm stratification with and without subsequent drying), chilling (with and without) and three cultivars. The treatments were applied in the sequence of soaking, warm stratification and then chilling. All soaking treatments lasted 24 hours. Warm stratification lasted one month at 25<sup>0</sup>C in 100 ppm GA<sub>3</sub> and 0.01M KNO<sub>3</sub>. Drying after warm stratification was done at approximately 15<sup>0</sup>C and 15 percent relative humidity for 24 hours. Seeds were chilled at 3-5<sup>0</sup>C for 2 weeks in 100 ppm GA<sub>3</sub> and 0.01M KNO<sub>3</sub>. All treatment combinations were then placed in germination test with 100 ppm GA<sub>3</sub> and 0.01M KNO<sub>3</sub>.

## RESULTS AND DISCUSSIONS

### Initial Germination Test

The seeds started to germinate after three months and continued until the test ended after six months. Depending on the cultivars, only 1 to 3.3 percent germination was observed (Table 1), in spite of the high (> 80percent) viability of the seeds. A high initial viability in all cultivars from the tetrazolium test meant it was possible to investigate the germination problem of large cardamom. This finding leads us to believe that large cardamom do not lose viability immediately after harvest in ambient condition.

**Table 1: Germination of large cardamom seeds after six months. Seeds were germinated in water at 20/30<sup>0</sup>C (8hrs/16hrs). Means of six replicates of seeds.**

Cultivar	Per cent	Initial viability	Final viability
Ramsahi	1	84	72
Dambarsahi	2	82	28
Kupringe	3.33	90	52

Viability of non-germinated seeds was assessed at the end of July. In contrast to the initial viability test, the X<sup>2</sup>-test of significance revealed the strong evidence (P<0.01) of poorer viability of *Dambarsahi* as compared to *Ramsahi* and *Kupringe* (Table 1). The initial germination study confirmed the extremely poor and irregular germination of large cardamom reported by Ojha (1992) and PAC (1994) and demonstrates the inherent problem associated with propagation by seeds.

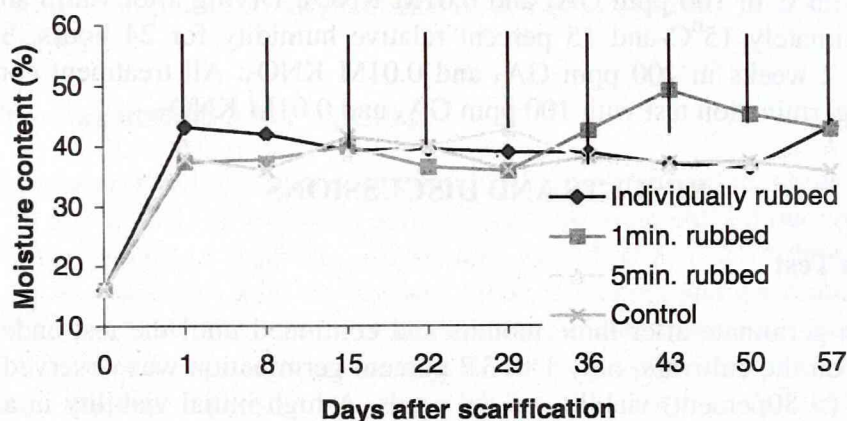
## Mechanical scarification

As in the initial germination test, germination from the mechanically scarified seeds was found low and irregular (Table 2).

**Table 2: The effect of mechanical scarification on the number of germinated seeds (out of 100).**

Cultivar	Treatment			
	Individually rubbed	5 minutes rubbed	1 minutes rubbed	unrubbed
Ramsahi	0	1	0	1
Dambarsahi	0	0	0	0
Kupringe	0	2	0	0

Germination obtained was so low that it is difficult to come to any conclusion. Although permeable to water, the failure of germination implies that there is embryonic or physiological dormancy. The moisture uptake was measured to determine whether or not the seeds were hard seeded. Even after 24 hours, water uptake was similar in all treatments and cultivars (Figure 1).



**Figure 1. Effect of mechanical scarification on moisture uptake by large cardamom seeds (mean result of three cultivars). The bars represent the LSD ( $P=0.05$ ) value for each date.**

The moisture content of the seed increased from 16.1 (mean of three cultivars) to 39.6 percent (mean of all treatments) in 24 hours. There is, although the increase was slight greater for rubbed seeds and especially for individually rubbed seeds, no evidence of hardseededness or impermeability to water. It was anticipated that scarification would lead to substantially higher rates of water absorption compared to unscarified seeds. By contrast, even individually rubbed seeds only showed slightly faster water absorption. This finding supports the insignificant difference in germination between intact and incised seeds reported by Paudyal *et al.* (1993). On the basis of these findings, it can be concluded that the stony seed coat does not hinder water uptake. The rapid uptake indicates that the seeds are permeable to water.

Damage caused by mechanical scarification was associated with seeds rotting. The hand rubbed seeds started to ooze out mucilage after a week of setting. Fungal attack was initially observed in exposed parts of individually rubbed seeds and at the point of caryopsis attachment in other treatments. The rotting of seeds was judged by gently pressing the seeds with forceps. The cumulative percentage of rotted seeds shows that rotting increased with increasing degree of scarification (Figure 2).

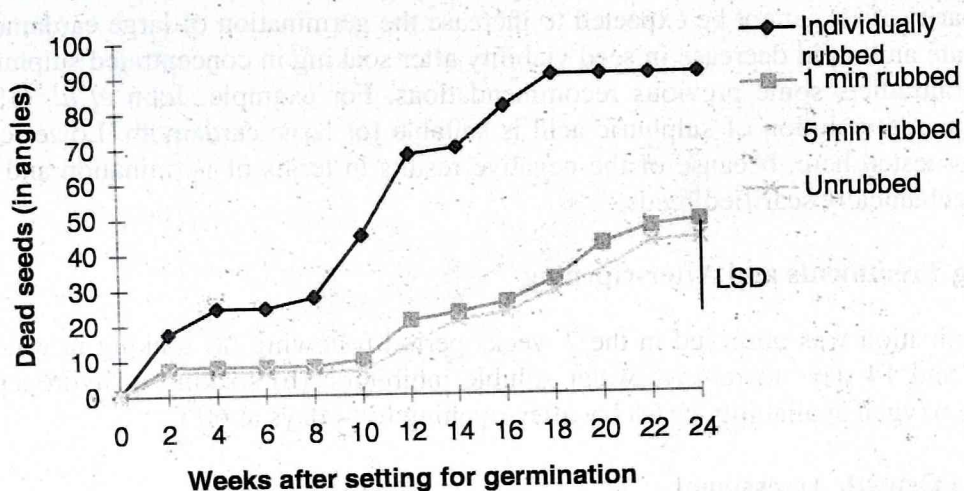


Figure 2: Rotting of large cardamom seeds in germination tests at 20°C/30°C after mechanical scarification. Mean results are presented for three cultivars. The LSD applies only to data after 24 weeks.

Rotting of seeds was negligible for the first two months of setting and increased sharply thereafter in all the treatments. Hundred percent rotting was recorded after 18 weeks of setting in individually rubbed seeds (Figure 2). After 24 weeks, rotting of seeds rubbed for 5 minutes was significantly higher than the control ( $P < 0.001$ ) and 1 minute rubbed ( $P < 0.01$ ) treatments. However, no significant difference was found between one minute rubbing. No significant difference was found between cultivars, as well.

### Acid Scarification

Seeds of cultivar *Ramsahi* were scarified in concentrated sulphuric acid for 5, 10, 15, 30, 60 and 120 minutes. Only, 16 percent seeds were viable after 5 minutes and none were viable after 10 minutes. In a second trial, viability again declined with increase of period of soaking in concentrated sulphuric acid (Figure 3).

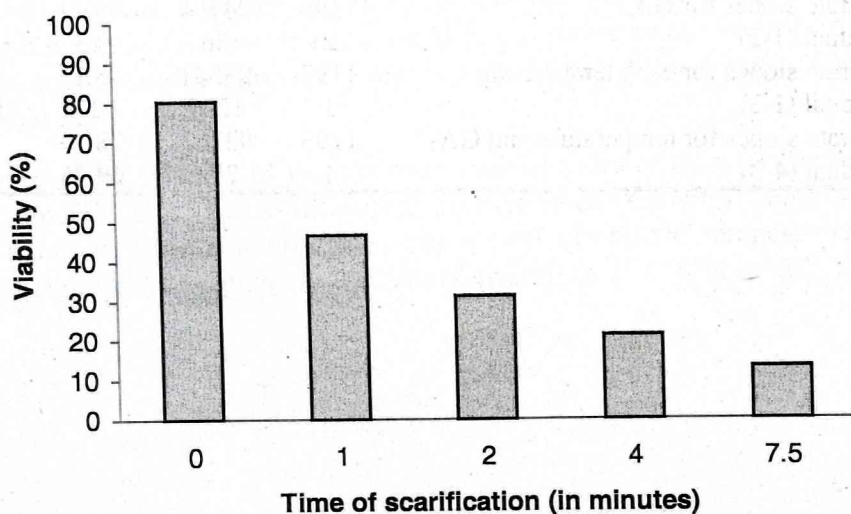


Figure 3: Effect of acid scarification on large cardamom seed viability (cv. *Ramsahi*). Seeds were dipped in concentrated sulphuric acid.

Acid scarification cannot be expected to increase the germination of large cardamom due to the immediate and rapid decrease in seed viability after soaking in concentrated sulphuric acid. This result contradicts some previous recommendations. For example, John *et al.* (1982) reported that 25percent solution of sulphuric acid is suitable for large cardamom. Lower concentrations were not tested here, because of the negative results in terms of germination and water uptake from mechanically scarified seeds.

### Soaking Treatments and After-ripening

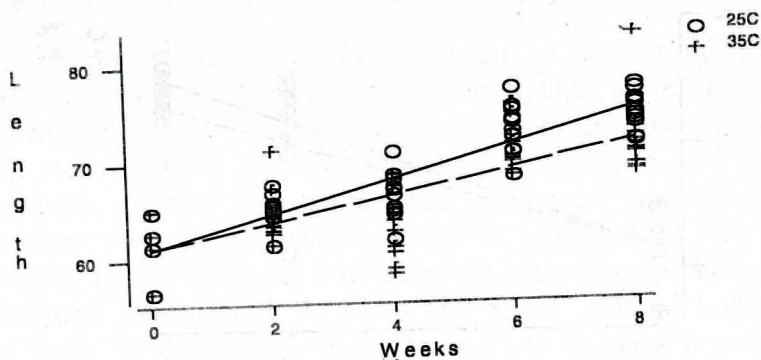
No germination was observed in the 7 weeks period following (a) soaking in water for periods of 3, 7 and 14 days to remove water soluble inhibitors; (b) soaking in hydrogen peroxide to increase oxygen availability or (c) fry after-ripening for 4 days at 60°C.

### Embryo Growth Assessment

In spite of variability, perhaps due to initial differences in embryo length, the embryos grew significantly over eight weeks in all treatments (Fig. 4)). Growth was faster at 25°C compared to 35°C (Figure 4) and there was a significant reduction in the residual sum of squares, if temperatures were considered separately than if there were a common line for all treatment combinations (Table 3). This difference is reflected in the slopes of the lines (Table 4). Gibberellic acid, however, did not significantly affected the rate of growth at either temperature (Table 3).

**Table 3:** Analysis of variance of regressions of embryo length against time. Analysis is based on embryo length measurement of 40 individual seeds after 0, 2, 4, 6 and 8 weeks of warm stratification at 25°C and 35°C in 0, 100 and 500 ppm GA<sub>3</sub>. Live and dead embryos are included in this analysis.

No	Source of variation	df	SS	MS	F-ratio
1	Total	1199	117402		
	Common line	1	22583		
	Residual	1198	94820	79.15	
2	Separate slopes for GA <sub>3</sub>	1196	94734	79.21	
	Residual (1-2)	2	86	43	0.54
3	Separate slopes for each temperature	1197	93541	78.15	
	Residual (1-3)	1	1279	1279	16.16***
4	Separate slopes for temperature and GA <sub>3</sub>	1193	93262	78.17	
	Residual (4-3)	4	279	69.75	0.88



**Figure 4:** Effect of temperature on growth of embryo of large cardamom cv. Ramsahi as a function of period of time at 25°C (o-o) or 35°C (+-+). Analysis is based on length of individual embryos and lengths represent the measurement taken from live and dead embryos. For clarity of presentation, replication means are plotted. Regression coefficients of the fitted lines are presented in Table 4. Unit of length on y-axis is 1/22 mm.

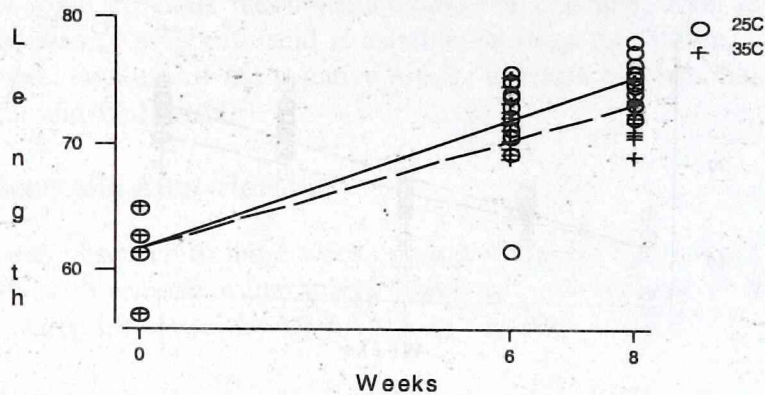
**Table 4:** A regression coefficients of relationships between embryo length and time. Data for live and dead embryos is shown in Fig. 4. Live embryos data is only for 0, 6, and 8 weeks and for 0 and 500 ppm GA<sub>3</sub>.

Common intercept	Live and dead embryo		Live embryo only		Units
	slope	s.e.	slope	s.e.	
25°C	1.744	0.104	1.773	0.131	mm/22 week <sup>-1</sup>
35°C	1.323	0.104	1.474	0.131	mm/22 week <sup>-1</sup>

Compared to an initial seed viability of 84 percent in cv. *Ramsahi*, only 76 to 42 percent of apparently healthy embryos were viable after 8 weeks at 25°C and 35°C, respectively. A further regression analysis was, therefore, performed for live embryos only including observations from 0, 6 and 8 weeks. A similar response (i.e. a positive linear growth of the embryo over time) was found and coefficients of the regressions did not differ significantly between the two analyses (Table 4).

The linear regression analysis of live embryos explained more than 80 percent of the variation in all fitted models. This clearly shows the positive linear growth of embryos over time. The calculation made in Table 5 demonstrates that the effect of 25°C temperature on the growth of embryos was significantly higher ( $P < 0.05$ ) as compared to 35°C (Figure 5).





**Figure 5:** Effect of temperature on growth of live embryos. Analysis is based on mean values of replicates. The solid line represents 25<sup>0</sup>C and long dash represents 35<sup>0</sup>C. Regression coefficients of the fitted lines are shown in Table 6. Unit of length on the y-axis is 1/22 mm.

The regression analysis shows that embryo growth at 25<sup>0</sup>C was 1.77 mm/22 week<sup>-1</sup>, that is, 0.08 mm week<sup>-1</sup> and was 16.25 percent lower at 35<sup>0</sup>C. On the other hand, exogenous GA<sub>3</sub> up to 500 ppm did not significantly increase embryo growth rate. The linear increase of embryo length during warm stratification at 25<sup>0</sup>C, however, gives some evidence of an immature embryo.

### Embryo Culture

Aseptically excised embryos placed in Murashige and Skoog medium did not show any changes in growth of embryos. In the period of 6 weeks, none of the embryos germinated and no any sign of contamination was observed. This result also supports the presence of embryo dormancy. The failure to germinate excised embryos in a growth medium supports the evidence of deep embryonic dormancy. Additionally, Bianco *et al.* (1994) mentioned that removal of the cotyledons did not make significant difference in germination in the case of embryo dormancy. Presence of growth inhibitors, or immature embryos, or both of these factors as in *Taxus baccata* (Zhiri *et al.*, 1994) might be the cause of embryonic dormancy in large cardamom.

### Application of more than Two Dormancy-relieving Treatments

No seeds germinated, but the results are still of value for future work because certain treatments caused a significant loss of viability. The following analysis concerns this loss of viability. A combined analysis of variance on seed viability of warm stratification and time (i.e. viability before and after the 4-10 weeks germination test) was carried out. Although some higher order interactions were significant, it is only considered necessary to mention those effects, which might influence the design of future experiments.

Among the five factors tested, warm stratification and the germination test period were found to reduce seed viability most significantly ( $P < 0.001$ ). Soaking and cultivars had smaller effects on viability ( $P < 0.01$ ) as compared to warm stratification. On the other hand, chilling had no effect ( $P > 0.05$ ). Among the three warm stratification treatments, loss of viability was lowest (41.58 angles) in unstratified seeds, followed by the drying after warm stratification (50.99 angles). Clearly, the 25<sup>0</sup>C temperature and/or 4 weeks of warm stratification was not suitable for large cardamom. However, it is interesting to note that a high rate of seed viability was found in drying after warm stratification as compared to without drying. The viability of large cardamom

seeds also declined from 56.25 to 44.92 (in angles) during the two months germination test at ( $P=0.001$ )  $5^{\circ}/25^{\circ}\text{C}$  (8h/16h). These effects occurred in all three cultivars though *Dambarasahi* was always found to have lower in viability in comparison to *Ramsahi* and *Kupringe*.

Large cardamom germination has also been related to temperature in a few reports (Karibasappa, 1990; Bhowmick and Chattopadhyay, 1960). Ojha (1992) mentioned that  $12^{\circ}\text{C}$  to  $20^{\circ}\text{C}$  is a suitable temperature for field germination of large cardamom. The effectiveness of dormancy breaking treatments in this study might be concealed due to an inappropriate germination temperature regime. The temperature difference between the maximum and minimum of alternating temperature regimes seems as important as the actual temperature (Murdoch *et al.*, 1989). In the same way, the effect of exogenous gibberellic acid and  $\text{KNO}_3$  supplied in a moistening solution did not show any effect. However, these chemicals were found effective in seeds of ornamental plants having the similar physical and germination (linear embryo with dormancy) behaviour (Atwater, 1980).

In the case of immature embryos, warm stratification should be effective and  $\text{GA}_3$  might be expected to promote growth. But quick germination did not take place following by warm scarification while  $\text{GA}_3$  had no effect. However, in *Cardamine concatenata* (also with embryonic dormancy) warm stratification followed by chilling decreased the length of cold stratification and germination period (Baskin and Baskin, 1995). In the field a relatively high rate of germination occurs where seeds are sown immediately after harvest. This could be due to a short period at warm temperatures (September to October) followed by cool temperatures (November to January). However, warm followed by cold stratification did not work in this experiment. In addition to the failure to hasten germination, loss of viability was also evident in all cases by warm stratification. Because the loss of viability was greater at  $35^{\circ}\text{C}$  compared to  $25^{\circ}\text{C}$ , lower temperatures need to be tested in any future work.

Physiological dormancy may be caused by properties of the coat or embryo (Ballard, 1973). This might be the reason why previous studies have basically concentrated on hard seed coats. The results obtained from mechanical scarification study did not give the indication of presence or absence of physiological dormancy. In *Amomum xanthioides*, among five layers in the seed coat, the third layer is composed of oily cells and the fourth layer by stony cells (Kimura *et al.*, 1958). This could be the same in large cardamom as seed structure is usually similar within families and their close relatives (Corner, 1976). These layers could hinder other physiological processes for germination. The mechanical strength of the coat or of surrounding covers may restrict germination and/or radicle penetration and/or expansion and development of embryos (Tran and Cavanagh, 1984). The beneficial effect of scarification (John *et al.*, 1982; Cardamom Board, undated) could be due to weakening of mechanical restraints of a hard seed coat. The poor germination in five minutes and one minutes rubbing treatments from this experiment might be due to undisturbance to the third and fourth layers, whereas poor germination of individually rubbed treatment seems to be due to early rotting.

Failure to show germination results by dormancy-relieving treatments like dry-after ripening, soaking (in hydrogen peroxide and water) and stratification (warm and cold) could be due to the short duration of the germination tests. According to Ellis *et al.* (1985), large cardamom needs at least three months period for germination. In this study, all dormancy-relieving treatments got less than 3 months duration for germination except mechanically scarified seeds.

The previous studies conducted on dormancy of large cardamom were extremely limited and of a preliminary nature and were principally concentrated on the hard seed coat. But this study tried to cover other possible aspects of dormancy, not limiting its scope to only one aspect. In this circumstance, the findings obtained from the study could be used as stepping stones for

future research. In particular, the failure to establish a relationship between scarification and water uptake and the positive linear growth of embryos at 25<sup>0</sup>C opens up new avenues for research in large cardamom. A high rate of viability loss during the course of germination tests also indicates inappropriate temperature regimes, even though these were lower than those recommended by Ellis, *et al.* (1985). The direct implication of this finding is to investigate the use of lower temperatures in germination tests.

## CONCLUSION

It is clear that large cardamom seeds give evidence of orthodox seed storage behaviour, are not hard seeded and the probable cause of dormancy is an immature linear embryo. The temperature regime for germination must be carefully selected because of the long germination period. Temperature regimes below 25<sup>0</sup>C are likely to be more appropriate for large cardamom. Similarly, warm stratification must be tested in below 25<sup>0</sup>C condition.

## REFERENCES

- Atwater, B. R. 1980. Germination, dormancy and morphology of the seeds of herbaceous ornamental plants. *Seed Science and Technology*, 8: 523-573.
- Ballard, L. A. T. 1973. Physical barriers to germination. *Seed Science and Technology*, 1: 285-303.
- Baskin, C. C. and J. M. Baskin. 1995. Warm plus cold stratification requirement for dormancy break in seeds of the woodland herb *Cardamine concatenata* (Brassicaceae), and evolutionary implications. *Canadian Journal of Botany*, 73(4): 608-612.
- Bhowmick, T.P. and S. B. Chattopadhyay 1960. Germination of seeds of larger cardamom. *Science and Culture*, 26: 185-186.
- Bianco, J., G. Garellko and M. T. Le Page-Degivry. 1994. Release of dormancy in sunflower embryos by dry storage: involvement of gibberellins and abscisic acid. *Seed Science Research*, 4: 57-62.
- Cardamom Board (undated). Large Cardamom Package and Practices. Cardamom Board, Ministry of Commerce, Government of India, Gangtok, Sikkim, India.
- Corner, E. J. H. 1976. The seeds of Dicotyledons. Vol. 1. Cambridge University Press, Cambridge.
- Ellis, R. H., T. D. Hong and E. H. Roberts. 1985. Handbook of Seed Technology for Gene Banks Vol. II, Compendium of Specific Germination Information and Test Recommendations. International Board for Plant Genetic Resources, Rome.
- Gautam, S.R., M. P. Thapa, K. K. Bimba, S. L. Sherpa, R. B. Paneru, G. B. Gurung, T. R. Shrestha and V. R. Duwadi. 1992. Samuhik Bhraman on Some Cardamom Growing Areas of Mechi and Koshi Hills PAC Working Paper No. 30, Pakhribas Agricultural Centre, Dhankuta, Nepal.
- Gupta, P. N. 1982. Variety selection and preparation of transplanting material for large cardamom. pp. 8-10 in Text of Training Courses on Large cardamom for the Officers of Government of Mizoram. The Training Institute of Agriculture Department, Gangtok, India.
- Hartkamp, A. D. 1993. Black Gold, Wageningen Agricultural University, The Netherlands
- ISTA. 1993. International Rules for Seed Testing 1993. *Seed Science and Technology* Vol. 21, ISTA.
- John, P. T., J. R. Subba and M. B. Subba. 1982. Large cardamom - Seedling Growing in Nurseries. pp 24-26 in Text of Training Courses on Large Cardamom for the Officers of

- Government of Mizoram. The Training Institute of Agriculture Department, Gangtok, India.
- Karibasappa, G. S. 1990. A Manual of Large Cardamom Cultivation, Indian Council for Agriculture Research, Gangtok, India.
- Kimura, Y., Y. Kobayashi and T. Kurosu. 1958. Pharmacognostical study of vegetable drugs of cardamom group. *Journal of Japanese Botany*, 33: 297-301.
- Mukherje, D. K. 1973. Large Cardamom. *World Crops*, 25 (1): 31-33.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15: 473-497.
- Murdoch, A. J., E. H. Roberts and C. O. Goerdert. 1989. A Model for germination response to alternating temperatures. *Annals of Botany*, 63: 97-111.
- Ojha, G. 1992. Alaichi Utpadan Tatha Bechbikhan (Large cardamom production and marketing), Centre for Environment, Agriculture, Policy, Research, Extension and Development, Nepal.
- PAC. 1994. Annual Report 1992/93, Pakhribas Agricultural Centre, Dhankuta, Nepal.
- Paudyal, B., P. R. Neupane and M. R. Bhattaraj. 1993. Preliminary Observation on Germinating Large Cardamom (*Amomum subulatum* Roxb.) in Laboratory. PAC Working Paper No. 43, Pakhribas Agricultural Centre, Dhankuta, Nepal.
- Purseglove, J.W., E. G. Brown, C. L. Green and S. R. J. Robbins. 1981). *Spices* vol. 2. Longman Group Limited.
- Sangraula, I. P. 1989. Nepal Ma Alaichi Kheti (Large cardamom cultivation in Nepal). Pakhribas Agricultural Centre, Dhankuta, Nepal.
- Tran, V. N. and A. K. Cavanagh. 1984. Structural aspects of dormancy. pp 1-44 Murray, D.R. (Ed.) *Seed Physiology, Germination and Reserve Mobilisation* Vol. 2, Academic Press, London.
- Zhiri, A., M. Jaziri, J. Homes, M. Vanhaelen and K. Shimomura. 1994. Factors affecting the in vitro rapid germination of *Taxus* embryos and the evaluation of taxol content in the plantlets. *Plant cell, Tissue and Organ Culture*, 39 (3): 261-263.