S. L. J. Tea Sci. 59 (2), 89-94, 1990. *Printed in Sri Lanka.*

633.72

Short Communication

GERMINATION AND DESICCATION TOLERANCE OF TEA (*CAMELLIA SINENSIS* (L.) O. KUNTZE) SEEDS AND FEASIBILITY OF CRYOPRESERVATION

Rekha Chaudhury, Suman Lakhanpaul, K. P. S. Chandel

(National Facility for Plant Tissue Culture Repository, NBPGR, New Delhi - 110012, India)

and

I. D. Singh*

(Tocklai Experimental Station, Jorhat - 785008, Assam, India)

Tea is a large seeded species. Moisture content of seeds was around 40% by fresh weight. The seeds could survive desiccation only up to 24%. Rehydration over a moist atmosphere could bring some increase in germination of seed samples desiccated further to about 23%. Seeds desiccated to different levels were unable to survive liquid nitrogen exposure. Cryoprotectants like glycerol and DMSO at concentrations of 10 - 40% and PEG '6000' at - 10 to – 20 bars did not prove beneficial. Based on these results, the recalcitrant nature of tea seeds has been discussed.

Tea (*Camellia sinensis* (L.) O. Kuntze) is an important plantation crop of India grown extensively on a commercial scale. However, due to uprooting of old seed grown plantations in several parts of the country, conservation of valuable tea germplasm in the form of seeds has assumed considerable importance (Singh, 1979). Some studies on tea seed maturation, germination, viability (Visser and Tillekeratne, 1958; Katsuo, Toyao and Kayumi, 1970; Barman, 1988) and on aspects of storage (Sebastiampillai and Anandappa, 1979; Amma and Watanabe, 1985) have been reported. Tea seeds have been shown to be short-lived and storage ranging from nine months to six years has been reported with more than 70% germination at the end of these periods by several workers (Sebastiampillai and Anandappa, 1979; Amma and Watanabe, 1985; Barman, 1988). All attempts to store tea seeds so far, have been by manipulating the storage temperature and relative humidity (RH). Cryopreservation of tea seeds can definitely bring about extended storage.

Tea has been listed by Chin and Roberts (1980) as the species in which recalcitrant behavior has been reported but not confirmed. Sebastiampillai and Anandappa (1979) also indicated possible recalcitrance of tea seeds. In the present studies, characterisation of tea seeds was, attempted by studying seed physiology in terms of their germination, desiccation and freezing response.

---

*The Institute does not necessarily endorse the views expressed in papers contributed by persons other than members of its staff.

Fully mature tea seeds harvested in the months of November and December (Barman, 1988) of stocks TS 449, 450, 463 and Rydak 105 at the Tea Research Association, Nagrakata, West Bengal, were, upon receiving, stored at 10-15° C in polythene bags till they were used in about five weeks time. All the cracked, infected and damaged seeds as well as light weight floating seeds were discarded. All the experiments were conducted on the decoated seeds, unless otherwise specified. Moisture content of decoated seeds was determined immediately after receiving, by hot air oven method at 103 ± 2° C for 17h and expressed as percentage of fresh weight. Germination was carried out by sowing seeds 1cm deep in sterilized sand in plastic germination trays (15 cm diameter) at 25° C in natural day and night conditions. All the experiments were repeated for two seasons. Each year, the experiments had three replicates each comprising of 20 seeds.

Seeds were desiccated in a seed drier (Model MVB 50 SD, Bry-Air India Ltd.) under forced air at 15°C and 25% RH and moisture content during drying was determined by progressive loss of weight at different intervals. Desiccated seeds were packed in 20 ml capacity cryovials and immersed in liquid nitrogen (-196°C). On retrieval after 24h of storage, seeds were thawed by immersion in a water bath maintained at 37-38°C. In one set of experiments, seeds with original moisture content and also with moisture content reduced by 5 and 11% of fresh weight, were soaked in different cryoprotectants for 2h before plunging in liquid nitrogen (LN). The cryoprotectants used were glycerol and dimethyl sulfoxide (DMSO) at concentrations of 10 , 20 , 30 and 40 % and polyethylene glycol (PEG) (mol. wt. 6000) at osmotic potential of -10, -15 and -20 bars at 25°C.

Moisture content, size and weight of seeds

Fresh tea seeds were found to possess high moisture contents (about 40%), though seed stocks received on different dates showed some variation in their moisture contents ranging from 39.7 to 42.3% (Table 1). All the fresh seed stocks showed 100% germination except for TS 450 which showed 80-100% germinability. Floater seeds had lower germinability (60%) and thus were discarded. It is notable that tea seeds are shed in moist conditions, a characteristic of recalcitrant seeds. They showed no dormancy as 38% of seeds began germination while still held at 10-15° C. Tea seeds showed variation in size: 0.70 to 1.20 cm in length (mean 0.98±0.14 cm) and 1.0 to 1.50 cm in width (mean 1.30±0.15 cm). They also showed variation in seed weight with mean values ranging from 14.9 to 18.4g for ten seeds considering all the four seed stocks (Table 1). Thus judging by the dimensions and weight of tea seeds, they are relatively large like most of the recalcitrant species (Chin and Roberts, 1980).

TABLE 1– *Moisture content, germination and weight of seeds of different tea seed stocks*

Seed stock	Moisture content (% of fresh weight)	Germination (%)	Weight (g) of 10 seeds
Rydak 105	42.0 ± 0.5	100	16.5 ± 1.1
TS 449	42.3 ± 0.3	100	18.4 ± 4.5
TS 450	39.7 ± 1.6	90 ± 10	17.6 ± 1.2
TS 463	42.1 ± 0.5	100	14.9 ± 0.9

Seed germination

The germination in tea is hypogeal and it took 2-3 weeks for the shoots to emerge from the sand. However, the position of the seeds while sowing in the sand determined the duration of emergence of the shoots without affecting the type of germination unlike the reports for some recalcitrant species (Singh and Rao, 1963). The emergence of the shoot was early with formation of a straight seedling when seeds were planted with the micropyle pointing upwards. The role of seed coat in tea as a mechanical barrier as suggested earlier (Visser and Tillekeratne, 1958; Sebastiampillai and Anandappa, 1979) has been confirmed in the present studies. Intact seeds (with coat) subjected to 24h water soaking before sowing in sand developed into seedlings with average shoot lengths of 2.4 ± 0.4 and 3.5 ± 0.5 cm after 32 and 40 days, respectively. Whereas, seeds pre-treated similarly but decoated just before sowing formed seedlings of average shoot lengths of 3.4 ± 0.9 and 5.6 ± 1.0 cm after 32 and 40 days, respectively.

Although, all four seed stocks were used for the subsequent sets of experiments as well, there were no differences in terms of their desiccation and LN tolerance between the stocks. Hence, the results reported here are for one seed stock only viz. stock TS 463.

Desiccation tolerance

In preliminary experiments, desiccation of seeds over dry silica gel in a desiccator at room temperature for 18h brought about 9.9% reduction in moisture without affecting the germinability. To obtain faster rates of drying as recommended for larger seeds (Farrant, Pammenter and Berjak, 1986), the seeds were desiccated in a seed drier (Table 2). About 11% moisture was lost within the first 4h of drying and it took 10h for the seeds to lose about 29% moisture. Table 3 shows the extent of desiccation tolerance of seeds and recovery of desiccated seeds following rehydration at 100% RH for varying periods. Seeds desiccated to about 24% moisture showed no loss of viability, whereas, those desiccated to 23.1% moisture showed first signs of desiccation injury as the germination was reduced by 50%. Rehydration for 48h brought about the highest recovery of germinability (80%). Further desiccation caused a further fall in viability until when at 13% moisture content the seeds lost their viability completely.

TABLE 2– *Desiccation of tea seeds in seed drier for varying periods*

Duration of desiccation (h)	Moisture content lost* (%)
1	3.1
2	5.0
4	10.9
6	17.5
8	23.8
10	29.4
4 days	29.5

• Average values of 3 replicates

Earlier attempts to determine the desiccation tolerance of tea seeds have been with intact seeds (Sebastiampillai and Anandappa, 1979). Such seeds were reported to tolerate desiccation in an oven at 35-38°C to 21% moisture by dry weight (equivalent to 11.5% moisture by fresh weight). In the present study, seeds without their coats i. e. cotyledons along with embryonic axes only, were used. Since the seed coat was found to act as a barrier for the loss as well as the uptake of moisture, seeds were always decoated before use. Such seeds could survive desiccation only up to 24% moisture level without any adverse effect on viability. It is known that desiccation causes some spatial changes in plasma membranes which results in progressive loss of seed viability. Membrane integrity in the partially viable desiccated tea seeds could, however, be recovered to some extent by rehydration over a moist atmosphere. In the present studies it is likely that the plasma membranes were irreversibly damaged at 13% moisture level of the cotyledons leading to a complete loss of germinability.

Liquid nitrogen tolerance

Seeds after desiccation to different moisture levels (Table 3) were unable to survive exposure to LN. Rehydration of seeds for 24h after retrieval from LN also did not bring about any improvement. Ice crystal formation and subsequent membrane injury are

TABLE 3– *Extent of desiccation tolerance of tea seeds and recovery of desiccated seeds following rehydration at 100% RH at room temperature for various periods*

Moisture content of seeds (%)	Period of rehydration (h)	Germination* (%)
36.4	0	100
	0.5	100
33.4	0	90
	0.5	90
23.9	0	100
	0.5	95
	48	100
23.1	0	50
	24	60
	48	80
	72	20
19.9	24	20
13.0	24	0
	96	0

*Average values of replicates of the two seasons apparently the causal factors. Cryoinjury to the cotyledons was visible in the form of blotches and white patches and often one of the cotyledons had its connection with the embryonic axis severed. Cryoprotectants used in the present study offered no special advantage during LN exposure as retrieved seeds showed symptoms of cryoinjury and complete loss of viability.

In conclusion, tea seeds are shed in highly moist conditions, are large seeded, exhibit no dormancy and are physiologically sensitive to desiccation and freezing. Since these characteristics observed in the present studies conform to the criteria laid down for the identification of the recalcitrant seeds (Chin, 1988), tea seeds can be categorised as recalcitrant in nature. In order to devise a long-term storage technique, especially by cryopreservation, it would be worthwhile to use the excised embryonic axes as has been successfully done earlier in several recalcitrant species (Withers, 1980; Grout, Shelton and Pritchard, 1983; Normah, Chin and Hor, 1986; Chin, Hor and Krishnapillay, 1989). Attempts are currently being made in our laboratory towards this end.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. R. S. Rana, Director, NBPGR for his encouragement and to the Department of Biotechnology for providing facilities to conduct this study.

REFERENCES

AMMA, S and WATANABE, A. (1985). Long-term storage of germplasm of tea (*Camellia sinensis* (L.) O. Kuntze). *JARQ* **19**, 196-201.

BARMAN, T. S. (1988). Maturation, germination and storage of tea seed. Proc. 30th Tocklai Conf., 1988, 102-105.

CHIN, H. F. (1988). Recalcitrant seeds: techniques for conservation. In *Crop Genetic Resources of East Asia, Proc. Int. Workshop on Crop Genetic Resources of East Asia* 1987, Tsukuba, Japan. ed. S. Suzuki IBPGR 1988.

CHIN, H. F., HOR, Y. L. and KRISHNAPILLAY, B. (1989). Report in IBPGR Newsletter for Asia and the Pacific **1**, 5.

CHIN, H. F. and ROBERTS, E. H. (1980). Recalcitrant Crop Seeds. Tropical Press, Malaysia.

FARRANT, J. M., PAMMENTER, N. W. and BERJAK, P. (1986). Recalcitrance. A current assessment. *Seed Sci. Technol.* **16**, 155-166.

GROUT, B. W. W., SHELTON, K. and PRITCHARD, H. W. (1983). Orthodox behaviour of oil palm seed and cryopreservation of the excised embryo for genetic conservation. *Ann. Bot.* **52**, 381-384.

KATSUO, K., TOYAO, T. and KAYUMI, S. (1970). The germination of tea seed. *Study of Tea*, No. **39**, (Parts 1 & 2) 14-19.

NORMAH, M. N., CHIN, H. F. and HOR, Y. L. (1986). Desiccation and cryopreservation of embryonic axes of *Hevea brasiliensis Muell-Arg. Pertanika* **9**, 299-303.

SEBASTIAMPILLAI, A. R. and ANANDAPPA, T. I. (1979). The influence of moisture and temperature on the germinability and longevity of tea (*Camellia sinensis* L.) seeds. *Tea Q.* **48**, 8-20.

SINGH, H. and RAO, A. N. (1963). Seed germination and seedling morphology in *Durio ziebethinus*. *The Malayah Forester*, **26**, 98-103.

SINGH, I. D. (1979). Indian tea germplasm and its contribution to the world tea industry. *Two and a Bud*, **26**, 23-26.

VISSER, T. and TILLEKERATNE, L. M. de W. (1958). Observations on the germination and storage of tea pollen and seed. *Tea Q.* **29**, 30-35.

WITHERS, L. A. (1980). Cryopreservation of plant cell and tissue cultures. In *Tissue Culture Methods for Plant Pathologists*. eds. D. S. Ingram and J. P. Helgeson, Blackwell Scientific Publications, Oxford. pp. 63-70.