Studies on curry leaf (*Murraya koenigii*) seeds

Curry leaf (*Murraya koenigii*, Rutaceae) is a small evergreen tree, growing 4–6 m tall, with a trunk diameter of 40 cm. The species name commemorates the botanist Johann König. It is an important leafy vegetable condiment in the South Indian cuisine and the leaves are widely used in Indian cookery for flavouring foodstuff. The leaves have a slightly pungent, bitter and feebly acidic taste, and they retain their flavour and other qualities even after drying. Curry leaf is also used in many of the Indian ayurvedic and unani prescriptions. The crop is usually propagated by seeds or suckers.

Polyembryony is the phenomenon of two or more embryos developing from a single fertilized egg, i.e. the phenomenon of more than one seedlings emerging from a single seed. Polyembryonic seeds occur in fruit crops, viz. mango, citrus, jamun, etc. Reports of polyembryony in curry leaf are scanty.

Seeds of curry leaf are chlorophyllous and poor storers. Generally the seeds are classified into 'orthodox' and 'recalcitrant' based on their desiccation and temperature sensitivity. Recalcitrance is the phenomenon of viability loss shown by some seeds to moisture loss[1]. Curry leaf seeds cannot resist the effects of drying or exposure to temperatures less than 10°C; thus, they cannot be stored for long periods like orthodox seeds because they can lose their viability. Hence the curry leaf seeds are considered recalcitrant.

The tetrazolium (TZ) test is one of the most reliable techniques used to estimate seed viability and vigour[2]. TZ is reduced in living cells and produces a coloured, insoluble salt, formazan, which makes it possible to distinguish the living embryos by their red colouration from the dead embryos that retain their original colour[3,4]. Every species requires its own specific method of dissection, staining and evaluation of TZ[5].

Fruits of *M. koenigii* collected from the Agricultural College and Research Institute, Madurai (9°58′N/78°12′E) were utilized for the study. The collected and weighed fruits were graded into eight groups based on fruit weight, i.e. fruit weight range 0.2–0.3 g/fruit, 0.31–0.4, 0.41–0.5, 0.51–0.6, 0.61–0.7, 0.71–0.8, 0.81–0.9 and >0.91 g/fruit.

Fruits selected randomly from each category were subjected to further scientific evaluation. Each group was divided into two sets. One set of fruits was weighed, followed by seed extraction and separation into embryos and cotyledons that were weighed to assess the fruit : seed ratio and seed : embryo ratio. The other set of fruits was used for extraction of seeds that were surface-sterilized with 0.01% mercuric chloride and TZ staining pattern was assessed by soaking the seeds in 1% TZ solution for 4 h at 40°C. A simple CRD design was arranged with eight fruit weights and replicated three times. An analysis of variance (ANOVA) was made using SAS software. Correlation was assessed by using Microsoft Excel software. Significance of mean difference of the variable means was separated using LSD at $P = 0.05$.

The results revealed that fruit weight ranged between 0.97 and 0.27 g (Figure 1 *a*) with mean seed weight ranging between 0.1 and 0.3 g (Figure 1 *b*). The contribution of seed weight to total fruit weight was 26%, but the contribution of embryo was negligible and 74% of the fruit was occupied by the fruit coat, pulp and moisture (data not shown). Smaller fruits were single-seeded and as the fruit weight increased, the number of seeds and embryos also increased. Variation of fruit weight within a category was less compared to seed within the same category. Some of the small fruits had embryoless seeds. As the fruit weight increased, the number of embryos per seed increased (Table 1), as revealed by the number of protruded root/s (Figure 2). Maximum number of embryos present in a curry leaf seed was three/seed. The weight of single embryo was least in small fruit, with single seed and increased with fruit size. Further studies are needed for fruit weight ranging more than 1 g. Seeds collected from dry fruits did not germinate indicating the recalcitrant nature of curry leaf seed. Figure 2 shows that fruit weight has the significant effect on seed weight and that the contribution of embryo to the seed is negligible.

As far as correlation study was concerned, fruit weight was highly significantly correlated with seed weight (0.830), the number of double-seeded fruits (0.951), total weight of embryos (0.906) and weight of single embryo (0.910), and had a significant negative correlation

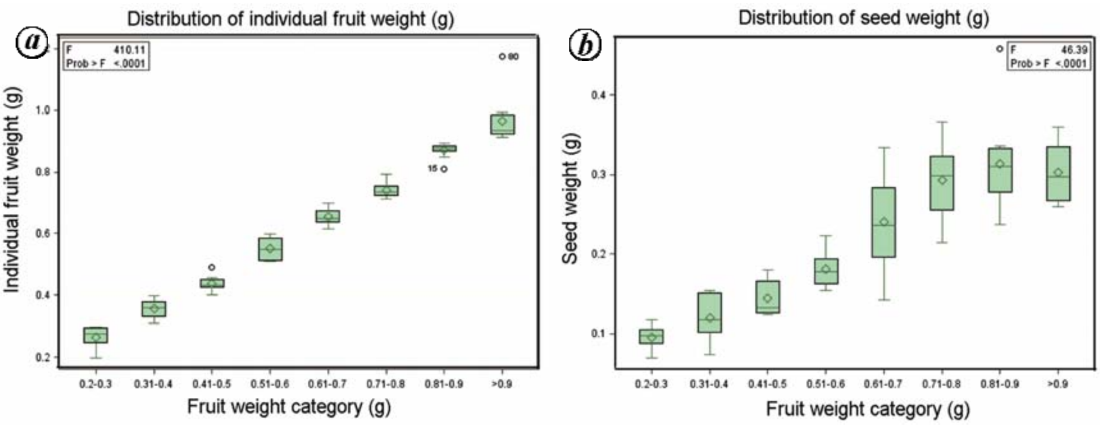

Figure 1. Mean fruit weight of curry leaf fruit categories.

Table 1. Relationship between fruit weight and polyembronic phenomenon

	Fruit weight (mg)	Seed weight (mg)	No. of single-seeded fruits	No. of double-seeded fruits	Seeds without embryo	Seeds with two embryos	Seeds with three embryos	Total number of embryos (from 10 seeds)	Total weight of embryos (mg) (extracted from 10 seeds)	Weight of single embryo (mg)
Fruit weight (mg)	1									
Seed weight (mg)	0.830*	1								
No. of single-seeded fruits	–0.951**	–0.928**	1							
No. of double-seeded fruits	0.951**	0.928**	–1.000**	1						
Seeds without embryo	–0.590	–0.462	0.520	–0.520	1					
Seeds with two embryos	0.109	0.202	–0.313	0.313	0.000	1				
Seeds with three embryos	0.547	0.289	–0.355	0.355	–0.488	–0.258	1			
Total no. of embryos (from 10 seeds)	0.483	0.492	–0.498	0.498	–0.707	0.407	0.609	1		
Total weight of embryos (mg) (extracted from 10 seeds)	0.906**	0.806*	–0.861**	0.861**	–0.843**	0.083	0.579	0.705	1	
Weight of single embryo (mg)	0.910**	0.757*	–0.835**	0.835**	–0.803*	–0.084	0.477	0.472	0.955**	1

*Significant at 5% level; **Significant at 1% level.

Figure 2. Polyembryonic nature of curry leaf seeds: ***a***, Single seedling; ***b***, Double seedlings; ***c***, Triple seedlings.

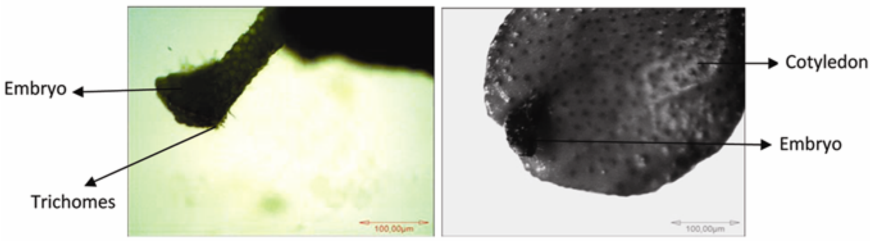

Figure 3. Trichomes in curry leaf embryo and cotyledons.

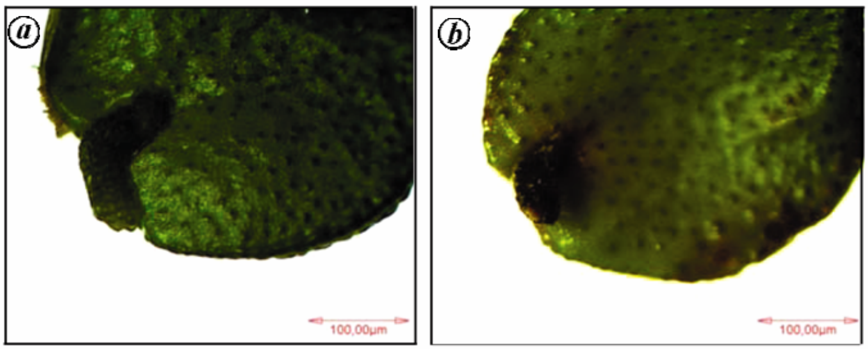

Figure 4. Tetrazolium staining. ***a***, Well stained; ***b***, Partially stained. Note: Cotyledons remain unstained.

with the number of single-seeded fruits (–0.951). Absence of embryon axis was noticed in single-seeded fruit with positive correlation. Seed weight was positively correlated with double-seeded fruit which also had higher embryo weight. Hence double-seeded fruits must be selected for higher seed and embryo weight (Table 1).

Microscopic analysis of curry leaf seed revealed that the embryo had trichomes, while cotyledons were bereft of trichomes (Figure 3).

The TZ test which is the measurement of viability was conducted with a part of the cotyledon enclosing the embryonic axis. Separation of embryonic axis was difficult as it was firmly embedded into the cotyledon. The curry leaf seeds incubated in 1% TZ solution for 4 h at 40°C showed a pink stain in the embryo part and there was no stain in the cotyledon parts (Figure 4). Except the embryo, all other parts of the curry leaf seed did not stain. This is unique as the cotyledon is viable, but still does not stain. This may be because the cotyledon surface cells may have physical barriers to the aqueous TZ solution.

In conclusion, curry leaf seed is a polyembryonic seed. As the fruit weight increases, the seed weight and polyembryony increases. Some authors have reported similar positive association between fruit size and seed quality character. It has been reported in mango that for varieties Espada and Uba, low-density seeds showed poor performance with respect to germinability and seedling growth[6]. In mango large seeds showed better performance than medium and small-sized seed nuts. Seed germination was higher as the seed weight increased in oilpalm[7]. The seed embryo possesses trichomes and during TZ staining only the plumle stained with unstained chlorophyllous cotyledon. Contribution of embryo to seed weight is negligible. This may be due to the barrier-like surface cells that could prevent the infusion of TZ solution. Further studies are needed for fruit weight ranging more than 1 g. Correlation studies revealed the positive nature of fruit size on seed size. Double-seeded fruits were superior in providing seeds of higher weight with no embryos.

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Wolves in Trans-Himalayas: 165 years of taxonomic confusion

Species recognition and systematics of canids has always been a subject of contention among the experts. Canid Action Plan, published by IUCN/SSC Canid Specialist Group in 2004, mentions that there is an existing argument among scientists regarding species number, which ranges from 34 to 38 species[1]. Many species and subspecies of wolves have been identified and reported from different parts of the world. Approximately 13 subspecies of Grey wolf (*Canis lupus*) are recognized, which may not be related so closely[2]. Presence of ecological, morphological and molecular continuum among the species and subspecies of canids makes it difficult to demarkate them. In recent years, discovery of a new wolf species from India has been reported, but the recognition and proper identification of this population as a different species is still ambiguous. This issue came into light with an article by Aggarwal *et al.*[3] showing that the wolf population found in the Indian region of Trans-Himalaya, earlier generally considered to be another population of Tibetan wolf (*Canis lupus chanco*) – a subspecies of the Grey wolf, could be an entirely different species or subspecies. Their results further showed that this population is the oldest lineage of the wolf-dog clan worldwide. Similar results were found in the case of the population from peninsular India, which was generally considered to be a population of another subspecies of the Grey wolf, viz. *Canis lupus pallipes*. This population was also found suitable for upgradation as a separate species or subspecies of wolves. These results were based upon the analysis of mitochondrial DNA and rRNA samples from different populations of canids from all over the world.

Major results of this study, however, were corroborated by similar studies almost at the same time[2,4]. The samples by Sharma *et al.*[4] were collected from much diverse sources and most of the canid populations of the world were represented. This study focused on marking the time of origin of the wolf lineages in India. It also applied the same genetic techniques using mitochondrial DNA and rRNA, and the species divergence was calculated based on fossil record estimates of the divergence time of the coyote and wolf lineages to calibrate sequence divergence rates for each gene. Results of this study show that samples of *C. lupus pallipes* (wolf from peninsular India, Iran, Iraq and parts of Arabia) and *C. lupus chanco* (wolf from Indian Trans-Himalaya, Tibet and Nepal) fall in three separate clades, viz. Indian *C. lupus pallipes* (eastern Pakistan and peninsular India), Himalayan clade of *C. lupus chanco* (Ladakh, Spiti, Tibet and Nepal) and wolf-dog clade (including *C. lupus chanco* from northwest Jammu and Kashmir, i.e. Gilgit and Baltistan). They, further, argue that Himalayan *C. lupus chanco* is the most ancestral and diverged at 800,000 years ago, when the Himalayan region was going through a major geologic and climatic upheaval. Indian *Canis lupus pallipes* is altogether diverged from wolf-dog clade 400,000 years ago. These lineages are the oldest of all wolf lineages in the world, hence it is postulated that India could have been the centre of origin of wolf-dog clan. In this study, dogs were reported to be in close relation with the wolves from Europe and America, therefore, wolves of India might have not been used for domestication. Dogs have originated from multiple wolf ancestors and they started to diverge about 150,000 years ago[5].

Although revising the status of wolves of the Himalayas as species or subspecies, distinct from the other populations, remains disputed, the Himalayan wolf (*C. lupus laniger*) was included in the agenda of Wolf Specialist Group of IUCN in 2005 (accessed from http://wolf-specialistgroup.org/resolutions/). Aggarwal *et al.*[6] published another paper improving on their previous paper. Moreover, they raised a few doubts on the results and methodology of Sharma *et al.*[4]. The new results again confirmed the distinctness of Himalayan and Indian wolves from the Trans-Himalaya and peninsular India respectively, as different species; and the lineage of the Himalayan wolf was confirmed to be the oldest. Following these