Induction of desiccation tolerance in recalcitrant curry leaf (*Murraya koenigii* (L.) Sprengel) seed

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Abstract

Curry leaf is an important leafy vegetable cum spice species grown widely in South India. It is mainly propagated by seeds. The seeds possess very short shelf life and display recalcitrant behavior which is desiccation and freezing sensitive. In order to induce the desiccation tolerance in the seed by seed treatment to enhance seed longevity, the seeds were treated with different desiccation tolerance induction chemicals *viz*., sodium nitroprusside, L-arginine and abscisic acid for 18 h and stored under ambient condition. The results showed that the seed germination was extended upto 20 days when the seeds were soaked in abscisic acid at 100 ppm for 18 h. However, untreated control seed lost its viability completely at 10th day of storage.

Keywords: Abscisic acid, Curry leaf, Desiccation tolerance, Recalcitrant seed, Seed viability.

Introduction

Curry leaf (*Murraya koenigii* (L.) Sprengel) is one of the important spices in India and widely grown in Southern States. It is a small spreading shrub grows with a height of about 2.5 m and grows up to 1500 m altitude. The leaves are the major economic part of the plant and it is added to make flavor in Indian dishes. The major constituents responsible for flavor and aroma have been identified as sabinene, pinene, cadinol, cadinene and caryophyllene. The leaves have a slightly pungent, bitter, feebly acidic taste and retain flavor even after drying. It contains essential oils which are used to make the byproducts like massage oils, scent, air fresheners, soap making ingredients, facial steams, hair treatments, potpourri, diffusers, bath oils, aroma therapy products, lotions etc. Also, the leaves contain the volatile oil and glycoside called "Koenin" which helps for easy digestion in human digestive system. In Indian ayurvedic and unani prescriptions, curry leaf plays an important role in curing many diseases (Singh et al., 2014).

Curry leaf is propagated through seeds and root stocks, but the root stocks are produced only in very few plants, compared to seeds. So, the seeds are mainly used as the propagating material for better plant population. However, curry leaf seeds show recalcitrant behaviour and it loses its viability very quickly (Raja et al., 2001 a). Generally, the seeds are classified into 'orthodox' and 'recalcitrant' based on their storage behavior (Roberts, 1973). The orthodox seeds can be stored with low moisture content and temperature (-196°C) for longer period. However, recalcitrant seeds cannot be stored for longer time with reduction of moisture or storage at low temperature (Roberts, 1973).

Usually, the recalcitrant seeds possess high moisture content ranging from 30 to 70 per cent (Chin, 1989). The reduction in moisture content leads to desiccation changes in cells which causes loss of viability. Generally, sudden decrease in germination of recalcitrant seeds has been noticed at certain moisture level which is known as 'Critical Moisture Content' (Poulsen and Eriksen, 1992) or 'Lowest

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Safe Moisture Content' (Tompsett, 1987). Curry leaf seed also loses its viability at 33.1 per cent moisture content (Arulmoorthy et al., 2019). This desiccation damage in recalcitrant seeds is due to metabolic 'switching off' and 'switching on' mechanism, absence or incomplete expression of physical characteristics of cells and intracellular constituents, accumulation of putative protective substances including Late Embryogenic Abundant (LEA) proteins, operation of repair systems, sucrose and other oligosaccharides as well as amphipathic molecules and presence of oleosins (Pammenter and Berjack, 1999). Due to desiccation sensitivity, storage is the major problem for recalcitrant seeds. So, any development in short-term storage will ease the problem of field collection and transportation to gene banks. The storage of seeds in sealed containers (Raja et al., 2001 b; Arulmoorthy et al., 2020) or waxing (Chin, 1989, Raja and Palanisamy, 2009) or desiccation and cryopreservation of embryos (Raja et al., 2003) has had some success. Therefore, the desiccation tolerance would enable the seed to retain its shelf life for longer period. This could be possible in desiccation tolerance orthodox seeds but not in recalcitrant species. In this regard, the present study has been attempted to induce desiccation tolerance in the recalcitrant curry leaf seeds by seed treatments.

Materials and Methods

The experiment was conducted in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2018-19. The curry leaf seeds were extracted from the freshly collected fruits and they were manually graded to get the uniform size. Then, the seeds were subjected into different desiccation tolerance treatments *viz*., T_1 - Control ,T_2 - Water soaking, T_3 - Sodium nitroprusside @ 50 µM, T_4 - Sodium nitroprusside @ 100 µM, T_5 - Sodium nitroprusside @ 150 µM, T_6 - L-Arginine @ 50 µM, T_7 - L-Arginine @ 100 µM, T_8 - L-Arginine @ 150 µM, T_9 - Abscisic acid @ 50 ppm, T_{10} - Abscisic acid @ 100 ppm and T_{11} - Abscisic acid @ 150 ppm for about 18 h to induce the desiccation tolerance. After that, the seeds were surface dried and stored in plastic trays under ambient condition (30±2°C, 60% RH). The seed samples were drawn from each treatment at once in five days and analyzed the physical, physiological and biochemical changes.

The seed moisture was determined by hot air oven method at 105°C for 16±1 h. Germination test was conducted in four replications with 100 seeds each in sterilized sand medium (ISTA, 2013) and the final evaluation was done at 50 days after sowing. The vigour index was calculated by multiplying the germination percentage and seedling length (Abdul-Baki and Anderson, 1973). Electrical conductivity of seed leachate was measured in the seeds placed for desiccation (Presley, 1958) and seed chlorophyll content was measured at 652 nm using UV-VIS spectrophotometer (Yoshida et al., 1971).

The intercellular changes in the control and treated seeds were also observed through the scanning electron microscope (SEM) (Model: QUANTA 250). The collected data were subjected into statistical analysis (Panse and Sukhatme, 1967) and the critical difference values were calculated at 5 % probability level.

Results and Discussion

Desiccation tolerance is the capacity of an organism to dehydrate below 10 per cent moisture content on fresh weight basis without accumulation of lethal damage (Alpert, 2005). It is the physiological process that takes place in seeds during its development. However, in some species it is absent and leading to sensitivity to desiccation. Therefore, the induction of desiccation tolerance by external stimuli has been studied (Goldbach, 1979; Stanwood, 1986; Costa et al., 2015). There was a gradual reduction in all the parameters tested over the period of storage for all treatments. Seeds of curry leaf when exposed to different treatments showed highly significant differences among the treatments over a period of storage. Among the

Table 1. Effect of desiccation tolerance treatment on seed germination (%) in curry leaf

Treatments	Days after storage					
	0	5	10	15	20	Mean
T_1 - Control	100(87.1)	82(64.9)	13(21.1)	0(2.8)	0(2.8)	39(38.7)
T_2 - Seed soaking in water	100(87.1)	86(68.0)	22(28.0)	0(2.8)	0(2.8)	42(40.4)
T_3 - Seed soaking in Sodium nitroprusside @ 50 µM	100(87.1)	95(77.1)	46(42.7)	0(2.8)	0(2.8)	48(43.9)
T_4 - Seed soaking in Sodium nitroprusside @ 100 µM	100(87.1)	92(73.6)	44(41.6)	0(2.8)	0(2.8)	47(43.3)
T_5 - Seed soaking in Sodium nitroprusside @ 150 µM	100(87.1)	89(70.6)	42(40.4)	0(2.8)	0(2.8)	46(42.7)
T_6 - Seed soaking in L- Arginine @ 50 µM	100(87.1)	92(73.6)	38(38.1)	0(2.8)	0(2.8)	46(42.7)
T_7 - Seed soaking in L-Arginine @ 100 µM	100(87.1)	87(68.9)	36(36.9)	0(2.8)	0(2.8)	45(42.1)
T_8 - Seed soaking in L-Arginine @ 150 µM	100(87.1)	84(66.4)	32(34.5)	0(2.8)	0(2.8)	43(41.0)
T_9 - Seed soaking in Abscisic acid @ 50 ppm	88(69.7)	70(56.8)	56(48.5)	22(28.0)	0(2.8)	47(43.3)
T_{10} - Seed soaking in Abscisic acid @ 100 ppm	86(68.0)	72(58.1)	59(50.2)	26(30.7)	7(15.3)	50(45.0)
T_{11} - Seed soaking in Abscisic acid @ 150 ppm	80(63.4)	64(53.1)	52(46.2)	18(25.1)	0(2.8)	43(41.0)
	T		P		T×P	
SEd	1.7		1.2		3.9	
CD (P=0.05)	3.5		2.3		7.8	

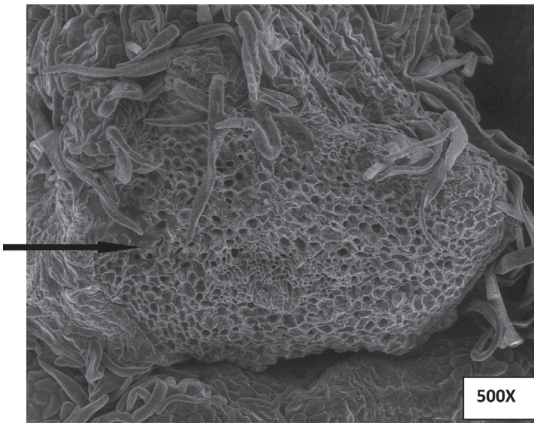
(Values in parenthesis indicate the arc sine transformed values)

Table 2. Effect of desiccation tolerance treatment on seed moisture (%) in curry leaf

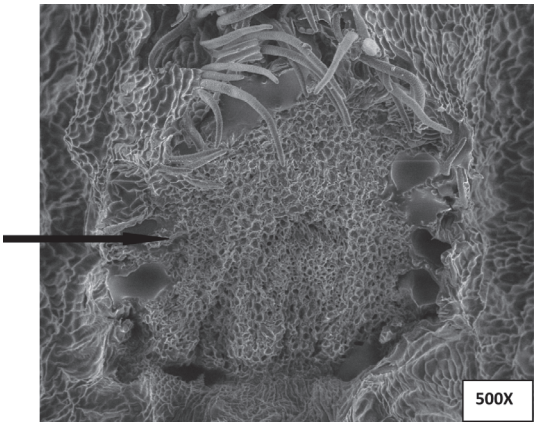
Treatments	Days after storage					
	0	5	10	15	20	Mean
T_1 - Control	47.3	35.6	23.2	15.6	11.3	26.6
T_2 - Seed soaking in water	50.2	42.5	27.2	16.2	12.1	29.6
T_3 - Seed soaking in Sodium nitroprusside @ 50 µM	48.7	44.5	30.6	17.6	14.8	31.2
T_4 - Seed soaking in Sodium nitroprusside @ 100 µM	48.9	44.3	30.3	17.3	14.7	31.1
T_5 - Seed soaking in Sodium nitroprusside @ 150 µM	48.5	43.8	30.1	17.2	14.4	30.8
T_6 - Seed soaking in L- Arginine @ 50 µM	47.9	42.6	29.6	17.1	13.8	30.2
T_7 - Seed soaking in L-Arginine @ 100 µM	48.8	41.7	28.5	16.9	13.5	29.9
T_8 - Seed soaking in L-Arginine @ 150 µM	48.3	41.4	28.1	16.5	13.2	29.5
T_9 - Seed soaking in Abscisic acid @ 50 ppm	48.6	42.3	34.9	24.5	15.6	33.2
T_{10} - Seed soaking in Abscisic acid @ 100 ppm	48.2	42.0	35.3	24.8	15.8	33.2
T_{11} - Seed soaking in Abscisic acid @ 150 ppm	48.9	41.6	34.6	24.2	15.2	32.9
	T		P		T×P	
SEd	0.2		0.1		0.4	
CD (P=0.05)	0.4		0.2		0.8	

treatments, the seeds treated with abscisic acid at 100 ppm recorded highest germination per cent ranging from 100 per cent to 7 per cent at the end of the storage period and the seed moisture content from 48 to 15.8 per cent at 20 days after storage under ambient storage condition (Table 1 & 2). While, untreated control seeds lost its viability completely by tenth day of storage recording a moisture content of 11.3 per cent. The seeds treated with ABA can maintain about 20 per cent germination for upto 15 days irrespective of the concentrations. Abscisic acid (ABA) and gibberellic acid (GA_3) are the most important plant growth regulators which controls many plant development processes including root and stem elongation, floral induction, anther development and seed germination in orthodox and recalcitrant seeds (Yamaguchi, 2008). Therefore, it can be concluded that ABA has desiccation tolerance effect on curry leaf seed.

The anatomical structures of curry leaf seeds viewed through SEM showed considerable differences in shoot and radicle cells of untreated and abscisic acid treated seeds (Figure 1 & 2). The ABA treated seed possessed intact cells in shoot and root portions of the embryonic axis whereas they were disintegrated and collapsed in the untreated seeds. Disintegration of the cells of the embryonic axis may be the reason for the reduced germination in the untreated seeds. The curry leaf seed possess chlorophyll in its

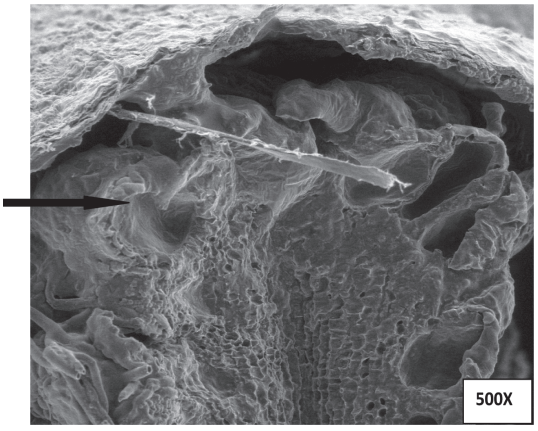


Abscisic acid treated seed (integrity in shoot cells)

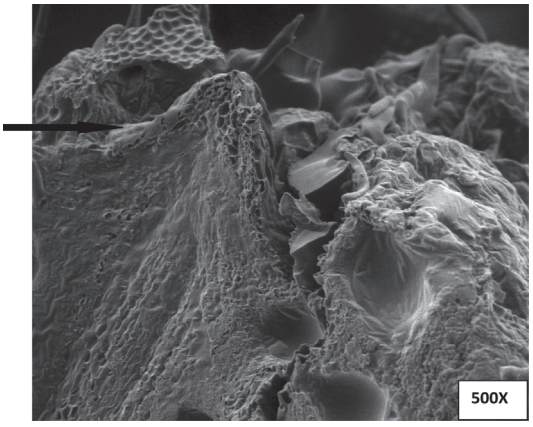


Control (untreated) seed (disintegration in shoot cells)

Figure 1. SEM view of abscisic acid treated and untreated shoot cells of curry leaf seed



Abscisic acid treated seed (integrity in radicle cells and presence of root cap)



Control (untreated) seed (disintegration in radicle cells and absence of root cap)

Figure 2. SEM view of abscisic acid treated and untreated radicle cells of curry leaf seed

Table 3. Effect of desiccation tolerance treatment on vigour index in curry leaf

Treatments	Days after storage					
	0	5	10	15	20	Mean
T_1 - Control	2410	1681	212	0	0	861
T_2 - Seed soaking in water	2470	1823	392	0	0	937
T_3 - Seed soaking in Sodium nitroprusside @ 50 µM	2690	2280	929	0	0	1180
T_4 - Seed soaking in Sodium nitroprusside @ 100 µM	2640	2153	867	0	0	1132
T_5 - Seed soaking in Sodium nitroprusside @ 150 µM	2600	2011	798	0	0	1082
T_6 - Seed soaking in L- Arginine @ 50 µM	2460	2098	752	0	0	1062
T_7 - Seed soaking in L-Arginine @ 100 µM	2430	1836	655	0	0	984
T_8 - Seed soaking in L-Arginine @ 150 µM	2400	1739	563	0	0	940
T_9 - Seed soaking in Abscisic acid @ 50 ppm	1681	1162	756	233	0	766
T_{10} - Seed soaking in Abscisic acid @ 100 ppm	1557	1094	767	302	62	756
T_{11} - Seed soaking in Abscisic acid @ 150 ppm	1360	915	588	166	0	606
	T		P		T×P	
SEd	13.4		9.0		30.0	
CD (P=0.05)	26.6		17.9		59.5	

Table 4. Effect of desiccation tolerance treatment on seed chlorophyll (mg g^{-1}) in curry leaf

Treatments	Days after storage					
	0	5	10	15	20	Mean
T_1 - Control	1.04	0.52	0.40	0.31	0.24	0.50
T_2 - Seed soaking in water	1.07	0.55	0.39	0.33	0.29	0.53
T_3 - Seed soaking in Sodium nitroprusside @ 50 mM	1.06	0.63	0.48	0.36	0.31	0.57
T_4 - Seed soaking in Sodium nitroprusside @ 100 mM	1.09	0.61	0.45	0.34	0.30	0.56
T_5 - Seed soaking in Sodium nitroprusside @ 150 mM	1.07	0.60	0.43	0.31	0.28	0.54
T_6 - Seed soaking in L- Arginine @ 50 mM	1.08	0.58	0.45	0.35	0.31	0.55
T_7 - Seed soaking inL-Arginine @ 100 mM	1.09	0.56	0.42	0.33	0.27	0.53
T_8 - Seed soaking inL-Arginine @ 150 mM	1.07	0.54	0.40	0.29	0.24	0.51
T_9 - Seed soaking in Abscisic acid @ 50 ppm	1.08	0.57	0.44	0.38	0.30	0.55
T_{10} - Seed soaking in Abscisic acid @ 100 ppm	1.09	0.58	0.47	0.40	0.33	0.57
T_{11} - Seed soaking in Abscisic acid @ 150 ppm	1.08	0.55	0.42	0.33	0.27	0.53
	T		P		T×P	
SEd	0.006		0.004		0.01	
CD (P=0.05)	0.013		0.009		0.03	

Table 5. Effect of desiccation tolerance treatment on electrical conductivity (µSm^{-1}) of seed leachate in curry leaf

Treatments	Days after storage					
	0	5	10	15	20	Mean
T_1 – Control	81	225	292	549	822	394
T_2 - Seed soaking in water	76	218	289	538	803	385
T_3 - Seed soaking in Sodium nitroprusside @ 50 µM	75	201	273	514	738	360
T_4 - Seed soaking in Sodium nitroprusside @ 100 µM	76	204	275	519	743	363
T_5 - Seed soaking in Sodium nitroprusside @ 150 µM	78	207	278	521	749	367
T_6 - Seed soaking in L- Arginine @ 50 µM	76	213	280	524	750	369
T_7 - Seed soaking inL-Arginine @ 100 µM	78	218	282	529	755	372
T_8 - Seed soaking inL-Arginine @ 150 µM	79	222	286	532	778	379
T_9 - Seed soaking in Abscisic acid @ 50 ppm	75	209	259	293	560	279
T_{10} - Seed soaking in Abscisic acid @ 100 ppm	76	206	256	290	558	277
T_{11} - Seed soaking in Abscisic acid @ 150 ppm	78	211	261	295	563	282
	T		P		T×P	
SEd	3.5		2.4		7.9	
CD (P=0.05)	7.0		4.7		15.8	

cotyledons and therefore, the seed is green in colour. Usually, fresh seed contains 1.04 mg g^{-1} of chlorophyll when it is 100 per cent viable. However, the seed turns brown to black colour when it is desiccated and becomes non-viable. The rate of reduction of chlorophyll content was high in the untreated seeds from 1.04 to 0.24 mg g^{-1}. The rate of reduction was lesser in the ABA @ 100 ppm treated seeds and therefore, chlorophyll content recorded was comparatively higher (0.33 mg g^{-1}) (Table 4). Similarly, electrical conductivity of the seed leachate measured was lower (558 µSm^{-1}) in ABA @ 100 ppm treated seeds than the untreated (822 µSm^{-1}) (Table 5). This lower leachate might be due to the integration of cell walls in the ABA treated seeds as showed in the SEM images. Similar findings reported that the exogenous application of ABA with 10^{-4} M concentration maintained 100 per cent germination upto 17 weeks in recalcitrant *Meliococcus bijiugatus* seeds while untreated control lost their viability within four weeks (Goldbach, 1979). The silver maple embryonic axes pre-treated with 20 µM and 60 µM ABA and tetcyclasis compound increased the seed germination and seedling length and also, it increases ABA content in embryonic axes (Beardmore and Whittle, 2005). Occurrence of similar process in curry leaf seed cannot be ruled out. Likewise, *Inga vera* sub sp. *affinis* seeds stored at polyethylene glycol + abscisic acid under 20°C

maintained 45 per cent germination upto 62 days (Faria et al., 2006). Costa et al. (2015) identified that the ABA treatment in *Arabidopsis thaliana* showed down-regulation of biotic responses including response to the chitin and induces desiccation tolerance in germinated seeds as evidenced in curry leaf seed. Therefore, ABA was found to induce desiccation tolerance in curry leaf seeds and may be used with further refinements for increasing the storage life and improved seed germination.

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