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Breaking of seed dormancy in ash gourd (*Benincasa pruriens*) through fruit storage and seed treatments

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ABSTRACT

The dormancy in seeds collected from freshly harvested ash gourd [*Benincasa pruriens f. hispida* (Thunb.) de Wilde & Duyfjes] fruits reduces the seed quality and field performance viz. early germination, faster seedling establishment and vigorous seedling growth. Post harvest storage of fruits and different physical and chemical methods are useful in breaking seed dormancy in cucurbits. The present study was carried out during winter (*rabi*) 2020–21 and rainy (*kharif*) seasons of 2021–22 at the Agricultural Research Station (Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu), Vaigai Dam, Tamil Nadu to identify the optimal duration of post-harvest fruit storage and dormancy-breaking method for reducing seed dormancy in ash gourd cv. CO 1. Experiment was conducted in a randomized block design (RBD) with 3 replications. Firstly, the results revealed that the seeds extracted from the fruits, which were stored for 60 days after harvest, exhibited the best field performance, viz. early emergence, higher field emergence, faster rate of emergence and more seedling length, seedling dry weight, seedling vigour index, and viability indicating that prolonged storage can effectively reduce seed dormancy. Secondly, among different dormancy-breaking treatments, warm stratification of seeds at 40–50°C for 5 days and soaking of seeds in 500 ppm ethrel for 16 h remarkably improved the field performance, indicating the usefulness of these methods in improving seed quality characteristics in ash gourd. Farmers can use ethrel to break dormancy in ash gourd.

Keywords: Ash gourd, Breaking treatments, Dormancy, Fruit storage, Germination

Ash gourd [*Benincasa pruriens f. hispida* (Thunb.) de Wilde & Duyfjes] is one of the popular cucurbitaceous vegetable crops grown in India has medicinal and nutritional benefits. In South India, the popular ash gourd cultivars are CO 1, CO 2 and TNAU Ash Gourd Hybrid CO 1 which were released by Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. Seed dormancy is a severe problem in cucurbit species (Gupta *et al.* 2021a), especially in ash gourd. Usually, the seeds are allowed to ripe on the plant and extracted from completely ripe fruits. However, due to varied maturing nature of fruits, seeds harvested from various fresh fruits are not uniformly mature (Gupta *et al.* 2021b, Meghwal *et al.* 2023). This leads to poor seedling establishment and ultimately poor seed yield.

Seed development and maturation in fleshy fruits of cucurbits usually continue even after the harvest until they get extracted from fruits. Dormancy in seeds extracted from freshly harvested ash gourd fruits prevents seed germination for a few months or even years (Rahman *et al.* 2014). Therefore, dormant seeds require time to complete the biochemical and physiological changes associated with seed maturation and gain full germination potential (Parihar *et al.* 2023). Cucurbits have proved that post-harvest storage of fruits helps seeds to attain physiological maturity to improve germination. The beneficial effects of pre-sowing seed dormancy breaking treatments on germination and stand establishment have been reported in various crops, viz. in tomato, rapeseed, ash gourd, bitter gourd and cabbage (Venketasubramanium and Umarani 2007, Bijanzadeh *et al.* 2010, Rahman *et al.* 2014, Saleem *et al.* 2014, Batool *et al.* 2015). So far, the research on seed dormancy in ash gourd is scanty. There is a necessity to find an easy and effective seed-dormancy breaking method (Rahman *et al.* 2014). Hence, in this study, we aimed to find an appropriate post-harvest fruit-storage duration and a suitable dormancy breaking treatment to improve the field performance of seeds of ash gourd cv. CO 1.

MATERIALS AND METHODS

The experiment was conducted during winter (*rabi*) 2020–21 and rainy (*kharif*) seasons of 2021–22 at Agricultural Research Station (Tamil Nadu Agricultural

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University, Coimbatore, Tamil Nadu), Vaigai Dam, Theni, Tamil Nadu in a randomized block design (RBD) with 3 replications. The variety of ash gourd used for our experiment was CO 1. During both *rabi* (2021) and *kharif* (2022) seasons, fully matured ash gourd fruits (70 days after anthesis) were harvested from the field and were used as the basic seed for conducting experiments.

Post-harvest fruit-storage duration: Fresh fruits were harvested from the field and subjected to different post-harvest fruit-storage durations under ambient conditions, viz. T_1, Control, (i.e., seeds are extracted immediately after the harvest of fruits without storage); T_2, 10 days of fruit storage; T_3, 20 days of fruit storage; T_4, 30 days of fruit storage; T_5, 40 days of fruit storage; T_6, 50 days of fruit storage; T_7, 60 days of fruit storage. The seeds from these fruits were extracted and sown in the field and data related to field performance were collected, viz. number of days to emerge, field emergence (%) at 7^{th}, 10^{th} and 14^{th} day, rate of germination, seedling length (cm), seedling dry weight (g) and vigour index.

Dormancy breaking treatments: Seeds were extracted from the freshly harvested fruits and were subjected to different seed dormancy treatments. The treatments include: T_1, Control; T_2, Warm stratification at 40–50°C for 2 days; T_3, Warm stratification at 40–50°C for 5 days; T_4, Drying of seeds in a hot air oven at 45°C for 24 h; T_5, Drying of seeds in a hot air oven at 45°C for 48 h; T_6, Soaking of seeds in warm water at 50°C for 30 min; T_7, Soaking of seeds in warm water at 50°C for 60 min; T_8, Soaking of seeds in 250 ppm ethrel for 16 h and T_9, Soaking of seeds in 500 ppm ethrel for 16 h. In warm stratification, the seeds were mixed with moist sand and incubated at 40–50°C for 2 days and 5 days before sowing and ensured that the medium was wet. In hot air oven treatment, the seeds were placed in a butter paper bag and subjected to heating at 45°C for 24 and 48 h in a hot air oven. At the end of the treatments, seeds were taken out and sown in the field and data related to field performance were collected, viz. number of days to emerge, field emergence (%) at the 7^{th}, 10^{th} and 14^{th} day, rate of emergence (%), seedling length (cm), seedling dry weight (g/10 seedlings) and vigour index. Observations were recorded for the field emergence (%), rate of emergence (%), seedling length (cm), seedling dry weight, vigor index and viability (%). The data collected from various experiments were analyzed statistically (Panse and Sukhatme 1999).

RESULTS AND DISCUSSION

Effect of post-harvest fruit-storage duration: Seed dormancy in seeds of ash gourd is mainly due to inadequate physiological maturation of seeds resulting from inadequate post-harvest fruit-storage duration (Gupta *et al.* 2021a). Our results indicate that different fruit storage durations influence the field performance of ash gourd seeds differently. With reference to the number of days to emergence, the seeds extracted from the 60-days stored fruits (i.e. treatment 7) took very less time to emerge (5.2 days), when compared to seeds extracted from the control (freshly harvested fruits) (7.5 days). Moreover, with reference to the field emergence on the 7^{th} day, 10^{th} day and 14^{th} day (Table 1), the seeds from the 60 days stored fruits exhibited a very high percentage of field emergence (16%, 51% and 64% respectively), when compared to control seeds (1%, 9% and 18% respectively). The rate of emergence exhibited by seeds from fruits stored for 60-days was significantly higher (7%) than that of seeds from fresh fruits (1.8%). Similarly, with reference to the seedling dry weight, seedling length and seedling vigour index, the seeds extracted from the 60-days stored fruits exhibited more seedling dry weight (0.383 g/10 seedlings), more seedling length (18.93 cm) and more seedling vigour index (1056), when compared to seeds from fresh fruits (0.229 g/10 seedlings, 10.18 cm and 178 respectively) (Table 2). Regarding the seed viability index, the seeds extracted from the 60-days stored fruits exhibited very high viability (83%), when compared to the control (34%) (Table 2). The superior field performance of seeds of the 60-days stored fruits may be attributed to the extended storage duration, which might have helped the seeds to fully complete their biochemical and physiological maturation.

Table 1 Effect of post-harvest fruit-storage period on days to emergence and field emergence of ash gourd cv. CO 1 under field condition during *rabi* 2021 and *kharif* 2022

Treatment	Number of days to emerge (Days)			Field emergence at 7^{th} day (%)			Field emergence at 10^{th} day (%)			Field emergence at 14^{th} day (%)		
	Rabi	*Kharif*	Mean	*Rabi*	*Kharif*	Mean	*Rabi*	*Kharif*	Mean	*Rabi*	*Kharif*	Mean
T_1	8	7	7.5	0	2	1	7	10	9	18	17	18
T_2	8	7	7.3	1	3	2	20	17	19	23	24	24
T_3	7	7	7.0	5	7	6	29	23	26	28	32	30
T_4	7	6	6.7	8	11	10	32	24	28	32	33	33
T_5	7	6	6.3	11	13	12	37	31	34	37	34	36
T_6	6	5	5.3	14	15	15	43	41	42	48	42	45
T_7	5	5	5.2	15	17	16	48	54	51	64	63	64
CD (P=0.05)		0.580			2.434			4.075			2.747	
SEd		0.282			1.182			1.995			1.345	

Treatment details are given under Materials and Methods.

Table 2 Effect of the post-harvest fruit-storage period on the rate of emergence, seedling dry-weight, seedling length, vigour and viability (%) of ash gourd cv. CO 1 under field condition during *rabi* 2021 and *kharif* 2022

Treatment	Rate of emergence (%)			Seedling dry weight (g/10 seedlings)			Seedling length (cm)			Seedling vigour index			Viability (%)		
	Rabi	*Kharif*	Mean	*Rabi*	*Kharif*	Mean	*Rabi*	*Kharif*	Mean	*Rabi*	*Kharif*	Mean	*Rabi*	*Kharif*	Mean
T_1	1.5	2.0	1.8	0.235	0.223	0.229	9.89	19.04	10.18	174	181	178	33	34	34
T_2	2.7	2.6	2.7	0.257	0.271	0.264	11.69	22.37	11.65	272	282	277	40	41	41
T_3	3.5	3.8	3.7	0.291	0.313	0.302	13.75	23.36	13.81	389	449	419	46	47	47
T_4	3.6	4.4	4.0	0.308	0.340	0.324	14.37	25.33	14.47	454	485	470	55	51	53
T_5	4.9	4.8	4.9	0.323	0.358	0.340	16.05	26.31	15.92	594	531	563	60	58	59
T_6	5.8	6.3	6.1	0.341	0.368	0.355	16.98	28.25	17.31	736	747	742	72	71	72
T_7	6.8	7.1	7.0	0.374	0.392	0.383	18.70	29.22	18.93	905	1207	1056	82	84	83
CD (P=0.05)		0.685			0.013			0.465			41.903			2.751	
SEd		0.335			0.006			0.226			20.517			1.347	

Treatment details are given under Materials and Methods.

Murugesan and Vanangamudi (2005) have recommended that ash gourd fruits should be stored for a period of 5 months under ambient conditions for proper post maturation of seeds and improved germination. They have observed that storage beyond 5 months has a deleterious effect on seed quality for the cultivars in Tamil Nadu Seed Production. However, studies conducted by Ganar *et al.* (2004) reported that under north Indian conditions, storage of ash gourd fruits for 30 days is enough to produce the best seed quality.

The seed dormancy, found in freshly harvested cucurbit seeds, could be broken by post-harvest storage (after ripening) for one to a few months (Baskin *et al.* 2020). One month of post-harvest ripening of seeds in ash gourd improved germination above minimum seed certification standards (Ganar *et al.* 2004). The reason for the observation of an increase in seedling vigour index in our studies might be due to an increase in the rate of metabolic activity and respiration, better mobilization, and utilization of metabolites to growing points and higher activity of enzymes. The results are in accordance with the findings of Ganar *et al.* (2004) and Murugesan and Vanangamudi (2005) in ash gourd and Yao *et al.* (2012) in bottle gourd. A thicker seed coat is reported to act as a mechanical barrier for water imbibition. The possible explanation for the observation of an increase in early emergence and higher rate of emergence in our studies might be owing to the natural increase in the permeability of the seed coat, which occurs after storage due to the opening of specialized cracks in the seed coat (Smykal *et al.* 2014).

Effect of dormancy-breaking treatments: With reference to the number of days to emergence (Table 3), the seeds warm-stratified at 40–50°C for 5 days took very less time to emerge (5.5 days), when compared to seeds extracted from control (untreated freshly harvested seeds) (8 days). Moreover, with reference to the field emergence on the 7th day, 10th day and 14th day (Table 3), the seeds warm-stratified at 40–-50°C for 5 days exhibited a very high percentage of field emergence (32%, 51% and 65% respectively), when compared to control seeds (1%, 13% and 14% respectively). The rate of emergence exhibited by seeds warm stratified at 40–50°C for 5 days was significantly higher (7.75%) than that of control seeds (1.5%).

Similarly, with reference to the seedling dry weight, seedling length and vigour index, the seeds warm-stratified at 40–50° C for 5 days exhibited more seedling dry weight (0.386 g/10 seedlings), more seedling length (14.87 cm) and more seedling vigour index (961), when compared to control seeds (0.253 g/10 seedlings, 10.77 cm and 151 respectively) (Table 3). Soaking of seeds for 16 h in 500 ppm ethrel also exhibited comparably better results, viz. early emergence (6.0 days), higher field emergence (32% at 7th day, 36% at 10th day and 63% at 14th day), faster rate of emergence (7.3%), and more seedling dry weight (0.371 g/10 seedlings), seedling length (12.96 cm) and seedling vigour index (810).

Studies have also demonstrated that techniques like seed coat puncture, scarification, seed treatment with

Table 3 Effect of pre-sowing dormancy-breaking treatments on days to emergence, rate of emergence field emergence, seedling dry weight, seedling length and seedling vigour index of ash gourd cv. CO 1 under field condition during *rabi* 2021 and *kharif* 2022

Treatment	Number of days to emerge (Days)			Rate of emergence (%)			Field emergence at 7th day (%)			Field emergence at 10th day (%)			Field emergence at 14th day (%)			Seedling dry weight (g/10 seedlings)			Seedling length (cm)			Seedling vigour index		
	Rabi	*Kharif*	Mean	*Rabi*	*Kharif*	Mean	*Rabi*	*Kharif*	Mean	*Rabi*	*Kharif*	24.855	*Rabi*	*Kharif*	Mean	*Rabi*	*Kharif*	Mean	*Rabi*	*Kharif*	Mean	*Rabi*	*Kharif*	Mean
T_1	8	8	8.0	1.7	1.3	1.50	1	0	1	14	11	13	14	13	14	0.238	0.267	0.253	10.71	10.82	10.77	157	144	151
T_2	7	6	6.5	4.0	3.9	3.95	17	21	19	32	23	28	34	33	34	0.338	0.357	0.348	11.69	11.19	11.44	397	375	386
T_3	6	5	5.5	7.9	7.6	7.75	32	31	32	50	52	51	66	63	65	0.378	0.394	0.386	14.48	15.26	14.87	956	966	961
T_4	9	8	8.5	1.8	1.2	1.50	1	1	1	14	10	12	18	15	17	0.233	0.261	0.247	9.41	9.69	9.55	166	144	155
T_5	9	8	8.5	1.5	1.1	1.30	2	1	2	4	4	4	16	11	14	0.219	0.247	0.233	9.43	10.17	9.80	154	115	135
T_6	9	8	8.5	1.9	1.3	1.60	5	3	4	6	8	7	21	18	20	0.322	0.325	0.324	8.65	10.70	19.35	181	194	188
T_7	8	8	8.0	2.1	1.5	1.80	1	4	3	4	7	6	23	16	20	0.319	0.337	0.328	9.70	10.70	10.20	220	170	195
T_8	7	6	6.5	6.6	5.6	6.10	20	23	22	16	24	20	48	51	50	0.347	0.366	0.357	11.72	12.17	11.95	562	621	592
T_9	6	6	6.0	7.4	7.2	7.30	30	33	32	37	35	36	63	62	63	0.357	0.384	0.371	12.2	13.72	12.96	769	851	810
CD (P=0.05)		0.457			0.337			3.723			3.578			2.742			0.011			0.465			50.234	
SEd		0.227			0.167			1.842			1.771			1.357			0.005			0.226			24.855	

Treatment details are given under Materials and Methods.

hydrochloric acid and sulphuric acid, chilling treatment, hot water treatment, etc. have reduced seed dormancy in many cucurbits (Paul *et al.* 2008, Hatwal *et al.* 2022, Padhiary *et al.* 2022). Rahman *et al.* (2014) have reported that the seed dormancy in ash gourd is due to a thick seed coat. They tested different methods to break the dormancy of ash gourd seeds and found that 0.4% potassium nitrate treatment gave the best results i.e. maximum seed germination (78.67%) and maximum vigour index (2.13) of ash gourd. Ma *et al.* (2023) reported that warm and cold stratification promotes the breaking of the physiological dormancy of seeds, by changing the physiology and growth potential of the embryo.

Thirdly, the soaking treatment of seeds for 16 h in 500 ppm ethrel also displayed comparably better field performance, indicating the usefulness of ethylene treatment in improving seed quality characters. The reasons for the better results in ethylene treatment may be due to the stimulation of a series of biochemical changes in the seed that are essential for initiating the emergence process like break down of dormancy, hydrolysis and removal of growth inhibitors, imbibition, and activation of enyzmes (Rahman *et al.* 2014, Mathad *et al.* 2003). Hilli *et al.* (2008) also reported that ethrel treatment increase the photosynthetic activity, synthesis and translocation of more metabolites to developing embryos for better development of seed and release growth promoting enzymes, which degrade macro-molecules into micro-molecules.

Ethrel, upon penetration into plant tissues, is decomposed to ethylene, which positively affects the growth process. Ethylene is involved in numerous plant developmental processes including seed germination and seedling establishment. Ethylene plays a key role in breaking dormancy in numerous species (Deepthi *et al.* 2020, Chaudhari *et al.* 2023). The hormonal balance between ethylene, abscisic acid (ABA) and gibberellins (GAs) regulates seed dormancy (Corbineau *et al.* 2014).

This study clearly demonstrates that seed dormancy, affects germination, seedling establishment and seedling growth in ash gourd. It can be easily overcome by storing the harvested fruit for at least 60 days. Alternatively, seed dormancy can be broken before sowing of seeds either using a warm stratification at 40–50°C for 5 days or soaking of seeds in 500 ppm ethrel for 16 h. Since ethrel treatment is relatively easier and cheaper than warm stratification, we suggest that the farmers of ash gourd could choose ethrel treatment for large-scale commercial applications.

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