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HALOPRIMING: A MEANS TO OFFSET SEED AGEING IN ASH GOURD

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ABSTRACT

An experiment was conducted to explicate the performance and longevity of the ash gourd seeds following halopriming. The seeds of ash gourd were treated with various priming solutions *viz.*, KNO_3 (0.4% and 0.7%), KH_2PO_4 (10^{-1} M), kinetin (10 ppm), salicylic acid (60 ppm), PEG 6000, vinegar (pH 3.7 for 2 hrs.), $CaCl_2$ (50 mM) and *Pseudomonas fluorescens* (1×10^6 cfu.ml^{-1}). Results of the study revealed that invigoration treatments significantly influenced the quality and longevity of seeds over the storage period. Thought seed treatment with salicylic acid and vinegar was found to be detrimental, other invigoration treatments registered higher seed quality parameters over the storage period. Treated seeds ($Cacl_2$@50 mM for 24 hrs.) had retained viability above Minimum Seed Certification Standard for a longer period (7 months) compared to two months by untreated seeds. Maximum seed germination and seedling vigour over the storage period were observed in the treatment of 50 Mm $CaCl_2$ for 12 hrs. followed by those treated with *Pseudomonas fluorescens* for 12 hrs. and $CaCl_2$ for 24 hrs. With the results of the study, we recommend seed treatment with 50 mM $CaCl_2$ or *Pseudomonas fluorescens* (1×10^6 cfu.ml^{-1} for 12 hrs.) for effective invigoration in ash gourd.

Key words : Halopriming, Ash gourd, Seed invigoration, Seed storage.

Introduction

Ash gourd (*Benincasa hispida* Thunb. Cogn.) also known as a white gourd, tallow gourd, wax gourd, fuzzy gourd and winter melon and is highly valued for its aesthetic, nutritive and medicinal properties throughout Asia. It finds a mention in the ancient Ayurvedic Indian texts *Charaka Samhita* and *Astanga Hridaya Samhita* for the treatment of ulcers, intestinal parasites, weight loss and many more. It is a highly preferred ingredient in low carb, fat, cholesterol and high fibre diets and also contain considerable amounts of vitamins B and C along with crucial minerals like calcium, potassium, iron and phosphorous. Ash gourd is also valued for its relation with a cultural ceremony in most Asian countries.

Usually grown in rainy and winter season in Kerala, ash gourd is a popular cucurbitaceous vegetable mostly eaten when mature. Despite its popularity, its cultivation is greatly affected by very low seed germination (Robinson and Deckers, 1997), invariably immediately after seed extraction. In addition, retention of seeds under high humid tropical regions like Kerala is also a major issue faced by the farming community.

Seed maturation in cucurbits usually continues until the fruit starts yellowing with senescence. The quality of seed in fleshy-fruited species is enhanced when they acquire maximum dry weight (Demir and Ellis, 1992) and the seeds continue to develop and mature in the fleshy fruits until they get extracted from fruits (Ahmed *et al.*, 1987). Seed coat-imposed dormancy has been attributed to be one of the reasons for low germination. Seed dormancy prevents the seeds from germinating after harvest for a few months or even years (Ganar, 2003). Dormancy in cucurbit seed exhibited usually immediately on harvest and can be broken by a month or more of

ripening (Robinson and Deckers, 1997).

To overcome low germination issue and increase the quality and viability of seeds, invigoration with an appropriate chemical and various osmotics have been suggested in several vegetable species. Seed invigoration treatments have been employed to improve both the rate and uniformity of germination in vegetable species. Pre-storage priming of seeds has been found effective in prolonging seed viability over storage. Such technologies could greatly benefit both the seed industry and the farming community. Considering the above, a study was conducted in ash gourd to identify an efficient way to enhance germination as well as seed viability and quality over the storage period.

Materials and Methods

An experiment was conducted in the Department of Seed Science and Technology, Kerala Agricultural University, Vellanikkara, Thrissur between April 2015 and February 2016 using the ash gourd variety 'KAU Local', which widely grown in Kerala. A completely randomised design with four replications was followed. Freshly extracted uniform sized seeds with the moisture of 15 per cent were subjected to various priming treatments for the respective duration (Table 1). Untreated seeds served as the control. The treated and untreated seeds dried to ≤8 per cent moisture and then packed in polythene bags (700 G) and stored in ambient condition. To record monthly observations (germination and other seed quality parameters) seed samples were drawn at random from each replication of each treatment. After sowing, observation on seedling emergence was recorded on daily basis for 14 days. The study was conducted for eight months.

Table 1 : Seed treatments.

Treatments	Details
T_1	Hydropriming (for 24 hrs.)
T_2	Thiourea (@0.5 % for 24 hrs.)
T_3	KNO_3 (@0.4 % for 24 hrs.)
T_4	KNO_3 (@0.7 % for 24 hrs.)
T_5	KH_2PO_4 (@10^{-1} M for 24 hrs.)
T_6	Vinegar (pH 3.7 for 2 hrs.)
T_7	Polyethylene glycol 6000 (@-0.5 MPa or 24 hrs.)
T_8	Salicylic acid (@60 ppm for 12 hrs.)
T_9	Salicylic acid (@60 ppm for 24 hrs.)
T_{10}	Cytokinin (@10 ppm for 12 hrs.)
T_{11}	Cytokinin (@10 ppm for 24 hrs.)
T_{12}	$Cacl_2$ (@50 mM for 12 hrs.)
T_{13}	$Cacl_2$ (@50 mM for 24 hrs.)
T_{14}	*Psuedomonas fluorescens* (1×10^6 cfu.ml^{-1} for 12 hrs.)
T_{15}	*Psuedomonas fluorescens* (1×10^6 cfu.ml^{-1} for 24 hrs.)
T_{16}	Control (Untreated)

Germination index (Speed of germination)

Four replicates of 100 seeds each were used to test the speed of germination from different treatments. The seeds showing radicle protrusion were counted every day from the third day after sowing up to 14 days. From the number of seeds germinated each day, the speed of germination was calculated using the following formula down below and the results were expressed as number (Maguire, 1962).

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots + \frac{X_n - X_{n-1}}{Y_n}$$

X_n- Number of seeds germinated on 1st, 2nd…. nth day

Y_n- Number of days from sowing to 1st, 2nd …. nth count

Mean time to germination (days)

Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981)

$$\text{Mean germination time (MGT)} = \frac{\Sigma Dn}{\Sigma n}$$

Where,

n = number of seeds, which were germinated on day D

D = number of days counted from the beginning of germination test

Vigour index- I

Vigour index-I was computed adopting the formula suggested by Abdul-Baki and Anderson (1972) and expressed as the whole number.

Vigour index-I = Germination (%) × Seedling length (cm)

Vigour index- II

Vigour index-II was computed, adopting the formula suggested by Bewly and Black (1994).

Vigour index-II = Germination (%) × Seedling dry weight (g).

Results and Discussion

Results revealed that germination and seed quality during storage was found to be significantly influenced by the seed invigoration treatment.

Germination declined with an increase in the storage period both in untreated control and in invigorated seeds (Table 2). In most treatments, the germination declined after one month of storage. However, in seeds treatments *viz*., KNO_3 (0.7% for 24 hrs.), *P. fluorescens* (*Pf*) (1×106 cfu.ml^{-1} for 24 hrs) and control, an initial increase was evident one month after storage (MAS). This initial increase can be explained by the dormancy break after a certain span of time (Robinson and Deckers, 1997; Bian *et al*., 2013). Invariably, the loss of germination over the storage period may be attributed to an increase in external osmotic pressure which influences the assimilation of water by the seed. It can also be due to the accumulation of Na^+ and Cl^- in the embryo which may lead to a change of the metabolic processes of germination and subsequently causing cells death in the embryo (Maher *et al*., 2013).

Seed invigoration with salicylic acid (12 hrs and 24 hrs), vinegar (for 12 hrs) and PEG 6000 for 24 hrs proved to be detrimental to the seeds. Germination in the above treatments failed to reach above the Minimum Seed Certification Standard (MSCS) (60%) required for ash gourd during the storage period. All other seed invigoration treatments exerted a positive influence on extending the seed viability (Fig. 1), while, the untreated seed had sustained germination (above 60 per cent) for two MAS only.

For the most part of the storage period, seeds treated with $CaCl_2$ (50 mM for 24 hrs.) and *Pf* and kinetin for 12 hrs. recorded high germination (Table 2). Germination in the treatment with $CaCl_2$ (50 mM for 24 hrs.) was maintained above MSCS for 7 MAS, while in the seeds treated with $CaCl_2$ (50 mM for 12 hrs) and *Pf* (for 24 hrs), viability was retained above MSCS up to 6 MAS. Seeds treated with thiourea, KNO_3, KH_2PO_4 and kinetin retained viability above MSCS for 4 MAS.

Seed quality parameters like germination per cent, germination index, mean time to germination, vigour index-I and vigour index-II had exhibited a general decline during the period of storage (Tables 2 and 3). The significant decrease in seed quality parameters during storage can possibly be attributed to either one or combination of the factors like accumulation of toxicants and corrosive action caused by acids (Zhana *et al*., 1993), membrane degradation, which resulted in greater leakage of sugars, amino acids and inorganic solutes from the seed (Abdul-Baki and Anderson, 1972), free radical damage formed due to lipid peroxidation (Rudrapal and Basu, 1982), imposed enzymatic activity (Chauhan *et al*., 1984; Zuo *et al*., 1988), increase in respiratory quotients (Harrington, 1973), *etc*.

Although, a marginal decrease in germination index,

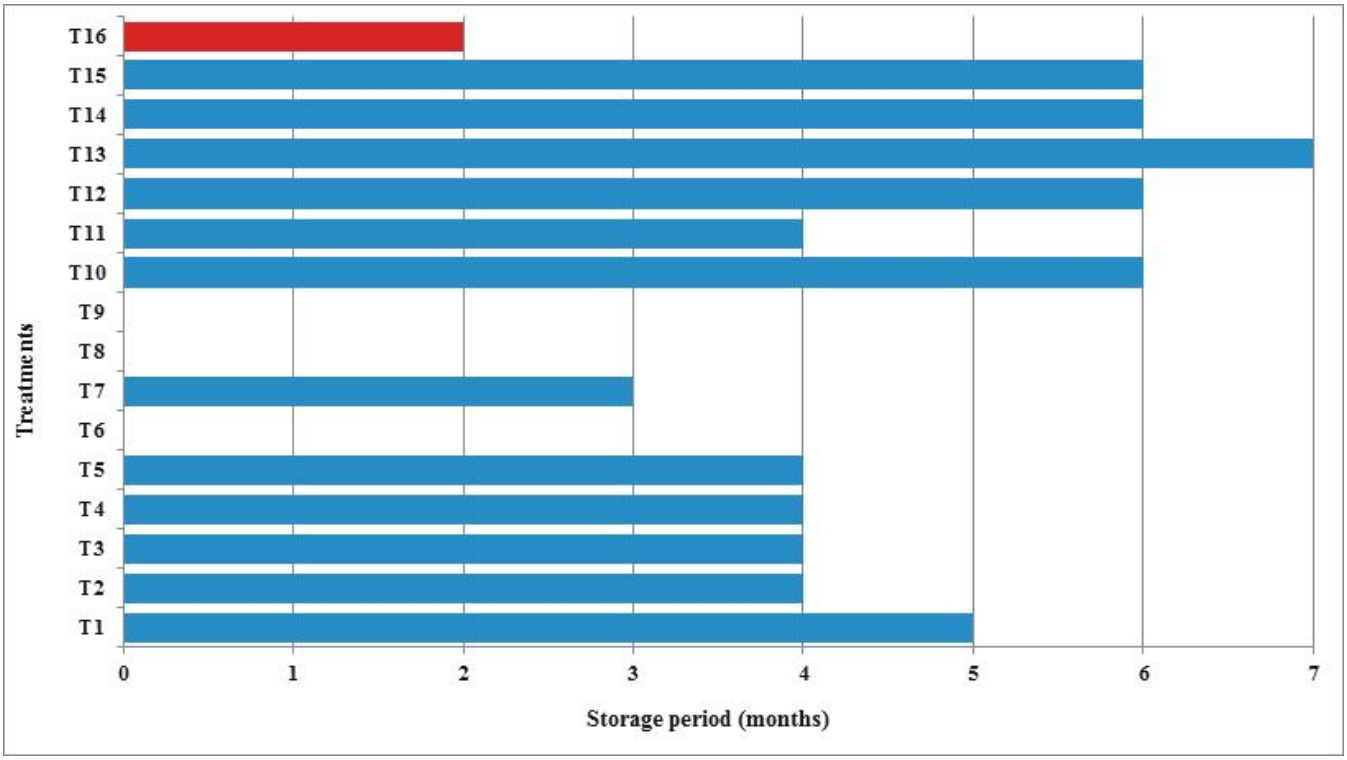

Fig. 1 : Impact of seed invigoration on retention of seed viability during storage.

Table 2 : Effect of seed invigoration on germination, germination index and mean time to germination in ash gourd seeds during storage.

Treatments	Germination (%)					Germination index					Mean time to germination				
	2 MAS	4 MAS	6 MAS	7 MAS	8 MAS	2 MAS	4 MAS	6 MAS	7 MAS	8 MAS	2 MAS	4 MAS	6 MAS	7 MAS	8 MAS
T_1	85.00	79.16	52.50	48.70	30.00	12.26	10.99	5.74	5.34	3.81	6.93	7.19	9.14	9.11	7.86
T_2	77.50	65.83	46.50	38.21	27.50	8.60	5.48	4.25	3.24	2.85	9.01	12.01	10.92	11.78	9.64
T_3	73.40	71.53	50.75	40.50	20.50	10.33	7.66	5.71	4.23	2.11	7.10	9.33	8.88	9.56	9.68
T_4	94.16	70.83	58.00	47.60	25.50	13.51	10.17	7.16	5.16	3.36	6.96	6.96	8.10	9.22	7.57
T_5	90.83	70.00	50.50	48.50	55.00	12.82	10.00	6.42	5.32	6.43	7.08	7.00	7.86	9.10	8.54
T_6	30.00	9.16	10.58	5.50	0.00	2.71	0.84	0.85	0.62	0.00	11.05	10.90	12.44	8.82	0.00
T_7	47.50	34.50	18.70	15.50	2.00	4.75	3.24	1.87	1.82	0.81	10.00	10.64	9.98	8.51	8.44
T_8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_{10}	85.00	69.16	62.20	43.70	35.00	12.28	9.80	7.47	8.20	4.37	6.91	7.05	6.75	5.32	6.60
T_{11}	82.50	70.00	56.00	47.50	39.50	11.69	10.00	7.31	5.86	4.93	7.05	7.00	7.65	6.91	6.73
T_{12}	95.00	87.50	68.50	58.00	40.50	13.52	12.69	8.68	7.32	5.06	7.02	6.89	7.88	7.92	6.74
T_{13}	92.50	90.83	70.50	62.00	55.50	13.68	12.84	8.45	7.43	6.93	6.75	7.07	6.75	6.77	6.93
T_{14}	98.30	84.23	72.00	55.50	49.50	14.12	12.15	8.96	6.55	6.18	6.95	6.93	6.80	6.68	6.78
T_{15}	84.16	62.50	67.50	52.20	42.50	12.20	8.69	8.33	6.33	5.31	6.89	7.19	6.84	6.83	6.58
T_{16}	69.16	35.00	26.50	10.50	0.00	6.39	3.26	4.41	1.66	0.00	10.81	10.71	6.00	6.32	0.00
CD @ 5%	3.93	2.34	2.21	2.25	3.32	1.20	0.66	0.40	0.42	0.65	1.80	1.67	1.90	1.67	2.72
SEm ±	3.58	3.24	2.69	2.44	2.62	1.25	0.89	0.54	0.56	0.89	0.25	0.31	0.31	0.28	0.41

vigour indices I and II was observed in both treated and untreated seeds as storage period increased, these parameters in the majority of invigoration treatments were higher than that in untreated control pointing to the beneficial effect of seed invigoration. Treatments with $CaCl_2$ (for 12 hrs and 24 hrs) and *Pf* for 12 hrs had registered high germination index as well as vigour indices I and II for the most part of storage period.

Negligible to a marginal increase in electrical conductivity (EC) of seed leachate was observed in all treatments over the period of storage (Table 3). The increase being invariably high in seeds treated with salicylic acid 60 ppm, vinegar, PEG 6000 and untreated seeds. However, the highest electrical conductivity of seed leachate at the end of storage was observed in untreated seeds. The differential EC values recorded among the seed treatments indicated that the nature and extent of membrane protection offered may not be same for all treatments, thus resulting in a difference in EC values (Kurdikeri, 1993). The lower EC in treated seeds may be because priming permits early DNA replication, increase RNA and protein synthesis, enhances embryo growth, repairs deteriorated seed parts and reduces leakage of metabolites (Adebisi *et al*., 2011). Increase in membrane repair during the hydration process may also be the cause of low EC of seed leachate as reported by Rudrapal and Nakamura (1988) in radish and eggplant, Pen-aloza and Eira (1993) in tomato and Basra *et al*. (2003) in fine rice.

Based on the findings of the present study enumerated above, halopriming with $CaCl_2$ can be adjudged the best seed invigoration in ash gourd. Seeds treatment with $CaCl_2$ (50 mM for 24 hrs) had retained viability for the maximum period after invigoration (7 MAS) compared to untreated seeds (2 MAS). Most of the haloprimed seed also recorded higher germination index, vigour indices I and II while registering lower mean time to germination and electrical conductivity, over the storage period.

In addition, halopriming with 50 mM $CaCl_2$ or bio-priming with *Pseudomonas fluorescens* (1×10^6 cfu.ml^{-1}) for 12 hrs *Pf* can also be recommended for seed invigoration in

Table 3 : Effect of seed invigoration on vigour index-I, II and mean electric conductivity in ash gourd seeds during storage.

Treatments	Vigour index-I					Vigour index-II					Mean EC
	2 MAS	4 MAS	6 MAS	7 MAS	8 MAS	2 MAS	4 MAS	6 MAS	7 MAS	8 MAS	
T_1	2163	1879	1128	1045	609	2.20	1.77	1.24	1.10	0.62	0.0019
T_2	1600	1497	841	635	456	1.83	1.31	0.91	0.62	0.40	0.0028
T_3	1588	1522	978	677	370	1.71	1.47	0.98	0.77	0.36	0.0028
T_4	2182	1703	1243	564	501	1.91	1.39	0.98	0.74	0.46	0.0027
T_5	2258	1693	1160	1168	1257	2.08	1.33	1.04	0.98	0.96	0.0025
T_6	612	199	203	79	0	0.11	0.074	0.24	0.076	0.00	0.0044
T_7	1195	831	365	266	28	0.74	0.72	0.41	0.13	0.01	0.0042
T_8	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.0045
T_9	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.0048
T_{10}	2044	1674	1202	868	685	2.26	1.56	1.12	0.80	0.59	0.0026
T_{11}	2070	1706	1188	1107	872	1.97	1.67	1.19	1.03	0.80	0.0025
T_{12}	2365	2212	1544	1273	873	2.24	1.80	1.25	1.25	0.85	0.0026
T_{13}	2231	2231	1499	1330	1212	2.21	2.42	1.43	1.28	1.05	0.0025
T_{14}	2306	1971	1507	1137	10102	1.99	1.79	1.41	1.05	0.88	0.0023
T_{15}	2093	1430	1429	1141	924	1.85	1.18	1.37	1.00	0.71	0.0025
T_{16}	1608	714	438	154	0	1.38	0.52	0.50	0.79	0.00	0.0052
CD @ 5%	105.17	98.75	75.16	85.76	102.21	0.18	0.18	0.12	0.20	0.14	NA
SEm ±	119.69	87.02	66.33	63.29	59.03	0.19	0.18	0.13	0.21	0.15	

ash gourd to offset deterioration of seed quality over storage.

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