IMPACT OF PRE-STORAGE SEED INVIGORATION IN ASH GOURD (*Benincasa hispida* (Thunb.) Cogn.)

By

ATHMAJA S.

(2016-11-017)

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF HORTICULTURE

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KERALA, INDIA

2018

DECLARATION

I, hereby declare that the thesis entitled **'Impact of pre-storage seed invigoration in ash gourd (B*enincasa hispida* (Thunb.) Cogn.),'** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that the thesis entitled **'Impact of pre-storage seed invigoration in ash gourd (B*enincasa hispida* (Thunb.) Cogn.),'** is a record of research work done independently by **Ms. Athmaja, S. (2016-11-017)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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Acknowledgement

It is with immense pleasure I wish to express my deep sense of whole hearted gratitude and indebtedness to my major advisor ***Dr. Rose Mary Francies****, Professor and Head, Department of Seed Science and Technology, College of Horticulture, Vellanikkara for her expert guidance, constant inspiration, affectionate advices, unreserved help, abiding patience and above all, the understanding and wholehearted co-operation rendered throughout the course of my study. This work would not have been possible without her unfailing support in the preparation of the manuscript.*

I would like to express my extreme indebtedness and obligation to ***Dr. Dijee Bastian****, Professor, Plant Breeding & Genetics, Department of Seed Science & and member of my advisory committee for her meticulous help, unwavering encouragement, forbearance, timely support and critical examination of the manuscript that has helped a lot for the improvement and preparation of the thesis.*

My deep sense of gratitude to ***Dr.Sarah T George,*** *Professor,Department of Vegetable Science, Vellanikkara and member of my Advisory Committee for her support, relevant suggestions and guidance throughout my studies and preparation of the thesis.*

I express my heartfelt thanks to ***Dr. Anita Cherian K,*** *Professor and Head, Department of Plant Pathology, Vellanikkara and member of my Advisory Committee for her timely help, constant support and valuable guidance for the successful completion of the research work. .*

I wish to express my deep sense of gratitude to ***Dr. Lally John*** *, Professor, Department of Department of Agricultural Statistics, College of Horticulture, Vellanikkara and* ***Dr. A. V. Santhosh Kumar****, Professor, Department of Tree Physiology and Breeding, College of Forestry, Vellanikkara for their valuable suggestions and timely help in the statistical analysis of the data.*

I also owe my special thanks to ***Dr. A. T. Francies****, Librarian, College of Horticulture and all other staff members of the library for their guidance during the period of course and* research work. I appreciate and acknowledge the facilities provided by the Library of College of Horticulture which assisted me in the preparation of the manuscript.

I express my deep obligation and gratitude to **Kerala Agricultural University** for the financial and technical support for persuasion of my study and research work.

I gratefully thank **Aravind chettan**, Computer club, COH for the technical assistance in the preparation of the manuscript.

It gives me great pleasure in acknowledging the support and help of **Mrs. Simi**, Teaching Assistant, Department of soil science and agricultural chemistry, for their boundless help accorded during the laboratory work. and help of all the non-teaching staff members **Hitha chechi, Smitha chechi, Jeena chechi, Shymol chechi, Revathy chechi, , Indira chechi**, whose constant help, love and support have helped me a lot to complete this venture successfully.

I am genuinely indebted to my seniors Ms.**Nishitha , Nagendra , Ms Shobha, Ms. Navya, Ajinkya** for their words of support and guidance during the entire period of my research.

It's my fortune to gratefully acknowledge the infinite affection, warm concern, constant encouragement and moral support of my friends **Reshma, Bennett, Adersh, Juby, Megha, Anju, Chakravarthi, Geethu, Shibana, Sreelekshmi, Aruna, Sijo, Bincy, Lekshmi, Aswathi and Stephy**. They were always besides me during the happy and hard moments of my life to push and motivate me.

I owe sincere thanks to my juniors **Agina, Athulya, Gayathri** and **Rosna** for their help and support.

I am deeply indebted to my father **Mr. Subhagan B**, my mother **Mrs. Jayalekshmi A B**; my sister **Ms. Gouri** and **other relatives** for their love, affection, sacrifices, moral support and prayers.

A word of apology to those whom I forgot to mention here. Once again I express my sincere gratitude to all those who supported me for the successful completion of my reseach.

Athmaja S

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List of abbreviation

AI	- Allometric index
cm	- centimetre
^{o}C	- degree Celsius
CVG	- Coefficient of velocity of germination
EC	- Electrical conductivity
g	- gram
GA_3	- Gibberellic acid
GE	- Energy of germination
GI	- Germination index
h	- hour
KAU	- Kerala Agricultural University
kg	- kilogram
MAS	- Months after storage
mg	- milligram
MGT	- Mean germination time
μSm^{-1}	- micro Siemens per metre
mm	- millimetre
%	- per cent
Th_n	- 'n' months after thawing, where 'n'= 1, 2, 3….,12
t/ha	- tonnes/hectare
T_{50}	- time taken for 50 per cent germination
VI-I	- Seedling vigour index- I
VI-II	- Seedling vigour index- II

Introduction

1. INTRODUCTION

Ash gourd (*Benincasa hispida* (Thunb.) Cogn.) (Syn. Wax gourd, white pumpkin, white gourd, winter melon) is an important warm season cucurbit. It is grown for its immature fruits which is used as vegetable. The fruits is acclaimed for its value added products such as candy, peda, pickle etc. Apart from the fruits, the young shoots, leaves and flowers are also used as food. Ash gourd is also valued for its medicinal properties as it is considered to be a good diuretic and antihelmenthic. The seeds are used as a vermifuge. It is also used as the root stalk for melon. Every 100g edible portion of ash gourd contains 96.30 per cent moisture, 1.9g carbohydrate, 0.4g protein, 0.1g fat, 0.8g fibre, 1.0mg vitamin C, 30mg calcium, 20mg phosphorous and 0.8mg iron (Pradeepkumar *et al.*, 2013).

Ash gourd is an important vegetable crop of Kerala. Total production of ash gourd in Kerala during 2016-17 was about 34 tons from an area of 974 ha with an average productivity of about 0.034 t/ha (DES, 2017). In Kerala, access to sufficient quantity of quality seed at the right time and location is a serious problem in most crops including ash gourd. In the state, prolonging seed longevity is a major concern as the prevailing hot humid conditions accelerates deterioration of seeds leading to rapid loss of viability during storage. Under this circumstance, storing of seeds under controlled environment is recommended to prolong viability. It is to be noted that in addition to the high recurring cost to maintain the seed under ideal controlled conditions, such seeds also undergo thawing when taken out for distribution. Usually one to three months elapses before the distributed seeds are used for sowing. Thawing or alternate low and high temperatures are reported to adversely affect the quality and viability of the stored seeds ((Jordan, 1982).

Ensuring the availability of superior quality of the seed to farming community is the basic objective of any seed production programme. Indeed seed deterioration due to ageing is inevitable and irreversible phenomenon, the best that can be done is to lower its rate (Coolbear, 1995). It is a serious problem in places where the storage of the seeds are taken up without a proper control of air humidity, temperature and O_2/CO_2 concentration. It is therefore evident that storage conditions have a profound influence on seed quality.

According to Shelar *et al.* (2008), a progressive decline in the seed performance due to the increasing deterioration rate is one of the basic reasons for low productivity. Thus information on storage of seeds that retain the vigour and viability from harvest to next planting season and for carryover purposes is of prime importance in any seed production programme. Uniformity and increased seedling emergence have major impact on final yield and quality (Gupta *et al.*, 2008). It is reported that seed priming technology helps in rapid and uniform germination, emergence of seeds and impart a great tolerance to adverse environmental conditions (Heydecker *et al.*, 1973). Although priming was found to be a useful technique to invigorate the seed, a lot many conflicting reports on the storability of the primed seeds have been widely reported across the world (Venkatasubramanian and Umarani, 2010). A study conducted at KAU on the storability of invigorated seeds of ash gourd variety KAU local revealed that viability of the primed seed was maintained above the minimum standards for seed certification (MSCS) for seven months after storage (MAS) under ambient condition (Shobha, 2016).

Considering all the above, the present study was formulated with the following objectives:

- To elucidate the effect of seed invigoration on viability of seeds stored under refrigerated condition.
- To analyse the impact of thawing on seed longevity in ash gourd.

Review of literature

2. Review of Literature

Seed being a living entity; it undergoes various cytological, physical, physiological and biochemical changes leading to the loss of viability and ultimate death (Jyothi and Malik, 2013). Ensuring high crop productivity demands for a physically and genetically pure, physiological sound and pathologically clean seed. It is estimated that annual losses due to deterioration of the seeds can be as much as 25 per cent of the harvested crop resulting in low seed productivity (Shelar *et al.*, 2008).

Use of vigorous and high quality seed catapults the advantages expected on the application of other production inputs such as watering and fertilizers (Hussain *et al.,* 2014). Unlike vigorous seed lot, low vigour seeds germinate and emerge poorly and give a poor crop stand (Ellis and Roberts, 1981). Seed invigoration is usually practiced to enhance its germination and field establishment. Despite immediate improvements in seed performance following invigoration treatments, there have been contrasting reports of seed storage potential following treatments. Liu *et al.* (1996) suggested that the seed storage potential in primed seeds will be reduced if a large number of cells in the radical tip have entered the G2 phase of cell cycle during the priming treatment.

A study conducted at Kerala Agricultural University revealed that seed invigoration was highly effective in prolonging viability of seeds of ash gourd variety KAU Local stored under ambient conditions. However, the impact of these treatments on longevity of seeds under refrigerated storage is wanting. In addition, the effect of the treatments on the quality of seed on thawing is also little known.

Considering the above, the present study was formulated to elucidate the effect of seed invigoration on viability of seeds stored under refrigerated condition and to analyse the impact of the period of thawing on seed longevity in ash gourd.

The literature related to the study is detailed below in brief under the following headings.

2.1. Invigoration for enhanced seed indices

2.1.1 Effect of halopriming with $CaCl_2$

2.1.2 Effect of osmopriming with KH_2PO_4

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2.2. Storability of invigorated seeds

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2.2.2. Effect of storage environment on seed longevity

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2.1. Invigoration for enhanced seed indices

Seed invigoration or seed enhancement techniques are in vogue to enhance the vigour of seeds (Basra *et al*., 2005; Farooq *et al*., 2008). Seed enhancement is done after harvest but prior to sowing for an improved germination, emergence and seedling growth by altering the physiological state of seed (Black and Peter, 2006). Generally, three general invigoration methods *viz.,* i). Pre-sowing hydration treatments (priming), ii). Seed coating technologies and iii). Integration of these methods to enhance seed quality, are practiced in order to shorten the time between the planting and emergence (Hussain *et al.,* 2014). Seed priming is a controlled hydration technique in which seeds are soaked in different solutions with high osmotic potential that initiates the germination related metabolic activities but prevents the seeds from absorbing enough water for radicle emergence, thus suspending the seeds in the lag phase (Farooq *et al*., 2007; Hussain *et al*., 2014).

2.1.1 Effect of halopriming with $CaCl_2$

Sl.no	Crop	Experimental details	Reference
1.	Sunflower	Seeds priming with 1% $CaCl_2$ enhanced the emergence and growth of seedlings.	Kathiresan *et al.* (1984)
2.	Wheat	Seeds treatment with 2% $CaCl_2$ significantly enhanced the number of seedlings per unit area, dry matter accumulation and seedling height.	Bhati and Rathore (1988)
3.	Maize	Seeds treated with 1% $CaCl_2$ exhibited significant increase in germination, speed of germination, emergence and seedling vigour, over untreated seed.	Kulkarni and Eshanna (1988)
4.	Maize	Seed priming with 50 mM $CaCl_2$ was found to enhance germination by reducing mean time to germination and higher germination index, energy of germination, final germination per cent.	Hocart *et al.* (1990)
5.	Pigeon pea and cowpea	An increased germination (97.8%) and vigour index (5007) was reported in the seeds primed with 0.4 % $CaCl_2$.	Purushotham *et al.* (1993)
6.	Jatropha	Increased germination and higher seedling survival was recorded through seed priming with $CaCl_2$ (1%).	Kathiravan (2004)
7	Maize	Priming the seeds with 1% $CaCl_2$ for 12h at 25^0C increased germination, germination index, energy of emergence, seedling root length, shoot length, seedling fresh weight and dry weight and vigour index-I.	Kumari *et al.* (2017)

8	Wheat	Priming with $CaCl_2$ (100 $mmolL^{-1}$) improved salt tolerance of wheat cultivars due to improved seedling vigour.	Iqbal *et al*. (2006) Afzal *et al*. (2008)
9	Melon	Osmopriming melon seeds with KNO_3 and 1% $CaCl_2$ resulted in improved germination per cent, energy of germination and shoot length compared with other treatments. However, priming with 1% $CaCl_2$ reduced root length. With the exception of 2% $CaCl_2$, all the priming treatments increased the shoot length.	Farooq *et al*. (2007)
10.	Marigold	Halo priming with 50mM $CaCl_2$ for 24 h resulted in maximum germination and germination index compared with non-treated and treated seeds (KNO3 and NaCl).	Afzal *et al*. (2009)
11.	Lettuce	Coefficient of velocity of germination (CVG) increased with priming. The highest CVG was recorded in $CaCl_2$ treated seeds.	Ahmed and Farag (2011)
12	Tomato	Maximum root and shoot length in tomato was observed on priming seeds with 50 mM $CaCl_2$	Afzal *et al*. (2011)
13.	Rice	Osmopriming with $CaCl_2$ (-1.00 MPa) increased energy of germination, coefficient of velocity of germination and reduced mean time to germination.	Yousof (2013)
14	Chilli	Seeds treated with 0.5% KH_2PO_4 and 1% $CaCl_2$ exhibited an increased germination per cent.	Vishwanath *et al*. (2014)
15.	Castor	Castor seeds treated with $CaCl_2$ took significantly less mean germination time but	Jamadar and

		exhibited significantly higher daily germination index, coefficient of velocity of germination.	Chandrashekar (2015)
16.	Ash gourd	Seeds of ash gourd invigorated with 50 Mm $CaCl_2$ recorded the highest germination and retained their germination above MSCS for 7 MAS.	Shobha (2016)

2.1.2 Effect of osmopriming with KH_2PO_4

Sl.no	Crop	Experimental details	Reference
1.	Sunflower	Seeds priming with KH_2PO_4 enhanced the emergence and seedling growth.	Kathiresan *et al.* (1984)
2.	Wheat	Seeds treated with KH_2PO_4 significantly enhanced the number of seedlings per unit area, dry weight and seedling height.	Bhati and Rathore (1988)
3.	Onion	A significant improvement in seedling size of tomato, capsicum and onion seeds was observed when primed in KH_2PO_4.	Jagadesh *et al*. (1994)
4.	Bitter gourd	Increased germination and seedling vigour index was observed in the seeds primed with 1.5% KH_2PO_4.	Renugadevi and Jacqueline (1994)
5.	Maize hybrid COH(M) 5	Reduction in cell phytate content and stored minerals *viz.,* boron, copper, magnesium, manganese, iron and zinc with concomitant increase in potassium was found in seeds	Sathish (2009)

		osmoprimed with 1% KH_2PO_4.	
6.	Okra	Seeds primed with 3% KH_2PO_4 at 25^0C and 30^0C registered the highest speed index	Sahib *et al*. (2013).
7.	Bitter gourd	Maximum seedling vigour index-II was recorded when the seeds were primed with KH_2PO_4.	Kumar and Singh, (2013)
8.	Chilli	Seeds priming with 0.5% KH_2PO_4 and 1% $CaCl_2$ increased the germination per cent.	Vishwanath *et al*. (2014)
10.	Cabbage	Germination and vigour of less vigorous cabbage seed was enhanced by priming with 1% KNO_3 and 2% KH_2PO_4. Vigour index of the cabbage cultivars was substantially improved in response to seed priming with KH_2PO_4 (1, 2 and 3%) and KCl (1%, 2% and 3%)	Batool *et al*. (2015)
11.	Cucumber	After priming treatment, an improvement from one to 11.90 per cent was observed in the seed lot that registered 88.00 to 93.00 per cent germination. The speed of germination increased from 3.70 per cent to 105.60 per cent while mean of germination decreased from 0.20 per cent to 41.50 per cent.	Krainart *et al* (2015)
12.	Chilli	Priming increased seed germination by 37.50 per cent compared to control.	Dutta *et al*. (2015)

2.1.3. Effect of bio-priming with *Pseudomonas fluorescens*

Sl.no	Crop	Experimental details	References

1	Bitter gourd	Significant increases in the levels of malondialdehyde (MDA) and total peroxides were observed in accelerated-aged seeds, suggesting that lipid peroxidation during imbibition was enhanced in aged bitter gourd seeds.	Hsu *et al.* (2003)
2	Sunflower	Bio-priming of sunflower seeds with *Pseudomonas fluorescens* (1×10^6 cfu.ml^{-1}) suspension for three hours was found beneficial for enhancement of seed indices and improvement of seedling growth.	Moeinzadeh *et al.* (2010)
3	Common bean	Seeds bio-primed with *T. harzianum* for four hours exhibited higher per cent of germination, shoot length, root length, dry weight, vigour index-I and vigour index-II and it was closely on par with the treatment with *P. fluorescens*. However in field condition the seeds bio-primed with *P. fluorescens* (40%) for 4h enhanced the plant growth attributes and lead to reduction in disease incidence and increased seed yield.	Monasila (2014)
4	Chilli	An increased values in rate of germination, germination per cent, root length, shoot length, biomass production and seedling vigour index was observed in seeds bio primed with *T. viride* or *P.fluorescens* at the concentration of 60% (w/v) for 3h and at 60% (w/v) for 12 h respectively.	Ananthi *et al.* (2014)
5	*Abies hickelii* *Abies religiosa*	A combination of hydropriming and bio-priming (*Pseudomonas fluorescens*, *P. putida* and *Bacillus subtilis)* improved the germination per cent upto 91.00 per	Zulueta-Rodríguez *et al.* (2015)

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		cent in *A. hickelii and* 68.00 per cent in *A. religiosa.*	
6	Chilli	Seeds bio-primed with *P. fluorescens* 60% for 12 h along with the spray of neem seed kernel extract 5% controlled the pest, increased the seed yield and quality in chilli during kharif and rabi seasons.	Ananthi *et al.* (2017)
7	Pumpkin	Under the field condition the seeds bio-primed with *Azospirillum* 10 % + Phosphobacteria 20 % + *Pseudomonas fluorescens* 20 % for 12 h was found to be the best in maximizing the plant growth and development, seed yield and quality of resultant seed. However no significant difference was observed between the primed and the unprimed seeds under laboratory condition.	Sivakalai and Krishnaveni (2017)
8	Tomato and brinjal	Seeds bio-primed with different dosages of red seaweed extracts exhibited a higher germination per cent, germination index, mean germination time, seedling length, radical length, plumule length, seedling vigour index and seed stamina index than hydroprimed seeds compared to control.	Rinku *et al.* (2017)

2.1.4. Effect of hormonal priming with Cytokinin

Sl.no	**Crop**	**Experimental details**	**References**
1	Eggpalnt and	Seed priming with cytokinin resulted in lower EC of seed leachates primarily due to	Rudrapal and Nakamura

	radish	improved membrane repair in treated seeds as reported in eggplant and radish.	(1988)
2	Cabbage, cotton and sunflower	Cabbage, cotton, sunflower, red clover and alfalfa when treated with 0.5mM kinetin exhibited increased emergence of shoot and seedling growth under saline conditions. However GA_3 was more effective in case of Poaceae seeds.	Kabar (1990)
3	Green gram and black gram	The fresh and dry weight increased in seedlings raised from seeds treated with kinetin and GA_3 as compared to untreated seeds	Patel and Saxena (1994)
4	Corn and soybean	At 10^0C GA_3 and kinetin stimulated corn and soybean seedling emergence and development (kinetin 0.1mM). However, GA_3 was more effective than kinetin.	Wang *et al*. (1996)
5	Spring maize	Maximum germination index and germination energy were found for kinetin followed by Morringa Leaf Extract (MLE)-priming. The greatest increase in seedling fresh weight, however was observed following priming with kinetin.	Afzal *et al*. (2012)
6	Wheat	Significant differences between seed priming (salicylic acid and cytokinin) and non-priming treatments were observed with respect to seed germination and seedling growth. Seed priming with 50 ppm cytokinin for 12h was significantly high in germination per cent, mean germination rate, shoot and root length, speed of germination and dry weight.	Ghobadi *et al*. (2012)
7	Onion	Priming with GA_3 increased root growth and time taken for germination unlike priming with IAA and kinetin. A similar decrease in shoot length with a concurrent increase in maximum dry weight was achieved with 10^{-10} M of kinetin for 10h.	Yarnia *et al*. (2012)
8	Tomato	Seed priming with 10 ppm kinetin for 24 h increased final germination per cent,	

		germination index, shoot length and seedling fresh weight. The pre-sowing seed priming was helpful in lessening electrolyte conductivity of seed leachates and significantly affected dry weight of seedlings, germination and seedling vigour. Maximum decrease in electrolyte leakage and increase in seed indices including seedling dry weight was induced by cytokinin at 10ppm followed by 50ppm cytokinin, 10ppm BAP, 10ppm Kinetin followed by priming with 50ppm Kinetin	Nawaz *et al*. (2013).
9	Maize	Priming with 10mM kinetin increased germination, radicle and hypocotyl length while increased concentration of kinetin and abscisic acid treatment was detrimental.	Bahrani (2015)
10	Rice	The physio-morphological traits (shoot and root length, fresh and dry weight) and biochemical traits like proline content, total chlorophyll continent and super oxide dismutase (SOD) activity, was significantly high in seeds treated with 2.5 ppm kinetin compared to higher and lower concentration of kinetin and untreated control.	Mondal *et al*. (2016)
11	Green gram	Kinetin 10^{-4} M enhanced germination, root length, shoot length, fresh and dry weight.	Marutirao (2016)
12	Tomato	Osmopriming of seeds with 10ppm, 50ppm and 100ppm kinetin for 24h at 25^0C resulted in improved germination and seedling vigour by dormancy breakdown compared with untreated seeds. Highest vigour was observed in seeds treated with 10 ppm kinetin followed by 100ppm and 50 ppm.	Zeb *et al*. (2018)

2.2. Storability of invigorated seeds

Good seed storage is one of the essential factors in a successful seed operation. Seed quality is maintained, but does not improve during storage. Proper storage of seeds is an essential way to preserve the viability and vigour of seed from harvest to sale thereby ensuring its effective use by farmers or breeders and also protecting the investment and profit of the seed men.

Seeds are normally stored for short periods, until the next growing season, but in certain circumstances, such as genetic conservation, a fairly long storage period have to be taken upon to maintain genetic variability for future use. Generally, the longer seed storage is achieved either by preserving seeds in climatic conditions favourable for storage or by modifying the environment around the seeds that favours the long storage of seed. Improper seed storage reduces seed viability and causes great losses within the farming community. According to Harrington Thumb Rules (1959), the storage life of the seed lot is doubled with every one per cent decrease in seed moisture and for a 5°C decrease in storage temperature and that the arithmetic sum of temperature in °F and per cent relative humidity should not exceed 100, with not more than half contributed by temperature. Walters (1998) stated that these rules are applicable for short-term storage of two or more years.

The effective storage of the seeds mainly depends on three factors *i.e.*, temperature, relative humidity of storage environment and moisture content of the seed lot to be stored. Low temperature and low seed moisture are the two most effective means of maintaining seed quality in storage. Low temperature is effective in preserving seed quality, especially in moisture-proof containers under humid conditions (Doijode, 2001). Seeds of tomato, cucumber and sweet pepper are viable for 36 months at 20°C and for 70 months at 0°C (Zhang and Kong, 1996).

Farmers, nurserymen, and breeders face many hardships in maintaining high seed quality during storage. Although priming was found to be a useful technique to invigorate the seed, a lot many conflicting reports on the storability of the primed seeds have been widely reported across the world (Venkatasubramanian and Umarani, 2010). Various studies on the storability of primed seeds show that there is a decline in the viability of the primed seeds upon storage. According to Saracco *et al.* (1995), the priming seeds increased their sensitivity to deteriorative factors imposed during storage as a consequence of advanced germinative events. Various reports on the enhanced storability of the primed seeds are also reported. An improvement in seed storability after osmotic priming in many species (e.g., *Allium cepa* L., *Capsicum annum* L., *Pisum sativum* and *Daucus carota*) has been reported by Savino *et al.* (1979), Dearman *et al.* (1986) and Georghiou *et al.* (1987). Pre-storage applications of anti-aging chemicals such as iodine, chlorine, bromine, methanol, ethanol, and propanol improve seed storability (Doijode, 2001).

According to Bradford (1986), the varying response in the longevity of the primed seed is result of the response of the seed lots to the priming treatments, the compound used as osmoticum, duration of priming etc. So, the effect of these invigoration treatments on the storability of the seeds plays a crucial role as far as the seed producers are concerned. This is important because the time that elapses between the production or the priming of the seeds to reach the farmers may vary between three to six months. Moreover, the unsold seeds will have to be stored until the next season. These being the pre-requisites, adopting any seed treatment whose effect does not last for long or that will not allow seeds to live longer will not be practicable.

2.2.1. Effect of storage environment on seed indices

Sl.no	Crop	Experimental details	References
1	Crimson clover and perennial rye grass	On prolonged storage of seeds under ambient conditions, an increase in seed leachate and free amino acid implying the deterioration of seed was recorded	Ching and School (1968)
2	Ash gourd	Ash gourd seeds stored in cloth bags under ambient condition showed more than 85 per cent germination even after 12 months of storage.	Araujo *et al*. (1982)
3	Poppy	Seeds of poppy stored in 700 gauge polyethylene cover retained the viability for eight months under ambient condition.	Verma *et al*. (1996)
4	Okra	Reduction in germination was observed in seeds stored in moisture impermeable containers under ambient (16°C to35°C) temperature for five years. At low temperature storage, seed retained the initial viability for 20 years both at 5^0C and -20°C. High vigour in terms of coefficient of germination, seedling growth, dry weight and vigour was also observed in seeds stored at low temperature.	Doijode (2005)
5	Soyabean	High germination (70% for 6 months of storage) and vigour index was maintained for 180 days in soybean seeds stored under refrigeration. Seedling vigour index	Sharma *et al*. (2005)

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		declined with storage of seeds with lower values at ambient temperature. The lipid peroxidation expressed as malondialdehyde content increased in the seeds stored either at ambient temperature or in refrigerated conditions up to 180 days of storage. However, the rise in MDA was rapid in seeds stored at ambient conditions. The increase in lipid peroxidation increased the leaching of sugar.	
6	Chilli	A decline of 11.5 per cent in germination of chilli seeds stored under cold condition was observed compared to a loss of 36.4 per cent in germination stored under ambient condition after 9.MAS	Jyothi *et al*. (2008)
7	Gaillardia	Maximum germination (70.33%) was recorded in seeds under ambient storage (20-22°C) during 1MAS, which was significantly higher than the cold condition. Later at 6 MAS, seeds under cold storage (0-4°C) conditions exhibited a higher germination (56.60%) than ambient conditions (50.00%). Similarly after 12 MAS, cold stored seeds showed 36.00 per cent germination, which was significantly higher than that of ambient stored seeds (20.80%).	Dhatt and Kumar (2010)
8	Soybean	In soybean stored under ambient conditions, the speed of germination increased gradually with the increasing of storage period. The vigour index was minimum in the seeds stored for one month and increased gradually with the increase in storage period.	Rahman *et al*. (2014).

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9	Pansy	Maximum viability of seed (41.40%) was recorded in seeds under cold storage (0°C - 4°C). This was significantly higher (29.89%) than that observed under ambient storage (20°C - 22°C) after 18 months.	Dhatt (2016)
10	Pigeon pea	Seeds stored in cold condition retained a high germination of 74.50 per cent seedling dry weight (65.75 mg), vigour (1096.25) compared to a low germination per cent of 37.33 per cent, seedling dry weight (50.83mg), vigour (398.2) under the ambient condition after 26 months of storage. However, the electrical conductivity of the seeds stored under cold condition (5°C - 7^0C) was found to be significantly low compared to the seeds stored under ambient storage.	Basavegowda *et al.* (2016)
11	Beans	Storage period increased electrical conductivity. However, seed stored at 10°C underwent only a few changes with low seeds electrolytes losses while, seeds that remained at 30ºC degraded easily and resulted in reduced vigour, quality and durability.	Camila *et al.* (2017)
12	*Nemesia strumosa*	Maximum seed viability (30.5%) was recorded in seed under cold storage followed by those in ambient storage (26.1%) for 18 months during a storage experiment conducted in three storage chambers *viz*., cold storage (0^0C to 4°C and 90.7% RH), incubator (20^0C to 22°C and 75% RH) and ambient conditions.	Dhatt *et al.* (2018)

2.2.2. Effect of storage environment on seed longevity

Sl.no	Crop	Experimental details	References
1.	Spinach	The improved germination performance of primed spinach seeds was retained after 30 days of storage at 5^0C.	Atherton and Farooqe (1983)
2.	Onion	The primed and dried onion seeds stored at 10^0C and nine per cent moisture content maintained a higher rate of germination after 18 months in storage.	Dearman *et al*. (1986)
3.	Tomato	The osmoprimed tomato seeds, dried to six per cent moisture content were stored at 10^0C, 20^0C and 30°C. Storage at 10 and 20°C had no influence on germination capacity for 18 months, while a decrease of the parameter was observed after 5 months storage at 30°C.	Alvaradon and Bradford (1988)
4.	Tomato	The non-primed tomato seeds maintained viability after one year of storage at 4^0C and 30°C. However, viability and germination rates of primed seeds were significantly reduced after six months storage at 30°C.	Argerich *et al*. (1989)
5.	Asparagus	The germination per cent and rate of primed asparagus and tomato seeds stored	Owen and Pill (1994)

		under 4°C were maximum up to three months of storage than the storage at 20^0C.	
6.	Muskmelon	When compared to non-primed seeds, primed muskmelon seeds lost viability more rapidly, germinated more slowly, and lost uniformity more quickly following controlled deterioration experiments.	Oluoch and Welbaum (1996)
7.	Leek and Onion	The invigorated onion and leek seeds maintained their viability after one year of storage at 10°C.	Drew *et al*. (1997)
8.	Canola	Primed canola seeds maintained their increased vigour for six months when stored under low temperature (8°C).	Basra *et al*. (2003)
9.	Snake gourd	The study on storability of invigorated snake gourd seeds revealed that the germination declined during storage under the ambient condition. The overall germination per cent was 62.83 at 1MAS which reached 39.66 per cent after 6 months.	Mohan (2005)
10.	Tomato	Long storage periods of osmoprimed tomato seeds up to six months indicated that priming benefits could be maintained till two months and then started declining.	Ismail *et al*. (2005).
11.	Soybean	Storage studies on soybean seeds treated with different fungicides and stored under ambient and cold condition revealed that the seed quality parameters such as germination, seedling length and vigour index declined gradually upto six	Sharma *et al*. (2006)

		months and there after a rapid decline was observed in ambient storage compared to cold storage.	
12.	Cashew	Seeds of cashew invigorated with KH_2PO_4 (2500ppm and 5000 ppm) and kinetin (20 and 40ppm) increased the storability up to 13 months under ambient condition.	Mini and Mathew (2008)
13.	Tomato Eggplant Chilli	Tomato seed hydroprimed for 48 h, eggplant and chilli seeds subjected to sand matric priming (80% water holding capacity) for 3 days and dried back to original moisture content could be stored in aluminium foil pouches for at least six months without losing the efficacy of the priming treatment.	Venkatasubramanian and Umarani (2010)
14.	Sunflower	Sunflower seeds were stored at different temperatures. It was found that at a temperature of 10°C (cold room), seed vigour was maintained during the twelve months of storage without any significant change.	De Souza Abreu. (2012)
15.	Amaranth	The invigorated seeds of amaranth stored under ambient conditions retained viability up to three months.	Kehinde *et al*. (2013)
16.	Onion	Osmoprimed seeds exhibited a higher energy of germination than non-primed seeds after storage at 4°C, but lower germination capacity after storage at 20°C. Values of germination rates (T_{10} and T_{50}) remained lower for primed than for non-primed seeds, during whole storage, especially at 4°C. The results showed that for	Dorna *et al*. (2013)

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		maximal seed viability and germination rate after 6 and 12 months storage, both hydroprimed and osmoprimed seeds should to be stored at 4°C rather than 20°C.	
17.	Sunflower	The seeds stored under ambient condition showed a sharp decline in germination from the third month of storage; fully losing their germinating power in the sixth months regardless of the type of packaging used for storage. In the dry cold room, the refrigerator and freezer, germination of sunflower seeds remained over 80 per cent throughout the storage period.	Lima *et al.* (2014)
18.	*Leucospermum cordifolium*	Low temperature stored seeds maintained a high viability and vigour for two years but ambient temperature storage led to a marked decline after 1 year ending exhibiting almost complete mortality after four years of shelf storage.	Brits *et al.* (2015)
19.	Sesame	The sesame seeds stored in refrigerated condition retained their viability and vigour for twelve months compared to seeds that retained viability only for six months under ambient storage	Lima *et al.* (2014)
20	Fodder sorghum	In an experiment conducted with six varieties of fodder sorghum in seed stored under ambient condition for 15 months, it was found that after nine months of storage, the standard germination and seedling vigour index ranged from 74.60 per cent to 77.33 per cent and 2113.09 to 2685.88, respectively while the EC increased	Verma *et al.* (2014)

		with the passage of time and it ranged from 0.433 dSm^{-1} to 0.893 dSm^{-1}.	
21.	Ash gourd	Seeds treated with $CaCl_2$ recorded the highest per cent increase in germination and the viability was retained for 7 MAS under the ambient storage compared to 2 MAS in untreated seeds.	Shobha (2016)
22.	Soybean	The seeds packed in high density polythene bags and stored under room temperature maintained minimum seed certification standards only up to 8 months, while the seeds stored under cold storage could retain their viability up to 14 months. The energy of germination of cold stored seeds was higher than ambient stored seeds.	Meena *et al.* (2017)
23.	Sorghum	After 12 months of ambient storage maximum germination (78.00%) was observed in the seeds treated with 1% KNO_3 followed by the seeds treated with 2% K_2HPO_4 (75.67%)	Bhuker *et al.* (2018)

2.3. Thawing period and its impact on seed quality.

Sl.no	Crop	Experimental details	References
1	Potato	Seed tubers of three potato cultivars stored at temperatures of 3^0C, 7^0C and -4.7^0C to - 10°C lost their viability when thawed rapidly at 18^0C to -20°C, while they retained their viability and ability to produce a crop when thawed slowly in melting snow or ice.	Lukyanenko (1967)
2	Red clover	Freshly harvested seed of red clover cv. Dollard, containing 96 per cent live seed, was stored in cotton bags at -5^0C to -15^0C. One lot was continuously frozen, other lots were allowed to thaw for 24 h annually, semi-annually, monthly or weekly. None of the lots showed lower viability than the original lot after 13 years of storage	Rincker (1974)
3	Paddy	Studies on quick *vs*. slow thawing of stored frozen grain at 0^0 C indicated that frequent (10 day intervals) and quick thawing reduced germination capacity significantly while slow thawing after 50 days showed no adverse effect.	Mukherjee *et al*. (1976)

4	*Setari italica*, *Vicia faba*, cotton, castor, and sunflower	When the seeds stored for three years at -196^{0}C was thawed fast by raising temp at 10^{0}C per second showed much higher seed viability was observed than in slow thawing at 1^{0}C /sec.	Fedosenko (1978)
5	*Setaria lutescens*	One freeze to −196 °C followed by a slow thaw, increased seed germination from 40 per cent to 70 per cent, in *Setaria lutescens*. However, additional freeze-thaw cycles reduced germination to 30 per cent.	Jordan (1982)
6	Papaya	The fast thawing process of papaya seeds with 5.3 per cent to 9.8 per cent moisture immersed in liquid N or maintained in N vapour for 48 h and thawed slowly at -20°C or quickly in water at 40°C led to a 64 per cent loss in seed viability.	Althoff and Carmona (1999)
7	Pepper and eggplant	There was no significant difference in germination per cent, mean germination time and per cent of abnormal seedlings of slow or rapid freezing-thawing cycles.	Quagliotti and Comino (2003)
8	Pumpkin	Refrigerated storage of pumpkin seeds frozen at -18°C for 36 h in PET bottle and stored for six months resulted in increased seed viability as compared to ambient storage.	Da Fonseca *et al.* (2017)

Materials & Methods

3. MATERIALS AND METHODS

The study on 'Impact of pre-storage seed invigoration in ash gourd seed (*Benincasa hispida* (Thunb.) Cogn.),' was conducted in Kerala Agricultural University (KAU) during 2016-18. The study was conducted with the objective of ascertaining the storage potential of treated seeds under refrigerated and ambient conditions as well as to elucidate the effect of invigoration on thawed seeds during storage in ash gourd variety KAU Local. The details regarding the materials and techniques that have been used for the research work are described below.

3.1 Location and climate

The experiment was conducted at the Department of Seed Science and Technology, College of Horticulture, Kerala Agricultural University (KAU), Vellanikkara P. O., Thrissur 680656. The location experiences humid tropical climate and is located 40 m above MSL at $10^{0}54'$ North latitude and $76^{0}28'$ East longitude. The relative humidity ranged between 51 per cent (February, 2017) and 85.00 per cent (July, 2018) while rainfall varied from 0.00 mm (February, 2017) to 793.20 mm (July, 2018) during the study. The monthly mean maximum temperatures ranged from 36^{0}C (February, 2017) to 33.2^{0}C (May, 2018), while the range in mean minimum temperature was between 23.2^{0}C (February, 2017) and 22.6^{0}C (May, 2018).

3.2 Experimental material

For the conduct of the experiment, the seed of ash gourd variety KAU Local was collected immediately after the extraction from Vegetable and Fruit Promotion Council, Keralam (VFPCK), Alathur, Palakkad, cleaned, primed and dried before storage.

3.3. Experimental method

3.3.1 Treatment details

The experiment was conducted as a completely randomized design, with seven treatments replicated thrice. The seed were primed following the invigoration treatments (I_1 to I_6) specified in Table 1. The untreated seeds (I_7) served as the control.

Table 1: Details of treatment

Treatment	Details
I_1	$CaCl_2$ (50 mM for 12 h)
I_2	$CaCl_2$ (50 mM for 24 h)
I_3	Kinetin (Cytokinin) (10 ppm for 12 h)
I_4	Kinetin (Cytokinin) (10 ppm for 24 h)
I_5	KH_2PO_4 (10^{-1} M for 24 hours)
I_6	*Pseudomonas fluorescens* ($1x10^6$ cfu.ml^{-1} for 12 h)
I_7	Absolute control

3.3.2. Seed treatment procedure

The seeds were invigorated with the respective priming agents (Table 1) in the ratio 1:2 on volume basis for the specified period. The invigorated and untreated seeds were shade dried at room temperature to $\leq$ 8 per cent moisture prior to packing.

3.3.3. Method of storage

The quantity (12.5 g) of dried seeds (7%) required for the monthly assessment (up to 14 months) of seed quality in individual replications of each of the seven treatment enumerated above, were packed in separate polyethylene bags of 700 gauge. Three replicates each of thus packed seeds were stored under two storage conditions *i.e.*, ambient storage (S_1) and refrigerated storage (S_2). The temperature under refrigerated storage was maintained between 4^0C to 6^0C throughout the storage period.

In addition, under refrigerated storage, sufficient quantity of seed required to assess the quality parameters on retrieval from refrigeration, were also set aside. At monthly intervals up to 10 months of storage (MAS), three replicates of seed stored under refrigerated condition were taken out and the seeds allowed to thaw under ambient conditions for a period of five months. In each case (*i.e.,* the seeds retrieved from refrigerated storage at monthly intervals up to 10 MAS), the effect of thawing on seed quality was assessed every month, up to five months from retrieval.

3.4. Observations recorded

The seed quality parameters of the invigorated seeds as well as the untreated seeds were recorded at the start of the storage and subsequently at monthly intervals up to ten months. In addition, monthly assessment of germination per cent was continued up to 14 MAS. At bimonthly intervals quantification of lipid peroxidation, sugar and amino acids leached out from the seeds and the seed micro flora infection was also done.

3.4.1. Germination (%)

The germination test was carried out in sand medium as per standard procedure (ISTA, 2010). Four replicates of 100 seeds each were germinated in a germination room maintained at 25±2°C temperature and 90±3% RH. The number of normal seedlings were counted in each replication at the end of germination period *i.e.,* on the 14th day and the per cent of germination was computed.

3.4.2. Root length of seedling (cm)

The length between the collar region and tip of the root of ten randomly selected normal seedlings were measured in cm on the final day of germination *i.e.,* on the 14th day and the average expressed in centimeter.

3.4.3. Shoot length of seedling (cm)

The length between the collar region and tip of the leaf of the seedlings used to estimate the shoot length were measured and the average expressed in centimetre.

3.4.5. Seedling dry weight (mg)

Ten normal seedlings were dried for six hours and then in hot air oven at 85^0C for 24 hours. The dried seedlings were cooled in a desiccator for 45 minutes and weighed and the average dry weight was computed and expressed in milligram.

3.4.6. Speed of germination (Germination index)

In each treatment, four replicates of twenty five seeds were used to assess the speed of germination. The seeds showing radicle protrusion were counted every day from third day after sowing up to 14 days. The speed of germination was calculated as per the following formula and were expressed as number (Maguire, 1962).

$$\text{Speed of germination} = \frac{X1}{Y1} + \frac{X2-X3}{Y2} + \cdots + \frac{Xn-X}{Yn}$$

X_1- Number of seeds germinated at first count

X_2- Number of seeds germinated at second count

Xn- Number of seeds germinated on nth day

Y_1- Number of days from sowing to first count

Y_2- Number of days from sowing to second count

Y_n- Number of days from sowing to nth count

3.4.7. Coefficient of velocity of germination (%)

The coefficient of velocity of germination was calculated and expressed in per cent in accordance with the formula suggested by Scott *et al.* (1984).

$$\text{Coefficient of velocity of germination (CVG)} = \frac{G1 + G2 + G3 + \cdots + Gn}{(1\,x\,G1) + (1\,x\,G2) + \cdots (n\,x\,Gn)}$$

G1-Gn: number of germinated seeds from the first to the last day.

3.4.8. Mean time to germination (days)

The mean germination time (MGT) was calculated in accordance with Ellis and Roberts (1981).

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

Where,

n = number of seeds which were germinated on day D

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

3.4.9. Energy of germination

The energy of germination was estimated as per Ruan *et al.* (2002) Fourth day after sowing was taken as the time period for recording. The per cent of germinating seeds on the fourth day after sowing relative to the total number of seeds tested was calculated as the energy of germination.

3.4.10. Vigour index-I

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

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

3.4.11. Vigour index-II

Vigour index-II was arrived at using the formula suggested by Bewly and Black, (1994).

Vigour index-II = Germination (%) x Seedling dry weight (mg)

3.4.12. Time taken for 50 per cent germination (T_{50}) (days)

T_{50} in days was calculated according to the following formula suggested by Coolbear *et al.* (1984) and modified by Farooq *et al.* (2006).

$$T50 = \frac{\mathrm{ti} + \{(\mathrm{N}/2) - \mathrm{ni}\}(\mathrm{ti} - \mathrm{tj})}{ni - nj}$$

Where N is the final number of germination and n_i, n_j are the cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

3.4.13. Electrical conductivity (EC) of seed leachate (dSm^{-1})

In accordance with the procedure suggested by Jackson (1973), three replicates of 25 seeds from each treatments were pre-washed thrice with the distilled water to remove the adhering dirt, soil and chemicals. The seeds were soaked in 25ml of distilled water in separate beakers for 24 hours at room temperature and was stirred occasionally. The beakers were covered in order to reduce evaporation and other contaminants. The seed leachate was collected in 50 ml beaker from which EC of seed leachate was measured and expressed as microSeimens per meter (dSm^{-1}).

3.4.14. Seed moisture content (%)

Five gram of ground seed materials was placed in a moisture weighing bottle and kept in a hot air oven maintained at 103±2°C for 16±1h for drying and then cooled in a desiccator for 30 minutes. The weight of the seeds before and after drying was recorded in gram. The moisture content of the seed was calculated as given below and expressed as per cent (ISTA, 2000).

$$\text{Moisture content (\%)} = \frac{M2 - M3}{M2 - M1} \times 100$$

Where,

M1 – Weight of moisture bottle alone

M2 – Weight of bottle + Seed sample before drying

M3 – Weight of bottle + Seed sample after drying.

3.4.15. Seed infection (%)

Storage fungi present on seeds were tested using blotter method as prescribed by International Seed Testing Association (ISTA, 2000). Ten seeds were placed equidistantly on three layered moistened blotter taken in sterilized petri plates. Each treatment was replicated four times. They were incubated at 20±2°C for seven days with alternate cycles of 12h in near ultraviolet light (NUV) range and for the remaining 12h in dark. On eighth day, the plates were examined under stereo binocular microscope (50X) for the presence of seed borne fungi. The number of infected seeds were counted and expressed in per cent. Besides, the kind of fungi present was also identified and documented.

3.4.16. Leakage of amino acid (µg leucine eqiv.ml^{-1})

The amino acid estimation was conducted as per the method advocated by Moore and Stein (1948) and Misra *et al.* (1975) with minor modifications. Twenty-five seeds from each replications of each treatments were soaked in 30ml distilled water for 24h at room temperature (26±1^{0}C) and then 0.1ml of leachate was taken in the test tube and 1ml of 2% Ninhydrin solution was added and mix thoroughly. The volume was made up to 2ml with distilled water and the mixture is heated in a boiling water bath for 20 minutes. To this 5ml of diluent (1 water: 1 propanol) was added and the intensity of colour was measured in spectrophotometer at 570 nm. The amount of amino acid leached out was expressed in µg leucine eqiv.ml^{-1} using the standard curve of leucine.

3.4.17. Leaching of sugar (µg glucose eqiv.ml^{-1})

The amount of sugar leached out from the seed was determined using the method described by Mc Cready *et al.* (1950) with minor modifications. Pre-cooled 2 ml leachate of ash gourd seeds from each replication of all treatments were taken separately in the test tube separately. To this 4ml of freshly prepared ice cold anthrone reagent (0.2% anthrone in 95% H_2SO_4) was added and kept in cold for 30 minutes for development of bluish green colour and the intensity of colour was measured by spectrophotometer at 580 nm. The amount of sugar leached out was expressed in µg glucose eqiv.ml^{-1} using the standard curve of glucose.

3.4.18. Lipid peroxidation (OD)

The magnitude of lipid peroxidation that occurred in ash gourd seeds over storage was estimated using the protocol suggested by Heath and Packer (1968). Seed tissue (0.1g) after the removal of seed coat was homogenised with 0.5ml of 0.1% Trichloroacetic acid. The homogenate was centrifuged at 15000 rpm at 4^0C for 10 minutes. From this 0.5ml of supernatant was collected and 1.5ml of 0.5% TBA diluted in 20% TCA was added and the mixture incubated in water-bath at 95^0C for 25 minutes The reaction was stopped by incubating in ice and the solution was centrifuged for 5 minutes (15000 x g, 4^0C) and absorbance measured at 532nm.

3.5 Statistical analysis

Statistical analysis of the data on various seed quality parameters was performed using OPSTAT and M STAT-C package for completely randomized design with two factors (Storage condition and invigoration treatments) that used Fisher's method of analysis of variance (Gomez and Gomez 1976). Arc sine transformation of data in per cent was done wherever applicable (Snedecor and Cochran, 1967).

3.5.1 ANOVA for factorial design

The data recorded in each experiment was analysed using Factorial ANOVA so as to estimate the effect of both storage condition and invigoration treatment on dependent variables. This helped us to determine whether there are interactions between the factors considered. The mean squares due to different sources of variation were worked out using the following analysis of variance (Gomez and Gomez 1976).

Source	**df**	**Mean square**	**Expected mean squares**
Replication	(r-1)	M_r	M_r/M_e
Main effect (A)	(a-1)	M_A	M_A/M_{e23}
Main effect (B)	(b-1)	M_B	M_B/M_e
Factor (AB)	(a-1) (b-1)	M_{AB}	M_{AB}/M_e
Error	ab (r-1)	M_e	

3.5.2 Pair-wise comparison using DMRT test

For experiments that require the evaluation of all possible pairs of treatment means, Duncan's multiple range test (DMRT) is useful. This is especially true when the total number of treatments is large.

DMRT involves the computation of numerical boundaries that allow for the classification of the difference between any two treatments or means as significant or non-significant. The procedure for applying the DMRT is similar to that for the LSD test. However, unlike the LSD test in which only a single value is required for any pair comparison at a prescribed level of significance, the DMRT requires computation of a series of values, each corresponding to a specific pair comparisons. The following steps are followed for ranking the data (Gomez and Gomez, 1976).

Step 1: The treatment means were ranked in decreasing (or increasing) order. It is customary to rank the treatment means according to the order of preference.

Step 2: The S_d value following the appropriate procedure

$$S_d = \sqrt{\frac{2s^2}{r}}$$

Step 3: Compute the (t - 1) values of the shortest significant ranges was computed as:

$$R_p = \frac{(r_p)(s_d)}{\sqrt{2}} \quad \text{for } p = 2, 3, \ldots, t$$

where't' is the total number of treatments,'s', is the standard error of the mean difference computed in step 2, 'r' values are the tabular values of the significant ranges, and 'p' is the distance in rank between the pairs of treatment means to be compared (*i.e.*, $p = 2$ for the two means with consecutive rankings and 'p' = t for the highest and lowest means).

Step 4: All treatment means that do not differ significantly from each other were identified and grouped together.

Step 5: According to the ranking to present the test results alphabet notation was used.

Results & Discussion

4. RESULTS AND DISCUSSION

Seed is a vehicle for genetic transformation and hence, it needs to be highly vigorous and viable for a long period of time to produce a healthy population generation after generation. Like all other living entities, seed too undergo the phenomenon of ageing, finally leading to the loss of vigour and viability. However, by keeping the factors that accelerates the deteriorative process under control, the longevity of the seed can be extended. Although various seed enhancement technologies have proven to be advantageous in improving and maintaining the performance of the seed under storage, an effective technique to extend the high vigour and viability of seeds over a long period of storage under the high humid conditions prevailing in Kerala is wanting. In addition, the effect of these treatments following a brief period of storage under refrigeration also needs attention.

Considering the above, the present study was formulated to evaluate the impact of pre-storage seed invigoration treatment and the storability of the invigorated seeds of ash gourd under ambient and refrigerated storage condition as well as their impact on seeds when subjected to thawing. The results obtained are detailed and discussed below.

4.1 Impact of storage environment on seed quality

4.1.1 Analysis of variance

Results indicated existence of wide variability in the impact of storage conditions, invigoration and their interaction on most of the seed indices studied before storage as well as during the entire period of seed storage.

4.1.2 Seed quality before storage

The seed quality parameters before storage are presented in Table 2. The seed quality parameters of the untreated seeds before storage were found to be inferior to the invigorated seeds. Irrespective of the invigoration treatments it was found that germination in all the invigorated seeds had reached above the minimum standards for seed certification (60%), whereas it had reached only 21.10 per cent in untreated seeds. At the start of the experiment, owing to seed invigoration, the per cent increase in germination varied from 197 per cent (kinetin 10 ppm for 12h) to 221 per cent (kinetin 10 ppm for 24h) in comparison to the untreated control.

Similarly, other seed quality parameters such as germination index, coefficient of velocity of germination (%), energy of germination (GE), vigour indices and allometric index were high for the invigorated seeds compared to the untreated seeds. The mean germination time (days), seed microflora (%) and time taken for 50 per cent germination (days) was high in untreated seeds compared to the invigorated seeds

4.1.3 Seed qualities during storage

4.1.3.1 Germination (%)

The results on germination (%) as influenced by storage condition, invigoration treatment and their interaction effects during the storage period are presented in Tables 3 and 4.

4.1.3.1 Due to Storage condition (S)

The germination of seeds stored under ambient storage (S_1), varied from 90.15 per cent at 1 MAS and 0.00 per cent at 14 MAS, while that of seeds stored under refrigerated storage (S_2) ranged between 70.12 per cent at 1 MAS to 43.65 per cent at 14 MAS.

Germination of seeds stored under the refrigeration was lower than that under ambient storage up to 3 MAS. However, cold stored seeds exhibited significant superior germination than that under ambient storage (S_1) from 5 MAS (S_1:67.61%; S2: 87.28%) to the end of storage period (14 MAS).

The germination in S_1 was retained above MSCS of 60 per cent up to 5 MAS (67.61) whereas, in S_2 it was retained above MSCS for 13 MAS (61.34%) (Fig.2). Similar reports on the extension of seed viability under the cold storage and its advantage over ambient storage were also observed by Doijode (2005) in okra, Jyothi *et al.* (2008) in chilli, Dhatt (2016) in pancy, Basavegowda *et al.* (2016) in pigeon pea and Dhatt *et al.* (2018) in *Nemesia strumosa.*

4.1.3.1.2 Due to Invigoration treatment (I)

Irrespective of the storage condition, the invigorated seeds exhibited significantly higher germination than the untreated control (I_7: 71.68%) at 4 MAS, the exception being I_3 (kinetin 10 ppm 12h; 75.00%) and I_4 (kinetin 10 ppm for 24h; 66.40%). From 5 MAS, germination in untreated control (I_7: 84.43%) was found to be on par with that in I_1 ($CaCl_2$ 50mM 12h; 86.37%) and I_2 ($CaCl_2$ 50mM 24h; 87.77%) up to 7 MAS (I_7: 77.22%, I_1: 72.77% and I_2: 75.57%). Germination of bio-primed seeds (I_6: *Pf* $1x10^6$ $cfu.ml^{-1}$12h) was also on par with I_1 ($CaCl_2$ 50mM 12h) and I_2 ($CaCl_2$ 50mM 24h) up to 7 MAS, except during 5 MAS.

Unlike the invigorated seeds which exhibited a germination per cent above 80 at 1 MAS, the germination of the untreated control (I_7) was below the MSCS at 1 MAS and gradually reached a maximum of 84.43 per cent at 5 MAS. This indicated that invigoration induced early germination. The finding is in consonance with that of earlier workers (Farooq *et al.*, 2007; Afzal *et al.*, 2008; Sathish, 2009; Moeinzadeh *et al.*, 2010; Afzal *et al.,* 2011 and Afzal *et al.*, 2012, Kumar and Singh, 2013; Sahib *et al.*, 2014; Vishwanath *et al.,* 2014; Jamadar and Chandrashekar, 2015; Shobha, 2016; Ananthi *et al.,* 2017; Zeb *et al.*, 2018).

Untreated (I_7) seeds retained viability above MSCS up to 9 MAS (66.93%) followed by 8 MAS in I_1 ($CaCl_2$ 50mM 12h; 70.55%) and I_2 ($CaCl_2$ 50mM 24h; 60.22%). Seeds invigorated with kinetin (I_3: 60.82% and I_4: 64.17%) retained viability above MSCS for 6 MAS while it was 7 MAS each for seeds invigorated with *Pf* (73.07%) and KH_2PO_4 10^{-1}M 24h (I_5: 70.57%). It was evident that irrespective of the storage condition, untreated seeds retained viability above MSCS for an additional one month compared to invigorated seeds. According to Venkatasubramanian and Umarani (2010), the probability for the reduced storability of the invigorated seeds under prolong storage may arise if duration of seed priming extends to an advanced stage where the seeds enter into the synthetic phase leading increase in DNA content.

Table 2: Seed quality parameters before storage

Parameter	Invigoration treatments						
	I_1	I_2	I_3	I_4	I_5	I_6	I_7
Germination (%)	64.70	66.63	62.73	67.73	66.67	66.07	21.10
Germination index	5.11	4.63	4.06	4.57	5.08	5.28	2.18
Coefficient of velocity of germination (%)	21.17	21.23	20.40	20.20	20.20	19.90	15.77
Mean time to germination (days)	8.78	8.36	8.45	9.39	8.03	7.79	11.10
Energy of germination (GE)	31.17	34.83	35.50	21.89	33.00	30.63	26.10
Time taken for 50 per cent germination (days)	3.54	3.55	3.90	3.96	3.55	3.63	6.57
Vigour index I	1490.00	1472.00	1125.00	1333.00	1412.00	1264.00	320.00
Vigour index II	0.86	0.99	0.88	1.01	1.27	0.99	0.30
Allometric index	0.56	0.57	0.39	0.61	0.53	0.42	0.49
Seed infection (%)	0.00	0.00	0.00	0.00	0.00	0.00	3.33

I_1: $CaCl_2$ (50 mM for 12h) I_2: $CaCl_2$ (50 mM for 24h) I_3: Kinetin (Cytokinin) (10 ppm for 12h) I_4: Kinetin (Cytokinin) (10 ppm for 24h)
I_5: KH_2PO_4 (10^{-1} M for 24h) I_6: *Pseudomonas fluorescens* ($1x10^6$ cfu.ml^{-1} for 12h) I_7: Absolute control

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4.1.3.1.2 Due to Invigoration treatment (I)

Irrespective of the storage condition, the invigorated seeds exhibited significantly higher germination than the untreated control (I_7: 71.68%) at 4 MAS, the exception being I_3 (kinetin 10 ppm 12h; 75.00%) and I_4 (kinetin 10 ppm for 24h; 66.40%). From 5 MAS, germination in untreated control (I_7: 84.43%) was found to be on par with that in I_1 ($CaCl_2$ 50mM 12h; 86.37%) and I_2 ($CaCl_2$ 50mM 24h; 87.77%) up to 7 MAS (I_7: 77.22%, I_1: 72.77% and I_2: 75.57%). Germination of bio-primed seeds (I_6: *Pf* $1x10^6$ cfu.ml^{-1}12h) was also on par with I_1 ($CaCl_2$ 50mM 12h) and I_2 ($CaCl_2$ 50mM 24h) up to 7 MAS, except during 5 MAS.

Unlike the invigorated seeds which exhibited a germination per cent above 80 at 1 MAS, the germination of the untreated control (I_7) was below the MSCS at 1 MAS and gradually reached a maximum of 84.43 per cent at 5 MAS. This indicated that invigoration induced early germination. The finding is in consonance with that of earlier workers (Farooq *et al.*, 2007; Afzal *et al.*, 2008; Sathish, 2009; Moeinzadeh *et al.*, 2010; Afzal *et al.,* 2011 and Afzal *et al.*, 2012, Kumar and Singh, 2013; Sahib *et al.*, 2014; Vishwanath *et al.,* 2014; Jamadar and Chandrashekar, 2015; Shobha, 2016; Ananthi *et al.,* 2017; Zeb *et al.*, 2018).

Untreated (I_7) seeds retained viability above MSCS up to 9 MAS (66.93%) followed by 8 MAS in I_1 ($CaCl_2$ 50mM 12h; 70.55%) and I_2 ($CaCl_2$ 50mM 24h; 60.22%). Seeds invigorated with kinetin (I_3: 60.82% and I_4: 64.17%) retained viability above MSCS for 6 MAS while it was 7 MAS each for seeds invigorated with *Pf* (73.07%) and KH_2PO_4 10^{-1}M 24h (I_5: 70.57%). It was evident that irrespective of the storage condition, untreated seeds retained viability above MSCS for an additional one month compared to invigorated seeds. According to Venkatasubramanian and Umarani (2010), the probability for the reduced storability of the invigorated seeds under prolong storage may arise if duration of seed priming extends to an advanced stage where the seeds enter into the synthetic phase leading increase in DNA content.

However, considering the significant superiority of seeds invigorated with I_1 ($CaCl_2$ 50mM 12h; 86.37%) and I_2 ($CaCl_2$ 50mM 24h; 87.77%) with respect to germination in the initial storage period (4 MAS) coupled with retention of germination above MSCS for 8 MAS, invigoration with $CaCl_2$ 50mM can be advocated. Similarly, bio-priming with *Pf* (I_6: *Pf* $1x10^6$ $cfu.ml^{-1}$ for 12h) can also be recommended if the storage period anticipated is less than 8 MAS.

As in the present study, the advantage of invigorating seeds of ash gourd with $CaCl_2$ 50mM was also reported by Shobha (2016). Similar findings have been reported by Kulkarni and Eshanna (1988) in maize, Kathiravan (2004) in jatropha, Afzal *et al.* (2009) in marigold and Vishwanath *et al.* (2014) in chilli. .

4.1.3.1.3 Due to Interaction (S x I)

Between 1 MAS and 3 MAS, germination of invigorated seeds under ambient storage (S_1) was higher than that of the invigorated and untreated seeds stored under cold storage (S_2). At 1 MAS, it varied between 53.11 per cent in S_2I_7 (Refrigerated storage - Control) and 99.10 per cent in S_1I_1 (Ambient - $CaCl_2$ 50mM 12h). S_1I_1 was found to be on par with S_1I_4 (Ambient – Kinetin 10ppm 24h: 98.33%) and S_1I_6 (Ambient-*Pf* $1x10^6$ cfu.ml-1 12h: 98.33%).

At 4 MAS, germination in treatments S_1I_1 (83.89%), S_1I_2 (Ambient - $CaCl_2$ 50mM 24h: 88.33%), S_1I_5 (Ambient - KH_2PO_4 10^{-1}M 24h: 93.33%), S_1I_6 (88.89%) under ambient storage were on par with invigorated seeds under refrigerated storage and significantly different from untreated seeds under both ambient (S_1I_7: 73.89%) and refrigerated storage (S_2I_7: 69.46%). Thereafter the invigorated seeds stored under refrigerated condition exhibited a significantly higher germination than the invigorated and untreated seeds under ambient storage

The results indicated that all the invigorated seeds stored under refrigeration except seeds invigorated with I_1 (S_2I_1: $CaCl_2$ 50mM 12h) retained viability above MSCS for 13 MAS whereas, under refrigeration the untreated seeds (S_2I_7) and invigorated seeds (S_2I_1) retained viability above MSCS for 12 MAS (Fig.2). The seeds under refrigeration invigorated with *Pf* $1x10^6$ $cfu.ml^{-1}$ for 12h (S_2I_6) recorded the maximum germination of 73.87 per cent and was significantly superior to other treatments at 13 MAS. At 13 MAS the treatments S_2I_2 (Refrigerated-$CaCl_2$ 50mM 24h: 60.0%), S_2I_3 (Refrigerated - Kinetin 10ppm 12h: 64.97%), S_2I_4 (Kinetin

10ppm 24h: 61.67%) and S_2I_5 (Refrigerated - KH_2PO_4 10^1M 24h: 62.23%) were on par with each other and found to be next best to S_2I_6.

In case of seeds under ambient storage, viability above MSCS was retained for a maximum period of 8 MAS in S_1I_1 (Ambient - $CaCl_2$ 50mM 12h; 61.10%) followed by 7 MAS S_1I_2 (Ambient - $CaCl_2$ 50mM 24h 63.89%) and S_1I_7 (Ambient - control: 70.00%).

As in the present study, Basra *et al.* (2003) also found that invigorated canola seeds under low temperature storage maintained their viability longer than untreated seeds. The result is also in confirmation with the works of Owen and Pill (1994) in asparagus and tomato and Lima *et al.* (2014) in sunflower.

The present study thus reveals that irrespective of seed invigoration, storing seeds under refrigeration at seven per cent moisture content is advantageous over ambient storage to prolong seed longevity and maintain seed quality. Germination above MSCS was retained for 13 MAS under refrigerator storage compared to 5 MAS in ambient stored seeds. The loss in viability and vigour in seed is rapid when stored under ambient condition due to prevailing high temperature and relative humidity (Doijode, 2006). A significant loss in seed quality after harvest occurs due to the active metabolic processes and respiration taking place in the grain. So, at most care is necessary during storage for reducing the deteriorative events that are taking place inside the seed (Brackmann *et al.*, 2002). According to Aguiar (1995), high temperatures cause increased respiration and depletion of accumulated reserves which was in conformity to the findings of De Alencar *et al.* (2006) where high respiration was reported in grains stored at temperatures of 30^0C and 40 °C.

The depletion of food reserve, increase in fat acidity, ultra structural changes, and reduced activity of enzymes and weakening of membrane integrity attribute towards the decline in germination over storage (Dhatt and Kumar, 2010). However, the reduced respiration rate, metabolic activities or the reduced level of pathogen which ensures the resources in seeds stored under cold storage and it could have been the reason attributed to a gradual decline in seed quality parameters of seeds in cold storage compared to a drastic decline in quality of seeds stored in an ambient condition (Rajasree and Jirali, 2017). This is in line with the findings of Filho (2005). He stated that the environments with lower relative humidity and temperatures, permits the chemical activity inside the seeds to occur at a slower rate.

Germination of seeds under the refrigerated storage was lower than that under ambient storage in the initial storage period. The germination in the cold stored seeds gradually spiked over and above that under ambient storage at the fourth month of storageand retained their superiority during the later phase. The result is in accordance with the work of Dhatt and Kumar (2010) in gaillardia where the germination of seeds stored under ambient condition was significantly higher (1 MAS: 70.33%, 2 MAS: 68.20%, 3 MAS: 65.40% and 4 MAS: 64.07%) than that stored in cold condition (1 MAS: 68.73%, 2 MAS: 67.33%, 3 MAS: 64.60% and 4 MAS: 63.40%) in the initial months. Thereafter, they observed that germination under ambient storage at 6 MAS decreased to 50.00 per cent, while the germination in cold stored seeds was 56.60 per cent.

Irrespective of the storage environment, untreated seeds retained viability above MSCS longer (9 MAS). Considering the significant superiority of seeds invigorated with I_1 ($CaCl_2$ 50mM 12h; 86.37%) and I_2 ($CaCl_2$ 50mM 24h; 87.77%) with respect to germination in the initial storage period (up to 4 MAS) coupled with retention of germination above MSCS for 8 MAS, seed invigoration with $CaCl_2$ 50mM can be advocated.

Considering the interaction between storage condition and invigoration treatment, seed invigoration followed by refrigerated storage is advantageous. If only ambient storage condition is feasible, it would be advantageous to prime the seeds with $CaCl_2$ 50mM for 12h (I_1) as viability above MSCS was retained for 8 MAS compared to untreated seeds (7 MAS). However, if provision for refrigerated storage is available, bio-priming with *Pf* $1x10^6$ $cfu.ml^{-1}$ for 12h (S_2I_6) would be most advantageous followed by invigoration with $CaCl_2$ 50mM for 24h (I_2), Kinetin 10ppm for 12h (I_3), Kinetin 10ppm for 24h (I_4) and KH_2PO_4 10^{-1}M for 24h (I_5).

4.1.3.2 Mean germination time (MGT) (days).

The results on mean germination time (days) as influenced by storage condition, invigoration treatment and their interaction effects during the storage period are presented in Tables 5 and 6.

4.1.3.2.1 Due to Storage condition (S)

Ambient storage (S_1) was significantly superior to the refrigerated storage (S_2) in terms of mean germination time up to 2 MAS. However, the trend revised from 3 MAS till the end of storage period (10 MAS). Mean germination time under both ambient and refrigerated storage was initially high (S_1: 7.51 days and S2: 8.93 days at 1MAS), decreased gradually between 3 MAS (S_1: 7.34 days and S_2: 5.10 days) and 6 MAS (S_1: 4.26 days and S_2: 3.77 days) and increased towards the end of storage period (S_1: 5.36 days and S_2: 4.15 days at 10 MAS).

Previous work on maize and other crops point out that mean germination time (MGT), is a possible alternative to assess the seed vigour. Low MGT is correlated with earliness and synchronicity in germination and is found to be proportional to the increased plant establishment (Matthews and Hosseini, 2006). Hence, lower the MGT better is the vigour and field establishment.

Table 3. Influence of storage condition and invigoration treatment on germination (%) during storage in ash gourd

Details	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Storage condition (S)										
S_1	90.15^{a} (74.99)	87.91^{a} (71.34)	85.43^{a} (68.58)	79.04 (63.95)	67.61^{b} (55.94)	57.05^{b} (49.15)	51.27^{b} (45.64)	35.705^{b} (36.25)	23.5^{b} (27.24)	12.286^{b} (16.92)
S_2	70.12^{b} (57.04)	74.10^{b} (59.63)	77.51^{b} (61.89)	80.56 (64.44)	87.28^{a} (70.78)	85.62^{a} (68.5)	86.59^{a} (69.11)	83.257^{a} (66.15)	79.28^{a} (63.04)	75.795^{a} (60.78)
SEm ±	0.684	0.55	0.611	1.124	1.135	1.345	1.133	0.87	0.656	1.04
CD(0.05)	1.992	1.6	1.78	NS	3.305	3.915	3.3	2.534	1.911	3.028
Invigoration treatment (I)										
I_1	89.49^{a} (74.48)	89.16^{a} (72.56)	88.44^{a} (71.30)	84.18^{a} (67.01)	86.67^{a} (70.66)	76.93^{a} (63.03)	72.77^{a} (59.11)	70.55^{a} (57.46)	58.35^{b} (50.18)	51.12^{a} (45.70)
I_2	86.32^{ab} (69.26)	88.83^{a} (70.90)	89.61^{a} (71.54)	89.72^{a} (71.54)	87.77^{a} (70.29)	75.28^{ab} (61.51)	75.57^{a} (61.05)	60.22^{b} (51.67)	56.65^{b} (49.31)	51.12^{a} (45.74)
I_3	82.38^{bc} (66.27)	79.55^{b} (63.17)	80.55^{b} (63.87)	75.00^{b} (60.97)	70.53^{cd} (60.87)	60.82^{c} (54.02)	57.78^{b} (52.06)	51.13^{c} (45.81)	43.32^{c} (39.71)	38.00^{b} (31.38)
I_4	81.89^{bc} (68.65)	77.50^{b} (62.07)	75.50^{c} (60.42)	66.40^{b} (54.69)	64.43^{d} (53.61)	64.17^{bc} (53.72)	55.55^{b} (48.87)	54.43^{bc} (48.38)	41.70^{c} (37.76)	39.45^{b} (34.42)
I_5	80.33^{c} (64.54)	81.00^{b} (64.72)	81.72^{b} (64.84)	87.79^{a} (70.45)	76.93^{bc} (62.93)	74.43^{ab} (62.09)	70.57^{a} (58.16)	53.62^{bc} (47.64)	46.95^{c} (42.55)	40.80^{b} (36.82)
I_6	84.27^{abc} (70.35)	85.50^{a} (70.92)	84.72^{ab} (68.02)	83.88^{a} (66.75)	71.37^{cd} (58.19)	70.82^{abc} (57.81)	73.07^{a} (60.58)	56.40^{bc} (50.11)	45.83^{c} (40.98)	43.35^{ab} (37.43)
I_7	56.27^{d} (48.59)	65.55^{c} (54.07)	69.83^{d} (56.66)	71.68^{b} (57.95)	84.43^{ab} (66.96)	76.95^{a} (61.62)	77.22^{a} (61.79)	70.02^{a} (57.32)	66.93^{a} (55.42)	44.45^{ab} (40.46)
SEm ±	1.28	1.028	1.144	2.103	2.123	2.515	2.12	1.628	1.228	1.946
CD(0.05)	3.726	2.994	3.33	6.122	6.183	10.30	6.174	4.741	3.575	5.666

*Values in parentheses are Arc sine transformed values

**Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Table 4. Interaction effect of storage condition and invigoration treatment on germination (%) during storage in ashgourd

Treatments	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
S_1I_1	99.10^{a} (85.60)	97.11^{a} (80.81)	94.77^{a} (77.62)	83.89^{abc} (66.95)	77.78^{cd} (61.84)	68.89^{d} (56.22)	62.77^{b} (52.63)	61.10^{c} (51.46)	41.12^{d} (39.86)	37.23^{d} (37.58)
S_1I_2	93.22^{bc} (75.49)	92.88^{b} (74.67)	92.99^{a} (74.84)	88.33^{abc} (70.03)	82.22^{bcd} (65.08)	62.23^{de} (52.06)	63.89^{b} (53.06)	37.67^{d} (37.79)	33.89^{e} (35.54)	25.57^{e} (30.28)
S_1I_3	92.88^{bc} (74.56)	82.55^{d} (65.29)	81.22^{bcd} (64.32)	64.43^{gh} (53.51)	44.46^{f} (41.78)	33.32^{g} ()35.12	27.22^{d} (31.22)	24.46^{e} (29.61)	10.54^{fg} (18.84)	0.43^{g} (3.18)
S_1I_4	98.33^{ab} (83.32)	85.66^{cd} (67.79)	80.55^{cde} (63.80)	60.57^{h} (51.12)	55.54^{e} (48.18)	48.32^{f} (44.02)	27.21^{d} (31.39)	20.54^{e} (26.74)	6.21^{g} (14.03)	3.33^{g} (8.48)
S_1I_5	90.40^{c} (72.17)	89.22^{bc} (70.92)	84.55^{bc} (67.01)	93.33^{a} (75.82)	63.90^{e} (53.10)	55.00^{ef} (47.85)	53.90^{c} (47.23)	22.79^{e} (28.46)	13.33^{f} (21.24)	4.97^{fg} (12.55)
S_1I_6	98.33^{ab} (83.78)	98.33^{a} (83.37)	93.77^{a} (75.56)	88.89^{ab} (70.80)	62.22^{e} (52.31)	60.53^{de} (51.22)	53.90^{c} (47.22)	24.46^{e} (29.56)	6.11^{g} (14.30)	1.69^{g} (7.48)
S_1I_7	58.77^{g} (50.03)	69.66^{f} (56.56)	70.22^{f} (56.90)	73.89^{def} (59.45)	87.22^{abc} (69.28)	71.12^{cd} (57.53)	70.00^{b} 56.76()	58.89^{c} (50.11)	53.33^{c} (46.88)	12.78^{f} (19.86)
S_2I_1	79.88^{d} (63.37)	81.22^{d} (64.32)	82.11^{bcd} (64.98)	84.46^{abc} (67.07)	95.56^{a} (71.47)	85.00^{ab} (67.84)	82.77^{a} (65.59)	80.00^{b} (63.49)	75.57^{b} (60.50)	65.00^{c} (53.82)
S_2I_2	79.44^{d} (63.03)	84.77^{d} (67.13)	86.22^{b} (68.23)	91.11^{ab} (73.06)	93.33^{a} (75.50)	88.33^{ab} (70.96)	87.22^{a} (69.04)	82.78^{ab} (65.54)	79.44^{b} (62.07)	76.67^{b} (61.19)
S_2I_3	71.88^{e} (57.97)	76.55^{e} (61.04)	79.88^{cde} (63.42)	85.54^{abc} (68.43)	96.66^{a} (79.97)	88.33^{ab} (72.91)	88.33^{a} (72.91)	77.78^{b} (62.02)	76.11^{b} (60.75)	75.56^{b} (60.59)
S_2I_4	65.44^{f} (53.97)	69.33^{f} (56.35)	70.44^{f} (57.04)	72.23^{efg} (58.26)	73.33^{d} (59.04)	80.00^{bc} (63.42)	83.89^{a} (66.36)	88.33^{a} (70.03)	77.21^{b} (61.49)	75.57^{b} (60.36)
S_2I_5	70.22^{ef} (56.90)	72.77^{ef} (58.52)	78.80^{de} (62.68)	82.22^{bcd} (65.08)	90.00^{ab} (72.76)	93.88^{a} (76.32)	87.22^{a} (69.10)	84.46^{ab} (66.83)	80.56^{ab} (63.87)	76.66^{b} (61.09)
S_2I_6	70.11^{ef} (56.91)	72.66^{ef} (58.46)	75.66^{e} (60.48)	78.89^{cde} (62.70)	80.56^{bcd} (64.08)	81.11^{bc} (64.41)	92.22^{a} (73.93)	88.33^{a} (70.66)	85.56^{a} (67.67)	85.00^{a} (67.37)
S_2I_7	53.11^{h} (47.15)	61.44^{g} (51.59)	68.50^{f} (56.42)	69.46^{fgh} (56.44)	81.67^{bcd} (64.63)	82.78^{ab} (67.71)	84.43^{a} (66.82)	81.11^{ab} (64.53)	80.56^{ab} (63.96)	76.11^{b} (61.06)
SEm ±	1.81	1.454	1.617	2.973	3.003	3.56	2.999	2.302	1.736	2.752
CD (0.05)	5.269	4.234	4.709	8.658	8.744	10.358	8.731	6.704	5.055	8.012

*Values in parentheses are Arc sine transformed values

**Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Contd…

Table 3: Influence of storage condition and invigoration treatment on germination (%) in ash gourd

Details	Period of storage (months)			
	11 MAS	12 MAS	13 MAS	14 MAS
Storage condition (S)				
S_1	7.36[b] (11.266)	2.97[b] (6.501)	0.87[b] (2.043)	0[b].00
S_2	72.7[a] (58.79)	70.24[a] (57.09)	61.34[a] (51.66)	43.65[a] (41.287)
SEm ±	1.028	0.731	0.686	0.742
CD (0.05)	2.992	2.129	1.998	2.159
Invigoration treatment (I)				
I_1	48.33[a] (44.08)	37.22[ab] (36.16)	29.17[b] (30.27)	20.00[bc] (19.58)
I_2	41.88[ab] (38.68)	40.40[a] (37.13)	30.00[b] (25.41)	20.00[bc] (19.58)
I_3	38.35[b] (34.34)	36.68[ab] (29.49)	32.48[ab] (26.84)	21.67[bc] 20.56
I_4	36.12[b] (30.95)	33.62[b] (30.02)	30.83[b] (25.86)	28.61[a] (24.59)
I_5	36.95[b] (39.74)	34.17[ab] (27.9)	31.12[b] (26.14)	20.56[bc] (19.89)
I_6	42.22[ab] (33.46)	39.72[ab] (31.56)	36.93[a] (29.64)	24.44[ab] (22.16)
I_7	36.38[b] (33.93)	34.47[ab] (30.28)	27.22[b] (23.77)	17.52[c] (18.11)
SEm ±	1.923	1.368	1.284	1.387
CD (0.05)	5.598	3.984	3.738	5.682

*Values in parentheses are Arc sine transformed values
**Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Contd…

Table 4. Interaction effect of storage condition and invigoration treatment on germination (%) during storage in ash gourd

Treatments	Period of storage (months)			
	11 MAS	12 MAS	13 MAS	14 MAS
Interaction (S x I)				
S_1I_1	32.77^{d} (34.87)	11.67^{e} (19.93)	6.13^{d} (14.30)	0.00^{e}
S_1I_2	9.33^{e} (17.71)	6.37^{ef} (14.59)	0.00^{e}	0.00^{e}
S_1I_3	2.80^{ef} (9.29)	0.00^{g}	0.00^{e}	0.00^{e}
S_1I_4	2.23^{ef} (4.99)	1.67^{fg} (5.98)	0.00^{e}	0.00^{e}
S_1I_5	0.00^{f}	0.00^{g}	0.00^{e}	0.00^{e}
S_1I_6	0.00^{f}	0.00^{g}	0.00^{e}	0.00^{e}
S_1I_7	4.43^{ef} (11.97)	1.13^{fg} (4.99)	0.00^{e}	0.00^{e}
S_2I_1	63.90^{c} (53.29	62.77^{d} (2.40)	52.20^{c} (46.24)	40.00^{cd} (39.16)
S_2I_2	74.43^{b} (59.65)	74.43^{ab} (59.67)	60.00^{b} (50.82)	40.00^{cd} (39.17)
S_2I_3	73.90^{b} (59.39)	73.37^{bc} (58.98)	64.97^{b} (53.69)	43.33^{bc} (41.12)
S_2I_4	70.00^{bc} (56.90)	65.57^{d} (54.07)	61.67^{b} (51.73)	57.22^{a} (49.18)
S_2I_5	73.90^{b} (59.48)	68.33^{cd} (55.80)	62.23^{b} (52.28)	41.11^{cd} (39.78)
S_2I_6	84.43^{a} (66.93)	79.43^{a} (63.12)	73.87^{a} (59.28)	48.89^{b} (44.33)
S_2I_7	68.33^{bc} (55.89)	67.80^{cd} (55.57)	54.45^{c} (47.55)	35.03^{d} (36.23)
SEm ±	2.719	1.935	1.816	1.962
CD (0.05)	7.917	5.634	5.287	5.682

*Values in parentheses are Arc sine transformed values

**Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Fig 1. Influence of storage condition on germination (%) during storage in ash gourd

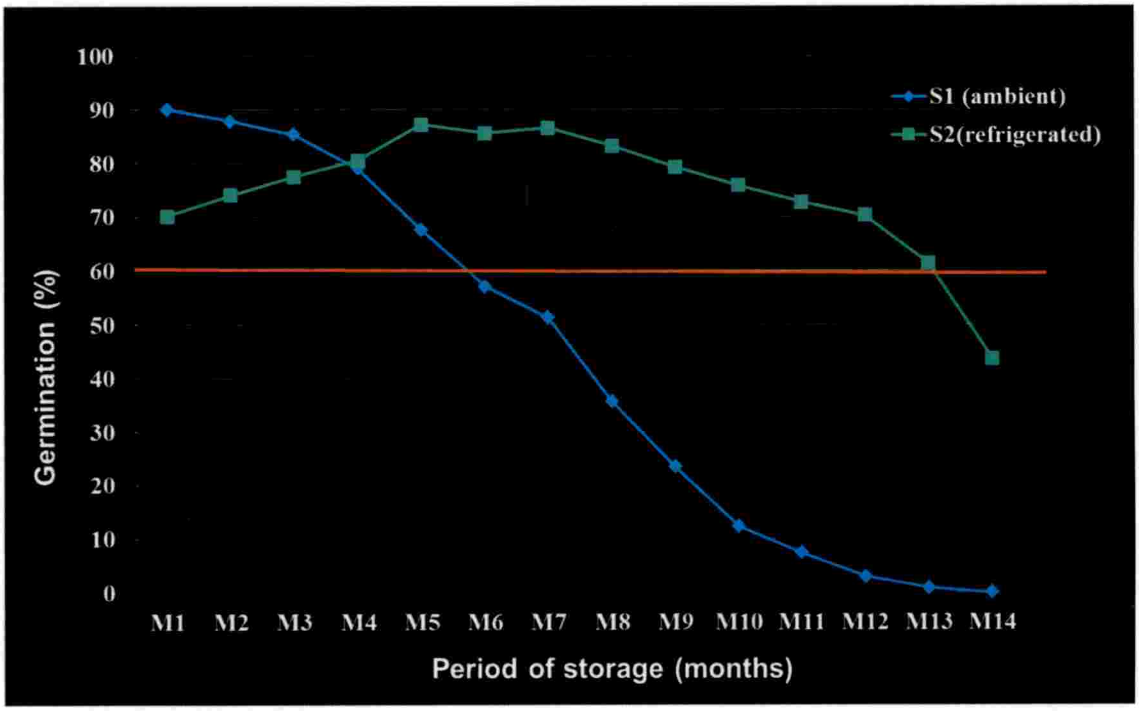
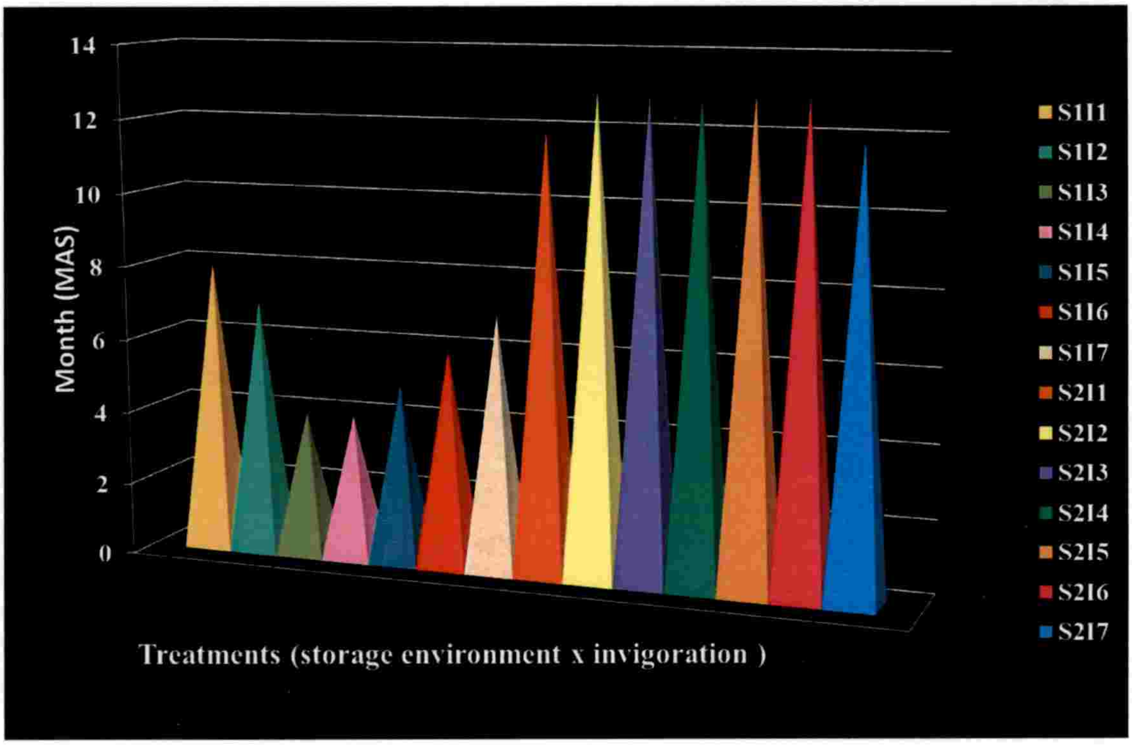


Fig 2. Seed longevity in ash gourd as influence by storage environment and invigoration treatment



Invigoration treatments I_2 ($CaCl_2$ 50mM 24h) with the lowest value (7.46 days) for MGT was found to be on par with I_1 ($CaCl_2$ 50mM 24h: 7.70 days) and significantly superior to the untreated seeds (I_7: 10.47 days) at 1 MAS. I_1 and I_2 were on par with each other over the storage period except at 2 MAS. Untreated seeds were inferior to all invigorated seeds throughout the storage period, except at 8 MAS.

Similar results on the reduced mean germination time of invigorated seeds were also reported by Kathiresan *et al.* (1984) in sunflower seeds when invigorated with 1% $CaCl_2$ and KH_2PO_4, Kaya *et al.* (2006) in sunflower seeds invigorated with NaCl, Krainart *et al.* (2015) in cucumber seeds invigorated with KH_2PO_4, Jamadar and Chandrashekar (2015) in castor when invigorated with 2% $CaCl_2$.

4.1.3.2.3. Due to Interaction (S x I)

During the start of storage period S_1I_2 (Ambient - CaCl2 50mM 12h), S_1I_3 (Ambient - Kinetin 10ppm 24h) and S_1I_6 (Ambient- *Pf* $1x10^6$ $cfu.ml^{-1}$ 12h) registered the least MGT of 6.68 days, 6.93 days and 6.91 days respectively compared to untreated seeds under ambient storage (S_1I_7: 10.64 days). From 3 MAS, seeds stored under refrigeration recorded significantly lower MGT compared to seeds under ambient storage. Both invigorated and untreated seeds under refrigeration were on par with each other from 3 MAS till the end of storage (10 MAS), exceptions being at 6 MAS and 8 MAS. Hence, it is evident that irrespective of the invigoration treatments, the seeds stored under refrigerated storage exhibited a lower value of MGT over the period of storage compared to that stored under ambient condition.

Mean germination time (MGT), which is the reciprocal of the rate of germination is considered as highly indicative of emergence performance in seed lots of pepper (Demir *et al.*, 2008) and maize (Matthews and Hosseini, 2006; Khajeh-Hosseini *et al.*, 2009). They reported significant correlations between MGT and rate of emergence, measured as mean emergence time (MET), final emergence and seedling size and uniformity. The MGT characteristics of seed lots in cucurbits have been indicative of seed vigour (Mavi *et al.,* 2010).

4.1.3.3. Time taken for 50% germination (days) (T_{50})

The results on T_{50} (days) as influenced by storage condition, invigoration treatment and their interaction effects during the storage period are presented in Tables 7 and 8.

4.1.3.3.1 Due to Storage condition (S)

Ambient storage (S_1) was significantly superior to the refrigerated storage (S_2) in terms of T_{50} up to 3 MAS. However, seeds under refrigerated storage proved to be superior from 4 MAS to the end of storage period . T_{50} under both ambient and refrigerated storage was initially low (S_1: 2.98 days and S_2: 3.47 days at 1 MAS) and increased towards end of storage reaching 21.87 days in S_1 and 3.77 days in S_2.

Our results are in line with that of Tzortzakis (2009) and Sarihan *et al.* (2005). Kato-Noguchi and Macías (2005) pointed out that lower T_{50} may be due to possible early activation or *de novo* synthesis of cell wall degrading enzymes.

4.1.3.3.2 Due to Invigoration treatment (I)

Irrespective of the storage condition, the invigorated seeds recorded significantly low values for T_{50} compared to untreated seeds, except between 6 MAS and 8 MAS. Treatment I_2 ($CaCl_2$ 50mM 24h) was on par with I_1 ($CaCl_2$ 50mM 12h) during most of the storage period, except during 3 MAS. In addition, I_1 ($CaCl_2$ 50mM 12h: 44.01 days) and I_2 ($CaCl_2$ 50mM 24h: 4.28 days) was also on par with I_6 (*Pf* $1x10^{-6}$ cfu.ml^{-1} 12h: 6.65 days.) till 8 MAS.

Table 5. Influence of storage condition and invigoration treatment on mean germination time (days) during storage in ash

Details	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Storage condition (S)										
S_1	7.51^{b}	7.38^{b}	7.34^{a}	3.74	4.04^{a}	4.26^{a}	4.50^{a}	4.73^{a}	4.90^{a}	5.36^{a}
S_2	8.93^{a}	8.09^{a}	5.10^{b}	3.88	3.83^{b}	3.77^{b}	3.879^{b}	3.94^{b}	4.09^{b}	4.15^{b}
SEm ±	0.03	0.04	0.11	0.05	0.07	0.03	0.06	0.05	0.06	0.07
CD (0.05)	0.10	0.11	0.33	NS	0.207	0.09	0.19	0.16	0.19	0.21
Invigoration treatment (I)										
I_1	7.70^{de}	7.33^{c}	6.10^{b}	3.79	4.01	4.11	4.19	4.20^{ab}	4.34^{b}	4.58
I_2	7.46^{e}	7.12^{cd}	5.88^{b}	3.81	4.09	4.02	4.12	4.22^{ab}	4.38^{b}	4.63
I_3	8.04^{bc}	6.98^{d}	6.16^{b}	3.76	3.85	3.97	4.16	4.47^{a}	4.61^{a}	4.85
I_4	8.30^{b}	7.93^{b}	6.33^{b}	3.83	3.91	3.97	4.08	3.90^{b}	4.22^{b}	4.43
I_5	7.90^{cd}	7.74^{b}	5.90^{b}	3.90	3.83	3.85	4.25	4.48^{a}	4.51^{a}	4.90
I_6	7.72^{de}	6.97^{d}	5.82^{b}	3.92	3.99	4.11	4.22	4.55^{a}	4.77^{a}	4.97
I_7	10.47^{a}	10.13^{a}	7.35^{a}	3.69	3.93	4.10	4.31	4.55^{a}	4.66^{a}	4.95
SEm ±	0.07	0.075	0.213	0.096	0.133	0.062	0.123	0.107	0.122	0.14
CD (0.05)	0.204	0.218	0.619	NS	NS	NS	NS	0.31	0.35	NS

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Table 6. Interaction effect of storage condition and invigoration treatment on mean germination time (days) during storage in ash gourd

Treatments	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
	Interaction (S x I)									
S_1I_1	7.19^{g}	7.10^{de}	6.92^{b}	3.76^{a}	4.34^{a}	4.40^{ab}	4.46^{abc}	4.49^{bcde}	4.56^{bc}	5.03^{bc}
S_1I_2	6.68^{h}	6.91^{ef}	7.06^{b}	3.86^{a}	4.18^{ab}	4.21^{abc}	4.21^{abcd}	4.37^{cdef}	4.56^{bc}	5.13^{bc}
S_1I_3	6.93^{gh}	6.74^{f}	7.05^{b}	3.78^{a}	4.00^{ab}	4.14^{bc}	4.40^{abc}	4.92^{ab}	5.19^{a}	5.51^{abc}
S_1I_4	7.13^{g}	7.22^{cde}	7.01^{b}	3.81^{a}	4.00^{ab}	4.18^{abc}	4.50^{ab}	4.52^{abcd}	4.52^{bcd}	4.94^{cd}
S_1I_5	7.16^{g}	7.07^{de}	7.10^{b}	3.92^{a}	3.92^{ab}	4.20^{abc}	4.60^{a}	5.00^{a}	5.07^{ab}	5.82^{a}
S_1I_6	6.91^{gh}	6.67^{f}	6.86^{b}	3.77^{a}	4.08^{ab}	4.46^{a}	4.67^{a}	5.00^{a}	5.42^{a}	5.63^{ab}
S_1I_7	10.64^{a}	10.00^{a}	9.36^{a}	3.29^{b}	3.80^{ab}	4.27^{ab}	4.67^{a}	4.83^{abc}	4.99^{ab}	5.50^{abc}
S_2I_1	8.21^{f}	7.55^{c}	5.29^{c}	3.82^{a}	3.67^{b}	3.82^{d}	3.92^{cd}	3.92^{f}	4.12^{cde}	4.14^{e}
S_2I_2	8.23^{f}	7.33^{cd}	4.69^{c}	3.77^{a}	3.99^{ab}	3.83^{d}	4.03^{bcd}	4.07^{def}	4.21^{cde}	4.14^{e}
S_2I_3	9.15^{d}	7.22^{cde}	5.27^{c}	3.73^{a}	3.70^{ab}	3.80^{d}	3.92^{cd}	4.02^{ef}	4.03^{cde}	4.19^{e}
S_2I_4	9.47^{c}	8.64^{b}	5.64^{c}	3.84^{a}	3.82^{ab}	3.76^{de}	3.66^{d}	3.27^{g}	3.92^{e}	3.92^{e}
S_2I_5	8.64^{e}	8.40^{b}	4.69^{c}	3.88^{a}	3.73^{ab}	3.51^{e}	3.89^{cd}	3.95^{f}	3.96^{de}	3.97^{e}
S_2I_6	8.53^{e}	7.26^{cd}	4.77^{c}	4.07^{a}	3.90^{ab}	3.76^{de}	3.78^{d}	4.09^{def}	4.12^{cde}	4.31^{e}
S_2I_7	10.3^{b}	10.26^{a}	5.34^{c}	4.09^{a}	4.06^{ab}	3.94^{cd}	3.96^{bcd}	4.26^{def}	4.34^{cde}	4.41^{de}
SEm ±	0.099	0.106	0.301	0.136	0.188	0.088	0.174	0.151	0.173	0.198
CD (0.05)	0.284	0.308	0.870	0.315	0.544	0.253	0.501	0.150	0.499	0.574

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

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Nawaz *et al.* (2011) observed that halopriming treatments resulted in lower T_{50}, MGT and higher Final germination per cent (FGP) in tomatoes. There was a significant decrease in time taken to 50% germination with invigoration which may be attributed to early reserve breakdown as well as reserve mobilization.

4.1.3.3.3 Due to Interaction (S x I)

During the start of storage period (*ie.*, at 1 MAS), S_1I_2 (Ambient - $CaCl_2$ 50mM 24h), S_1I_5 (Ambient- KH_2PO_4 10^{-1}M 24h) and S_1I_6 (Ambient - *Pf* $1x10^6$ cfu.ml^{-1} 12h) registered low T_{50} of 2.48 days, 2.68 days and 2.47 days respectively compared to the highest T_{50} values registered by untreated seeds under ambient storage (S_1I_7: 4.00 days). The untreated seeds and most of the invigorated seeds registered significantly low T_{50} from 6 MAS. However, at the end of storage period (10 MAS) S_1I_6 (Ambient - *Pf* $1x10^6$ cfu.ml^{-1} 12h; 57.00 days) followed by S_1I_7 (Ambient – untreated: 25.50 days) and S_1I_5 (Ambient - KH_2PO_4 10^{-1}M 24h: 24.60 days) registered significantly higher T_{50} values while S2I4 (Refrigerated - Kinetin 10ppm 24h: 3.52 days) registered the least T_{50} value.

T_{50} is considered inversely proportional to seedling vigour. The earliness in time taken to attain 50 per cent germination (T_{50}) by invigorated seeds as evident in the study is in conformity with the results of Dezfuli *et al* (2008) in maize. The effect of kinetin in reducing the germination time has been proved by Nawaz *et al.* (2013) in tomato where seeds invigorated with 10 ppm kinetin for 24h recorded the least T_{50} value.

4.1.3.4. Energy of germination (GE)

The results on Energy of germination (GE) as influenced by storage condition, invigoration treatment and their interaction effects during the storage period are presented in Tables 9 and 10.

4.1.3.4.1 Due to Storage condition (S)

Except at 1 MAS, throughout the storage period seeds stored under the refrigerated condition (S_2: 36.58) registered significantly high GE compared to those stored under ambient condition (S_1: 42.08). Under both storage conditions, GE increased initially and decreased towards the end of storage (S_1: 1.33; S_2: 50.04). Under both storage conditions, GE increased initially and decreased towards the end of storage (S_1: 1.33; S_2: 50.04). The results are in consonance with the findings of Mrda *et al.* (2011). Rajic *et al.* (2005) found that the GE and germinability of sugar beet seeds was significantly increased six months after harvesting. Tatic *et al.* (2008), furthermore, determined that the quality of soybean seeds was significantly influenced not only by the length of storage but by the method of storing as well. Ghasemnezhad and Honermeier (2009) on the other hand found that storage duration had no effect on seed GE, which is in opposition to the findings of the present study.

4.1.3.4.2 Due to Invigoration treatment (I)

During 1 MAS, I_1 ($CaCl_2$: 50mM 12h: 47.98) and I_6 (*Pf* $1x10^6$ cfu.ml^{-1} 12h: 45.70) registered high significantly GE over all other treatments. Untreated seeds (I_7) were inferior to most invigorated treatments during the storage period. However, towards the end of storage (9 MAS and 10 MAS) no significant difference between treatments was observed with respect to GE. The invigoration treatments I_2 ($CaCl_2$ 50mM 24h) and I_3 (Kinetin 10 ppm 12h) were on par with each other from 3 MAS to 8 MAS.

Indicators of seed vitality (germination energy and germinability as well as emergence in field conditions) play a direct role and are the key factor in determining plant number per hectare, which is one of the three main components of yield (Mrda *et al.*, 2011). Seed quality also affects the rate and uniformity of emergence as well as the rate of initial plant growth. The weather conditions can have great influence on seed quality during the growing season (Mihailovic *et al.,* 2002). Also, when seeds are kept at higher temperatures and higher relative humidity, their quality may become reduced (Simic *et al.,* 2006).

As found in the present study, seed invigoration with 1% $CaCl_2$ was found to increase the energy of germination in marigold (Hocart *et al.*, 1990; Kumari *et al.,* 2017), in tomato, in capsicum and onion when invigorated with KH_2PO_4

(Jagadesh *et al.*, 1994), in melon when with KNO_3 and 1% $CaCl_2$ (Farooq *et al.*, 2007).

4.1.3.4.3 Due to Interaction (S x I)

During the start of storage period (1 MAS), S_1I_1 (Ambient - $CaCl_2$ 50mM 12h), S_1I_6 (Ambient - *Pf* $1x10^6$ $cfu.ml^{-1}$ 12h) and S_2I_1 (Refrigerated - $CaCl_2$ 50mM 12h) registered high GE of 47.73, 51.67 and 48.23 respectively compared to all other invigorated seeds as well as untreated seeds both under refrigeration and ambient storage. The seeds (both invigorated and untreated) under refrigeration recorded, significantly higher GE from 5 MAS to end of storage, indicating that invigoration followed by refrigeration is more advantageous to produce vigorous seedling when storage is prolonged. Our results are in concomitance with that of Meena *et al.* (2017). They found that soybean seeds packed in high density polythene bags and stored under room temperature maintained minimum seed certification standards (72.5 and 75.5 %) only up to 8 months, while the seeds stored under cold storage could retain their viability up to 14 months. The energy of germination of cold stored seeds was higher than ambient stored seeds. Similar findings were also reported by Dearman *et al.* (1986), Owen and Pill (1994), Dorna *et al.* (2013).

4.1.3.5 Germination index (GI)

The results on Germination index (GI) as influenced by storage condition, invigoration treatment and their interaction effects during the storage period are presented in Tables 11 and 12.

4.1.3.5.1 Due to Storage condition (S)

The germination index gradually decreased from 12.436 at 1 MAS to 0.96 at 10 MAS in ambient storage (S_1), while it decreased from 12.72 at 4 MAS to 9.70 at 10 MAS in refrigerated storage (S_2). During the initial two months of storage, seeds under ambient storage registered significant higher values of germination index and hence forth, from 4 MAS to 10 MAS, seeds stored under refrigerated condition exhibited significant high GI irrespective of invigoration treatment.

Table 7. Influence of storage condition and invigoration treatment on time taken for 50% germination (days) during storage in ash gourd

Details	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Storage condition (S)										
S_1	2.98^{b}	3.00	3.14	3.27	3.64	4.42	5.09	8.16	11.52	21.87
S_2	3.47^{a}	3.43	3.38	3.29	3.13	3.31	3.33	3.52	3.63	3.77
SEm ±	0.054	0.04	0.059	0.065	0.078	0.052	0.093	0.341	1.978	1.156
CD (0.05)	0.157	0.117	0.17	NS	0.228	0.153	0.27	0.992	5.761	3.365
Invigoration treatment (I)										
I_1	3.19^{bc}	3.16^{bc}	3.17^{ab}	3.21	3.22	3.81^{b}	3.98^{b}	4.01^{b}	3.91	4.64^{c}
I_2	2.83^{c}	2.95^{c}	3.03^{b}	3.10	3.26	3.94^{b}	3.96^{b}	4.28^{b}	4.49	6.88^{bc}
I_3	3.19^{bc}	3.14^{bc}	3.20^{ab}	3.28	3.78	4.50^{a}	4.95^{a}	6.04^{b}	8.25	9.36^{bc}
I_4	3.35^{b}	3.32^{b}	3.50^{ab}	3.61	3.74	3.76^{b}	4.77^{a}	5.61^{b}	7.87	9.51^{bc}
I_5	3.04^{bc}	3.15^{bc}	3.26^{ab}	3.21	3.22	3.51^{b}	4.06^{b}	10.05^{a}	10.93	14.16^{bc}
I_6	3.03^{bc}	3.05^{bc}	3.10^{ab}	3.10	3.26	3.93^{b}	3.94^{b}	6.65^{b}	13.16	30.42^{a}
I_7	4.00^{a}	3.77^{a}	3.60^{a}	3.49	3.27	3.61^{b}	3.84^{b}	4.27^{b}	4.45	14.81^{b}
SEm ±	0.101	0.075	0.109	0.122	0.147	0.098	0.173	0.637	3.701	2.162
CD (0.05)	0.294	0.219	0.319	NS	NS	0.286	0.504	1.855	NS	6.295

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Table 8. Interaction effect of Storage condition and Invigoration treatment on Time taken for 50% germination (days) during storage in ashgourd

Treatments	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Interaction (S x I)										
S_1I_1	2.95^{ef}	2.92^{efg}	3.07^{de}	3.15^{cd}	3.36^{c}	3.82^{cdef}	4.18^{c}	4.22^{ef}	4.45^{d}	5.58^{c}
S_1I_2	2.48^{g}	2.71^{fg}	2.81^{ef}	2.96^{cd}	3.22^{c}	4.56^{b}	4.60^{bc}	5.21^{d}	5.44^{d}	9.82^{bc}
S_1I_3	3.00^{def}	3.10^{Cdef}	3.23^{cd}	3.47^{abc}	4.93^{a}	5.63^{a}	6.53^{a}	8.66^{c}	13.00^{c}	15.15^{bc}
S_1I_4	3.33^{bcd}	3.28^{bcde}	3.68^{ab}	3.93^{a}	4.27^{b}	4.35^{bc}	6.46^{a}	8.45^{c}	12.33^{c}	15.50^{bc}
S_1I_5	2.68^{fg}	2.93^{defg}	3.23^{cd}	3.27^{bcd}	3.31^{c}	3.97^{bcde}	4.68^{b}	16.5^{a}	18.19^{b}	24.60^{b}
S_1I_6	2.47^{g}	2.51^{g}	2.67^{f}	2.83^{d}	3.19^{c}	4.58^{b}	4.64^{b}	9.58^{b}	22.50^{a}	57.00^{a}
S_1I_7	4.00^{a}	3.57^{abc}	3.33^{cd}	3.29^{bcd}	3.21^{c}	4.03^{bcd}	4.56^{bc}	4.53^{de}	4.79^{d}	25.50^{b}
S_2I_1	3.43^{bc}	3.40^{bcd}	3.28^{cd}	3.27^{bcd}	3.07^{cd}	3.80^{cdef}	3.77^{d}	3.79^{efg}	3.38^{d}	3.69^{c}
S_2I_2	3.18^{cde}	3.19^{bcde}	3.25^{cd}	3.23^{bcd}	3.30^{c}	3.31^{efg}	3.32^{e}	3.35^{gh}	3.53^{d}	3.93^{c}
S_2I_3	3.37^{bc}	3.18^{bcde}	3.17^{d}	3.09^{cd}	2.62^{d}	3.37^{defg}	3.37^{de}	3.41^{gh}	3.51^{d}	3.56^{c}
S_2I_4	3.37^{bc}	3.35^{bcde}	3.32^{cd}	3.29^{bcd}	3.20^{c}	3.17^{fg}	3.08^{e}	2.77^{h}	3.41^{d}	3.52^{c}
S_2I_5	3.41^{bc}	3.36^{bcde}	3.29^{cd}	3.16^{cd}	3.12^{cd}	3.05^{g}	3.43^{de}	3.60^{fg}	3.67^{d}	3.73^{c}
S_2I_6	3.60^{b}	3.59^{ab}	3.53^{bc}	3.37^{bc}	3.32^{c}	3.27^{fg}	3.25^{e}	3.72^{fg}	3.81^{d}	3.84^{c}
S_2I_7	3.99^{a}	3.97^{a}	3.86^{a}	3.69^{ab}	3.32^{c}	3.19^{fg}	3.11^{e}	4.01^{efg}	4.11^{d}	4.12^{c}
SEm ±	0.14	0.10	0.15	0.17	0.20	0.13	0.24	0.90	5.23	3.05
CD (0.05)	0.41	0.30	0.45	0.50	0.60	0.40	0.71	2.62	2.61	8.90

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

The result is in accordance with the work of Vertucci *et al.* (1994). They observed a rapid decline in germination index in pea seeds stored in ambient storage compared to those under cold storage (5^0C). Hussain *et al.* (2015) noticed a reduced germination index of rice seeds stored at 25°C compared to the seeds stored at -4^0C.

According to Maguire (1962), a higher germination index of a sample in relation to another is an indicative of high seedling vigour. It is used to predict the relative vigour of samples, especially for cultivated species, since samples with the same quantity of seeds germinated gives different values for this index. Although this parameter is not presented with a unit it denotes the number of normal seedlings per day (Ranal and Santana, 2006).

4.1.3.5.2 Due to Invigoration treatment (I)

Irrespective of the treatments, the germination index showed a decreasing trend over the storage period. Treatment I_1 ($CaCl_2$ 50mM 12h) was on par with I_2 ($CaCl_2$ 50mM 24h) throughout the storage period irrespective of storage condition. In addition, the treatments I_1 and I_2 were also found to be on par with I_3 (kinetin 10 ppm 12h), I_4 (kinetin 10 ppm 24h), I_5 (KH_2PO_4 10-1 M 24h) and I_6 (*Pf* $1x10^6$ cfu.ml^{-1} 12h) at 1 MAS, 2 MAS, 3 MAS and 10 MAS. Irrespective of the storage condition over the storage period, the untreated seed were significantly inferior to all other treatments except at eighth and ninth month after storage, indicating the advantage of invigoration before seed storage.

Similar to the present study, an increased seed performance of seeds invigorated with $CaCl_2$ was reported by Hocart *et al.*, (1990) in maize seeds invigorated with $CaCl_2$ (50 mM), Yousof (2013) in rice and Shobha (2016) in ash gourd. Shobha (2016) had found that seeds treated with $CaCl_2$ recorded the highest per cent increase in germination, germination index and the seed viability for 7 MAS under the ambient storage compared to the untreated seeds.

4.1.3.5.3 Due to Interaction (S x I)

Under refrigerated storage, irrespective of the treatments, the germination index registered an initial increase and then exhibited a decreasing trend over the storage period. This is in agreement with the findings of Lima (2014) in sunflower. A sharp decline in rate of emergence was observed in ambient stored seeds over storage while, the index increased up to the ninth month and declined as storage period prolonged to twelfth month in seeds stored under refrigerated condition.

The performance of untreated seeds under ambient (S_1I_7) was significantly inferior to the invigorated seeds throughout the storage period. The germination index of invigorated seeds under ambient except S_1I_5 (Ambient - KH_2PO_4 10^{-1}M 24h; 12.57) was significantly superior to both invigorated and untreated seeds under refrigeration during 1 MAS. Further, it was observed that during the initial three month of storage S_1I_1 (Ambient - $CaCl_2$ 50mM 12h), S_1I_2 (Ambient - $CaCl_2$ 50mM 24h), S_1I_3 (Ambient - kinetin 10 ppm 12h), S_1I_4 (Ambient-kinetin 10 ppm 24h) and S_1I_6 (Ambient- *Pf* $1x10^6$ cfu.ml^{-1} 12h) were on par with each other. These were also found to be on par with S_2I_1 (Refrigerated - $CaCl_2$ 50mM 12h), S_2I_2 (Refrigerated - $CaCl_2$ 50mM 24h), S_2I_3 (Refrigerated - Kinetin 10ppm 12h) and S_1I_5 (Refrigerated - KH_2PO_4 12h) from 2 MAS to 4 MAS and thereafter a progressive decline in germination index of ambient stored seeds were observed.

The treatment S_2I_2 (Refrigerated - $CaCl_2$ 50mM 12h: 12.36) was on par with S_2I_6 (Refrigerated - *Pf* $1x10^6$ cfu.ml^{-1} 12h: 10.42) but significantly superior to all other treatments. It was observed that GI in S_2I_2 was on par with S_2I_1 (Refrigerated - $CaCl_2$ 50mM 12h) from 2 MAS to 9 MAS. The germination index of untreated seeds under refrigerated storage (S_2I_7) were significantly inferior to the invigorated seeds during the initial period of storage (1 MAS to 6 MAS) and also at the end of storage period.

Table 9. Influence of storage condition and invigoration treatment on energy of germination (GE) during storage in ash gourd

Details	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Storage condition (S)										
S_1	39.95^{a}	49.29^{a}	44.37^{b}	52.19^{b}	43.57^{b}	31.58^{b}	15.79^{b}	7.22^{b}	4.24^{b}	1.33^{b}
S_2	36.58^{b}	39.08^{b}	57.28^{a}	64.29^{a}	71.35^{a}	73.97^{a}	70.20^{a}	58.73^{a}	51.97^{a}	50.04^{a}
SEm ±	0.685	1.417	0.795	1.547	1.466	2.397	1.813	1.502	1.558	1.124
CD (0.05)	1.995	4.127	2.314	4.504	4.269	6.979	5.279	4.374	4.536	3.272
Invigoration treatment (I)										
I_1	47.98^{a}	54.35^{a}	56.40^{ab}	65.73^{a}	59.72^{abc}	49.31	36.84	30.14^{b}	27.09	21.94
I_2	42.30^{bc}	47.45^{ab}	48.68^{c}	71.39^{a}	68.20^{a}	57.42	44.17	33.75^{ab}	31.25	28.72
I_3	37.28^{c}	48.52^{ab}	60.95^{a}	65.83^{a}	57.50^{abc}	45.42	36.67	35.00^{ab}	25.56	22.50
I_4	32.00^{d}	43.84^{ab}	47.68^{c}	46.53^{b}	48.47^{c}	50.23	41.94	45.69^{a}	30.56	29.72
I_5	37.63^{c}	38.97^{b}	52.58^{bc}	66.11^{a}	65.42^{ab}	61.94	41.11	31.94^{b}	29.97	28.06
I_6	45.70^{ab}	49.64^{ab}	50.75^{bc}	50.00^{b}	47.50^{c}	58.33	49.17	25.42^{b}	25.2	24.72
I_7	24.98^{e}	26.57^{c}	38.75^{d}	42.12^{b}	55.42^{bc}	46.81	51.11	28.89^{b}	27.08	24.17
SEm ±	1.282	2.651	1.487	2.894	2.743	4.484	3.392	2.810	2.910	2.103
CD (0.05)	3.732	7.72	4.33	8.427	7.987	NS	NS	8.183	NS	NS

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Table 10. Interaction effect of storage condition and invigoration treatment on energy of germination (GE) during storage in ashgourd

Treatments	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
	Interaction (S x I)									
S_1I_1	47.73^{ab}	57.03^{a}	55.03^{bc}	70.55^{abcd}	45.00^{ef}	42.78^{cd}	19.44^{de}	11.11^{d}	9.44^{c}	0.00^{d}
S_1I_2	45.33^{bc}	54.90^{ab}	42.77^{de}	66.11^{bcd}	54.17^{def}	32.50^{de}	26.67^{d}	12.50^{d}	8.33^{c}	4.87^{d}
S_1I_3	39.77^{de}	54.27^{ab}	48.00^{d}	51.67^{e}	22.50^{h}	16.67^{e}	8.33^{e}	5.83^{d}	0.56^{c}	0.000^{d}
S_1I_4	32.97^{fg}	54.90^{ab}	37.03^{e}	32.50^{f}	32.50^{gh}	30.83^{de}	8.33^{e}	7.50^{d}	1.67^{c}	1.67^{d}
S_1I_5	41.20^{cd}	43.77^{bcd}	44.60^{d}	59.44^{de}	51.67^{ef}	43.89^{cd}	16.67^{de}	1.67^{d}	1.67^{c}	1.11^{d}
S_1I_6	51.67^{a}	57.07^{a}	54.83^{bc}	49.17^{e}	41.67^{fg}	35.00^{de}	11.67^{de}	0.83^{d}	0.56^{c}	0.00^{d}
S_1I_7	21.03^{h}	23.13^{f}	28.33^{f}	35.90^{f}	57.50^{de}	19.44^{e}	19.44^{de}	11.11^{d}	7.50^{c}	1.67^{d}
S_2I_1	48.23^{ab}	51.67^{abc}	57.76^{b}	60.91^{cde}	74.44^{bc}	55.83^{bc}	54.22^{c}	49.17^{c}	44.75^{b}	43.89^{c}
S_2I_2	39.27^{de}	40.00^{cde}	54.60^{bc}	76.67^{ab}	82.22^{ab}	82.33^{a}	61.67^{bc}	55.00^{bc}	54.17^{ab}	52.56^{abc}
S_2I_3	34.80^{ef}	42.78^{bcd}	73.89^{a}	80.00^{a}	92.50^{a}	74.17^{ab}	65.00^{bc}	64.17^{b}	50.56^{ab}	45.00^{c}
S_2I_4	31.03^{fg}	32.78^{def}	58.33^{b}	60.55^{cde}	64.44^{cd}	69.63^{ab}	75.55^{ab}	83.89^{a}	59.44^{a}	57.78^{a}
S_2I_5	34.07^{efg}	34.17^{def}	60.56^{b}	72.78^{abc}	79.17^{b}	80.00^{a}	65.56^{bc}	62.22^{b}	58.27^{a}	55.00^{ab}
S_2I_6	39.73^{de}	42.22^{cd}	46.67^{d}	50.83^{e}	53.33^{def}	81.67^{a}	86.67^{a}	50.00^{c}	50.00^{ab}	49.44^{abc}
S_2I_7	28.93^{g}	30.00^{ef}	49.17^{cd}	48.33^{e}	53.33^{def}	74.17^{ab}	82.78^{a}	46.67^{c}	46.67^{ab}	46.67^{bc}
SEm ±	1.812	3.75	2.103	4.093	3.879	6.341	4.797	3.975	4.121	2.973
CD (0.05)	5.277	10.918	6.123	11.917	11.296	18.464	13.968	11.573	11.94	8.61

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

The high values of germination index in invigorated and refrigerated stored seeds indicated the retention of seed quality over extended period of storage. Storing $CaCl_2$ invigorated seeds under refrigeration was found to be the best to acquire higher GI. The advantage of storing invigorated seeds under refrigerated condition over ambient storage was confirmed by Kanwar (2013). It was found that seed invigoration (solid-matrix invigoration, hydropriming and halopriming with KNO_3 and $CaCl_2$) in bitter gourd can improve storability and germination index up to six months under refrigerated storage and three months under ambient conditions

4.1.3.6 Coefficient of velocity of germination (CVG) (%)

The results on coefficient of velocity of germination as influenced by storage condition, invigoration treatment and their interaction effects during the storage period are presented in Tables 13 and 14.

4.1.3.6.1 Due to Storage condition (S)

The coefficient of velocity of germination in ambient stored seeds decreased over the period of storage while it increased initially and declined towards end of storage in seeds stored under refrigeration. The CVG of ambient stored seeds was 25.77 per cent at 1 MAS and 17.09 per cent at 10 MAS while that of seeds under refrigeration was 22.47 per cent at 1 MAS, increasing to 26.47 per cent at 5 MAS and thereafter gradually decreasing to 23.65 per cent at 10 MAS.

It was observed that ambient stored seeds were superior to refrigerated seeds upto 3 MAS and thereafter seeds stored in refrigerated condition were significantly superior to the seeds under ambient storage. Our result is in agreement with the results of Rehman *et al.* (1999) in acasia, Lanteri *et al.* (1996) in chilli and Khaje- Hoseini *et al.* (2003) in soybean. A reduced CV in seeds stored at ambient storage compared to those stored in cold storage was also reported by Rastegar *et al.* (2011) in soybean. According to Ghassemi-Golezani *et al.* (2010), a decrease in germination per cent and other indices could be related to physiological and biochemical changes during seed aging.

4.1.3.6.2 Due to Invigoration treatment (I)

Reduction in coefficient of velocity of germination was observed towards end of storage period in all treatments irrespective of storage condition. The untreated seed was significantly inferior to invigorated seeds throughout the storage period of ten months. Seeds invigorated with I_1 ($CaCl_2$ 50mM 12h) and I_2 ($CaCl_2$ 50mM 24h) were found to be significantly superior throughout the storage period. These treatments were found to be on par with the treatments I_5 (KH_2PO_4 10^{-1} M 24h) and I_6 (*Pf* $1x10^6$ $cfu.ml^{-1}$ 12h) at 1 MAS and 6 MAS.

Moghadam and Mohammadi (2013) also reported significant increased daily germination parameters and germination per cent and reduced mean germination time in invigorated seeds of safflower. Similarly, a reduction in germination time *i.e.,* CVG was also reported by Jamadar and Chandrashekhar (2015) in castor seeds invigorated with 2% $CaCl_2$. According to Mewael *et al.* (2010), invigoration accounts for the early replication of DNA, increased RNA production, protein synthesis, enhanced enzyme activity and greater availability of ATP and repair of deteriorated seed parts. These activities might have accelerated the early protrusion of radicle and thereby shortening the germination time (Elouaere and Hannachi, 2013).

4.1.3.6.3 Due to Interaction (S x I)

Irrespective of the treatment at 1 MAS seeds under ambient storage registered a high significant coefficient of velocity of germination over refrigerated storage. The CVG of all ambient stored seeds gradually declined from the start to the end of storage period while over the storage period there was an initial increase in CVG followed by a decline in case of both invigorated and untreated seeds stored under refrigeration.

Table 11. Influence of storage condition and invigoration treatment on germination index (GI) during storage in ash gourd

Details	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Storage condition (S)										
S_1	12.43^{a}	12.41^{a}	12.01	12.07^{b}	10.49^{b}	8.17^{b}	6.00^{b}	4.162^{b}	2.72^{b}	0.96^{b}
S_2	8.29^{b}	10.77^{b}	11.79	12.72^{a}	13.08^{a}	12.65^{a}	12.39^{a}	11.87^{a}	10.69^{a}	9.70^{a}
SEm ±	0.197	0.308	0.191	0.211	0.211	0.278	0.34	0.317	0.188	0.212
CD (0.05)	0.573	0.896	NS	0.614	0.615	0.81	0.989	0.924	0.548	0.617
Invigoration treatment (I)										
I_1	12.35^{a}	12.21^{a}	12.76^{a}	13.43^{a}	13.99^{a}	10.85	9.017	8.00^{ab}	7.18^{abc}	5.10^{a}
I_2	10.31^{a}	12.31^{a}	12.83^{a}	13.18^{a}	13.56^{ab}	9.23	9.447	9.34^{a}	7.74^{ab}	4.62^{a}
I_3	11.21^{a}	12.31^{a}	12.36^{a}	12.21^{ab}	9.858^{c}	9.28	7.733	7.04^{b}	5.40^{c}	4.06^{a}
I_4	11.26^{a}	10.94^{a}	11.23^{ab}	10.50^{c}	9.77^{c}	9.99	8.183	7.93^{ab}	6.30^{bc}	4.56^{a}
I_5	10.58^{a}	11.75^{a}	11.96^{a}	13.66^{a}	11.90^{abc}	11.85	9.542	7.46^{b}	6.25^{bc}	5.08^{a}
I_6	11.03^{a}	12.24^{a}	12.42^{a}	12.64^{ab}	11.92^{abc}	11.04	10.00	7.20^{b}	5.67^{c}	5.28^{a}
I_7	5.802^{b}	9.423^{b}	9.78^{b}	11.17^{bc}	11.53^{bc}	10.64	10.47	9.12^{a}	8.37^{a}	2.18^{b}
SEm ±	0.368	0.576	0.357	0.394	0.395	0.52	0.636	0.594	0.352	0.396
CD (0.05)	1.072	1.677	1.04	1.148	1.15	NS	NS	1.43	1.026	1.154

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

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Table 12. Interaction effect of storage condition and invigoration treatment on germination index (GI) during storage in ash gourd

Treatments	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Interaction (S x I)										
S_1I_1	14.30^{a}	13.23^{ab}	13.16^{a}	12.98^{abc}	12.79^{bcde}	9.81^{cdef}	7.05^{cd}	5.41^{cd}	4.19^{d}	1.44^{cd}
S_1I_2	13.33^{ab}	13.33^{ab}	13.20^{a}	13.38^{abc}	13.39^{abcd}	7.43^{fg}	6.79^{cde}	5.53^{cd}	5.04^{cd}	3.19^{c}
S_1I_3	13.70^{ab}	12.50^{abc}	11.43^{abc}	9.93^{de}	5.47^{g}	5.32^{g}	4.14^{de}	2.75^{d}	0.98^{e}	0.90^{d}
S_1I_4	13.37^{ab}	11.53^{abc}	11.23^{abc}	9.38^{e}	8.02^{f}	7.36^{fg}	3.42^{e}	2.57^{d}	0.49^{e}	0.15^{d}
S_1I_5	12.57^{b}	12.50^{abc}	12.32^{a}	14.33^{a}	10.86^{e}	8.76^{ef}	6.12^{cde}	2.55^{d}	1.36^{e}	0.37^{d}
S_1I_6	13.30^{ab}	14.13^{a}	13.25^{a}	13.27^{abc}	11.13^{de}	9.20^{def}	6.25^{cde}	2.68^{d}	0.65^{e}	0.15^{d}
S_1I_7	6.49^{fg}	9.68^{c}	9.54^{c}	11.23^{cde}	11.84^{cde}	9.31^{def}	8.27^{bc}	7.65^{bc}	6.35^{c}	0.57^{d}
S_2I_1	10.40^{c}	11.18^{abc}	12.36^{a}	13.89^{ab}	15.19^{a}	11.89^{bcd}	10.98^{ab}	10.60^{ab}	10.18^{ab}	8.78^{b}
S_2I_2	7.30^{ef}	11.28^{abc}	12.47^{a}	12.98^{abc}	13.72^{abc}	11.03^{bcde}	12.10^{a}	13.15^{a}	10.45^{ab}	12.36^{a}
S_2I_3	8.72^{de}	12.11^{abc}	13.28^{a}	14.50^{a}	14.25^{ab}	13.24^{ab}	11.33^{ab}	11.33^{a}	9.82^{b}	8.78^{b}
S_2I_4	9.16^{cd}	10.34^{bc}	11.23^{abc}	11.61^{cd}	11.53^{cde}	12.63^{abc}	12.94^{a}	13.31^{a}	12.13^{a}	9.53^{b}
S_2I_5	8.59^{de}	11.00^{abc}	11.60^{ab}	12.99^{abc}	12.95^{bcde}	14.93^{a}	12.97^{a}	12.38^{a}	11.15^{ab}	9.54^{b}
S_2I_6	8.77^{de}	10.34^{bc}	11.59^{ab}	12.01^{bcd}	12.72^{bcde}	12.87^{ab}	13.76^{a}	11.73^{a}	10.70^{ab}	10.42^{ab}
S_2I_7	5.12^{g}	9.17^{c}	10.03^{bc}	11.12^{cde}	11.22^{de}	11.98^{bcd}	12.67^{a}	10.59^{ab}	10.41^{ab}	8.680^{b}
SEm ±	0.521	0.814	0.505	0.558	0.559	0.736	0.899	0.839	0.498	0.561
CD (0.05)	1.516	2.359	1.471	1.624	1.627	2.142	2.129	2.444	1.451	1.439

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

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Both invigorated and untreated seeds under refrigeration were found to be on par with each other between4 MAS and 10 MAS, except at 6 MAS and 8 MAS. At 10 MAS, these were also found to be on par with S_1I_1 (Ambient - $CaCl_2$ 50mM 12h) and S_1I_2 (Ambient - $CaCl_2$ 50mM 24h), S_1I_4 (Ambient - kinetin 10 ppm 24h) and untreated seeds (S_1I_7). Doijode (2005) found that high vigour in terms of coefficient of velocity germination was observed in okra seeds stored for 20 years at low temperature.

4.1.9 Vigour index-I (VI-I)

The results on seedling vigour index I as influenced by storage condition, invigoration treatment and their interaction during the storage period are presented in Tables 15 and 16.

4.1.3.7.1 Due to Storage condition

The seedling vigour decreased from 2374.00 (1 MAS) to 228.00 (10 MAS) under ambient storage, while under refrigerated storage it varied from 1532.00 (1 MAS) and 1544.00 (10 MAS). It was also observed that the VI-I of ambient stored seeds declined over the period of storage, while that of refrigerated stored seeds increased initially up to 5 MAS and thereafter declined.

Seeds under ambient storage exhibited significant high VI-I than refrigerated stored seeds during the initial three months of storage (Fig.3). Henceforth, from 5 MAS seedling vigour index I of seeds under refrigerated storage was significantly superior to that of ambient stored seeds.

According to Heydecker (1972), the reduced seedling vigour in stored seeds was associated with weakening of cell membrane. High rate of lipid peroxidation at high temperature results in membrane destruction thereby adversely affecting the seed quality parameters (Rajasree and Jirali, 2017). Powell *et al.* (2000) stated that the decreased growth of root and shoot is associated with the seed deterioration from the loss of viability. Similar results on high vigour indices exhibited by the seeds of musk melon after 15 months of storage in 5^0C and -20^0C was reported by

Doijode, (2017). In addition, storage studies in beans in ambient and cold storage by Camila (2017) revealed that seed storage at 10°C provided better seed conservation whereas temperature of 30°C accelerated the deterioration and reduced the vigour. Vishnurammethi (1996), Jasper (1998) and Ananthi (2001) in cowpea, Doijode (2005) in okra and Basavegowda *et al.* (2016) in pigeon pea, also reported similar results.

4.1.3.7.2 Due to Invigoration treatment (I)

Seedling vigour index I decreased towards the end of period of storage in both invigorated and untreated seeds. Seed invigoration with I_1 ($CaCl_2$ 50mM 12h) and I_2 ($CaCl_2$ 50mM 24h) were on par with each other throughout the storage period except at 7 MAS and 9 MAS.

Untreated seeds were significantly inferior to seeds invigorated with $CaCl_2$ 50mM 12h (I_1) between 1 MAS and 4 MAS and thereafter, they were found to be on par with each other. The results thus pointed out that it was advantageous to haloprime seeds with $CaCl_2$ for improved vigour during storage.

According to Jie *et al.* (2002), halopriming helps to release enzymes and accelerate seed metabolism and physiological activities which ultimately increase the germination of seeds. High vigour in the seed invigorated with $CaCl_2$ as observed in the present study is in agreement with the studies of Singh *et al.* (2017). They observed maximum seedling vigour) in seeds invigorated with $CaCl_2$ for 12hours followed by seeds invigorated with 1% $CaCl_2$ for 8 hours compared to the untreated seeds. It has also been reported that invigorated seeds exhibited enhanced germination pattern and higher seedling vigour compared to untreated seeds (Ruan *et al.*, 2002). Tabrizian and Osareh (2007) observed that the increase in vigour index by invigoration becomes evident as invigoration improves the capability of the plants to compete for the basic needs of nutrients, water and light

4.1.3.7.3 Due to Interaction (S x I)

Seedling vigour index I of invigorated seeds stored under ambient condition progressively decreased over the storage period, while, the VI-I of invigorated seeds under cold storage increased initially and then gradually declined towards the end of storage. Under ambient, all the invigorated seeds were significantly superior to untreated seeds throughout the storage period.

In case of seeds stored under refrigeration, invigoration with $CaCl_2$ 50mM for 24h (S_2I_2) and kinetin 10 ppm for 12h (S_2I_3) were found to be on par with each other at 5 MAS as well as towards the end of storage (S_2I_2: 1621.00 and S_2I_3:1540.00). Untreated seeds (I_7) was on par with these treatments at 9 MAS and 10 MAS only. At the end of storage period, invigoration with Kinetin 10ppm for 24h (S_2I_4; 1488.00), KH_2PO_4 10^{-1}M for 24h (S_2I_5: 1591.00), *Pf* $1x10^6$ cfu.ml^{-1} for 12h (S_2I_6: 1730.00) were also on par with I_2, I_3 and I_7.

Hence, it was evident that storage of seeds under the refrigerated condition irrespective of invigoration treatments was optimum to deduce high VI-I. Seed invigoration with $CaCl_2$ was beneficial to realise higher VI-I both under ambient and refrigerated storage.

4.1.3.8 Vigour index II (VI-II)

The results on seedling vigour index II as influenced by storage condition, invigoration treatment and their interaction during the storage period are presented in Tables 17 and 18.

4.1.3.8.1 Due to Storage condition (S)

The seedling vigour decreased from 2.20 (1 MAS) to 0.29 (10 MAS) under ambient storage, while under refrigerated storage it varied from 1.26 (1 MAS) to 1.43 (10 MAS). It was also observed that the VI-II of ambient stored seeds declined over the period of storage, while that of refrigerated stored seeds increased initially upto 5 MAS and thereafter declined (Fig. 4).

Table 13. Influence of storage condition and invigoration treatment on coefficient of velocity of germination (%) during storage in ash gourd

Details	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Storage condition (S)										
S_1	25.77^{a}	25.75^{a}	25.36^{a}	24.33	24.54^{b}	22.72	21.52	20.01^{b}	18.71	17.09
S_2	22.47^{b}	23.65^{b}	24.60^{b}	25.83	26.47^{a}	26.07	25.89	25.68^{a}	24.55	23.65
SEm ±	0.512	1.076	0.273	0.989	0.81	0.731	1.303	0.933	0.957	2.582
CD (0.05)	0.176	0.369	0.721	NS	2.142	NS	NS	2.469	NS	NS
Invigoration treatment (I)										
I_1	25.25^{a}	25.38	26.23^{a}	25.29	25.00	24.60^{ab}	24.11	23.80^{ab}	22.70	21.96
I_2	24.54^{a}	25.32	25.70^{a}	25.15	26.22	24.54^{ab}	24.28	23.32^{ab}	21.94	21.60
I_3	24.46^{a}	25.64	25.80^{a}	25.19	25.54	24.70^{ab}	22.41	21.92^{b}	21.15	14.83
I_4	24.31^{a}	23.90	24.28^{b}	25.04	24.40	23.43^{b}	23.86	24.91^{a}	22.02	21.94
I_5	25.28^{a}	24.98	24.68^{b}	24.84	26.16	25.90^{a}	23.79	22.76^{ab}	21.49	21.16
I_6	24.45^{a}	24.88	24.91^{b}	25.67	25.60	24.26^{ab}	24.15	21.46^{b}	20.90	20.34
I_7	20.58^{b}	22.84	23.30^{c}	24.42	25.63	23.35^{b}	23.41	21.77^{b}	21.24	20.77
SEm ±	0.329	0.691	0.35	1.271	1.04	0.94	1.675	1.199	1.23	3.317
CD (0.05)	1.34	NS	0.717	NS	NS	1.922	NS	2.45	NS	NS

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Table 14. Interaction effect of storage condition and invigoration treatment on coefficient of velocity of germination (%) during storage in ashgourd

Treatments	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Interaction (S x I)										
S_1I_1	27.23^{b}	26.62^{ab}	26.78^{a}	24.31^{ab}	22.75^{de}	22.58^{de}	22.58^{bcdefg}	22.04^{bcd}	21.09^{bc}	20.06^{abc}
S_1I_2	25.58^{cd}	26.20^{abc}	26.03^{abc}	24.15^{ab}	25.92^{abc}	23.77^{cd}	23.76^{abcdef}	22.01^{bcd}	19.68^{cd}	19.60^{abc}
S_1I_3	25.25^{d}	26.48^{abc}	26.37^{ab}	23.34^{b}	24.28^{cde}	22.71^{de}	19.29^{g}	18.97^{d}	17.44^{d}	8.330^{d}
S_1I_4	25.92^{bcd}	24.57^{abcd}	23.95^{fg}	23.76^{ab}	22.22^{e}	22.11^{de}	20.23^{fg}	18.82^{d}	18.47^{cd}	18.33^{abc}
S_1I_5	26.86^{abc}	25.62^{abc}	24.23^{f}	23.63^{b}	25.54^{abc}	23.85^{cd}	21.76^{cdefg}	19.75^{cd}	17.66^{d}	16.99^{c}
S_1I_6	27.39^{a}	27.43^{a}	26.76^{a}	26.76^{a}	24.66^{bcd}	22.55^{de}	21.67^{defg}	18.46^{d}	17.39^{d}	17.46^{bc}
S_1I_7	22.17^{fg}	23.34^{bcd}	23.44^{gh}	24.39^{ab}	26.41^{abc}	21.48^{e}	21.42^{efg}	20.05^{cd}	19.25^{cd}	18.84^{abc}
S_2I_1	23.27^{ef}	24.13^{bcd}	25.67^{bcd}	26.26^{ab}	27.26^{a}	25.64^{bc}	25.64^{abc}	25.56^{b}	24.30^{a}	23.86^{abc}
S_2I_2	23.50^{ef}	24.43^{abcd}	25.37^{cde}	26.26^{ab}	26.51^{abc}	25.14^{bc}	24.79^{abcde}	24.64^{b}	24.20^{a}	23.60^{abc}
S_2I_3	23.67^{e}	24.80^{abcd}	25.23^{de}	26.26^{ab}	26.81^{ab}	26.32^{ab}	25.53^{abcd}	24.86^{b}	24.85^{a}	21.33^{abc}
S_2I_4	22.70^{efg}	23.23^{cd}	24.60^{ef}	26.26^{ab}	26.58^{abc}	26.60^{ab}	27.50^{a}	30.99^{a}	25.56^{a}	25.55^{a}
S_2I_5	23.70^{e}	24.33^{abcd}	25.13^{de}	26.26^{ab}	26.77^{ab}	27.84^{a}	25.82^{ab}	25.77^{b}	25.33^{a}	25.32^{ab}
S_2I_6	21.50^{g}	22.33^{d}	23.07^{h}	26.26^{ab}	26.55^{abc}	25.68^{bc}	26.63^{a}	24.47^{b}	24.41^{a}	23.21^{abc}
S_2I_7	19.00^{h}	22.33^{d}	23.17^{h}	26.26^{ab}	24.84^{abcd}	25.28^{bc}	25.39^{abcd}	23.49^{bc}	23.23^{ab}	22.69^{abc}
SEm ±	0.465	0.977	0.248	0.899	0.736	0.665	1.184	0.848	0.87	2.346
CD (0.05)	1.355	2.828	0.094	0.34	0.278	0.251	0.448	0.321	0.329	0.887

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Seeds under ambient storage exhibited significant high VI-II than refrigerated stored seeds during the initial three months of storage. Henceforth, from 4 MAS seedling vigour index II of seeds under refrigerated storage was significantly superior to that of ambient stored seeds. This result is in consonance with the findings of Lima *et al.* (2014) in sunflower. He found that seedling vigour index II decreased from 2.20 to 0.29 in S_1 (ambient storage) while it decreased from 2.04 at 4 MAS to 1.43 at 10 MAS in S_2 (refrigerated storage). The trend observed was attributed to the trend observed in dry weight of seedlings over storage period. The dry weight of seedlings under refrigerated storage was lower than that of ambient stored seeds over nine months of storage followed by an increase in the 12^{th} month for seeds stored under refrigerated condition, while the dry weight for the seeds stored under ambient condition gradually reduced up to third month followed by a sharp decline at the sixth

4.1.3.8.2 Due to Invigoration treatment (I)

Seeds invigorated with $CaCl_2$ 50mM 24h (I_2) and $CaCl_2$ 50mM 12h (I_1) were to be on par with each other throughout the storage period except at 7 MAS (I_1: 1.29; I_2: 1.46) and 10 MAS (I_1: 0.85; I_2: 1.01). In addition, I_2 was found to be on par with I_5 (KH_2PO_4 10^{-1} M for 24h) and I_6 (*Pf* $1x10^6$ cfu.ml^{-1} for 12h) from 1 MAS to 7 MAS. The VI-II of untreated seeds (I_7) was on par with I_2 from 4 MAS to 10 MAS. Considering the above, seed invigoration with $CaCl_2$ 50mM for 12h (I_2) was found ideal to obtain vigourous seedling during storage.

As in the present study, invigoration treatments proved to increase the seedling vigour compared to the untreated seeds (Ashraf and Rauf, 2001; Toklu *et al.*, 2015 Arun *et al.*, 2017; Singh *et al.*, 2017). According to Dasgupta and Austerson (1973) seedling vigour, characterised by the weight of seedling is essentially a physiological phenomenon influenced by reserve metabolites and enzyme activation. A reduction in dry matter content of the seeds is due to the increased metabolic activity and the associated reduction in the seed reserves (Bewely and Black, 1982).

4.1.3.8.3 Due to Interaction (S x I)

The VI-II of both invigorated and untreated seeds under ambient storage was significantly inferior to that of refrigerated stored seeds from 4 MAS except with respect to seeds invigorated with $CaCl_2$ 50mM for 12h (S_1I_1). S_1I_1 was found to be on par with seeds treated with $CaCl_2$ 50mM for 24h under refrigeration (S_2I_2) at 4 MAS. SV-II of S_2I_2 was significantly high and on par with S_2I_5 (KH_2PO_4 10^{-1}M 24h), S_2I_6 (*Pf* $1x10^6$ cfu.ml^{-1} 12h) and untreated seeds (S_2I_7) from 6 MAS to 10 MAS.

Considering the above, seed invigouration with $CaCl_2$ 50mM for 24h (I_2) followed by refrigeration was found ideal to obtain high seedling vigour index II while, seed invigouration with $CaCl_2$ 50mM for 12hours (I_1) was found be the best for ambient storage. This is in conformity with the results of Basavegowda *et al.* (2016) in pigeon pea where, a high value of seed parameters in terms of seedling dry weight (65.75 mg), vigour (1096.25) was exhibited by the seeds of pigeon pea stored under the cold condition compared to a low value of seedling dry weight (50.83mg) and vigour (398.2) in ambient stored seeds after 26 months of storage. Similar results were also reported by Sharma *et al.* (2006) in soybean. Bezerra *et al.* (2004), opined that in moringa seeds stored in ambient condition, the initial dry weight of seedling was maintained up to six months and thereafter showed a drastic drop at 24^{th} month of storage, however the variation in seedling dry weight of the seeds under controlled storage was less pronounced.

4.1.3.9 Allometric index (AI)

The results on seedling allometric index as influenced by storage condition, invigoration treatment and their interaction during the storage period are presented in Tables 19 and 20, Plates 1 and 2. The data on seedling shoot and root observed at monthly intervals are detailed in Appendices I to IV.

Table 15. Influence of storage condition and invigoration treatment on seedling vigour index-I during storage in ash gourd

Details	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Storage condition (S)										
S_1	2374.00^{a}	2256.00^{a}	2147.00^{a}	1948.00	1610.00^{b}	1299.00^{b}	1098.00^{b}	734.00^{b}	452.00^{b}	228.00^{b}
S_2	1532.00	1665.85^{b}	1785.50^{b}	1890.90	2141.00^{a}	2094.00^{a}	2071.00^{a}	1825.00^{a}	1658.00^{a}	1544.00^{a}
SEm ±	18.51	13.47	19.89	40.47	38.77	42.10	36.26	27.62	29.05	24.14
CD (0.05)	53.90	39.21	57.90	NS	112.90	122.59	105.57	80.41	84.59	70.28
Invigoration treatment (I)										
I_1	2319.00^{a}	2261.00^{a}	2225.00^{ab}	2104.00^{ab}	2164.00^{a}	1840.00^{a}	1630.00^{b}	1477.00^{a}	1233.00^{ab}	1008.00
I_2	2215.00^{ab}	2264.00^{a}	2262.00^{a}	2295.00^{a}	2270.00^{a}	1895.00^{a}	1739.00^{ab}	1324.00^{ab}	1079.00^{bc}	1044.00
I_3	1942.00^{bc}	1901.00^{b}	1936.00^{cd}	1798.00^{cd}	1781.00^{bc}	1406.00^{c}	1307.00^{c}	1083.00^{b}	892.00^{c}	773.00
I_4	1944.00^{bc}	1819.00^{b}	1727.00^{de}	1476.00^{e}	1424.00^{d}	1403.00^{c}	1224.00^{c}	1275.00^{ab}	862.00^{c}	772.00
I_5	1910.00^{c}	1971.00^{b}	2019.00^{abc}	2160.00^{ab}	1865.00^{bc}	1923.00^{a}	1540.00^{b}	1165.00^{b}	987.00^{bc}	832.00
I_6	2135.00^{abc}	2050.00^{ab}	1984.00^{bc}	1948.00^{bc}	1621.00^{cd}	1597.00^{b}	1774.00^{ab}	1186.00^{b}	946.00^{bc}	879.00
I_7	1205.00^{d}	1462.00^{c}	1612.00^{e}	1655.00^{de}	2007.00^{ab}	1814.00^{a}	1884.00^{a}	1450.00^{a}	1388.00^{a}	893.00
SEm ±	34.63	25.19	37.20	75.71	72.54	78.76	67.83	51.66	54.35	45.16
CD (0.05)	100.83	73.35	108.32	220.44	211.21	229.34	197.50	150.43	158.26	NS

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Table 16. Interaction effect of storage condition and invigoration treatment on vigour index I during storage in ash gourd

Treatments	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Interaction (S x I)										
S_1I_1	2693.00^{a}	2557.00^{a}	2415.00^{a}	2083.00^{bcd}	1855.00^{cd}	1622.00^{def}	1385.00^{de}	1304.00^{c}	913.00^{b}	715.00^{c}
S_1I_2	2469.00^{ab}	2427.00^{ab}	2365.00^{a}	2223.00^{ab}	2018.00^{bc}	1513.00^{ef}	1426.00^{de}	799.00^{d}	411.00^{c}	467.00^{d}
S_1I_3	2344.00^{ab}	2069.00^{de}	2020.00^{c}	1600.00^{ef}	1077.00^{f}	778.00^{h}	632.00^{f}	503.00^{e}	221.00^{cd}	6.00^{e}
S_1I_4	2564.00^{ab}	2216.00^{cd}	2016.00^{c}	1459.00^{f}	1293.00^{ef}	1045.00^{gh}	559.00^{f}	392.00^{e}	141.00^{d}	57.00^{e}
S_1I_5	2382.00^{ab}	2348.00^{bc}	2225.00^{b}	2376.00^{a}	1521.00^{de}	1198.00^{g}	1106.00^{e}	453.00^{e}	259.00^{cd}	74.00^{e}
S_1I_6	2815.00^{a}	2567.00^{a}	2333.00^{a}	2165.00^{b}	1406.00^{e}	1329.00^{fg}	1109.00^{e}	486.00^{e}	127.00^{d}	29.00^{e}
S_1I_7	1349.00^{cd}	1609.00^{fg}	1658.00^{e}	1731.00^{e}	2102.00^{bc}	1608.00^{def}	1475.00^{d}	1203.00^{c}	1097.00^{b}	246.00^{de}
S_2I_1	1945.00^{bc}	1966.00^{e}	2036.00^{c}	2125.00^{bc}	2473.00^{a}	2058.00^{bc}	1876.00^{c}	1651.00^{b}	1552.00^{a}	1301.00^{b}
S_2I_2	1961.00^{bc}	2100.00^{de}	2158.00^{b}	2368.00^{a}	2521.00^{a}	2276.00^{b}	2051.00^{bc}	1849.00^{b}	1746.00^{a}	1621.00^{a}
S_2I_3	1540.00^{cd}	1733.00^{f}	1853.00^{d}	1995.00^{cd}	2486.00^{a}	2034.00^{bc}	1982.00^{bc}	1662.00^{b}	1564.00^{a}	1540.00^{ab}
S_2I_4	1324.00^{cd}	1421.00^{hi}	1439.00^{f}	1494.00^{f}	1554.00^{de}	1762.00^{cde}	1888.00^{c}	2157.00^{a}	1583.00^{a}	1488.00^{ab}
S_2I_5	1438.00^{cd}	1594.00^{fg}	1813.00^{d}	1944.00^{d}	2209.00^{ab}	2647.00^{a}	1974.00^{bc}	1877.00^{ab}	1716.00^{a}	1591.00^{a}
S_2I_6	1455.00^{cd}	1532.00^{gh}	1634.00^{e}	1731.00^{e}	1837.00^{cd}	1865.00^{cd}	2438.00^{a}	1885.00^{ab}	1765.00^{a}	1730.00^{a}
S_2I_7	1061.00^{d}	1314.00^{i}	1565.00^{e}	1580.00^{ef}	1912.00^{bc}	2020.00^{bc}	2293.00^{ab}	1698.00^{b}	1680.00^{a}	1541.00^{ab}
SEm ±	142.60	103.73	153.19	311.75	298.70	324.34	279.30	212.74	223.81	185.95
CD (0.05)	48.97	35.62	52.61	107.06	102.58	111.39	95.92	73.06	76.86	63.86

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

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Table 17. Influence of storage condition and invigoration treatment on vigour index II during storage in ash gourd

Details	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Storage condition (S)										
S_1	2.20^{a}	1.98^{a}	1.87^{a}	1.84^{b}	1.66^{b}	1.35^{b}	1.03^{b}	0.71^{b}	0.35^{b}	0.29^{b}
S_2	1.26^{b}	1.397^{b}	1.57^{b}	2.04^{a}	2.17^{a}	2.08^{a}	1.92^{a}	1.81^{a}	1.56^{a}	1.43^{a}
SEm ±	0.044	0.055	0.044	0.058	0.066	0.058	0.066	0.058	0.045	0.06
CD (0.05)	0.127	0.16	0.129	0.169	0.193	0.168	0.192	0.168	0.13	0.173
Invigoration treatment (I)										
I_1	2.040^{a}	2.00^{a}	1.98^{a}	2.240^{a}	2.08^{a}	1.77^{abc}	1.29^{b}	1.44^{ab}	1.19^{ab}	0.85^{bc}
I_2	1.91^{ab}	1.83^{a}	1.93^{a}	2.26^{a}	2.40^{a}	1.90^{a}	1.46^{ab}	1.34^{ab}	1.27^{a}	1.01^{ab}
I_3	1.69^{ab}	1.70^{a}	1.66^{ab}	1.85^{ab}	1.51^{bc}	1.36^{c}	1.27^{b}	1.15^{ab}	0.85^{bc}	0.76^{bc}
I_4	1.56^{b}	1.57^{ab}	1.55^{b}	1.43^{b}	1.36^{c}	1.40^{bc}	1.21^{b}	1.18^{ab}	0.69^{c}	0.65^{c}
I_5	1.93^{ab}	1.74^{a}	1.81^{ab}	1.95^{a}	2.05^{a}	1.94^{a}	1.55^{ab}	1.06^{b}	0.94^{abc}	0.85^{bc}
I_6	2.00^{a}	1.83^{a}	1.66^{ab}	1.93^{a}	1.95^{ab}	1.83^{abc}	1.90^{a}	1.09^{ab}	0.82^{bc}	0.76^{bc}
I_7	0.99^{c}	1.17^{b}	1.52^{b}	1.95^{a}	2.07^{a}	1.85^{ab}	1.67^{ab}	1.58^{a}	0.97^{abc}	1.18^{a}
SEm ±	0.082	0.103	0.083	0.109	0.124	0.108	0.123	0.108	0.084	0.111
CD (0.05)	0.238	0.299	0.242	0.316	0.36	0.314	0.359	0.313	0.243	0.324

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

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Table 18. Interaction effect of storage condition and invigoration treatment on vigour index II during storage in ash gourd

Treatments	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
	Interaction (S x I)									
S_1I_1	2.77^{a}	2.65^{a}	2.52^{a}	2.41^{ab}	1.97^{bc}	1.71^{bcde}	1.13^{cde}	1.19^{bc}	0.94^{cd}	0.69^{de}
S_1I_2	2.14^{b}	1.97^{bcd}	1.95^{b}	1.95^{c}	1.95^{bc}	1.72^{bcde}	1.06^{de}	0.85^{cd}	0.75^{d}	0.43^{ef}
S_1I_3	2.15^{b}	1.80^{cd}	1.68^{bcd}	1.57^{def}	0.88^{f}	0.75^{g}	0.67^{ef}	0.44^{d}	0.19^{e}	0.06^{g}
S_1I_4	1.83^{bc}	1.77^{cde}	1.70^{bcd}	1.40^{f}	1.27^{ef}	0.97^{fg}	0.53^{f}	0.47^{d}	0.12^{e}	0.06^{g}
S_1I_5	2.53^{a}	2.06^{bc}	1.98^{b}	1.86^{cd}	1.79^{cd}	1.32^{ef}	1.19^{cd}	0.43^{d}	0.27^{e}	0.09^{fg}
S_1I_6	2.89^{a}	2.30^{ab}	1.87^{bcd}	1.78^{cde}	1.69^{cde}	1.46^{def}	1.58^{bc}	0.41^{d}	0.12^{e}	0.04^{g}
S_1I_7	1.12^{ef}	1.33^{fg}	1.44^{cd}	1.92^{cd}	2.10^{bc}	1.54^{cde}	1.06^{e}	1.20^{bc}	0.09^{e}	0.72^{de}
S_2I_1	1.31^{e}	1.35^{fg}	1.44^{d}	2.05^{bc}	2.20^{bc}	1.85^{bcde}	1.44^{bcd}	1.72^{ab}	1.45^{ab}	1.04^{cd}
S_2I_2	1.68^{cd}	1.70^{cdef}	1.90^{bc}	2.57^{a}	2.85^{a}	2.08^{abc}	1.85^{ab}	1.83^{a}	1.78^{a}	1.59^{ab}
S_2I_3	1.23^{ef}	1.60^{def}	1.64^{bcd}	2.13^{bc}	2.14^{bc}	1.96^{bcd}	1.88^{ab}	1.86^{a}	1.51^{ab}	1.45^{ab}
S_2I_4	1.28^{e}	1.36^{fg}	1.39^{d}	1.46^{ef}	1.46^{de}	1.83^{bcde}	1.89^{ab}	1.89^{a}	1.26^{bc}	1.23^{bc}
S_2I_5	1.33^{de}	1.41^{ef}	1.62^{bcd}	2.04^{bc}	2.31^{b}	2.55^{a}	1.92^{ab}	1.69^{ab}	1.62^{ab}	1.60^{ab}
S_2I_6	1.12^{ef}	1.36^{fg}	1.45^{cd}	2.08^{bc}	2.20^{bc}	2.20^{ab}	2.21^{a}	1.76^{a}	1.52^{ab}	1.48^{ab}
S_2I_7	0.86^{f}	1.00^{g}	1.60^{bcd}	1.98^{c}	2.03^{bc}	2.15^{ab}	2.29^{a}	1.94^{a}	1.84^{a}	1.63^{a}
SEm ±	0.116	0.145	0.118	0.153	0.175	0.152	0.175	0.152	0.118	0.157
CD (0.05)	0.350	0.350	0.419	0.342	0.51	0.444	0.44	0.50	0.445	0.459

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Fig 3. Influence of storage condition on vigour index I in ash gourd

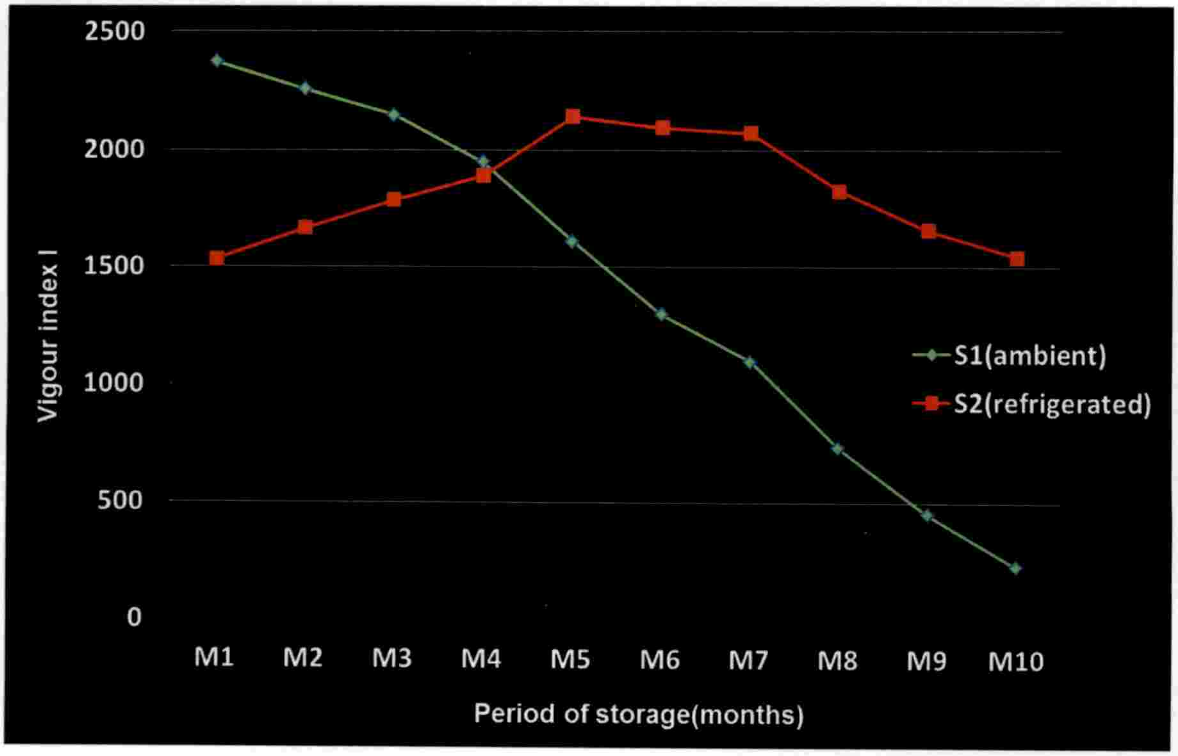

Fig 4. Influence of Storage condition on vigour index II in ash gourd

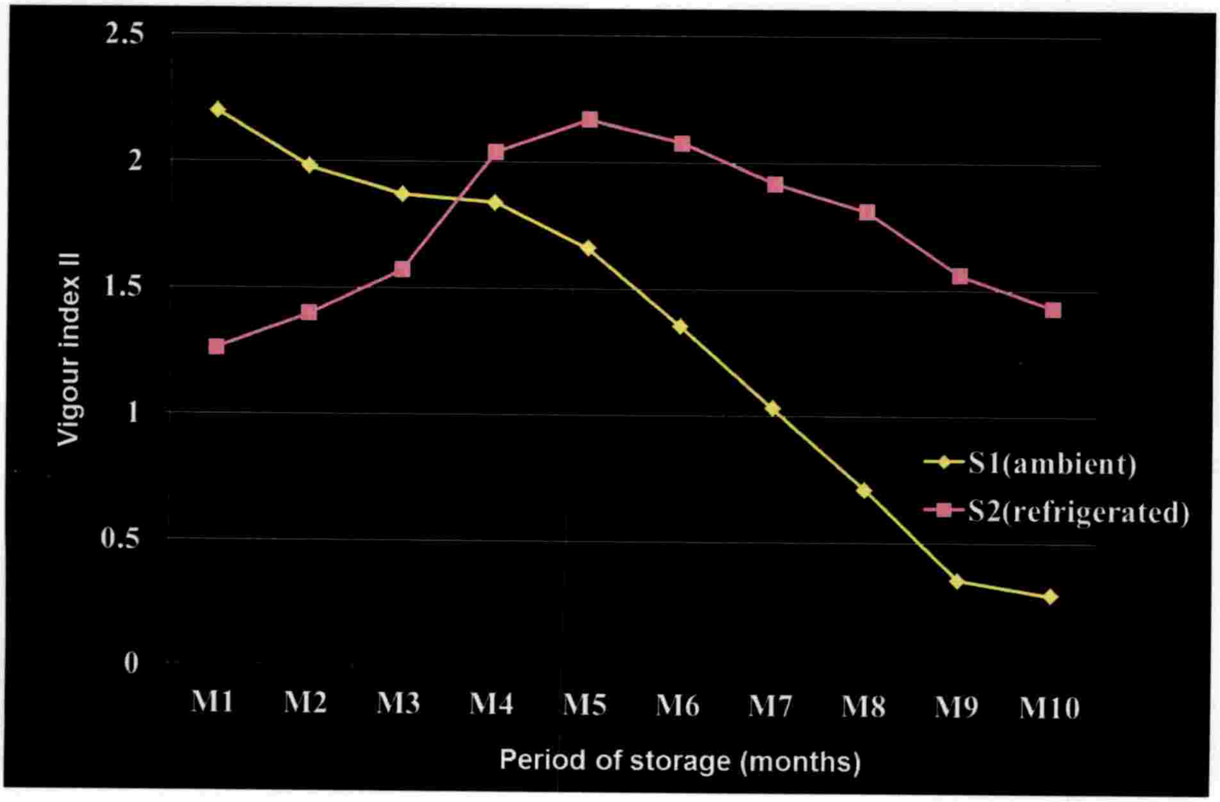

Table 19. Influence of storage condition and invigoration treatment on allometric index during storage in ash gourd

Details	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
	Storage condition (S)									
S_1	0.35^{a}	0.36^{a}	0.35^{a}	0.35^{a}	0.31	0.30	0.24	0.19^{b}	0.16	0.15^{b}
S_2	0.25^{b}	0.24^{b}	0.25^{b}	0.26^{b}	0.29	0.30	0.27	0.22^{a}	0.20	0.18^{a}
SEm ±	0.005	0.005	0.005	0.014	0.016	0.031	0.019	0.012	0.029	0.014
CD (0.05)	0.015	0.015	0.014	0.041	NS	NS	NS	0.035	NS	0.039
	Invigoration treatment (I)									
I_1	0.37^{a}	0.36^{a}	0.34^{a}	0.33	0.32	0.31	0.26	0.21	0.19	0.17
I_2	0.35^{ab}	0.34^{ab}	0.32^{ab}	0.33	0.33	0.31	0.23	0.22	0.18	0.15
I_3	0.25^{cd}	0.27^{c}	0.28^{bc}	0.28	0.33	0.27	0.24	0.19	0.19	0.15
I_4	0.30^{bc}	0.30^{bc}	0.25^{c}	0.28	0.25	0.27	0.23	0.22	0.18	0.16
I_5	0.29^{cd}	0.30^{bc}	0.32^{ab}	0.32	0.28	0.36	0.23	0.21	0.18	0.17
I_6	0.25^{cd}	0.26^{c}	0.27^{bc}	0.28	0.24	0.25	0.27	0.19	0.17	0.16
I_7	0.24^{d}	0.26^{c}	0.28^{bc}	0.29	0.32	0.28	0.30	0.19	0.18	0.18
SEm ±	0.01	0.01	0.009	0.027	0.03	0.058	0.035	0.023	0.055	0.036
CD (0.05)	0.028	0.028	0.026	NS	NS	NS	NS	NS	NS	NS

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Table 20. Interaction effect of storage condition and invigoration treatment on allometric index during storage in ash gourd

Treatments	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Interaction (S x I)										
S_1I_1	0.41^{a}	0.39^{a}	0.36^{ab}	0.33^{abcd}	0.31^{abc}	0.31	0.26	0.26	0.21	0.17
S_1I_2	0.38^{abc}	0.36^{ab}	0.33^{b}	0.33^{abcd}	0.31^{abc}	0.30	0.25	0.25	0.18	0.14
S_1I_3	0.26^{ef}	0.33^{bc}	0.33^{b}	0.31^{abcd}	0.30^{abc}	0.31	0.27	0.21	0.24	0.16
S_1I_4	0.41^{a}	0.41^{a}	0.34^{b}	0.40^{a}	0.31^{abc}	0.31	0.23	0.19	0.21	0.19
S_1I_5	0.39^{ab}	0.39^{a}	0.40^{a}	0.38^{ab}	0.30^{abc}	0.29	0.25	0.22	0.19	0.19
S_1I_6	0.28^{de}	0.33^{bc}	0.35^{ab}	0.36^{abc}	0.25^{abc}	0.26	0.23	0.20	0.20	0.19
S_1I_7	0.28^{de}	0.30^{c}	0.34^{b}	0.35^{abc}	0.38^{a}	0.30	0.22	0.20	0.19	0.20
S_2I_1	0.34^{bc}	0.33^{bc}	0.33^{b}	0.34^{abc}	0.34^{ab}	0.31	0.26	0.17	0.17	0.18
S_2I_2	0.33^{cd}	0.32^{bc}	0.32^{b}	0.33^{abcd}	0.36^{ab}	0.33	0.23	0.19	0.18	0.16
S_2I_3	0.25^{efg}	0.22^{d}	0.24^{c}	0.25^{cde}	0.37^{ab}	0.25	0.22	0.17	0.14	0.14
S_2I_4	0.20^{g}	0.18^{d}	0.17^{d}	0.18^{e}	0.20^{c}	0.24	0.24	0.25	0.16	0.13
S_2I_5	0.20^{g}	0.22^{d}	0.24^{c}	0.26^{bcde}	0.27^{abc}	0.44	0.22	0.21	0.17	0.15
S_2I_6	0.22^{fg}	0.20^{d}	0.20^{cd}	0.21^{de}	0.24^{bc}	0.25	0.31	0.18	0.15	0.14
S_2I_7	0.21^{fg}	0.22^{d}	0.24^{c}	0.24^{cde}	0.27^{abc}	0.27	0.39	0.19	0.18	0.16
SEm ±	**0.018**	0.018	0.0182	0.036	0.040	0.081	0.051	0.031	0.077	0.036
CD (0.05)	0.05	0.052	0.052	0.105	0.118	NS	NS	NS	NS	NS

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

4.1.3.9.1 Due to Storage condition (S)

The allometric index decreased from 0.35 (1 MAS) to 0.15 (10 MAS) under ambient storage, while under refrigerated storage it varied from 0.25 (1 MAS) to 0.18 (10 MAS). It was also observed that the index of ambient stored seeds declined over the period of storage, while that of refrigerated stored seeds increased initially up to 6 MAS and thereafter declined.

Seeds under ambient storage exhibited significant high allometric index than refrigerated stored seeds during the initial four months of storage. Henceforth, there was no significant difference between seeds under ambient storage and refrigerated storage except at 8 MAS and 10 MAS. At 10 MAS the index was 0.18 in refrigerated storage and 0.15 in ambient stored seeds.

Poor seedling establishment is a major deterrent in most vegetable crops. Allometric index is an indication of seedling field establishment. Higher the AI greater is the seedling establishment.

4.1.3.9.2 Due to Invigoration treatment (I)

Irrespective of the storage condition the seeds invigorated with I_1 ($CaCl_2$ 50mM 12h) was found to be significantly superior to all treatments upto 3 MAS. It was found to be on par with I_2 during 2 MAS and 3 MAS. No significant difference was observed between invigorated and untreated seeds from 4 MAS onwards.

Farooq *et al.* (2006) reported that seed invigoration improved germination emergence and allometry. They attributed faster and uniform emergence to improved α-amylase activity, which increased the level of soluble sugars in the invigorated kernels. The results suggest that physiological changes produced by osmohardening enhanced the starch hydrolysis and made more sugars available for embryo growth, vigorous seedling production and, later on, improved allometric, kernel yield and quality attributes. The results also indicated that seed invigoration could be an attractive approach to obtain better crop stand.

a) **Ambient Storage**



T_1- $CaCl_2$ (50Mm 12h) **T_7- Absolute control** **T_5- KH_2PO_4 (10^1 M 24h)**

b) Refrigerated storage



T_1- $CaCl_2$ (50Mm 12h) **T_7- Absolute control** **T_5- KH_2PO_4 (10^{-1} M 24h)**

Plate 1: Growth of seedling from seeds stored under ambient and refrigerated storage at 10 MAS

4.1.3.9.3 Due to Interaction (S x I)

The results indicated that seed invigoration and storage environment did not significantly influence allometric index from 6 MAS till the end of storage.Both invigorated and untreated seeds under ambient storage as well seeds under refrigerated storage after invigoration with $CaCl_2$ 50mM for 12h (S_2I_1), or for 24h (S_2I_2) exhibited significantly high AI over other treatments at 4 MAS. These treatments were found to be on par with S_2I_3 (Refrigerated - Kinetin 10ppm 12h), S_2I_5 (Refrigerated - KH_2P0_4 10^{-1}M 24h) and untreated seeds under refrigeration (S_2I_7) at 5 MAS. Hence, it was clear that seed invigoration with $CaCl_2$ 50mM was beneficial to sustain AI over storage.

Unlike the results of the present study, Rauf *et al.* (2007) reported existence of negative and no significant correlation between germination, shoot length and root length with root/shoot length ratio in wheat.

4.1.3.10 Electrical conductivity (EC) (μSm^{-1})

The results on electrical conductivity (μSm^{-1}) as influenced by storage condition, invigoration treatment and their interaction during the storage period are presented in Tables 21 and 22.

4.1.3.10.1 Due to Storage condition (S)

The electrical conductivity of seed leachate was observed to increase with increase in storage period irrespective of storage condition (Fig. 5). The electrical conductivity of seed leachate of seeds stored in S_2 (cold storage) was found to be least and significantly superior over S_1 (ambient) throughout storage. The electrical conductivity of seed leachate increased from 16.77 μSm^{-1} to 149.98 μSm^{-1} in S_1 (ambient condition) while, the increase was from 11.14 μSm^{-1} to 115.49 μSm^{-1} in S_2 (refrigerated storage).

The prolonged storage of seeds results in the deterioration of seed as a result of lipid peroxidation and it leads to the loss of membrane integrity and thereby increase in seed leachate. The electrical conductivity of the seed leachate as a measure of membrane integrity is a good index for seed viability and vigour. The increase in the electrical conductivity of seeds under ambient storage was more pronounced than in the seeds under controlled condition. Loss of membrane integrity during storage is the main reason for increased electrical conductivity (Delouche and Baskin, 1973). The rapid increase in electrical conductivity of seeds stored in ambient condition compared to those seeds in refrigerated condition is in good agreement with the results of Basavegowda *et al.* (2016) in pigeon pea, where the electrical conductivity of the seeds stored under cold condition was found to be significantly low compared to the seeds stored under ambient storage after 26 months of storage.

4.1.3.10.2 Due to Invigoration treatment (I)

Irrespective of storage conditions, seed invigoration with kinetin 10 ppm for 12h (I_3) was found to be the least throughout the storage period (Fig.6). The electrical conductivity of I_3 increased from 10.33 μSm^{-1} to 67.98 μSm^{-} at 1 MAS to 10 MAS respectively. I_3 (kinetin 10 ppm 12h) was also found to be on par with the invigoration treatments I_4 (kinetin 10 ppm 24h) and I_5 (KH_2PO_4 10^{-1}M 24h) between 1 MAS to 8 MAS. At the end of the storage period I_4 (90.05 μSm^{-1}) and untreated seeds (90.02 μSm^{-1}) registered low electrical conductivity next to I_3. The invigoration treatment I_1 ($CaCl_2$ 50mM 12h), I_4 (kinetin 10 ppm 24h) and untreated seeds (I_7) were on par with I_3, unto 6 MAS. The results indicated that seed deterioration was the least in seeds invigorated with kinetin 10 ppm for 12h. This may be due to improved membrane repair in treated seeds as reported by Rudrapal and Nakamura, (1988) in eggplant and radish. Similar report on the lower electrical conductivity was also reported by Nawaz *et al.* (2013) in tomato.

4.1.3.10.3 Due to Interaction (S x I)

The treatment S_2I_3 (Refrigerated - Kinetin 10ppm 12h) registered the least EC throughout the storage period. The electrical conductivity of seed leachate in S_2I_3 (Refrigerated - Kinetin 10ppm 12h) varied from 9.76 μSm^{-1}(1 MAS) to 51.60 μSm^{1} (10 MAS). S_2I_3 was on par with S_2I_1 (1 MAS to 7 MAS) and S_2I_2 (1 MAS to 6 MAS), S_21_6 (2 MAS to 4 MAS) and with S_2I_4 between 1 MAS to 8 MAS except at 5 MAS.

Table 21. Influence of storage condition and invigoration treatment on electrical conductivity (μSm^{-1}) during storage in ash gourd

Details	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Storage condition (S)										
S_1	16.77^{a}	26.35^{a}	37.63^{a}	45.77^{a}	53.15^{a}	65.71^{a}	78.64^{a}	104.54^{a}	131.96^{a}	149.98^{a}
S_2	11.14^{b}	13.65^{b}	17.64^{b}	21.14^{b}	21.60^{b}	31.93^{b}	60.91^{b}	80.94^{b}	97.13^{b}	115.49^{b}
SEm ±	0.78	0.77	0.91	1.60	1.69	1.40	1.97	2.05	1.50	2.33
CD (0.05)	2.27	2.26	2.65	4.6	4.93	4.09	5.74	5.98	4.37	6.79
Invigoration treatment (I)										
I_1	13.85^{ab}	18.00^{bc}	27.83^{bc}	30.51^{bc}	31.86^{c}	46.83^{bc}	69.83^{b}	93.75^{c}	134.1^{b}	156.0^{b}
I_2	12.53^{ab}	17.90^{bc}	30.38^{b}	34.88^{abc}	37.65^{bc}	49.30^{b}	70.53^{b}	97.54^{c}	122.9^{b}	149.4^{b}
I_3	10.33^{b}	13.94^{c}	19.67^{d}	24.10^{c}	26.75^{c}	36.48^{c}	40.25^{c}	56.85^{d}	62.87^{d}	67.98^{d}
I_4	14.66^{ab}	17.70^{bc}	20.90^{cd}	28.53^{bc}	34.43^{bc}	39.78^{bc}	48.47^{c}	67.77^{d}	81.86^{c}	90.05^{c}
I_5	18.02^{a}	33.10^{a}	41.58^{a}	47.33^{a}	51.91^{a}	63.15^{a}	108.00^{a}	156.20^{a}	165.60^{a}	185.80^{a}
I_6	16.59^{ab}	22.12^{b}	30.92^{b}	41.58^{ab}	46.42^{ab}	65.67^{a}	102.80^{a}	115.80^{b}	155.00^{a}	189.90^{a}
I_7	11.70^{ab}	17.26^{bc}	22.17^{cd}	27.22^{c}	32.60^{bc}	40.50^{bc}	48.54^{c}	61.31^{d}	79.43^{c}	90.02^{c}
SEm ±	2.06	2.05	2.41	4.25	4.48	3.71	5.21	5.43	3.97	6.17
CD(0.05)	5.93	5.95	6.99	12.32	12.99	10.77	15.11	15.75	11.50	17.88

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Table 22. Interaction effect of storage condition and invigoration treatment on electrical conductivity (μSm^{-1}) during storage in ash gourd

Treatments	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10
Interaction (S x I)										
S_1I_1	19.06^{ab}	23.67^{bc}	39.00^{b}	42.00^{cd}	51.33^{bc}	68.27^{b}	88.67^{cd}	107.6^{d}	162.6^{c}	179.3^{c}
S_1I_2	16.77^{abc}	23.47^{bc}	44.00^{b}	50.10^{bc}	52.30^{bc}	69.27^{b}	80.50^{d}	113.3^{cd}	139.4^{d}	170.5^{c}
S_1I_3	10.91^{cde}	16.54^{de}	24.33^{cd}	31.67^{def}	42.00^{cd}	54.33^{c}	44.00^{efgh}	66.90^{efgh}	75.13^{gh}	84.37^{g}
S_1I_4	18.40^{ab}	23.07^{bc}	22.47^{de}	34.00^{de}	42.67^{cd}	52.67^{c}	58.97^{ef}	76.53^{efg}	95.12^{ef}	107.0^{f}
S_1I_5	21.03^{a}	47.20^{a}	62.13^{a}	72.00^{a}	84.33^{a}	89.07^{a}	113.90^{a}	180.40^{a}	198.80^{a}	219.30^{a}
S_1I_6	17.23^{abc}	27.00^{b}	41.17^{b}	58.83^{b}	60.07^{b}	79.00^{ab}	110.30^{ab}	124.20^{bc}	179.30^{b}	200.50^{b}
S_1I_7	13.97^{bcde}	23.52^{bc}	30.33^{c}	31.77^{def}	39.33^{cde}	47.33^{cd}	54.05^{efg}	62.95^{fghi}	73.33^{h}	88.97^{fg}
S_2I_1	8.64^{e}	12.33^{e}	16.67^{def}	19.01^{fg}	12.38^{gh}	25.40^{ef}	51.00^{efgh}	79.93^{ef}	105.7^{e}	132.7^{e}
S_2I_2	8.30^{e}	12.33^{e}	16.77^{def}	19.67^{fg}	23.00^{fgh}	29.33^{ef}	60.57^{e}	81.83^{e}	106.4^{e}	128.3^{e}
S_2I_3	9.76^{de}	11.33^{e}	15.00^{ef}	16.54^{g}	11.49^{h}	18.62^{f}	36.50^{h}	46.80^{i}	50.60^{i}	51.60^{h}
S_2I_4	10.91^{cde}	12.33^{e}	19.33^{def}	23.07^{efg}	26.20^{efg}	26.90^{ef}	37.97^{gh}	59.00^{hi}	68.60^{h}	73.10^{g}
S_2I_5	15.00^{abcde}	19.00^{cd}	21.03^{def}	22.67^{efg}	19.49^{fgh}	37.23^{de}	102.00^{abc}	132.00^{b}	132.30^{d}	152.40^{d}
S_2I_6	15.95^{abcd}	17.23^{cde}	20.67^{def}	24.33^{efg}	32.77^{def}	52.33^{c}	95.27^{bcd}	107.30^{d}	130.70^{d}	179.30^{c}
S_2I_7	9.42^{de}	11.00^{e}	14.00^{f}	22.67^{efg}	25.87^{efg}	33.67^{e}	43.03^{fgh}	59.67^{ghi}	85.53^{fg}	91.07^{fg}
SEm ±	**2.06**	2.05	2.44	4.25	4.48	3.71	5.21	5.43	3.97	6.17
CD (0.05)	5.98	5.95	6.99	12.32	12.99	10.77	15.11	15.75	11.50	17.88

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Fig 5. Influence of Storage condition on electrical conductivity (μSm^{-1}) in ash gourd

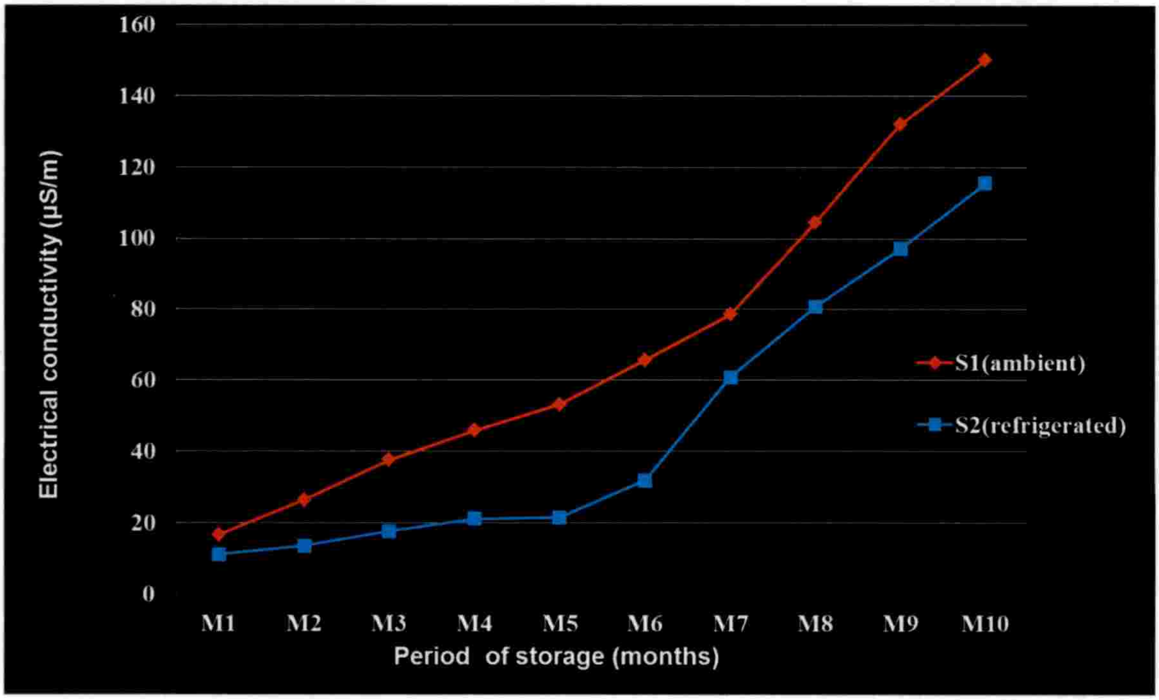

Fig 6. Influence of invigoration treatment on electrical conductivity (μSm^{-1}) in ash gourd

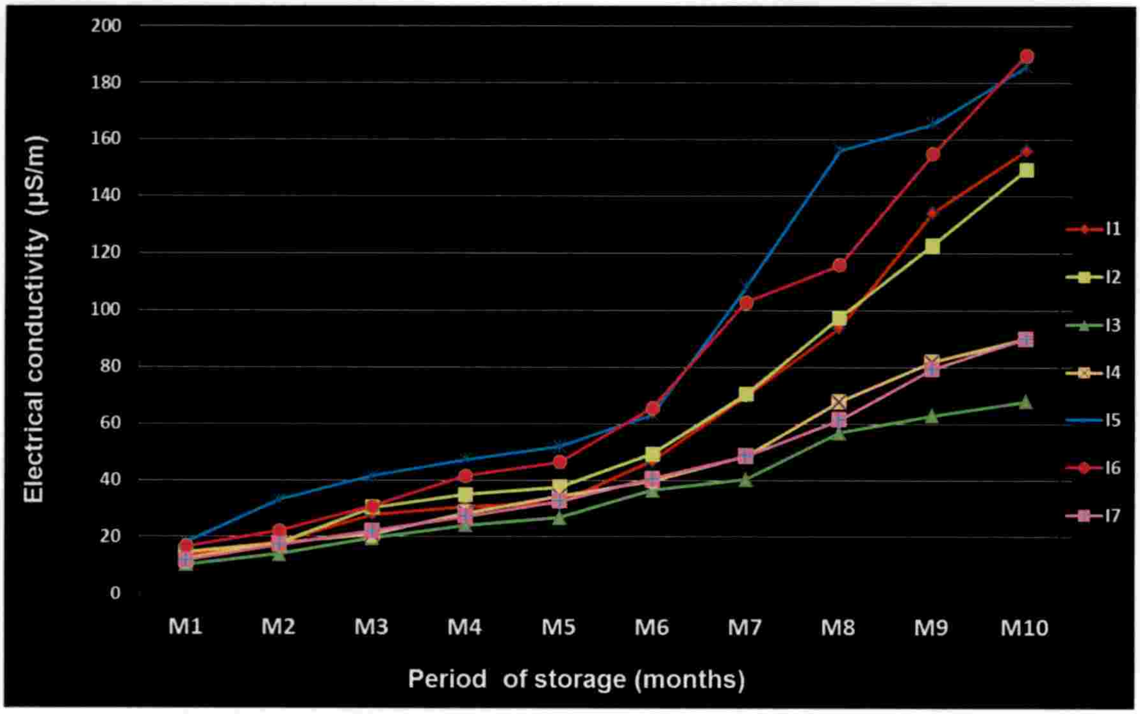

Table 23. Influence of storage condition and invigoration treatment on seed moisture content (%) during storage in ashgourd

Details	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Storage condition (S)										
S_1	7.00	7.01	7.02	7.03	7.03	7.04	7.07	7.07	7.09	7.12
S_2	7.00	7.01	7.02	7.03	7.03	7.05	7.06	7.08	7.09	7.13
SEm ±	0.001	0.002	0.004	0.004	0.003	0.003	0.004	0.003	0.002	0.003
CD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Invigoration treatment (I)										
I_1	7.00	7.01	7.02	7.03	7.04	7.04	7.06	7.07	7.09	7.11
I_2	7.00	7.00	7.02	7.03	7.02	7.04	7.06	7.08	7.09	7.12
I_3	7.00	7.01	7.01	7.02	7.03	7.05	7.07	7.07	7.09	7.12
I_4	7.00	7.01	7.03	7.03	7.04	7.05	7.06	7.08	7.09	7.13
I_5	7.00	7.00	7.03	7.03	7.03	7.05	7.07	7.08	7.09	7.13
I_6	7.00	7.01	7.01	7.04	7.03	7.05	7.06	7.08	7.09	7.13
I_7	7.00	7.01	7.01	7.02	7.03	7.04	7.08	7.07	7.09	7.12
SEm ±	0.004	0.004	0.007	0.007	0.006	0.006	0.008	0.006	0.004	0.006
CD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 24. Interaction effect of storage condition and invigoration treatment on seed moisture content (%) during storage in ash gourd

Treatments	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
	Interaction (S x I)									
S_1I_1	7.00	7.00	7.02	7.04	7.05	7.05	7.06	7.06	7.08	7.11
S_1I_2	7.01	7.00	7.02	7.03	7.02	7.03	7.07	7.08	7.09	7.12
S_1I_3	7.00	7.01	7.01	7.01	7.04	7.03	7.06	7.08	7.09	7.12
S_1I_4	7.01	7.01	7.04	7.03	7.04	7.05	7.06	7.08	7.09	7.13
S_1I_5	7.00	7.00	7.04	7.04	7.04	7.04	7.07	7.07	7.10	7.13
S_1I_6	7.00	7.01	7.01	7.02	7.03	7.06	7.07	7.07	7.09	7.12
S_1I_7	7.00	7.00	7.01	7.02	7.02	7.04	7.09	7.07	7.09	7.11
S_2I_1	7.00	7.02	7.01	7.03	7.02	7.03	7.06	7.08	7.09	7.12
S_2I_2	7.00	7.00	7.02	7.04	7.02	7.04	7.04	7.08	7.09	7.12
S_2I_3	7.00	7.00	7.00	7.03	7.02	7.06	7.08	7.07	7.09	7.11
S_2I_4	7.00	7.00	7.01	7.03	7.03	7.05	7.06	7.07	7.08	7.13
S_2I_5	7.00	7.00	7.01	7.03	7.03	7.05	7.08	7.08	7.08	7.14
S_2I_6	7.00	7.00	7.02	7.05	7.03	7.04	7.05	7.08	7.09	7.14
S_2I_7	7.00	7.01	7.02	7.02	7.03	7.04	7.07	7.07	7.09	7.12
SEm ±	0.002	0.006	0.01	0.009	0.008	0.009	0.011	0.008	0.005	0.008
CD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Similar reports of low EC in invigorated seeds has been reported by Camila *et al.* (2017) in beans and Arun *et al.* (2017) in cowpea. They attributed the reduced electrolyte leakage in invigorated seeds to enhanced repair mechanism following invigoration treatments.

4.1.3.11. Leakage of amino acid (µg leucine eqiv.ml^{-1})

The results on leakage of amino acid (µg leucine eqiv.ml^{-1}) as influenced by storage condition, invigoration treatment and their interaction during the storage period are presented in tables 23 and 24.

4.1.3.11.1 Due to Storage condition (S)

The amount of amino acid in seed leachate increased with increase in storage period irrespective of storage condition (Fig.7). The seeds stored under ambient condition recorded the highest amount of amino acid in seed leachate over the entire storage period compared to the seeds stored in cold condition. The amount of amino acid leached from the seeds stored under ambient condition varied from 7.14 µg leucine eqiv.ml^{-1} to 8.25 µg leucine eqiv.ml^{-1} while it varied from 7.17 µg leucine eqiv.ml^{-1} to 8.42 µg leucine eqiv.ml^{-1} in seeds stored under cold storage.

The increase in the amount of amino acid leached from the seed is not a desirable character as it is an indication of seed deterioration. Hence, it was evident that storing seeds under refrigerated condition was more advantageous in checking seed deterioration during storage.

The result is in conformation with the study of Ching and School (1968) in clover and perennial rye grass. The increase in seed leachate and free amino acid with an increase in temperature was observed in clover, however seed leachate and amino acid was high irrespective of storage condition in rye grass. Similar report on increased amino acid leakage was reported by Bhattacharya *et al.* (2015) in soybean. The release of exudation such as sugars and amino acids directly affects respiration and enzymatic activities and reduce the macromolecular synthesis.

(Arun *et al.,* 2017). Increased respiratory activity and the advancement in deterioration process results in the oxidation of amino acid which results in the decrease in protein content of the seed (Manonmani *et al.*, 2013; Sun and Leopold, 1995).

4.1.3.11.2 Due to Invigoration treatment (I)

As observed in case of electrical conductivity of seed leachate, the seeds treated with I_4 (kinetin 10 ppm 24h) recorded the low amount of amino acid leakage over the period of storage. The treatments I_4 (kinetin 10 ppm 24h) and I_3 (kinetin 10 ppm 12h) were on par with each other between 1 MAS to 9 MAS, the exception being at 5 MAS. The amount of amino acid leached from the seeds treated with I_4 (kinetin 10 ppm 24h) ranged from 7.17 μg leucine eqiv.ml^{-1} at 1 MAS to 8.10 μg leucine eqiv.ml^{-1} at 9 MAS. At the end of storage (10 MAS), I_3 was on par with I_4 and significantly superior to all other invigorated seeds as well as untreated seeds indicating their usefulness during seed storage. As observed in the present study, the reduced leakage of amino acid in invigorated seeds was also reported by Battacharya *et al.* (2015) in soybean. Rajjou *et al.* (2012) reported that the regulation of the synthesis and turnover of protein, post-translational modifications and translational activity reduction at the time of germination in dry seeds during storage resulted in the loss of seed vigour of stored seeds.

4.1.13.3 Due to Interaction (S x I)

In general, seeds stored under refrigerated conditions registered lower leakage of amino acid compared to those stored under ambient storage. The treatment S_2I_3 (Refrigerated - Kinetin 10ppm 12h) and S_2I_4 (Refrigerated - Kinetin 10ppm 24h) registered low leakage of amino acid in seed leachate throughout the storage period. S_2I_4 recorded the least value for amino acid leakage at the end of storage and was also significantly superior to all other treatments. The amount of leakage of amino acid in S_2I_4 ranged from 7.15 μg leucine eqiv.ml^{-1} (1 MAS) to 8.05 μg leucine eqiv.ml^{-1} (9 MAS). These treatments had also registered a low EC of seed leachate. The results indicated the existence of a direct correlation between leakage of amino acid in seed leachate and EC of seed leachate.

4.1.14 Lipid peroxidation (OD)

The results on lipid peroxidation (OD) as influenced by storage condition, invigoration treatment and their interaction effects during the storage period are presented in Tables 27 and 28.

4.1.14.1 Due to Storage condition (S)

Lipid peroxidation increased with increase in storage period irrespective of invigoration treatment. Throughout the storage period the seeds stored under the cold condition exhibited a low rate of lipid peroxidation ranging from an OD value of 0.038 to 0.921, while the seeds under ambient condition recorded a highest value of 0.44 to 2.22 irrespective of the invigoration treatment (Fig.8). Hence, it was evident that storing seeds under refrigerated condition was more advantageous in checking lipid peroxidation during storage. This is in confirmation with the findings of Sharma *et al.* (2006) in soybean. It was found that the lipid peroxidation which is expressed as malondialdehyde (MDA) content increased in the seeds stored either at ambient condition or in refrigerated conditions up to 180 days of storage. The rise in MDA was rapid in seeds stored at ambient conditions than in seeds stored in refrigerated condition (Sharma *et al.*, 2006).

According to Sathiyamoorthy and Nakamura (1995) degradation of lipids in deteriorating seeds releases free fatty acids which initiates oxidative deterioration processes by providing substrate for lipoxygenase. Membranes are primary targets of free radical attack and the study revealed that low-vigor embryos contained significantly higher levels of free radicals than high-vigor ones, while embryos from medium-vigor lots showed intermediate values.

Table 25. Influence of storage condition and invigoration treatment on Leakage of amino acid (μg leucine eqiv.ml^{1}) during storage in ash gourd

Details	Period of storage (months)				
	1 MAS	3 MAS	5 MAS	7 MAS	9 MAS
Storage condition (S)					
S_1	7.17^{a}	7.44^{a}	7.85^{a}	8.19^{a}	8.42^{a}
S_2	7.14^{b}	7.25^{b}	7.62^{b}	7.96^{b}	8.25^{b}
SEm ±	0.01	0.01	0.01	0.01	0.01
CD (0.05)	0.01	0.04	0.04	0.04	0.04
Invigoration treatment (I)					
I_1	7.18^{a}	7.36^{bc}	7.66^{bc}	7.88^{c}	8.22^{b}
I_2	7.15^{ab}	7.29^{cd}	7.71^{b}	8.17^{b}	8.50^{a}
I_3	7.12^{b}	7.23^{de}	7.57^{c}	7.74^{d}	8.07^{c}
I_4	7.17^{ab}	7.19^{e}	7.37^{d}	7.88^{c}	8.10^{c}
I_5	7.18^{a}	7.45^{ab}	7.73^{b}	8.24^{ab}	8.51^{a}
I_6	7.14^{ab}	7.52^{a}	8.05^{a}	8.31^{a}	8.47^{a}
I_7	7.12^{b}	7.39^{b}	8.06^{a}	8.32^{a}	8.50^{a}
SEm ±	0.01	0.02	0.02	0.02	0.03
CD (0.05)	0.03	0.07	0.07	0.07	0.07

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Table 26. Interaction effect of storage condition and invigoration treatment on leakage of amino acid (µg leucine eqiv.ml^{1}) during storage in ash gourd

Treatments	Period of storage (months)				
	1 MAS	3 MAS	5 MAS	7 MAS	9 MAS
	Interaction (S x I)				
S_1I_1	7.20^{ab}	7.49^{bc}	7.77^{def}	7.96^{f}	8.26^{ef}
S_1I_2	7.20^{ab}	7.40^{cd}	7.85^{bcd}	8.35^{bc}	8.44^{d}
S_1I_3	7.10^{d}	7.29^{ef}	7.70^{ef}	7.93^{f}	8.26^{ef}
S_1I_4	7.19^{abc}	7.19^{g}	7.45^{h}	8.02^{f}	8.15^{fg}
S_1I_5	7.22^{a}	7.53^{b}	7.78^{cde}	8.22^{de}	8.56^{bc}
S_1I_6	7.16^{bcd}	7.71^{a}	8.17^{a}	8.37^{b}	8.59^{ab}
S_1I_7	7.12^{d}	7.48^{bc}	8.25^{a}	8.48^{a}	8.69^{a}
S_2I_1	7.16^{bcd}	7.24^{fg}	7.55^{g}	7.81^{g}	8.17^{f}
S_2I_2	7.11^{d}	7.18^{g}	7.58^{g}	7.99^{f}	8.56^{bc}
S_2I_3	7.14^{bcd}	7.16^{g}	7.44^{h}	7.56^{h}	7.88^{h}
S_2I_4	7.15^{bcd}	7.19^{g}	7.29^{i}	7.75^{g}	8.05^{g}
S_2I_5	7.15^{bcd}	7.37^{de}	7.68^{f}	8.26^{cd}	8.46^{cd}
S_2I_6	7.13^{cd}	7.32^{def}	7.94^{b}	8.25^{d}	8.35^{de}
S_2I_7	7.12^{d}	7.31^{def}	7.87^{bc}	8.15^{e}	8.31^{e}
SEm ±	0.01	0.03	0.03	0.03	0.04
CD (0.05)	0.04	0.10	0.10	0.10	0.10

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

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4.1.14.2 Due to Invigoration treatment (I)

Irrespective of the storage condition the treatment I_1 ($CaCl_2$ 50mM 12h) showed the least rate of lipid peroxidation throughout the storage (0.17 at 1 MAS to 1.14 at 9 MAS). The treatments I_2 ($CaCl_2$ 50mM 24h) and I_3 (kinetin 10 ppm 12h) was found to be on par with I_1 ($CaCl_2$ 50mM 12h) at 3 MAS, 5 MAS and 7 MAS. Untreated seeds (I_7) exhibited a higher rate.

Similar results on the reduced lipid peroxidation rate in invigorated seeds were reported by Rudrapal and Basu (1981) in mustard, Vasantha (1995) in COPH1 hybrid pigeon pea, Hsu *et al.* (2003) in bitter gourd , Layek *et al.* (2012) in urd bean and Siri *et al.* (2013) in sweet pepper. According to Chauhan *et al.* (1984), leaching of toxic metabolites, germination advancement, antipathogenic impact, repair of biochemical lesions, quenching and counter activity of free radicals and prevention of lipid peroxidation could be the probable reasons for the reduced rate of deterioration of invigorated seeds during storage.

4.1.14.3 Due to Interaction (S x I)

In general, the seeds stored under refrigerated conditions registered lower rate of lipid peroxidation compared to those stored under ambient storage. The seeds invigorated with S_2I_1 (Refrigerated - $CaCl_2$ 50mM 12h) and S_2I_2 (Refrigerated - $CaCl_2$ 50mM 24h) registered low levels of lipid peroxidation throughout storage and were on par with each other.

The invigorated seeds under refrigeration except S_2I_4 (Refrigerated-Kinetin 10ppm 24h), S_2I_5 (Refrigerated - KH_2PO_4 10^{-1}M 24h) and S_2I_6 (Refrigerated - *Pf* $1x10^6$ cfu.ml^{-1} 12h) and were on par with each other with respect to lipid peroxidation throughout storage. However, lipid peroxidation in the untreated seeds was on par with these treatments only at 9 MAS. This indicated that invigoration the seeds before refrigeration was beneficial to reduce the rate of seed deterioration. Siri *et al.* (2013) reported that the antioxidant mechanism is activated by invigoration and these reduces the lipid peroxidation in seeds. Hsu *et al.* (2002) stated that in order to maintain high viability during long-term storage, the seeds receiving these pre-sowing treatments should be stored under favorable conditions. Krainart *et al.* (2015) attributed the reduction in total peroxide and malondialdehyde (a product of lipid oxidation and peroxidation) in cucumber to the effect of invigoration treatments.

4.1.3.13 Leakage of sugar (μg glucose eqiv.ml^{-1})

The results on leakage of sugar (μg glucose eqiv.ml^{-1}) as influenced by storage condition, invigoration treatment and their interaction effects during the storage period are presented in Table 12.

4.1.3.13.1 Due to Storage condition (S)

Leakage of sugar increased with increase in storage period irrespective of invigoration treatment. The findings of Arun *et al.* (2017) in cowpea proved that the total soluble sugars and protein in seed leachate was lower in fresh seeds (157.56 and 141.04 μgml^{-1} of seed leachate, respectively) and it was higher (253.5 and 253.2 μgml^{-1} of seed leachate, respectively) in low vigour accelerated aged seeds.

Throughout the storage period, the seeds stored under the cold condition exhibited the least leakage of sugar ranging from 1.04 μg glucose eqiv.ml^{-1} at 1 MAS to 1.68 μg glucose eqiv.ml^{-1} at 10 MAS, while the seeds under ambient condition recorded a higher value of 1.11 μg glucose eqiv.ml^{-1} to 2.66 μg glucose eqiv.ml^{-1} respectively at the start and end of storage period. Hence, it was evident that storing seeds under refrigerated condition was more advantageous in checking leakage of sugar during storage.

Similar to the findings of the present study, Hussain *et al.* (2015) reported an increase in seed leachate with an increase in storage temperature which was attributed to high deterioration rate of seed reserves during storage at high temperature.

According to Rajasree and Jirali (2017), an increase in temperature leads to high rate of lipid peroxidation and other biochemical changes in the seed under storage resulting in high seed leachate and ultimately the loss of seed quality parameters. This is in agreement with the findings of the present study.

4.1.3.13.2 Due to Invigoration treatment (I)

Irrespective of storage condition, seed treatment with I_1 ($CaCl_2$ 50mM 12h.), I_2 ($CaCl_2$ 50mM 24h.) and I_3 (kinetin 10 ppm 12h) registered low leakage of sugar. These were on par with each other at 9 MAS and also during most of the storage period. The untreated seeds registered significantly higher leakage of sugar throughout the storage period, indicating the beneficial effect of seed invigoration. The reduced amount of sugar leachate in invigorated seeds was also reported by Bhattacharya *et al.* (2015) in soybean.

4.1.3.13.3Due to Interaction (S x I)

In general, seeds stored under refrigerated conditions registered lower leakage of sugars compared to those stored under ambient storage. At 8 MAS and 9 MAS, treatment S_2I_3 (Refrigerated - kinetin 10 ppm 12h) recorded the least leakage of sugars (1.30 and 1.34 µg glucose eqiv.$ml^{-1}$of sugar leached from seed respectively) followed by the treatment S_2I_1 (1.58 µg glucose eqiv.ml^{-1}). This indicated that invigoration the seeds with kinetin 10 ppm for 12h and $CaCl_2$ 50mM for 12h (S_2I_1) before refrigeration was beneficial to reduce the amount of sugar leached from the seed.

The refrigerated storage of seeds irrespective of invigoration treatment has been proven to be best in controlling the seed microflora over a long period of storage. Hewett (1987) reported that the pathogen retained their viability for fourteen years in seed stored under deep freeze condition (-20^{0}C). The increase in seed infection over storage was also reported by Saxena and Karan (1991) in sesame and sunflower seeds and Kavitha (2007) in chilli, Shobha (2016) in ash gourd and Navya (2016) in chilli.

Table 27. Influence of storage condition and invigoration treatment on lipid peroxidation (OD) during storage in ash gourd

Details	Period of storage (months)				
	1 MAS	3 MAS	5 MAS	7 MAS	9 MAS
Storage condition (S)					
S1	0.446^{a}	0.537^{a}	1.204^{a}	1.427^{a}	2.225^{a}
S2	0.038^{b}	0.073^{b}	0.25^{b}	0.545^{b}	0.921^{b}
SEm ±	0.034	0.019	0.036	0.025	0.124
CD (0.05)	0.013	0.056	0.106	0.073	0.36
Invigoration treatment (I)					
I_1	0.17^{c}	0.29^{ab}	0.37^{b}	0.57^{c}	1.14
I_2	0.25^{ab}	0.29^{ab}	0.33^{b}	0.54^{c}	1.26
I_3	0.24^{ab}	0.30^{ab}	0.52^{b}	0.46^{c}	1.67
I_4	0.28^{a}	0.21^{b}	1.17^{a}	1.45^{a}	1.78
I_5	0.22^{bc}	0.29^{ab}	0.60^{b}	1.13^{b}	1.49
I_6	0.28^{a}	0.43^{a}	0.98^{a}	1.29^{ab}	1.73
I_7	0.26^{ab}	0.33^{ab}	1.13^{a}	1.47^{a}	1.95
SEm ±	0.017	0.036	0.068	0.047	0.231
CD (0.05)	0.006	0.104	0.198	0.136	NS

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Table 28. Interaction effect of storage condition and invigoration treatment on lipid peroxidation (OD) during storage in ash gourd

Treatments	Period of storage (months)				
	1 MAS	3 MAS	5 MAS	7 MAS	9 MAS
	Interaction (S x I)				
S_1I_1	0.31^{d}	0.49^{bc}	0.58^{ef}	0.75^{ef}	1.59^{bcde}
S_1I_2	0.44^{bc}	0.51^{b}	0.56^{ef}	0.85^{e}	1.95^{abc}
S_1I_3	0.45^{bc}	0.55^{b}	0.89^{cd}	0.69^{efg}	1.65^{bcde}
S_1I_4	0.52^{a}	0.35^{c}	2.06^{a}	2.39^{a}	2.84^{a}
S_1I_5	0.41^{c}	0.53^{b}	1.15^{c}	1.63^{c}	2.22^{ab}
S_1I_6	0.51^{a}	0.77^{a}	1.63^{b}	1.83^{b}	2.54^{ab}
S_1I_7	0.48^{ab}	0.56^{b}	1.56^{b}	1.86^{b}	2.78^{a}
S_2I_1	0.03^{e}	0.09^{d}	0.15^{g}	0.39^{hi}	0.69^{de}
S_2I_2	0.05^{e}	0.07^{d}	0.10^{g}	0.22^{i}	0.57^{e}
S_2I_3	0.02^{e}	0.06^{d}	0.15^{g}	0.24^{i}	1.68^{bcd}
S_2I_4	0.04^{e}	0.07^{d}	0.28^{fg}	0.50^{gh}	0.72^{de}
S_2I_5	0.03^{e}	0.05^{d}	0.04^{g}	0.62^{fg}	0.75^{de}
S_2I_6	0.05^{e}	0.09^{d}	0.33^{fg}	0.76^{ef}	0.92^{cde}
S_2I_7	0.04^{e}	0.09^{d}	0.70^{de}	1.08^{d}	1.12^{cde}
SEm ±	0.012	0.05	0.096	0.066	0.327
CD (0.05)	0.004	0.147	0.28	0.193	0.946

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Fig 7. Impact of storage environment on leakage of amino acid (µg leucine eqiv.ml^{-1}) in ash gourd

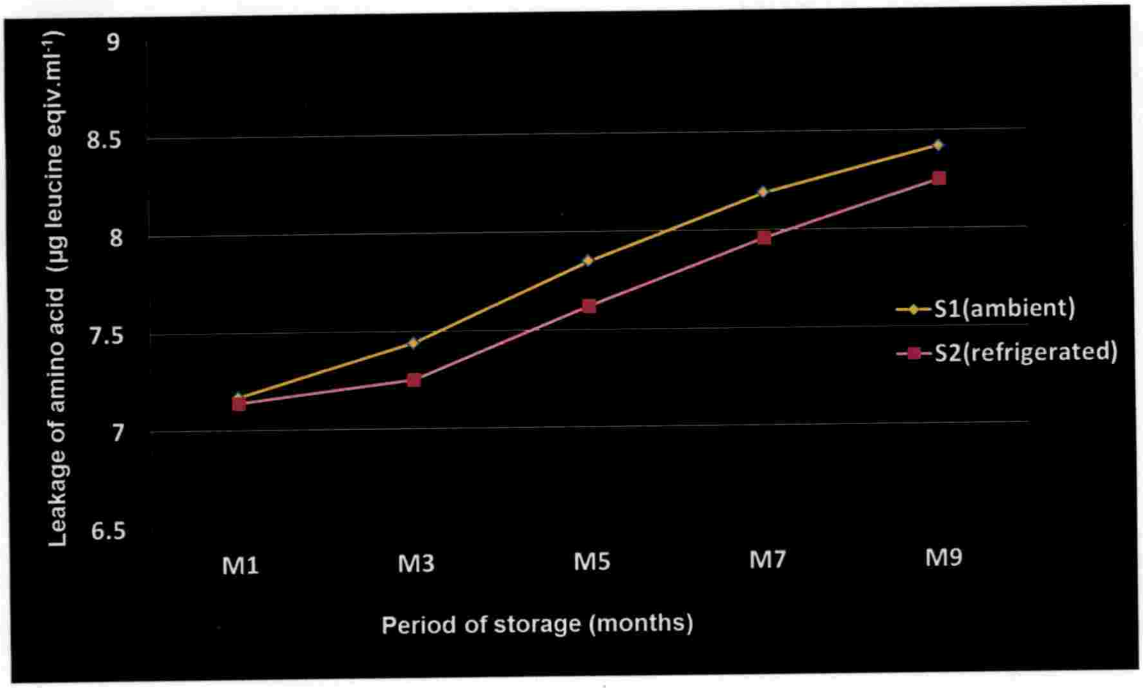
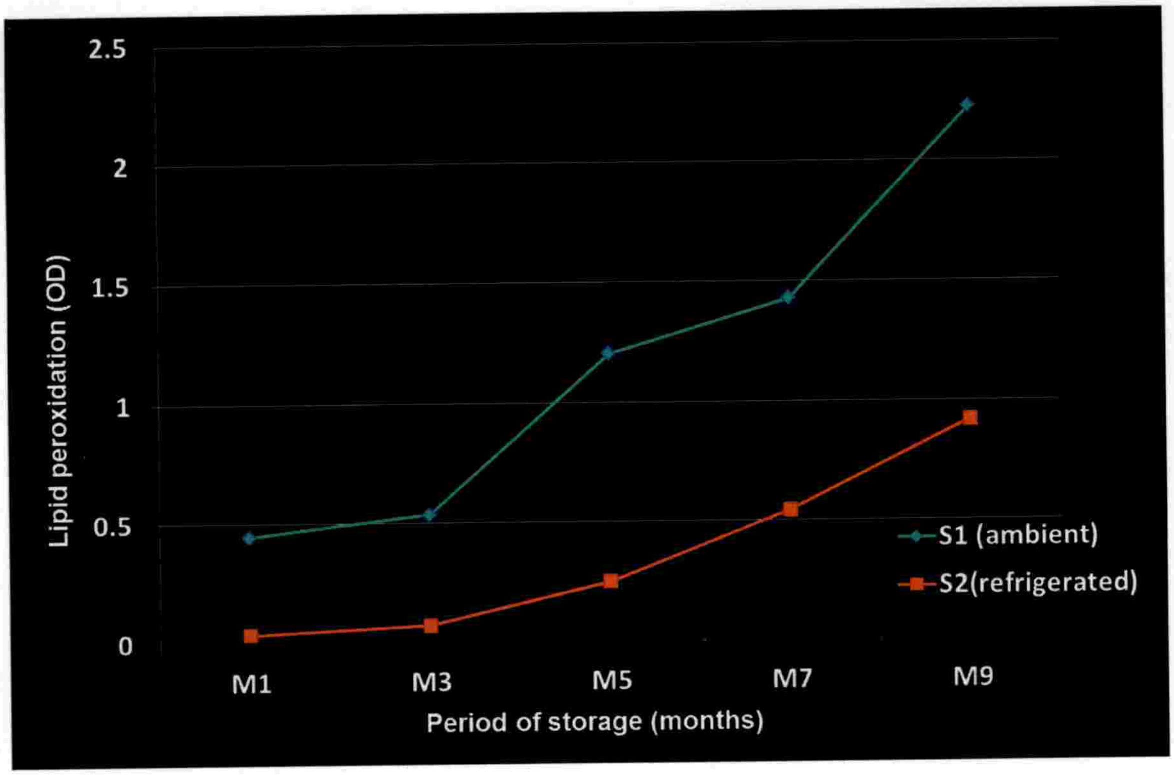


Fig 8. Influence of Storage condition on lipid peroxidation (OD) in ash gourd



4.1.3.14 Seed microflora (%)

The results on Seed micro flora (%) as influenced by storage condition, invigoration treatment and their interaction effects during the storage period are presented in Tables 31 and 32.

4.1.3.14.1 Due to Storage condition (S)

The per cent infection on the seed increased with increase in storage period irrespective of invigoration treatment. The per cent infection on the seed at the end of the storage (10 MAS) was high in seeds stored under ambient condition (41.43%) compared to the seeds under refrigerated storage (26.67 %) irrespective of the invigoration treatment (Fig.9). This is in accordance with the study of Bhattacharya and Subrata (2002). They reported a high incidence of *Aspergillus sp* in seeds of maize and groundnut stored under ambient condition.

The decreased incidence of seed microflora in cold condition (Plate 3 and 4) as reported in the present study is inconformity with the findings of Malaker *et al.* (2008) in wheat. They reported highest population of storage fungi in seeds stored in ambient condition unlike in those under refrigerated storage. The low temperature (10^{o} C) and unavailability of external moisture might have been the possible reason for the reduced activity of fungus.

The reduced microbial activity in seeds stored under refrigerated storage is in agreement with the results of Sumner and Lee (2009). They stated that the development of the fungus usually stops when temperatures are below 65^{0}F, and the moisture of the maize is below 12 per cent. Similar report on the high per cent of *Aspergillus sp* (Plate 5) in seeds stored in ambient storage condition as a result of a combination of heat and high humidity was also reported by Hell *et al.* (2010).

4.1.3.14.2 Due to Invigoration treatment (I)

The untreated seeds and seeds treated with KH_2PO_4 10^{-1} M for 24h (I_5) recorded significantly high infection throughout the storage period. Seeds invigorated with $CaCl_2$ 50mM 12h (I_1), $CaCl_2$ 50mM 24h (I_2) and *Pf* $1x10^6$

cfu.ml^{-1} for 12h (I_6) registered significantly less infection than the above treatments (Fig. 10). Treatment I_5 (KH_2PO_4 10^{-1} M 24h) recorded the highest seed microflora infection towards the end of storage period (9 MAS). The seed microflora infection in untreated seeds treatments (I_7), and seeds treated with kinetin 10 ppm for 12h (I_3) and kinetin 10 ppm for 24h (I_4) were on par with I_5.

4.1.3.14.3 Due to Interaction (S x I)

The treatment S_1I_5 (Ambient - KH_2PO_4 10^{-1}M 24h) registered significantly high per cent of seed infection over the storage period (10 % at 1 MAS to 96.7 % at 9 MAS) although untreated seeds (13.33%) under refrigeration had recorded high microbial infection at 1 MAS.

In general, seeds under ambient and refrigerated conditions treated with $CaCl_2$ 50mM for 12h under ambient storage (S_1I_1), as well as seeds treated with *Pf* 1x10^6 cfu.ml^{-1} for 12h (S_1I_6) and $CaCl_2$ 50mM for 24h under refrigeration (S_2I_2) and registered lower microflora infection compared to all other treatments including untreated seeds. As in the present study, the reduced rate of microflora infection in bio-primed seeds was also reported by Alemu and Alemu (2013) in faba bean. They observed that faba bean seeds bio-primed with isolates of *P. fluorescens* 9 (*Pf* 9) and *P. fluorescens* 10 (*Pf* 10) resulted in an inhibitory effects to the pathogen.

Table 29. Influence of storage condition and invigoration treatment on leaching of sugar (μg glucose eqiv.ml^{-1}) during storage in ash gourd

Details	Period of storage (months)				
	1 MAS	3 MAS	5 MAS	7 MAS	9 MAS
Storage condition (S)					
S_1	1.11^{a}	1.45^{a}	1.72^{a}	2.34^{a}	2.66^{a}
S_2	1.04^{b}	1.25^{b}	1.36^{b}	1.52^{b}	1.68^{b}
SEm ±	0.01	0.02	0.02	0.03	0.02
CD (0.05)	0.03	0.06	0.05	0.09	0.05
Invigoration treatment (I)					
I_1	1.02^{c}	1.10^{d}	1.56^{b}	1.78^{b}	1.98^{d}
I_2	0.97^{cd}	1.28^{bc}	1.43^{c}	1.98^{ab}	2.08^{cd}
I_3	0.97^{cd}	1.24^{cd}	1.36^{c}	1.83^{ab}	1.96^{d}
I_4	0.93^{d}	1.43^{b}	1.58^{ab}	2.06^{a}	2.27^{ab}
I_5	0.93^{d}	1.23^{cd}	1.56^{b}	1.89^{ab}	2.38^{a}
I_6	1.11^{b}	1.41^{b}	1.60^{ab}	1.89^{ab}	2.34^{a}
I_7	1.58^{a}	1.77^{a}	1.70^{a}	2.08^{a}	2.17^{bc}
SEm ±	0.02	0.04	0.03	0.06	0.03
CD (0.05)	0.06	0.10	0.09	0.17	0.10

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Table 30. Interaction effect of storage condition and invigoration treatment on leaching of sugar (µg glucose eqiv.ml^{-1}) during storage in ash gourd

Treatments	Period of storage (months)				
	1 MAS	3 MAS	5 MAS	7 MAS	9 MAS
			Interaction (S x I)		
S_1I_1	1.14^{c}	1.21^{ef}	1.74^{ab}	2.04^{bc}	2.38^{c}
S_1I_2	1.05^{d}	1.48^{cd}	1.63^{bc}	2.47^{a}	2.56^{b}
S_1I_3	0.98^{def}	1.34^{de}	1.59^{c}	2.35^{a}	2.59^{b}
S_1I_4	0.86^{h}	1.52^{c}	1.78^{a}	2.52^{a}	2.82^{a}
S_1I_5	1.01^{de}	1.43^{cd}	1.81^{a}	2.36^{a}	2.84^{a}
S_1I_6	0.93^{efgh}	1.43^{cd}	1.83^{a}	2.35^{a}	2.84^{a}
S_1I_7	1.79^{a}	1.69^{b}	1.70^{abc}	2.26^{ab}	2.59^{b}
S_2I_1	0.90^{fgh}	0.98^{g}	1.37^{d}	1.51^{de}	1.58^{f}
S_2I_2	0.89^{gh}	1.07^{fg}	1.24^{de}	1.49^{de}	1.60^{f}
S_2I_3	0.95^{efg}	1.14^{fg}	1.14^{e}	1.30^{e}	1.34^{g}
S_2I_4	1.00^{de}	1.33^{de}	1.37^{d}	1.59^{d}	1.72^{ef}
S_2I_5	0.85^{h}	1.03^{g}	1.31^{d}	1.43^{de}	1.92^{d}
S_2I_6	1.30^{b}	1.38^{cd}	1.36^{d}	1.43^{de}	1.83^{de}
S_2I_7	1.37^{b}	1.84^{a}	1.70^{abc}	1.90^{c}	1.75^{e}
SEm ±	0.03	0.05	0.04	0.08	0.05
CD (0.05)	0.08	0.15	0.12	0.24	0.14

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Table 31. Influence of storage condition and invigoration treatment on seed microflora during storage in ash gourd

Details	Period of storage (months)				
	1 MAS	3 MAS	5 MAS	7 MAS	9 MAS
Storage condition (S)					
S1	1.90	4.76	14.76	28.10^{a}	41.43^{a}
S2	1.90	4.76	10.00	17.69^{b}	26.67^{b}
SEm ±	0.476	1.992	2.451	2.897	4.179
CD (0.05)	NS	NS	NS	8.434	12.167
Invigoration treatment (I)					
I_1	0.00^{b}	6.67	10.00	13.33^{b}	16.67^{b}
I_2	0.00^{b}	0.00	6.670	11.67^{b}	13.33^{b}
I_3	1.67^{b}	5.00	15.00	23.33^{ab}	36.67^{ab}
I_4	0.00^{b}	0.00	10.00	16.67^{b}	43.33^{ab}
I_5	5.00^{a}	8.22	23.33	43.33^{a}	58.33^{a}
I_6	0.00^{b}	0.00	3.330	13.33^{b}	16.67^{b}
I_7	6.67^{a}	11.67	16.67	33.33^{ab}	46.67^{ab}
SEm ±	0.891	3.727	4.586	5.419	7.817
CD (0.05)	3.64	NS	NS	22.20	28.74

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Table 32. Influence of storage condition and invigoration treatment on seed microflora during storage in ash gourd

Treatments	Period of storage (months)				
	1 MAS	3 MAS	5 MAS	7 MAS	9 MAS
	Interaction (S x I)				
S_1I_1	0.00^{b}	0.00^{b}	3.33^{b}	6.67^{c}	10.00^{c}
S_1I_2	0.00^{b}	0.00^{b}	10.00^{b}	13.33^{c}	16.67^{bc}
S_1I_3	3.33^{b}	6.67^{ab}	23.33^{b}	26.67^{bc}	46.67^{b}
S_1I_4	0.00^{b}	0.00^{b}	6.67^{b}	20.00^{bc}	43.33^{b}
S_1I_5	10.00^{a}	16.67^{a}	43.33^{a}	63.33^{a}	96.67^{a}
S_1I_6	0.00^{b}	0.00^{b}	6.67^{b}	26.67^{bc}	30.00^{bc}
S_1I_7	0.00^{b}	10.00^{ab}	10.00^{b}	40.00^{b}	46.67^{b}
S_2I_1	0.00^{b}	13.33^{ab}	16.67^{b}	20.00^{bc}	23.33^{bc}
S_2I_2	0.00^{b}	0.00^{b}	3.33^{b}	10.00^{c}	10.00^{c}
S_2I_3	0.00^{b}	3.33^{ab}	6.67^{b}	20.00^{bc}	26.67^{bc}
S_2I_4	0.00^{b}	0.00^{b}	13.33^{b}	13.33^{c}	20.00^{bc}
S_2I_5	0.00^{b}	0.00^{b}	3.33^{b}	23.33^{bc}	43.33^{b}
S_2I_6	0.00^{b}	3.33^{ab}	3.33^{b}	10.0^{c}	16.67^{bc}
S_2I_7	13.33^{a}	13.33^{ab}	23.33^{b}	26.67^{bc}	46.67^{b}
SEm ±	1.26	5.27	6.486	7.664	11.055
CD (0.05)	3.64	14.13	18.43	22.20	28.74

*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Fig 9. Influence of Storage condition on seed microflora (%) in ash gourd

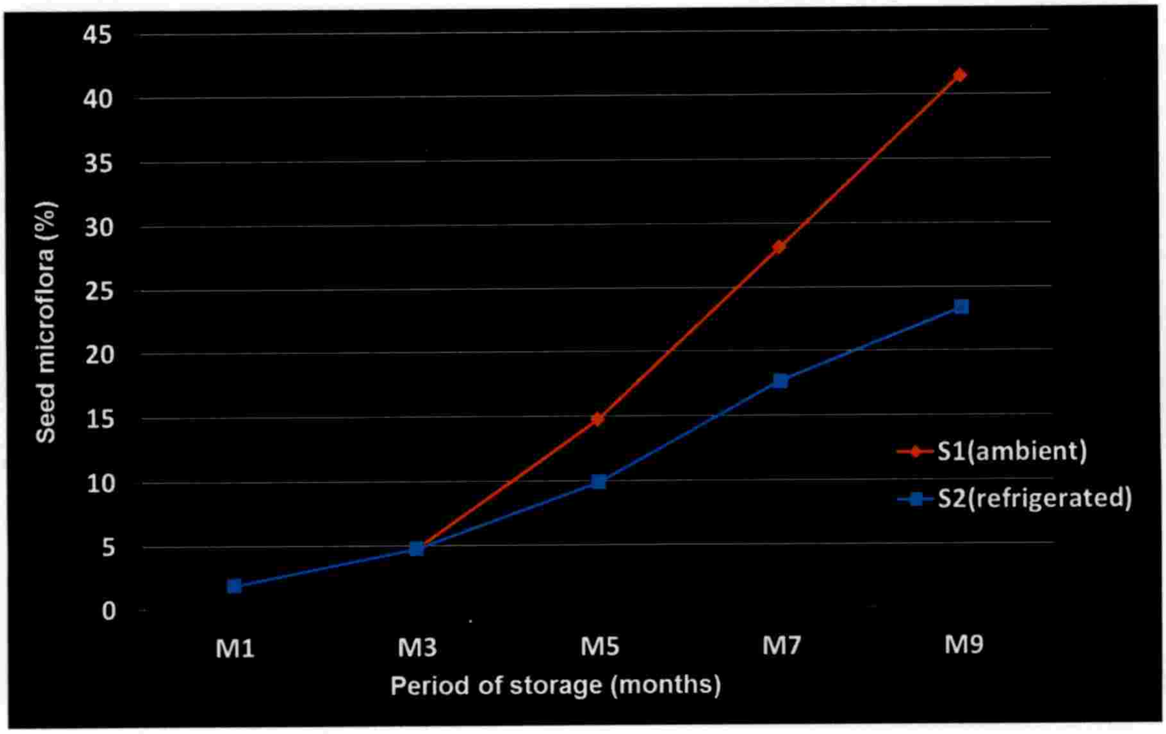


Fig 10. Influence of invigoration treatment on seed microflora (%) in ash gourd

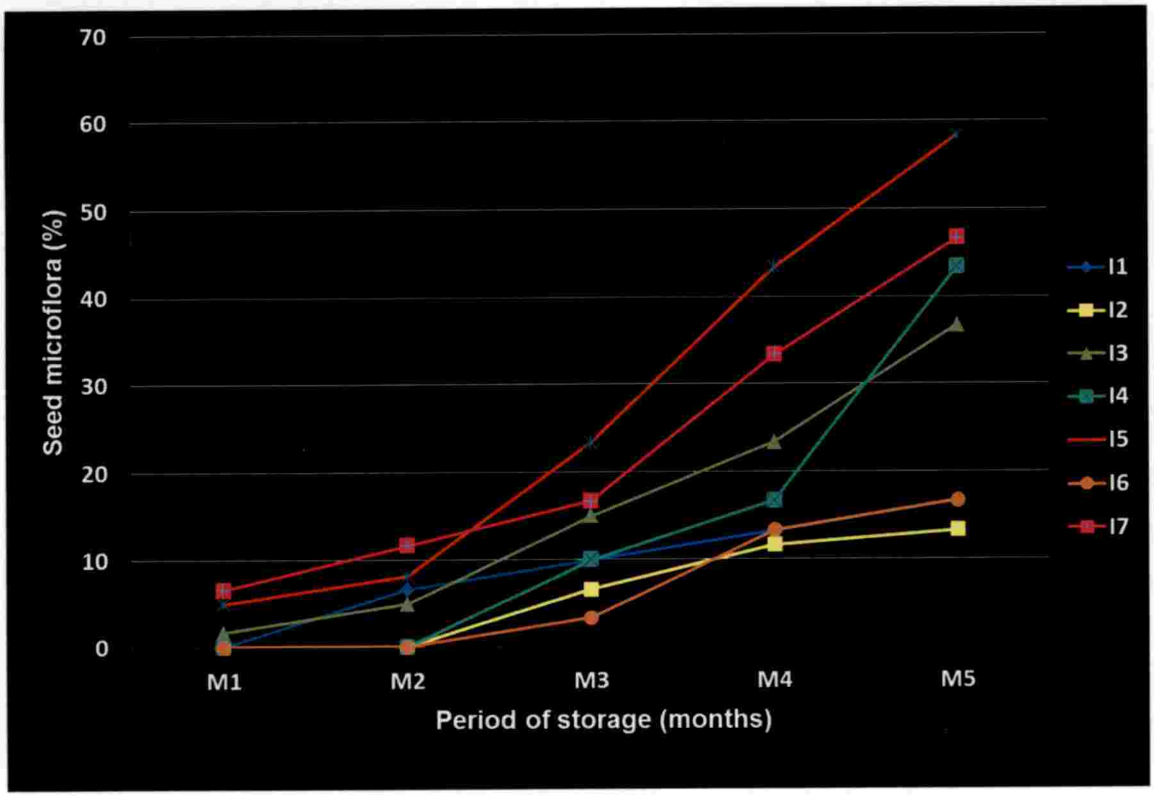
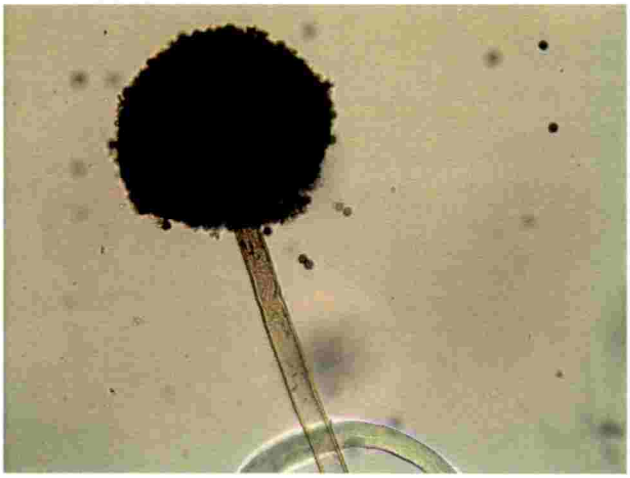


Plate 2. Seed microflora infection of seeds under ambient storage at 9 MAS

Plate 3. Seed microflora infection of seeds under refrigerated storage at 9 MAS



Aspergillus sp

Plate 4. Seed microflora found on seeds of ash gourd during storage

To summarise, it was clearly evident that refrigerated storage (S_1) of seeds conferred a clear advantage over ambient storage (S_2). The germination in S1 was retained above MSCS of 60 per cent up to 5 MAS whereas in S2 it was retained above MSCS for 13 MAS. Refrigerated storage not only prolonged seed longevity but also reduced the mean germination time and time to 50 per cent germination as the storage time increased. Seeds stored under refrigeration exhibited higher energy of germination, germination index, coefficient of velocity, vigour index I and II and allometric index as storage duration prolonged. Low electrical conductivity of seed leachate, leakage of amino acid, lipid peroxidation, leakage of sugars in seed leachate and seed microflora was evident in seeds stored under refrigeration. Considering the impact of storage environment on seed quality discussed above, it can be concluded that refrigerated storage is the best to prolong seed longevity as well as maintain seed quality.

Seed invigoration induced early germination. Irrespective of the storage condition, the invigorated seeds exhibited significantly higher germination than the untreated control up to 5 MAS. Seeds invigorated with $CaCl_2$ 50mM for12h (I_1) and $CaCl_2$ 50mM for 24h (I_2) not only registered high germination throughout the storage period but also exhibited higher magnitude of energy of germination, germination index, coefficient of velocity, vigour index I and II and allometric index. In addition, the mean germination time, time to 50 per cent germination, electrical conductivity of seed leachate, leakage of amino acid, lipid peroxidation, leakage of sugars in seed leachate and seed microflora were also invariably low in these treatments throughout the storage period unlike in untreated seeds. Thus, although the untreated seeds retained viability for 9 MAS against 8 MAS in seeds invigorated with $CaCl_2$ 50mM for 12h (I_1) and $CaCl_2$ 50mM for 24h (I_2) treated seeds, owing to the significant superiority of seeds invigorated with I_1 and I_2 with respect to germination in the initial storage period (4 MAS) coupled with higher magnitude of seed quality, invigoration with $CaCl_2$ 50mM can be advocated.

Focussing on the interaction between storage condition and invigoration treatment on germination and other seed indices, it can be concluded that, seed invigoration followed by refrigerated storage is advantageous. If only ambient storage condition is feasible, it would be advantageous to invigorate the seeds with $CaCl_2$ 50mM for 12h (S_1I_1) as viability above MSCS was found to be retained for 8 MAS compared to untreated seeds (7 MAS). If provision for refrigerated storage is available, bio-priming with *Pf* $1x10^{-6}$ $cfu.ml^{-1}$ for 12h (S_2I_6) followed by invigoration with $CaCl_2$ 50mM for 24h (S_2I_2), Kinetin 10 ppm for 12h (S_2I_3), Kinetin 10 ppm for 24h (S_2I_4) and KH_2PO_4 10^{-1} M for 24h (S_2I_5) would be most advantageous. These treatments helped retain viability above MSCS for 13 MAS while it was retained for 12 MAS on invigoration with $CaCl_2$ 50mM for 12h (I_1). However, considering the high germination per cent, energy of germination, germination index, coefficient of velocity, vigour index I and II and allometric index and lower mean germination time, time to 50 per cent germination, electrical conductivity of seed leachate, leakage of amino acid, lipid peroxidation, leakage of sugars in seed leachate and seed microflora throughout the storage period seed invigoration with either $CaCl_2$ 50mM for 12h (I_1) or $CaCl_2$ 50mM for 24h (I_2) would be most advantageous for storage of ash gourd seeds both under ambient and refrigerated storage.

4.2 Impact of thawing on seed viability

4.2.1 Analysis of variance

Results indicated existence of wide variability in the impact of storage conditions, invigoration and their interaction on seed viability when the seed stored under refrigerated condition undergoes thawing.

4.2.2 Germination (%)

The results on germination (%) as influenced by storage condition, invigoration treatment and their interaction effects during the period of thawing are presented in Table 14 and Plate 6.

4.2.2.1 Due to thawing period (Th)

Irrespective of the invigoration treatment, the seeds taken out of the refrigerated condition after a short storage period *i.e.,* in the initial period of cold storage (1 MAS and 2 MAS), was subjected to thawing, exhibited an increase in germination after 1 to 2 months of thawing (Th_1 and Th_2). Germination of seeds stored under refrigeration (S_2) at 1 MAS and 2 MAS was 70.12 per cent and 74.11 per cent respectively. The germination of the same lot at Th_1, Th_2 and Th_3 was 87.11 per cent, 78.65 per cent and 64.51 respectively. In case of seeds retrieved after 2 MAS under cold storage, the germination at Th_1, Th_2 and Th_3 was 78.65 per cent, 68.26 per cent and 50.64 per cent respectively, while its initial germination immediately after retrival fromcold storage (*i.e,* at 2 MAS) was 74.11 per cent.

However, as the seeds under refrigeration aged (*i.e.,* from 3 MAS to 10 MAS), germination decreased irrespective of the period of thawing. Germination of seeds stored under refrigeration (S_2) at 3 MAS and 10 MAS was 77.52 per cent and 75.79 per cent respectively while the corresponding value at Th_1 was 65.87 per cent and 69.90 per cent respectively.

When subjected to thawing, no seed retrieved from the refrigerated storage except in case of those retrived at 1 MAS, 4 MAS and 7 MAS retained viability above MSCS at Th_3. The corresponding germination of the lots at Th_3 was 64.51 per cent (1 MAS), 71.35 per cent (2 MAS) and 70.24 (3 MAS) per cent respectively. The longevity of seeds on thawing with respect to seeds retrieved from cold storage at 5 MAS and 10 MAS was the least *i.e.,* for Th_1 only (69.06% and 69.90% respectively). In all other instances *i.e.,* at 2 MAS, 3 MAS, 6 MAS, 8 MAS and 9 MAS seed viability was retained above MSCS for two months.

Considering the above, it was evident that the seeds retrieved from refrigerated storage up to 10 MAS would retain viability above MSCS for a minimum period of one month. Further retention of viability above a period of one month of thawing was subjective. The retention of seed longevity varied in both invigorated and untreated seeds when exposed to further thawing. The variation in ambient storage environment that prevailed during the period of thawing may have influenced the seed viability during the thawing period.

It was also evident that none of the treatments could help retain seed viability for five months after retrieval from refrigeration. The increased velocity of some enzyme and hydraulic reactions, which accelerates the deterioration process was well pronounced at high temperature (Oskouei, B. and Sheidaie, S., 2013). According to Agha *et al.* (2004) the increased temperature caused increase in respiration thus affecting seed vigour. Increase in lipid peroxidation in stored seed enhanced the seed deterioration. According to Limbird (2017), a reduced viability was exhibited by the seeds of the exotic *Euonymus* during a freeze following a warm spell. The swelling and seed coat splitting exposing the embryo in these thawed seeds. Sharma *et al.* (2006) observed decreased activities of catalase and peroxidase was observed in soybean, seeds stored in refrigerated condition. They observed rapid deterioration due to high lipid peroxidation in seeds under ambient condition.

The reduced viability of seeds under increasing temperature was also reported by Buitink *et al.* (2000). It was reported that high viscosity and low molecular mobility of the cytoplasm of the seeds stored under refrigerated condition could prevent or inhibit many deleterious processes while at increasing temperature, the low viscosity and enhanced molecular mobility would permit certain deteriorative reactions to proceed rapidly resulting in the reduced longevity of seeds under high temperature storage.

4.2.2.2 Due to Invigoration treatment (I)

Irrespective of the thawing period and the duration of storage under refrigeration, germination in seeds invigorated with I_1 ($CaCl_2$ 50mM 12h) was found to be on par with that of untreated seeds (Plate 6) except at 9 MAS. The seeds invigorated with $CaCl_2$ 50mM for 12h (I_1) and retrieved from cold storage at 9 MAS recorded significantly higher germination (74.69%) compared to untreated seeds (64.61%). Although the germination of both invigorated and untreated seeds did not show consistency in retention of viability above MSCS after the period of cold storage, it was observed that irrespective of period of thawing when seeds were retrieved from refrigerated storage at monthly intervals (upto 10 MAS), the germination in seeds treated with $CaCl_2$ 50mM for 12h was retained above MSCS for eight out of 10 months in comparison with untreated seeds that retained viability above MSCS for seven out of 10 months only (Fig.11). Hence, it can be concluded that invigoration with $CaCl_2$ 50mM for 12h (I_1) is beneficial in instances when it is anticipated that seeds stored under refrigeration needs to be retrieved and stored under ambient storage before sowing.

According to Tabrizian and Osareh (2007) the essential role of calcium in cell division, membrane functions, the activation of protein kinases and calmodulin-mediated processes might be the reason for enhanced germination and emergence in marigold seeds treated with $CaCl_2$. Similar results on the positive effect of Ca^{+2} on cell membranes and thereby decreasing the leakage of solute in $CaCl_2$ treated seeds than other invigoration treatments was proved by Shannon and Francois (1977) in cotton.

4.2.2.3 Due to Interaction (Th x I)

As observed earlier, the germination of both invigorated and untreated seeds retrieved from cold storage one month of storage (1 MAS) under refrigeration increased after one month of thawing (Th_1). One month after retrieval from refrigerated storage (Th_1) the germination per cent in seeds invigorated with I_1 ($CaCl_2$ 50mM 12h), I_2 ($CaCl_2$ 50mM 24h), I_5 (KH_2PO_4 10^{-1} M 24h) and I_6 (*Pf* $1x10^6$ $cfu.ml^{-1}$ 12h), was 95.00 (Th_1I_1), 92.23 (Th_1I_2), 94.90 (Th_1I_5), 96.67 (Th_1I_6) respectively. Similarly, two months after retrieval from refrigerated storage (Th_2), the germination per cent in seeds invigorated with I_1 ($CaCl_2$ 50mM 12h), I_5 (KH_2PO_4 10^{-1} M 24h) and I_6 (*Pf* $1x10^6$ $cfu.ml^{-1}$ 12h), was 92.20% (Th_2I_1),89.43 (Th_2I_5), 90.00 (Th_2I_6) and 93.00 (Th_2I_7) respectively. The germination in the above instances was high and significantly superior to all treatments exposed to thawing during the corresponding period. The germination of the seeds retrieved 1 MAS was retained above MSCS even after three months of thawing (Th_3) when invigorated with $CaCl_2$ 50mM for 12h (Th_3I_1: 63.37%), $CaCl_2$ 50mM for 24h (Th_3I_2: 64.33%), Kinetin 10 ppm for 12 h (Th_3I_3: 72.23%), KH_2PO_4 10^{-1} M for 24h (Th_3 I_5: 60.53%), *Pf* 1x106 cfu.ml-1 for 12 h (Th_3I_6: 65.00%) as well as in untreated seeds (Th_3I_7: 81.10%).

It was observed that when the seeds were retrieved from refrigeration at varying intervals (*i.e.,* 1 to 10 MAS) and subjected to thawing for a period of one month (Th_1), the seeds invigorated with $CaCl_2$ 50mM for 12h (Th_1I_1), $CaCl_2$ 50mM 24h (Th_1I_2), *Pf* 1 x 10^{-6} cfu.ml^{-1} for 12h (Th_1I_6) and untreated seeds (Th_1I_7), had retained viability above MSCS.

When retrieved from refrigeration at varying intervals (*i.e.,* 1 to 10 MAS), and subjected to thawing under ambient storage for a period of two months (*i.e.,* at Th_2), the seeds invigorated with $CaCl_2$ 50mM for 12h (Th_2I_1) had retained viability above MSCS throughout except when retrieved at 2 MAS. At Th_2, in seeds invigorated with $CaCl_2$ 50mM for 24h (Th_2I_2), the germination fell below MSCS when retrived from refrigeration at 5 MAS. However, in case of seeds invigorated with *Pf* 1x10^6 cfu.ml^{-1} for 12h (Th_2I_6) and untreated seeds (Th_2I_7), germination fell below MSCS when retrived from refrigeration at 5 MAS as well as 10 MAS.

After three months of ambient storage on retrieval from refrigeration (*i.e.,* at Th_3) the germination in seeds invigorated with $CaCl_2$ 50mM for 12h (Th_3I_1) fell below MSCS in seeds retrieved at 2 MAS, 5 MAS and 10 MAS. Unlike in I_1, in seeds invigorated with $CaCl_2$ 50mM for 24h (I_2) germination fell below MSCS when retrieved at 2 MAS, 5 MAS, 8 MAS and 10 MAS while in seeds invigorated with *Pf* 1x10^6 cfu.ml^{-1} for 12h (I_6), it fell below MSCS when the seeds were retrieved at 2 MAS, 3 MAS, 4 MAS, 5 MAS, 9 MAS and 10 MAS. In untreated seeds (I_7) at Th_3, germination fell below MSCS when retrieved at 2 MAS, 5MAS and 10 MAS.

When exposed to thawing for a period of four months (Th_4) *i.e.,* four months after retrieval from refrigeration, the seeds invigorated with $CaCl_2$ 50mM for 12h (Th_4I_1) retained viability above MSCS only with respect to seeds retrieved from cold storage at 4 MAS, 6 MAS and 8 MAS. In seeds invigorated with *Pf* 1x10^6 cfu.ml^{-1} for 12h (I_6), germination was above MSCS when retrievd from cold storage at 4 MAS and 6 MAS while in untreated seeds (I_7) it was retained above MSCS when retrievd from cold storage at 1 MAS, 4 MAS, 6 MAS and 8 MAS. It was obbserved that when seeds where exposed to thawing for a period of five months (Th_5), both invigorated and untreated seeds did not retain viability above MSCS.

Table 33. Influence of thawing period and invigoration treatment on germination (%) in ash gourd

Period of thawing	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Thawing period(Th)										
Th_0	70.12^b (57.04)	74.11^a (59.63)	77.52^a (61.89)	80.56^a (64.44)	87.29^a (70.78)	85.63^a (68.8)	86.59^a (69.11)	83.26^a (66.15)	79.28^a (63.04)	75.79^a (60.78)
Th_1	87.11^a (70.89)	78.65^a (63.19)	65.87^b (54.82)	80.87^a (65.10)	69.06^b (57.08)	80.23^a (64.35)	80.23^{ab} (63.75)	72.77^b (58.83)	73.84^a (59.78)	69.90^a (57.20)
Th_2	84.68^a (68.38)	68.26^b (56.32)	60.72^c (51.31)	77.87^{ab} (62.58)	56.19^c (48.70)	69.44^b (56.85)	75.63^{bc} (60.54)	67.46^b (55.48)	62.75^b (52.64)	56.74^b (48.95)
Th_3	64.51^b (53.68)	50.64^c (45.34)	52.82^d (46.59)	71.35^b (57.88)	44.63^d (41.84)	59.92^c (50.85)	70.24^c (57.18)	58.41^c (49.99)	57.95^b (49.69)	46.13^b (42.63)
Th_4	47.69^c (43.53)	43.49^d (41.16)	32.23^e (34.35)	56.12^c (48.64)	32.85^e (34.86)	52.62^d (46.48)	44.07^d (47.00)	46.59^d (42.71)	38.65^c (38.11)	
Th_5	30.08^d (32.76)	17.08^e (23.94)	14.96^f (21.68)	24.52^d (29.25)	24.84^f (29.91)	31.03^e (32.58)	26.59^e (30.46)	31.18^e (32.24)		
SEm ±	0.923	0.862	0.665	0.976	0.902	0.944	1.157	0.891	1.033	1.474
CD (0.05)	2.601	2.428	1.873	2.749	2.542	2.659	3.259	2.511	2.92	4.187
Invigoration treatment (I)										
I_1	69.61^a (58.76)	58.17^a (50.04)	58.04^a (49.90)	73.07^a (59.82)	63.99^a (55.39)	75.92^a (61.53)	69.95^a (57.35)	71.25^a (57.90)	74.69^a (60.41)	64.63^{ab} (53.62)
I_2	69.99^a	58.57^a	59.46^a	67.78^{ab}	57.22^{ab}	67.22^b	65.36^{ab}	58.24^{cd}	64.17^{bc}	68.07^a

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	(57.93)	(50.73)	(51.16)	(56.93)	(50.06)	(56.49)	(54.80)	(50.08)	(53.66)	(55.84)
I_3	64.47^{ab} (53.87)	57.12^{a} (48.87)	46.64^{c} (42.51)	60.84^{b} (51.78)	50.58^{bc} (46.52)	63.14^{bc} (53.71)	58.80^{b} (50.60)	53.71^{d} (47.21)	57.31^{c} (49.30)	56.22^{ab} (40.780
I_4	53.03^{c} (46.78)	46.28^{b} (42.28)	32.95^{d} (34.31)	50.28^{c} (45.03)	38.14^{d} (37.28)	48.06^{d} (43.99)	59.54^{b} (50.82)	45.69^{e} (42.29)	47.53^{d} (45.54)	53.89^{b} (47.34)
I_5	57.62^{bc} (50.78)	53.80^{a} (47.20)	46.43^{c} (42.44)	69.26^{a} (57.44)	51.38^{bc} (46.46)	59.81^{c} (51.06)	63.51^{ab} (53.32)	61.19^{bc} (59.92)	66.37^{b} (54.83)	63.72^{ab} (53.43)
I_6	66.33^{a} (56.40)	58.68^{a} (50.41)	52.89^{b} (45.43)	65.64^{ab} (54.15)	47.31^{c} (47.97)	58.52^{c} (49.78)	66.94^{ab} (55.88)	61.84^{bc} (52.56)	62.79^{bc} (53.08)	64.81^{ab} (54.22)
I_7	67.18^{a} (56.16)	54.97^{a} (48.31)	58.35^{a} (50.01)	69.64^{a} (57.29)	58.71^{a} (50.52)	69.35^{ab} (56.62)	63.15^{ab} (52.92)	67.69^{ab} (55.72)	64.61^{bc} (53.76)	63.67^{ab} (53.67)
SEm ±	0.997	0.931	0.718	1.054	0.975	1.019	1.25	0.963	1.222	1.95
CD (0.05)	2.809	2.622	2.023	2.969	2.746	2.872	3.521	2.713	3.455	5.539

*Values in parentheses are Arc sine transformed values
*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Table 34: Interaction effect of Thawing period and Invigoration treatment on germination (%) during storage in ashgourd

Period of thawing	Treatments	Period of storage (months)									
		1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
		Interaction (Th x I)									
0 months (Immediately after retrieval from refrigeration)	Th_0I_1	79.88^{cde} (63.37)	81.22^{cd} (64.32)	82.11^{bc} (64.98)	84.47^{bcd} (67.07)	95.57^{a} (79.47)	85.00^{bcde} (67.84)	82.77^{abcde} (65.59)	80.00^{bcd} (63.45)	75.57^{bcd} (60.50)	65.00^{bcdef} (53.82)
	Th_0I_2	79.44^{cdef} (63.04)	84.77^{abc} (67.13)	86.22^{ab} (68.23)	91.10^{a} (73.06)	93.33^{a} (75.50)	88.33^{abcd} (70.96)	87.23^{abc} (69.04)	82.77^{abc} (65.54)	79.43^{abc} (63.07)	76.67^{ab} (61.19)
	Th_0I_3	71.88^{fghi} (57.97)	76.55^{defg} (61.04)	79.88^{cd} (63.42)	85.57^{abc} (68.43)	96.63^{a} (79.97)	88.33^{abcd} (72.91)	88.33^{ab} (72.91)	77.80^{bcde} (62.02)	76.10^{bcd} (60.75	75.57^{abc} (60.59)
	Th_0I_4	65.44^{hijk} (53.97)	69.33^{g} (56.35)	70.44^{efg} (57.04	72.23^{fghi} (58.2)	73.33^{de} (59.04)	80.00^{ef} (63.42)	83.90^{abcd} (66.36)	88.33^{a} (70.03)	77.20^{abc} (61.49)	75.57^{abc} (68.36)
	Th_0I_5	70.22^{ghij} (56.90)	72.77^{efg} (58.52)	78.88^{cd} (62.68)	82.23^{bcde} (65.08)	89.97^{ab} (72.76)	93.87^{a} (76.32)	87.23^{abc} (69.10)	84.43^{ab} (64.38)	80.57^{abc} (63.870	76.63^{ab} (61.09)
	Th_0I_6	70.22^{ghij} (56.91)	72.66^{efg} (58.46)	75.66^{de} (60.48)	78.87^{cdefg} (62.70)	80.53^{c} (66.08)	81.10^{def} (64.41)	92.23^{a} (73.93)	88.36^{a} (68.23)	85.53^{a} (67.67)	85.00^{a} (67.37)
	Th_0I_7	53.77^{l} (47.15)	61.44^{h} (51.59)	69.44^{g} (56.42)	69.47^{hij} (56.44)	81.63^{c} (64.63)	82.77^{cde} (65.71)	84.43^{abcd} (66.82)	81.1^{abcd} (70.66)	80.57^{abc} (63.96)	76.13^{abc} (61.06)
1 month (One month after retrieval from refrigeration)	Th_1I_1	95.00^{a} (79.20)	77.77^{cdef} (62.22)	70.00^{fg} (56.77)	88.33^{ab} (70.53)	91.13^{ab} (77.73)	92.77^{ab} (74.38)	80.57^{bcde} (64.06)	80.00^{bcd} (64.53)	84.43^{ab} (67.97)	75.33^{abc} (60.21)
	Th_1I_2	92.23^{a} (74.57)	81.67^{cd} (65.40)	89.47^{a} (71.11)	85.57^{abc} (68.44)	70.53^{e} (57.11)	89.43^{abc} (71.34)	81.10^{bcde} (64.43)	76.67^{bcde} (63.42)	77.77^{abc} (62.01)	75.33^{abc} (60.20)
	Th_1I_3	82.73^{bcd} (65.62)	70.57^{fg} (57.19)	59.97^{i} (50.75)	70.57^{ghij} (57.19)	56.67^{fg} (48.22)	78.33^{efg} (62.47)	83.30^{abcde} (66.41)	60.53^{ghi} (61.22)	61.33^{efg} (51.53)	60.67^{def} (51.20)
	Th_1I_4	76.10^{defg} (60.75)	69.43^{g} (56.55)	36.13^{m} (36.84)	61.10^{kl} (59.43)	47.23^{hi} (43.37)	77.77^{efg} (61.96)	77.80^{cdef} (61.91)	60.00^{ghij} (51.12)	60.57^{efg} (51.12)	68.00^{bcde} (55.67)
	Th_1I_5	94.43^{a} (76.89)	82.23^{bcd} (65.24)	57.77^{ij} (49.47)	88.33^{ab} (30.96)	62.77^{f} (52.39)	85.53^{bcde} (68.07)	83.30^{abcde} (66.01)	77.20^{bcde} (50.73)	73.90^{cd} (59.35)	71.33^{bcd} (58.70)
	Th_1I_6	96.67^{a} (80.74)	88.90^{ab} (71.90)	68.90^{g} (56.11)	84.43^{abcd} (66.74)	70.03^{e} (56.89)	66.13^{ijk} (54.41)	79.43^{bcdef} (63.05)	76.10^{cde} (61.46)	82.77^{abc} (65.71)	75.33^{abc} (60.22)

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	Th_1I_7	72.63^{efgh} (58.46)	80.00^{cde} (63.86)	78.87^{cd} (62.68)	87.77^{ab} (70.40)	85.03^{bc} (67.27)	71.67^{ghi} (57.84)	76.13^{def} (68.78)	78.89^{bcde} (60.89)	76.13^{bcd} (60.78)	63.33^{cdef} (54.91)

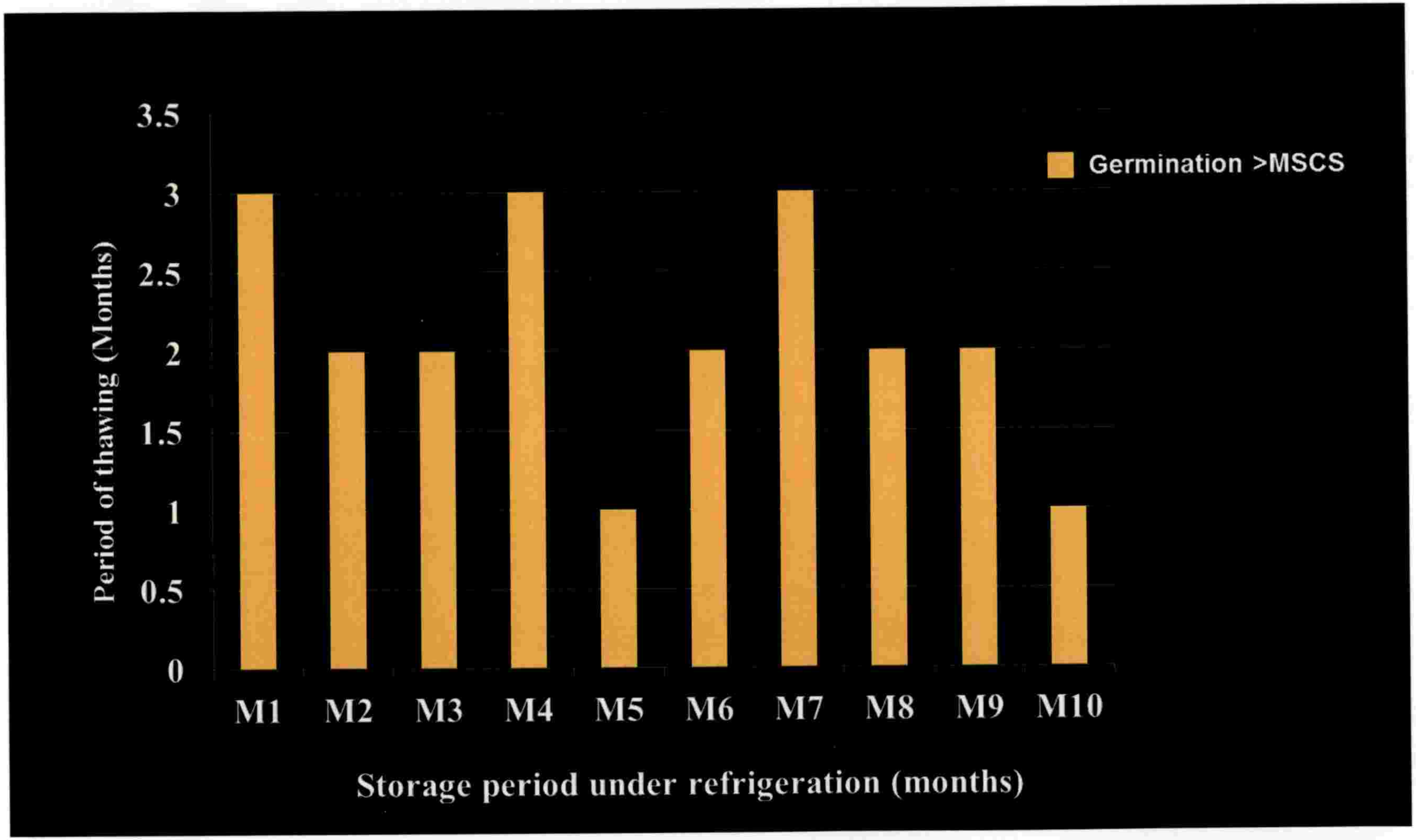
Thawing period	**Treatments**	**1 MAS**	**2 MAS**	**3 MAS**	**4 MAS**	**5 MAS**	**6 MAS**	**7 MAS**	**8 MAS**	**9 MAS**	**10 MAS**
2 months (Two months after retrieval from refrigeration)	**TH_2I_1**	92.20^{a} (74.66)	57.23^{hi} (49.15)	68.90^{g} (56.17)	87.80^{ab} (69.56)	79.47^{cd} (63.05)	81.67^{cdef} (64.710	78.90^{bcdef} (62.84)	76.67^{bcde} (62.95)	80.00^{abc} 67.460	60.67^{def} (51.15)
	TH_2I_2	84.47^{bc} (66.94)	80.00^{cde} (63.40)	75.00^{def} (60.05	85.00^{abc} (67.96)	57.23^{fg} (49.15)	83.90^{cde} (66.61)	76.67^{def} (61.22)	75.57^{cde} (61.22)	65.33^{e} (54.29)	62.00^{def} (52.23)
	TH_2I_3	75.53^{defg} (60.35)	69.43^{g} (56.44)	57.20^{ij} (49.150	68.37^{ijk} (56.86)	51.10^{gh} (45.69)	66.10^{ijk} (54.39)	77.20^{cdef} (61.46)	50.57^{k} (60.72)	60.57^{efg} (59.10)	58.67^{def} (50.20)
	TH_2I_4	67.23^{hijk} (55.06)	55.27^{hij} (47.87)	29.47^{n} (32.14)	61.13^{kl} (51.42)	36.63^{jklmn} (37.19)	44.43^{p} (41.77)	73.33^{ef} (48.98)	56.10^{hijk} (44.30)	37.33^{h} (37.60)	45.33^{gh} (42.3)
	TH_2I_5	89.43^{ab} (73.84)	52.23^{ijk} (46.26)	56.13^{ijk} (48.55)	87.77^{ab} (69.50)	52.77^{gh} (46.56)	74.43^{fgh} (59.60)	70.00^{fg} (56.80)	75.00^{cde} (48.49)	68.00^{de} (50.56)	54.67^{efg} (47.66)
	TH_2I_6	90.00^{ab} (71.86)	73.90^{efg} (59.39)	67.23^{g} (55.08)	76.10^{defghi} (60.92)	57.20^{fg} (49.18)	64.43^{ijkl} (53.44)	78.90^{bcdef} (62.87)	71.67^{ef} (60.00)	60.00^{efg} (55.67)	56.67^{efg} (48.22)
	TH_2I_7	93.90^{a} (76.02)	90.00^{a} (79.69)	71.10^{efg} (57.47)	78.90^{cdefg} (62.82)	58.90^{fg} (50.12)	71.10^{ghi} (57.46)	74.43^{def} (59.60)	66.67^{fg} (57.90)	68.00^{de} (61.61)	59.20^{def} (50.28)
3 months (Three months after retrieval from refrigeration)	**TH_3I_1**	63.37^{jk} (52.740)	53.33^{ijk} (46.89)	65.57^{gh} (54.22)	74.47^{efghi} (59.64)	51.67^{gh} (45.93)	74.43^{fgh} (59.67)	76.67^{def} (61.22)	74.43^{de} (54.71)	77.33^{abc} (52.75)	57.50^{efg} (49.29)
	TH_3I_2	64.33^{ijk} (53.79)	42.77^{lm} (40.79)	60.57^{hi} (51.09)	73.33^{fghi} (59.13)	51.10^{gh} (45.63)	66.67^{hijk} (54.77)	76.67^{def} (61.40)	55.00^{hijk} (59.97)	63.33^{ef} (47.65)	58.30^{def} (49.78)
	TH_3I_3	72.23^{efghi} (58.30)	57.23^{hi} (49.17)	50.53^{l} (45.28)	63.33^{jkl} (52.71)	37.43^{jklm} (37.70)	59.43^{klmn} (50.44)	69.47^{fg} (56.46)	52.78^{jk} (47.86)	54.67^{fg} (44.23)	30.00^{i} (33.14)
	TH_3I_4	45.00^{m} (42.11)	40.00^{mn} (39.20)	27.80^{n} (31.78)	57.23^{l} (49.18)	32.77^{lmno} (34.82)	33.33^{q} (35.24)	62.20^{gh} (52.02)	36.67^{l} (46.58)	35.33^{hi} (64.23)	26.67^{i} (31.02)

	TH_3I_5	60.53^{kl} (51.09)	52.23^{ijk} (46.26)	51.10^{kl} (46.61)	80.00^{bcdef} (63.49)	40.00^{ijkl} (39.20)	52.23^{no} (46.23)	56.67^{hi} (48.82)	53.33^{ijk} (37.240	56.00^{fg} (48.42)	52.23^{fgh} (46.26)
	TH_3I_6	65.00^{hijk} (53.75)	55.57^{hij} (48.19)	53.33^{jkl} (46.89)	73.87^{efghi} (59.28)	42.77^{ij} (40.80)	63.90 ^{ijkl} (53.11)	76.67^{def} (61.31)	71.10^{ef} (46.89)	5(04.00^{g} 47.28	42.23^{h} (40.49)
	TH_3I_7	81.10 ^{cd} (64.43)	53.33^{ijk} (46.89)	60.83^{hi} (59.26)	77.23^{cdefgh} (61.77)	56.70^{fg} (48.84)	69.43^{hij} (56.44)	73.33^{ef} (58.93)	65.57^{fg} (57.48)	65.00^{e} (53.72)	56.00^{efg} (48.30)
Thawing period	**Treatments**	**1 MAS**	**2 MAS**	**3 MAS**	**4 MAS**	**5 MAS**	**6 MAS**	**7 MAS**	**8 MAS**	**9 MAS**	**10 MAS**
4 months (Four months after retrieval from refrigeration)	**TH_4I_1**	45.00^{m} (42.11)	52.23^{ijk} (43.34)	35.57 ^{m} (36.56)	68.37^{ijk} (55.86)	33.33^{klmno} (35.24)	62.77^{jklm} (52.40)	56.90^{hi} (48.99)	65.56^{fg} (54.09)	56.13^{fg} (48.51)	
	TH_4I_2	55.00^{l} (47.86)	35.00^{no} (46.27)	28.30^{n} (32.06)	46.67^{m} (43.06)	39.43^{jklm} (38.85)	43.87^{p} (41.42)	38.30^{kl} (38.19)	40.00^{l} (54.08)	35.00^{hi} (36.15)	
	TH_4I_3	46.10^{m} (42.74)	56.13^{hij} (36.22)	22.23^{o} (28.07)	47.23^{m} (43.39)	35.57^{jklmn} (36.48)	55.57^{mno} (48.19)	17.78^{o} (22.68)	52.23^{k} (39.07)	33.87^{hi} (35.47)	
	TH_4I_4	38.87^{m} (38.49)	34.47^{no} (48.55)	24.43^{no} (29.6)	27.77^{nop} (31.77)	32.20^{lmno} (34.55)	31.67^{q} (35.17)	32.20^{lm} (34.53)	18.89^{n} (46.26)	27.20^{i} (31.07)	
	TH_4I_5	18.33^{op} (25.24)	47.20^{kl} (35.92)	25.00^{no} (29.93)	57.77^{l} (49.46)	36.10^{jklmn} (29.66)	48.33^{op} (44.02)	57.77^{hi} (49.46)	50.55^{k} (25.150	53.37^{g} (46.93)	
	TH_4I_6	55.00^{l} (47.85)	49.43^{jkl} (43.37)	49.43^{l} (44.65)	71.13^{ghij} (57.53)	24.43^{p} (32.42)	62.23^{jklm} (54.06)	53.87^{hi} (47.20)	37.22^{l} (045.29	31.67^{hi} (33.93)	
	TH_4I_7	75.57^{defg} (60.40)	30.00^{op} (44.67)	40.67^{m} (39.58)	73.90^{efghi} (59.39)	28.87^{nop} (34.89)	63.90^{ijkl} (53.11)	51.67^{ij} (45.93)	61.67^{gh} (37.39)	33.33^{hi} (34.68)	
5 months (Five months after retrieval from refrigeration)	**TH_5I_1**	42.23^{m} (40.51)	27.23^{p} (33.10)	26.10^{no} (30.69)	35.00^{n} (36.24)	32.77^{lmno} (34.14)	58.87^{klmn} (50.14)	43.89^{jk} (41.41)	50.83^{k} (51.29)		
	TH_5I_2	44.47^{m} (41.78)	27.23^{p} (31.42)	17.23^{p} (24.43)	25.00^{opq} (29.91)	31.67^{mnop} (30.55)	31.13^{q} (33.80)	32.22^{lm} (34.54)	19.43^{n} (45.45)		
	TH_5I_3	38.33^{m} (38.23)	12.80^{q} (31.44)	10.00^{q} (18.38)	30.00^{nop} (33.12)	26.10^{op} (14.75)	31.10^{q} (33.87)	16.70^{o} (24.11)	28.33^{m} (26.06)		
	TH_5I_4	25.57^{n} (30.28)	9.470^{q} (20.84)	9.430^{q} (17.86)	22.23^{pq} (28.09)	6.67^{q} (30.97)	21.13^{r} (27.35)	27.78^{mn} (31.05)	14.17^{n} (31.98)		

	TH_5I_5	12.77[p] (20.72)	16.13[q] (17.80)	10.00[q] (18.42)	19.43[q] (26.14)	26.67[op] (17.28)	4.43[t] (12.09)	26.11[mno] (29.75)	26.63[m] (22.09)		
	TH_5I_6	21.10[no] (27.30)	11.63[q] (23.55)	2.80[r] (9.29)	9.43[r] (17.70)	8.90[q] (39.81)	13.33[s] (21.24)	20.56[no] (26.89)	26.63[m] (31.03)		
	TH_5I_7	26.10[n] (30.48)	15.03[q] (22.73)	29.17[n] (32.67)	30.57[no] (33.54)	41.10[ijk] (36.48)	57.23[lmn] (49.15)	18.89[no] (25.46)	52.23[k] (31.03)		
	SEM ±	2.442	2.28	1.759	2.582	2.387	2.497	3.061	2.358	2.733	3.9
	CD (0.05)	6.881	6.423	4.956	7.273	6.726	7.035	8.623	6.644	7.726	17.12

*Values in parentheses are Arc sine transformed values
*Means in each column with atleast one letter in common are not significantly different at 5% level of probability

Fig 11. Impact of period of thawing on seed longevity in ash gourd



I$_1$: $CaCl_2$ 50Mm for 12h

I$_7$: untreated control

Th$_1$

Th$_2$

Th$_3$

Th$_4$

Th$_5$

Plate 5. Effect of thawing on seedling growth of seeds retrieved at 1 MAS.

From the above, it can be concluded that when the seeds are retrieved after a short period of refrigerated storage (1 MAS), germination in both invigorated and untreated seeds increased when exposed to thawing up to a period of one month (Th_1) and thereafter declined during thawing with each passing month of cold storage.

Irrespective of the storage period under refrigeration (1 MAS to 10 MAS), it would be advisable to use the untreated seeds or seeds invigorated with $CaCl_2$ 50mM for 12h (I_1) or CaCl2 50mM for 24h (I_2) or *Pf* $1x10^{-6}$ $cfu.ml^{-1}$ for 12h (I_6) within one month of retrieval after refrigeration. Viability retention of invigorated and untreated seeds during further periods of thawing is unpredictable.

However, if prolonged periods (>1 month) of ambient storage after retrieval from cold storage is unavoidable, invigoration with $CaCl_2$ 50mM for 12h (I_1) or $CaCl_2$ 50mM for 24h (I_2) would be advisable.

Considering the impact of storage environment, invigoration treatment and their interaction on seed viability and quality as well as their influence on seed quality during thawing, it can be summarised that invigoration seeds with $CaCl_2$ 50mM for 12h (I_1) or 24h (I_2) would be most advantageous to obtain good germination and crop stand. According to Farooq *et al.* (2006), the positive impact of the $CaCl_2$ on germination and other seed characters is due to the advantage of Ca^{2+} ions in improving cell water status, essential role as cofactors in the activities of numerous enzymes which are active during reserve mobilization and radical protrusion. Seed invigoration with calcium chloride resulted in the effective control of peroxidation and free radical damage either by stabilizing the cellular membrane or by converting the free radicals into non-harmful products had led to an increased germination and seedling vigour (Prabhu *et al.*, 2006).

Summary

6. SUMMARY

A study on the impact of seed invigoration on viability and quality of seeds in ash gourd variety KAU Local was conducted at College of Horticulture, Vellanikkara, Thrissur, during 2016-2018. The study was conducted to elucidate the effect of seed invigoration on viability of seeds stored under refrigerated condition and to analyse the impact of thawing on seed longevity in ash gourd. The results obtained are summarized below.

I. **Seed quality and longevity of ash gourd variety KAU Local as influenced by storage condition**

1. Seed quality during storage and seed longevity were found to be significantly influenced by storage environment throughout the storage period.

2. Irrespective of the invigoration treatments, it was found that germination in all the invigorated seeds had reached above the minimum standards for seed certification (MSCS) while the germination in untreated seeds was only 21.10 per cent. At the start of the experiment, owing to seed invigoration, the per cent increase in germination varied from 197 per cent (kinetin 10 ppm for 12h) to 221 per cent (kinetin 10 ppm for 24h) in comparison to the untreated control.

3. Germination of seeds stored under the refrigerated storage was lower than that under ambient storage during the initial storage period of storage (up to 3 MAS). Henceforth, refrigerated seeds exhibited significant superior germination than that under ambient storage till the end of storage period (14 MAS).

4. Germination above MSCS was retained for 13 MAS in seeds under refrigerated storage compared to only 5 MAS in ambient stored seeds.

5. Apart from germination, seeds stored under refrigeration were significantly superior to that under ambient storage with respect to seed quality parameters *viz*, germination index, coefficient of velocity of germination, energy of germination and vigour indices I and II.

6. The mean germination time, time taken for 50 per cent germination, electrical conductivity, lipid peroxidation, leakage of sugar and amino acid and infection by seed micro flora in the refrigerated seeds were significantly lower than that of ambient stored seeds at the end of storage.

7. The present study thus revealed that irrespective of seed invigoration treatments, to prolong seed longevity and maintain seed quality, storing seeds dried to seven per cent moisture content in 700 gauge polyethylene bags, under refrigeration is advantageous over ambient storage.

II. Seed quality and longevity of ash gourd variety KAU Local as influenced by invigoration treatment

1. The seed quality parameters of the invigorated seeds before storage were found to be superior to untreated seeds.

2. Seed quality and longevity during storage were found to be significantly influenced by invigoration treatment throughout the storage period

3. Irrespective of the invigoration treatment, germination and other seed quality parameters such as germination index, coefficient of velocity of germination, energy of germination, vigour indices I and II, in both treated and untreated seeds decreased progressively over the storage period.

4. There was an increase in mean time to germination, time taken for 50 per cent germination, allometric index, electrical conductivity of seed leachate, seed

infection per cent, leachate of sugar, amino acid and lipid peroxidation, towards the end of storage period.

5. Invigoration induced early germination. Unlike the invigorated seeds which exhibited a germination per cent above 80 at 1 MAS, the germination of the untreated control (I_7) was below the MSCS at 1 MAS and gradually reached a maximum of 84.43 per cent at 5 MAS. At 1 MAS, the increase in germination of treated seeds varied between 43 per cent (KH_2PO_4 10^{-1} M for 24h) to 59 per cent ($CaCl_2$ 50 mM for 12h) over untreated seeds.

6. Untreated (I_7) seeds retained viability above MSCS up to 9 MAS while the seeds invigorated with $CaCl_2$ 50mM (for 12h or 24h) retained viability for 8 MAS. Seeds invigorated with kinetin 10 ppm (for 12h or 24h) retained viability above MSCS for 6 MAS while it was 7 MAS each for seeds invigorated with *Pf* $1x10^6$ $cfu.ml^{-1}$ for 12h and KH_2PO_4 $10^{-1}M$ for 24h.

7. Seeds invigorated with $CaCl_2$ 50mM for12h (I_1) and CaCl2 50mM for 24h (I_2) not only registered high germination throughout the storage period but also exhibited higher magnitude of energy of germination, germination index, coefficient of velocity, vigour index I and II and allometric index. In addition, the mean germination time, time to 50 per cent germination, electrical conductivity of seed leachate, leakage of amino acid, lipid peroxidation, leakage of sugars in seed leachate and seed microflora were also invariably low in these treatments throughout the storage period unlike in untreated seeds.

8. Considering the significant superiority of seeds invigorated with I_1 ($CaCl_2$ 50mM 12h) and I_2 ($CaCl_2$ 50mM 24h) with respect to germination during the initial storage period (up to 4 MAS), retention of superior seed qualities during storage as well as retention of viability above MSCS for a period of 8 MAS, seed invigoration with $CaCl_2$ 50mM before storage can be advocated.

III. Seed quality and longevity of ash gourd variety KAU Local as influenced by interaction between storage condition and invigoration treatment

1. Results indicated existence of wide variability in the impact of interaction between storage environment and invigoration treatment and on all the seed indices during seed storage.

2. Based on the influence of interaction between storage condition and invigoration treatment on germination and other seed indices, it can be concluded that, seed invigoration followed by refrigerated storage is advantageous.

3. If only ambient storage condition is feasible, it would be advantageous to invigorate the seeds with $CaCl_2$ 50mM for 12h (I_1) since viability above MSCS was retained for 8 MAS compared to 7 MAS in untreated seeds.

4. If provision for refrigerated storage is available, bio-priming with *Pf* $1x10^{-6}$ cfu.ml^{-1} for 12h (S_2I_6) or invigoration with $CaCl_2$ 50mM for 24h (I_2), Kinetin 10 ppm for 12h (I_3), Kinetin 10 ppm for 24h (I_4) and KH_2PO_4 10^{-1} M for 24h (I_5) would be most advantageous. These treatments helped retain viability above MSCS for 13 MAS while it was retained for 12 MAS on invigoration with $CaCl_2$ 50mM for 12h (I_1).

5. However, considering the high germination per cent, energy of germination, germination index, coefficient of velocity, vigour index I and II and allometric index and lower mean germination time, time to 50 per cent germination, electrical conductivity of seed leachate, leakage of amino acid, lipid peroxidation, leakage of sugars in seed leachate and seed microflora throughout the storage period seed invigoration with either $CaCl_2$ 50mM for 12h (I_1) or $CaCl_2$ 50mM for 24h (I_2) would be most advantageous for storage of ash gourd seeds both under ambient and refrigerated storage.

IV. Seed quality and longevity of ash gourd variety KAU Local as influenced by thawing period and invigoration treatment

1. Results indicated that the storage environment, invigoration treatment and their interaction significantly influenced seed viability when a seed stored under refrigerated condition undergoes thawing.

2. Irrespective of the invigoration treatment, the seeds taken out after a short storage period of storage under refrigeration (*i.e.,* 1 MAS or 2 MAS), when subjected to thawing, exhibited an increase in germination after 1 or 2 months of thawing (Th_1 and Th_2). However, as the seeds under refrigeration aged (*i.e.*, from 3 MAS to 10 MAS), germination decreased irrespective of the period of thawing.

3. Irrespective of the storage period under refrigeration (*i.e.,* storage period varying between 1 MAS and 10 MAS), the seeds were found to retain viability above MSCS for a minimum period of one month after retrieval from refrigerated storage. Viability retention of invigorated and untreated seeds during further periods of thawing was unpredictable.

4. When retrieved from refrigeration at varying intervals (*i.e.,* 1 to 10 MAS), and subjected to thawing under ambient storage for two months (*i.e.,* at Th_2), the seeds invigorated with $CaCl_2$ 50mM for 12h (I1) retained viability above MSCS throughout, except at 2 MAS, unlike the untreated seeds (I_7). Results thus revealed that seed invigoration with $CaCl_2$ 50mM for 12h (I_1) is advantageous, if one or two months of ambient storage after retrieval from cold storage is unavoidable

5. Irrespective of the thawing period, germination in seeds invigorated with $CaCl_2$ 50mM 12h (I_1) was found to be on par with untreated seeds, except at 9 MAS. At 9 MAS the seeds invigorated with $CaCl_2$ 50mM 12h when retrieved from

cold storage recorded significantly higher germination (74.69%) compared to untreated seeds (64.61%).

6. It was observed that irrespective of the thawing period, when seeds were retrieved from refrigerated storage at monthly intervals (*i.e.,* 1 to 10 MAS), the germination in seeds invigorated with $CaCl_2$ 50mM 12h was retained above MSCS throughout, except at 2 MAS and 3 MAS. In, the untreated seeds however, the germination was fell below MSCS when retrieved from cold storage at 2 MAS, 3 MAS and 5 MAS. Hence, it can be concluded that invigoration with $CaCl_2$ 50mM for 12h (I_1) is beneficial in instances when it is anticipated that seeds stored under refrigeration needs to be retrieved and stored under ambient storage before sowing.

7. None of the invigoration treatments helped retain seed viability for five months after retrieval from refrigeration.

8. Considering the impact of storage environment, invigoration treatment and their interaction on seed viability and quality as well as their influence on seed quality on thawing, it can be summarised that invigoration seeds with $CaCl_2$ 50mM for 12h (I1) or 24h (I2) would be most advantageous to realise good germination and seed quality during storage.

9. The increase in cost of seed invigoration with calcium chloride amounts to Rs.52.86 per kilogramme of seed. Hence, a 4.40 per cent escalation in the cost of production of seeds invigorated with calcium chloride occurs over untreated control. However, by incurring this increase in expenditure, the viability of the seed can be extended over one month compared to the untreated contro

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IMPACT OF PRE-STORAGE SEED INVIGORATION IN ASH GOURD SEED (*Benincasa hispida* (Thunb.) Cogn.)

By

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(2016-11-017)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement

for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2018

ABSTRACT

A study to elucidate the effect of seed invigoration on viability and quality of seeds in ash gourd variety KAU Local was conducted at College of Horticulture, Vellanikkara, Thrissur, during 2016-2018. The impact of seed invigoration on seed viability and seed quality parameters under ambient (S_1) and refrigerated storage (S_2) was assessed following a completely randomized design with seven invigoration treatments (I_1 to I_7) and three replications. Seeds were separately invigorated using $CaCl_2$ (50 m M) for 12h (I_1), $CaCl_2$ (50 mM) for 24h (I_2), kinetin (10 ppm) for 12h (I_3), kinetin (10 ppm) for 24h (I_4), KH_2PO_4 (10^{-1} M) for 24h (I_5), *Pseudomonas fluorescens* ($1x10^6$ cfu.ml^{-1}) for 12h (I_6). Untreated seeds (I_7) served as control. Both treated and untreated seeds were dried to < 8 per cent moisture content and packed in polythene bags (700 gauge). The seed quality parameters were recorded immediately after treatment and subsequently at monthly intervals for a period of 10 months, while, germination of stored seeds was assessed up to 14 months after storage (MAS). At bimonthly intervals, quantification of lipid peroxidation, sugar and amino acids leached out from the seeds and the seed micro flora infection was also done.

Seed quality during storage and seed longevity were found to be significantly influenced by storage environment, invigoration treatment and their interaction throughout the storage period. The results revealed that germination and other seed quality parameters such as germination index, coefficient of velocity of germination, energy of germination, vigour indices I and II, in both treated and untreated seeds decreased progressively over the storage period. However, there was an increase in mean time to germination, time taken for 50 per cent germination, allometric index, electrical conductivity of seed leachate, seed infection per cent, leachate of sugar, amino acid and lipid peroxidation, towards the end of storage period.

Germination of seeds stored under the refrigerated storage was lower than that under ambient storage in the initial storage period (upto 3 MAS). Henceforth,

refrigerated seeds exhibited significant superior germination than that under ambient storage till the end of storage period (14 MAS). Germination of seeds under refrigeration was retained above 60 per cent (the minimum seed certification standards required for ash gourd) for 13 MAS compared to 5 MAS in ambient stored seeds. The study thus revealed that irrespective of seed invigoration treatments, to prolong seed longevity and maintain seed quality, storing seeds under refrigeration is advantageous over ambient storage.

Irrespective of storage environment, priming induced early germination. The seed quality parameters of the invigorated seeds before storage were found to be superior to untreated seeds. The invigorated seeds had also exhibited a germination per cent above 80 at 1 MAS, while, the germination in untreated control (I_7) during the corresponding period was below the MSCS. Seeds invigoration with calcium chloride for 12h (I_1) and 24h (I_2) recorded significantly high germination and other seed quality parameters during the storage period of ten months. Owing to the significant superiority of seeds invigorated with I_1 ($CaCl_2$ 50mM 12h) and I_2 ($CaCl_2$ 50mM 24h) with respect to germination in the initial period of storage (up to 4 MAS), superior seed qualities during storage as well as retention of germination above MSCS for 8 MAS, seed invigoration with $CaCl_2$ 50mM before storage can be advocated to help retain seed qualities and prolonging seed longevity during storage.

The interaction between storage condition and invigoration treatment on germination and other seed indices pointed out that it was most advantageous to treat seeds with $CaCl_2$ 50mM for 12h (I_1) before storing under ambient conditions. If provision for refrigerated storage is available, bio-priming with *Pf* $1x10^{-6}$ $cfu.ml^{-1}$ for 12h (S_2I_6) or priming with $CaCl_2$ 50mM for 24h (I_2), kinetin 10 ppm for 12h (I_3) or kinetin 10 ppm for 24h (I_4) or KH_2PO_4 10^{-1} M for 24h (I_5) would be most advantageous.

Analysis of the impact of pre-storage seed invigoration treatment on seed longevity subsequent to retrieval of seeds from refrigerated storage revealed that, irrespective of the storage period under refrigeration, the seeds were found to retain viability above MSCS for a minimum period of one month after retrieval from refrigerated storage. Viability retention of invigorated and untreated seeds during further periods of thawing was unpredictable. It was also evident that none of the treatments could help retain seed viability above MSCS for five months after retrieval from refrigeration. Results also revealed that seed invigoration with $CaCl_2$ 50mM 12h (I_1) is advantageous, if one or two months of ambient storage after retrieval from cold storage is unavoidable.

Hence, considering the impact of storage environment, invigoration treatment and their interaction on seed longevity and quality, as well as their influence on seed longevity during thawing, it can be summarised that seed invigoration with $CaCl_2$ 50mM for 12h (I_1) or 24h (I_2) would be beneficial.

Appendices

Appendix I

Influence of invigoration treatment on seedling shoot length of seeds stored under ambient condition.

Invigoration treatment	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
	Seedling shoot length (cm)									
I_1	19.23	18.97	18.72	18.70	18.20	18.01	17.42	16.99	16.85	16.37
I_2	19.23	19.17	19.14	19.00	18.77	18.72	17.94	17.09	16.00	15.99
I_3	19.98	18.84	18.73	18.86	18.64	18.30	18.20	17.05	14.13	13.75
I_4	18.47	18.30	18.20	17.25	17.83	16.51	16.87	16.00	14.60	14.40
I_5	18.97	18.91	18.82	18.48	18.25	16.90	16.63	16.30	15.75	12.52
I_6	22.34	19.70	18.37	17.96	17.77	17.53	16.65	16.61	15.89	14.55
I_7	17.87	17.77	17.65	17.41	17.47	17.51	17.27	16.90	16.76	15.85

Appendix II

Influence of invigoration treatment on seedling shoot length of seeds stored under refrigerated condition

Invigoration treatment	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
	Seedling root length (cm)									
I_1	18.13	18.20	18.62	18.78	19.37	18.45	17.95	17.62	17.57	16.96
I_2	18.53	18.77	18.90	19.48	19.88	19.44	19.19	18.77	18.63	18.28
I_3	17.17	18.58	18.65	18.68	18.82	18.46	18.30	18.17	18.08	18.04
I_4	16.83	17.33	17.40	17.57	17.65	17.73	18.10	19.60	17.70	17.41
I_5	17.07	18.01	18.47	18.69	19.34	19.60	18.55	18.40	18.24	18.10
I_6	17.03	17.56	18.03	18.12	18.48	18.50	20.13	18.09	18.08	17.97
I_7	16.30	17.50	18.17	18.34	18.50	19.07	19.50	17.66	17.60	17.46

Appendix III

Influence of invigoration treatment on seedling root length (cm) of seeds stored under ambient condition

Invigoration treatment	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
	Seedling root length (cm)									
I_1	7.93	7.37	6.75	6.08	5.64	5.57	4.54	4.43	3.49	2.83
I_2	7.27	6.97	6.28	6.18	5.77	5.60	4.40	4.23	3.50	2.21
I_3	5.27	6.23	6.14	5.90	5.65	5.60	4.93	3.55	3.42	2.25
I_4	7.60	7.57	6.36	6.93	5.53	5.14	3.81	3.10	3.07	2.51
I_5	7.37	7.42	7.49	7.00	5.55	4.86	4.13	3.55	3.05	2.43
I_6	6.30	6.41	6.51	6.38	4.53	4.53	3.91	3.33	3.25	2.80
I_7	5.07	5.33	5.96	6.10	6.61	5.19	3.80	3.45	3.18	3.14

Appendix IV

Influence of invigoration treatment on seedling root length (cm) of seeds stored under refrigerated condition

Invigoration treatment	Period of storage (months)									
	1 MAS	2 MAS	3 MAS	4MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
	Seedling root length (cm)									
I_1	6.20	6.01	6.17	6.35	6.52	5.75	4.74	3.00	3.00	3.01
I_2	6.15	6.02	6.13	6.50	7.14	6.30	4.33	3.55	3.35	2.91
I_3	4.25	4.06	4.53	4.65	6.89	4.57	4.11	3.17	2.48	2.45
I_4	3.40	3.17	3.03	3.08	3.53	4.30	4.40	4.83	2.80	2.30
I_5	3.40	3.90	4.52	4.95	5.21	8.61	4.10	3.84	3.10	2.65
I_6	3.70	3.51	3.56	3.83	4.35	4.48	6.32	3.27	2.57	2.40
I_7	3.42	3.89	4.37	4.40	4.93	5.25	7.60	3.36	3.25	2.85