SEED INVIGORATION TO OVERCOME DORMANCY IN ASH GOURD (*Benincasa hispida* (Thunb.) Cogn.)

By

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(2014-11-231)

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR – 680 656

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THESIS

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DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

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DECLARATION

I, hereby declare that this thesis entitled **'Seed invigoration to overcome dormancy in ash gourd (*Benincasa hispida* (Thunb.) Cogn.)'** is a bonafide record of the research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of my degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellanikkara **Shobha, K. V.**
Date: **(2014-11-231)**

CERTIFICATE

Certified that this thesis entitled **'Seed invigoration to overcome dormancy in ash gourd (*Benincasa hispida* (Thunb.) Cogn.)'** is a bonafide record of the research work done independently by **Ms. Shobha, K. V.** Under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of advisory committee of **Ms. Shobha, K. V.**, a candidate for the degree of **Master of Science in Agriculture** with major field in **Seed Science and Technology**, agree that the thesis entitled **'Seed invigoration to overcome dormancy in ash gourd (*Benincasa hispida* (Thunb.) Cogn),'** may be submitted by **Ms. Shobha, K. V**. **(2014-11-231)**, in partial fulfilment of the requirement for the degree.

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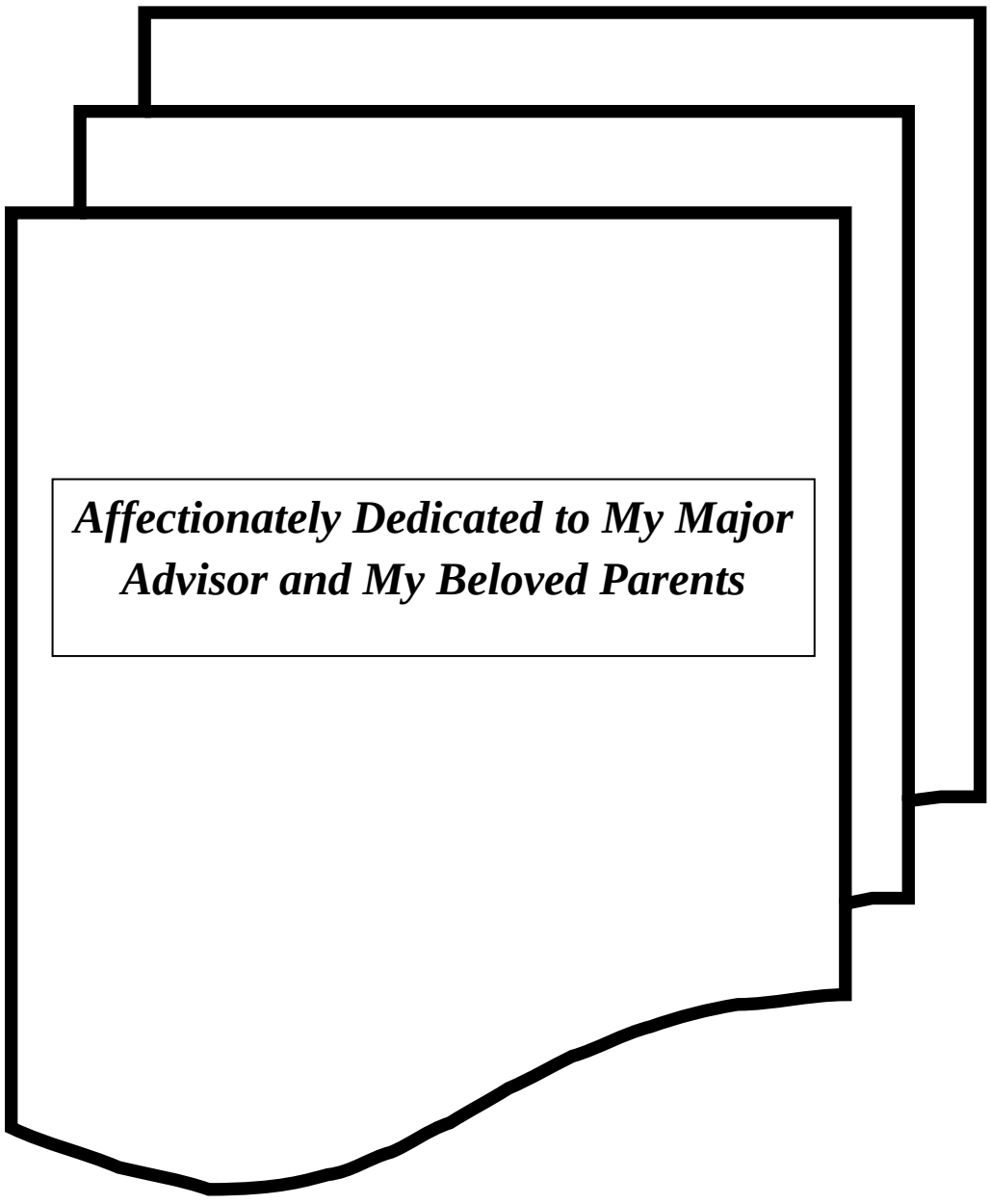
Affectionately Dedicated to My Major Advisor and My Beloved Parents

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LIST OF ABBREVIATIONS

g	- gram
KAU	- Kerala Agricultural University
^{o}C	- degree Celsius
mM	- milliMolar
M	- Molar
MPa	- Mega Pascal
ppm	- parts per million
cfu.ml^{-1}	- colony forming unit per millilitre
%	- per cent
cm	- centimetre
dSm^{-1}	- desi Siemens per metre
µm	- micrometer
mg	- milligram
Pf	- *Pseudomonas flourescens*

Introduction

1. INTRODUCTION

Ash gourd (*Benincasa hispida* (Thunb.) Cogn.)**;** syn ash gourd, wax gourd, white pumpkin) locally known as '***Kumbalanga***' belongs to the family Cucurbitaceae. It is an important vegetable crop mainly valued for its long storage life and good scope for value addition. Green immature fruits, young branches and sometimes mature fruits of white gourd are used as vegetables. The fruits are consumed as baked, fried, boiled, prickled or candied preserved (Ali *et al.,* 2010). Every 100g edible portion of the fruit contains 96.10g water, 13 kcal energy, 3g carbohydrates, 0.4g protein, 0.2g fat, 160 mg minerals and 13.71 mg vitamins (USDA, 2016).

Ash gourd is a popular vegetable crop of Kerala. In Kerala, the production of vegetables is high during winter (September-December) and most districts produce marketable surplus during this season (KAU, 2011). Total production of ash gourd in Kerala during 2014-15 was about 25245 metric tons from an area of 3,822 ha and an average productivity of about 6.7 t/ha.

Although a single fruit of ash gourd contains large number of seed, germination of seeds under natural field conditions is highly variable, erratic, and very poor. Seed germination in ash gourd is very low immediately after extraction and seed dormancy is one of the reasons for the poor germination observed (Robinson and Deckers, 1997; Malik *et al.,* 2001; Gopalakrishnan, 2004; Nerson 2007; Doody and Conor, 2011; Bian *et el*., 2013). Dormancy prevents the seeds from germinating for few months or even years after harvest (Ganar, 2003). It is nature's way of setting a time clock that allows seeds to germinate when conditions become normally favourable (Evans and Frank, 1999). Breakdown of dormancy in natural course is a gradual process, and may extend for a period of five to six months. Storage studies revealed that dormancy in ash gourd extended up to 6 to 11 months

after harvest and was present universally in the seeds produced in all crop seasons (Rahman *et al*., 2014).

The effects of dormancy on emergence and field establishment can be critical. Delayed emergence or missing plants in crop plants that are directly sown in the field may reduce yield and necessitate alternations in cultural practices. This forces the vegetable growers to incur more money in purchasing larger quantity of seed in order to maintain adequate plant stand in their field while, seed traders face the problem of selling such seeds (Rahman *et al*., 2014).

The inactive conditions of seeds under dormancy may result from unfavourable environmental conditions and sometimes through internally imposed germination blocks or chemical blocks (Unnikrishnan, 2005). Ash gourd seeds are characterized by the presence of hard seed coat. They exhibit dormancy, resulting in poor germination and seedling establishment. Germination of several vegetable species has been shown to increase after seed treatment with chemicals and various osmotica (Sridhar *et al.,* 2013). Seed invigoration treatments were initially developed to improve both the rate and uniformity of germination in vegetable species. By standardizing the invigoration technique that could break the dormancy in seeds of freshly harvested fruits of ash gourd, it would be possible to use the seeds immediately after extraction by the farmers negating the wait for the natural process of dormancy breaking.

Despite the immediate improvements in seed performance following invigoration treatments, there have been contrasting reports of seed storage potential following the treatment. By studying the storability of invigorated seeds, it would be possible to know whether the initial advantage gained through invigoration is retained even after storage. Such information will play a significant role in the farming sector

as well as seed industry where seed supply and distribution can be managed with a lot of flexibility.

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

- To elucidate the effect of seed invigoration on dormancy in ash gourd
- To ascertain the anatomical changes in seed coat on seed treatment
- To assess the storage potential of treated seeds under ambient conditions.

Review of Literature

2. REVIEW OF LITERATURE

The eventual function of the surviving seed is germination. A dormant seed is one that does not have an ability to grow in a predefined time frame under any combination of normal physical environmental factors, that are generally is favourable for its germination (Baskin and Baskin, 2004). A viable seed passes from a dormant, quiescent state to one of active growth, allowing the embryo to break through its seed coat in the process of germination (Baskin and Baskin, 2004). For germination to occur; a seed requires moisture, suitable temperature and in most cases an aerobic atmosphere. If one or more of these requirements are not met, germination will fail to occur, and in this condition the seeds may be regarded as being in a condition of imposed dormancy (Roberts, 1972). Seed dormancy is however a temporary failure of a viable seed to germinate. After a specific length of time and in a particular set of environmental conditions the restrictive state becomes terminated by either natural or artificial conditions which then allows germination to occur (Simpson, 1990).

The proportion of dormant seeds in a seed lot, the intensity and duration of dormancy will effectively determine the crop stand with far-reaching implications in the returns realised from the crop. Dormancy is a considered as a force to reckon with among the players of seed industry. The various aspects of seed dormancy in vegetable crops especially in ash gourd are briefly reviewed here under.

2.1 Seed dormancy in vegetables – the causes

Causes of dormancy are many and varied. Impermeability of seed coat to water and gases, immaturity of the embryo, special requirement for temperature and light, vicinity of inhibitors and mechanical restriction to embryo growth are the major reasons (Tran and Cavanagh, 1984). Primary dormancy encompassed dormancies

occurring due to pre-harvest or pre-dispersal changes in seeds and the one induced following harvest or dispersal, by natural or artificial means (Khan and Karssen, 1980).

2.1.1 Impermeable membrane and seed coat imposed dormancy

Dormancy imposed by the seed coat had been a subject of interest since third century B.C. The Greek writer Theophrastus stated that the seeds of certain pulses were 'hard' and requires soaking in 'nitre' for proper germination. Stier (1938) revealed that the seed coat is responsible for restricting the oxygen supply to the developing embryos and it was responsible for dormancy in potato.

In cucurbitaceous crops like *Cucurbita pepo*, regardless of the less permeable external membrane, controlled gaseols exchange by the inner membrane such as nucellar membrane has been considered to cause dormancy (Brown 1940 and Suryawanshi *et al*., 1996). Works on cucurbits have demonstrated that seed dormancy is related to seed coat impermeability to gases (Thornton, 1968).

During seed ontogeny, the outer integument gives rise to several distinct layers transforming itself into the testa, while in many species the inner integument disappears (Esau, 1977 and Miller *et al*., 1999). Inwards from the surface they are: the waxy cuticle layer, the epidermis, the hypodermis, and the interior stellate parenchyma (Swanson *et al*., 1985). The outermost waxy cuticlar layer which is of variable thickness represents the first barrier to imbibition. The epidermis, a layer of thick-walled palisade cells called macrosclereids and hypodermal pillar cells constitute the next layer. Meryl and Moore (1959) found that water enters the cotton seed by an opening in the palisade layer at the chalazal end in hard seed. This opening is made impervious by a chalazal cap and a seal between the cap and palisade layer.

Seed coverings that impose exogenous dormancy are the endosperm, perisperm, outer integuments of the seed coat or the remnant of the fruit pericarp. The most common form of exogenous dormancy occurs in seeds with "hard" seed coats that get to be suberized and are impenetrable to water. Macrosclereid cells of the outer integument get to be enhanced, combine, consolidate suberin stores, and develop external cutin coverings (Rolston, 1978).

Nada *et al*. (1994) reported that okra seeds exhibit dormancy resulting from a hard, water resistant seed coat and chalazal plug, causing very slow water uptake. According to Bewley (1997) thick seed coats can act as a barrier against radicle protrusion. Radicles will therefore, take longer to emerge from the seed coat thence appearing shorter and reducing root: shoot ratio.

Baskin (2000) reported that physical dormancy is caused by one or more water impermeable layers of palisade cells in the seed coat. The cuticle layer will become gelatinous or mucilaginous when wet and thus forms the first barrier for water imbibition. Palisade layer consists mainly of pectin and hemi-cellulose and is very hard and hydrophobic during the later stages of seed maturation (Werker, 1981).

In cucumber (*Cucumis*) and spinach (*Spinacia*), adhesive layers on the seed coverings can restrict gaseous exchange (Bewley and Black, 1982). These layers of integument and leftovers of the endosperm and nucellus remain physiologically active during ripening and after the seed are detached from the plant. Such physiologically active layers maintain primary dormancy, mainly because their semipermeable in nature, limits air circulation and inhibits development.

Coat-imposed dormancy is reported, in seeds of *Sida acuta*, *Urena lobata* and *Commelina bengalensis* (Egley, 1989; Seal and Gupta, 2000; Wang *et al*., 2009). However, it has become clear that seed coat dormancy is a more complex

phenomenon than anticipated. Hence it is imperative to detail the sequence of events leading to dormancy in the seed, to identify the dormancy mechanism in the seed coat and finally to build up the precise location and nature of the barrier to germination. Developmental studies (anatomical and ultra structural) have turned out to be indispensable in recognizing the kind of seed coat dormancy and in giving information as to how it is broken naturally.

Quagliotti *et al*. (1981) reported that in okra seeds dormancy results from a hard, water resistant seed coat and a chalazal plug, causing very slow water uptake. For a number of species, the embryo can be removed from the seed coat of a dormant seed and grown normally. In such instances, the seed coverings are the primary barrier to germination. The physical quality of the endosperm, perisperm or seed covers appeared to limit germination in cultivated crops like *Beta*, *Capsicum*, *Lactuca*, *Lycopersicon* and *Cucumis* (Dutta *et al*., 1994; Watkins and Cantliffe 1983; and Welbaum *et al*., 1995).

Physical obstructions, such as, seed coats impermeable to water and/or gasses, is accounted for dormancy in Fabaceae, Malvaceae (okra), and various other families (Copeland and McDonald, 2001).

Mohan (2005) reported that snake gourd exhibit delayed and uneven germination for a period of four months due to the presence of hard seed coat that restricts water imbibition. In other words, the dormancy of snake gourd seeds is coat imposed or physical dormancy. Baskin and Baskin (2006) observed that seeds with physical dormancy have a water gap in the seed coat that opens in response to an environmental signal, thereby allowing water to enter.

Seed germination of ash gourd is very low and seed dormancy is one of the reasons for low germination (Robinson and Deckers, 1997; Malik *et al.,* 2001;

Nerson 2007; Doody and Conor, 2011; Bian *et al*. 2013). Seed dormancy of ash gourd due to thick seed coat lowers the seed germination (Rahman *et al*., 2014).

2.1.2 Dormancy due to chemical inhibitors

In many cases seed dormancy has been ascribed to the effect of substances that inhibit metabolic processes and inhibit growth. According to the promoter-inhibitor hypothesis of dormancy control, which was initially proposed for potato tubers by Hemberg (1949) and Amen (1968), the relative dormancy is given by a critical balance between the level of ABA, CK, and GA.

Among the most commonly reported is abscisic acid (ABA), which has been found to inhibit the RNA synthesis (Walbot *et al*., 1975; Ho and Varner, 1976), and to interact with gibberellins (GA) and cytokinins (CK) (Sussex *et al*., 1975; Karssen, 1976; Dunlap and Morgan, 1977; Khan, 1980/81). ABA can counteract the promotion of germination by red light and gibberlic acid (GA_3) in light-requiring seeds, and also the germination in darkness of some other seeds (Karssen, 1976). In *Chenopodium album* ABA has been found to hinder the embryo growth necessary to penetrate the coverings of the seed, although the initial events of embryo expansion are not prevented (Karssen, 1976).

Holm (1972) found acetaldehyde, ethanol, and acetone as volatiles that were delivered by *Abutilon theophrasti*, *Ipomoea purpurea* and *Brassica kaber* seeds, that where capable of restraining their germination.

Newton and Egly (1977) found that both dormant and non-dormant *Sida spinosa* seeds contained water-soluble inhibitors. Chemical exogenous dormancy caused by germination inhibitors in the seed coat can also be seen in vegetable crops, such as, spinach and parsley (Leskovar *et al*., 1999; Wien, 1997).

Inhibitors have been found in the seeds of such vegetable and flower families as Polygonaceae, Brassicaceae, Chenopodiaceae, Linaceae (Linum), Lamiaceae (Lavendula), Portulaceae (Portulaca), and Violaceae (Atwater, 1980).

Chemicals that accumulate in fruit and seed covering tissues during development and remain with the seed after harvest can likewise go about as germination inhibitors (Evenari, 1949). Fleshy fruits, or juices from them, can firmly repress seed germination as in *Cucumis*, and *Lycopersicon* species. Some of the substances connected with dormancy are various phenols, coumarin, and abscisic acid (Bewley and Black, 1982). Williams and Hoagland (1982) reported the combination of coumarin with p-hydroxybenzaldehyde to inhibit the germination of *Sesbania exaltata* and *Sida spinosa* to a greater extent than either compound alone.

Soaking of seeds with impermeable seed coat may result in substances leaching from the seed coat. If seeds germinate on leahing, an inhibitory substance may have been present. Very often inhibitors are present concurrently with other dormancy mechanisms. These substances need not be hormonal. *Sesbania punicea* seeds exude an orange substance upon imbibitions, which could be the inhibitory substance (Graaff and Stade, 1984).

Felix and Harr (1987) assessed the polyamine content in 15 wild species and 15 crop species from 13 families including Malvaceae, before and after germination and found a marked increase in polyamine content in the cotyledon or endosperm on germination.

According to Fischer *et al*. (1989) reported that seed germination of the dicotyledons and monocotyledons was both inhibited and promoted by compounds at concentrations as low as 1 μM.

Colorado *et al*. (1994) reported that expression of germination in the ABA-regulated clones was dependent on the presence of calcium ions, suggesting that calcium was involved in the response of the seeds to ABA. Presence of ABA led to 96 per cent dormancy in fresh seeds of brinjal var. Arkaneelkant (Yogeesha *et al*., 2002).

Hassan *et al.* (2003) reported that inhibitors present in the fruits of *Terminalia laxiflora* have inhibitory effect on the seed germination.

Alhadi *et al*. (2012) suggested that amino acid reserves in dry seeds are major determinants for germination capacity and germination behaviour in the germination of pomegranate.

Negi *et al.* (2014) reported that Nickel (Ni) when in excess, inhibits seed germination and reduces seedling growth in *Triticum aestivum*.

2.1.3 External factors (gas, light, temperature, RH, soil factors)

An inhibition of normal gas exchange may be an inhibition of oxygen entry into the seed or it may be of carbon dioxide exit from the seed into the atmosphere (Leggatt, 1948). Leggatt went as far as suggesting that the inability of the intact seeds to germinate could be explained in the first case by the lack of the necessary oxygen and in the second case by the presence of carbon dioxide which produces dormancy. Forward (1949) found that carbon dioxide induced dormancy in oats and that the dormant condition of freshly harvested grains was caused by the accumulation of carbon dioxide.

Wesson and Wareing (1969) found that a gaseous inhibitor, other than (CO_2), emanated from the seeds *Spergula arvensis* and accumulate in the soil atmosphere, thereby inducing dormancy in seeds. Carbon dioxide (CO_2) may be accumulated

within the seed as a result of its own metabolism, and may account for the inhibition of germination, although it is unlikely to be a primary cause of dormancy, and rather may be one of a complex of factors affecting the metabolism of dormant tissues (Villiers, 1975). Roberts and Smith (1977) stated that breaking of seed dormancy often requires easy availability of oxygen, which was provided by oxidants like KNO_3.

Seed dormancy may be imposed by mechanisms that require particular light conditions in order to be counteracted. This phenomenon has been referred to as positive photoblastism (Evenari *et al*., 1955). The suppression of germination of *Phacelia tanacetifolia* by white light increased with intensity and the inhibition was proportional to the duration of illumination (Schulz and Klein, 1963). Light may either inhibit or promote germination of dormant seeds. But not all light-sensitive seeds are advanced by white light. Some seeds, like *Nemophila*, *Nigella* and *Phacelia* are light-inhibited. This is apparently because of the far-red component of the white light (Black, 1970). Photoblastism is common among small seeded species such as *Amananthus retroflexus*, *Chenopodium album*, and many other annual weeds (Holzner *et al*., 1982; Grime, 1982; Smith and Morgan, 1983 and Gutterman, 1985).

When a few seeds are held at high temperature for a long time they become thermo-dormant. This dormancy instigated by high temperature can be overcome by special treatments before seeds will germinate at lower temperature (Vidaver and Hsiao, 1975). This damaging impact of high temperature may be the result of changes in the properties of membranes and compounds and protein denaturation (Hendricks and Taylorson, 1978; Christiansen and Foy, 2008; Levitt, 1969). Ladeira (1997) reported that freshly collected seeds of nightshade (*Solanum americanum*) exhibited dormancy at a temperature of 25^0C. In *Solanum physalifolium* freshly harvested seeds appeared to be dormant (Monte-del and Tarquis, 1997; Bithell *et al*., 2002; Andersson and Yahya, 2003). According to Baskin and Baskin (1998) *S. nigrum*

seeds are restrictively dormant and the sort of seed dormancy in *S. nigrum* and *S. sarrachoides* is non-deep physiological depending on conditions required to release dormancy

Ganar (2003) revealed that seeds extracted from fresh ash gourd fruits exhibited dormancy and showed a very low germination of five percent during *kharif* season and 10 percent during spring summer. Unnikrishnan (2005) also revealed endogenous dormancy in ash gourd, which is due to certain chemical blocks, caused by the presence of growth factors or due to deficiency of some essential compounds.

2.2 Seed invigoration to overcome seed dormancy

The invigoration implies an improvement in seed performance by any post-harvest treatments (physical, chemical and physiological) results in improved germinability, better field stand than the corresponding untreated seeds. Reduced seed vigour as a result of ageing can have a considerable effect on the timing of harvest, quality and yield of vegetable crops (Finch-Savage, 1994). Seed invigoration treatments were initially developed to overcome these problems and to improve both the rate and uniformity of germination in vegetable species.

The invigoration process was found to be independent of the cultivar, but seems to be best expressed in highly viable but low vigour seed lots. Several workers have reviewed the beneficial effects of seed invigoration or priming or pre-soaking treatments wherein bio-ingredients such as growth regulators, nutrients, antioxidants, osmoticums etc. are incorporated into the seed in order to improve its performance.

2.2.1 Effect of seed invigoration on germination

Ganar (2003) reported that seed invigoration treatment with 2% KNO_3 for 2 days and PEG (6000) 30% for 2 days improved per cent germination in ash gourd during climatic conditions of spring- summer season.

Caseiro *et al.* (2004) found that pre-sowing soaking was the most effective method for improving seed germination of onion, especially when the seeds were hydrated for 96 h compared to 48 h.

Invigoration treatment of Italian ryegrass (*Lolium multiflorum*) and sorghum (*Sorghum bicolor*) seeds with 20% PEG-8000 for 2 d at 10°C increased germination percentage, germination rate, seedling establishment and dry matter production under water stress, water logging, cold stress and saline conditions (Golezani *et al*., 2008).

Brinjal seeds when exposed to 12 hours of white light followed by 12 hours of red light, registered maximum germination percentage, root length, shoot length and vigour index (Menaka and Vanagamudi, 2008).

Pandita *et al*. (2010) reported that pre-sowing soaking enhanced the germination percentage of many field crops such as onion, carrot, muskmelon and sorghum.

Priming with low concentration salt (10 mM NaCl and 10 mM KNO_3) improved germination potential of tomato cultivars by decreasing MGT and increasing FGP, as well as GI (Nawaz *et al*., 2011).

Tomato seeds primed with KNO_3, ascorbic acid, salicylic acid and acetyl salicylic acid showed the maximum value of final germination percentage compared to unprimed seeds (Mirabi and Hasanabadi, 2012)

In bitter gourd maximum germination (%) was obtained in cultivar Punjab 14 (84.00 %) when the seeds were treated with KNO_3 followed by the cultivar Punjab 14 (83.67 %) and Green Special Long (83.67 %) (Kumar and Singh, 2013).

Vishwanath *et al*. (2014) reported that seed invigoration with NaH_2PO_4 (1%), KH_2PO_4 (0.5%), CaCl2 (1%) and PEG-6000 improved per cent germination in chilli. Chrysiansen and Foy (2008) and Hecht-Buchholz (1979) revealed that seed calcium fixation and germination rate were positively correlated which suggests the role of calcium as an important segment in membrane stabilization and as an enzyme co-factor.

Seed invigorated with KNO_3 (1%), KH_2PO_4 (1 and 2%), KCl (1%) and $MgCl_2$ (2%) significantly improved germination in cabbage (Batool *et al*., 2015).

According to Dutta *et al*. (2015) KH_2PO_4 (2 and 6 %) priming increased seed germination by 37.50 and 24.46 % respectively compared to control.

Nego *et al*. (2015) reported that the maximum value of standard germination was in statistical parity with standard germination values obtained in response to priming the onion seeds with distilled water for 12, 24 and 48 hrs, with 1% KNO_3 for 24 hrs, with 2% and 3% KNO_3 for 12 and 24 hrs as well as with standard germination recorded for the unprimed seeds (control treatment).

Okra seed priming with (osmo-priming with 5% PEG) for 24 hrs duration lead to better germination and tolerate to adverse environmental effects by increasing

activities of antioxidant enzymes of okra can be recommended to farmers (Kaur *et al*., 2015).

2.2.2 Effect of seed invigoration on speed of germination (germination index)

Nerson and Govers (1986) subjected the seeds of muskmelon to salt priming and found that 2-3% solutions of KH_2PO_4 + KNO_3 (1:1) for 1-5 days significantly increased and synchronization and emergence rate.

Germination index (GI), was significantly improved in rye seeds primed with 25 ppm salicylic acid at 10°C for 12 h (Ansari *et al*., 2013).

Kaya *et al*. (2006) and Basra *et al*. (2006) reported that the seeds of sunflower and wheat soaked in water could sprout faster and produced longer seedling under salinity stress, compared with untreated seeds.

Osmopriming of melon seeds with 1% KNO_3 resulted in maximum emergence index, to that of control (Farooq *et al*., 2007).

In soybean the highest germination index was attained from -1.2 osmotic potential and 12 h seed priming duration treatments. Mean while germination index decreased by osmotic potential reduction and increment of seed hydro priming duration (Sadeghi *et al*., 2011). Tavili *et al*. (2011) reported that speed of germination in *Bromus* spp. increased with seed priming treatments rather than that of control seeds while lettuce seeds treated with $CaCl_2$ showed maximum germination index and reduced mean time to emergence (Ahmed and Farag, 2011).

Canola seeds primed with PEG at -2 and -10 bars potential recorded the highest germination index (Aboutalebian *et al*., 2012). Osmo priming at 15±1°C for 24 h increased germination index (GI) as compared to the unprimed and other

treatments Ansari and Zadeh (2012) in rye. Hydropriming had also increased germination index (GI) in rye seeds (Ansari and Zadeh, 2012).

Pretreatment of wheat seeds with salicylic acid (14%) increased the germination index (16%) (Fateh *et al*., 2012).

The highest speed index was obtained from okra seeds primed with 3 % KH_2PO_4 solution at 25 and 30^0C (Sahib *et al*., 2013).

Dastanpoor (2013) revealed that maximum germination index was recorded for sage seeds hydroprimed for 12 h at 30°C, followed by hydropriming for 24 h at 10°C compared to unprimed seeds. This was due to, water uptake rate in priming period is slow and seeds had enough time to finish the pre-germination process (Varier *et al*., 2010).

The highest emergence index was noted in tomato seeds treated with 10 ppm Kinetin followed by priming with 10 ppm Kinetin (Nawaz *et al*., 2013).

Nezhad *et al*. (2013) stated that maximum germination index was observed for treatments with distilled water and 5% PEG during 12 hours in corn seeds.

Vishwanath *et al*. (2014) reported that speed of germination was highest in chilli seeds invigorated with KNO_3 (1%).

2.2.3 Effect of seed invigoration on coefficient of velocity of germination

According to Giri and Schillinger (2003) maximum coefficient of velocity of germination and vigour index were recorded from wheat seeds soaked in water for 12 h.

Coefficient of velocity of germination increase with priming treatments. In lettuce seeds the highest CVG were recorded by $CaCl_2$ treatment seeds (Ahmed and Farag, 2011).

Aboutalebian *et al*. (2012) reported that Hydropriming increased coefficient of velocity of germination in all of the cultivars of canola seeds under stress levels, from 44.61% in no-primd to 47.5% in hydroprimed seeds.

In rye seeds the highest seedling vigour index (SVI) and coefficient of velocity of germination (CVG) were attained from treatment, 75 ppm SA at 10°C for 12 h (Ansari *et al.,* 2013).

The highest CVG was obtained in tomato seeds, primed with KNO_3 in 50mM (Ebrahimi *et al*., 2014).

Jamadar and Chandrashekar (2015) revealed that castor seeds treated with CaCl2 @ 2.0 per cent solution took significantly less mean germination time but exhibited significantly higher daily germination index, coefficient of velocity of germination.

2.2.4 Effect of seed invigoration on mean time to germination

The reduction in mean time to germination in primed seeds might be due to increased rate of cell division (Bose and Mishra, 1992) and stimulation of metabolites activities during early phases of seed germination (Bradford, 1986; Taylor and Harman, 1990).

Kester *et al*. (1997) reported that priming with 3% KNO_3 reduced the mean time to germination in aged and non aged tomato seeds.

The earliness in emergence and lower mean emergence time due to seed priming induced a range of biochemical changes such as hydrolysis, DNA replication; increase RNA and protein synthesis enhanced the embryo growth, activation of enzymes, and reduced leakage of metabolites (McDonald, 2000).

Priming with PEG in wild rye resulted in higher superoxide dismutase and peroxidase activity that ultimately resulted in lower germination time (Jie *et al*., 2002).

Water-melon seeds treated with 3% KNO_3 solution increased germination per cent and decreased MGT (Demir and Mavi, 2004).

Gharib and Hegazi (2010) in bean (*Phaseolus vulgaris* L.) and Rouhi *et al*., (2012) in tall wheat grass demonstrated that priming reduced mean time to germination. Osmo priming enhances seedling development in various plant species by increasing the expression of aquaporins (Gao *et al*., 1999), change of ATP ase action, RNA and corrosive posphathase combination (Fu *et al*., 1998), and also by enhancing amylases, lipases and protease synthesis (Ashraf and Foolad, 2005).

The reason for early emergence of the primed seed was due to the completion of pre-germination metabolic activities making the seed ready for radical protrusion and the primed seed germinated soon after planting compared with unprimed seed (Arif, 2005). Ruan *et al*. (2002) also reported higher EI rates in rice seeds treated with $CaCl_2$. Similarly enhanced EI and reduced seedling emergence time has been reported in seeds primed with $CaCl_2$ (Farooq *et al*., 2006).

Guzman and Olave (2006) reported that seed priming with KNO_3 improved germination rate and germination index.

Dezfuli *et al*. (2008) reported that maize seeds hydroprimed for 36 h had the lowest values (T_{50} and MGT), followed by 24 h and 48 h seed treatments.

Dezfuli *et al*. (2008) revealed hydroprimed seeds for 36 hours had lowest values (T_{50} and MGT) while Farooq *et al*., (2008) reported that seed priming decreased the mean time to germination.

Primed seeds had lower mean germination time than control seeds (Sadeghi *et al*., 2011, Saglam *et al*., 2010, and Dezfuli *et al*., 2008). Such positive effects were due to stimulatory impacts of seed priming on biochemical activities and meiosis during primary phases of germination (Sivriteps *et al*., 2000).

Mohseni *et al*. (2010) observed that corn seeds treated with 2% exhibited the least minimum mean time to germination. .

Cotton seeds priming with 3 g KNO_3 showed reduced MGT compared to unprimed seeds (Ahmadvand *et al.,* 2012). The accelerated germination of treated seeds is due to increased rate of cell division (Bose *et al*., 1992) and stimulation of metabolic activities during early cycles of seed germination (Taylor *et al*., 1990 and Bradford, 1986)

Afzal *et al*. (2011) revealed that halopriming with 50 mM $CaCl_2$ and 1% KNO_3 triggered higher germination and reduced MGT in tomato cv. Roma, while, Nawaz *et al*., (2013) observed lowest MET in tomato seeds primed with 10 ppm Kinetin followed by 50 ppm kinetin. Rehman *et al*., (2011) reported that seed priming enhanced the crop stand and seedling vigour and osmopriming with $CaCl_2$ by re-drying was the most effective to enhance the seedling establishment as evident from reduced values for the MET and higher EI in rice.

Lentil seeds soaked in water for 24 h increased the germination percentage and decreased mean germination time compared with seeds primed for 12 h. This outcome demonstrated that longer priming time may overcome antagonistic impacts of diminished water potential in osmo-priming treatments (Yucel, 2012).

Aboutalebian *et al*. (2012) reported that hydropriming reduced mean germination time in all canola cultivars and stress levels from 2.28 to 2.16 days (about 5.5%).Osmopriming reduced mean time to germination (MTG) in rye seeds as compared to the unprimed seeds (Ansari and Zadeh, 2012).

Dastanpoor (2013) stated that lowest MGT (4.76 days) was recorded in sage seeds hydroprimed for 12 h at 30°C compared to untreated seeds of sage (*Salvia officinalis* L.).

The minimum mean germination time was observed for treatment with 1%KNO3 during 24 hours. Mean germination time for treated seeds is less than untreated seeds (Nezhad *et al*., 2013) in corn.

The minimum mean time to germination (MTG) in rye seeds was recorded in seeds primed with 75 ppm salicylic acid at 10°C for 12 h (Ansari *et al*., 2013).

Kaur *et al*. (2015) reported that okra seeds soaked in water for 6 hrs duration recorded minimum germination time when compared to unprimed seeds.

Mahboob *et al*. (2015) revealed that maize seed osmoprimed with $CaCl_2$, under late sown conditions resulted in minimum mean time to germination over control.

2.2.5 Effect of seed invigoration on energy of germination

Farooq *et al*. (2007) revealed that osmopriming melon seeds with KNO_3 and 1% $CaCl_2$ resulted in improved germination percentage and energy of germination compared with other treatments.

In soybean the highest energy of germination (EG) was obtained from -1.2 osmotic potential and seed hydroprimed for 12 h (Sadeghi *et al*., 2011).

Marigold seeds 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. This improvement in germination and emergence performance of primed seeds was due to repair mechanism, mobilization of storage reserves for utilization during germination and dormancy breakdown (Burgass and Powell, 1984 and Hocart *et al*., 1990).

Yousof (2013) found that rice seed osmopriming with $CaCl_2$ (-1.00 MPa) increased energy of germination, coefficient of velocity of germination and reduced mean time to germination.

Halopriming seeds of judas tree with KNO_3 increased seed germination percentage, speed, and energy of germination and decreasing mean time to germination (Haroni *et al*., 2015).

2.2.6 Effect of seed invigoration on seedling growth

Hopper *et al*. (1979) reported that in primed seeds, radical and plumule appeared faster because of more water uptake efficiency and metabolic activity during germination.

Jagadesh *et al*. (1994) observed significant improvement in seedling size of tomato, capsicum and onion seeds when primed in KH_2PO_4.

Muskmelon seed priming with KNO_3 increase root and shoot length compared to other priming treatments like PEG and mannitol (Nascimento, 2005), while, The significant increase in shoot and root length in primed seeds may be due to its involvement in cell elongation or cell division and meristematic growth (Khan *et al*., 2006).

Under non-saline conditions radicle length and shoot length increased significantly when snake gourd seeds were pre-treated with KNO_3 or NaCl (Gharahlar *et al*., 2010).

Osmopriming with KNO_3 and 3% $CaCl_2$ resulted in improved root length compared with untreated seeds but 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).

The osmopriming and hydro priming have been reported to promote vigorous root growth and shoot growth in soybean and cumin (Yuan-Yuan *et al*., 2010).

Seed priming with 25 mM KNO_3 gave lower values of E50, MET and higher values of FEP root and shoot length and seedling fresh and dry weights as compared with other primed or nonprimed seeds of both cultivars (Nawaz *et al*., 2011).

Priming improved the shoot length, results showed the same pattern for shoot length as well as root length is maximum in seeds primed in potassium nitrate (2.56 cm) followed by those primed with acetylic salicylic acid (2.43 cm) and PEG-6000 (2.21 cm), statistically at par with each other (Mirabi and Hasanabadi, 2012).

The maximum speed of emergence (34.85) and longest root length (5.36 cm) and shoot length was recorded in chilli seeds halo primed with KNO_3 (Maiti *et al*., 2013).

The cytokinins treatments showed significant effect on shoot length. The maximum shoot was found in the seeds treated with 10 ppm Kinetin followed by priming with 50 ppm Kinetin (Nawaz *et al*., 2013).

Sahib *et al*. (2014) stated that seed priming caused significant increase in seedling growth. Increased osmo priming concentration (3% KH_2PO_4) at both 25 and 30^0C temperatures produced highest seedling length of okra 13, 14.83cm respectively compared to control 5.33cm.

Radicle length of cabbage was increased significantly in response to seed priming with KH_2PO_4 (1, 2 and 3%) and KCl (1, 2 and 3%) (Batool *et al*., 2015).

2.2.7 Effect of seed invigoration on seedling vigour

Yogananda *et al*. (2004) found that bell pepper seeds invigorated with KNO_3 (1.0%) recorded higher seedling vigour index over control.

Seedling vigour index of germinating seeds have profound influence on the establishment and yield of crops. Priming increases the vigour index by enhancing the capability of the plants to compete for the basic needs of nutrients, water and light (Tabrizian and Osareh, 2007).

Lentil seed germination rate and seed vigour index were significantly affected by seed priming. The highest germination rate and vigour index obtained for seeds primed with water (Golezani *et al*., 2008).

Maiti *et al.* (2009) studied the effect of priming on seedling vigour and productivity of tomato, chilli, cucumber and cabbage during post-rainy seasons demonstrating that priming improved germination and seedling development and yield of these vegetable species.

In bitter gourd the maximum seedling vigour index-II was recorded in cultivar Jamunpuri Long, when the seeds were primed with KH_2PO_4 followed by Katahi with GA_3 and Green Special Long with KH_2PO_4, which were significantly higher than other seedling vigour index-II value in interaction (Kumar and Singh, 2013)

Nawaz *et al.* (2013) stated that germination and seedling vigour may be enhanced by seed pretreatment with cytokinins in both the cultivars of tomato (Nagina and Pakit) by dormancy breakdown. However kinetin with 10 ppm was more effective than all other cytokinins treatments.

The improvements in germination rate and vigour index of KNO_3 priming compared with nonprimed controls were possibly caused by the decreased lipid peroxidation and increased antioxidative activities (Dong *et al*., 2014).

Rahman *et al*. (2014) suggested that the vigour index was minimum (0.82) in the seeds of 1 month storage and increased gradually with the increasing of storage period. Finally, after 7 months storage seed showed maximum vigour index and it was 1.69. The reason for increasing germination and vigour index might be due to the elimination of dormancy of the ash gourd seeds with the increase of their storage period.

Vigour index of cabbage cultivars was not significantly enhanced in response to priming except with KNO_3 (1%) and KH_2PO_4 (2%) which substantially improved seedling vigour of Green Ball and Golden Acre (Batool *et al*., 2015).

Seed priming and soaking durations had significant effect on seedling vigour index. In okra maximum seedling vigour index as recorded in (osmo-priming with 5% Polyethylene glycol), followed by (hydropriming), osmopriming with 10% polyethylene glycol while it was minimum in unprimed seeds. (Kaur *et al*., 2015).

Vigour index was significantly influenced by both the main effect of priming duration as well as its interaction with the concentration of the priming salt (KNO_3). Increasing the concentration of the potassium nitrate salt as well as the priming duration resulted in significant decrease trend in vigour Index. Thus, in okra the highest vigour index II was recorded for seedlings raised from seeds primed with distilled water for 48 and 72 hrs, seeds with 1% KNO_3 for only 12 hrs, with 2% KNO_3 for 24 and 48 hrs and with 3% for 12 and 24 hrs (Nego *et al*., 2015).

2.2.8 Effect of seed invigoration on seedling dry weight

Patel and Saxena (1994) reported that fresh and dry weight increased in seedlings raised from seeds treated with kinetin and GA_3 as compared to untreated seeds.

Snake melon seed priming with KNO_3 decreased MGT and increased seedling dry weight under salinity stress compared to other priming treatments (Gharahlar, 2010). Osmopriming with 1% KNO_3 resulted in increased seedling dry weight (Farooq *et al*., 2013).

Dry weight of seedlings was significantly affected by different cytokinins priming treatments. Hormonal priming with 10 ppm cytokinin gave maximum dry weight followed by priming with 10 ppm BAP (Nawaz *et al*., 2013).

Sahib *et al*. (2014) observed that okra seeds primed with 3% KH_2PO_4 at 25 and 30^0C temperatures had significant increase in dry weight (0.0202g, 0.0201g) when compared to control. Similar results were also observed by Saleem *et al*. (2014) in varieties Green Special Long (1.54 g) and Katahi (1.53 g).

Highest seedling dry weights were recorded for seedlings of onion seeds primed with distilled water for 48 and 72 hours, with 1% KNO_3 for the duration of 12 hours, with 2% KNO_3 for all durations, and with 3% KNO_3 for the durations of 12, 24, and 72 hrs (Nego *et al*., 2015).

2.2.9 Effect of seed invigoration on electrical conductivity of seed leachate

The increase in electrical conductivity values with increase in storage period. This increase in electrical conductivity values with increase in period of storage was attributed to membrane aberrations seeds (Berjak and Villiers, 1972).

Seed priming with cytokinin resulted in lower EC of seed leachates primarily due to improved membrane repair in treated seeds as reported by Rudrapal and Nakamura, (1988) for eggplant and radish.

According to Borji *et al*. (2007) an increase in seed coat thickness resulted in an increase in the availability of physiochemicals (water soluble compounds) within the seed coat that play an important role in the seed and its integrity when soaked in water. Therefore, seeds with thicker seed coats exude more physiochemicals thus giving off a high EC value.

Electrical conductivity of seed leachates was also influenced by seed priming. Conductivity of lentil seeds primed with water and PEG was lower than that of the

other primed and unprimed seeds. The highest conductivity was observed for seeds primed with KNO_3 (Golezani, 2008).

Bijanzadeh *et al*. (2010) observed that hydropriming and solid matrixpriming had a positive effect on membrane stability and minimized the seed electrical conductivity in rapeseed.

The electrolyte leakage increased with increasing imbibition period including all treatments and control. After a longer period of imbibition from 1 to 24 h, all the priming treatments lowered down the electrolyte leakage in the seeds of tomato cultivars. However, significantly lower electrolyte leakage was observed in seeds exposed to 25 mM KNO_3 on all measuring periods (Nawaz *et al*., 2011).

Pre-sowing cytokinins seed treatments were helpful in lessening electrolyte conductivity of seed leachates. Maximum decrease in electrolyte leakage was induced by 10 ppm cytokinin on all measuring periods. Seed priming with 10 ppm cytokinin followed by 50 ppm cytokinin was successful in decreasing electrolyte leakage (Nawaz *et al*., 2013).

2.2.10 Effect of seed invigoration on seed coat parameters

The seed coat imposed dormancy of *Acacia negrii* can be broken by mechanical scarification, acid treatment, boiling water and dry heat treatment (Demel, 1997).

Baptisia australis seeds subjected to acid scarification or immersion in hot water reduced thickness of testa and improved the germination percentage (Boyle, 2005).

Maiti *et al*. (2009) submerged seeds in water for 12-24 hours, to successfully break dormancy in sunflower seeds. Priming works first by initiating the germination process. Further, it works by washing away ABA and other compounds that have negative effects on germination. Lastly, imbibition weakens teguments and hard structures, thereby removing physical dormancy, while, Pallavi *et al*. (2010) applied temperature (60^{o}C) for 15 minutes to break dormancy of sunflower seeds. Temperature treatments increased germination over control by desiccating waxes, weakening the impermeable layer, and allowing water to be absorbed.

In *Cassia fistula* Soliman and Abbas (2013) found that acid scarification of seeds for 2 minutes and then soaking in hot water at 100^{0}C for 6 minutes resulted in reduced seed dormancy through softening the seed coat.

2.3 Storage potential of invigorated seeds

The storability of a seed of a given kind is primarily determined by the vigour of the seeds at maturity and level of deterioration at the time it enters storage. Loss of storage potential after invigoration of seed hence should be widened to include the compliex subject of seed deterioration (Ching and Schoolcraft, 1968).

Black and Bewley (2000) reported that seed invigoration will cause seeds to be repaired and be protected from deterioration injuries like mitochondria and enzymes inactivity, membrane damages during seed storage and aging.

Araujo *et al*. (1982) found increased seed germination with increasing fruit age and storage length in cucurbits.

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 to reduce the rate of deterioration of invigorated seeds during storage (Chauhan *et al*., 1984).

Oluoch and Welbaum (1996) reported that osmotic priming had a deleterious effect on the seed storage life of muskmelon seeds, where, Drew *et al*., (1997) opined that invigorated onion and leek seeds stored at 10°C, maintained viability after one year of storage.

Gayathri and Kalappa (2002) invigorated the seed lots of tomato hybrid, Akash differing in germination percentage using different methods. Invigoration with selected chemicals, humidification (24 hours at 95% RH) and hydration-dehydration for two hours at 1:1 (wt/vol.) seed to water ratio followed by shade drying (43 hours) was tried before storing at room temperature (25^0C) for four months period. Seed invigoration with one per cent KNO_3 showed enhanced performance even after storage.

Gurusinghe *et al*. (2002) reported that when primed seeds are slowly dried back, it induces synthesis of LEA (late embryogenesis abundant) proteins, while rapid drying at higher temperatures may induce heat shock proteins, promoting protective mechanisms which increased the seed storage life.

The study on storability of invigorated snake gourd seeds revealed that the germination percentage showed a declining trend during storage. The overall germination percentage was 62.83 at 1MAS which reached 39.66 per cent after 6 months. There was a significant decrease in seed quality parameters of invigorated treatments in the storage which could be attributed to either one or a combination of the factors like accumulation of toxicants and corrosive action caused by acids, membrane degradation which resulted in greater leakage of sugars, amino acids and

inorganic solutes from the seed, free radicle damage formed due to lipid peroxidation, impaired enzymatic activity and increase in respiratory quotient (Mohan, 2005).

Schwember and Bradford (2005) reported that irrespective of drying rates, longevity of lettuce seeds were lower than untreated seeds.

Mubshar *et al*. (2006) stated that improvement in priming is largely dependent on factors such as plant species, water potential of priming agent, priming duration, temperature, seed vigour and storage condition of primed seed.

Hydropriming and solid matrixpriming had a positive effect on membrane stability and minimized the seed electrical conductivity in rapeseed (Bijanzadeh *et al*., 2010).

According to Olasoji *et al*. (2012) seed deterioration in *Hibiscus cannabinus* seeds during storage could be minimized by harvesting at optimum stage. Seeds harvested five weeks after flowering and stored at 10^0C showed the highest seed viability.

Amaranth seeds stored under ambient conditions retained viability upto three months on seed invigoration (Kehinde *et al*., 2013)

Kuppusamy and Ranganathan (2014) observed that improvement in seed quality with good storability can be obtained in okra by subjecting the seeds to sand matricpriming (60 % WHC) for 3 h while for beet root seeds, hydropriming for 12 h in water (double the volume of seeds) was found to be feasible.

In ash gourd the speed of germination increased gradually with the increasing of storage period and it might be due to decrease of seed dormancy with the

advancement of storing time at ambient temperature. However, the increment of seed germination percentage after 7 months of storage was 204.20 per cent higher compared to 1 month after storage (Rahman *et al*., 2014).

Sheidae *et al*. (2015) stated that high osmotic potentials had a negative effect on germination of soybean seeds, probably due to oxygen deficiency and inadequacy of water adsorption and -8 bars osmo-priming is a proper treatment for stored soybean seeds. On the other hands it seems that by increasing the storage duration, initiation of germination processes and water absorption will delay, so more time is required for osmo-priming treatment of seeds. Therefore 18 and 30 months stored seeds indicated the best efficiency at 18 and 24 hours of osmo-priming treatment.

Materials and methods

3. MATERIALS AND METHODS

The present investigation 'Seed invigoration to overcome dormancy in ash gourd (*Benincasa hispida* (Thunb.) Cogn.)' was conducted in Kerala Agricultural University (KAU) during 2014-16. The study involved assessing the effect of seed invigoration treatments on dormancy in ash gourd variety KAU Local. The impact of the treatments on viability, seed quality and performance of seedlings over storage was also assessed. The details of materials used and the techniques adopted for the study are described hereunder.

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. Vellanikkara 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 56.00 per cent (January, 2016) and 85.00 per cent (June and July, 2015) while rainfall varied from 9.00 mm (Febrauary, 2016) to 629.8 mm (May, 2015) respectively during the study. The monthly mean maximum temperatures ranged from 30.3^{0}C (July, 2015) to 35.3^{0}C (February, 2016), while the range in mean minimum temperature was between 23^{0}C (January, 2016) and 24.7^{0}C (May, 2015).

3.2 Experimental material

Seed of ash gourd variety KAU Local was collected immediately after extraction from the winter crop (December to March, 2015) raised at Krishi Vigyan Kendra, Thrissur. The seeds were manually extracted from the fruit without causing mechanical damage. The seeds were washed thoroughly with clean water to remove any mucilage and fruit pulp adhering to them before initiating the study.

3.3 Experimental method

3.3.1 Treatment details

The experiment was conducted following a completely randomized design with 15 treatments replicated thrice.

The seeds were subjected to seed invigoration treatment as specified in Table 1. The primed seeds were compared to unprimed seeds that served as the control.

Table 1: Treatment details

Treatments	Details
T_1	Hydropriming (for 24 hours)
T_2	Thiourea (0.5% for 24 hours)
T_3	KNO_3 (0.4% (8 mM) for 24 hours)
T_4	KNO_3 (0.7% (15 mM) for 24 hours)
T_5	KH_2PO_4 (10^{-1} M for 24 hours)
T_6	Vinegar pH 3.7 for 2 hours
T_7	Polyethylene glycol (PEG 6000 @-0.5 MPa for 24 hours)
T_8	Salicylic acid (60 ppm for 12 hours)
T_9	Salicylic acid (60 ppm for 12 hours)
T_{10}	Cytokinin (kinetin) (10 ppm for 12 hours)
T_{11}	Cytokinin (kinetin) (10 ppm for 24 hours)
T_{12}	$CaCl_2$ (50 mM for 12 hours)
T_{13}	$CaCl_2$ (50 mM for 24 hours)
T_{14}	*Pseudomonas fluorescens* ($1x10^6$ cfu.ml^{-1} for 12 hours)
T_{15}	*Pseudomonas fluorescens* ($1x10^6$ cfu.ml^{-1} for 24 hours)
T_{16}	Absolute control

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3.3.2 Seed treatment procedure

During priming, seeds were soaked in the respective priming agent in the ratio 1:2 on volume basis for the period specified (Table 1). In case of hydropriming, water was used as the priming agent. The primed as well as unprimed seeds were shade dried at room temperature to $\leq$ 8 per cent moisture.

3.3.3 Method of storage

After seed invigoration treatments, seeds were dried to 8 per cent moisture. Seeds required for recording observations at monthly intervals in each replication of a treatment were packed in separate polythene bag (400 guage) in order to avoid moisture fluctuations. The packed seeds were stored under ambient condition.

3.4 Observations recorded

Seed samples were drawn and evaluated for quality parameters immediately after extraction, as well as immediately after seed treatment. Observations were also recorded on alternative days after seed invigoration upto a 35 day period. Seed quality parameters were recorded at the start of storage period and subsequently at monthly intervals up to ten months. Histochemical studies to analyse the changes that occur in the dimensions of embryo and proportions of different fractions of seed coat were studied using microtomy and image analysis (stereo and light microscopy) at bimonthly intervals.

3.4.1 Germination (%)

The germination test was conducted by following the procedure outlined in ISTA (2010) rules using sand medium. Four replicates of 100 seeds each were germinated in a germination room maintained at 25±2°C temperature and 90±3% RH.

At the end of germination period, the number of normal seedlings in each replication were counted and the germination was calculated and expressed in percentage.

3.4.2 Root length of seedling (cm)

At the end of germination test period *i.e*., on the 14^{th} day, ten normal seedlings were carefully uprooted at random from the test sample and the length between collar region and tip of the root was measured as root length and expressed in cm.

3.4.3 Shoot length of seedling (cm)

From the same sample, after measuring root length, the length between collar region and tip of the leaf was measured in cm and the mean value was recorded as shoot length.

3.4.4 Seedling dry weight (mg)

Five normal seedlings were dried at first for six hours and that in hot air oven kept at 85^0C for 24 hours. Dried seedlings were cooled in desiccators for 45 minutes and the dry weight of single seedling was expressed in milligram.

3.4.5 Speed of germination (Germination index)

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

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

X_1- Number of seeds germinated at first count

X_2- Number of seeds germinated at second count

X_n- Number of seeds germinated on n^{th} 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 n^{th} count

3.4.6 Coefficient of velocity of germination (%)

This is another index of seed germination speed and velocity (Scott, *et al*., 1984), and calculated by:

Coefficient of velocity of germination (CVG) $= \frac{G1+G2+G3+--+Gn}{(1xG1)+(1xG2)+--+(nxGn)} x100$

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

3.4.7. Mean time to germination (days)

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

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

Where,

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

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

3.4.8 Energy of germination

Energy of germination was recorded fourth day after planting. It is the percent of germinating seeds 4 days after planting relative to the total number of seeds tested (Ruan et *al*., 2002).

3.4.9 Vigour index- I

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

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

3.4.10 Vigour index- II

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

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

3.4.11 Electrical conductivity of seed leachate (dsm^{-1})

Three replicates of twenty five seeds from each treatment were placed in beakers and prewashed well with distilled water to remove the adhering dirt, soil and chemicals. The seeds were then soaked in 25 ml of distilled water for 24 h by occasionally stirring the contents at room temperature. The beakers containing soaked seeds were covered to reduce evaporation and other probable contamination. The seed leachate was filtered and collected in 50 ml beaker from which EC was estimated. The electrical conductivity of seed leachate was measured in an advanced conductivity meter (EVTECH CON-510) with cell constant of terminal 0.1. The electrical conductivity of seed leachate was expressed as desi Simons per meter (dSm^{-1}) (Jackson, 1973).

3.4.12 Seed moisture content (%)

Five gram of ground seed materials were 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, they were cooled in a desiccator for 30 min. The weight of the seeds before and

after drying was recorded in gram. The moisture content of the seed was calculated using the following formula and expressed as percentage (ISTA, 2000).

$$\text{Moisture content (\%)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

Where,

M_1 – Weight of moisture bottle alone

M_2 – Weight of bottle + Seed sample before drying

M_3 – Weight of bottle + Seed sample after drying.

3.4.13 Seed infection (%)

Storage fungi present on seeds were tested using blotter method as prescribed by ISTA. 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 percentage. Besides, the kind of fungi present was also identified and documented (ISTA, 2000).

3.4.14 Seed coat thickness (µm)

Uniform, bold, undamaged and healthy seeds from each treatment were selected. Further seed length and embryo length of selected seeds were determined using Vernier calliper and image analyser using the software 'Labomed Digipro-2' at 4X magnification. Ten uniform seeds were selected for further studies.

Due to hard seed coat the selected seeds were subjected to tissue processing and embedding before taking microscopic thin (2-5 μm) serial sections of seeds. The procedure involving fixing, tissue processing and staining is detailed below.

3.4.14.1. Killing, fixing and aspiration

FAA (Formalin Acetic acid Alcohol) was used for killing and fixing of samples. The samples were aspirated in an aspirator (samples kept in tubes with FAA and suction given. Bubbles will be seen coming and when they stop coming it marks the end of aspiration) and kept in FAA itself for 24 hours and then subject to infiltration in a tissue processor.

3.4.14.2. Dehydration in alcohol series

After aspiration process, seeds were soaked in ethyl alcohol series (30%, 50%, 70%, 95% and absolute alcohol) for one hour.

3.4.14.3. Infiltration of paraffin (58-60^{0}C) in the paraffin solvent media

After dehydration, seeds were infiltrated in tertiary butyl alcohol (TBA) and paraffin wax for three hours (TBA, TBA+ Wax (3:1), (1:1) and Paraffin wax).

3.4.14.4. Sectioning/ microtomy

Sections were taken using a sliding microtome, Leica SM 2000 R at the Department of Wood Science, College of Forestry, Vellanikkara.

3.4.14.5. Staining and mounting

Before staining the wax has to be completely removed from the sections using xylene and water. The slides were dewaxed, the slides were transferred to a mixture

of absolute alcohol and xylene, then to absolute alcohol and to water. After wax has been completely removed the sections were stained and treated with xylene. A thin coat of Dibutyl Phathalate Xylene (DPX) was placed on top of the slide to make permanent slides.

The slides were examined under a digital microscope (LABOMED Digi 2 1500) attached to an image analyser system using the software DigiPro, version 4.0. The seed coat thickness was noted by measuring thickness of testa and tegmen layers separately as well as total thickness of these layers. Embryo length was also recorded. Three measurements were taken from three sections of each seed and averages of ten seeds were noted from each treatment. The thickness was expressed in μm, under 4X magnification.

3.5 Statistical analysis

Statistical analysis of the data on seed quality parameters was performed using SPSS package.

3.5.1 Anova for completely randomized design

The data recorded in each experiment was analyzed using one way ANOVA. The mean squares due to different sources of variation were worked out using the following analysis of variance (Gomez and Gomez, 1976).

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.

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

Step 1: Rank all the treatments means in decreasing (or increasing) order. It is customary to rank the treatments means according to the order of preference.

Step 2: Compute the s_d value following the appropriate procedure

$$Sd = \sqrt{\frac{2S^2}{r}}$$

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

$$Rp = \frac{(rp)\ (sd)}{\sqrt{2}} \quad \text{for } p = 2, 3,..., t$$

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 two means with consecutive rankings and p = t for the highest and lowest means).

Step 4: identify and group together all treatment means that do not differ significantly from each other.

Step 5: Use the alphabet notation according to the ranking to present the test results.

3.5.3 Correlation studies

Correlation coefficients were calculated using the method by Panse and Sukhatme, (1978).

Correlation coefficient, $r_{(X.Y)} = \frac{\delta XY - \frac{(\delta X)(\delta Y)}{n}}{\sqrt{(SS_x)(SS_y)}}$

Where,

$\partial_{(X.Y)}$ = Covariance between X and Y

$r_{(X.Y)}$ = Coefficient of variation between traits X and Y

Results

4. RESULTS

The present investigation 'Seed invigoration to overcome dormancy in ash gourd (*Benincasa hispida* (Thunb.) Cogn.)' was carried out in ash gourd variety KAU Local during 2014-16 immediately after seed extraction to elucidate the effect of seed invigoration on dormancy in ash gourd and to ascertain the anatomical changes in seed coat on seed treatment. Assessment of the storage potential of treated seeds under ambient conditions was also envisaged in the study. The results are presented in this chapter.

4.1 Effect of seed invigoration treatments on seed dormancy

The seed quality parameters before and upto 35 days after seed invigoration is enumerated below.

4.1.1 Seed quality before seed invigoration

The seed quality parameters prior to seed invigoration are presented in Table 2. The moisture content of seeds immediately after extraction was 15 per cent and it exhibited a low germination per cent of 7.00. The seedling shoot length, seedling root length, seedling dry weight, vigour index I, vigour index II and electrical conductivity of seed leachate were 18.50 cm, 4.78 cm, 0.026g, 9162.96, 0.18 and 0.038 dSm^{-1} respectively. The thickness of seed coat was 453.30 µm, with testa and tegmen measuring 390.80 µm and 62.50 µm respectively. The length of embryo (EL) measured 4061.70µm against a seed length (SL) of 11447µm, the ratio of EL/SL being 0.354µm. The per cent seed infection by microflora was 1.50 per cent.

4.1.2 Seed quality parameters within 35 days of seed invigoration

The results of the impact of invigoration treatments on seed quality parameters within 35 days of seed invigoration are presented in Tables 2 to 9 and detailed below.

Table 2. Seed quality parameters before invigoration

Parameter	Details
Germination (%)	7.00
Germination index	0.83
Coefficient of velocity of germination (%)	12.84
Mean time to germination (days)	8.01
Seedling shoot length (cm)	18.50
Seedling root length (cm)	4.78
Seedling dry weight (mg)	0.0261
Vigour index-I	9162.96
Vigour index-II	0.18
Electrical conductivity of seed leachate (dsm^{-1})	0.0287
Total seed coat thickness (µm)	453.30
Thickness of testa (µm)	390.80
Thickness of tegmen (µm)	62.50
Embryo length (µm)	4061.70
Seed length (µm)	11447
Embryo length / seed length ratio (µm)	0.354
Seed infection (%)	1.50
*Moisture content (%)	15.00

* Seeds were dried to $\leq$8.00 per cent moisture content before packing

4.1.2.1 Analysis of variance

The analysis of variance revealed that, there existed significant differences in seed quality parameters like germination per cent, germination index, mean time to germination, coefficient of velocity of germination, energy of germination, seedling vigour index I and II among various seed treatments within 35 days of invigoration.

4.1.2.1.1 Germination (%)

The effect of seed invigoration on germination was found to be highly significant upto 35 days after invigoration (DAI). Germination (Table 3) was improved by 35 days of seed invigoration in both treated and untreated seeds. In most treatments germination initially increased progressively and later decreased marginally, the exceptions being T12 ($CaCl_2$ 50 mM for 12 hours), T11 (kinetin 10 ppm for 24 hours) and T14 (*Pf* $1x10^6$ $cfu.ml^{-1}$ for 12 hours). Germination in these treatments (T12, T11 and T14) exhibited an increasing trend during the 35 day period after invigoration. However, seeds treated with salicylic acid (T8: salicylic acid 600 ppm for 12 hours and T9: salicylic acid 600 ppm for 24 hours), germinated only on 31^{st} and 35^{th} DAI, while in those treated with vinegar at pH 3.7 for 2 hours (T6), there was a sharp decline in germination after the initial increase. Germination in these treatments was inferior to control throughout the period of observation. Between 1^{st} DAI and 35^{th} DAI, germination in T6 varied from 5.00 per cent to 28.00 per cent.

Germination reached above the minimum standards for seed certification (MSCS) prescribed for ash gourd i.e., 60 per cent on the 11^{th} day after invigoration (DAI) in treatments T5 (KH_2PO_4 0.4% for 24 hours) and T11 (kinetin 10 ppm for 24 hours), while in majority of the treatments MSCS was reached on the 13^{th} DAI. These included seed invigoration with KNO_3 0.4 % for 24 hours (T3), KNO_3 0.7% for 24 hours (T4), kinetin 10 ppm for 12 hours (T10), $CaCl_2$ 50 mM for 12 hours (T12), $CaCl_2$ 50 mM for 24 hours (T13), *Pf* ($1x10^6$ $cfu.ml^{-1}$ for 12 hours: T14) and *Pf* ($1x10^6$

cfu.ml^{-1} for 12 hours: T15). In treatments T1 (hydropriming (12 hours), T2 (thiourea (0.5% for 24 hours), T7 (polyethylene glycol 6000 @-0.5 MPa for 24 hours) and untreated seeds (T16), germination reached above MSCS on the 15th, 17th, 19th and 35th DAI respectively. However, in seed treatments with salicylic acid (T8: salicylic acid 60 ppm for 12 hours and T9: salicylic acid 60 ppm for 24 hours), and those treated with vinegar at pH 3.7 for 2 hours (T6), germination did not reach the MSCS within this period. Germination in these treatments was inferior to control throughout the period of observation.

Higher germination was observed on seed invigoration compared to untreated control over the 35 day period, the exceptions being T8, T9 and T6. The average germination in seeds treated with salicylic acid 60 ppm for 24 hours (T9: 5.00%), salicylic acid 60 ppm for 12 hours (T8: 7.12%), vinegar at pH 3.7 for 2 hours (T6: 26.75%) and control (T16: 34.84%) was less than 50 per cent. The mean germination over 35 days of invigoration was high in seeds treated with $CaCl_2$ 50 mM for 12 hours (T12: 74.40%), *Pf* 1x10^6 cfu.ml^{-1} for 12 hours (T14: 73.86%), $CaCl_2$ 50 mM for 24 hours (T13: 73.46%), KH_2PO_4 10^{-1}M for 24 hours (T5: 73.11%) and T11 (72.28%). Germination in T12 had increased from 15.00 to 82.50 per cent, from 25.00 to 80.40 per cent in T13, from 20.00 to 79.80 per cent in T14, from 20.00 to 76.60 per cent in T11 and from 15.00 to 68.50 per cent in T5 within 13 DAI while the increase was from 5.00 to 27.00 per cent in control.

Seeds treated with salicylic acid (T8: salicylic acid 600 ppm for 12 hours and T9: salicylic acid 600 ppm for 12 hours) ceased to germinate on 35th DAI, while in T6 it decreased to 28.00 per cent by 35th DAI. Germination at 35th DAI in seed invigoration treatments other than T8, T9 and T6, varied between 62.60 per cent (T7: polyethylene glycol 6000 @ -0.5 MPa for 24 hours) and 100.00 per cent (T14: *Pf* 1x10^6 cfu.ml^{-1} for 12 hours) in treated seeds while it was 68.80 per cent in untreated seeds.

Table 3. Germination in ash gourd after seed invigoration

Treatments	Germination (%)																		Mean
	1st DAI	3rd DAI	5th DAI	7th DAI	9th DAI	11th DAI	13th DAI	15th DAI	17th DAI	19th DAI	21st DAI	23rd DAI	25th DAI	27th DAI	29th DAI	31st DAI	33rd DAI	35th DAI	
T_1	10.00^{d} (18.43)	10.00^{g} (18.43)	20.00^{f} (26.56)	30.00^{d} (33.21)	34.80^{e} (36.15)	44.00^{f} (41.55)	55.00^{g} (47.86)	75.00^{f} (60.00)	80.00^{e} (63.43)	80.00^{d} (63.43)	85.00^{d} (67.21)	95.00^{a} (77.07)	94.50^{b} (76.43)	94.00^{c} (75.82)	96.50^{c} (79.21)	95.83^{c} (78.21)	95.20^{c} (77.34)	95.50^{c} (77.75)	66.12 (54.41)
T_2	15.00^{c} 922.78	15.00^{f} (22.78)	20.00^{f} (26.56)	24.60^{e} (29.73)	25.00^{g} (30.00)	32.50^{i} (34.75)	53.00^{h} (46.71)	56.50^{g} (48.73)	69.63^{f} (56.55)	75.00^{e} (60.00)	85.00^{d} (67.21)	90.00^{b} (71.56)	90.00^{c} (71.56)	89.50^{f} (71.09)	89.00^{f} (70.63)	80.00^{f} (63.43)	82.50^{e} (65.27)	81.00^{f} (64.15)	59.62 (50.54)
T_3	15.00^{c} (22.78)	25.00^{a} (30.00)	27.50^{c} (31.62)	30.00^{d} (33.21)	33.00^{f} (35.06)	35.60^{h} (36.63)	68.94^{e} (56.12)	75.00^{f} (60.00)	85.00^{d} (67.21)	85.00^{c} (67.21)	85.00^{d} (67.21)	85.00^{c} (67.21)	82.00^{e} (64.89)	82.50^{h} (65.27)	80^{h} (63.43)	78.33^{g} (62.25)	78.00^{f} (62.02)	78.50^{g} (62.37)	62.74 (52.38)
T_4	22.00^{b} (26.56	22.00^{b} (27.92)	30.00^{b} (33.21)	30.00^{d} (33.21)	35.00^{e} (36.27)	40.20^{g} (39.34)	64.2^{f} (53.24)	80.00^{e} (63.43)	90.00^{c} (71.56)	100^{a} (90.00)	95.00^{b} (77.07)	90.00^{b} (71.56)	90.50^{c} (72.04)	91.20^{e} (72.74)	91.50^{e} (73.04)	91.66^{d} (73.21)	91.50^{d} (73.04)	91.76^{d} (73.31)	69.14 (56.25)
T_5	15.00^{c} (22.78)	20.80^{bc} (27.13)	35^{a} (36.27)	38.21^{a} (38.18)	42.00^{b} (40.39)	65.00^{a} (53.72)	68.5^{e} (55.85)	90.00^{c} (71.56)	95.00^{b} (77.09)	100^{a} (90.00)	100^{a} (90.00)	95.00^{a} (77.07)	95.50^{ab} (77.75)	92.70^{cd} (74.32)	91.00^{e} (72.54)	90.83^{d} (72.37)	91.00^{d} (72.54)	90.50^{d} (72.04)	73.11 (58.76)
T_6	**	**	5.00^{h} (12.92)	**	15.00^{i} (22.78)	25.00^{j} (30.00)	25.00^{k} (30.00)	10.00^{j} (18.43)	45.00^{h} (42.13)	40.00^{f} (39.23)	35.00 (36.27)	25.00^{f} (30.00)	28.50^{h} (32.26)	28.00^{k} (31.94)	31.5^{k} (34.14)	30.83^{j} (33.72)	29.50^{i} (32.89)	28.00^{j} (31.94)	26.75 (31.14)
T_7	**	10.00^{g} (18.43)	10.00^{g} (18.43)	15.70^{f} (23.34)	25.00^{g} (30.00)	15^{k} (22.78)	35^{i} (36.27)	35^{h} (36.27)	40^{i} (39.23)	75.00^{e} (60.00)	55.00^{e} (47.86)	60.3^{d} (50.94)	66.50^{f} (54.63)	58.50^{i} (49.89)	55.8i (49.33)	63.33^{h} (52.73)	62.80^{g} (52.41)	62.60^{i} (52.29)	43.85 (41.47)
T_8	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	9.25^{k} (17.70)	5.00^{j} (12.92)	**	7.12 (15.47)
T_9	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	5.00^{l} (12.92)	5.00^{j} (12.92)	**	5.00 (12.92)
T_{10}	15.00 (22.78)	17.50^{e} (24.72)	20.50^{f} (26.92)	25.40^{e} (26.92)	33.00^{f} (35.06)	40.80^{g} (39.69)	70.50^{d} (57.10)	90.00^{c} (71.56)	100^{a} (90.00)	95.00^{b} (77.07)	95.00^{b} (77.07)	85.00^{c} (67.21)	85.50^{d} (67.61)	92.50^{de} (74.10)	92.00^{e} (73.57)	95.83^{c} (78.21)	95.5^{c} (77.75)	95.50^{c} (77.75)	69.14 (56.25)
T_{11}	20.00^{b} (26.56)	20.00^{cd} (26.56)	20.70^{ef} (27.06)	30.02^{d} (33.22)	38.00^{d} (38.05)	62.80^{b} (52.41)	76.60^{c} (61.07)	95.00^{b} (77.07)	90.00^{c} (71.56)	85.00^{c})67.21)	85.00^{d} (67.21)	95.00^{a} (77.07)	96.50^{a} (79.21)	96.00^{b} (78.46)	97.50^{bc} (80.90)	97.50^{b} (80.90)	97.00^{b} (80.02)	98.50^{b} (82.96)	72.28 (58.23)
T_{12}	15.00^{c} (22.78)	19.50^{cd} (26.20)	25.31^{d} (30.20)	30.00^{d} (33.21)	40.00^{c} (39.23)	50.5^{e} (45.28)	82.50^{a} (65.27)	100^{a} (90.00)	100^{a} (90.00)	95.00^{b} (77.07)	100^{a} (90.00)0	90.00^{b} (71.56)	95.50^{ab} (77.75)	98.00^{a} (81.86)	98.50^{ab} (82.96)	100^{a} (90.00)	100^{a} (90.00)	99.50^{ab} (85.94)	74.40 (59.60)
T_{13}	25.00^{a} (30.00)	19.20^{d} (26.02)5	25.00^{d} (30.00)	32.10^{c} 34.51)	40.10^{c} (39.28)	51.25^{e} (45.71)	80.40^{b} (63.72)	100^{a} (90.00)	95.00^{b} (77.07)	95.00^{b} (77.07)	95.00^{b} (77.07)	95.00^{a} (77.07)	95.50^{ab} (77.75)	94.00^{c} (75.82)	94.80^{d} (76.81)	95.00^{c} (77.07)	95.00^{c} (77.07)	95.00^{c} (77.07)	73.46 (58.99)
T_{14}	20.00^{b} (26.56)	20.1^{cd} (26.36)	22.00^{e} (27.92)	35.20^{b} (36.39)	42.10^{b} (40.45)	55.00^{d} (47.86)	79.80^{b} (63.29)	85.00^{d} (67.21)	95.00^{b} (77.07)	95.00^{b} (77.07)	90.00^{c} (71.56)	95.00^{a} (77.07)	96.80^{a} (79.69)	99.00^{a} (84.26)	99.50^{a} (85.94)	100^{a} (90.00)	100^{a} (90.00)	100^{a} (90.00)	73.86 (59.25)
T_{15}	20.00^{b} (26.56)	20.54^{cd} (26.94)	30.00^{b} (33.21)	38.00^{a} (38.05)	45.00^{a} (42.13)	58.00^{c} (49.60)	77.70^{c} (61.82)	80.00^{e} (63.43)	100^{a} (90.00)	95.00^{b} (77.07)	90.00^{c} (71.56)	95.00^{a} (77.07)	89.50^{c} (71.09)	88.00^{g} (69.73)	84.50^{g} (66.81)	83.33^{e} (65.90)	83.50^{e} (66.03)	83.80^{e} (66.26)	70.10 (56.85)
T_{16}	5.00^{e} (12.92)	10.00^{g} (18.43)	20.00^{f} (26.56)	17.00^{f} (24.35)	23.00^{h} (28.65)	25.00^{j} (30.00)	27.00^{j} (31.30)	31.00^{i} (33.83)	33.50^{i} (35.36)	35.8^{g} (36.75)	35.40^{f} (36.51)	40.00^{e} (39.23)	38.50^{g} (39.35)	51.00^{j} (45.57)	52.00^{j} ?(46.14)	58.70^{i} (50.01)	55.50^{h} (48.15)	68.80^{h} (56.04)	34.84 (36.17)
Mean	16.25 (23.73)	17.66 (24.85)	22.21 (28.12)	26.87 (31.22)	33.64 (35.45)	42.90 (40.92)	61.72 (51.78)	71.60 (57.80)	79.86 (63.33)	82.20 (65.04)	80.74 (63.97)	81.09 (64.22)	76.48 (64.75)	82.49 (65.26)	82.43 (65.22)	73.46 (58.99)	72.93 (58.64)	83.33 (65.90)	
SEm±	0.86	0.77	1.18	1.08	1.31	2.28	2.99	4.21	3.54	3.10	3.34	3.41	3.32	3.19	3.14	4.99	4.60	2.96	
CD (0.05)	1.61	1.39	1.54	1.53	1.67	1.31	1.31	1.54	1.47	1.54	1.54	1.67	1.67	1.61	1.39	1.60	1.30	1.39	
CD (0.01)	2.18	1.88	2.08	2.08	2.26	1.78	1.78	2.08	1.99	2.08	2.08	2.26	2.26	2.18	1.88	2.17	1.75	1.88	

DAI: Days after seed invigoration *Means in each column with at least one letter in common are not significantly different at 5% level of probability
**All the replicate values having zero are not included in the analysis Values in parentheses are Arc Sine transformed values

4.1.2.1.2 Germination index

The effect of seed invigoration on germination index was highly significant except at 1st DAI and 3rd DAI. Speed of germination (Table 4) was observed to increase in both treated and untreated seeds by 35th DAI.

Average germination index in majority of invigorated seeds was observed to be higher than that of untreated control. However, in treatments T7 (4.90), T6 (2.96), T8 (0.78) and T9 (0.55), the germination index was inferior to control throughout the period upto 35 DAI. Over 35 DAI average germination index was high (>10.00) in seeds treated with *Pf* $1x10^6$ $cfu.ml^{-1}$ for 12 hours (T14: 12.05), $CaCl_2$ 50 mM for 24 hours (T13: 11.98), kinetin (10 ppm for 24 hours) (T11: 11.69) and kinetin 10 ppm for 12 hours (T10: 11.22). The increase in germination index observed between the 1st day (DAI) and 35th DAI in T14 ranged from 2.85 to 16.00, from 3.57 to 15.83 in T13, from 2.85 to 16.41 in T11 and from 2.14 to 15.91 in T10 while the increase in control (T16) was between 0.71 and 10.11.

By 13th DAI, germination index in T13 increased from 3.57 to 13.40, from 2.85 to 13.30 in T14, from 2.14 to 11.78 in T12 and from 2.85 to 11.10 in T15 while the increase was from 0.71 to 3.85 in control.

On 35th DAI, treatments T11 (16.41) and T14 (16.00) registered the highest germination index at 35 DAI and were found to be on par with treatments T10 and T13 (15.91 and 15.83). Treatment T6 had registered the least value (3.11) while treatments T8 and T9 had failed to germinate on 35th DAI.

4.1.2.1.3 Coefficient of velocity of germination (%)

The effect of seed invigoration on coefficient of velocity of germination was highly significant except at 1st DAI and 3rd DAI. An increase in coefficient of velocity of germination (Table 5) was observed in both treated and untreated seeds by 35th DAI. The mean coefficient of velocity of germination in majority of invigoration

Table 4. Germination index in ash gourd after seed invigoration

Treatments	Germination index																	
	1st DAI	3rd DAI	5th DAI	7th DAI	9th DAI	11th DAI	13th DAI	15th DAI	17th DAI	19th DAI	21st DAI	23rd DAI	25th DAI	27th DAI	29th DAI	31st DAI	33rd DAI	35th DAI	Mean
T_1	1.42^{cd}	1.42^{bcde}	2.85^{bc}	4.28^{abc}	4.97^{ab}	6.28^{cd}	7.85^{fg}	10.71^{cde}	11.42^{de}	11.42^{d}	12.14^{de}	13.57^{bc}	13.50^{c}	13.42^{cd}	13.78^{cde}	13.69^{c}	13.6^{cd}	13.64^{cd}	9.44
T_2	1.87^{bcd}	1.87^{abcd}	2.50^{bc}	3.07^{cd}	3.12^{cde}	4.06^{fg}	6.60^{g}	7.06^{f}	8.70^{f}	9.37^{e}	10.62^{e}	10.62^{d}	11.25^{ef}	11.18^{ef}	11.12^{fg}	10.00^{f}	10.31^{f}	10.12^{f}	7.45
T_3	1.87^{bcd}	3.12^{a}	3.43^{ab}	3.75^{bc}	4.12^{bcd}	4.45^{efg}	8.61^{fg}	9.37^{e}	10.62^{e}	12.14^{cd}	12.14^{de}	12.14^{c}	11.71^{de}	11.78^{def}	11.42	11.19^{ef}	11.14^{ef}	11.21^{ef}	8.57
T_4	2.85^{ab}	3.14^{a}	4.28^{ab}	4.28^{abc}	5.00^{ab}	5.74^{def}	9.17^{ef}	11.57^{c}	12.28^{d}	14.28^{ab}	13.50^{bcd}	12.85^{bc}	12.92^{cd}	13.02^{cde}	13.07^{def}	13.09^{cd}	13.07^{cd}	13.10^{cd}	9.87
T_5	2.14^{bc}	2.97^{a}	5.00^{a}	5.45^{a}	6^{a}	6.42^{cd}	9.28^{def}	9.78^{de}	12.85^{cd}	13.57^{bc}	14.28^{abc}	13.57^{bc}	13.64^{c}	13.24^{cd}	13.00^{def}	12.97^{cd}	13^{cd}	12.92^{cde}	10.44
T_6	**	**	0.55^{d}	**	1.66^{ef}	2.77^{gh}	2.77^{hi}	1.11^{h}	5.00^{g}	4.44^{f}	3.88^{h}	2.77^{g}	3.16^{h}	3.11^{h}	3.50^{i}	3.42^{h}	3.27^{h}	3.11^{h}	2.96
T_7	**	1.11^{cde}	1.11^{cd}	1.74^{ef}	2.77^{de}	1.66^{hi}	3.88^{h}	3.88^{g}	4.44^{g}	8.33^{e}	6.11^{g}	6.70^{f}	7.38^{g}	6.50^{g}	6.75^{h}	7.03^{g}	6.97^{g}	6.95^{g}	4.90
T_8	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	1.02^{i}	0.55^{i}	**	0.78
T_9	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	0.55^{i}	0.55^{i}	**	0.55
T_{10}	2.14^{bc}	2.5^{abc}	2.92^{b}	3.62^{bc}	4.71^{abc}	5.82^{de}	10.07^{cde}	15.00^{ab}	16.66^{a}	15.83^{a}	15.83^{a}	14.16^{b}	14.25^{bc}	15.41^{ab}	15.33^{abc}	15.97^{ab}	15.91^{ab}	15.91^{ab}	11.22
T_{11}	2.85^{ab}	2.85^{ab}	2.95^{b}	4.28^{abc}	5.42^{ab}	8.97^{a}	10.94^{bcd}	15.83^{ab}	15.00^{b}	14.16^{ab}	14.16^{abc}	15.83^{a}	16.08^{a}	16.00^{a}	16.25^{a}	16.25^{a}	16.16^{a}	16.41^{a}	11.69
T_{12}	2.14^{bc}	2.78^{ab}	3.61^{ab}	4.28^{abc}	5.71^{ab}	7.21^{bcd}	11.78^{ab}	14.28^{b}	14.28^{bc}	13.57^{bc}	14.28^{ab}	12.85^{bc}	13.64^{c}	14.00^{bc}	14.07^{bcd}	14.28^{bc}	14.28^{bc}	14.21^{bc}	10.62
T_{13}	3.57^{a}	2.75^{ab}	3.57^{ab}	4.58^{abc}	5.72^{ab}	7.32^{abcd}	13.40^{a}	16.66^{a}	15.83^{ab}	15.83^{a}	15.83^{a}	15.83^{a}	15.91^{a}	15.66^{ab}	15.8^{ab}	15.83^{ab}	15.83^{ab}	15.83^{ab}	11.98
T_{14}	2.85^{ab}	2.87^{ab}	3.14^{b}	5.02^{ab}	6.01^{a}	7.85^{abc}	13.30^{a}	14.16^{b}	15.83^{ab}	15.83^{a}	15.00^{ab}	15.83^{a}	16.13^{a}	16.00^{a}	16.58^{a}	16.66^{a}	16.66^{a}	16.00^{a}	12.05
T_{15}	2.85^{ab}	2.93^{a}	4.28^{ab}	5.42^{a}	6.42^{a}	8.28^{ab}	11.10^{bc}	11.42^{cd}	14.28^{bc}	13.57^{bc}	12.85^{cd}	13.57^{bc}	12.78^{cde}	12.^{cde}	12.07^{def}	11.90	11.92^{de}	11.97^{de}	10.01
T_{16}	0.71^{de}	1.42^{bcde}	2.85^{bc}	2.42^{de}	3.28^{cde}	3.57^{g}	3.85^{h}	4.42^{g}	4.78^{g}	5.11^{f}	7.91^{f}	8.57^{e}	9.78^{f}	10.14^{f}	10.17^{g}	10.23^{ef}	10.21^{f}	10.11^{f}	4.97
Mean	2.27	2.44	3.07	4.01	4.63	5.74	8.75	10.37	11.56	11.96	12.03	12.06	12.29	12.32	12.35	10.88	10.83	12.14	
SEm±	0.18	0.17	0.22	0.22	0.25	0.35	0.53	0.73	0.65	0.58	0.59	0.57	0.56	0.58	0.57	0.75	0.77	0.59	
CD (0.05)	NS	NS	1.98	1.82	1.88	1.88	1.79	1.88	1.68	1.84	1.88	1.68	1.58	1.88	1.88	1.71	1.71	1.88	
CD (0.01)	NS	NS	2.69	2.46	2.55	2.55	2.43	2.55	2.28	2.49	2.55	2.28	2.15	2.55	2.55	2.30	2.30	2.55	

DAI: Days after seed invigoration *Means in each column with at least one letter in common are not significantly different at 5% level of probability

**All the replicate values having zero are not included in the analysis

treatments was higher than that of untreated control (12.95%). It ranged from 11.00 per cent in T9 (salicylic acid 60 ppm for 24 hours) to 15.73 per cent in T13 ($CaCl_2$ 50 mM for 24 hours). Treatments T13 (15.73%), T10 and T11 (15.59% each), and T14 (14.40%) had exhibited high mean coefficient of velocity of germination. Apart from treatments T8 (salicylic acid 60 ppm for 12 hours: 11.02%) and T9 (salicylic acid 60 ppm for 24 hours: 11.00%) that ceased to germinate on 35th DAI, treatments T6 (vinegar pH 3.7 for 2 hours: 11.08%), T7 (polyethylene glycol 6000 @-0.5 MPa for 24 hours: 11.15%) and T2 (thiourea 0.5% for 24 hours: 12.44%) had registered a lower mean coefficient of velocity of germination than the control. Coefficient of velocity of germination in these treatments was inferior to control throughout the period of observation.

By 13th DAI coefficient of velocity of germination in T11 increased from 14.25 to 14.28% per cent per cent while it was constant in treatments T13 and T5 (14.28%). The variation in T4 between 1st and 1st DAI was 14.26 to 14.27 per cent, 14.25 to 14.27 per cent in T15 and T12. However in control coefficient of velocity of germination reduced from 12.19 to 11.99 per cent by 13th DAI.

At 35 DAI, treatments T13 (16.66%), T10 (16.65%) and T11 (16.65%) registered the highest coefficient of velocity of germination and were on par with each other and found to be significantly superior to all other treatments. Other treatments except T6 (11.08%), T7 (11.09%) and T2 (12.49%) were found to be on par with each other while treatments T8 and T9 had failed to germinate on 35th DAI.

4.1.2.1.4 Mean time to germination

The effect of seed invigoration on mean time to germination was significant except at 3rd DAI. Mean time to germination (Table 6) was observed to decrease in all treatments between 1st day after invigoration (DAI) and 35th DAI. The average mean time to germination over 35 DAI varied between 6.33 days in T13 ($CaCl_2$ 50 mM for 24 hours) and 9.09 days in T9 (salicylic acid 60 ppm for 24 hours). It was found to be

Table 5. Coefficient of velocity of germination in ash gourd after seed invigoration

Treatments	Coefficient of velocity of germination (%)																		
	1st DAI	3rd DAI	5th DAI	7th DAI	9th DAI	11th DAI	13th DAI	15th DAI	17th DAI	19th DAI	21st DAI	23rd DAI	25th DAI	27th DAI	29th DAI	31st DAI	33rd DAI	35th DAI	Mean
T_1	14.20 (22.13)	14.20 (22.13)	14.20^{a} (22.13)	14.25^{a} (22.17)	14.26^{a} (22.19)	14.28^{a} (22.20)	14.27^{a} (22.19)	14.27^{b} (22.19)	14.28^{b} (22.20)	14.27^{b} (22.19)	14.27^{b} (22.19)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{a} (22.20)	14.27^{b} (22.20)	14.27^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.26 (22.19)
T_2	12.46 (20.67)	12.46 (20.67)	12.46^{b} (20.67)	12.50^{b} (20.70)	12.47^{bc} (20.68)	12.48^{b} (20.68)	12.49^{b} (20.69)	12.45^{cd} (20.66)	12.49^{c} (20.70)	12.49^{b} (20.70)	12.49^{cd} (20.69)	12.49^{c} (20.70)	11.80^{c} (20.09)	12.50^{c} (20.70)	12.49^{c} 20.69)	12.49^{c} (20.70)	12.50^{c} (20.70)	12.49^{c} (20.70)	12.44 (20.65)
T_3	12.46 (20.67)	12.46 (20.67)	12.48^{b} (20.68)	12.47^{b} (20.68)	12.50^{b} (20.70)	12.48^{b} (20.69)	12.50^{b} (22.70)	14.28^{b} (20.69)	14.29^{b} (20.69)	14.29^{b} (20.70)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.27^{b} (22.20)	14.27^{b} (22.19)	14.28^{b} (22.20)	14.28^{b} (22.20)	13.28 (21.36)
T_4	14.25 (22.17)	14.26 (22.17)	14.27^{a} (22.19)	14.26^{a} (22.19)	14.26^{a} (22.19)	14.28^{a} (22.20)	14.27^{a} (22.20)	14.28^{b} (22.20)	14.46^{b} (22.35)	13.64^{bc} (21.67)	14.28^{b} (22.20)	14.21^{b} (22.14)	14.27^{b} (22.20)	14.27^{b} (22.20)	14.27^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.24 (22.17)
T_5	14.26 (22.19)	14.25 (22.19)	14.27^{a} (22.20)	14.28^{a} (22.20)	14.28^{a} (22.18)	14.28^{a} (22.20)	14.28^{a} (22.20)	13.54^{bc} (21.59)	10.86^{d} (19.24)	13.52^{bc} (21.57)	13.57^{bc} (21.61)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.27^{b} (22.20)	14.28^{b} (22.20)	13.96 (21.93)
T_6	**	**	11.00^{b} (19.37)	**	11.06^{bc} (19.43)	11.06^{bc} (19.43)	11.08^{bc} (19.44)	11.08^{def} (19.44)	11.10^{d} (19.46)	11.11^{d} (19.47)	11.10^{d} (19.46)	11.08^{c} (19.44)	11.08^{c} (19.44)	11.08^{c} (19.45)	11.10^{c} (19.46)	11.11^{c} (19.47)	11.09^{d} (19.45)	11.08^{c} (19.44)	11.08 (18.35)
T_7	**	11.10 (19.46)	11.10^{b} (19.46)	11.11^{c} (19.47)	11.08^{bc} (19.44)	11.08^{bc} (19.44)	11.06^{bc} (19.43)	11.08^{def} (19.44)	11.08^{d} (19.44)	11.10^{d} (19.46)	11.10^{d} (19.46)	11.10^{c} (19.46)	11.11^{c} (19.47)	11.09^{c} (19.45)	11.11^{c} (19.47)	12.09^{c} (20.35)	11.10^{d} (19.46)	11.09^{c} (19.46)	11.15 (19.50)
T_8	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	11.02^{d} (19.39)	11.02^{d} (19.39)	**	11.02 (19.39)
T_9	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	11.00^{d} (19.37)	11.00^{d} (19.37	**	11.00 (19.37
T_{10}	14.26 (22.19)	14.26 (22.19)	14.28^{a} (22.20)	14.24^{a} (22.17)	14.25^{a} (22.18)	14.27^{a} (22.19)	14.26^{a} (22.19)	14.28^{b} (22.20)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.65^{a} (24.08)	16.66^{a} (24.09)	16.65^{a} (24.08)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.65^{a} (24.08)	15.59 (23.24)
T_{11}	14.25 (22.17)	14.26 (22.17)	14.25^{a} (22.17)	14.25^{a} (22.17)	14.25^{a} (22.18)	14.26^{a} (22.18)	14.28^{a} (22.20)	14.28^{b} (22.20)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.65^{a} (24.08)	16.65^{a} (24.08)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.65^{a} (24.08)	15.59 (23.24)
T_{12}	14.26 (22.19)	14.26 (22.19)	14.25^{a} (22.18)	14.26^{a} (22.18)	14.26^{a} (22.19)	14.27^{a} (22.19)	14.27^{a} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.27 (22.20)
T_{13}	14.28 (22.20)	14.28 (22.20)	14.28^{a} (22.20)	14.28^{a} (22.20)	14.26^{a} (22.19)	14.26^{a} (22.19)	14.28^{a} (22.20)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.65^{a} (24.08)	16.65^{a} (24.08)	16.66^{a} (24.09)	16.66^{a} (24.09)	16.66^{a} 24.09)	15.73 (23.35)
T_{14}	14.25 (22.17)	14.25 (22.17)	14.27^{a} (22.20)	14.27^{a} (22.19)	14.26^{a} (22.89)	14.27^{a} (22.19)	14.27^{a} (22.19)	14.28^{b} (22.20)	16.65^{a} (24.08)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.40 (22.30)
T_{15}	14.25 (22.17)	14.25 (22.17)	14.26^{a} (22.19)	14.26^{a} (22.19)	14.26^{a} (22.18)	14.26^{a} (22.19)	14.27^{a} (22.20)	14.28^{b} (22.20)	14.27^{a} (22.19)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.27^{b} (22.20)	14.28^{b} (22.20)	14.27^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.27^{b} (22.19)	14.27 (22.19)
T_{16}	11.11 (19.47)	11.11 (19.47)	12.19^{b} (20.43)	12.34^{b} (20.57)	11.83^{bc} (20.12)	12.50^{b} (20.70)	11.99^{bc} (20.59)	12.82^{c} (20.98)	12.50^{c} (20.70)	12.50^{cd} (20.70)	12.50^{cd} (20.70)	14.00^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	14.28^{b} (22.20)	12.95 (21.09)
Mean	13.69 (21.71)	12.53 (20.73)	13.40 (21.47)	12.62 (20.81)	13.38 (21.45)	13.43 (21.50)	13.40 (21.47)	13.58 (21.62)	13.89 (21.88)	13.85 (21.85)	14.03 (21.99)	14.18 (22.12)	14.16 (22.10)	14.21 (22.14)	14.21 (22.14)	14.75 (22.40)	13.81 (22.10)	14.09 (22.05)	
SEm±	0.40	0.38	0.22	0.21	0.22	0.22	0.22	0.23	0.33	0.28	0.27	0.26	0.27	0.28	0.27	0.24	0.25	0.26	
CD (0.05)	NS	NS	1.53	1.61	1.53	1.53	1.53	1.48	1.43	1.43	1.43	1.43	1.43	1.48	1.48	1.37	1.37	1.43	
CD (0.01)	NS	NS	2.08	2.18	2.08	2.08	2.08	2.01	1.94	1.94	1.94	1.94	1.94	2.01	2.01	1.86	1.86	1.96	

DAI: Days after seed invigoration *Means in each column with at least one letter in common are not significantly different at 5% level of probability

**All the replicate values having zero are not included in the analysis Values in parentheses are Arc Sine transformed values

high and more than 8.00 days in treatments T6 (vinegar pH 3.7 for 2hours), T7 (polyethylene glycol PEG 6000 @-0.5 MPa for 24 hours) and T2 (thiourea 0.5% for 24 hours) while it was low (≤ 7 days) in case of T13 ($CaCl_2$ 50 mM for 24 hours), T10 (kinetin 10 ppm for 12 hours), T11 (kinetin 10 ppm for 24 hours), T14 (*Pf* $1x10^6$ $cfu.ml^{-1}$ for 12hours), T15 (*Pf* $1x10^6$ $cfu.ml^{-1}$ for 24 hours), T12 ($CaCl_2$ 50 mM for 12 hours) and T1 (hydropriming 12 hours). Treatments T13 (6.33), T10 (6.39) and T11 (6.39) had registered the minimum mean time to germination.

Mean time to germination by 13th DAI in decreased from 7.00 to 6.00 in T13, from 7.01 to 6.00 in T14, from 7.01 to 7.00 in T11 and T15 and from 7.04 to 7.00 in T1, while the decrease was from 9.00 to 7.80 in control.

At 35 DAI, T13, T10 and T11 with an estimate of 6.00 days each were found to be on par with each other and also significantly superior to all other treatments. Other treatments except T6 (Acetic acid pH 3.7 for 2hours), T7 (polyethylene glycol @-0.5 MPa for 24 hours) and T2 (thiourea 0.5% for 24 hours) were found to be on par with other. Apart from the seeds treated with salicylic acid i.e., T8 and T9 that ceased to germinate by 35th DAI, treatments T6 and T7 with a value of 9.00 days each, registered the highest mean time to germination on 35 DAI. These treatments were found to be significantly inferior to all other treatments including control (7.00), while T13, T14, T10 and T11 with a value of 6.00 days each were significantly superior to all other treatments at 35th DAI.

4.1.2.1.7 Energy of germination

Significant impact of seed treatment on energy of germination was evident throughout 35 DAI. Energy of germination (Table 7) increased in both treated and untreated seeds by 35th DAI. The average energy of germination in majority of invigoration treatments was higher than that of untreated control (13.61). It ranged from 7.02 in T6 (vinegar pH 3.7 for 2 hours) to 40.83 in T14 (*Pf* $1x10^6$ $cfu.ml^{-1}$ for 12 hours). Treatments T12 (39.55), T13 (38.40), T15 (37.35) and T5 (34.72) had

Table 6. Mean time to germination in ash gourd after seed invigoration

Treatments	Mean time to germination																		Mean
	1st DAI	3rd DAI	5th DAI	7th DAI	9th DAI	11th DAI	13th DAI	15th DAI	17th DAI	19th DAI	21st DAI	23rd DAI	25th DAI	27th DAI	29th DAI	31st DAI	33rd DAI	35th DAI	
T_1	7.04^{b}	7.01^{b}	7.01^{b}	7.00^{a}b	7.00^{c}	7.00^{abc}	7.00^{ed}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{a}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00
T_2	8.02^{ab}	8.02^{ab}	8.00^{ab}	8.01^{ab}	8.01^{b}	8.00^{ab}	8.03^{bc}	8.00^{b}	8.00^{b}	8.00^{b}	8.00^{b}	8.47^{b}	8.00^{b}	8.00^{b}	8.00^{b}	8.00^{b}	8.00^{b}	8.00^{b}	8.03
T_3	8.02^{ab}	8.01^{ab}	8.01^{ab}	8.00^{ab}	8.00^{b}	8.00^{ab}	8.00^{bc}	8.00^{b}	8.00^{b}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.50
T_4	7.01^{a}	7.00^{b}	7.00^{b}	7.00^{b}	7.00^{c}	7.00^{abc}	7.00^{ed}	6.93^{cd}	6.77^{cd}	7.00^{c}	7.03^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.01
T_5	7.00^{a}	7.00^{b}	7.00^{b}	7.01^{b}	7.00^{c}	7.00^{abc}	7.38^{cd}	6.84^{cd}	7.01^{a}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.06
T_6	**	**	9.09^{a}	**	9.03^{a}	9.02^{a}	9.02^{a}	9.00^{ab}	9.00^{a}	9.00^{a}	9.02^{a}	9.02^{a}	9.01^{a}	9.00^{a}	9.00^{a}	9.01^{a}	9.02^{a}	9.00^{a}	9.02
T_7	**	9.00^{a}	9.00^{a}	9.02^{a}	9.02^{a}	9.03^{a}	9.02^{ab}	9.02^{ab}	9.00^{a}	9.00^{a}	9.00^{a}	9.00^{a}	9.01^{a}	9.00^{a}	8.26^{b}	9.00^{a}	9.01^{a}	9.00^{a}	8.46
T_8	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	9.06^{a}	9.09^{a}	9.07
T_9	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	9.09^{a}	9.09^{a}	9.09
T_{10}	7.00^{b}	7.00^{b}	7.02^{b}	7.01^{b}	7.00^{c}	7.01^{abc}	7.00^{ed}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{b}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.39
T_{11}	7.01^{b}	7.01^{b}	7.01^{v}	7.01^{b}	7.01^{c}	7.01^{abc}	7.00^{ed}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{b}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.39
T_{12}	7.00^{a}	7.01^{b}	7.01^{b}	7.00^{b}	7.00^{c}	7.00^{abc}	7.00^{cd}	7.00^{c}	7.00^{a}	7.00^{c}	7.00^{c}	7.00^{ab}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00
T_{13}	7.00^{a}	7.00^{b}	7.00^{b}	7.00^{b}	7.01^{c}	7.00^{abc}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{b}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.33
T_{14}	7.01^{b}	7.00^{b}	7.00^{b}	7.01^{b}	7.00^{c}	7.00^{abc}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{b}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.00^{d}	6.02^{d}	6.94
T_{15}	7.01^{b}	7.01^{b}	7.00^{b}	7.01^{b}	7.00^{c}	7.00^{abc}	7.00^{ed}	7.00^{c}	7.00^{c}	6.00^{d}	7.00^{c}	6.00^{b}	7.00^{a}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00^{c}	7.00
T_{16}	9.00^{a}	8.20^{a}	8.10^{ab}	8.45^{ab}	8.00^{b}	8.34^{a}	7.80^{c}	8.00^{b}	8.00^{b}	8.00^{b}	7.00^{c}	8.10^{ab}	8.20^{b}	7.00^{c}	8.45^{b}	7.00^{c}	8.34^{b}	7.00^{c}	7.94
Mean	7.34	6.88	7.52	6.89	7.50	7.53	7.44	7.36	7.33	7.24	7.14	7.25	7.23	7.14	7.19	7.14	7.24	7.20	
SEm±	0.14	0.13	0.15	0.14	0.13	0.19	0.16	0.17	0.17	0.16	0.15	0.22	0.17	0.15	0.15	0.15	0.16	0.15	
CD (0.05)	NS	1.04	1.68	1.23	0.97	1.23	0.97	0.97	0.97	0.97	0.68	0.74	1.08	0.48	0.48	0.48	0.48	0.48	
CD (0.01)	NS	1.40	0.93	NS	1.31	1.67	1.31	1.31	1.31	0.31	0.93	1.01	1.47	0.65	0.65	0.65	0.65	0.65	

DAI: Days after seed invigoration *Means in each column with at least one letter in common are not significantly different at 5% level of probability

**All the replicate values having zero are not included in the analysis

exhibited maximum energy of germination. Apart from treatments T8 (salicylic acid 60 ppm for 12 hours) and T9 (salicylic acid 60 ppm for 24 hours) that ceased to germinate by 35th DAI, treatments T6 (acetic acid pH 3.7 for 2 hours: 7.02) and T7 (polyethylene glycol 6000 @-0.5 MPa for 24 hours: 13.48) had registered lower mean energy of germination than control. Energy of germination in both these treatments was also inferior to control throughout the period of observation.

Energy of germination by 13th DAI increased from 7.00 to 47.00 in T15, from 6.50 to 42.00 in T14, from 8.00 to 40.00 in T12, from 10.00 to 39.50 in T13 and from 7.00 to 38.00 in T5, while the increase was from 0.75 to 5.42 in control.

At 35 DAI, treatments T12 (55.50), T10 and T14 (53.00), T11 (48.00) and T13 (46.80) registered the highest energy of germination and were found to be significantly superior to all other treatments. All other treatments except T6 (7.50) and T7 (16.00) were found to be superior to control (T16: 17.00). Treatments T8 and T9 had failed to germinate on the 4th day after sowing throughout the study, thereby the energy of germination could not be arrived at.

4.1.2.1.8 Vigour index-I

The effect of seed treatment on vigour index-1 was found to be highly significant throughout the 35 days after invigoration. An increase in vigour index-I (Table 8) was observed in both treated and untreated seeds by 35th DAI. The average vigour index-I over the 35 day period in majority of invigoration treatments was higher than that of untreated control (806). It values varied from 101.50 in T9 (salicylic acid 60 ppm for 24 hours) to 1936.38 in T14 (*Pf* 1x10^{6} cfu.ml^{-1} for 12 hours). Treatments T12 (1815.38), T13 (1788.83), T11 (1726.88) and T5 (1709.27) had exhibited the highest average vigour index-I. Apart from treatments T9 (salicylic acid 60 ppm for 24 hours: 101.50), T8 (salicylic acid 60 ppm for 12 hours: 146.50), T6 (vinegar pH 3.7 for 2hours: 551.13) had registered lower average vigour index-I

Table 7. Energy of germination in ash gourd after seed invigoration

Treatments	Energy of germination																	
	1st DAI	3rd DAI	5th DAI	7th DAI	9th DAI	11th DAI	13th DAI	15th DAI	17th DAI	19th DAI	21st DAI	23rd DAI	25th DAI	27th DAI	29th DAI	31st DAI	33rd DAI	35th DAI	Mean
T_1	2.00^{d}	3.00^{e}	5.00^{c}	4.50^{ef}	6.00^{h}	17.40^{h}	22.00^{g}	35.41^{e}	35.50^{g}	36.00^{h}	33.60^{g}	38.53^{f}	38.50^{f}	41.00^{f}	42.10^{d}	46.80^{c}	43.32^{ef}	43.70^{e}	27.46
T_2	1.00^{de}	1.00^{f}	1.50^{e}	3.70^{f}	3.50^{j}	4.71^{j}	7.10^{j}	12.24^{h}	25.00^{h}	33.00^{i}	30.70^{h}	28.50^{g}	25.50^{h}	28.50^{i}	30.00^{f}	31.60^{g}	25.50^{h}	28.00^{h}	17.83
T_3	1.01^{de}	5.00^{d}	8.50^{b}	5.51^{e}	8.00^{g}	7.60^{i}	18.30^{h}	25.00^{f}	48.00^{e}	42.00^{e}	42.80^{e}	45.00^{d}	38.90^{f}	36.00^{g}	35.00^{e}	38.10^{f}	35.00^{g}	35.00^{g}	26.37
T_4	6.20^{c}	7.00^{c}	11.00^{a}	11.00^{cd}	13.30^{e}	20.01^{g}	35.00^{e}	44.60^{b}	50.00^{d}	54.80^{a}	45.00^{d}	50.00^{b}	46.50^{c}	41.50^{f}	47.00^{b}	45.00^{d}	43.00^{f}	46.00^{d}	34.27
T_5	7.00^{bc}	4.50^{d}	8.00^{b}	17.00^{a}	15.50^{e}	26.70^{f}	38.00^{d}	36.00^{e}	48.00^{e}	45.00^{d}	45.50^{d}	54.00^{a}	40.60^{e}	52.00^{b}	48.00^{b}	44.00^{d}	54.20^{b}	41.00^{f}	34.72
T_6	**	**	**	**	**	2.00^{k}	5.00^{k}	3.50^{j}	13.00^{j}	9.80^{j}	5.00^{k}	5.00^{i}	8.00^{j}	8.00^{j}	12.00^{i}	10.47^{j}	2.00^{k}	7.50^{j}	7.02
T_7	**	**	**	1.00^{g}	4.00^{ij}	2.00^{k}	3.50^{l}	7.00^{i}	17.00^{i}	37.70^{g}	15.04^{j}	17.00^{h}	11.00^{i}	3.00^{k}	21.00^{h}	25.00^{h}	22.00^{i}	16.00^{i}	13.48
T_8	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
T_9	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
T_{10}	6.00^{c}	8.00^{bc}	5.50^{c}	12.00^{c}	11.00^{f}	28.00^{f}	33.00^{f}	42.00^{c}	46.50^{e}	47.00^{c}	49.00^{c}	40.50^{e}	43.00^{d}	55.00^{a}	50.00^{a}	48.00^{bc}	44.00^{def}	53.00^{b}	34.52
T_{11}	6.00^{c}	9.00^{ab}	9.00^{b}	10.00^{d}	10.00^{f}	32.00^{e}	35.00^{e}	40.00^{d}	42.00^{f}	40.00^{f}	41.00^{f}	45.00^{d}	45.50^{c}	48.00^{c}	47.00^{b}	45.00^{d}	44.40^{de}	48.00^{c}	33.16
T_{12}	8.00^{b}	8.00^{bc}	9.00^{b}	11.00^{cd}	17.00^{c}	36.00^{d}	40.00^{c}	55.00^{a}	56.00^{a}	54.50^{a}	57.00^{a}	48.00^{c}	46.00^{c}	47.00^{cd}	50.00^{a}	56.00^{a}	58.00^{a}	55.50^{a}	39.55
T_{13}	10.00^{a}	9.00^{ab}	8.50^{b}	11.00^{cd}	15.00^{d}	38.00^{c}	39.50^{c}	55.00^{a}	54.00^{bc}	54.00^{a}	50.00^{c}	51.00^{b}	58.00^{a}	46.00^{d}	48.00^{b}	49.00^{b}	48.40^{c}	46.80^{cd}	38.40
T_{14}	6.50	10.00^{a}	11.00^{a}	15.00^{b}	22.00^{b}	43.00^{b}	42.00^{b}	45.00^{b}	53.00^{c}	55.00^{a}	53.00^{b}	50.00^{b}	58.50^{a}	56.00^{a}	50.00^{a}	55.00^{a}	57.00^{a}	53.00^{b}	40.83
T_{15}	7.00^{bc}	8.50^{b}	9.00^{b}	16.00^{ab}	25.00^{a}	45.00^{a}	47.00^{a}	43.00^{c}	55.00^{ab}	50.00^{b}	49.50^{c}	48.00^{c}	51.80^{b}	44.00^{e}	43.50^{c}	41.10^{e}	45.00^{d}	44.00^{e}	37.35
T_{16}	**	1.00^{f}	3.00^{d}	3.00^{f}	5.00^{hi}	7.00^{i}	12.00^{i}	17.00^{g}	10.00^{k}	11.00^{j}	17.00^{i}	18.00^{h}	28.00^{g}	32.00^{h}	25.00^{g}	22.00^{i}	17.00^{j}	17.00^{i}	13.61
Mean	5.41	6.50	8.05	9.28	11.94	22.10	26.95	32.91	39.50	40.70	38.15	38.46	63.55	38.42	39.18	39.79	38.48	38.17	
SEm±	0.46	0.45	0.43	0.82	1.07	2.34	2.24	2.40	2.36	1.92	2.04	1.92	2.07	2.19	1.61	1.63	2.03	2.08	
CD (0.05)	1.54	1.61	1.55	1.55	1.55	1.77	1.62	1.49	1.55	1.67	1.84	1.67	1.75	1.61	1.67	1.55	1.55	1.67	
CD (0.01)	2.08	2.17	2.09	2.09	2.09	2.39	2.18	2.01	2.09	2.25	2.48	2.25	2.36	2.17	2.25	2.09	2.09	2.25	

DAI: Days after seed invigoration *Means in each column with at least one letter in common are not significantly different at 5% level of probability

**All the replicate values having zero are not included in the analysis

than control. Vigour index-I in these treatments was inferior to control throughout the period of observation.

The increase in vigour index-I between 1st DAI and 13th DAI in treatment T14 was from 609 to 2323, from 608 to 2113 in T13, from 379 to 2103 in T12, from 524 to 2079 in T15 and from 473 to 2039 in T11, while the increase was from 124 to 728 in control (T16).

At 35th DAI, treatments T14 (2527), T12 (2470), T1 (2368) and T11 (2301) registered maximum vigour index-I and were found to be significantly superior to all other treatments. All other treatments except T6 (505), T7 (1429), T16 (1578) and T2 (1706) were superior to untreated seeds at 35th DAI. Treatments T8 and T9 had failed to germinate on 35th DAI.

4.1.2.1.9 Vigour index-II

The effect of seed treatment on vigour index-II was found to be significant throughout 35 DAI, the exceptions being 11th DAI to 23rd DAI and 27th DAI to 33rd DAI. An increase in vigour index- II (Table 9) was observed in both treated and untreated seeds by 35th DAI. The average vigour index-II in majority of invigoration treatments was higher than that of untreated control (0.94). It ranged from 0.30 in T6 (vinegar pH 3.7 for 2 hours) to 1.79 in T12 ($CaCl_2$ 50 mM for 24 hours). Treatments T13 (1.70), T10 and T14 (1.69 each), T11 (1.66) and T1 (1.65) had exhibited the highest average vigour index- II. Apart from treatments T9 (salicylic acid 60 ppm for 24 hours: 0.12), T8 (salicylic acid 60 ppm for 12 hours: 0.14) T6 (vinegar pH 3.7 for 2hours: 0.30) and T7 (polyethylene glycol 6000 @-0.5 MPa for 24 hours: 0.78) had also registered lower mean vigour index- II than control. Vigour index- II in both these treatments was inferior to control throughout the period of observation.

Table 8. Vigour index-I in ash gourd within 35 days of seed invigoration

Treatments	Vigour index-I																		
	1st DAI	3rd DAI	5th DAI	7th DAI	9th DAI	11th DAI	13th DAI	15th DAI	17th DAI	19th DAI	21st DAI	23rd DAI	25th DAI	27th DAI	29th DAI	31st DAI	33rd DAI	35th DAI	Mean
T_1	237^{d}	227^{l}	451^{j}	696^{d}	845^{g}	932^{h}	1312^{j}	1639^{i}	1900^{h}	1904^{i}	1955^{h}	2232^{e}	2192^{f}	2256^{e}	2441^{b}	2376^{c}	2351^{a}	2368^{c}	1573
T_2	337^{c}	312^{i}	435^{k}	574^{e}	567^{l}	689^{k}	1136^{k}	1203^{j}	1400^{j}	1687^{k}	1795^{k}	1908^{k}	1962^{j}	1808^{k}	1870^{j}	1563^{i}	1733^{cdef}	1706^{k}	1260.27
T_3	333^{c}	557^{a}	644^{d}	731^{cd}	805^{i}	752^{ij}	1568^{h}	1700^{h}	1793^{i}	1837^{j}	1896^{j}	1998^{j}	1927^{k}	1851^{j}	1858^{k}	1841^{h}	1821^{bcde}	1808^{j}	1428.88
T_4	459^{b}	453^{f}	703^{b}	710^{d}	831^{h}	912^{i}	1562^{i}	1770^{g}	2102^{f}	2224^{g}	2014^{g}	2016^{i}	2081^{h}	2126^{g}	1944^{i}	1954^{g}	2034^{abcd}	1973^{i}	1548.22
T_5	346^{c}	435^{g}	782^{a}	876^{a}	1020^{b}	1563^{b}	1618^{g}	2056^{e}	2131^{e}	2350^{b}	2534^{a}	2179^{g}	2313^{b}	2124^{h}	2097^{g}	2134^{e}	2128^{abc}	2081^{g}	1709.27
T_6	**	**	104^{j}	**	324^{n}	582^{m}	614^{n}	215^{m}	997^{k}	801^{n}	704^{n}	498^{n}	607^{n}	535^{n}	657^{n}	620^{l}	504^{g}	505^{n}	551.13
T_7	**	239^{k}	236^{l}	336^{g}	581^{k}	357^{n}	803^{ll}	815^{k}	860^{l}	1500^{l}	1199^{l}	1305^{l}	1400^{l}	1258^{l}	1172^{m}	1412^{j}	1386^{ef}	1429^{m}	958.11
T_8	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	194^{m}	99^{h}	**	146.50
T_9	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	102^{n}	99^{h}	**	101.50
T_{10}	354^{c}	409^{h}	493^{h}	574^{e}	768^{j}	948^{g}	1763^{f}	2254^{c}	2388^{d}	2232^{f}	2365^{i}	2032^{h}	1998^{i}	2187^{f}	2151^{f}	2253^{c}	2292^{ab}	2243^{e}	1650.22
T_{11}	472^{b}	470^{d}	488^{i}	641^{cd}	877^{f}	1571^{a}	2039^{e}	2195^{d}	2029^{g}	2041^{h}	1939^{d}	2280^{c}	2267^{d}	2449^{b}	2337^{d}	2449^{b}	2221^{def}	2301^{d}	1726.88
T_{12}	379^{c}	468^{e}	632^{e}	702^{d}	969^{c}	1142^{f}	2103^{c}	2500^{b}	2416^{c}	2299^{d}	2400^{b}	2298^{b}	2296^{c}	2403^{c}	2414^{c}	2428^{b}	2358^{a}	2470^{b}	1815.38
T_{13}	608^{b}	467^{e}	597^{f}	722^{d}	929^{e}	1295^{e}	2113^{b}	2630^{a}	2475^{a}	2308^{c}	2290^{e}	2185^{f}	2261^{a}	2345^{a}	2209^{e}	2236^{d}	2297^{ab}	2232^{f}	1788.83
T_{14}	609^{a}	488^{c}	519^{f}	805^{bc}	945^{d}	1438^{c}	2323^{a}	2257^{c}	2418^{c}	2616^{a}	2388^{c}	2589^{a}	2561^{e}	2644^{d}	2595^{a}	2725^{a}	2408^{a}	2527^{a}	1936.38
T_{15}	524^{b}	533^{b}	689^{c}	873^{ab}	1046^{a}	1435^{d}	2079^{d}	1961^{f}	2444^{b}	2290^{e}	2115^{f}	2259^{d}	2099^{g}	2114^{i}	2073^{i}	1952^{f}	2051^{abc}	1998^{h}	1696.38
T_{16}	124^{e}	241^{j}	488^{i}	395^{f}	508^{m}	596^{l}	728^{m}	689^{l}	738^{m}	806^{m}	825^{m}	872^{m}	901^{m}	1192^{m}	1195^{l}	1351^{k}	1281^{f}	1578^{l}	806
Mean	398.5	407.61	518.64	664.23	786.78	1015.14	1388.42	1579.35	1863.64	1921.07	1887.07	1903.64	1918.92	1949.42	2099.78	1724.37	1691.43	1944.21	
SEm±	22.19	17.79	27.14	23.36	31.84	59.11	83.87	107.46	92.24	82.87	86.60	88.37	83.68	87.74	82.75	82.99	82.45	78.95	
CD (0.05)	2.13	1.74	1.60	1.48	2.14	2.27	1.91	1.54	3.28	1.78	1.58	1.95	1.67	1.66	1.56	1.61	1.61	1.61	
CD (0.01)	2.89	2.35	2.35	2.01	2.89	3.07	2.58	2.08	4.43	2.41	2.13	2.63	2.25	2.24	2.11	2.17	2.17	2.17	

DAI: Days after seed invigoration *Means in each column with at least one letter in common are not significantly different at 5% level of probability

**All the replicate values having zero are not included in the analysis

Table 9. Vigour index-II in ash gourd after seed invigoration

Treatments	Vigour index-II																		
	1st DAI	3rd DAI	5th DAI	7th DAI	9th DAI	11th DAI	13th DAI	15th DAI	17th DAI	19th DAI	21st DAI	23rd DAI	25th DAI	27th DAI	29th DAI	31st DAI	33rd DAI	35th DAI	Mean
T_1	0.23^{de}	0.24^{bc}	0.50^{b}	0.77^{bcd}	0.83^{ab}	1.06	1.56	1.84	1.94	1.92	2.08	2.35	2.37^{a}	2.25	2.44	2.53	2.47	2.31	1.65
T_2	0.36^{c}	0.36^{ab}	0.49^{b}	0.63^{d}	0.60^{abc}	0.77	1.23	1.55	2.33	1.99	2.04	2.18	2.20^{a}	2.11	2.14	1.92	1.89	1.94	1.48
T_3	0.34^{cd}	0.46^{a}	0.61^{ab}	0.65^{cd}	0.73^{abc}	0.64	1.19	1.74	1.81	1.96	1.7	2.04	1.62^{ab}	1.68	1.92	1.63	1.87	1.57	1.34
T_4	0.41^{bc}	0.38^{a}	0.60^{ab}	0.46^{e}	0.77^{ab}	0.66	1.23	1.85	2.04	2.15	1.78	1.80	1.81^{a}	1.91	2.01	1.87	1.83	1.83	1.41
T_5	0.33^{cd}	$0.36a^{b}$	0.49^{b}	0.95^{a}	0.65^{abc}	0.50	0.92	1.13	1.25	1.57	1.98	2.09	1.91^{a}	1.96	2.00	2.07	2.09	1.88	1.34
T_6	**	**	0.04^{d}	**	0.14^{e}	0.67	0.20	0.21	1.15	0.3	0.25	0.21	0.28^{bc}	0.19	0.20	0.24	0.23	0.21	0.30
T_7	**	0.12^{c}	0.10^{d}	0.19^{f}	0.28^{cde}	0.15	0.38	0.82	0.74	1.57	0.64	1.21	1.36^{abc}	1.21	1.21	1.10	1.13	1.17	0.78
T_8	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	0.18	0.11	**	0.14
T_9	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	0.15	0.10	**	0.12
T_{10}	0.37^{c}	0.42^{a}	0.52^{b}	0.62^{d}	0.79^{ab}	0.96	1.70	2.16	2.40	2.28	2.28	2.13	2.12^{a}	2.34	2.26	2.39	2.30	2.32	1.69
T_{11}	0.51^{ab}	0.47^{a}	0.51^{b}	0.72^{bcd}	0.91^{ab}	0.96	1.81	2.26	1.93	1.87	2.08	2.31	2.28^{a}	2.31	2.38	2.24	2.14	2.26	1.66
T_{12}	0.37^{c}	0.45^{a}	0.62^{ab}	0.73^{bcd}	0.97^{a}	1.35	1.92	2.25	2	2.56	2.35	2.25	2.36^{a}	2.37	2.46	2.38	2.51	2.42	1.79
T_{13}	0.60^{a}	0.45^{a}	0.61^{ab}	0.73^{bcd}	0.95^{ab}	1.36	1.79	2.2	2.09	1.99	2.20	2.28	2.29^{a}	2.25	2.27	2.09	2.23	2.28	1.70
T_{14}	0.49^{ab}	0.47^{a}	0.53^{b}	0.80^{abc}	0.98^{a}	1.42	1.74	1.78	2.28	2.07	1.84	2.09	2.22^{a}	2.27	2.53	2.35	2.31	2.30	1.69
T_{15}	0.49^{ab}	0.47^{a}	0.73^{a}	0.87^{ab}	1.03^{a}	1.82	1.65	1.72	2.56	2.01	1.96	2.03	1.87^{a}	1.84	1.94	1.83	1.83	1.92	1.59
T_{16}	0.13^{e}	0.24^{bc}	0.33^{c}	0.39	0.51^{bcd}	0.57	0.60	0.66	0.72	0.78	1.16	1.40	1.54^{ab}	1.64	1.63	1.65	1.66	1.85	0.94
Mean	0.41	0.37	0.47	0.65	0.72	0.92	1.28	1.58	1.80	1.78	1.73	1.88	1.87	1.88	1.95	1.88	1.85	1.85	
SEm±	0.02	0.02	0.03	0.04	0.05	0.09	0.13	0.14	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	
CD (0.05)	0.13	0.14	0.15	0.17	0.48	NS	NS	NS	NS	NS	NS	NS	1.18	NS	NS	NS	NS	NS	
CD (0.01)	0.18	0.20	0.21	0.23	NS	NS	NS	NS	NS	NS	NS	NS	1.59	NS	NS	NS	NS	NS	

DAI: Days after seed invigoration *Means in each column with at least one letter in common are not significantly different at 5% level of probability
**All the replicate values having zero are not included in the analysis

vigour index-II in T15 increased from 0.49 to 1.03, in T14 (0.49 to 0.98), T13 (0.60 to 1.79), T12 (0.37 to 0.97) and T13 (0.60 to 0.95) respectively within 9 DAI while the increase was from 0.13 to 0.57 in control.

At 25th DAI, treatments T1 (2.37), T12 (2.36), T13 (2.29), T11 (2.28), T14 (2.22) and T2 (2.20) had registered maximum vigour index- II and were on par with each other. These treatments were also found to be on par with all other treatments excluding T6 (0.28) at 25th DAI.

4.2 Effect of seed invigoration treatments on seed viability during storage

The results of impact of invigoration treatments on seed quality parameters upto ten months after seed invigoration are presented in Appendix II and Tables 10 to 24. The results obtained are enumerated below.

4.2.1 Analysis of variance

The analysis of variance revealed that, there existed significant differences in seed quality parameters like germination per cent, germination index, mean time to germination, coefficient of velocity of germination, energy of germination, seedling vigour index I and II, electrical conductivity of seed leachate, thickness of seed coat, thickness of testa and tegmen, ratio of embryo length to seed length and seed microflora, among various seed treatments for most of the storage period (10 months) after seed invigoration.

4.2.2 Germination (%)

The effect of seed invigoration on germination was found to be high and significant throughout the storage period of 10 months. Germination (Table 10) decreased over storage in all treatments. The average germination over the storage

period varied between 5.00 per cent in seeds treated with salicylic acid 60 ppm for 24 hours (T9) and 74.54 per cent in T14 (*Pf* $1x10^6$ $cfu.ml^{-1}$ for 12 hours). Average germination was high (>70.00%) in seed invigoration treatments T14, T13 and T12. Apart from treatments T8 (salicylic acid 60 ppm for 12 hours) and T9 (salicylic acid 60 ppm for 24 hours) were seeds failed to germinate after one month of storage after seed invigoration (MAS), mean germination over storage was found to be low (< 40%) in treatments T6 and T7. Germination in these treatments was lower than that in the control (40.91).

Germination in all the treatments decreased below the MSCS of 60 per cent at 9 MAI. Germination in T6 did not reach above the minimum standards for seed certification (MSCS) of 60 per cent required for ash gourd even after 1 MAS while in T7 (polyethylene glycol 6000 @-0.5 MPa for 24 hours: 63.40%), it was retained for 1 MAS. Untreated seed (T16: 69.16) registered a germination above 60 per cent for 2 MAS. The untreated seeds as well as seeds treated with vinegar failed to germinate from 8 MAS, while seeds treated with PEG 6000 ceased to germinate at 9 MAS.

Seed germination in majority of invigorated seeds reached below MSCS by 5 MAS (T2: 33.75%, T3: 51.25%, T4: 55.00%, T11: 56.25%, T5: 57.50% and T10: 58.75%). Seeds invigorated with $CaCl_2$ (50 mM for 24 hours (T13) was the only treatment in which seed viability was retained above MSCS for 7 MAS. Germination in treatments T10 (62.20%), T12 (68.50%), T14 (72.00%) and T15 (67.50%) was above MSCS upto 6 MAS while in T1 (65.00%), it was retained upto 5 MAS.

4.2.3 Germination index

Significant differences among seed invigoration treatments existed throughout the storage period. It (Table 11) decreased over storage irrespective of invigoration treatment. Average germination index in majority of invigorated seeds was observed to be higher than that of untreated control. The exceptions were treatments T7 (3.40),

Table 10. Effect of seed invigoration on germination in ash gourd during storage

Treatments	Germination (%)									
	1MAS	2MAS	3MAS	4MAS	5MAS	6MAS	7MAS	8MAS	9MAS	Mean
T_1	95.83^{bc} (78.27)	85.00^{d} (67.24)	79.16^{ef} (62.78)	79.16^{d} (62.87)	65.00^{b} (53.74)	52.50^{ef} (46.43)	48.7^{de} (44.25)	30.00^{e} (33.16)	20.00^{f} (26.54)	61.70 (51.76)
T_2	80.00^{ef} (63.45)	77.50^{ef} (61.69)	75.00^{fg} (60.01)	65.83^{fg} (54.23)	33.75^{f} (35.51)	46.50^{f} (42.99)	38.21^{g} (38.17)	27.50^{ef} (31.58)	17.50^{f} (24.70)	51.31 (45.75)
T_3	78.33^{f} (62.29)	73.40^{fg} (61.69)	72.5^{gh} (58.39)	71.53^{e} (57.76)	51.25^{e} (45.71)	50.75^{e} (46.57)	40.5^{fg} (39.52)	20.50^{g} (26.90)	18.50^{f} (25.45)	53.02 (46.73)
T_4	91.7^{d} (73.33)	94.16^{b} (76.37)	91.67^{b} (73.30)	70.83^{e} (57.31)	55.00^{d} (47.87)	58.00^{d} (49.60)	47.60^{e} (43.62)	25.50^{f} (30.25)	13.40^{g} (21.43)	60.87 (51.27)
T_5	90.83^{d} (72.43)	90.83^{c} (72.43)	85.83^{c} (67.89)	70.00^{e} (56.79)	57.50^{cd} (49.32)	50.50^{de} (45.28)	48.50^{e} (44.13)	55.00^{a} (48.06)	27.00^{e} (29.98)	63.99 (53.12)
T_6	30.83^{i} (33.71)	30.00^{h} (33.20)	33.40^{j} (35.29)	9.16^{i} (17.60)	5.80^{h} (13.93)	10.58^{i} (18.92)	5.50^{j} (13.52)	**	**	17.89 (25.02)
T_7	63.40^{h} (52.77)	47.50^{h} (43.56)	68.40^{h} (55.80)	34.5^{h} (35.96)	1.25^{i} (6.41)	18.70^{h} (25.60)	15.50^{h} (23.12)	2.00^{h} (7.09)	**	31.40 (34.08)
T_8	9.25^{j} (17.64)	**	**	**	**	**	**	**	**	9.25 (17.70)
T_9	5.00^{k} (12.82)	**	**	**	**	**	**	**	**	5.00 (12.92)
T_{10}	95.83^{bc} (78.45)	85.00^{d} (68.07)	80.83^{de} (64.05)	69.16^{ef} (56.28)	58.75^{c} (50.05)	62.20^{c} (52.06)	43.70^{f} (41.37)	35.00^{d} (36.26)	28.50^{d} (32.25)	62.10 (52.00)
T_{11}	97.5^{b} (81.53)	82.50^{de} (65.29)	80.00^{e} (63.45)	70.00^{e} (56.79)	56.25^{cd} (48.59)	56.00^{de} (48.44)	47.50^{e} (43.56)	39.50^{cd} (38.93)	30.00^{d} (33.20)	62.13 (52.02)
T_{12}	100^{a} (90.00)	95.00^{b} (77.25)	95.83^{a} (78.45)	87.50^{b} (69.33)	70.00^{a} (56.79)	68.50^{b} (55.86)	58.00^{b} (49.60)	40.50^{c} (39.52)	38.00^{bc} (38.05)	72.59 (58.42)
T_{13}	95.00^{c} (77.25)	92.50^{bc} (74.19)	93.34^{b} (75.30)	90.83^{a} (72.38)	72.50^{a} (58.38)	70.50^{ab} (57.11)	62.00^{a} (51.94)	55.50^{a} (48.15)	38.50^{b} (38.53)	74.51 (59.67)
T_{14}	100^{a} (90.00)	98.30^{a} (82.70)	93.34^{b} (75.15)	84.23^{c} (66.62)	72.50^{a} (58.38)	72.00^{a} (58.06)	55.50^{bc} (48.15)	49.50^{b} (44.71)	45.50^{a} (42.14)	74.54 (59.69)
T_{15}	83.40^{e} (65.98)	84.16^{d} (66.53)	84.16^{cd} (66.57)	62.50^{g} (52.24)	72.50^{a} (58.38)	67.50^{b} (55.25)	52.20^{cd} (46.26)	42.50^{c} (40.68)	35.00^{c} (36.26)	64.88 (53.65)
T_{16}	58.70^{g} (50.01)	69.16^{g} (56.27)	43.34^{i} (41.17)	35.00^{h} (36.26)	30.21^{g} (33.34)	26.50^{h} (30.97)	10.50^{i} (18.85)	**	**	40.91 (39.76)
Mean	73.47 (58.52)	78.92 (62.66)	76.91 (61.28)	64.30 (53.30)	50.16 (45.09)	50.76 (45.43)	40.99 (39.80)	35.25 (36.42)	28.35 (32.17)	
SEm±	3.32	3.58	3.44	3.24	3.02	2.69	2.44	2.62	2.28	
CD (0.05)	3.39	3.93	3.16	2.34	2.25	2.21	2.25	3.32	2.43	
CD (0.01)	4.56	5.30	4.27	3.16	3.04	2.98	3.04	4.51	3.30	

MAS: Months after storage
*Means in each column with at least one letter in common are not significantly different at 5% level of probability
**All the replicate values equalling zero are not included in the analysis
Values in parentheses are arc sine transformed values

T6 (1.60), T8 (0.90) and T9 (0.50). The germination index in the above treatments was also inferior to untreated control throughout the period of storage.

Germination index over the storage period of 10 months after seed invigoration (MAI) varied between 0.50 in seeds treated with salicylic acid 60 ppm for 24 hours (T9) to 10.11 in T13 ($CaCl_2$ 50 mM for 24 hours). The average speed of germination over the storage period was high (>9.00) in seed invigoration treatments T13 (10.11), T14 (10.07) and T12 (9.94). Apart from treatments T8 (salicylic acid 60 ppm for 12 hours) and T9 (salicylic acid 60 ppm for 24 hours), where seeds failed to germinate after 1 MAI, average germination index over storage was found to be low (1.60 and 3.40) in treatments T6 and T7. The estimates in these treatments were lower than in the control (4.21).

Treatments T14 (14.12), T13 (13.68), T12 (13.52) and T4 (13.51) had registered the highest germination index at 2 MAS and were found to be on par with each other. These treatments were also found to be significantly superior to all other treatments. Treatment T6 had registered the least value (2.71).

At 7 MAS, treatments T10 (8.20), T13 (7.43) and T12 (7.32) registered the maximum germination index and were found significantly superior to other treatments. Seeds treated with vinegar pH 3.7 for 2 hours had registered the least value (0.62) while T8 and T9 failed to germinate after 1 MAS.

4.2.4 Coefficient of velocity of germination (%)

The effect of seed invigoration on coefficient of velocity of germination was highly significant except at 9 MAS (Table 12). Coefficient of velocity of germination declined progressively over the period of storage irrespective of invigoration treatments. The average coefficient of velocity of germination in majority of invigoration treatments was higher than that of untreated control (11.49%). It varied from 9.80 per cent in T8 (salicylic acid 60 ppm for 12 hours) to 13.47 per cent in T10

Table 11. Effect of seed invigoration on germination index in ash gourd seeds during storage

Treatments	Germination index									
	1MAS	2MAS	3MAS	4MAS	5MAS	6MAS	7MAS	8MAS	9MAS	Mean
T_1	13.71^{ab}	12.26^{bc}	11.1^{c}	10.99^{c}	9.25^{b}	5.74^{e}	5.34^{d}	3.81^{de}	2.21^{e}	8.27
T_2	8.46^{d}	8.60^{e}	8.34^{d}	5.48^{g}	3.73^{e}	4.25^{f}	3.24^{f}	2.85^{f}	1.62^{f}	5.17
T_3	7.77^{d}	10.33^{d}	7.15^{e}	7.66^{f}	7.31^{d}	5.71^{e}	4.23^{e}	2.11^{g}	1.74^{f}	6.00
T_4	12.93^{b}	13.51^{a}	13.18^{ab}	10.17^{d}	6.87^{d}	7.16^{c}	5.16^{d}	3.36^{ef}	1.42^{f}	8.19
T_5	12.73^{b}	12.82^{b}	12.32^{bc}	10.00^{d}	8.30^{c}	6.42^{d}	5.32^{d}	6.43^{ab}	3.16^{d}	8.61
T_6	2.83^{f}	2.71^{h}	2.79^{g}	0.84^{i}	0.60^{f}	0.85^{h}	0.62^{h}	**	**	1.60
T_7	6.38^{e}	4.75^{g}	8.20^{d}	3.24^{h}	0.15^{f}	1.87^{g}	1.82^{g}	0.81^{h}	**	3.40
T_8	0.90^{g}	**	**	**	**	**	**	**	**	0.90
T_9	0.50^{g}	**	**	**	**	**	**	**	**	0.50
T_{10}	13.73^{ab}	12.28^{bc}	11.63^{c}	9.80^{d}	8.43^{c}	7.47^{c}	8.20^{a}	4.37^{d}	3.24^{d}	8.79
T_{11}	13.46^{ab}	11.69^{c}	11.36^{c}	10.00^{d}	8.15^{c}	7.31^{c}	5.86^{d}	4.93^{cd}	3.35^{d}	8.46
T_{12}	14.09^{a}	13.52^{a}	13.61^{a}	12.69^{ab}	10.1^{a}	8.68^{ab}	7.32^{b}	5.06^{c}	4.42^{b}	9.94
T_{13}	13.69^{ab}	13.68^{a}	13.18^{ab}	12.84^{a}	10.46^{a}	8.45^{b}	7.43^{b}	6.93^{a}	4.34^{b}	10.11
T_{14}	13.78^{ab}	14.12^{a}	13.26^{ab}	12.15^{b}	10.56^{a}	8.96^{a}	6.55^{c}	6.18^{b}	5.06^{a}	10.07
T_{15}	11.80^{c}	12.20^{bc}	12.32^{bc}	8.69^{e}	10.30^{a}	8.33^{b}	6.33^{c}	5.31^{c}	3.89^{c}	8.80
T_{16}	6.38^{e}	6.39^{f}	3.79^{f}	3.26^{h}	3.56^{e}	4.41^{f}	1.66^{g}	**	**	4.21
Mean	9.57	10.63	10.16	8.41	6.98	6.11	4.93	4.34	3.13	
SEm±	0.69	0.54	0.54	0.57	0.53	0.37	0.35	0.30	0.24	
CD (0.05)	0.85	0.66	0.95	0.66	0.65	0.40	0.42	0.65	0.51	
CD (0.01)	1.15	0.89	1.28	0.89	0.88	0.54	0.56	0.89	0.70	

MAS: Months after storage

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

**All the replicate values having zero are not included in the analysis

(kinetin 10 ppm for 12 hours). Treatments T10 (13.47%), T12 (13.43), T11 (13.33), T14 (13.30%) and T13 (13.29) had exhibited high average coefficient of velocity of germination. Apart from treatments T8 (salicylic acid 60 ppm for 12 hours: 9.80%) and T9 (salicylic acid 60 ppm for 24 hours: 10.00%) that ceased to germinate after 1 MAS, treatments T6 (vinegar pH 3.7 for 2hours: 9.36%), T2 (thiourea 0.5% for 24 hours: 9.94%), T7 (polyethylene glycol 6000 @-0.5 MPa for 24 hours: 10.03%) and T3 (KNO_3 0.4% for 24 hours: 11.14%) had registered a lower average coefficient of velocity of germination than control. Coefficient of velocity of germination in treatments T6 and T7 was inferior to control throughout the period of observation.

Most of the invigorated seeds registered the maximum coefficient of velocity of germination (>14.00%) between 2 MAS and 5 MAS whereas, it was 16.65 per cent T16 at 6 MAS. Maximum velocity in treatments T2 (11.09%), T6 (11.33 %) and T7 (11.99%) was however less than 12 per cent.

At 7 MAS, treatments T16 (15.80%), T10 (12.77%), T12 (12.62%), T11 (12.34%) and T15 (12.12%) registered the maximum coefficient of velocity of germination. These were found to be on par with each other and were also significantly superior to other treatments. Seeds treated with thiourea 0.5% for 24 hours (T2) had registered the least value (8.48%) at 7MAS while T8 and T9 had failed to germinate after 1 MAS.

4.2.5 Mean time to germination

The effect of seed invigoration on mean time to germination was significant. Mean time to germination (Table 13) increased progressively over the period of storage. The average mean time to germination over ten months varied between 7.25 days in T10 (kinetin 10 ppm for 12 hours) and 10.81 days in T6 (vinegar pH 3.7 for 2 hours). Apart from T6, the average mean time to germination over storage period was found to be high and more than 10.00 days in treatments T8 (salicylic acid 60 ppm

Table 12. Effect of seed invigoration on coefficient of velocity of germination in ash gourd during storage

Treatments	Coefficient of velocity of germination (%)									
	1MAS	2MAS	3MAS	4MAS	5MAS	6MAS	7MAS	8MAS	9MAS	Mean
T1	14.31^{a} (22.22)	14.42^{a} (22.32)	14.02^{a} (21.99)	13.89^{a} (21.88)	14.23^{a} (22.16)	10.93^{de} (19.31)	10.97^{bc} (19.34)	12.72^{a} (20.89)	11.06 (19.43)	12.95 (21.09)
T2	10.57^{b} (18.97)	11.09^{b} (19.45)	11.12^{bc} (19.48)	8.32^{b} (16.76)	11.06^{bc} (19.42)	9.15^{fg} (17.61)	8.48^{c} (16.93)	10.36^{c} (18.77)	9.29 (17.75)	9.94 (18.38)
T3	9.92^{bc} (18.36)	14.07^{a} (22.03)	9.86^{cd} (18.30)	10.70^{b} (19.10)	14.26^{a} (22.19)	11.25^{cde} (19.59)	10.45^{bc} (18.86)	10.32^{c} (18.74)	9.42 (17.87)	11.14 (19.50)
T4	14.10^{a} (22.50)	14.35^{a} (22.26)	14.37^{a} (22.28)	14.36^{a} (22.27)	12.49^{b} (20.69)	12.34^{bcd} (20.57)	10.84^{bc} (19.22)	13.20^{b} (21.30)	10.64 (19.04)	12.96 (21.10)
T5	14.01^{a} (21.98)	14.11^{a} (22.07)	14.35^{a} (22.26)	14.28^{a} (22.20)	14.43^{a} (22.32)	12.71^{bcd} (20.88)	10.98^{bc} (19.35)	11.70^{b} (20.00)	11.72 (20.02)	13.14 (21.26)
T6	9.19^{bc} (17.64)	9.04^{c} (17.50)	8.36^{d} (16.81)	9.17^{b} (17.62)	10.40^{c} (18.81)	8.03^{g} (16.46)	11.33^{bc} (19.67)	**	**	9.36 (17.81)
T7	10.06^{bc} (18.49)	10.00^{b} (18.43)	11.99^{bc} (20.26)	9.39^{b} (17.84)	12.26^{b} (20.50)	10.01^{ef} (18.45)	11.74^{b} (20.03)	4.83^{d} (12.69)	**	10.03 (19.16)
T8	9.80^{bc} (18.24)	**	**	**	**	**	**	**	**	9.80 (18.24)
T9	10.00^{bc} (18.43)	**	**	**	**	**	**	**	**	10.00 (18.43)
T10	14.33^{a} (22.24)	14.45^{a} (22.34)	14.38^{a} (22.29)	14.17^{a} (22.12)	14.34^{a} (22.25)	12.01^{bcd} (20.28)	12.77^{b} (25.67)	12.48^{bc} (20.69)	11.36 (19.70)	14.03 (22.00)
T11	13.81^{a} (21.81)	14.16^{a} (22.11)	14.20^{a} (22.14)	14.28^{a} (22.20)	14.50^{a} (22.38)	13.06^{b} (21.19)	12.34^{b} (20.56)	12.48^{bc} (20.69)	11.17 (19.53)	13.33 (21.42)
T12	14.09^{a} (22.05)	14.23^{a} (22.16)	14.20^{a} (22.14)	14.50^{a} (22.38)	14.42^{a} (22.32)	12.68^{bc} (20.86)	12.62^{b} (20.80)	12.48^{bc} (20.69)	11.63 (19.94)	13.43 (21.50)
T13	14.40^{a} (22.31)	14.79^{a} (22.62)	14.12^{a} (22.07)	14.13^{a} (22.08)	14.43^{a} (22.33)	11.99^{bcd} (20.26)	11.99^{b} (20.26)	12.48^{bc} (20.69)	11.29 (19.63)	13.29 (21.38)
T14	14.28^{a} (22.20)	14.37^{a} (22.27)	14.20^{a} (22.14)	14.42^{a} (22.32)	14.57^{a} (22.44)	12.45^{bcd} (20.66)	11.81^{b} (20.10)	12.48^{bc} (20.69)	11.13 (19.49)	13.30 (21.39)
T15	14.15^{a} (22.10)	14.50^{a} (22.38)	14.64^{a} (22.50)	13.90^{a} (21.89)	14.21^{a} (22.15)	12.35^{bcd} (20.57)	12.12^{b} (20.37)	12.49^{bc} (20.70)	11.11 (19.47)	13.27 (21.37)
T16	10.88^{bc} (19.26)	9.24^{c} (17.70)	8.74^{d} (17.20)	9.33^{b} (17.78)	11.80^{bc} (20.09)	16.65^{a} (24.08)	15.80^{a} (23.42)	**	**	11.78 (20.08)
Mean	12.73 (20.59)	13.06 (21.18)	12.75 (20.92)	12.49 (20.70)	13.39 (21.46)	11.83 (20.12)	12.16 (20.41)	11.50 (19.82)	10.89 (19.27)	
SEm ±	0.34	0.37	0.36	0.41	0.25	0.32	0.39	0.39	0.19	
CD(0.05)	1.55	1.63	1.67	2.78	1.67	1.63	1.67	1.58	NS	
CD (0.01)	2.09	2.20	2.25	3.61	2.25	2.20	2.25	2.14	NS	

MAS: Months after storage

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

**All the replicate values having zero are not included in the analysis Values in parentheses are Arc Sine transformed values

for 12 hours: 10.20), T2 (thiourea 0.5% for 24 hours: 10.17), and T9 (salicylic acid 60 ppm for 24 hours: 10.00), while it was low (< 7.5 days) in case of T10 (kinetin 10 ppm for 12 hours: 7.25) and T12 ($CaCl_2$ 50 mM for 12 hours: 7.48).

At 7 MAS, treatments T10 (5.32), untreated seeds (T16: 6.32), T14 (*Pf* $1x10^6$ $cfu.ml^{-1}$ for 12hours: 6.68), T13 ($CaCl_2$ 50mM for 24 hours: 6.77), T15 (*Pf* $1x10^6$ $cfu.ml^{-1}$ for 24 hours: 6.83) and T11 (kinetin 10 ppm for 24 hours: 6.91) registered the minimum mean time to germination and were found to be on par with each other. These treatments were also found significantly superior to all other treatments. Seeds treated with thiourea 0.5% for 24 hours had registered the highest value (11.78 days) followed by T1 (hydropriming 12 hours: 9.11), T3 (KNO_3 0.4% for 24 hours: 9.56), T4 (KNO_3 0.7% for 24 hours: 9.22) and T5 (KH_2PO_4 10^{-1} M for 24 hours: 9.10), while T8 and T9 had failed to germinate after 1 MAS.

4.2.7 Energy of germination

The effect of seed invigoration on energy of germination was found to be highly significant. Energy of germination (Table 14) decreased over storage irrespective of invigoration treatment.

The average energy of germination over storage was generally higher in invigorated seeds than that in untreated control (13.69). It ranged from 8.33 in T6 (vinegar pH 3.7 for 2 hours) to 37.72 in T13 ($CaCl_2$ 50 mM for 24 hours). Treatments T13 (37.72), T14 (37.13), and T12 (36.96) exhibited maximum (> 35.00) energy of germination. Apart from treatments T8 (salicylic acid 60 ppm for 12 hours) and T9 (salicylic acid 60 ppm for 24 hours) that ceased to germinate for most part of storage, treatments T6 (vinegar pH 3.7 for 2 hours: 8.33) had also registered a lower energy of germination than control. Energy of germination in these treatments was inferior to control throughout the period of observation. At 35 DAI, treatments T12 (55.50), T10 and T14 (53.00), T11 (48.00) and T13 (46.80) registered the highest energy of germination and found to be significantly superior to all other treatments. Other

Table 13. Effect of seed invigoration on mean time to germination in ash gourd during storage

Treatments	Mean time to germination (days)									
	1MAS	2MAS	3MAS	4MAS	5MAS	6MAS	7MAS	8MAS	9MAS	Mean
T_1	6.98^{b}	6.93^{c}	7.13^{de}	7.19^{cd}	7.02^{cd}	9.14^{bc}	9.11^{b}	7.86^{bc}	9.03^{ab}	7.82
T_2	9.45^{a}	9.01^{b}	8.98^{bc}	12.01^{a}	9.04^{ab}	10.92^{ab}	11.78^{a}	9.64^{a}	10.75^{a}	10.17
T_3	10.07^{a}	7.10^{c}	10.13^{b}	9.33^{b}	7.00^{cd}	8.88^{cd}	9.56^{b}	9.68^{a}	10.61^{a}	9.15
T_4	7.09^{b}	6.96^{c}	6.95^{de}	6.96^{cd}	8.00^{bcd}	8.10^{cd}	9.22^{b}	7.57^{bc}	9.39^{ab}	7.80
T_5	7.13^{b}	7.08^{c}	6.96^{de}	7.00^{cd}	6.92^{ef}	7.86^{cde}	9.10^{b}	8.54^{ab}	8.52^{bc}	7.68
T_6	10.88^{a}	11.05^{aq}	11.95^{a}	10.90^{ab}	9.61^{a}	12.44^{a}	8.82^{bc}	**	**	10.81
T_7	9.90^{a}	10.00^{ab}	8.33^{cd}	10.64^{ab}	8.15^{abcd}	9.98^{bc}	8.51^{bc}	8.44^{ab}	**	9.36
T_8	10.20^{a}	**	**	**	**	**	**	**	**	10.20
T_9	10.00^{a}	**	**	**	**	**	**	**	**	10.00
T_{10}	6.97^{b}	6.91^{c}	6.95^{de}	7.05^{cd}	6.96^{cd}	6.75^{ef}	5.32^{e}	6.60^{c}	6.63^{d}	7.25
T_{11}	7.24^{b}	7.05^{c}	7.04^{de}	7.00^{cd}	6.89^{d}	7.65^{cde}	6.91^{cde}	6.73^{c}	6.53^{d}	7.54
T_{12}	7.09^{b}	7.02^{c}	7.03^{de}	6.89^{cd}	6.93^{cd}	7.88^{cde}	7.92^{bcd}	6.74^{c}	6.81^{d}	7.48
T_{13}	6.93^{b}	6.75^{c}	7.07^{de}	7.07^{cd}	6.92^{cd}	6.75^{ef}	6.77^{de}	6.93^{c}	6.97^{cd}	7.59
T_{14}	6.99^{b}	6.95^{c}	7.03^{de}	6.93^{cd}	6.86^{cd}	6.80^{ef}	6.68^{de}	6.78^{c}	6.76^{d}	7.58
T_{15}	7.06^{b}	6.89^{c}	6.82^{de}	7.19^{c}	7.03^{bc}	6.84^{ef}	6.83^{de}	6.58^{c}	6.80^{d}	7.59
T_{16}	10.88^{a}	10.81^{ab}	11.43^{a}	10.71^{ab}	8.47^{abc}	6.00^{ef}	6.32^{de}	**	**	9.28
Mean	6.19	6.51	6.62	6.58	6.21	6.38	5.98	5.96	6.15	
SEm ±	0.25	0.25	0.28	0.31	0.19	0.31	0.28	0.41	0.42	
CD (0.05)	1.66	1.80	1.67	1.67	1.67	1.90	1.67	2.72	1.61	
CD (0.01)	2.23	2.43	2.25	2.25	2.25	2.57	2.25	3.69	NS	

MAS: Months after storage

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

**All the replicate values having zero are not included in the analysis

Table 14. Effect of seed invigoration on energy of germination in ash gourd during storage

Treatments	Energy of germination									
	1MAS	2MAS	3MAS	4MAS	5MAS	6MAS	7MAS	8MAS	9MAS	Mean
T_1	45.00^{c}	35.00^{e}	29.60^{g}	30.00^{d}	28.00^{d}	22.90^{b}	19.00^{d}	10.00^{g}	5.50^{f}	25.00
T_2	29.00^{e}	23.00^{g}	25.00^{h}	23.00^{e}	20.20^{f}	12.70^{c}	7.00^{e}	3.50^{i}	1.00^{h}	16.04
T_3	42.00^{d}	36.60^{de}	33.00^{f}	37.00^{b}	24.00^{e}	21.80^{b}	19.00^{d}	6.00^{h}	3.00^{g}	24.71
T_4	45.00^{c}	44.50^{c}	45.90^{bc}	33.20^{c}	29.00^{cd}	23.00^{b}	19.00^{d}	12.00^{f}	4.00^{g}	28.40
T_5	44.00^{c}	45.00^{c}	41.50^{d}	33.00^{c}	30.00^{c}	22.50^{b}	21.00^{c}	25.00^{b}	10.00^{e}	30.22
T_6	10.00^{g}	8.00^{i}	7.00^{j}	**	**	**	**	**	**	8.33
T_7	28.00^{e}	33.00^{f}	16.00^{i}	10.50^{f}	**	1.00^{d}	1.00^{f}	**	**	14.91
T_8	**	**	**	**	**	**	**	**	**	0
T_9	**	**	**	**	**	**	**	**	**	0
T_{10}	54.00^{b}	48.00^{b}	40.00^{de}	33.00^{c}	29.50^{cd}	29.00^{a}	20.80^{c}	19.00^{d}	11.00^{e}	31.58
T_{11}	55.00^{b}	37.00^{de}	33.00^{f}	33.00^{c}	24.50^{e}	23.00^{b}	18.40^{d}	17.10^{e}	13.00^{d}	28.22
T_{12}	57.00^{a}	55.00^{a}	48.00^{ab}	45.00^{a}	34.40^{b}	30.00^{a}	31.30^{a}	17.00^{e}	15.00^{c}	36.96
T_{13}	55.00^{b}	43.50^{c}	45.00^{c}	44.00^{a}	45.00^{a}	30.00^{a}	32.00^{a}	28.00^{a}	17.00^{b}	37.72
T_{14}	58.50^{a}	55.00^{a}	49.00^{a}	38.20^{b}	33.50^{b}	30.00^{a}	28.00^{b}	23.00^{c}	19.00^{a}	37.13
T_{15}	44.00^{c}	38.00^{d}	36.00^{e}	28.50^{d}	34.70^{b}	29.00^{a}	22.00^{c}	19.50^{d}	17.00^{b}	29.85
T_{16}	23.50^{f}	21.00^{h}	18.00^{i}	11.00^{f}	8.00^{g}	0.66^{d}	**	**	**	13.69
Mean	36.93	37.32	33.35	28.52	24.34	19.77	17.03	15.00	10.50	
SEm ±	2.03	1.99	2.31	2.27	2.01	2.33	2.16	2.11	1.79	
CD (0.05)	1.99	1.96	2.27	2.18	1.88	2.15	2.02	1.91	1.61	
CD (0.01)	2.69	2.65	3.07	2.95	2.55	2.90	2.745	2.59	2.19	

MAS: Months after storage

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

**All the replicate values having zero are not included in the analysis

treatment except T6 (7.50), T7 (16.00), T16 (17.00) and T2 (28.00) while treatments T8 and T9 had failed to germinate throughout the study.

Treatments T12 and T14 (55.00 each) had registered the higher energy of germination at 2 MAS and were found to be significantly superior to all other treatments. These were also found to be on par with each other. Apart from treatments T8 and T9, T6 with an estimate of 8.00) was significantly inferior to untreated seeds (T16: 21.00) at 2 MAS.

At 7 MAS, treatments T13 (32.00), T12 (31.30) and T14 (28.00) registered the maximum energy of germination and found significantly superior to other treatments. Seeds treated with PEG 6000 (T7) had registered the least value of 1.00 while T6, T8, T9 and untreated seeds (T16) had failed to germinate at 7 MAS.

4.2.8 Vigour index-I

The effect of seed invigoration on vigour index-I was found to be highly significant. Vigour index-I (Table 15) decreased over storage irrespective of invigoration treatment. A decrease in vigour index-I was observed in both treated and untreated seeds at the end of storage period of 10 months. The average vigour index-I in majority of invigoration treatments was higher than that of untreated control (886). It varied from 98 in T9 (salicylic acid 60 ppm for 24 hours) to 1747 in T13 ($CaCl_2$ 50 mM for 24 hours). Treatments T13 (1747), T12 (1739.11), T14 (1718.77) and T5 (1532.22) had exhibited high mean vigour index-I. Apart from treatments T9 and T8 (Salicylic acid 60 ppm for 12 hours: 171) that ceased to germinate after 1 MAS, treatments T6 (vinegar pH 3.7 for 2 hours: 338.42) and T7 (polyethylene glycol 6000 @-0.5 MPa for 24 hours: 748) had registered a low average vigour index-I than control (886) over the storage period. Vigour index-I in treatments T6 and T7 was found to be inferior to control throughout the period of observation.

Table 15. Effect of seed invigoration on vigour index-I in ash gourd during storage

Treatments	Vigour index-I									
	1MAS	2MAS	3MAS	4MAS	5MAS	6MAS	7MAS	8MAS	9MAS	Mean
T_1	2469^{c}	2163^{cde}	1953^{f}	1879^{b}	1407^{c}	1128^{e}	1045^{c}	609^{d}	401^{f}	1450.44
T_2	1656^{hi}	1601^{f}	1560^{h}	1497^{d}	561^{f}	840^{g}	644^{e}	456^{ef}	286^{gh}	1011.22
T_3	1898^{g}	1588^{f}	1770^{g}	1522^{d}	944^{e}	978^{f}	677^{e}	370^{f}	329^{g}	1119.55
T_4	1979^{fg}	2182^{bcd}	2278^{bc}	1703^{c}	1237^{d}	1243^{c}	864^{d}	501^{e}	254^{h}	1360.11
T_5	2154^{e}	2258^{abc}	2179de	1693^{c}	1374^{c}	1159de	1168^{b}	1257^{a}	548^{e}	1532.22
T_6	483^{j}	612^{h}	676^{j}	199^{g}	118^{g}	202^{j}	79^{gh}	**	**	338.42
T_7	1570^{i}	1194^{g}	1703^{g}	831^{e}	27^{h}	365^{i}	266^{f}	28^{g}	**	748.00
T_8	171^{k}	**	**	**	**	**	**	**	**	171.00
T_9	98^{k}	**	**	**	**	**	**	**	**	98.00
T_{10}	2338^{d}	2044^{e}	2009^{f}	1674^{c}	1159^{d}	1202^{cd}	868^{d}	685^{d}	523^{e}	1388.11
T_{11}	2407^{cd}	2069de	1917^{f}	1706^{c}	1334^{c}	1188^{cde}	1106^{bc}	871^{c}	630^{d}	1469.77
T_{12}	2576^{b}	2365^{a}	2365^{b}	2212^{a}	1668^{a}	1544^{a}	1273^{a}	873^{c}	776^{bc}	1739.11
T_{13}	2319^{d}	2231^{bc}	2507^{a}	2230^{a}	1573^{b}	1499^{a}	1330^{a}	1212^{a}	822^{b}	1747.00
T_{14}	2870^{a}	2305^{ab}	2243^{cd}	1971^{b}	1515^{b}	1506^{a}	1137^{b}	1012^{b}	910^{a}	1718.77
T_{15}	2055^{ef}	2092de	2119^{e}	1430^{d}	1542^{b}	1429^{b}	1141^{b}	924^{c}	748^{c}	1497.77
T_{16}	1675^{h}	1608^{f}	1012^{i}	714^{f}	601^{f}	438^{h}	154^{g}	**	**	886.00
Mean	1794.87	1877.92	1877.92	1518.64	1075.71	1051.50	839.42	733.16	566.09	
SEm±	119.69	76.28	77.81	87.02	81.98	66.33	63.26	59.03	44.48	
CD (0.05)	105.17	137.30	105.84	98.75	91.55	75.16	85.76	102.21	66.36	
CD (0.01)	141.36	185.20	142.80	133.20	123.50	101.39	115.69	138.50	90.19	

MAS: Months after storage

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

**All the replicate values having zero are not included in the analysis

Treatments T12 (2365), T14 (2305) and T5 (2258) registered high vigour index-I at 2 MAS. These treatments were found to be on par with each other and superior to untreated control (1608). Treatments T6 (612), T7 (1194), T2 (1601) and T3 (1588) had registered low values and were found to be on par with each other.

At 7 MAS, treatments T12 (1273) and T13 (1330) registered the maximum vigour index-I and found significantly superior to other treatments. T15 (1141) and T14 (1137) had also exhibited high vigour index-I and found significantly superior to other treatments. Seeds treated with vinegar (T6) had registered the least value (79) while untreated control recorded the next lower value of 1542. T8 and T9 had failed to germinate after 1 MAS.

4.2.9 Vigour index-II

The effect of seed invigoration on vigour index-II was found to be highly significant throughout storage. Vigour index-II (Table 16) decreased over storage irrespective of invigoration treatment.

The average vigour index-II over storage in majority of invigoration treatments was higher than that of untreated control (0.78). It varied from 0.036 in T9 (salicylic acid 60 ppm for 24 hours) to 1.68 in T13 ($CaCl_2$ 50 mM for 24 hours). Treatments T13 (1.68), T12 (1.60), T1 (1.48), T14 (1.47) and T5 (1.32) had exhibited high mean vigour index-II. Apart from treatments T9 and T8 (salicylic acid 60 ppm for 12 hours: 0.075) that ceased to germinate after 1 MAS, treatments T6 (vinegar pH 3.7 for 2hours: 0.15) and T7 (polyethylene glycol 6000 @ -0.5 MPa for 24 hours: 0.58) had registered a lower mean vigour index-II than control (0.78). Vigour index-II in both these treatments was inferior to control throughout the period of observation.

Table 16. Effect of seed invigoration on vigour index-II in ash gourd during storage

Treatments	Vigour index-II									
	1MAS	2MAS	3MAS	4MAS	5MAS	6MAS	7MAS	8MAS	9MAS	Mean
T_1	2.48^{a}	2.20^{a}	1.92^{bc}	1.77^{b}	1.58^{a}	1.24^{cd}	1.10^{abc}	0.62^{ef}	0.37^{e}	1.48
T_2	1.94^{de}	1.83^{cd}	1.50^{e}	1.31^{ef}	0.50^{h}	0.91^{g}	0.62^{gh}	0.40^{g}	0.23^{f}	1.03
T_3	2.03^{d}	1.71^{d}	1.74^{d}	1.47^{de}	0.99^{ef}	0.98^{fg}	0.77^{efg}	0.36^{g}	0.33^{e}	1.15
T_4	1.92^{de}	1.91^{bc}	1.83^{bcd}	1.39^{de}	0.86^{f}	0.98^{fg}	0.74^{fg}	0.46^{g}	0.22^{f}	1.15
T_5	2.05^{cd}	2.08^{ab}	1.97^{b}	1.33^{ef}	1.02^{e}	1.04^{ef}	0.98^{cdef}	0.96^{ab}	0.47^{d}	1.32
T_6	0.24^{h}	0.11^{g}	0.17^{g}	0.074^{i}	0.14^{i}	0.24^{h}	0.076^{i}	**	**	0.15
T_7	1.20^{g}	0.74^{f}	1.39^{e}	0.72^{g}	0.028^{i}	0.41^{h}	0.13^{h}	0.01^{h}	**	0.58
T_8	0.075^{hi}	**	**	**	**	**	**	**	**	0.075
T_9	0.036^{i}	**	**	**	**	**	**	**	**	0.036
T_{10}	2.48^{a}	2.26^{a}	1.96^{b}	1.56^{cd}	1.09^{de}	1.12^{de}	0.80^{defg}	0.59^{f}	0.48^{d}	1.37
T_{11}	2.27^{b}	1.97^{bc}	1.75^{d}	1.67^{bc}	1.24^{cd}	1.19^{cd}	1.03^{abcd}	0.80^{cd}	0.37^{e}	1.37
T_{12}	2.66^{a}	2.24^{a}	2.23^{a}	1.80^{b}	1.30^{bc}	1.25^{bc}	1.25^{ab}	0.85^{bc}	0.80^{a}	1.60
T_{13}	2.24^{bc}	2.21^{a}	2.30^{a}	2.42^{a}	1.44^{ab}	1.43^{a}	1.28^{a}	1.05^{a}	0.73^{b}	1.68
T_{14}	2.23^{bc}	1.99^{bc}	1.98^{b}	1.79^{b}	1.13^{de}	1.41^{a}	1.05^{abcd}	0.88^{bc}	0.77^{ab}	1.47
T_{15}	1.75^{ef}	1.85^{cd}	1.76^{cd}	1.18^{f}	1.52^{a}	1.37^{ab}	1.00^{bcde}	0.71^{de}	0.59^{c}	1.30
T_{16}	1.60^{f}	1.38^{e}	0.79^{f}	0.52^{h}	0.67^{g}	0.50^{h}	0.79^{defg}	**	**	0.78
Mean	1.70	1.75	1.66	1.36	0.96	1.00	0.77	0.64	0.49	
SEm±	0.19	0.19	0.16	0.18	0.16	0.13	0.21	0.15	0.08	
CD (0.05)	0.19	0.18	0.15	0.18	0.16	0.12	0.20	0.14	0.08	
CD (0.01)	0.26	0.25	0.21	0.24	0.21	0.17	0.28	0.19	0.10	

MAS: Months after storage

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

**All the replicate values having zero are not included in the analysis

At 2 MAS, treatments T10 (2.26), T12 (2.24), T13 (2.21) and T1 (2.20) registered the maximum vigour index-II. These were found to be on par with other treatments and also significantly superior to all other treatments. Except treatments T6 (0.11) and T7 (0.74) had the least value all other treatments were found to be superior to the untreated seeds. Treatments T8 and T9 had ceased to germinate after 1 MAS.

At 7 MAS, treatment T13 (1.28) was found to be significantly superior to other treatments. T12 (1.25), T1 (1.10), T14 (1.05) and T11 (1.03) were the next best treatments. Seeds treated with vinegar had registered the least value (0.076) while T8 and T9 had failed to germinate after 1 MAS. These were inferior to untreated seeds (0.79) at 7 MAS.

4.2.10 Electrical conductivity of seed leachate (dSm^{-1})

The effect of seed invigoration on electrical conductivity was highly significant throughout the storage period. Electrical conductivity of seed leachate (Table 17) increased over storage irrespective of invigoration treatment.

The average electrical conductivity of seed leachate (EC) over ten months varied between 0.019 dsm^{-1} days in T1 (hydropriming 12 hours) and 0.0052 dsm^{-1} in T16 (control). It was also found to be high in treatments with salicylic acid (T8 and T9), PEG 6000 (T7), and vinegar (T6), EC of seed leachate was low in case of T1 (hydropriming for 12 hours), T14 (*Pf* $1x10^6$ $cfu.ml^{-1}$ for 12 hours), T11 (kinetin 10 ppm for 24 hours), T13 ($CaCl_2$ 50 mM for 24 hours) and T15 (*Pf* $1x10^6$ $cfu.ml^{-1}$ for 24 hours).

Treatments T1 (0.0017 dsm-1), T3 (0.0018 dsm^{-1}), T4 and T15 (0.0020 dsm^{-1} each), registered lower EC at 2 MAS and found to be significantly superior to control (0.0046 dsm^{1}). Untreated seeds were found to be significantly inferior to all other treatments at 2 MAS.

Table 17. Effect of seed invigoration on electrical conductivity in ash gourd during storage

Treatments	Electrical conductivity (dsm^{-1})										Mean
	0MAS	1MAS	2MAS	3MAS	4MAS	5MAS	6MAS	7MAS	8MAS	9MAS	
T_1	0.0017^{c}	0.0017^{ef}	0.0017^{g}	0.0017^{f}	0.0018^{j}	0.0018^{e}	0.0020^{e}	0.0020^{h}	0.0022^{f}	0.0024^{i}	0.0019
T_2	0.0014^{d}	0.0015^{fg}	0.0025^{d}	0.0031^{d}	0.0032^{e}	0.0026^{bc}	0.0031^{b}	0.0036^{d}	0.0031^{d}	0.0045^{d}	0.0028
T_3	0.0017^{c}	0.0027^{b}	0.0018^{fg}	0.0020^{e}	0.0026^{fg}	0.0028^{b}	0.0024^{d}	0.0031^{ef}	0.0043^{c}	0.0045^{d}	0.0028
T_4	0.0012^{d}	0.0022^{cd}	0.0020^{efg}	0.0022^{e}	0.0024^{ghi}	0.0025^{bcd}	0.0027^{cd}	0.0039^{d}	0.0041^{c}	0.0045^{d}	0.0027
T_5	0.0021^{b}	0.0021^{cd}	0.0024^{de}	0.0023^{e}	0.0026^{fg}	0.0024^{cd}	0.0026^{cd}	0.0027^{g}	0.0027^{e}	0.0035^{ef}	0.0025
T_6	0.0021^{b}	0.0023^{cd}	0.0035^{c}	0.0038^{c}	0.0043^{d}	0.0049^{a}	0.0053^{a}	0.0054^{c}	0.0064^{ab}	0.0063^{bc}	0.0044
T_7	0.0018^{bc}	0.0023^{cd}	0.0021^{defg}	0.0041^{bc}	0.0049^{c}	0.0028^{b}	0.0052^{a}	0.0064^{a}	0.0064^{ab}	0.0064^{b}	0.0042
T_8	0.0018^{bc}	0.0027^{b}	0.0032^{c}	0.0043^{b}	0.0052^{b}	0.0052^{a}	0.0053^{a}	0.0054^{c}	0.0061^{b}	0.0062^{bc}	0.0045
T_9	0.0021^{b}	0.0030^{b}	0.0041^{b}	0.0041^{bc}	0.0056^{a}	0.0050^{a}	0.0050^{a}	0.0064^{a}	0.0067^{a}	0.0060^{c}	0.0048
T_{10}	0.0014^{d}	0.0013^{gh}	0.0022^{def}	0.0023^{e}	0.0031^{e}	0.0026^{bcd}	0.0026^{cd}	0.0032^{e}	0.0030^{de}	0.0044^{d}	0.0026
T_{11}	0.0020^{b}	0.0020^{cde}	0.0022^{def}	0.0021^{e}	0.0022^{hi}	0.0024^{cd}	0.0025^{cd}	0.0032^{e}	0.0032^{d}	0.0037^{e}	0.0025
T_{12}	0.0020^{b}	0.0024^{bc}	0.0023^{de}	0.0023^{e}	0.0027^{f}	0.0025^{bcd}	0.0027^{c}	0.0028^{fg}	0.0030^{de}	0.0033^{fg}	0.0026
T_{13}	0.0021^{b}	0.0024^{bc}	0.0023^{de}	0.0024^{e}	0.0025^{fgh}	0.0025^{bcd}	0.0026^{cd}	0.0026^{g}	0.0029^{de}	0.0028^{h}	0.0025
T_{14}	0.0019^{bc}	0.0020^{cde}	0.0021^{defg}	0.0020^{e}	0.0022^{hi}	0.0025^{bcd}	0.0026^{cd}	0.0026^{g}	0.0026^{e}	0.0031^{gh}	0.0023
T_{15}	0.0019^{bc}	0.0019^{de}	0.0020^{efg}	0.0021^{e}	0.0021^{ij}	0.0022^{d}	0.0031^{b}	0.0031^{ef}	0.0032^{d}	0.0032^{fg}	0.0025
T_{16}	0.0037^{a}	0.0040^{a}	0.0046^{a}	0.0047^{a}	0.0046^{d}	0.0051^{a}	0.0052^{a}	0.0058^{b}	0.0063^{ab}	0.0085^{a}	0.0052
Mean	0.0019	0.0023	0.0025	0.0028	0.0032	0.0031	0.0034	0.00391	0.0041	0.0046	
SEm±	8.18E**05	9.33E**05	0.00012	0.00014	0.00017	0.00016	0.00017	0.00021	0.00023	0.00023	
CD (0.05)	0.00081	0.00093	0.00012	0.00014	0.00017	0.00016	0.00017	0.00020	0.00023	0.00023	

MAS: Months after storage

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

**All the replicate values having zero are not included in the analysis

At 7 MAS, treatments T1 with a low EC of 0.0020 $dsm^{-1}$was significantly superior to all other treatments. T13 and T14 (0.0026 dsm^{-1} each) and T5 (0.0027 dsm^{-1}) were the next best and found significantly superior to other treatments. These treatments were on par with each other treatments. Seeds treated with PEG 6000 and salicylic acid 60 ppm for 24 hours (0.0064 dsm^{-1}) followed by untreated control (T16: 0.0064 dsm^{-1}) had registered higher electrical conductivity at 7 MAS.

4.2.11 Seed moisture content

Throughout storage period no significant difference was observed in moisture content of seed between treatments.

4.2.12 Total seed coat thickness (µm)

The effect of seed invigoration on total seed coat thickness was highly significant. Freshly extracted untreated seeds showed the high seed coat thickness of 453.3µm.

The average total seed coat thickness over ten months (Table 18) varied between 422.76 µm in T14 (*Pf* $1x10^6$ $cfu.ml^{-1}$ for 12 hours) and 470 µm in T9 (salicylic acid 60 ppm for 24 hours). It was found to be high (> 455 µm) in seeds treated with vinegar (T6), PEG 6000 (T7), salicylic acid (T8 and T9) and untreated control (T16), while it was low (< 425 µm) in the case of seeds treated with *Pf* $1x10^6$ $cfu.ml^{-1}$ for 12 hours (T14), T12 ($CaCl_2$ 50 mM for 12 hours) and T13 ($CaCl_2$ 50 mM for 24 hours).

Treatment of seeds with vinegar (T6), PEG 6000 (T7), salicylic acid (T8 and T9) and untreated control (T16) also registered higher seed coat thickness throughout storage period. Treatments T12 (419.70 µm), T14 (420.60 µm), T14 T4 (421.50 µm) possessed lower total seed coat thickness at 9 MAS.

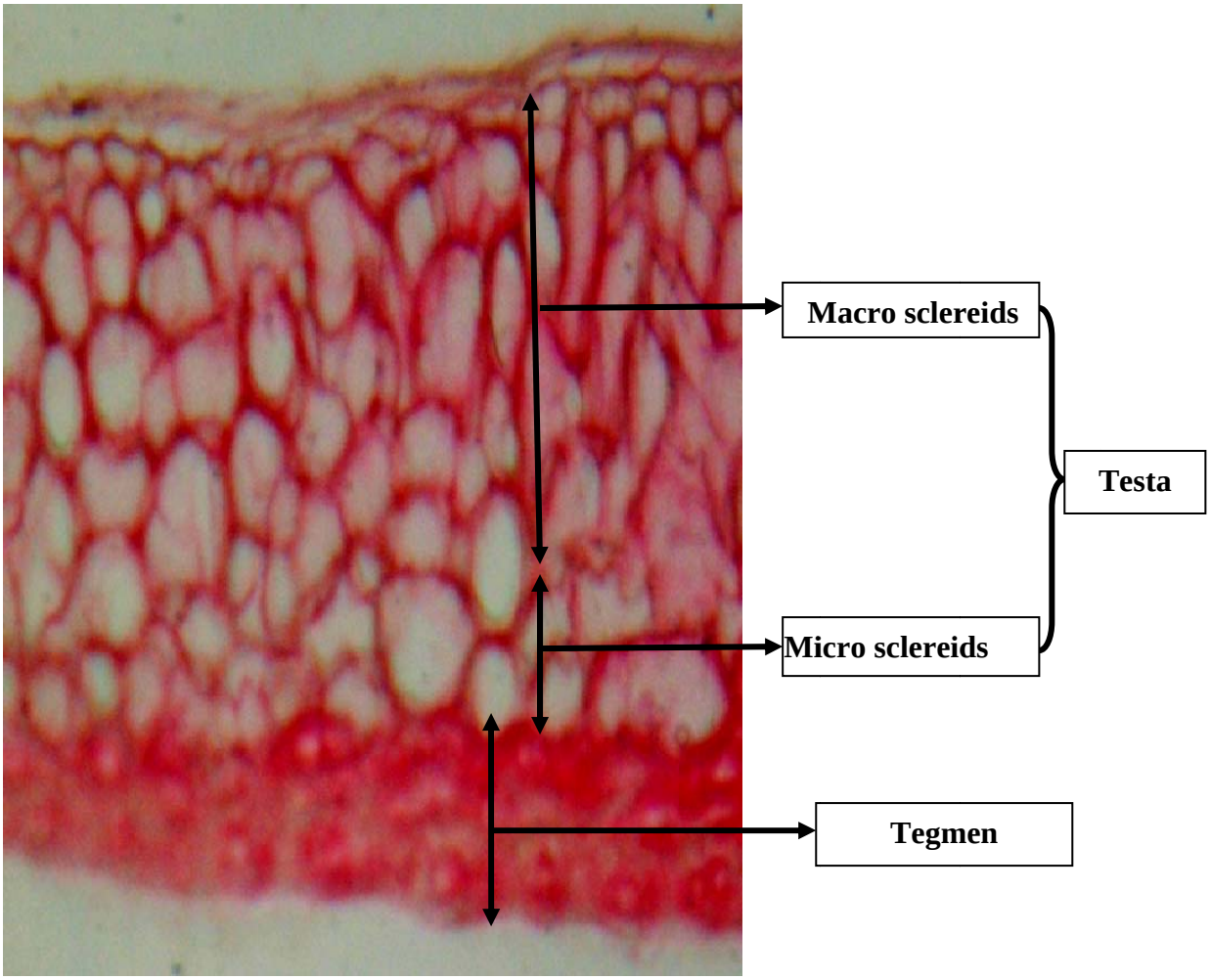
Table 18. Effect of seed invigoration on total seed coat thickness in ash gourd during storage

Treatments	Total seed coat thickness (µm)						
	0MAS	1MAS	3MAS	5MAS	7MAS	9MAS	Mean
T_1	421.20^{gh}	422.76^{fg}	424.79^{g}	427.08^{de}	429.98^{fg}	436.05^{f}	426.97
T_2	429.70^{ef}	430.16^{de}	432.90^{de}	439.25^{c}	441.86^{e}	447.09^{d}	436.82
T_3	431.90^{e}	433.00^{d}	435.60^{d}	436.76^{c}	439.50^{e}	442.05^{de}	436.46
T_4	420.01^{ghij}	422.10^{fg}	421.50^{ghi}	424.05^{def}	427.05^{g}	436.30^{f}	425.16
T_5	420.71^{ghi}	423.20^{fg}	424.30^{gh}	427.30^{de}	429.20^{fg}	432.30^{fg}	426.16
T_6	457.75^{b}	458.70^{b}	458.60^{b}	461.40^{b}	464.80^{cd}	468.00^{c}	461.54
T_7	449.60^{d}	450.20^{c}	452.20^{c}	458.20^{b}	461.40^{d}	466.01^{c}	456.26
T_8	464.80^{a}	466.00^{a}	466.30^{a}	467.60^{a}	471.70^{b}	475.95^{a}	468.72
T_9	465.90^{a}	466.30^{a}	467.30^{a}	470.40^{a}	476.70^{a}	474.35^{ab}	470.15
T_{10}	422.40^{g}	423.40^{f}	425.50^{fg}	426.00^{def}	430.16^{fg}	437.25^{ef}	427.45
T_{11}	419.3^{hijk}	420.20^{fgh}	429.30^{ef}	428.30^{d}	432.30^{f}	436.37^{f}	427.62
T_{12}	416.78^{k}	417.60^{h}	419.70^{i}	424.60^{def}	427.80^{g}	432.28^{fg}	423.12
T_{13}	418.60^{ijk}	419.30^{gh}	424.30^{gh}	424.90^{def}	427.32^{g}	433.08^{fg}	424.58
T_{14}	417.50^{ik}	417.90^{h}	420.60^{hi}	423.10^{ef}	426.90^{g}	430.57^{g}	422.76
T_{15}	427.60^{f}	428.50^{e}	421.80^{ghi}	422.00^{f}	430.20^{fg}	435.17^{fg}	427.54
T_{16}	452.50^{c}	453.00^{c}	456.50^{b}	460.10^{b}	466.84^{c}	470.03^{abc}	459.82
Mean	433.51	434.52	436.32	438.81	442.73	447.05	
SEm±	2.55	2.53	2.48	2.56	2.63	2.45	
CD (0.05)	2.52	3.98	4.18	4.77	4.26	5.18	
CD (0.01)	3.40	5.35	5.62	6.41	5.73	6.96	

MAS: Months after storage

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

Plate 1: Components of seed coat in ash gourd



At 7 MAS, treatments T14 (426.90 µm), T4 (427.05 µm), T13 (427.32 µm) and T12 (427.80 µm) had registered lower total seed coat thickness and were found to be significantly superior to other treatments. These treatments were on par with each other. Treatments T9 (476.70 µm), T8 (471.70 µm), T16 (466.84 µm) and T6 (464.80 µm) registered the highest total seed coat thickness at 7 MAS.

4.2.13 Thickness of testa (µm)

The effect of seed invigoration on total seed coat thickness was highly significant. The average thickness of testa (Table 19) over ten months varied between 369.51 µm in T14 (*Pf* $1x10^6$ $cfu.ml^{-1}$ for 12 hours) and 400.00 µm in T8 (salicylic acid 60 ppm for 12 hours). Apart from control (T8), total seed coat thickness was found to be high (> 390 µm) in treatments T9 (salicylic acid 60 ppm for 24 hours: 400.02), T6 (vinegar: 395.16), control (T16: 393.54) and T7 (PEG 6000: 393.34). However, it was low (< 370 µm) in case of T14 (*Pf* $1x10^6$ $cfu.ml^{-1}$ for 12 hours) and T12 ($CaCl_2$ 50mM for 12 hours).

At 7 MAS, treatments T13 (371.02 µm) and T14 (371.10 µm) registered lower thickness of testa and were found to be significantly superior to other treatments. These treatments were at par with each other. In contrast, the treatments T9 (402.50 µm), T8 (402.00 µm), T16 (396.82 µm), T6 (396.50 µm) and T7 (394.90 µm) had registered higher thickness of testa at 7 MAS**.**

4.2.14 Thickness of tegmen (µm)

The effect of seed invigoration on total seed coat thickness was highly significant throughout storage. The average thickness of tegmen (Table 20) over ten months varied between 52.73 µm in T4 (KNO_3 0.7% for 24 hours) and 69.80 µm in T9 (salicylic acid 60 ppm for 24 hours). Apart from T9, tegmen was thicker (> 66 µm) in treatments with salicylic acid for 12 hours (T8: 68.38 µm), untreated control (T16: 66.28 µm), PEG 6000 (T7: 62.65 µm) and vinegar (T6: 62.20 µm) while it was

Table 19. Effect of seed invigoration on thickness of testa in ash gourd during storage

Treatments	Thickness of testa (µm)						
	0MAS	1MAS	3MAS	5MAS	7MAS	9MAS	Mean
T_1	370.20^{ef}	370.60^{ef}	371.72^{e}	372.03^{ef}	373.78^{de}	377.35^{ef}	372.61
T_2	376.50^{d}	376.46^{d}	378.00^{d}	381.85^{d}	382.66^{c}	384.59^{c}	380.01
T_3	377.10^{d}	377.50^{d}	378.50^{d}	380.56^{d}	380.70^{c}	381.65^{d}	379.33
T_4	370.00^{ef}	371.60^{e}	370.60^{ef}	371.55^{efg}	372.53^{def}	377.32^{ef}	372.26
T_5	370.01^{ef}	371.80^{e}	371.90^{e}	372.80^{ef}	374.00^{d}	376.37^{ef}	372.81
T_6	394.00^{b}	394.60^{b}	393.70^{b}	395.60^{b}	396.50^{b}	396.60^{b}	395.16
T_7	391.53^{bc}	391.60^{c}	392.50^{bc}	393.20^{bc}	394.90^{b}	396.31^{b}	393.34
T_8	398.00^{a}	398.80^{a}	398.80^{a}	399.30^{a}	402.00^{a}	403.15^{a}	400.00
T_9	397.90^{a}	398.10^{a}	398.60^{a}	400.20^{a}	402.50^{a}	402.85^{a}	400.02
T_{10}	370.30^{e}	370.90^{ef}	371.70^{e}	372.70^{ef}	373.96^{d}	377.85^{e}	372.90
T_{11}	368.80^{efg}	369.40^{fg}	372.90^{e}	373.40^{e}	374.50^{d}	377.77^{e}	372.79
T_{12}	367.00^{g}	367.50^{g}	367.80^{g}	370.80^{fg}	372.30^{def}	374.52^{fg}	369.98
T_{13}	368.00^{efg}	368.20^{g}	369.10^{fg}	370.50^{fg}	371.02^{f}	374.58^{fg}	370.23
T_{14}	367.50^{fg}	367.60^{g}	368.10^{g}	369.30^{g}	371.10^{ef}	373.47^{g}	369.51
T_{15}	375.30^{d}	375.70^{d}	371.70^{e}	370.30^{fg}	373.50^{def}	375.30^{efg}	373.63
T_{16}	390.50^{c}	390.90^{c}	391.20^{c}	392.90^{c}	396.82^{b}	398.95^{b}	393.54
Mean	378.29	378.82	379.17	380.43	382.04	384.28	
SEm±	1.66	1.65	1.63	1.65	1.70	1.58	
CD (0.05)	2.76	2.17	2.42	2.54	2.73	2.88	
CD (0.01)	3.72	2.92	3.25	3.41	2.73	3.87	

MAS: Months after storage

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

Table 20. Effect of seed invigoration on thickness of tegmen in ash gourd during storage

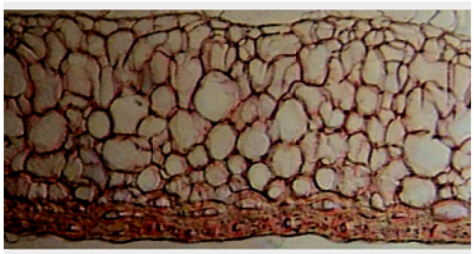
Treatments	Thickness of tegmen (µm)						
	0MAS	1MAS	3MAS	5MAS	7MAS	9MAS	Mean
T_1	51.00^{ef}	52.16^{ef}	53.07^{fg}	55.05^{cde}	56.20^{efg}	58.70^{def}	54.19
T_2	53.20^{de}	53.70^{de}	54.90^{def}	57.40^{c}	59.20^{d}	62.50^{c}	56.65
T_3	54.80^{d}	55.50^{d}	57.10^{cd}	56.20^{cd}	58.80^{de}	60.40^{cd}	56.96
T_4	50.01^{f}	50.50^{f}	50.90^{gh}	52.50^{e}	54.52^{g}	58.98^{def}	52.73
T_5	50.70^{ef}	51.40^{ef}	52.40^{fgh}	54.50^{cde}	55.20^{fg}	55.93^{f}	53.02
T_6	63.75^{b}	64.10^{b}	64.90^{b}	65.80^{b}	68.30^{bc}	71.40^{ab}	66.20
T_7	58.40^{c}	58.60^{c}	59.70^{c}	65.00^{b}	66.50^{c}	69.70^{b}	62.65
T_8	66.80^{a}	67.20^{a}	67.50^{ab}	68.30^{ab}	69.70^{b}	72.80^{a}	68.38
T_9	68.00^{a}	68.20^{a}	68.70^{a}	70.20^{a}	74.20^{a}	71.50^{ab}	69.80
T_{10}	52.10^{def}	52.50^{def}	53.80^{efg}	53.30^{de}	56.20^{efg}	59.40^{de}	54.21
T_{11}	50.50^{ef}	50.80^{ef}	56.40^{de}	54.90^{cde}	57.80^{def}	58.60^{def}	54.50
T_{12}	49.78^{f}	50.10^{f}	51.90^{gh}	53.80^{de}	55.50^{fg}	57.76^{def}	52.97
T_{13}	50.60^{ef}	51.10^{ef}	55.20^{def}	54.40^{cde}	56.30^{efg}	58.50^{def}	54.01
T_{14}	50.00^{f}	50.30^{f}	52.50^{fgh}	53.80^{de}	55.80^{fg}	57.10^{ef}	52.91
T_{15}	52.30^{def}	52.80^{def}	50.10^{h}	51.70^{e}	56.70^{defg}	59.87^{cde}	53.91
T_{16}	62.00^{b}	62.10^{b}	65.30^{b}	67.2^{ab}	70.02^{b}	71.08^{ab}	66.28
Mean	53.87	55.69	57.14	58.37	60.68	62.76	
SEm±	0.92	0.91	0.89	0.94	0.95	0.89	
CD (0.05)	2.72	3.03	2.95	3.48	2.82	3.09	
CD (0.01)	2.72	3.03	2.95	4.68	3.79	4.15	

MAS: Months after storage

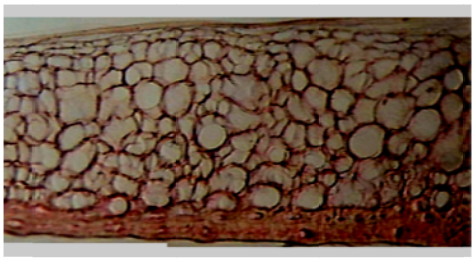
*Means in each column with at least one letter in common are not significantly different at 5% level of probability

Plate 2: Seed coat thickness in ash gourd

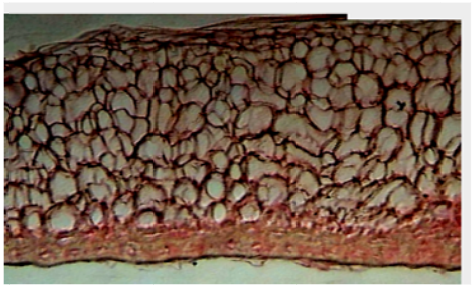
a) Seed coat thickness immediately after invigoration



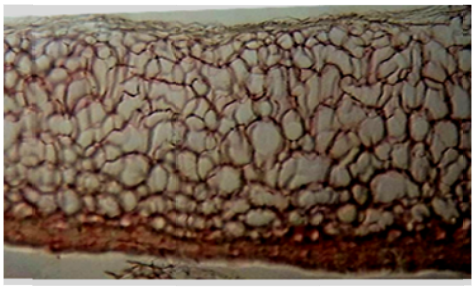
T14 (*Pseudomonas flourescens* for 12 h.)



T13 (50mM $CaCl_2$ for 24 h.)

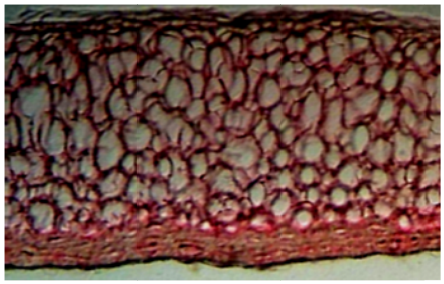


T9 (Salicylic acid for 24 h)

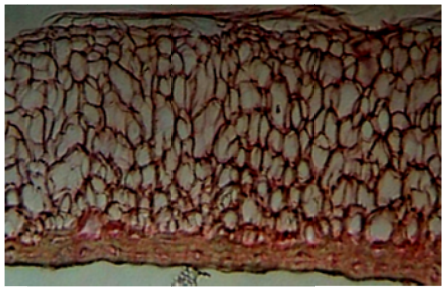


T16 (untreated seeds)

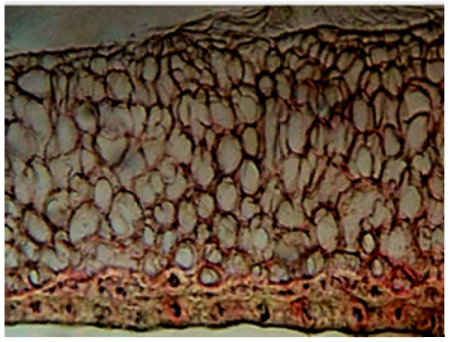
b) Seed coat thickness at seven months of storage



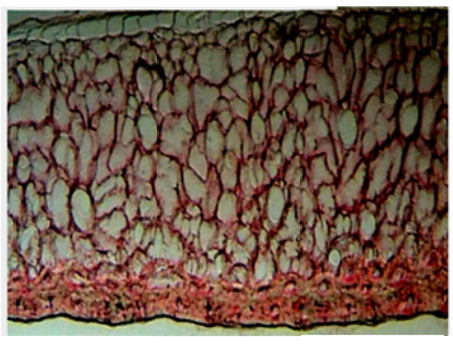
T14 (*Pseudomonas flourescens* for 12 h.)



T13 (50mM $CaCl_2$ for 24 h.)



T16 (untreated seeds)



T9 (Salicylic acid for 24h.)

the least in low (<53 µm) in case of T14 (*Pf* 1x10^6 cfu.ml^{-1} for 12 hours: 52.91 µm), T12 ($CaCl_2$ 50 mM for 12 hours : 52.97 µm) and T4 (KNO_3 0.7% for 24 hours: 52.73 µm).

Treatments T9, T8, T7, T8 and T16 (control) invariably recorded high tegmen thickness throughout the storage period. Similarly in treatments T12, T13, T14, T1 and T4, thickness of tegmen was found to be thinner than in other treatments throughout the storage period.

At 7 MAS, treatments T4 (54.52 µm) with the least tegmen thickness was superior to all other treatments. Treatments T14 (55.80 µm), T5 (55.20 µm), T12 (55.50 µm), and T12 (55.80 µm) had registered the next lower tegmen thickness. These treatments were at par with each other but significantly superior to other treatments while treatments T9 (74.20 µm), followed by T16 (70.02 µm), T8 (69.70 µm), T6 (68.30 µm) and T7 (66.50 µm) had registered higher thickness of tegmen**.**

4.2.15 Embryo length / seed length (EL/SL) ratio

Significant differences were observed in EL/SL ratio between various seed invigoration treatments throughout storage. The average EL/SL ratio (Table 21) over the storage period in majority of invigoration treatments was higher than that of untreated control (0.355 µm). The estimate varied from 0.353 µm in T4, T9, T11 and T14 to 0.358 µm in T15 (*Pf* 1x10^6 cfu.ml^{-1} for 24 hours). Treatment T13 (0.356 µm) followed by T15 exhibited high EL/SL ratio.

At 7 MAS, T13 with a EL/SL ratio of 0.365 µm was superior to all other treatments while T9 (0.347) recorded the least value and was found inferior to all other treatments.

Table 21. Effect of seed invigoration on Embryo length/seed length ratio (EL/SL) in ash gourd during storage

Treatments	EL/SL ratio						
	0MAS	1MAS	3MAS	5MAS	7MAS	9MAS	Mean
T_1	0.354^{d}	0.355^{cd}	0.354^{cd}	0.355^{b}	0.354^{cd}	0.355^{a}	0.354
T_2	0.354^{d}	0.355^{cd}	0.354^{cd}	0.355^{b}	0.355^{c}	0.355^{a}	0.354
T_3	0.354^{d}	0.356^{c}	0.356^{a}	0.355^{b}	0.354^{cd}	0.355^{a}	0.355
T_4	0.349^{h}	0.354^{de}	0.354^{cd}	0.354^{bc}	0.353^{de}	0.354^{a}	0.353
T_5	0.355^{c}	0.354^{de}	0.355^{b}	0.356^{a}	0.355^{c}	0.352^{b}	0.354
T_6	0.356^{b}	0.357^{b}	0.355^{b}	0.355^{b}	0.354^{cd}	0.355^{a}	0.355
T_7	0.351^{f}	0.354^{de}	0.355^{b}	0.354^{bc}	0.355^{c}	0.355^{a}	0.354
T_8	0.358^{a}	0.355^{cd}	0.355^{b}	0.354^{bc}	0.355^{c}	0.355^{a}	0.355
T_9	0.355^{c}	0.355^{cd}	0.354^{cd}	0.355^{b}	0.347^{e}	0.355^{a}	0.353
T_{10}	0.350^{g}	0.355^{cd}	0.354^{cd}	0.354^{bc}	0.354^{cd}	0.355^{a}	0.354
T_{11}	0.354^{d}	0.346^{e}	0.354^{cd}	0.354^{bc}	0.355^{c}	0.355^{a}	0.353
T_{12}	0.354^{d}	0.355^{cd}	0.354^{cd}	0.355^{b}	0.354^{cd}	0.355^{a}	0.354
T_{13}	0.354^{d}	0.355^{cd}	0.352^{d}	0.355^{b}	0.365^{a}	0.355^{a}	0.356
T_{14}	0.346^{i}	0.355^{cd}	0.354^{cd}	0.353^{cd}	0.355^{c}	0.354^{a}	0.353
T_{15}	0.353^{e}	0.374^{a}	0.354^{cd}	0.355^{b}	0.357^{b}	0.355^{a}	0.358
T_{16}	0.355^{c}	0.355^{cd}	0.356^{a}	0.355^{b}	0.355^{c}	0.355^{a}	0.355
Mean	0.353	0.355	0.354	0.355	0.355	0.355	
SEm±	0.00040	0.00076	0.00012	0.0001	0.00049	0.00011	
CD (0.05)	0.001	0.001	0.001	0.001	0.001	0.001	
CD (0.01)	0.001	0.001	0.001	0.001	0.001	0.001	

MAS: Months after storage

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

4.2.16 Seed microflora

The effect of seed treatment on seed infection was found to be highly significant. Seed infection by microflora (Table 22) increased over storage irrespective of invigoration treatment.

The average seed infection per cent over the period of storage in majority of invigoration treatments were lowering than that of untreated control (26.06%). It varied from 13.54 per cent in T14 (*Pf* $1x10^6$ $cfu.ml^{-1}$ for 12 hours) to 26.06 per cent in T16 (control). Treatments T14 (13.54%), T3 (13.75%) and T1 (13.93%) had also exhibited low seed infection over storage. Apart from treatment T16 (control: 26.06%), T8 (salicylic acid 60 ppm for 12 hours: 24.68%) and T9 (salicylic acid 60 ppm for 24 hours: 22.82%) registered high mean seed infection per cent.

Treatment T14 (12.50%) had registered the lowest seed infection per cent at 3 MAS while seed infection was highest in T16 (24.84%). At 7 MAS, treatment T7 (18.20%) and T14 (18.46%) registered low infection while the infection was highest in untreated control (T16: 35.50%) followed by T8 (34.33%) and T9 (32.11%). These three treatments were inferior to all other treatments with respect to microbial infection of seed.

4.3 Correlation study

Correlation coefficients were estimated to understand the inter-relationship between germination and seed quality and anatomical features in treated ash gourd seeds recorded immediately after seed invigoration as well as during storage period at 1 MAS, 3 MAS and 7MAS. The results are summarised in Table 23 to Table 26.

4.3.1 Correlation among seed quality parameters immediately after invigoration

Correlation coefficients between germination and seed quality and anatomical features recorded immediately after seed invigoration are summarised in Table 23.

Table 22. Effect of seed invigoration on seed infection in ash gourd during storage

Treatments	Seed infection (%)						
	0 MAS	1MAS	3MAS	5MAS	7MAS	9MAS	Mean
T_1	4.00^{bcde} (11.47)	8.00^{e} (16.40)	11.00^{j} (19.35)	15.26^{j} (22.99)	20.16^{h} (26.68)	25.16^{e} (30.11)	13.93 (21.91)
T_2	1.50^{fg} (6.96)	4.66^{g} (12.41)	13.53^{h} (21.58)	18.71^{i} (25.63)	23.50^{d} (28.99)	28.30^{c} (32.13)	15.03 (22.81)
T_3	1.00^{g} (5.37)	3.50^{h} (10.76)	14.13^{gh} (19.49)	18.16^{i} (25.91)	20.33^{h} (26.80)	25.40^{e} (30.26)	13.75 (21.76)
T_4	2.70^{ef} (9.34)	4.50^{g} (12.23)	13.50^{h} (21.55)	20.05^{f} (26.60)	21.93^{gh} (27.92)	24.66^{ef} (29.77)	14.55 (22.43)
T_5	5.10^{abcd} (13.01)	12.33^{c} (20.55)	20.20^{cd} (26.70)	23.25^{d} (28.83)	23.00^{e} (28.65)	27.3^{d} (31.49)	18.53 (25.49)
T_6	3.10^{de} (10.05)	7.60^{ef} (16.00)	10.53^{i} (18.93)	16.50^{j} (23.96)	20.66^{h} (27.03)	27.83^{d} (31.84)	14.37 (22.27)
T_7	3.00^{ef} (9.54)	12.5^{c} (20.66)	15.44^{g} (23.13)	19.43^{gh} (26.15)	18.20^{j} (25.25)	27.43^{d} (31.58)	16.00 (23.57)
T_8	5.50^{abc} (13.52)	14.60^{b} (22.45)	21.66^{b} (27.73)	33.33^{b} (35.26)	34.33^{b} (35.86)	38.66^{a} (38.44)	24.68 (29.79)
T_9	5.75^{abc} (13.73)	15.00^{b} (22.77)	18.40^{ef} (25.40)	28.6^{c} (32.32)	32.11^{c} (34.52)	37.1^{b} (37.52)	22.82 (28.54)
T_{10}	4.81^{abcd} (12.62)	11.4^{cd} (19.72)	17.37^{f} (24.63)	21.46^{e} (27.60)	21.33^{gh} (27.50)	23.40^{f} (28.92)	16.62 (24.06)
T_{11}	3.86^{cde} (11.05)	12.08^{c} (20.34)	19.00^{e} (25.84)	22.22^{e} (28.12)	23.30^{de} (28.86)	24.50^{ef} (29.66)	17.49 (24.72)
T_{12}	6.07^{ab} (14.23)	14.16^{b} (22.10)	15.49^{g} (23.18)	18.80^{h} (25.69)	19.62^{hi} (26.29)	23.66^{f} (29.10)	16.30 (23.81)
T_{13}	4.00^{bcde} (11.47)	11.16^{cd} (19.51)	13.20^{h} (21.30)	16.4^{j} (23.88)	19.10^{hi} (25.91)	23.73^{f} (29.15)	14.60 (22.46)
T_{14}	4.25^{abcde} (12.19)	10.4^{d} (18.81)	12.50^{hi} (20.70)	15.30^{k} (23.02)	18.46^{i} (25.45)	20.33^{g} (26.80)	13.54 (21.59)
T_{15}	3.31^{de} (10.40)	6.50^{f} (14.76)	15.63^{g} (23.29)	20.06^{g} (26.60)	21.10^{gh} (27.34)	23.66^{g} (29.11)	15.04 (22.82)
T_{16}	6.90^{a} (15.20)	17.00^{a} (24.34)	24.84^{a} (29.89)	34.33^{a} (35.86)	35.50^{a} (36.57)	37.83^{ab} (37.95)	26.06 (30.70)
Mean	4.05 (11.61)	10.33 (18.75)	16.02 (23.60)	21.37 (27.53)	23.29 (28.85)	27.43 (31.58)	
SEm±	0.42	0.59	0.65	0.57	0.53	0.40	
CD (0.05)	3.03	1.91	0.82	0.60	0.63	0.86	
CD (0.01)	4.07	1.42	1.10	0.80	0.84	1.15	

MAS: Months after storage
*Means in each column with at least one letter in common are not significantly different at 5% level of probability
**All the replicate values equalling zero are not included in the analysis
Values in parentheses are arc sine transformed value

Plate 3.a) Detection of seed microflora (Blotter method)

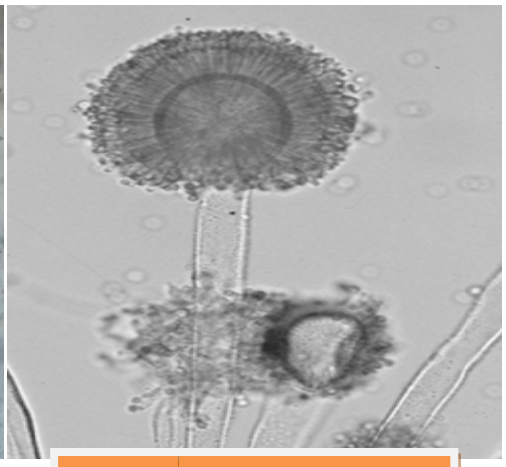
T14 (*Pseudomonas fluorescens*) **T16 (control)**

Plate 3.b) Detection of seed microflora (Agar plate method)

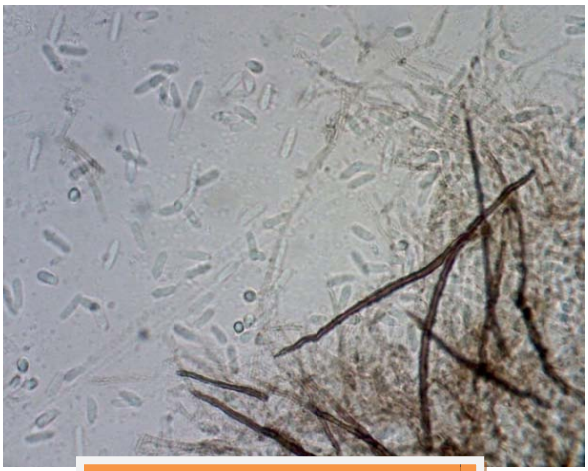
T14 (*Pseudomonas fluorescens*) **T16 (control)**

Plate 4: Seed microflora detected during storage

Aspergillus niger



Aspergillus flavus



Colletotrichum Sp.

4.3.1.1 Association of seed quality and anatomical features with germination immediately after invigoration

Germination recorded a high significant positive correlation with germination index (r_g = 0.986), vigour index-II (r_g = 0.984), vigour index-I (r_g = 0.970), coefficient of velocity of germination (r_g = 0.695) and energy of germination (r_g = 0.695). The correlation between germination and thickness of tegmen (r_g = -0.758) and total seed coat thickness (r_g = -0.743), thickness of testa (r_g = -0.731), electrical conductivity of seed leachate (r_g = -0.729) and mean time to germination (r_g = -0.711), recorded significant and negative values. However, no significant association was evident between germination and seed microflora and embryo length to seed length ratio immediately after seed invigoration.

4.3.1.2 Inter-correlation among seed quality parameters immediately after invigoration

4.3.1.2.1 Germination index

Germination index recorded a significant to high significant, positive correlation with germination per cent (r_g = 0.986), vigour index-II (r_g = 0.969), vigour index-I (r_g = 0.967), energy of germination (r_g = 0.824) and coefficient of velocity of germination (r_g = 0.730) and negative correlation with mean time to germination (r_g = -0.737), thickness of tegmen (r_g = -0.739), total seed coat thickness (r_g = -0.731), thickness of testa (r_g = -0.723) and electrical conductivity of seed leachate (r_g = -0.653).

4.3.1.2.2 Mean time to germination

Mean time to germination was found to have a significant positive correlation with total seed coat thickness (r_g = 0.941), thickness of tegmen (r_g = 0.938), thickness of testa (r_g = 0.937) and electrical conductivity of seed leachate (r_g = 0.800). It recorded negative correlation with coefficient of velocity of germination

(r_g = -0.999), energy of germination (r_g = -0.795), vigour index-I (r_g = -0.743) and vigour index-II (r_g = -0.734), germination index (r_g = -0.737) and germination per cent (r_g = -0.711). .

4.3.1.2.3 Coefficient of velocity of germination

Coefficient of velocity of germination recorded a high significant, positive correlation with energy of germination (r_g = 0.809), vigour index-I (r_g = 0.731), germination index (r_g = 0.730), vigour index-II (r_g = 0.718) and germination per cent (r_g = 0.695) and negative correlation with mean time to germination (r_g = -0.999), total seed coat thickness (r_g = -0.927) thickness of testa (r_g = -0.924), thickness of tegmen (r_g = -0.922) and electrical conductivity of seed leachate (r_g = -0.768).

4.3.1.2.4 Energy of germination

Significant to high significant positive correlation was observed with germination index (r_g = 0.824), coefficient of velocity of germination (r_g = 0.809), vigour index-I (r_g = 0.767), germination per cent (r_g = 0.756) and vigour index-II (r_g = 0.753) and negative correlation with mean time to germination (r_g = -0.795), thickness of testa (r_g = -0.718), total seed coat thickness (r_g = -0.711), thickness of tegmen (r_g = -0.689) and electrical conductivity of seed leachate (r_g = -0.465).

4.3.1.2.5 Vigour index-I

Correlation between vigour index-I was found to be significant and positive correlation with vigour index-II (r_g = 0.971), germination per cent (r_g = 0.970), speed germination index (r_g = 0.967), energy of germination (r_g = 0.767) and coefficient of velocity of germination (r_g = 0.731) and negative correlation with thickness of tegmen (r_g = -0.773), total seed coat thickness (r_g = -0.764), thickness of testa (r_g = -0.755), mean time to germination (r_g = -0.743) and electrical conductivity of seed leachate (r_g = -0.711).

4.3.1.2.6 Vigour index-II

Correlation between vigour index-II was found significant and positive correlation with germination per cent (r_g = 0.984), vigour index-I (r_g = 0.971), germination index (r_g = 0.969), energy of germination (r_g = 0.753) and coefficient of velocity of germination (r_g = 0.718) and negative correlation with thickness of tegmen (r_g = -0.774), total seed coat thickness (r_g = -0.768), thickness of testa (r_g = -0.759), electrical conductivity of seed leachate (r_g = -0.747) and mean time to germination (r_g = -0.734).

4.3.1.2.7 Electrical conductivity of seed leachate

Electrical conductivity was found to have a significant and positive correlation with thickness of tegmen (r_g = 0.912), total seed coat thickness (r_g = 0.890), thickness of testa (r_g = 0.873) and mean time to germination (r_g = 0.800) and negative correlation with coefficient of velocity of germination (r_g = -0.768), vigour index-II (r_g = -0.747), germination per cent (r_g = -0.729), vigour index-I (r_g = -0.711), germination index (r_g = -0.653) and energy of germination (r_g = -0.465).

4.3.1.2.8 Thickness of testa

Thickness of testa was found to have a significant and positive correlation with total seed coat thickness (r_g = 0.998), thickness of tegmen (r_g = 0.982), mean time to germination (r_g = 0.937) and electrical conductivity (r_g = 0.873) and negative correlation with coefficient of velocity of germination (r_g = -0.924), vigour index-II (r_g = -0.759), germination index (r_g = -0.758), vigour index-I (r_g = -0.755), germination per cent (r_g = -0.731) and energy of germination (r_g = -0.718).

4.3.1.2.9 Thickness of tegmen

Thickness of tegmen recorded a significant positive correlation with total seed coat thickness (r_g = 0.992), thickness of testa (r_g = 0.982), mean time to germination

Table 23. Correlation among germination and seed quality parameters in ash gourd immediately after seed invigoration

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1	1												
X2	0.986**	1											
X3	-0.711**	-0.737**	1										
X4	0.695**	0.730**	-0.999**	1									
X5	0.756**	0.824**	-0.795**	0.809**	1								
X6	0.970**	0.967**	-0.743**	0.731**	0.767**	1							
X7	0.984**	0.969**	-0.734**	0.718**	0.753**	0.971**	1						
X8	-0.729**	-0.653*	0.800**	-0.768**	-0.465**	-0.711**	-0.747**	1					
X9	-0.731**	-0.723**	0.937**	-0.924**	-0.718**	-0.755**	-0.759**	0.873**	1				
X10	-0.758**	-0.739**	0.938**	-0.922**	-0.689*	-0.773**	-0.774**	0.912**	0.982**	1			
X11	-0.743**	-0.731**	0.941**	-0.927**	-0.711**	-0.764**	-0.768**	0.890**	0.998**	0.992**	1		
X12	-0.487	-0.458	0.374	-0.363	-0.232	-0.579	-0.455	0.453	0.419	0.440	0.428	1	
X13	-0.007	-0.141	-0.273	0.317	0.518	0.045	0.037	0.293	-0.083	0.005	-0.053	0.186	1

***** significant at 5% level; ****** significant at 1% level

X1: Germination (%)
X2: Germination index
X3: Mean time to germination (days)
X4: Coefficient of velocity of germination (%)
X5: Energy of germination
X6: Vigour index-I
X7: Vigour index-II
X8: Electrical conductivity (dsm^{-1})
X9: Thickness of testa (µm)
X10: Thickness of tegmen (µm)
X11: Total seed coat thickness (µm)
X12: Embryo length/seed length (µm)
X13: Seed microflora

(r_g = 0.938) and electrical conductivity (r_g = 0.912) and and negative correlation with coefficient of velocity of germination (r_g = -0.922), vigour index -II (r_g = -0.774), vigour index-I (r_g = -0.773), germination per cent (r_g = -0.758), germination index (r_g = -0.739), and energy of germination (r_g = -0.689).

4.3.1.2.10 Total seed coat thickness

The attribute recorded a significant positive correlation with mean time to thickness of testa (r_g = 0.998), thickness of tegmen (r_g = 0.992), germination (r_g = 0.941) and electrical conductivity (r_g = 0.890) and negative correlation with coefficient of velocity of germination (r_g = -0.927), vigour index-II (r_g = -0.768), vigour index-I (r_g = -0.764), germination per cent (r_g = -0.743), germination index (r_g = -0.731) and energy of germination (r_g = -0.711).

4.3.1.2.11 Embryo length to seed length ratio (EL/SL)

There was no significant correlation between embryo length to seed length ratio and other parameter studied immediately after invigoration.

4.3.1.2.12 Seed microflora

Seed microflora did not exhibit significant correlation with the other seed quality and anatomical features studied immediately after invigoration.

4.3.2 Correlation among seed quality parameters one month after invigoration (1 MAS)

The inter-correlation between germination and other seed quality, seed anatomical features are summarised in Table 24.

4.3.2.1 Association of seed quality and anatomical features with germination at 1 MAS

The attribute recorded a significant positive relationship with vigour index-I (r_g = 0.930), energy of germination (r_g = 0.850), germination index (r_g = 0.823),

vigour index-II (r_g = 0.755) and coefficient of velocity of germination (r_g = 0.668) and negative correlation with thickness of testa (r_g = -0.941), total seed coat thickness (r_g = -0.925), thickness of tegmen (r_g = -0.816) and mean time to germination (r_g = -0.667), .

4.3.2.2 Inter-correlation among seed quality parameters at 1 MAS

4.3.2.2.1 Germination index

Germination index recorded a high significant, positive correlation with coefficient of velocity of germination (r_g = 0.960), germination per cent (r_g = 0.823), vigour index-I (r_g = 0.790) and energy of germination (r_g = 0.786) and negative correlation with mean time to germination (r_g = -0.959), total seed coat thickness (r_g = -0.910), thickness of testa (r_g = -0.897), thickness of tegmen (r_g = -0.864), and seed microflora (r_g = -0.627).

4.3.2.2.2 Mean time to germination

Mean time to germination was found to have a significant positive correlation with total seed coat thickness (r_g = 0.799), thickness of tegmen (r_g = 0.818) and thickness of testa (r_g = 0.761). It recorded negative correlation with coefficient of velocity of germination (r_g = -0.999), germination index (r_g = -0.959), seed microflora (r_g = -0.700), germination per cent (r_g = -0.667), vigour index-I (r_g = -0.664) and energy of germination (r_g = -0.663).

4.3.2.2.3 Coefficient of velocity of germination

Coefficient of velocity of germination recorded a significant to high significant, positive correlation with germination index (r_g = 0.960), energy of germination (r_g = 0.675), vigour index-I (r_g = 0.672) and germination per cent (r_g = 0.668) and negative correlation with mean time to germination (r_g = -0.999),

thickness of tegmen (r_g = -0.809), thickness of testa (r_g = -0.762) and total seed coat thickness (r_g = -0.696).

4.3.2.2.4 Energy of germination

Significant to high significant positive correlation was observed with vigour index-I (r_g = 0.873), germination per cent (r_g = 0.850), germination index (r_g = 0.786), coefficient of velocity of germination (r_g = 0.675) and vigour index-II (r_g = 0.625) and negative correlation with thickness of testa (r_g = -0.847), total seed coat thickness (r_g = -0.810), thickness of tegmen (r_g = -0.666) and mean time to germination (r_g = -0.663).

4.3.2.2.5 Vigour index-I

Correlation between vigour index-I was found significant and positive correlation with germination per cent (r_g = 0.930), energy of germination (r_g = 0.873), germination index (r_g = 0.790), vigour index-II (r_g = 0.692) and coefficient of velocity of germination (r_g = 0.672) and negative correlation with, thickness of testa (r_g = -0.861), total seed coat thickness (r_g = -0.820), thickness of tegmen (r_g = -0.668) and mean time to germination (r_g = -0.664).

4.3.2.2.6 Vigour index-II

Correlation between vigour index-II was found significant and positive correlation with germination per cent (r_g = 0.755), vigour index-I (r_g = 0.692), energy of germination (r_g = 0.625), negative correlation with thickness of testa (r_g = -0.701) and total seed coat thickness (r_g = -0.616).

4.3.2.2.7 Electrical conductivity of seed leachate

There was no significant correlation between electrical conductivity and other parameter studied at 1 MAS.

4.3.2.2.8 Thickness of testa

Thickness of testa was found to have a significant and positive correlation with total seed coat thickness (r_g = 0.988), thickness of tegmen (r_g = 0.883) and mean time to germination (r_g = 0.761) and negative correlation with germination per cent (r_g = -0.941), germination index (r_g = -0.897), vigour index-I (r_g = -0.861), energy of germination (r_g = -0.847), coefficient of velocity of germination (r_g = -0.762), and vigour index-11 (r_g = -0.701).

4.3.2.2.9 Thickness of tegmen

Thickness of tegmen recorded a significant positive correlation with total seed coat thickness (r_g = 0.945), thickness of testa (r_g = 0.883) and mean time to germination (r_g = 0.818) and negative correlation with germination index (r_g = -0.864), germination per cent (r_g = -0.816), coefficient of velocity of germination (r_g = -0.809), vigour index-1 (r_g = -0.668) and energy of germination (r_g = -0.666).

4.3.2.2.10 Total seed coat thickness

The attribute recorded a significant positive correlation with thickness of testa (r_g = 0.988), thickness of tegmen (r_g = 0.945) and mean time to germination (r_g = 0.799) and negative correlation with germination per cent (r_g = -0.925), germination index (r_g = -0.910), vigour index-I (r_g = -0.820), energy of germination (r_g = -0.810), coefficient of velocity of germination (r_g = -0.797) and vigour index-II (r_g = -0.616).

4.3.2.2.11 Embryo length to seed length ratio (EL/SL)

There was no significant correlation between embryo length to seed length ratio and other parameters studied at 1MAS.

Table 24. Correlation among germination and seed quality parameters in ash gourd at 1 month after storage

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1	1												
X2	0.823**	1											
X3	-0.667*	-0.959**	1										
X4	0.668*	0.960**	-0.999**	1									
X5	0.850**	0.786**	-0.663*	0.675*	1								
X6	0.930**	0.790**	-0.664*	0.672*	0.873**	1							
X7	0.755**	0.582	-0.360	0.367	0.625*	0.692*	1						
X8	-0.007	-0.153	0.205	-0.191	0.212	0.019	-0.105	1					
X9	-0.941**	-0.897**	0.761**	-0.762**	-0.847**	-0.861**	-0.701**	-0.030	1				
X10	-0.816**	-0.864**	0.818**	-0.809**	-0.666*	-0.668*	-0.384	-0.018	0.883**	1			
X11	-0.925**	-0.910**	0.799**	-0.797**	-0.810**	-0.820**	-0.616*	-0.027	0.988**	0.945**	1		
X12	-0.380	-0.174	-0.049	0.055	-0.237	-0.224	-0.481	-0.033	0.438	0.291	0.402	1	
X13	-0.449	-0.627*	-0.700*	-0.696*	0.551	0.520	0.242	-0.114	-0.450	-0.474	-0.470	0.355	1

***** significant at 5% level; ****** significant at 1% level

X1: Germination (%)
X2: Germination index
X3: Mean time to germination (days)
X4: Coefficient of velocity of germination (%)
X5: Energy of germination
X6: Vigour index-I
X7: Vigour index-II
X8: Electrical conductivity (dsm^{-1})
X9: Thickness of testa (µm)
X10: Thickness of tegmen (µm)
X11: Total seed coat thickness (µm)
X12: Embryo length/seed length (µm)
X13: Seed microflora

4.3.2.2.12 Seed microflora

Seed microflora did not exhibit significant correlation with the other seed quality and anatomical features at 1 MAS.

4.3.3 Correlation among seed quality parameters three month after invigoration (3 MAS)

The inter-correlation between germination and other seed quality, seed anatomical features are summarised in Table 25.

4.3.3.1 Association of seed quality and anatomical features with germination at 3 MAS

The attribute recorded a significant positive relationship with vigour index-I (r_g = 0.913), energy of germination (r_g = 0.905), germination index (r_g = 0.876), vigour index-II (r_g = 0.766) and coefficient of velocity of germination (r_g = 0.611) and negative correlation with thickness of testa (r_g = -0.891), total seed coat thickness (r_g = -0.837) and mean time to germination (r_g = -0.638).

4.3.3.2 Inter-correlation among seed quality parameters at 3 MAS

4.3.3.2.1 Germination index

Germination index recorded a high significant, positive correlation with coefficient of velocity of germination (r_g = 0.916), vigour index-I (r_g = 0.890), germination per cent (r_g = 0.876), and energy of germination (r_g = 0.800) while the association was significant and positive with vigour index-II (r_g = 0.703). High significant negative correlation was observed between germination index and thickness of testa (r_g = -0.966), total seed coat thickness (r_g = -0.947) and mean time to germination (r_g = -0.921) while it was significant and negative with thickness of tegmen (r_g = -0.658).

4.3.3.2.2 Mean time to germination

Mean time to germination was found to have a significant positive correlation with thickness of testa (r_g = 0.892), total seed coat thickness (r_g = 0.865) and el/sl ratio (r_g = 0.609). It recorded negative correlation with coefficient of velocity of germination (r_g = -0.988), germination index (r_g = -0.921), vigour index-I (r_g = -0.737), germination per cent (r_g = -0.638) and vigour index-II (r_g = -0.617).

4.3.3.2.3 Coefficient of velocity of germination

Coefficient of velocity of germination found to be significant, positive correlation with germination index (r_g = 0.916), \vigour index-I (r_g = 0.694) and germination per cent (r_g = 0.611) and negative correlation with mean time to germination (r_g = -0.988), total seed coat thickness (r_g = -0.861), thickness of testa (r_g = -0.855) and thickness of tegmen (r_g = -0.635).

4.3.3.2.4 Energy of germination

Significant to high significant positive correlation was observed with germination per cent (r_g = 0.905), vigour index-I (r_g = 0.890), germination index (r_g = 0.800) and vigour index-II (r_g = 0.754) and negative correlation with thickness of testa (r_g = -0.821) and total seed coat thickness (r_g = -0.755).

4.3.3.2.5 Vigour index-I

Correlation between vigour index-I was found significant and positive correlation with germination per cent (r_g = 0.913), germination index (r_g = 0.890), energy of germination (r_g = 0.890), vigour index-II (r_g = 0.868) and coefficient of velocity of germination (r_g = 694) and negative correlation with thickness of testa (r_g = -0.893) total seed coat thickness (r_g = -0.820) and mean time to germination (r_g = -0.737).

4.3.3.2.6 Vigour index-II

Correlation between vigour index-II was found significant and positive correlation with vigour index-I (r_g = 0.868), germination per cent (r_g = 0.766), energy of germination (r_g = 0.754) and germination index (r_g = 0.703) negative correlation with thickness of testa (r_g = -0.800) and total seed coat thickness (r_g = -0.627) and mean time to germination (r_g = -0.617).

4.3.3.2.7 Electrical conductivity of seed leachate

There was no significant correlation between electrical conductivity and other parameter studied at 3 months after storage.

4.3.3.2.9 Thickness of testa

Thickness of testa was found to have a significant and positive correlation with total seed coat thickness (r_g = 0.935) and mean time to germination (r_g = 0.892) and negative correlation with germination index (r_g = -0.966), vigour index-I (r_g = -0.893), germination per cent (r_g = -0.891), coefficient of velocity of germination (r_g = -0.855), energy of germination (r_g = -0.821), and vigour index-II (r_g = -0.800).

4.3.3.2.10 Thickness of tegmen

Thickness of tegmen recorded a significant positive correlation with total seed coat thickness (r_g = 0.829) and negative correlation with germination index (r_g = -0.658) and coefficient of velocity of germination (r_g = -0.635).

4.3.3.2.11 Total seed coat thickness

The attribute recorded a significant positive correlation with thickness of testa (r_g = 0.935), thickness of tegmen (r_g = 0.892) and mean time to germination (r_g = 0.865), and negative correlation with germination index (r_g = -0.947),

Table 25. Correlation among germination and seed quality parameters in ash gourd at 3 months after storage

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1	1												
X2	0.876**	1											
X3	-0.638*	-0.921**	1										
X4	0.611*	0.916**	-0.988**	1									
X5	0.905**	0.800**	-0.581	0.549	1								
X6	0.913**	0.890**	-0.737**	0.694**	0.890**	1							
X7	0.766**	0.703*	-0.617*	0.529	0.754**	0.868**	1						
X8	-0.048	-0.211	0.374	-0.298	-0.173	-0.253	-0.279	1					
X9	-0.891**	-0.966**	0.892**	-0.855**	-0.821**	-0.893**	-0.800**	0.319	1				
X10	-0.522	-0.658*	0.587	-0.635*	-0.445	-0.480	-0.183	0.112	0.576	1			
X11	-0.837**	-0.947**	0.865**	-0.861**	-0.755**	-0.820**	-0.627*	0.267	0.935**	0.829**	1		
X12	-0.448	-0.555	0.609*	-0.559	-0.270	-0.542	-0.595	-0.030	0.586	0.075	0.433	1	
X13	-0.106	0.092	-0.145	0.229	0.057	-0.008	-0.029	0.130	0.042	0.055	0.053	0.210	1

* significant at 5% level; ** significant at 1% level

X1: Germination (%)
X2: Germination index
X3: Mean time to germination (days)
X4: Coefficient of velocity of germination (%)
X5: Energy of germination
X6: Vigour index-I
X7: Vigour index-II
X8: Electrical conductivity (dsm^{-1})
X9: Thickness of testa (µm)
X10: Thickness of tegmen (µm)
X11: Total seed coat thickness (µm)
X12: Embryo length/seed length (µm)
X13: Seed microflora

coefficient of velocity of germination (r_g = -0.861), germination per cent (r_g = -0.837), vigour index-I (r_g = -0.820), energy of germination (r_g = -0.755) and vigour index-II (r_g = -0.627).

4.3.3.2.12 Embryo length to seed length ratio

There was no significant correlation between embryo length to seed length ratio and other parameter studied 3 MAS.

4.3.3.2.13 Seed microflora

Seed microflora did not exhibit significant correlation with the other seed quality and anatomical features at 3 MAS.

4.3.4 Correlation among seed quality parameters seven month after invigoration (7 MAS)

The inter-correlation between germination and other seed quality and seed anatomical features are summarised in Table 26.

4.3.4.1 Association of seed quality and anatomical features with germination at 7 MAS

The attribute recorded a significant positive relationship with vigour index-II (r_g = 0.958), vigour index-I (r_g = 0.946), germination index (r_g = 0.820) and coefficient of velocity of germination (r_g = 0.659) and negative correlation with electrical conductivity of thickness of testa (r_g = -0.953), total seed coat thickness (r_g = -0.942), thickness of tegmen (r_g = -0.893) and seed leachate (r_g = -0.878). However, no significant association was evident between germination and seed microflora and embryo length to seed length ratio at 7 MAS.

4.3.4.2 Inter-correlation among seed quality parameters at 7 MAS

4.3.4.2.1 Germination index

Germination index recorded a significant to high significant, positive correlation with germination per cent (r_g = 0.820), vigour index-I (r_g = 0.812), vigour index-II (r_g = 0.796) and coefficient of velocity of germination (r_g = 0.726), and negative correlation with thickness of testa (r_g = -0.859), and total seed coat thickness (r_g = -0.835), thickness of tegmen (r_g = -0.760), mean time to germination (r_g = -0.709) and electrical conductivity of seed leachate (r_g = -0.682).

4.3.4.2.2 Mean time to germination

Mean time to germination was found to have a significant negative correlation with germination index (r_g = -0.70) and coefficient of velocity of germination (r_g = -0.593).

4.3.4.2.3 Coefficient of velocity of germination

Coefficient of velocity of germination recorded to be significant, positive correlation with vigour index-I (r_g = 0.747), germination index (r_g = 0.726), germination per cent (r_g = 0.695) and vigour index-II (r_g = 0.651) and negative correlation with thickness of testa (r_g = -0.744), total seed coat thickness (r_g = -0.723), thickness of tegmen (r_g = -0.657) and mean time to germination (r_g = -0.593).

4.3.4.2.4 Energy of germination

Energy of germination did not exhibit significant correlation with the other seed quality and anatomical features at 7 MAS.

4.3.4.2.4 Vigour index-I

Correlation between vigour index-I was found significant and positive correlation with vigour index-II (r_g = 0.967), germination per cent (r_g = 0.946),

germination index (r_g = 0.812) and coefficient of velocity of germination (r_g = 0.747) and negative correlation with thickness of testa (r_g = -0.907), total seed coat thickness (r_g = -0.884), electrical conductivity of seed leachate (r_g = -0.821) and thickness of tegmen (r_g = -0.812).

4.3.4.2.5 Vigour index-II

Correlation between vigour index-II was found significant and positive correlation with vigour index-I (r_g = 0.967), germination per cent (r_g = 0.958), germination index (r_g = 0.796) and coefficient of velocity of germination (r_g = 0.651) and negative correlation with electrical conductivity of seed leachate (r_g = -0.898), thickness of testa (r_g = -0.895), total seed coat thickness (r_g = -0.875) and thickness of tegmen (r_g = -0.806).

4.3.4.2.6 Electrical conductivity of seed leachate

Electrical conductivity was found to have significant and positive correlation with total seed coat thickness (r_g = 863), thickness of testa (r_g = 0.859) and thickness of tegmen (r_g = 848) and negative correlation with vigour index-II (r_g = -0.898), germination per cent (r_g = -0.878), vigour index-I (r_g = -0.821) and germination index (r_g = -0.682).

4.3.4.2.7 Thickness of testa

Thickness of testa was found to have a significant and positive correlation with total seed coat thickness (r_g = 0.996), thickness of tegmen (r_g = 0.960) and electrical conductivity (r_g = 0.859) and negative correlation with germination per cent (r_g = -0.953), vigour index-I (r_g = -0.907), vigour index-II (r_g = -0.895), germination index (r_g = -0.859) and coefficient of velocity of germination (r_g = -0.744).

Table 26. Correlation among germination and seed quality parameters in ash gourd at 7 months after storage

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1	1												
X2	0.820**	1											
X3	-0.211	-0.709**	1										
X4	0.659*	0.726**	-0.593*	1									
X5	0.209	0.277	-0.316	0.428	1								
X6	0.946**	0.812**	-0.304	0.747**	0.300	1							
X7	0.958**	0.796**	-0.248	0.651*	0.323	0.967**	1						
X8	-0.878**	-0.682*	0.097	-0.464	-0.094	-0.821**	-0.898**	1					
X9	-0.953**	-0.859**	0.345	-0.744**	-0.188	-0.907**	-0.895**	0.859**	1				
X10	-0.893**	-0.760**	0.221	-0.657*	-0.094	-0.812**	-0.806**	0.848**	0.960**	1			
X11	-0.942**	-0.835**	0.308	-0.723**	-0.159	-0.884**	-0.875**	0.863**	0.996**	0.981**	1		
X12	0.354	0.313	-0.100	0.069	0.093	0.386	0.355	-0.163	-0.190	-0.027	-0.140	1	
X13	-0.430	-0.311	0.162	-0.371	-0.198	-0.487	-0.528	0.246	0.280	0.086	0.221	-0.478	1

***** significant at 5% level; ****** significant at 1% level

X1: Germination (%)
X2: Germination index
X3: Mean time to germination (days)
X4: Coefficient of velocity of germination (%)
X5: Energy of germination
X6: Vigour index-I
X7: Vigour index-II
X8: Electrical conductivity (dsm^{-1})
X9: Thickness of testa (µm)
X10: Thickness of tegmen (µm)
X11: Total seed coat thickness (µm)
X12: Embryo length/seed length (µm)
X13: Seed microflora

4.3.4.2.8 Thickness of tegmen

Thickness of tegmen recorded a significant positive correlation with total seed coat thickness (r_g = 0.981), thickness of testa (r_g = 0.960) and electrical conductivity (r_g = 0.848) and negative correlation with germination per cent (r_g = -0.893), vigour index-I (r_g = -0.812), vigour index-II (r_g = -0.806), germination index (r_g = -0.760) and coefficient of velocity of germination (r_g = -0.657).

4.3.4.2.9 Total seed coat thickness

The attribute recorded a significant positive correlation with thickness of testa (r_g = 0.996), thickness of tegmen (r_g = 0.981) and electrical conductivity (r_g = 0.863) and negative correlation with germination per cent (r_g = -0.942), vigour index-I (r_g = -0.884), vigour index-II (r_g = -0.875), germination index (r_g = -0.835) and coefficient of velocity of germination (r_g = -0.723).

4.3.3.2.12 Embryo length to seed length ratio

There was no significant correlation between embryo length to seed length ratio and other parameters studied 7 MAS.

4.3.3.2.13 Seed microflora

Seed microflora did not exhibit significant correlation with the other seed quality and anatomical features at 7 MAS.

Discussion

5. DISCUSSION

Seed unquestionably occupies a pivotal place in any agricultural system. Obviously, seed dormancy, uneven germination, excessively low germination, vitality and low seed vigour can all lead to great economic loss in agriculture.

Ash gourd (*Benincasa hispida* (Thunb.) Cogn.) seeds characterized by the presence of hard seed coat, exhibit delayed and uneven germination initially after extraction. Specific information on the seed dormancy and germination behaviour of the popular ash gourd cultivar in Kerala (KAU Local) is unavailable and information on the techniques to enhance its germination and vigour is presently very limited. Hence, the present investigation was carried out at the Department of Seed Science and Technology, College of Horticulture, Vellanikkara to identify the most suitable seed invigoration technique to break dormancy in ash gourd, to understand the anatomical changes in seed coat on seed invigoration and to evaluate the storage potential of invigorated seeds under ambient conditions. The results obtained are discussed hereunder.

5.1 Effect of seed invigoration treatments on seed dormancy

Germination was found to increase within 35 days of seed extraction in both treated and untreated seeds. In untreated control and majority of seed invigoration treatments (Fig. 1), a progressive increase in germination was observed initially which however, decreased marginally later.

As per the Indian minimum standards for seed certification (MSCS), the minimum germination prescribed for ash gourd is sixty per cent. It was observed that germination reached above the MSCS earliest (Fig. 2) *i.e.*, on the 11^{th} day after seed invigoration (DAI) in seeds treated with 10^{-1} M KH_2PO_4 for 24 hours and also in those treated with kinetin 10 ppm for 24 hours, while in majority of the treatments, it was attained on the 13^{th} DAI. Seed priming with KH_2PO_4 10^{-1} M for 24 hours was

Fig. 1. Germination in ash gourd after seed invigoration

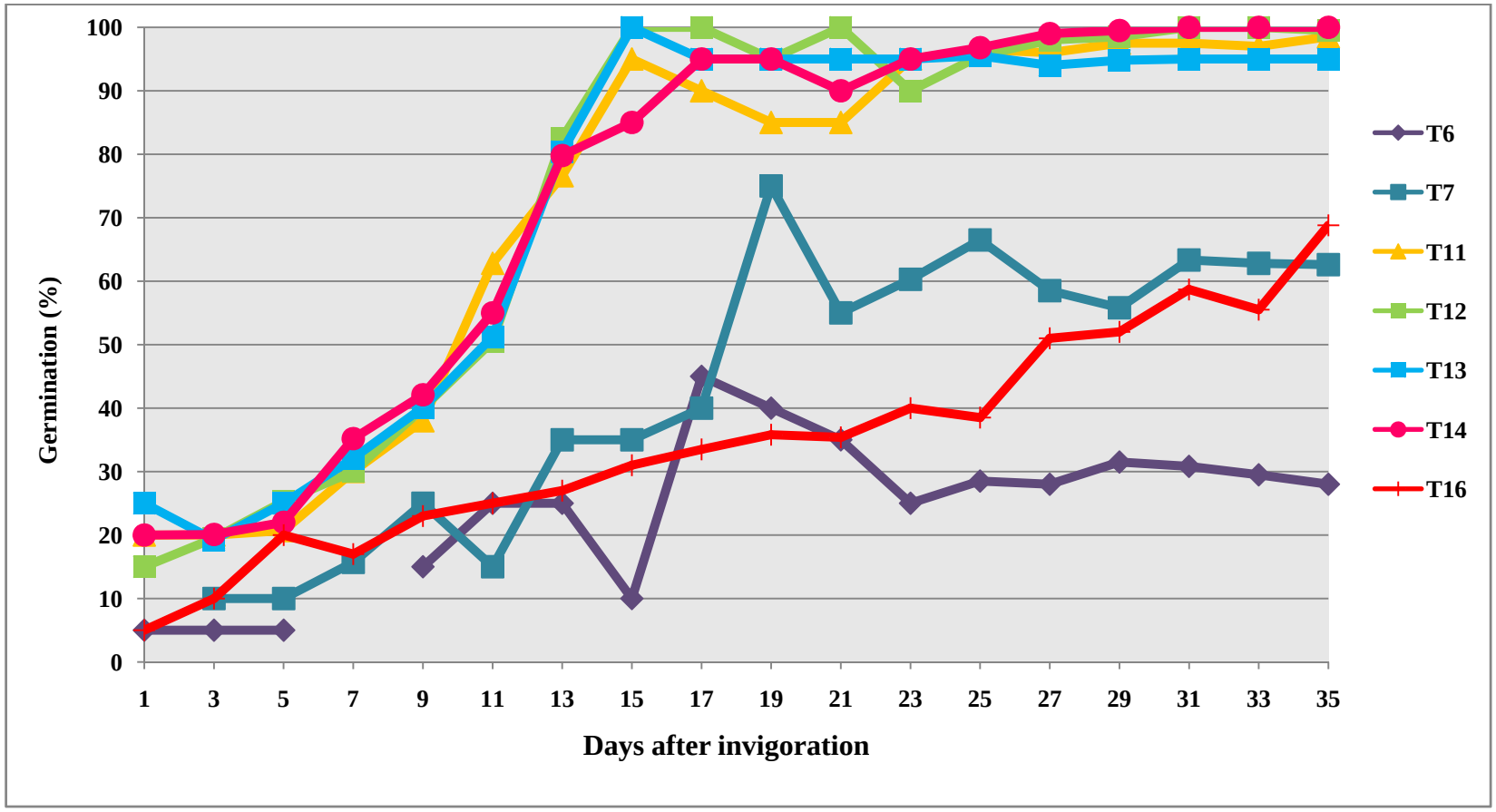
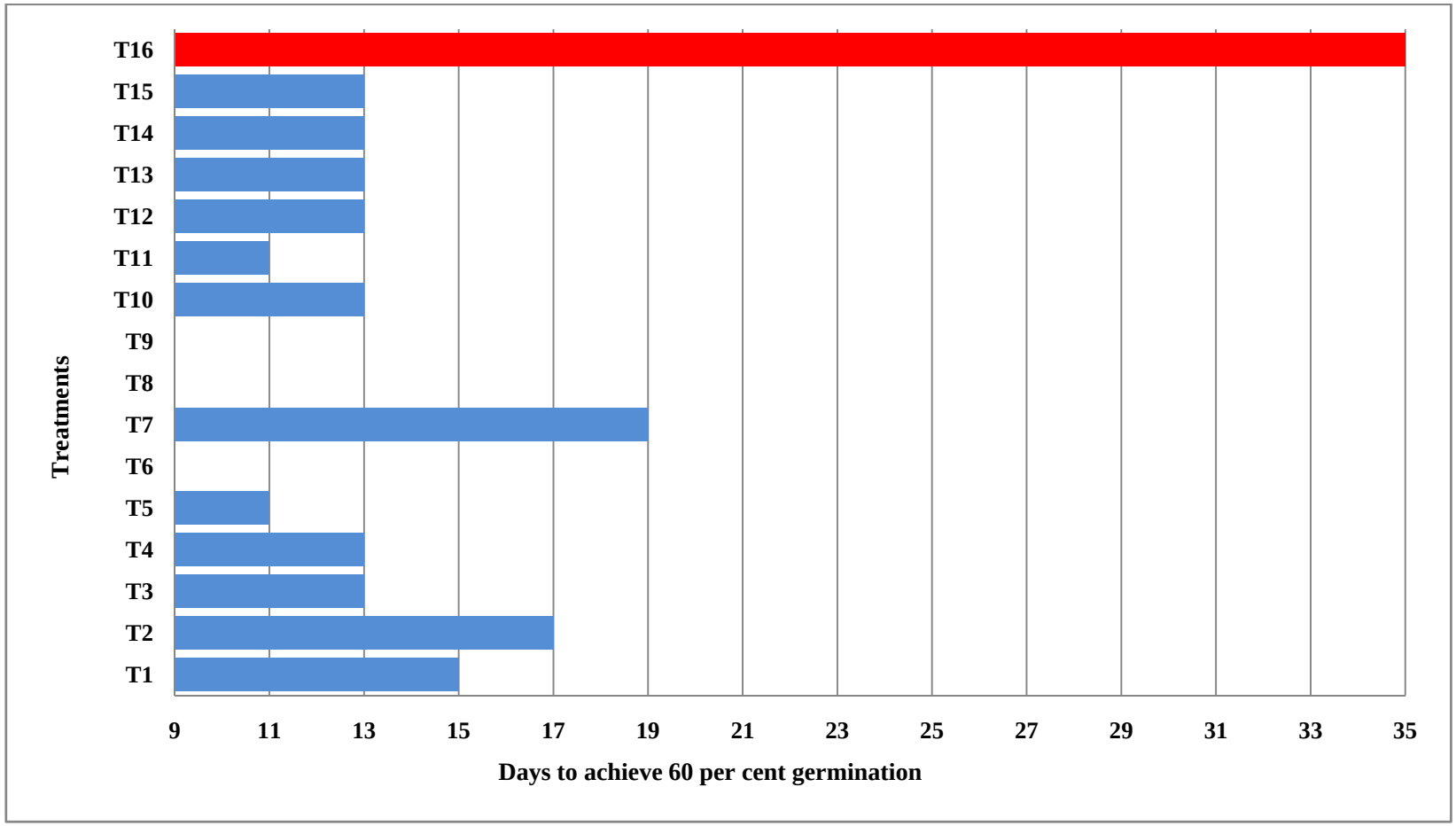


Fig. 2. Effect of seed invigoration in breaking dormancy (DG_{60})



found to enhance germination in bitter gourd seeds while in bottle gourd, seeds treated with KH_2PO_4 10^{-3} M for 48 hours proved to be highly beneficial in improving germination and crop growth (AICRP (VC), 2014). Sahib *et al*. (2014) reported that hydropriming okra seeds as well as priming with KH_2PO_4 caused significant increase in germination compared the control. The highest germination was observed in 3% KH_2PO_4 at 25 and 30 ºC.

Similarly, cytokinins are known to break seed dormancy in many species (Cohn and Butera, 1982). During the conditioning of parasitic *Orobanche* and *Striga* species and the release of lettuce thermo-inhibition, cytokinins appeared to contribute to the promotion of dormancy release and subsequent germination by enhancing ethylene biosynthesis (Saini *et al*., 1989 and Matilla, 2000). Nawaz *et al*. (2013) had also reported that cytokinins can be used as priming agent as they promoted cell division in the embryo. According to them, seed priming with 10 ppm kinetin for 24 hours in tomato cultivars, significantly reduced the time taken to 50% emergence and mean emergence, increased final seedling emergence, and seedling growth.

In seed invigoration treatments such as hydropriming and treatment with thiourea, germination above MSCS was attained on the 15^{th} and 17^{th} DAI respectively. Untreated seeds took the longest period to reach MSCS (35^{th} day after extraction) indicating that seed invigoration was highly effective in breaking dormancy in ash gourd. Causes of dormancy are many and varied. Impermeability of seed coat to water and gases, immaturity of the embryo, special requirement for temperature and light, vicinity of inhibitors and mechanical restriction to embryo growth are the major reasons (Tran and Cavanagh, 1984). The advantage of seed invigoration treatments in breaking dormancy has been reported by several earlier workers (Malarkodi *et al*., 2002 in muskmelon; Singh *et al.,* 2004 in watermelon; Mohan, 2005 in snake gourd; Venketasubramaniam and Umarani 2007 in tomato;

Bijanzadeh *et al.,* 2010 in rapeseed; Divya, 2013 in chilli; Rahman, 2014 in ash gourd; Saleem *et al*., 2014 in bitter gourd and Batool *et al*., 2015 in cabbage).

Seed invigoration with salicylic acid for 12 hours and 24 hours however proved to be detrimental to seed germination. Seeds treated with salicylic acid did not germinate until 31st day after invigoration and further no germination was observed from 35th DAI. Tilebani *et al*. (2013) had also found that seed treatment with salicylic acid did not help to break seed dormancy. According to Wu *et al*. (1998), increasing salicylic acid concentration enhanced ABA synthesis which can stop the seed germination. However, an experiment conducted by Mirabi and Hasanabadi (2012) to investigate the effect of seed priming on characteristic of seed and seedling vigour in tomato revealed that seed priming with salicylic acid (60 ppm) was beneficial. Priming with salicylic acid was also found to increase enzyme activity and neutralize the effects of seed ageing.

Germination in seeds treated with vinegar for 2 hours was poor and a sharp decline in germination was observed after the initial increase. In seeds treated with vinegar, germination failed to reach the required MSCS within the 35th DAI. However, unlike the result obtained in the present study, Sholberg and Gaunce (1996) in wheat and Gonzales (2015) in eggplant had found that seed treatment with household vinegar resulted in higher seed germination. Fonseka and Fonseka (2009) on priming of bitter gourd seeds had observed that treatment with household vinegar (pH -3.7) for 12 hours period induced higher germination and vigour.

Although germination in seeds invigorated with PEG 6000 (-0.5 MPa for 24 hours) reached 75.00 per cent on the 19th DAI, it declined to 62.60 per cent by 35th DAI. The germination in these treatments was also found to be inferior to untreated seeds throughout the period of observation. This result is in contrast with the reports of KAU (2009) and Divya (2013). The study conducted in Kerala Agricultural

University (2009) illustrated that improvement in germination and vigour of bittergourd could be achieved by priming with PEG 6000 (-0.5 MPa for 24 hours) (KAU, 2009). Divya (2013) had reported that osmopriming of chilli seeds in polyethylene glycol (PEG 6000; -1.5 MPa) for 3 hours in darkness improved germination, vigour and longevity. However, as in the present treatment Dodd and Donavon (1999) had also observed that reduction in germination can result from PEG treatments. They attributed the decrease in water potential gradient between seeds and their surrounding media to be the cause for poor germination. They also found that a great deal of genetic variation existed among cultivars in their response to osmotic stress treatments.

According to Hartmann and Kester (1975) although seed treatment to break dormancy usually modifies the hard seed coat in order to make them permeable to water and gases, the length of treatment depends upon temperature, concentration of acid, the kind of seed. The poor germination in seeds treated with salicylic acid, vinegar and PEG 6000 as evident in the present study may, therefore be attributed to the concentration and duration of seed treatment. Hence, standardization of seed treatment with salicylic acid, vinegar or PEG 6000 based on the thickness of seed coat in ash gourd may prove to be more effective.

Apart from early break of dormancy, seed invigoration was also found to significantly influence the seed quality parameters *viz*., germination index, mean time to germination, coefficient of velocity of germination, energy of germination, seedling vigour index I and II were by the seed invigoration methods within 35 days of treatment. In majority of invigorated seeds, these parameters were observed to be higher than that of untreated control. As observed earlier, seed treatment with salicylic acid, vinegar and PEG 6000 had a negative impact on the seed quality and seedling performance. The performance of seeds in these treatments was inferior to the untreated seeds. Detrimental effects of PEG uptake on seeds and seedling

performance was reported by Lagerwerff *et al*. (1961); Jackson, (1962) and Lawlor, (1970) in vegetables.

Results pointed out that germination index increased in both treated and untreated seeds by 35th DAI. High germination index was observed in treatments with high germination *viz*., seeds treated with *Pf* for 12 hours (T14), $CaCl_2$ 50 mM for 12 hours or 24 hours and KH_2PO_4 10^{-1} M for 24 hours etc. Biopriming of pearl millet seeds with *Pf* was found to increase the germination, seedling vigour, plant height, leaf area, tillering capacity, seed weight and yield and also resistance against doweny mildew disease caused by the fungus *Sclerospora graminicola* (Raj *et al.,* 2004) while Moeinzadeh *et al*. (2010) had reported the beneficial effect of biopriming of sunflower seeds with *Pf* on seed indices and improvement of seedling growth. As in the present study, priming of seeds with KH_2PO_4 (10^{-1} M for 24 hours) was also reported to improve germination and vigour in bittergourd (KAU, 2009), while, seed priming with varying concentration of 50 mM $CaCl_2$ and KNO_3 for 24 h was found to significantly improve final germination, root and shoot lengths which was attributed to enhanced chlorophyll contents and better germination pattern (Afzal *et al*., 2011).

A marginal increase in coefficient of velocity of germination was observed in both treated and untreated seeds by 35th DAI. Similar to the trend observed in germination index, in most seed invigoration treatments that recorded higher germination, coefficient of velocity of germination was also found to be high. Seeds treated with $CaCl_2$ 50 mM for 12 hours had registered high coefficient of velocity of germination. Coefficient of velocity of germination was found to increase in lettuce seeds on treatment with $CaCl_2$ (Ahmed and Farag, 2011). Jamadar and Chandrashekar (2015) revealed that castor seeds treated with $CaCl_2$ @ 2.0 per cent solution exhibited significantly higher daily germination index and coefficient of velocity of germination but significantly less mean germination time. Conversely, in treatments that exhibited low germination, the coefficient of velocity of germination

was also low. The primary effect of seed treatment with $CaCl_2$ is attributed to certain enzymatic activities occurring in the seed, while it is being held in moist condition (Austin *et al.,* 1969 and Hydecker, 1974).

A negligible decrease in mean time to germination was observed in all treatments between 1st DAI and 35th DAI. The average mean time to germination over 35 DAI was low (≤ 7 days) in case of seeds treated with *Pf* for 12 hours or 24 hours, kinetin 10 ppm for 12 hours or 24 hours, $CaCl_2$ 50 mM for 12 hours or 24 hours and hydropriming for 12 hours. Thus, it was evident that mean time to germination of a seed lot exert high influence on the actual germination per cent perceived. Dezfuli and Zadeh (2008) in maize, Nawaz *et al*. (2013) in tomato, Mahboob *et al*. (2014) in maize have reported the positive effect of seed invigoration in reducing the mean time to germination.

Significant reduction in mean germination time was noticed in seeds treated with $CaCl_2$ which might be attributed to the hydration and dehydration of seeds during priming, which hastens the germination process (Airin and Khosro, 2013). Nawaz *et al*. (2013) and Zeb (2006) in tomato had also reported that seed treatment with kinetin increased the coefficient of velocity of germination and minimised the mean time to germination. A significant reduction in mean time to germination and improvement in germination index might be because seed priming stimulates an array of biochemical changes such as hydrolysis, activation of enzymes and dormancy breaking in the seed (Aziza *et al*., 2004; Farooq *et al*., 2010) which are essential to start the germination process. According to Mewael *et al*. (2010), early DNA replication, increased RNA production and protein synthesis, increased enzyme activity, greater ATP availability (speedier embryo growth and efficient repair of deteriorated seed parts due to seed priming have been reported to reduce the mean time to germination.

Energy of germination increased progressively in all treatments including the control over 35 DAI. Similar to the impact of seed invigoration on germination, germination index and coefficient of velocity of germination, seed treatment with *Pf* or $CaCl_2$ for either 12 hours or 24 hours also increased the energy of germination. These treatments were also effective in improving seedling vigour indices. Similar to the findings of the present study, energy of germination was found to increase on halopriming seeds (Farooq *et al*., 2007 in melon; Burgass and Powell, 1984 and Hocart *et al*., 1990 in marigold; Yousof , 2013 in rice and Haroni *et al*., 2015 in judas tree seeds).

However, seed treatment with *Pf* for 24 hours though reduced mean time to germination was not beneficial in improving the seedling vigour of treated seeds. Similarly although kinetin reduced mean time to germination, no consistency was observed in its impact on energy of germination and seedling vigour indices. Therefore it was obvious that seed treatment with $CaCl_2$ for 24 hours or 12 hours was most beneficial seed invigoration treatment followed by treatment with *Pf* for 12 hours. Higher seedling vigour index-II on treatment with $CaCl_2$ has been reported in rice (Yousof, 2013), Dezfuli and Zadeh (2008) in maize, Jamadar and Chandrashekar (2015) in castor. According to Prabhu *et al*. (2006) the increased germination and seedling vigour observed in calcium chloride seed priming was due to the effective control of peroxidation and free radical damage, either by stabilizing the cellular membrane or by facilitating the recombination of free radicals into non-destructive products.

Beneficial effect of seed invigoration with $CaCl_2$ was also reported by Narayanaswamy and Shambulingappa (1998) in groundnut, Singh and Rao (1993) in sunflower, Narayanaswamy and Channarayappa (1996) and Narayanareddy and Biradarpatil (2012) in sunflower, Rehman *et al*. (2011) and Afzal *et al*. (2012) in rice. The advantage of pre-sowing invigoration seed treatment with $CaCl_2$ have been

primarily attributed to an advancement of germination procedure (Austin *et al.,* 1969; Hydecker, 1974) and change in other seed quality characteristics. Chrysiansen and Foy (1979) and Hecht-Buchholz (1979) reported that seed calcium fixation and germination rate were positively correlated and also suggested that calcium played a vital in membrane stabilization and as an enzyme co-factor. Seeds treated with $CaCl_2$ had an advantage in stabilizing germination due to the influence of Ca^{+2} on membranes (Shannon and Francis, 1977) and improved antioxidant proteins like SOD enzyme.

Research by Adebisi (2011) in cordia seeds, Reddy *et al*. (2008) in chickpea, Kade *et al*. (2013) in cocoa, Reetha *et al*. (2014) in onion have pointed to the positive effect of *Pf* in breaking dormancy and improving seed germination. *Pf* was capable of producing IAA, gibberellins and kinetins, nitrogen fixation and dissolved phosphate (Ashrafuzzaman *et al*., 2009; Mehrab *et al*., 2010; Ahmed and Farag, 2011) while Nawaz *et al*., (2011) in tomato had reported the advantage of seed invigoration with kinetin.

5.2 Effect of seed invigoration treatments on seed viability during storage

Results revealed that seed quality and longevity during storage were found to be significantly influenced by the seed invigoration treatment throughout the storage period.

Germination declined (Fig. 3) with increase in the storage period both in untreated control as well and invigorated treatments. However, in most treatments the decline set in after an initial increase as evident at one month after storage (MAS). The reduction in final germination rate can be explained by the expansion of external osmotic pressure which influences the assimilation of water by the seed and can be also due to the accumulation of Na+ and Cl^- in the embryo which may lead to a

Fig. 3. Effect of seed invigoration on germination in ash gourd during storage

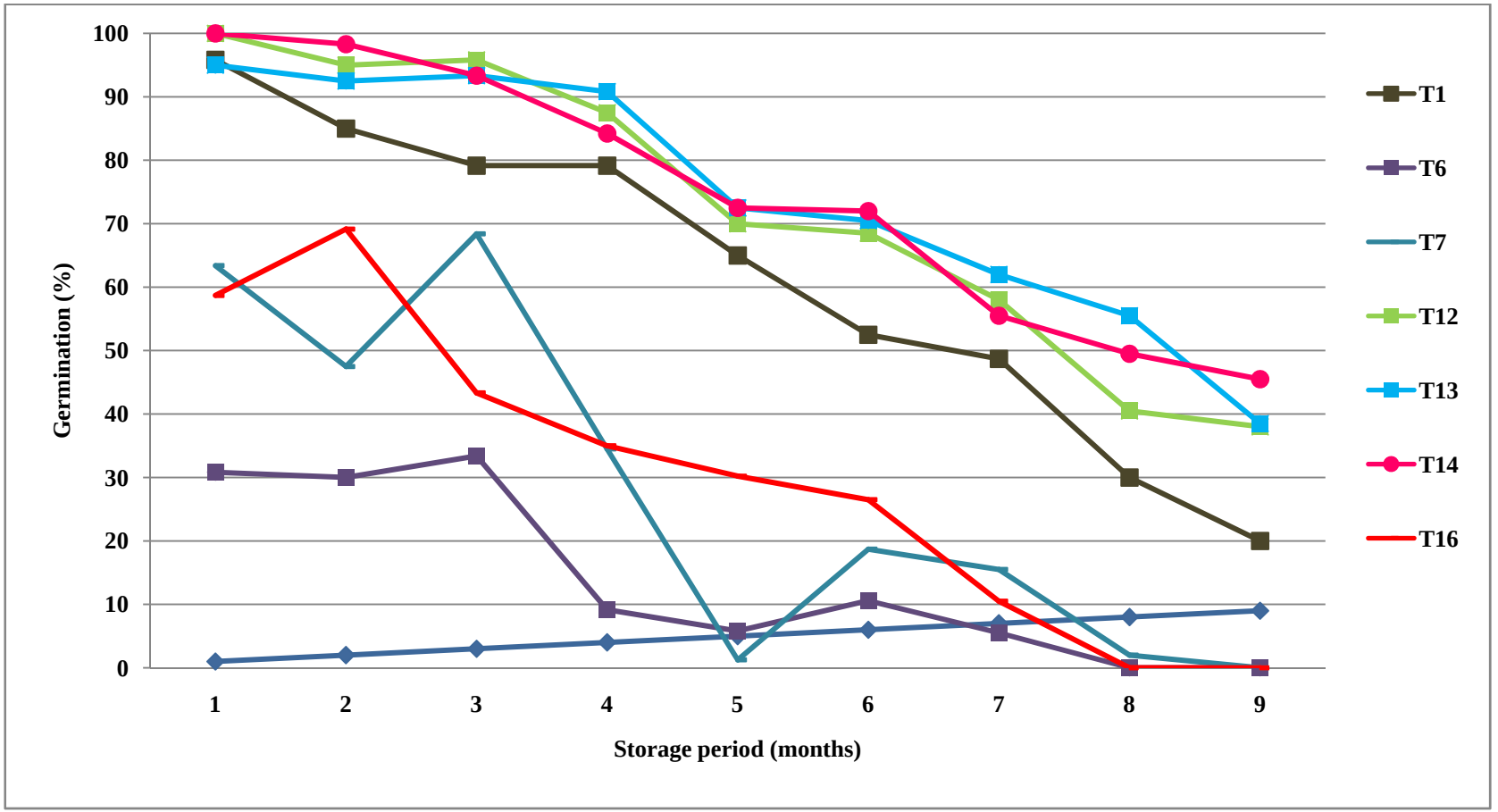
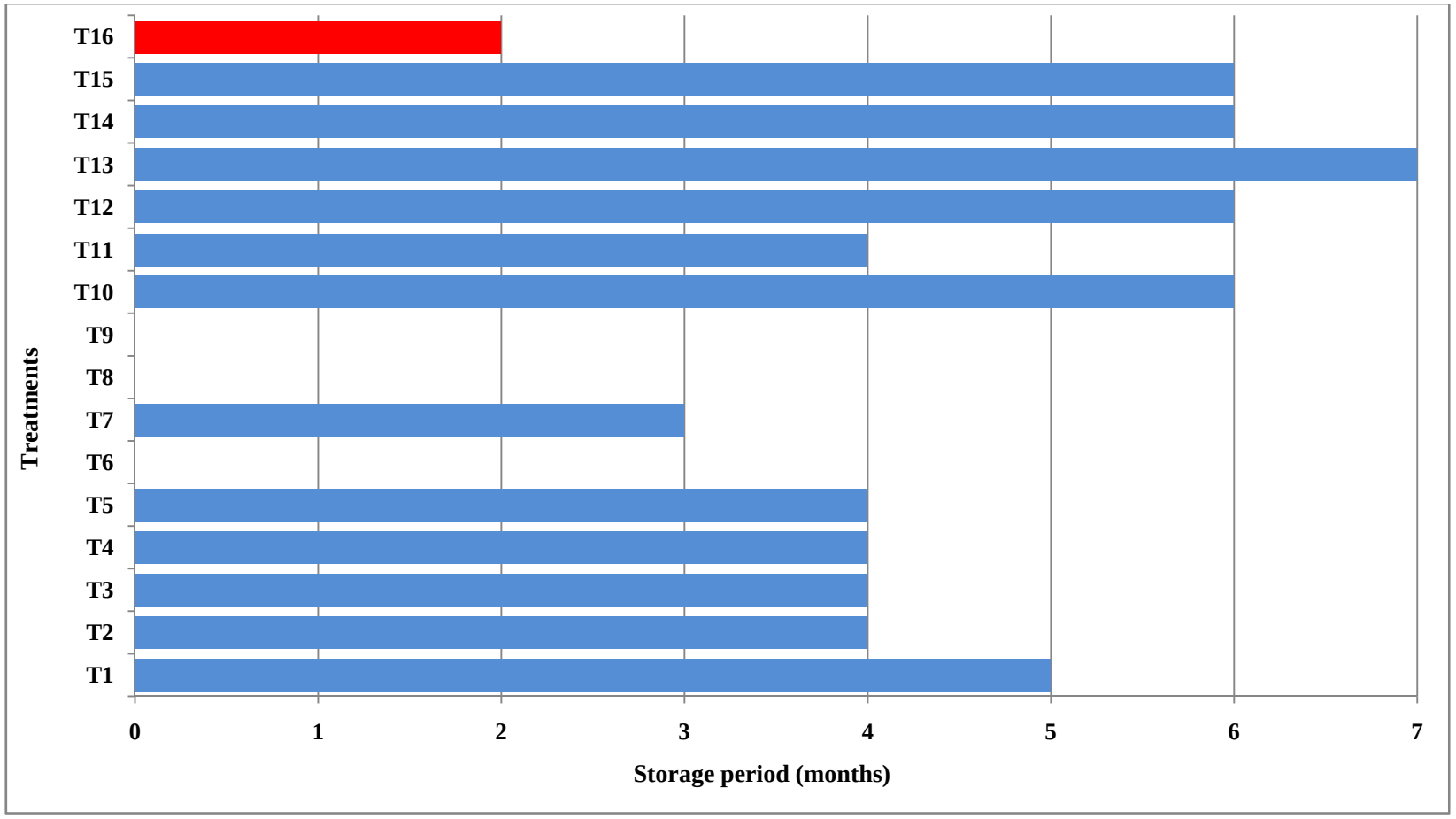


Fig. 4. Impact of seed invigoration on retention of seed viability during storage



change of the metabolic processes of germination and subsequently causing cells death in the embryo (Maher *et al*., 2013).

All the seed invigoration treatments exerted a positive influence on extending the period of seed viability (Fig. 4), while the untreated seed had sustained germination above 60 per cent for two MAS only. However, as discussed earlier (section 5.1), seed invigoration with salicylic acid for 12 hours or 24 hours, and those treated with vinegar for 2 hours and PEG 6000 for 24 hours proved to be detrimental to the seeds. Germination in seeds treated with salicylic acid and vinegar failed to reach above MSCS (60 %) required for ash gourd during the storage period. Seeds treated with salicylic acid also failed to germinate after one month of storage (MAS) while in seeds treated with polyethylene glycol 6000 (@-0.5 Mpa for 24 hours), although MSCS was reached on 19th DAI, germination was poor and not sustained above MSCS beyond 1 month.

For most part of storage period, seeds treated with $CaCl_2$ (50 mM for 24 hours) and *Pf* or kinetin for 12 hours recorded high germination. It was evident that seed invigoration with $CaCl_2$ was the best among the seed invigoration treatment. Germination in the treatment was maintained above MSCS for 7 MAS, while in the seeds treated with $CaCl_2$ for 12 hours or *Pf* for 24 hours, viability was retained above MSCS up to 6 MAS. Seed treatment with thiourea, KNO_3, KH_2PO_4 and kinetin were helpful in retaining viability retained above MSCS for 4 MAS.

Seed quality parameters like germination, germination index, coefficient of velocity of germination, mean time to germination, energy of germination, vigour index-I and vigour index-II had shown a general decline during the period of storage. The significant decrease in seed quality parameters during storage can possibly be attributed to either one or combination of the factors like accumulation of toxicants and corrosive action caused by acids (Zhang *et al*., 1993), membrane degradation which resulted in greater leakage of sugars, amino acids and inorganic solutes from

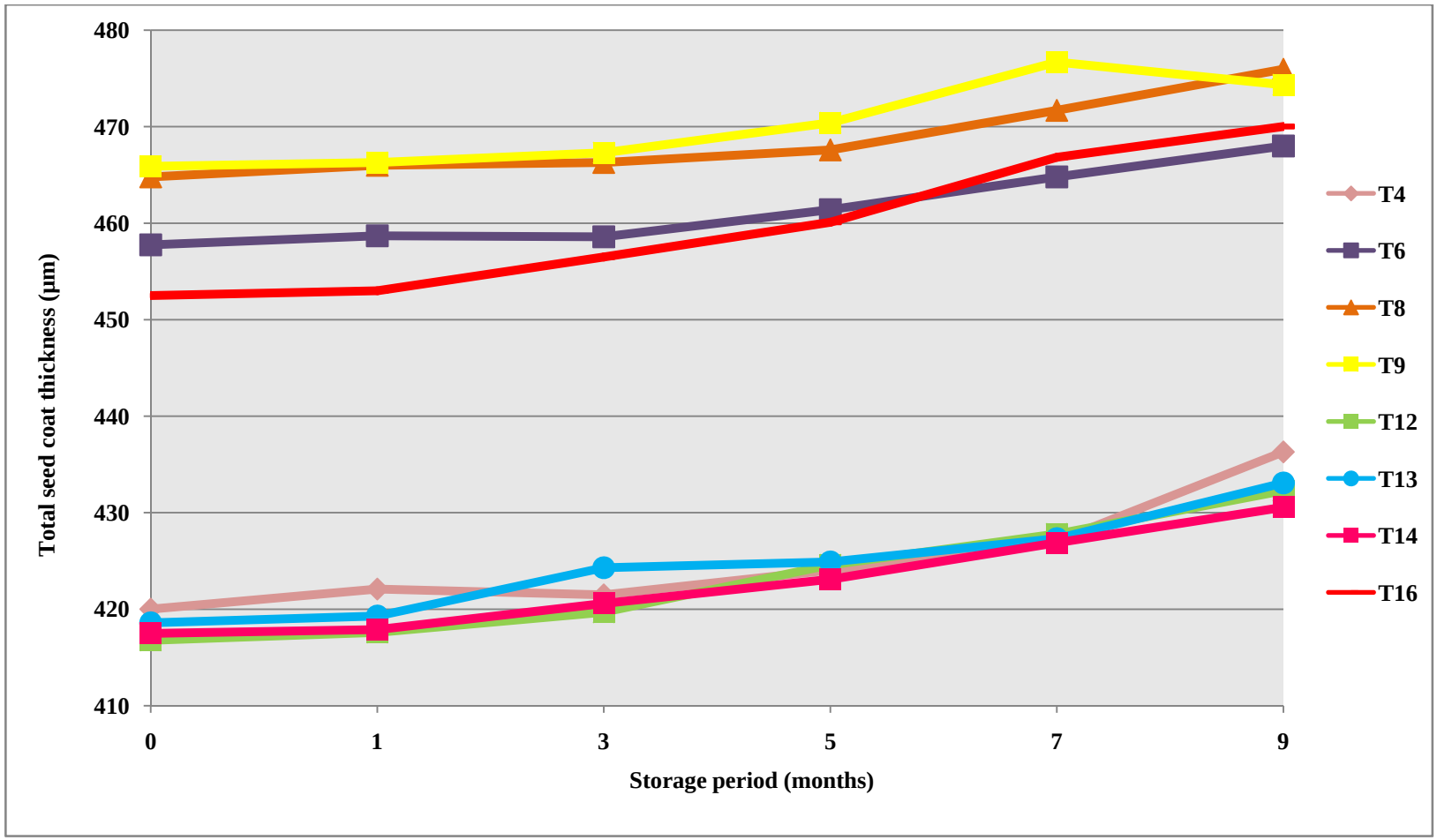
the seed (Abdul-Baki and Anderson, 1972), free radical damage formed due to lipid peroxidation (Rudrapal and Basu, 1982), imposed enzymatic activity (Chauhan *et al*., 1984: Zuo *et al*., 1988) and increase in respiratory quotients (Harrington, 1973).

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

Negligible to marginal increase in mean time to germination, electrical conductivity of seed leachate, total thickness of seed coat, thickness of testa and tegmen, embryo length to seed length ratio and microbial infection of seed was observed in all treatments over the period of storage, the increase being invariably high in seeds treated with salicylic acid 60 ppm, vinegar, PEG 6000 and untreated seeds. However, low values for these parameters were observed in seeds with high germination namely those treated with $CaCl_2$ and *Pf*.

Thickening of seed coat (Fig. 5) was observed over the period of storage although seeds that registered higher germination had thinner seed coat compared to untreated seeds as well as those exhibiting poor or no germination. Thicker seed coat is reported to act as a mechanical barrier for water imbibitions. However, it has been suggested that the seeds become permeable naturally after storage and this pattern of loss of impermeability are due to opening of specialized cracks in seed coat and general deterioration of its seed coat due loss of turgor pressure in cells (Raghus, 1987). Tran and Cavanagh (1984) had suggested that thick seed coat is slower in

Fig. 5. Effect of seed invigoration on total seed coat thickness in ash gourd during storage



absorbing water than thinner ones and old age seed has higher speed than the freshly harvested seeds and coat imposed dormancy breaks down during storage resulting in rapid water uptake.

The differential EC values recorded among the seed treatments indicated that the nature and extent of membrane protection offered may not be same for all treatments, thus resulting in difference in EC values (Kurdikeri, 1991). The highest electrical conductivity of seed leachate was observed in untreated seeds while the least was observed in hydroprimed seeds. The lower electrical conductivity of seed leachate in hydroprimed seeds may be because priming permits early DNA replication, increase RNA and protein synthesis, enhances embryo growth, repairs deteriorated seed parts and reduces leakage of metabolites (Adebisi *et al*., 2011). Increase in membrane repair during the hydration process may also be the cause of low EC of seed leachate as reported by Rudrapal and Nakamura (1998) in radish and eggplant, Pen-azola and Eira (1993) in tomato and Basra *et al*. (2003) in fine rice.

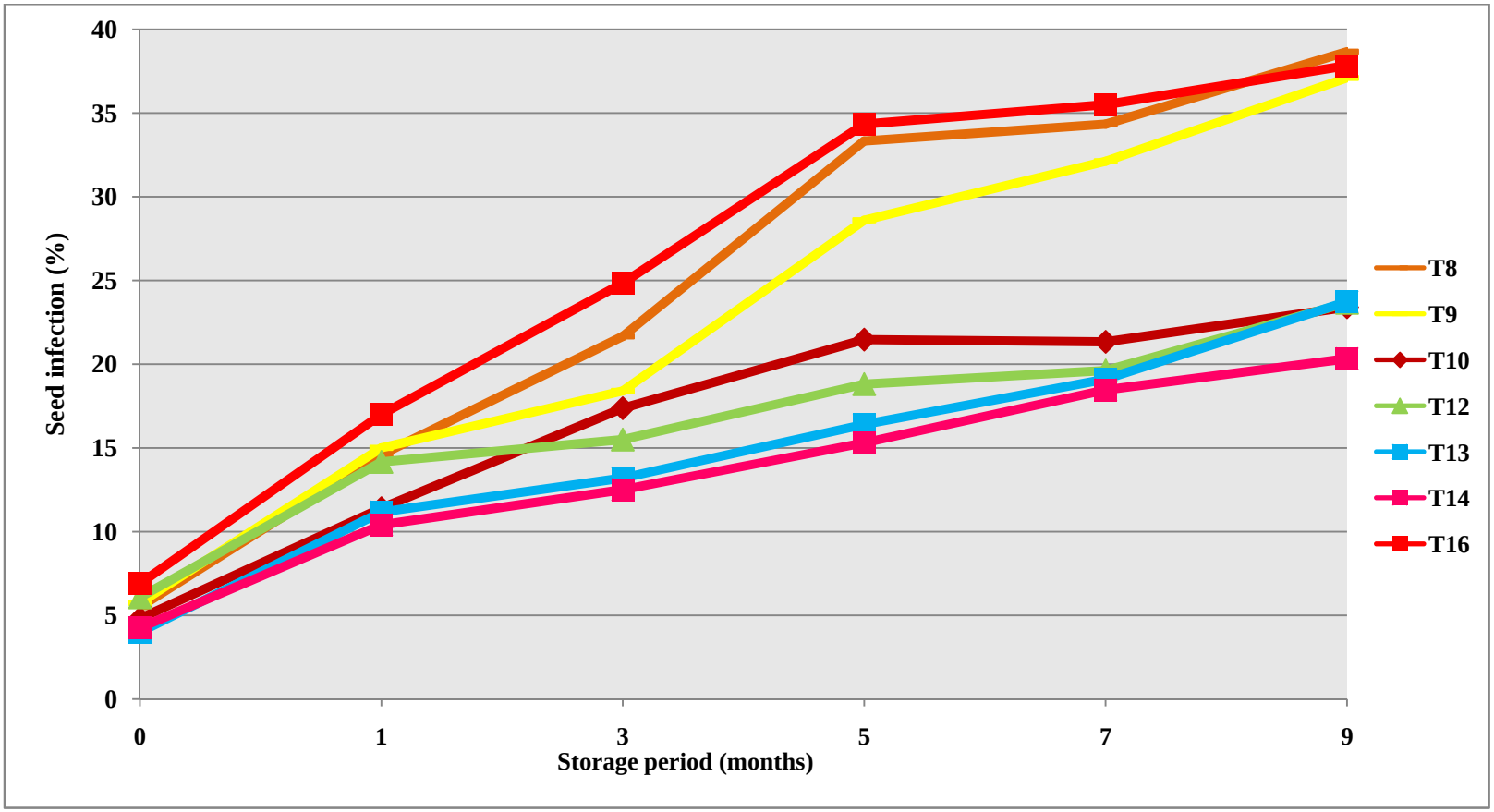
The infection by seed microflora (Fig. 6) was found to be the highest in untreated control throughout the storage period while it was the least in seeds treated with *Pf* for 12 hours and KNO_3 for 24 hours. The results once again reaffirmed the advantage of seed invigoration treatments with either $CaCl_2$ for 24 hours or *Pf* for 12 hours.

5.4 Correlation study

Existence of significant correlation between germination, seed quality and anatomical features was evident immediately after seed invigoration as well as at 1 MAS, 3 MAS and 7 MAS (Fig. 7, 8, 9 and 10).

High and significant positive correlation was found to exist between germination and speed of germination, coefficient of velocity of germination, vigour

Fig. 6. Effect of seed invigoration on seed infection in ash gourd during storage



indices I and II, while, it was highly significant and negative with total thickness of seed coat and thickness of testa immediately after seed treatment as well as throughout the storage period.

Towards the end of storage period (7 MAS), no significant correlation was evident between germination and mean time to germination as well as energy of germination indicating that as seed ages, mean time to germination and energy of germination does not affect germination. However, although EC of seed leachate and thickness of tegmen did not exhibit a significant influence on germination, mid-way between storage, towards end of storage, germination was negatively and significantly influenced by these factors. Several earlier workers had reported the existence of positive association between germination and coefficient of velocity of germination, seedling vigour and negative significant correlation with mean time to germination was reported by Sharafizad *et al*. (2013) in wheat (Odabas *et al*., 2006 and Sheidaei *et al*., 2014 in soybean). Similar to the findings of the study, a negative relation between germination and electrical conductivity was reported by Hill *et al*. (1988) in jute and Hossain *et al*. (2013) in cabbage. There was a significant and negative correlation between germination and thickness of testa in coloured beans seeds (Balkaya and Odabas, 2002).

The inter-relation between EC of seed leachate with speed of germination, coefficient of velocity of germination, energy of germination, vigour indices I and II was negative and significant while a positive and high significant correlation was evident between EC of seed leachate and mean time to germination, thickness of testa, tegmen and total seed coat for most part of storage. However, the relationship of EC of seed leachate with all the above factors and with germination was found to be non-significant during 1 and 3 MAS. Therefore an increased EC will decrease germination only with an accompanied increase in thickness of testa, tegmen and total seed coat. Borji *et al*. (2007) had reported existence of positive correlation

Fig. 7. Correlation among germination and seed quality parameters in ash gourd immediately after seed invigoration

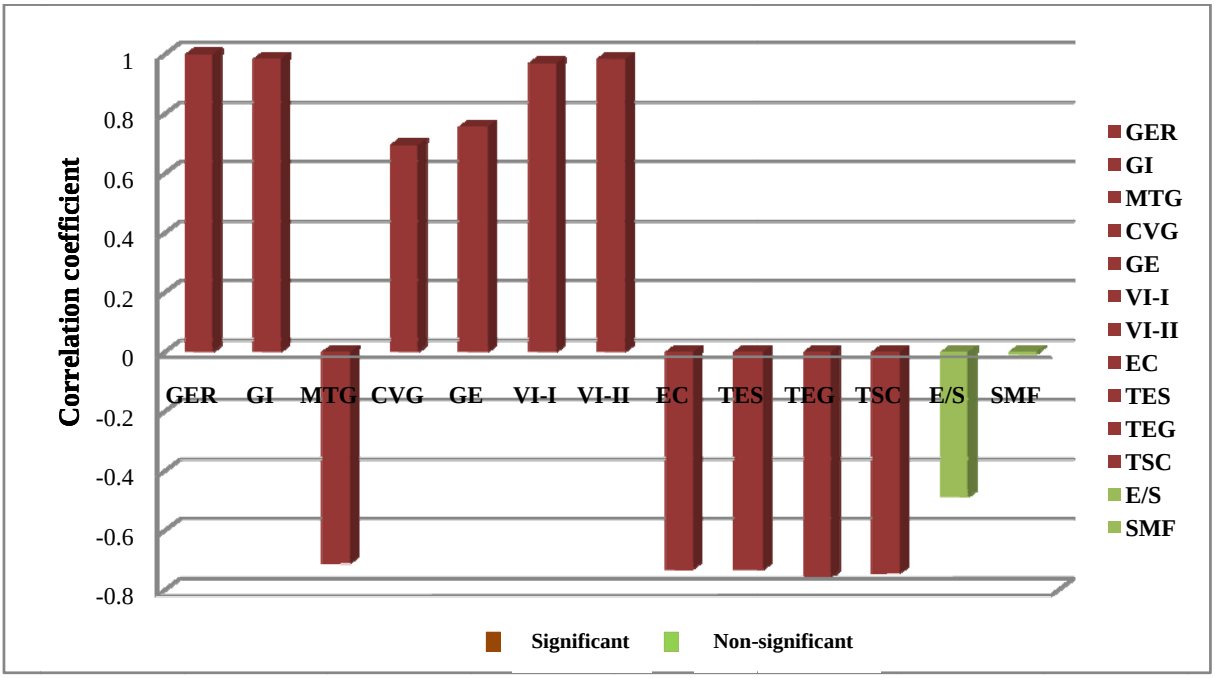
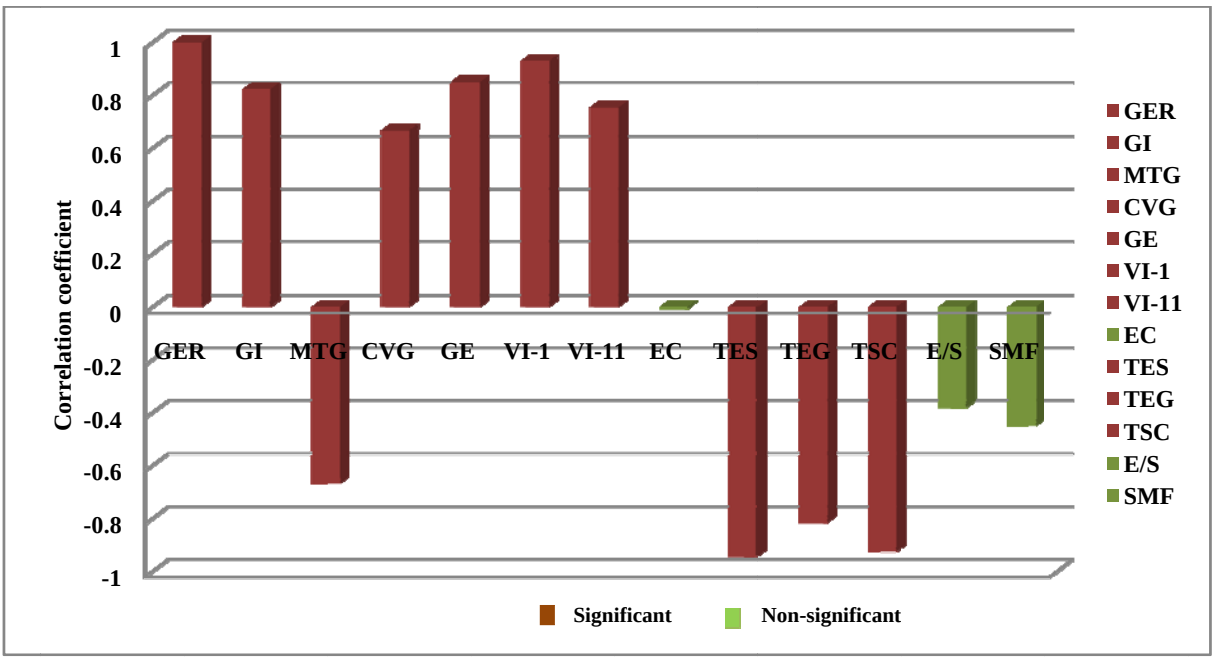


Fig. 8. Correlation among germination and seed quality parameters in ash gourd at 1 month after storage



between EC and total seed coat thickness in white bean. He stated that an increase in seed coat thickness resulted in an increase in the availability of physiochemicals within the seed coat. Therefore seeds with thicker seed coats exude more water soluble compounds thus giving off a high EC value. The finding of the study is also in consonance with Thanh *et al*. (2006) who elucidated the existence of a negative association between EC and seed coat thickness in soybean. Findings of Chimonyo and Modi (2013) had also pointed to a negative relation between EC and germination and speed of germination as found in the present study.

In addition, towards end of storage (7 MAS), no significant association was evident between EC and mean time to germination, coefficient of velocity of germination and energy of germination. This strongly indicated that an increase in EC of seed leachate over storage will be accompanied by a reduction in speed of germination and vigour indices I and II. Generally, electrical conductivity of seed leachate is negatively correlated with the seed viability and vigour. As seed ages the cell membrane and cell organelle become leaky on account of decrease in phospholipids content either due to enzymatic activity or non enzymatic lipid auto oxidation or due to fungal and insect activity (Ching and Schoolcraft, 1968; Koostra and Harrington, 1969; Pammenter *et al*., 1974). Ghasseemi *et al*., (2010) and Jyothi and Malik (2013) also found that lipid peroxidation not only destroys lipids but can damage the cell membrane and other cellular components which in turn leads to leaching loss and seed deterioration.

Invariably a significant positive relationship existed between germination index and velocity of germination, energy of germination, vigour indices I and II while it was negatively associated with mean time to germination, EC of seed leachate, thickness of testa, tegmen and total thickness of seed coat. However, correlation between the speed and energy of germination, vigour index-II and EC of seed leachate was found to be non-significant at 7 MAS, at 1 MAS and at 3 MAS

respectively. Thus the result indicated that germination index was less influenced by energy of germination, vigour index-II and EC of seed leachate. The germination index increased with an increase in coefficient of velocity of germination, vigour index-I and decreased with a decrease in mean time to germination, thickness of testa, tegmen and total thickness of seed coat. Similar to the findings of the study, a positive correlation between germination index and energy of germination was reported by Willian (1985) in forest seeds. Similarly, a positive and significant inter-relation among germination index, energy of germination, vigour index was reported by Adebisi *et al*. (2011) in rice.

Energy of germination had exhibited a high positive significant correlation with speed of germination, coefficient of velocity of germination, vigour index I and II while the relationship was high and negatively significant with mean time to germination, EC, thickness of testa, tegmen and total seed coat invariably during storage. However, at the end of storage (7 MAS), the association of energy of germination was found to be non-significant with most of these traits including germination. In addition, no significant correlation existed between energy of germination and traits mean time to germination, coefficient of velocity of germination EC and thickness of tegmen at 3 MAS. Hence, it can be concluded that energy of germination is strongly influenced by speed of germination, vigour indices I and II, thickness of testa and total seed coat rather than mean time of germination, coefficient of velocity of germination, EC of leachate and thickness of tegmen.

Germination was also positively influenced by coefficient of velocity of germination which in turn was found to be positively affected by speed of germination and vigour index-I. An increase in coefficient of velocity of germination also occurred with a decrease in mean time to germination, thickness of testa, tegmen and total seed coat.

Fig. 9. Correlation among germination and seed quality parameters in ash gourd at 3 months after storage

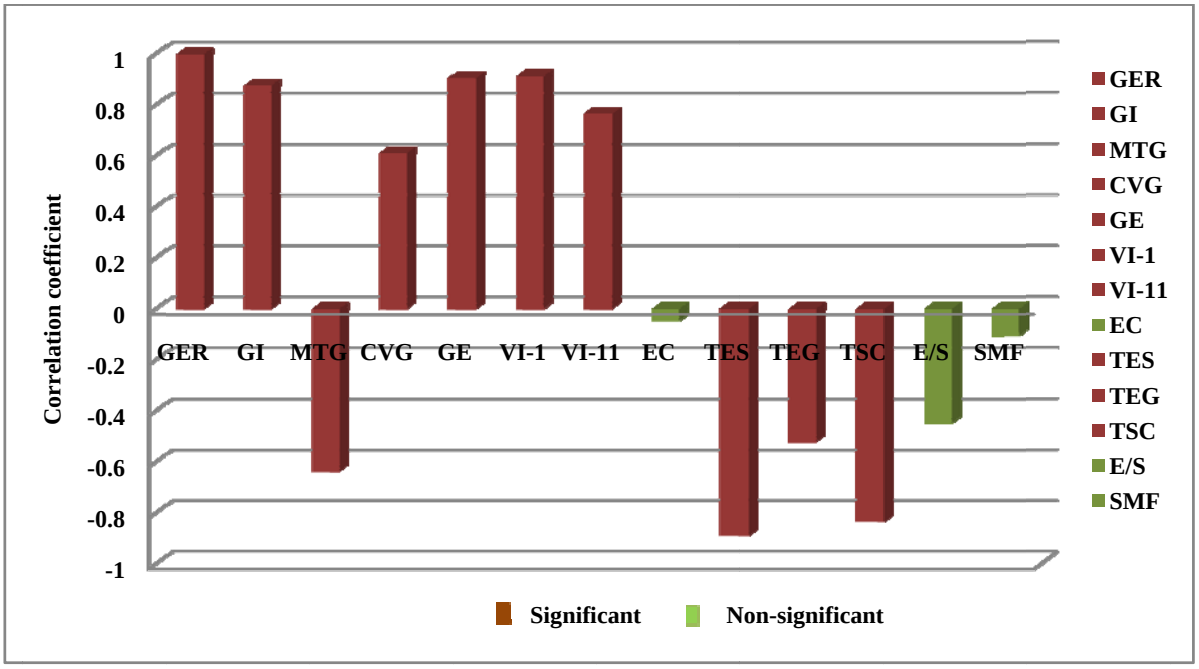
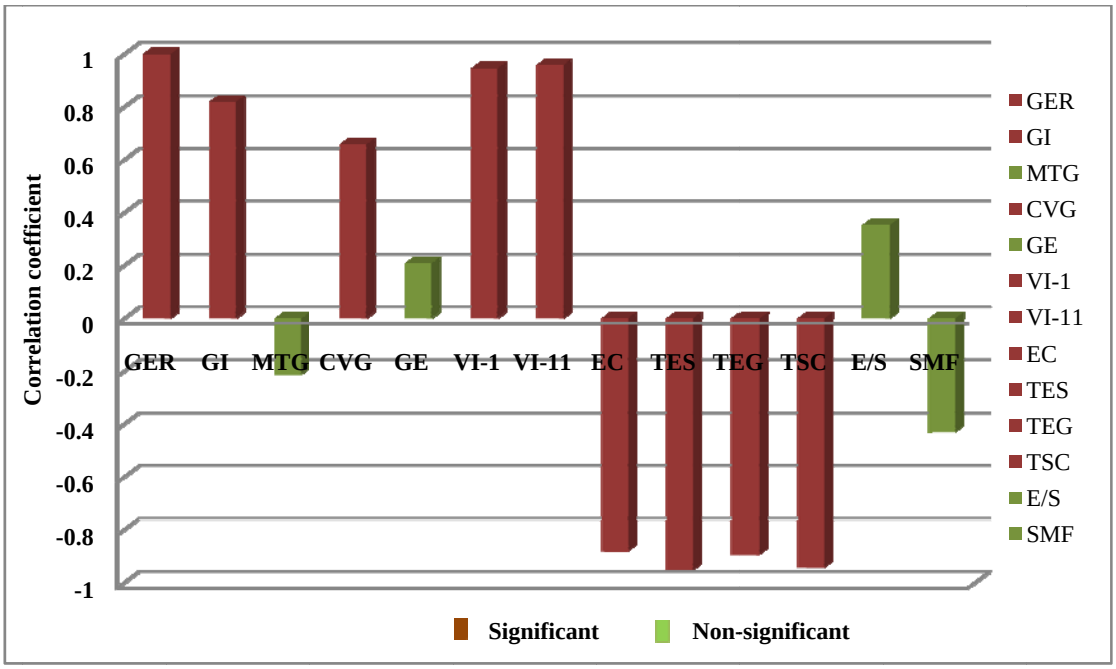


Fig. 10. Correlation among germination and seed quality parameters in ash gourd at 7 months after storage



Significant positive correlation was found to exist between vigour indices I, II and germination, speed of germination, coefficient of velocity of germination, energy of germination. Vigour indices exhibited high significant negative correlation with, mean time to germination, EC of seed leachate, thickness of testa, tegmen and total thickness of seed coat. However, the relation with mean time of germination, energy of germination was found to be non-significant towards end of storage (7 MAS). Therefore it may be deduced that higher the vigour indices better the germination. An increase in vigour indices will therefore be accompanied by an increase in the germination index, coefficient of velocity of germination, energy of germination while, a decrease in seedling vigour occurs upon a decrease in EC of seed leachate, thickening of testa, tegmen and total thickness of seed coat.

Over the storage period, an increase in mean time to germination was found to reduce germination. At 7 MAS however, the association was found to be non-significant. The association between mean time to germination and germination index, coefficient of velocity of germination, energy of germination, vigour indices I and II were highly significant and negative while it was significant and positive with EC of seed leachate, thickness of testa, tegmen and total seed coat. Similar to germination, towards the end of storage (7 MAS), correlation between mean time to germination and most of these traits were non-significant. Results thus indicated that mean time to germination decreases with an increase in speed of germination, coefficient of velocity of germination, energy of germination, vigour indices I and II while an increase in mean time to germination occurs with an increase in EC of seed leachate, thickness of testa, tegmen and total seed coat occurs.

A thick testa, tegmen and total seed coat thickness were found to decrease the germination. However, the correlation of germination with thickness of tegmen at 3 MAS was found to be non significant. It was also evident that inter-correlation between thickness of testa, tegmen and total seed coat thickness, germination index,

coefficient of velocity of germination, vigour indices I and II was negative. The association among EC, thickness of testa, tegmen and total seed coat thickness was found to be positive but not significant at 1 MAS and 3 MAS. Hence, it can be concluded that, germination will be strongly affected with an increase in thickness of total seed coat or its outer layer-the testa. The thickening of seed coat owing to the thickening of testa will be accompanied with an obvious decrease in germination index and coefficient of velocity of germination, vigour indices I and II. Results thus indicated that poor germination in seeds of ash gourd was may be due to a water impermeable seed coat (physical dormancy) conspicuous by thickening of outer testa which can be overcome by invigoration treatment for good germination.

No significant correlation was evident between germination and embryo length to seed length ratio and microbial infection of seed throughout the storage period. The non-significant correlation between germination and microbial infection may be because the seeds were packed at 8 per cent moisture in 400G polyethylene bags. Several earlier reports on the beneficial effect on enhancing seed viability by packing seeds in moisture proof containers at optimum moisture content have been reported (Mettananda *et al*., 2001 in maize; Kamara *et al*., 2011 in cowpea and Rahman and Ahmmad, 2014 in soybean).

5.5 Ranking of seed treatments at 7 MAS

For the selection of seed treatment best suited for breaking dormancy and maintaining seed quality during storage, ranking of treatments was done considering the parameters at 7 MAS, with slight modification to the procedure advocated by Arunachalam and Bandopadhyay (1984). Each treatment was ranked in serratum based on the magnitude of seed quality parameters *viz.,* germination per cent, germination index, coefficient of velocity of germination, energy of germination, vigour index-I and II in consideration of the DMRT test values *i.e*., individuals with

DMRT annotation ‘a’ were assigned rank 1, 2 for treatments with DMRT annotation ‘ab’, 3 for ‘abc’ and so on, for example, higher the germination lower will be the numerical value of the rank.

Total seed coat thickness, thickness of testa, thickness of tegmen and seed microflora, EC of leachate and mean time to germination was found to be negatively affecting germination. Therefore, for ranking individuals based on these seed qualities, the reverse format was followed *i.e*., the individuals were ranked in descending order of magnitude. A treatment with least magnitutde for the parameter and corresponding DMRT annotations was assigned rank 1, the next 2 and so on. Therefore, treatments with the least EC of leachate, mean time to germination, total seed coat thickness, thickness of testa, thickness of tegmen and seed microflora treatment were ranked 1, 2 and so on.

Final ranking of treatments were done considering the summation of score obtained by the treatment for each of the above criterion. Treatments with the least total score were therefore assigned final rank 1. Ranking of seed invigoration treatments (Table 27) pointed out that seed treatment with $CaCl_2$ for 24 hours or 12 hours, and *Pf* for 12 hours were found to rank high considering the earliness in breaking dormancy, improving germination, maintaining viability and higher seed quality and seedling performance during storage. The lower rank of a treatment is an indication that it is highly effective in breaking dormancy in ash gourd, maintaining higher seed quality and seedling performance during storage.

The advantage of seed treatment with $CaCl_2$ in increasing seed quality parameters *viz.,* speed of germination, energy of germination, vigour index-I and vigour index-II during storage has been reported in several crops (Farooq *et al*., 2007; Afzal *et al*., 2011; Ahmed and Farag, 2011; Shahzad *et al*., 2012; Azirloo *et al*., 2013; Mukthar *et al*., 2013; Hussain *et al*., 2014; Sneideris *et al*., 2015). Similar to the study, Moeinzadeh *et al*. (2010) had reported the advantage of *Pf* in obtainin

Table 27. Ranking of seed treatments at 7th MAS

Treatments	Earliness in breaking dormancy	Germination (%)	Germination index	Coefficient of velocity of germination (%)	Mean time to germination	Energy of germination	Vigour index-I	Vigour index-II	Electrical conductivity	Total seed coat thickness	Thickness of testa	Thickness of tegmen	Total	Rank
T_1	3	5	4	3	6	4	4	3	1	2	4	3	42	6
T_2	4	9	6	4	7	5	6	7	6	4	6	7	71	9
T_3	2	8	5	3	6	4	6	6	4	4	6	6	60	8
T_4	2	6	4	3	6	4	5	6	6	2	3	1	60	8
T_5	1	6	4	3	6	3	2	5	2	3	5	1	41	5
T_6	7	12	8	3	5	7	9	9	7	6	7	9	89	11
T_7	5	10	7	2	5	6	7	8	9	5	7	8	79	10
T_8	7	13	9	5	8	7	10	10	7	8	8	10	102	12
T_9	7	13	9	5	8	7	10	10	9	9	8	11	106	13
T_{10}	2	7	1	2	1	3	5	5	5	2	5	3	41	5
T_{11}	1	6	4	2	3	4	3	5	5	3	5	5	43	7
T_{12}	2	2	2	2	4	1	1	2	3	1	3	2	25	2
T_{13}	2	1	2	2	2	1	1	1	2	1	1	3	19	1
T_{14}	2	3	3	2	2	2	2	3	2	1	2	2	26	3
T_{15}	2	4	3	2	2	3	2	4	4	2	3	2	33	4
T_{16}	6	11	7	1	2	7	8	5	8	7	7	10	79	10

higher germination in sunflower, Jahanian *et al*. (2012) in artichoke (*Cynara scolymus*) and Rodriguez *et al*. (2015) in *Abies hickelii*.

Based on the findings of the present study enumerated above, seed treatment with $CaCl_2$ @ 50 mM for 24 hours can be adjudged as the best seed invigoration in ash gourd. This treatment had broken dormancy at 13^{th} DAI and had retained the viability for the maximum period after invigoration (7 MAS). The treatment had invariably exhibited higher germination per cent over the storage period, high estimates of speed of germination, coefficient of velocity of germination, energy of germination, vigour indices I and II and also lower estimates of mean time to germination, electrical conductivity of seed leachate, total thickness of seed coat, thickness of testa and tegmen, embryo length to seed length ratio and microbial infection of seed. Seed invigoration treatment with 50 mM $CaCl_2$ or *Pf* ($1x10^6$ cfu.ml^{-1}) for 12 hours can also be recommended for seed invigoration in ash gourd.

Summary

6. SUMMARY

The present investigation on 'Seed invigoration to overcome dormancy in ash gourd (*Benincasa hispida* (Thunb.) Cogn.)' was carried out at the Department of Seed Science and Technology, College of Horticulture, Kerala Agricultural University (KAU), Vellanikkara, Thrissur during 2014-2016. The experiment envisaged to elucidate the impact of seed invigoration techniques on breaking dormancy, seed storability and seedling performance as well as to assess the anatomical changes in seed coat following seed treatment. The salient findings of the study are summarized here under.

The salient findings of the study are summarized below:

1. Effect of seed invigoration treatments on seed dormancy

1. Untreated seeds of ash gourd var. KAU Local exhibited delayed and uneven germination for a period of 34 days.

2. Germination in the untreated seeds of var. KAU Local reached above minimum standards for seed certification (MSCS) prescribed for ash gourd (*i.e.,* 60 per cent) only on the 35th day after extraction. In other words, the inherent dormancy period in ash gourd var. KAU Local is 34 days after extraction.

3. Results indicated that all treatments except seed invigoration with salicylic acid (@ 60 ppm (for 12 hours or 24 hours) and vinegar at pH 3.7 for 2 hours were highly effective in breaking dormancy in ash gourd.

4. Although germination in seeds invigorated with PEG 6000 (-0.5 MPa for 24 hours) reached 75.00 per cent on the 19th DAI, it declined to 62.60 per cent by

35th DAI. The germination was also found to be inferior to control throughout the period of observation.

5. Germination reached above MSCS earliest *i.e.*, on the 11th DAI in seeds treated with KH_2PO_4 (10^{-1} M for 24 hours) and those treated with kinetin 10 ppm for 24 hours, while, in majority of the treatments it was attained on the 13th DAI. In seed invigoration treatments such as hydropriming and treatment with thiourea, germination above MSCS was attained on the 15th and 17th DAI respectively.

6. Within 35 days of extraction, seeds treated with *P f* @ $1x10^6$ cfu.ml^{-1} for 12 hours, kinetin 10 ppm for 12 hours and $CaCl_2$ 50 mM for 24 hours (T13) recorded significantly higher germination per cent, germination index, coefficient of velocity of germination, energy of germination, vigour index-I and vigour index-II and reduced mean time to germination compared to other treatments.

7. As observed earlier, seed treatment with salicylic acid, vinegar and PEG 6000 had also exhibited a negative impact on the seed quality and seedling performance. The performance of seeds in these treatments was inferior to the untreated seeds.

8. Considering the earliness in breaking dormancy as well as the impact on seed quality parameters, invigoration of seeds with 50 mM $CaCl_2$ for 12 hours or 24 hours, or *Pf* for 12 hours, or kinetin 10 ppm for 24 hours can be adjudged the best seed invigoration treatment that can be resorted to break seed dormancy in ash gourd.

2. Effect of seed invigoration treatments on seed viability during storage

1. A general decline in seed quality parameters *viz.*, germination, germination index, coefficient of velocity of germination, energy of germination, vigour index-I and vigour index-II were observed during the period of storage in both treated and untreated seeds.
2. All the seed invigoration treatments exerted a positive influence on extending the period of seed viability while in the untreated seeds, germination was sustained above 60 per cent for two months only.

3. However, seed invigoration with salicylic acid for 12 hours or 24 hours, and those treated with vinegar for 2 hours and PEG 6000 for 24 hours proved to be detrimental.

4. Germination in seeds treated with salicylic acid 60 ppm and vinegar failed to reach MSCS of 60 per cent required for ash gourd even during the storage period. Seeds treated with salicylic acid also failed to germinate after one month of storage (MAS) while although MSCS was reached on 19^{th} DAI in the seeds treated with PEG 6000 (@-0.5 MPa for 24 hours), the germination was poor and not sustained above MSCS beyond 1 month.

5. For most part of storage period, seeds treated with $CaCl_2$ recorded the highest per cent increase in germination. Seed viability was maintained above MSCS for 7 MAS.

6. Germination in the seed treatment with *Pf* or $CaCl_2$ or kinetin for 12 hours had retained above MSCS upto 6 MAS while seed treatment with thiourea, KNO_3, KH_2PO_4 and kinetin were helpful in retaining viability above MSCS for 4 MAS.

7. Negligible to marginal increase in mean time to germination, electrical conductivity of seed leachate, total thickness of seed coat, thickness of testa and tegmen, embryo length to seed length ratio and microbial infection of seed was observed in all treatments over the period of storage, the increase being invariably lower in treated seeds than in untreated seeds. However, performance of seeds treated with salicylic acid 60 ppm, vinegar and PEG 6000 (T16) were poor in comparison to untreated seeds.

8. Treatments with $CaCl_2$ for 24 hours followed by treatment with *Pf* or $CaCl_2$ for 12 hours had also registered high per cent increase in germination, germination index, coefficient of velocity of germination, energy of germination, as well as vigour index I and vigour index II and low mean time to germination, electrical conductivity of seed leachate, total thickness of seed coat, thickness of testa and tegmen, embryo length to seed length ratio and microbial infection of seed for most part of storage period.

9. Considering the higher retention period of viability above MSCS, and higher germination per cent over the storage period, high estimates of germination index, coefficient of velocity of germination, energy of germination, vigour indices I and II and finally lower estimates of mean time to germination, electrical conductivity of seed leachate, total thickness of seed coat, thickness of testa and tegmen, embryo length to seed length ratio and microbial infection of seed, seed treatment with $CaCl_2$ 50 mM for 24 hours could be adjudged as the best seed invigoration in ash gourd. This treatment had broken dormancy at 13 MAI and retained the viability for the maximum period after invigoration (7 MAS).

10. Seed invigoration treatment with *Pf* ($1x10^6$ $cfu.ml^{-1}$) or $CaCl_2$ for 12 hours were found to be the next best treatments that can also be recommended for seed invigoration in ash gourd.

3. Correlation study

1. High and significant positive correlation was found to exist between germination and germination index, coefficient of velocity of germination, vigour indices I and II at all instances while it was significant and negative with total thickness of seed coat and thickness of testa immediately.

2. However, towards the end of storage period (7 MAS), no significant correlation was evident between germination and mean time to germination as well as energy of germination indicating that as seed ages, mean time to germination and energy of germination does not affect germination.

3. Although EC of seed leachate and thickness of tegmen did not exhibit a significant influence on germination, mid-way between storage, towards end of storage, germination was negatively and significantly influenced by these factors.

4. The result indicated that germination index (germination index) was less influenced by energy of germination, vigour index II and EC of seed leachate. The germination index increases with an increase in coefficient of velocity of germination, vigour index I and decreases with a decrease in mean time to germination, thickness of testa, tegmen and total thickness of seed coat.

5. Energy of germination was found to be strongly influenced by germination index, vigour indices I and II, thickness of testa and total seed coat rather than

mean time of germination, coefficient of velocity of germination, EC of leachate and thickness of tegmen

6. An increase in coefficient of velocity of germination also occurred with a decrease in mean time to germination, thickness of testa, tegmen and total seed coat. Coefficient of velocity of germination was found to be positively affected by germination index and vigour index-I.

7. An increase in vigour indices was accompanied by an increase in the germination index, coefficient of velocity of germination, energy of germination as well as a decrease in seedling vigour occurs upon a decrease in EC of seed leachate and thickening of testa, tegmen and total thickness of seed coat.

8. Mean time to germination was found to decrease with an increase in germination index, coefficient of velocity of germination, energy of germination, vigour indices I and II while an increase in mean time to germination was observed when an increase in EC of seed leachate, thickness of testa, tegmen and total seed coat occurred.

9. An increased EC decreased germination only when accompanied with an increase in thickness of testa, tegmen and total seed coat while an increase in EC over storage will be accompanied by a reduction in germination index and vigour indices I and II.

10. No significant correlation was evident between germination and embryo length to seed length ratio and microbial infection of seed throughout the storage period. The non-significant correlation between germination and

microbial infection may be because the seeds were packed at 8 per cent moisture in 400G polyethylene bags.

11. Germination was strongly affected with an increase in thickness of total seed coat or its outer layer-the testa. The thickening of seed coat owing to the thickening of testa was accompanied with an obvious decrease in germination, germination index and coefficient of velocity of germination, vigour indices I and II.

12. Results thus indicated that poor germination in seeds of ash gourd may be due to a water impermeable seed coat (physical dormancy) mainly owing to thickening of outer layer the testa which can be overcome by invigoration treatment for good germination.

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SEED INVIGORATION TO OVERCOME DORMANCY IN ASH GOURD (*Benincasa hispida* (Thunb.) Cogn.)

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ABSTRACT OF THE THESIS

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ABSTRACT

An experiment 'Seed invigoration to overcome dormancy in ash gourd (*Benincasa hispida* (Thunb.) Cogn.),' was conducted during 2014-2016 in the Department of Seed Science and Technology, College of Horticulture, Kerala Agricultural University (KAU), Vellanikkara, Thrissur, following a completely randomized design with 16 treatments and three replications. The experiment aimed to elucidate the effect of seed invigoration on dormancy in ash gourd, to ascertain the anatomical changes in seed coat on seed treatment and to assess the storage potential of treated seeds under ambient conditions. Seed invigoration was resorted to by soaking seeds of variety KAU Local in water (hydro priming) for 24 hours, thiourea (0.5%) for 24 hours, KNO_3 (0.4%) for 24 hours, KNO_3 (0.7%) for 24 hours, KH_2PO_4 (10^{-1} M) for 24 hours, vinegar (pH 3.7) for 2 hours, polyethylene glycol 6000 (-0.5 MPa) for 24 hours, salicylic acid (60 ppm) for 12 hours, salicylic acid (60 ppm) for 24 hours, kinetin (10 ppm) for 12 hours, kinetin (10 ppm) for 24 hours, $CaCl_2$ (50 mM) for 12 hours, $CaCl_2$ (50 mM) for 24 hours, *Psuedomonas fluorescens* ($1x10^6$ cfu.ml^{-1}) for 12 hours, *Psuedomonas fluorescens* ($1x10^6$ cfu.ml^{-1}) for 24 hours. Untreated seeds served as control (T16).

Both treated and untreated seeds, dried to $\leq$ 8 per cent moisture content were packed in polythene bags (400 guage) and stored under ambient conditions upto ten months. The seed quality parameters *viz*., germination, germination index, coefficient of velocity of germination, energy of germination, mean time to germination, vigour indices I and II, and electrical conductivity of seed leachate were recorded at monthly intervals during storage. Seed microflora as well as histochemical studies to analyse the changes occurring in the dimensions of embryo and proportions of different fractions of seed coat were assessed at bimonthly intervals.

Results pointed out the existence of significant differences in the impact of various invigoration treatments on the seed quality and histochemical parameters observed both within 35 days of invigoration as well as during storage.

Majority of the seed invigoration treatments were effective in breaking dormancy in ash gourd. Dormancy was first broken in seeds treated with KH_2PO_4 and kinetin. In the above treatments germination reached above 60 per cent (the minimum standards for seed certification (MSCS) prescribed for ash gourd) on the 11^{th} day after invigoration (DAI). Dormancy in untreated seed was broken only on the 35^{th} day after extraction. However, seed invigoration with salicylic acid, vinegar and PEG 6000, proved to be detrimental.

Seed invigoration also helped in extending the viability of seeds. Germination in seeds treated with $CaCl_2$ for 24 hours, was found to be retained above MSCS for seven months during storage, whereas, in untreated seeds, germination above 60 per cent was observed for two months only. Seeds treated with salicylic acid failed to germinate by 35^{th} DAI while those treated with vinegar and polyethylene glycol (PEG) failed to attain MSCS throughout the period of study. The viability of seeds treated with kinetin or $CaCl_2$ for 12 hours and those treated with *Pf* for either 12 or 24 hours was found to be retained for six months of storage.

Germination index, coefficient of velocity of germination, energy of germination, vigour indices I and II in both treated and untreated seeds, increased by 35^{th} DAI and marginally decreased over the period of storage. In all the invigoration treatments, the above mentioned parameters were invariably higher than that of untreated control. However, the performance of seeds treated with salicylic acid, vinegar and PEG 6000 was lower than the untreated seeds. Invariably, the germination index, coefficient of velocity of germination, energy of germination, vigour indices I and II were high in seeds treated with $CaCl_2$ for 24 hours followed by treatment with *Pf* for 12 hours and $CaCl_2$ for 12 hours. The mean time to germination, electrical conductivity of seed leachate, the thickness of seed coat and its component layers as well as the microbial infection of seed was also observed to be low in these treatments.

High and significant positive correlation was found to exist between germination and speed of germination, coefficient of velocity of germination, vigour indices I and II. It became evident that germination in ash gourd will be strongly affected with an increase in thickness of total seed coat or its outer layer-the testa. The thickening of seed coat owing to the thickening of testa will be accompanied with an obvious decrease in germination, germination index and coefficient of velocity of germination, vigour indices I and II.

Results thus indicated that poor germination in seeds of ash gourd may be due to water impermeable seed coat (physical dormancy) mainly resulting from thickening of the outer layer (testa). Such dormancy in ash gourd can be best overcome by seed invigoration with $CaCl_2$ (50 mM) for 24 hours. In addition, enhancement of seed viability, seed quality and seedling performance over storage was also achieved. Seed treatment with $CaCl_2$ (50 mM) or *Psuedomonas fluorescens* ($1x10^6$ $cfu.ml^{-1}$), for 12 hours were found to be the next best to treatment with $CaCl_2$ (50 mM) for 24 hours. Hence, these can also be recommended for breaking dormancy and maintaining high seed quality in ash gourd.