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# Effect of Surface Microbiome and Osmo-Conditioning on Restoration of Storage-Induced Losses of Seed Viability in Muskmelon (*Cucumis melo* L.)

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## ABSTRACT

**Seed priming can restore age-induced loss of seed viability or longevity; however, these invigoration responses may vary with priming agents and seed aging duration. The present study investigated the effect of two potassium salts (K-salts) individually and in combination (1:1) on different lots of muskmelon genotype, MS-1 seeds stored for four consecutive years, from 2013 to 2016 under ambient conditions. The combination of K-salts significantly enhanced the percent germination of seeds stored for two years (from 2015 to 2016). Further, it also improved root morpho-traits of 10-day-old seedlings. The storage duration had significant effect on the seed surface bacterial and fungal populations. A significantly higher cfu $mL^{-1}$ microbial counts were recorded for 2013 harvested seeds on three different agar-based media compared to 2014 to 2016 stored seeds. Further, the Scanning EM and FT-IR study revealed the surface microbiological status and functional groups variations, respectively. Thus, aging-related seed coat microflora is responsible for deterioration of the seed coat. Osmo-conditioning cannot restore viability of seeds stored under ambient conditions for more than two years.**


**Keywords**: Electron microscopy, Melon, Osmolyte, Seed priming, Storage time, Surface microbes.

## INTRODUCTION

Uniform seedling establishment and optimum plant stand are essential for realization of potential yield, enhanced quality, and profitability of direct seeded crops, such as muskmelon. Rapid and uniform emergence are inherent to seed health and environmental conditions during emergence period. Slow emergence results in weak seedlings and poor crop stand (Marcos-Filho, 2005; Ozden *et al.*, 2018). The extended emergence period predisposes the seedlings to an array of phyto-pathogens (McGee *et al.*, 1980; Lamichhane *et al.*, 2017). Moreover, muskmelon is very sensitive to a variety of abiotic stresses such as drought, salinity, temperature extremes, and biotic stresses such as fungal, viral and insect attacks, particularly during establishment period. Thus, a uniform and vigorous plant stand establishment (Nerson and Govers, 1986; Finch-Savage and Bassel, 2016) is a major challenge of direct seeded muskmelon, specifically under low temperature conditions of early spring in north India. This irregular and delayed seedling emergence in a widely spaced crop such as muskmelon incurs heavy penalties on final yield.

Seed viability and germination potential is also influenced by the diversity and total load of the epiphytic microbiome on the

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surface of the stored seeds (da Costa *et al*., 2013). The seed moisture content and storage temperature considerably affect the phenological conditions for storage associated seed microflora (Francisco and Usberti, 2008). Higher initial seed moisture content enhances the occurrence of attack by *Aspergillus* or *Penicillium*, the storage-associated fungi (Cardoso *et al*., 2004). This microflora invariably contributes to storage-associated seed viability and longevity besides deterioration through seed coat damage and foraging on seed food reserves and the embryo.

'Seed priming', the pre-sowing treatment with osmolytes, improves the seed germination rate, and seedling emergence and uniformity in vegetable crops (Parera and Cantliffe, 1994; Varier *et al*., 2010). Furthermore, seed priming can also improve the ability of the primed seeds to resist or tolerate a variety of abiotic stresses such as temperature extremes, particularly the low temperature stress (Nerson and Gover, 1986; Wang *et al.*, 2016), water stress or drought (Wang *et al*., 2003) and chemical or osmotic stress due to high amounts of salts in soil (Sivritepe *et al*., 2003; Demir and Guloksuz, 2003; Galvan-Ampudia and Testerink, 2011; de Souza *et al*., 2016; Gebreegziabher and Qufa, 2017).

Seed priming involves altered moisture content profile in the primed seeds (Nascimento and West, 1998; Nascimento, 2003). Therefore, it is essential to assess the seed health before priming. Majority of published studies focused on the effect of seed priming on various aspects of seed germination and percent seedling emergence, besides benefits imparted for combating abiotic stresses (Singh *et al.*, 1999). However, there are no specific reports available particularly referring to the role of the storage microflora and its effects in relation to seed osmo-conditioning in muskmelon.

In muskmelon breeding programs, the maintenance of germplasm through years is a very challenging and resource demanding practice. Since melon seeds lose their viability very quickly, accessions in the repository have to be replaced with a fresh seed lot produced through self-pollination after every two-years (Vashisht, 2016; Personal communication). In hybrid seed production, the seeds of parental lines have to be provided every year. Thus, a standard protocol for the storage of muskmelon seed for several years can help to save substantial time and resources of breeders, spent on annual maintenance of parental lines/ germplasm. In this study, muskmelon genotype, MS-1, a male sterile line, which is a female parent of three commercial hybrids of muskmelon, namely, Punjab Hybrid (Nandapuri *et al.*, 1982), Punjab Anmol (Lal *et al*., 2007), and MH-27 (Kaur *et al*., 2017), has been used to assess the differential effect of osmo-conditioning on microflora, percent germination, and seedling root traits of seeds stored for varying durations. Further, the scanning electron microscopy of the seed coat and the infra-red spectroscopy analysis of both coat and cotyledon can provide a valuable method for identification of the extent of microbial damage caused to the stored seeds. The information generated in the current investigation will definitely be useful for academics and researches on muskmelon improvement and seed storage.

## MATERIALS AND METHODS

### Evaluation of Microbiological Status of Muskmelon Seeds

The seeds of muskmelon genotype MS-1 used in the present study were obtained from Department of Vegetable Science, Punjab Agricultural University, Ludhiana, Punjab, India. The samples were drawn from the seed stocks stored at ambient room temperature for four consecutive years (from 2013 to 2016 i.e. Y1 to Y4). . These seeds were evaluated for their surface microbiological status. The average viable cell counts of bacteria and fungi in the wash waters of the seeds were evaluated as described by Prokopowich and Blank

(1991). In brief, the seeds were placed in autoclaved normal saline solution of known volume and stirred for 10 to 15 minutes on a rotary incubator (Remi Cooling Orbital Shaking Incubator, model-CIS-24 Plus, capacity 215 Ltr, Remi Elektrotechnik Limited, Maharashtra, India) at 25±2ºC temperature. The wash water of the seeds was dilution plated on nutrient agar, potato dextrose agar and Rose Bengal agar media and incubated in BOD incubator (Remi BOD Incubator, Remi Elektrotechnik Limited, Maharashtra, India) at 25±2 ºC temperature for 3 to 5 days for the appearance of microbial growth. The experiment was performed in triplicate and the number of bacterial, fungal, and yeast colonies were counted after 5 days of incubation. The seeds were also directly placed on nutrient agar and Rose Bengal agar media and incubated up to 10 days at 25±2ºC temperature. The microbial growth was recorded at 5 and 10 days after incubation.

Fourier Transform Infra-Red (FT-IR) Spectroscopy of Muskmelon Seeds

The FT-IR spectra of the seed samples were collected by using Attenuated Total Reflectance (Smart Miracle assembly) attached on FT-IRS (Thermo Nicolet 6700, Thermo Scientific, USA) for mid-IR region (750 to 4000 $cm^{-1}$ wavenumbers) with 32 scans performed at spectral resolution of 4 $cm^{-1}$. The appropriate correction for the background absorbance was performed by subtracting the spectrum of the empty ATR crystal (Wang *et al*., 2017).

**Osmo-Priming of Muskmelon Seeds**

Osmo-conditioning of the muskmelon seeds was performed with 0.1 M potassium salts; potassium dihydrogen orthophosphate ($KH_2PO_4$) and potassium nitrate ($KNO_3$) alone and in combination of 1:1 ratio. These osmolyte solutions were used at the rate of 10 mL $g^{-1}$ of the seeds to soak for 12 hours in dark at room temperature (25±2ºC). The control seeds were soaked in deionized water for similar period of time. After priming, the seeds were dab dried on autoclaved Whatman filter paper no. 1. The dry seeds were placed on sterilized Whatman filter paper no. 1 in 124 mm sterilized Petri dishes for germination in BOD incubator at 25±2ºC temperature. Twenty-five seeds per treatment were taken for the study in three replicates. The number of germinated seeds were recorded daily according to the seedling evaluation Handbook of Association of Official Seed Analysts (1990) until a constant count was achieved. The root growth parameters *viz*., root length, diameter, surface area, and volume at 10 Days After Treatment (DAT) and number of lateral roots were recorded at both 5 and 10 DAT.

**Scanning Electron Microscopy of Osmo-Primed Seed Surface**

Osmo-primed muskmelon seeds were fixed in 2.5% buffered glutaraldehyde solution for 24 hours followed by secondary fixation in 1% osmium tetraoxide. The secondary fixative was drained and seeds were rinsed with rinsing buffer thrice. Later, the samples were dehydrated in graded series of ethanol (30 to 100%) and sputtered coated in Hitachi E1010 gold ion sputter coater to view at 15 kV acceleration voltage in Hitachi s-3400N Scanning electron microscope (Bozzola and Russell, 1999).

**Root Scanning Studies of Seedlings**

The roots of the 10-days old seedlings were stained with aqueous 1% methylene blue stain by dipping the roots in stain for 5 minutes followed by thorough rinsing in distilled water till no stain appeared in the wash water. Washed root samples were then scanned on a flatbed scanner (Reagent Instruments Inc.; STD 4800, Epson Perfection V700 Photo) at 450 dpi resolution. Root length, Root Surface Area (RSA; $cm^2$), and Average Diameter (AVD;

mm) were determined using WinRhizo software version 2009 (Reagent Instruments Inc., Quebec, Canada) at a 235-threshold value (Sharma *et al*., 2014).

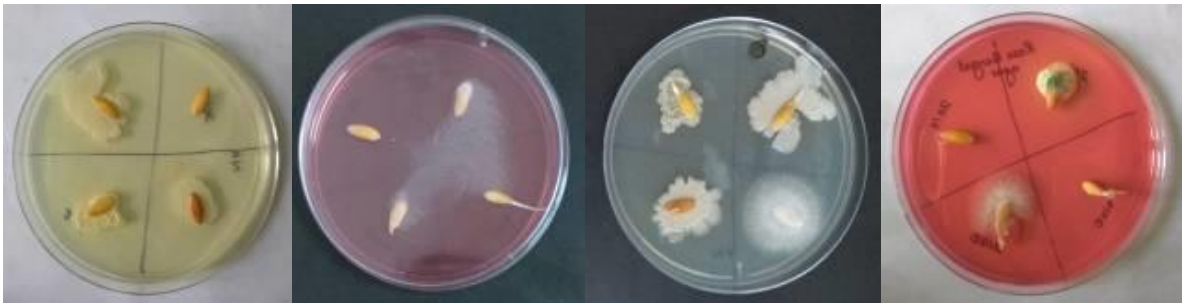
### Statistical Analysis

The data was subjected to analysis of variance using generalized linear model procedures of SAS software (version 9.3, Cary NC, US). All the experiments were carried out in triplicate.

## RESULTS

After placing muskmelon seeds on the surface of the nutrient agar and Rose Bengal agar media, the bacterial colonies appeared after 5 days of incubation while the fungal colonies appeared on RBA after 10 days of incubation (Figure 1). The seed wash water study showed that storage duration had significant effect on the surface microbial load of the muskmelon seeds. A statistically higher number of cfu $mL^{-1}$ of the bacteria ($25.0\pm1.8\times10^5$) and fungi ($10.0\pm0.7$, $8.0\pm0.57\times10^3$) were observed as a function of the time duration of stored seeds (Table 1). However, the seed surface bacterial load was significantly higher in Y4 seeds compared to Y2 and Y3 seeds (Table 1).

The effect of storage time duration on alterations in the occurrence of chemical functional groups of muskmelon seeds was also evaluated through FT-IR spectroscopy. The FT-Mid IR spectra of the outer seed coat of the muskmelon seeds exhibited distinct spectral bands representing specific functional groups with variations in the intensity of peaks (Figure 2-a). The peaks within the range of 3,800 to 3,300 $cm^{-1}$ indicated the stretching vibration modes of hydroxyl functional group on the seed coat surface. These vibrational bands were quite distinct in both 2013 and 2014 stored seeds while the FT-IR spectra of the 2015 and 2016 stored seed coat surface indicated subdued peaks in this region. This variation



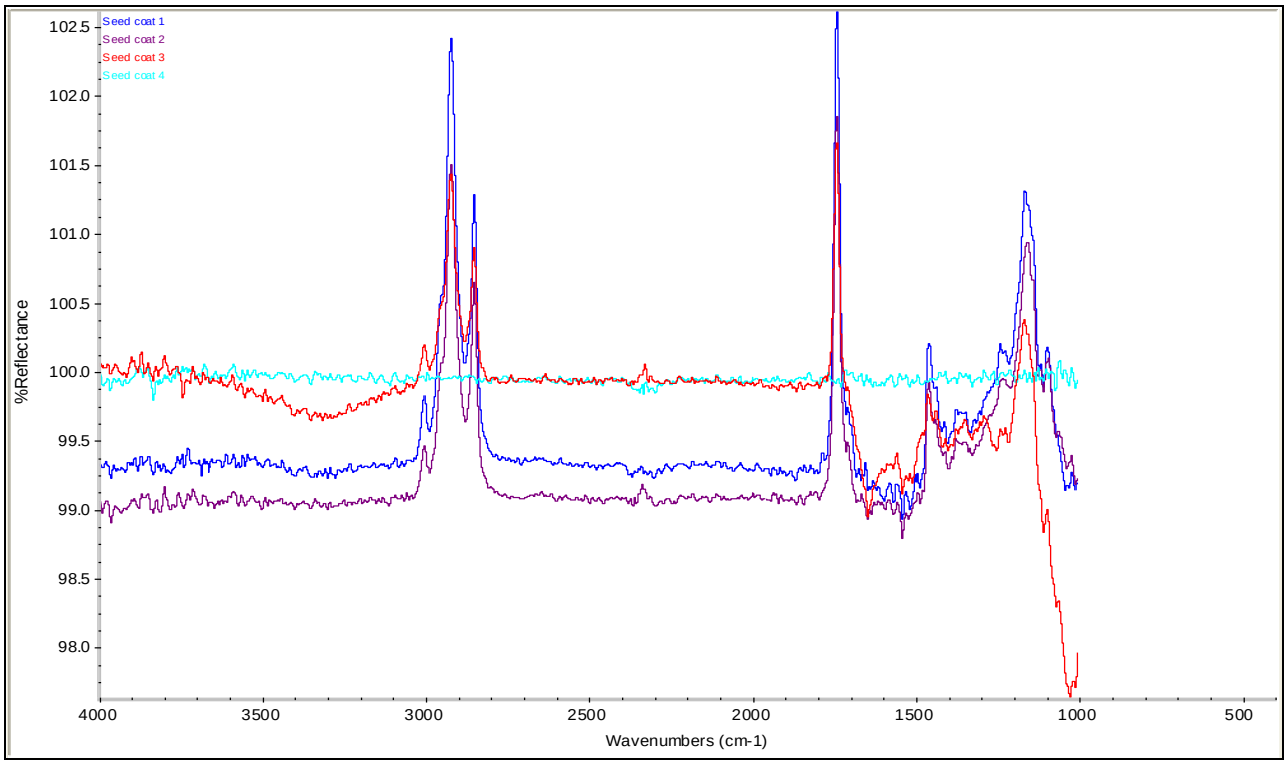
(a) (b)

**Figure 1.** Microbiological status of the surface of whole seeds of muskmelon genotype, MS-1 at 5 and 10 Days After Incubation (DAI) on nutrient agar and Rose Bengal agar. Panel a: 5 DAI, Panel b: 10 DAI. The panel includes the nutrient agar and rose bengal agar media imaged 5 and 10 days after incubation showing the growth of bacteria and fungi.

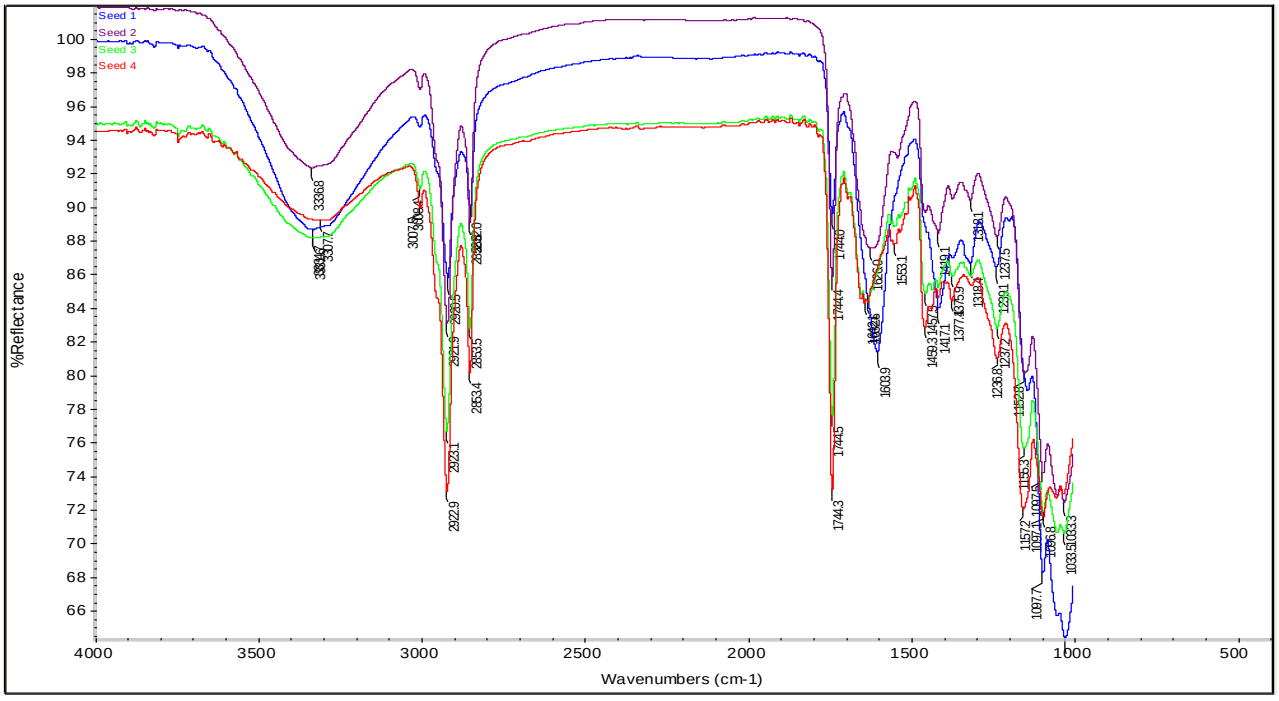
**Table 1**. Effect of storage duration on the surface microbial load (cfu $mL^{-1}$) in wash waters of seeds of muskmelon genotype (MS-1).[a]

| Year | Bacteria ($\times10^5$) | Yeast and molds ($\times10^3$) | |
|---|---|---|---|
| | Nutrient agar | Potato dextrose agar | Rose Bengal agar |
| 2013 | $25.0\pm1.8^a$ | $10.0\pm0.7^a$ | $8.0\pm0.57^a$ |
| 2014 | $7.0\pm0.5^c$ | $2.0\pm0.14^b$ | $1.0\pm0.07^b$ |
| 2015 | $2.0\pm0.1^d$ | $2.0\pm0.14^b$ | $0.0\pm0.0^c$ |
| 2016 | $14.0\pm1.0^b$ | $1.0\pm0.07^c$ | $0.0\pm0.0^c$ |

[a] Means followed by the same letter in a column are not significantly different at $p\leq 0.05$ using Least significant difference method.




(a)




(b)

**Figure 2.** Variabilities in the FT mid-IR spectra of the seed coat (a) and seed cotyledon (b) of *Cucumis melo* var. *reticulates* MS-1.

might be attributed to erosion of the seed coat surface and generation of reactive oxygen species due to heavy contamination of the 2013 and 2014 seeds by different microbes (Shetty *et al.*, 2008). Further, the vibrational peaks within a range of 3,000 to 2,800 $cm^{-1}$ indicated the aliphatic C-H stretching modes for fatty acids. These peaks indicate the decay of the seed coat and contamination of the fatty acids derived from the seed cotyledons. Contrarily, the seed coat of the 2015 and 2016 stored seed sample exhibited none of these vibrational peaks.

Muskmelon seed cotyledons were also evaluated with FT-IR spectroscopy. The spectra indicated the characteristic peaks for hydroxyl, aliphatic C-H bonds in fatty acids and protein amide I and II bands (Figure 2-b). As the muskmelon seed cotyledon contains high amount of fat components (Milovanovic and Pićurić-Jovanović, 2005; Petkova and Antova, 2015), the characteristic peak at 1,744 $cm^{-1}$ indicated the stretching vibrations of the C=O functional group of lipids, which was observed in all the cotyledons. The more prominent absorption peaks in the region 1,200 to 1,000 $cm^{-1}$ wavenumbers for the 2013 and 2014 stored seeds indicated formation of free reducing sugars and thus the loss or deterioration of the seed cotyledon reserves. Moreover, the peaks pertaining to 1,750 to 1,600 $cm^{-1}$ further indicated changes in the protein structure helix and beta-sheet among the 2013-2014 and 2015-2016 stored seeds. Therefore, storage under ambient conditions affected not only the seed coat but also the seed cotyledon nutritional contents.

The Scanning EM study of the seed surface further accentuated the occurrence of fungal hyphae and bacterial cell debris in 2013 and 2014 stored seeds. However, the 2015 and 2016 seed surface showed a greater number of bacterial cells than the presence of fungal hyphae (Figure 3). This indicated that fungal population of the seed surface had greater detrimental effects than the bacterial load. Further, osmo-priming cannot revert the detrimental effects of microbial attack on the seed coat. The SEM analysis of the surface of the osmo-primed muskmelon seeds showed variation in the surface topography and the exposed pores for the imbibition of water by the muskmelon seeds (Figure 4). The 2016 harvested seed surface showed presence of increased number of surface pores and cracks through which water can move inside. However, the surface of the three- and four-year-old seeds showed presence of debris and deposits, desiccation and crystallization of the surface proteins. Among the osmolyte treatments, the maximum number of pores were observed in seeds that were treated with potassium nitrate alone and in $KNO_3$+$KH_2PO_4$ combination.

The application of osmolytes i.e. potassium nitrate or potassium dihydrogen phosphate alone resulted in root growth similar to the distilled water alone i.e. hydropriming treatment for 2015 and 2016 seeds (Figure 5). However, an additive and statistically significant effect on root growth was observed on combined treatment of the osmolytes. The osmo-priming treatment enhanced several root parameters. The root length, volume, and surface area increased linearly in four treatments *viz*., distil water, 0.1M $KH_2PO_4$, 0.1M $KNO_3$ and $KH_2PO_4$:$KNO_3$ (1:1) for the year 2016 harvested muskmelon seeds (Table 2). However, priming with 0.1M $KH_2PO_4$ resulted in a decrease in all the four test root parameters in year 2015 harvested muskmelon seeds w.r.t. hydro primed (distilled water treatment) control treatment. The combination treatment resulted in the highest values for the abovementioned three root morphometric traits for both years, though a significant increase in abovementioned root parameters was observed for 0.1M $KNO_3$ treatment for 2016 harvested seeds. A significant decrease in root diameter was also recorded in 2016 harvested seeds, representing formation of thinner or fine roots. (Table3)


**Figure 3**. Scanning electron microscopy of the seeds of muskmelon genotype, MS-1 treated with osmo-salts showing occurrence of fungal hyphae ramifying the shrivelled and desiccated outer coat surface of 2013 and 2014 seeds. Y1 to Y4: 2013 to 2016 (years), T2 to T4 [T2=$KH_2PO_4$, T3= $KNO_3$, T4= $KH_2PO_4$+$KNO_3$ (1:1 ratio)]. White arrow indicates the presence of fungal hyphae on the seed coat surface.

**Table 3.** Effect of priming with potassium salt solutions on number of lateral roots per seedling of *Cucumis melo* var. *reticulates* MS-1.[a]

| Year | Treatment | Number of lateral roots per plant | |
|---|---|---|---|
| | | 5 DAT | 10 DAT |
| 2015 | T1 | 0 | 1.8±0.13[b] |
| | T2 | 0 | 0[c] |
| | T3 | 0 | 0[c] |
| | T4 | 0 | 3.4±0.24[a] |
| 2016 | T1 | 0.4±0.03[c] | 0.4±0.03[a] |
| | T2 | 0.4±0.03[c] | 0.4±0.03[a] |
| | T3 | 1.6±0.1[b] | 2.6±0.18[b] |
| | T4 | 4.6±0.33[a] | 6.4±0.45[c] |

[a] Data followed by the same letter in a column are not significantly different at P≤ 0.05. T1= Distilled water, T2= $K_2HPO_4$, T3= $KNO_3$, T4= $K_2HPO_4$+$KNO_3$ (1:1 ratio), DAT= Days After Treatment.

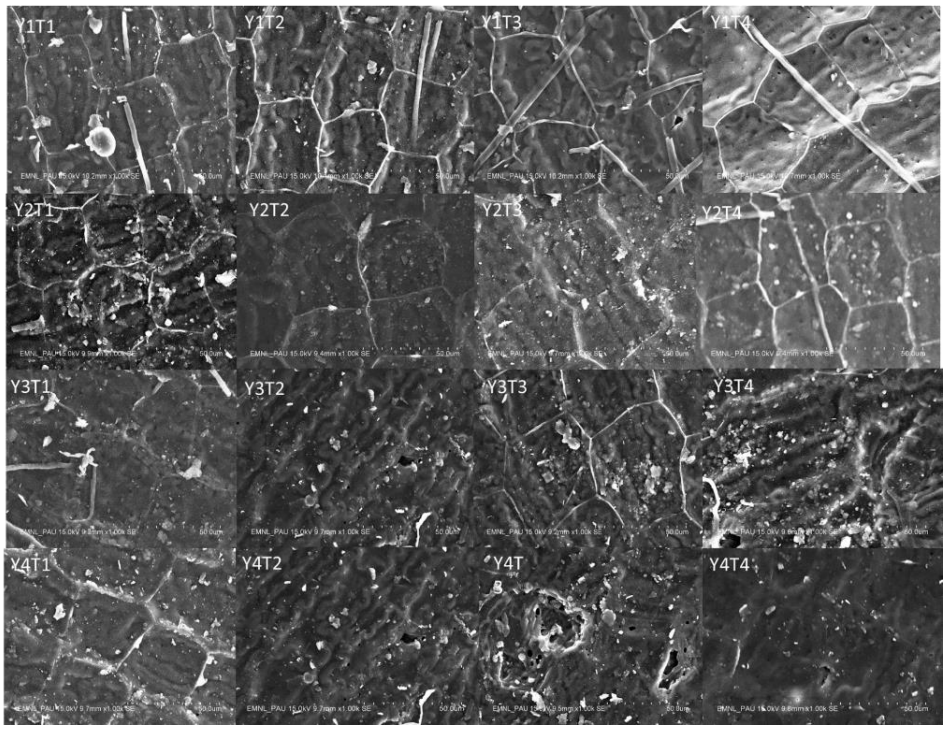
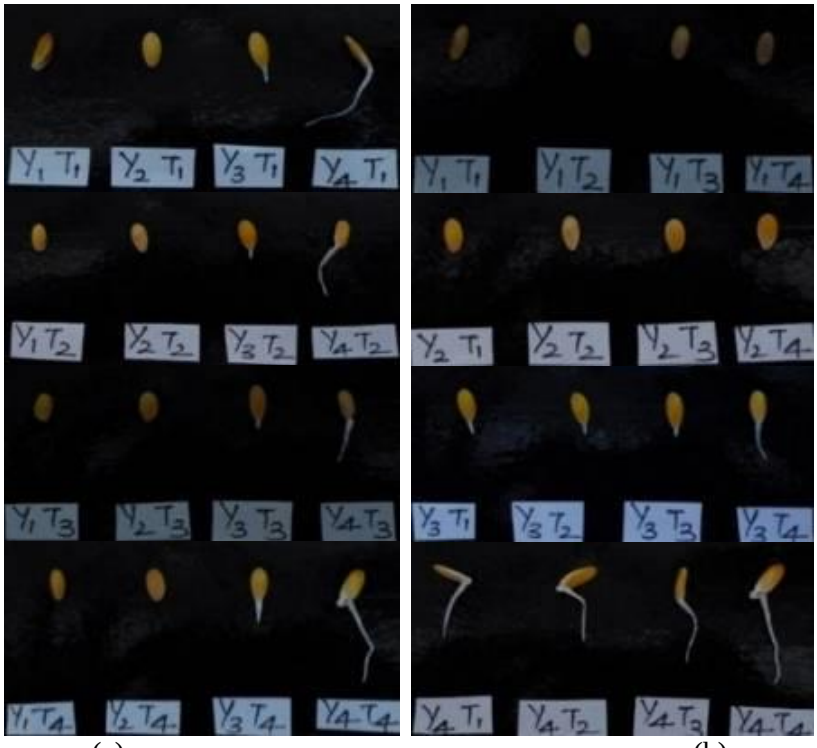



**Figure 4**. Effect of osmo-conditioning on seed coat surface hydration characteristics and surface topography of muskmelon genotype, MS-1. Y and T symbols are defined under Figure 3.



(a) (b)

**Figure 5**. Effect of osmopriming on seeds of muskmelon genotype, MS-1 harvested in four consecutive years 5 days after treatment. Panel A compares four years per treatment and panel B compares the treatment among single year seeds. Y and T symbols are defined under Figure 3.

## DISCUSSION

The microbiological status of stored seed is governed by two major factors: storage duration and temperature during storage, which are quite critical for germination in muskmelon (Bankole *et al*., 2005; Adeleke *et al*., 2012). The seed surface microbial diversity encompasses seed borne phyto-pathogens, contamination by human pathogenic microbes during handling/ processing and storage stages and thus the 'surrogate microbiota' acquired during the post-harvest storage period. The common seed-borne pathogens include *Didymella bryoniae* / (fungal gummy stem blight (Sudisha *et al*., 2006), *Colletotrichum orbiculare* /fungal anthracnose (Dean *et al*., 2012), and *Acidovor axavenae* subsp. *Citrulli*/bacterial fruit blotch (Bahar *et al*., 2009). However, during storage, melon seeds may be deteriorated by occurrence and secretions of molds such as *Aspergillus* and *Penicillium* (Chiejina, 2006).

Seed surface wash water study revealed the occurrence of a variety of bacteria. Similar results have also been reported by Kim *et al.* (2012) on the seed surface wash water analysis of alfalfa and rapeseed samples. Another report showed presence of different fungi that belong to genera of *Aspergillus*, *Fusarium*, *Rhizopus* and *Burgoa* (Adeleke *et al*., 2012) on surface of the stored melon seeds, which is in concurrence to the results of the present study. However, the presence of a significantly greater count of molds or filamentous fungi on seed surface of 2013 stored seeds may have resulted in decreased percent germination of the stored seeds owing to deterioration of these seeds and production of toxic metabolites (Basra *et al*., 2000; Adeleke *et al*., 2012).

On functional group characterization of seed coat and cotyledon of these samples, variable FT-IR spectra were obtained for both the outer surface of the seed coat and the cotyledon samples. The 2013-14 seed coat FT-IR spectra exhibited the marked spectra indicating hydroxyl group predominance, which may be attributed to moisture absorbing cellulosic seed coat of muskmelon. As the fundamental components of the muskmelon seed coat include the long chain aliphatic compounds (lingo-cellulose rich), the characteristic absorption peaks at 2925.0, 2852.2 and 1426.0 $cm^{-1}$ indicated the symmetric and asymmetric C–H stretching and bending vibrations of $CH_3$ and $CH_2$ functional groups, respectively (Fasasi *et al*., 2015; Nyakuma *et al*., 2016). The specific peaks in the region of 1234 to 1235 $cm^{-1}$ wavenumbers indicated the C–O stretching vibrations for the lignin and xylan compounds for the seed coats of 2013 and 2014 stored seeds (Turker-Kayam and Huck, 2017). These peaks get subdued in FT-IR spectra of the 2015 and 2016 stored seeds. Further, the intensity of the peaks that appeared in the FT-IR spectra varied among the seed samples. Similar observation of variability in the peak intensities has been reported in ATR-FTIR spectroscopy analysis of plasma treated cotton seeds (Wang *et al*., 2017). The variations in the intensity of the spectral peaks may be attributed to enhanced seed damage due to higher number of fungal contaminants leading to generation of reactive oxygen species and hydroxyl ions (Wang *et al*., 2017).

Due to lipid rich nature of the seed cotyledon, the FT-IR spectra obtained from all four samples indicated a characteristic absorption peak for fatty acid acyl group at 1,744.0 $cm^{-1}$ (Lu *et al*., 2011; Angaye and Inengite, 2018). Also, the absorption peak at 1,632.0 $cm^{-1}$ in 2016 stored seed cotyledon showed the occurrence of intramolecular β-sheets as secondary structure for the majority of proteins (Fasasi *et al*., 2015). However, the other seed cotyledon FT-IR spectra did not show this peak, indicating the alterations in the protein secondary structures (Fasasi *et al*., 2015). Altered protein secondary structures can, therefore, moderately to severely affect their functional properties.

The SEM of the seed coat surface of melon seeds stored for four years duration indicated that the loss of the seed viability and germination potential was due to variation in the surface topography, seed coat pores and fissures, and microflora inhabiting the seed surface. In addition, there occurred a variation among the osmo-priming treatments evaluated in the study. A similar observation indicated modification of the topography of the test seeds with enhanced appearance of micro-depression and fissures on priming in SE micrographs due to priming of the seed coat surface of *Trifolium repens* (Galhaut *et al*., 2014). Likewise, cold stratification and priming treatment with different concentrations of gibberellic acid of tallow tree (*Triadica sebifera* L.) seeds significantly enhanced the germination due to topological and chemical changes that occurred in the seed coat surface (Li *et al*., 2012). Another report on zinc sulphate (0.5% w/v) priming of onion seeds showed rapid emergence of radicle due to elongation of the seed coat surface (Saranya *et al*., 2017). Therefore, priming can induce changes on the surface of the seed coat. The surface topography of the seed coat, particularly the seed coat indentations and surface ribbings, also play a substantial role (Lopez‐Ribera and Vicient, 2017). A change in the appearance of the indentations on the seed coat occurred due to cavitation force generated due to ultrasonic wave treatment of *Arabidopsis* seeds and altered water imbibition and germination potential of the seeds (LopezRibera and Vicient, 2017).

Osmo-treatment of the muskmelon seeds stored for more than two years, i.e. 2013 and 2014, exhibited no response. However, the 2015 and 2016 stored seeds exhibited enhanced root growth on osmo-priming treatment. This variation in response may be attributed to the microbiological status of the stored seeds. Similar results for increase in root growth by osmo-treatment have been reported by Nerson and Govers (1986). The root length was maximum on K-salt combination (1:1) for the 2015 and 2016 stored seeds. Likewise, enhancement and additive effect of application of potassium nitrate and potassium dihydrogen phosphate on muskmelon seeds has also been reported by Nerson and Govers (1986).

## CONCLUSIONS

The storage duration exhibited a significant impact on seed surface bacterial and fungal populations, which increased as a function of storage time and caused a gradual deterioration of seed health. The FT-IR spectroscopy of the seed coat surface and the cotyledon also indicated the decline of seed health triggered by the microbial attack. The SEM analysis of the seed surface revealed positive effect of osmo-priming on hydration and outer spermosphere microbiota. The combined treatment of the two potassium osmolytes invariably enhanced the seed germination, besides increasing the root morpho-metric traits in muskmelon genotype, MS-1 seeds stored at ambient temperature for up to two years duration. Thus, high incidence of microorganisms can reduce the benefits of osmo-priming. Therefore, osmo-conditioning can be useful to ensure rapid germination and uniform seedling emergence of two-year stored seeds of muskmelon genotype, MS-1.

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## اثر میکروبیوم سطحی و آماده سازی اسمزی بر ترمیم صدمات ناشی از انبارداری روی زیوایی بذر طالبی (*Cucumis melo* L.)

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### چکیده

آماده سازی بذر می تواند صدمات به زیوایی یا ماندگاری بذر را که ناشی از سالخوردگی باشد ترمیم کند، با این همه، واکنش های تقویتی ممکن است بسته به عامل آماده سازی ( priming agents) و طول دوره سالخوردگی بذر تغییر کند. در پژوهش حاضر، اثر دو نمک پتاسیم (K-salt) به طور مجزا و با همدیگر به نسبت (۱:۱) روی انباشته های مختلف از بذر ژنوتیپ طالبی MS-1 که در ۴ سال پیاپی درسالهای ۲۰۱۳ تا ۲۰۱۶ در شرایط محیطی موجود (ambient) انبار شده بود بررسی شد. تیمار ترکیبی K-salt به طور معناداری در صد جوانه زنی بذر های انبارشده در دو سال (از ۲۰۱۵ تا ۲۰۱۶ )را بهبود بخشید. همچنین، این تیمار صفات شکلی ریشه گیاهچه ده روزه را بهتر کرد. طول دوره انبارداری بر جمعیت باکتری ها و قارچ های روی سطح بذر تاثیر معناداری داشت. نیز، شمارش میکروبی بر حسب $cfu\ mL^{-1}$برای بذر های برداشت شده در۲۰۱۳ که روی سه بستر متفاوت مبتنی بر آگار بودند به طور معناداری بیشتراز بذر های انبار شده ۲۰۱۴ تا ۲۰۱۶ بود. افزون بر این، اسکن EM و آزمون FT-IRبه ترتیب موقعیت میکروبیولوژیکی سطح (بذر) و تغییرات گروه های عامل (functional groups) را آشکار کرد . به این قرار، میکروفلورای پوسته بذر که پدیدآمده از سالخوردگی است مسول تخریب پوسته بذر می باشد. نتیجه دیگر اینکه، آماده سازی-اسمزی نمی تواند زیوایی(viability) بذر های انبارشده در شرایط محیطی موجود را که بیشتر از دوسال انبار شده اند ترمیم کند.