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# POSSIBLE ROLE OF MUCILAGE IN DESICCATION AND GERMINATION OF CITRUS ROOTSTOCK SEEDS

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Studies were conducted to assess the role of mucilage in seed desiccation, germination, and its interaction with fungus in Rough lemon, Sachtion citrumelo and Yuma citrange rootstock seeds of citrus fruits. Water loss percentage was minimum (with or without mucilage) in Rough lemon seeds (20.28%) and (31.25%) at 25°C and 35°C respectively, with high germination percentage (3.33%) while maximum water loss (29.50%) was observed in Sachtion citrumelo at 25°C having low germination percentage (1.33%). However, Yuma citrange showed maximum water loss (34.39%) at 35°C with germination percentage (1.67%). The pathogenic fungus which infected the seeds was isolated, purified and identified as *Aspergillus niger*. Fungus growth on seeds of Rough lemon, Sachtion citrumelo and Yuma citrange was 14.08 mm, 10.65 mm and 9.36 mm, respectively on 6$^{th}$ day of inoculation. It is obvious from the results that mucilage played significant role in desiccation, germination as well as fungal habitation.
**Keywords:** Citrus rootstocks, dehydration, seed storage, seed pathogen

## INTRODUCTION

Seed banks play an important role in the conservation of plant diversity (Wagner and Mitschunas, 2008). Seed longevity is the major challenge for the conservation of plant biodiversity (Rajjou and Debeaujon, 2008) because seeds behaviour in response to dehydration and storage is different (Masetto *et al*., 2008). Major factors that contribute in seed deterioration during storage are seed ageing, moisture contents, temperature and microflora (McDonald, 2004). Usually seeds deteriorate during storage, lose vigour, become more sensitive to stresses during germination and ultimately lose ability to germinate (Rajjou and Debeaujon, 2008).

Moisture content of the seed is the single most important factor controlling the rate of deterioration (Kulkarni, 2004). The viability of citrus seeds is adversely affected by desiccation (King *et al*., 1981) so drying of citrus seeds caused considerable delay in radicle emergence (Doijode, 2002). Seed coat also influences the seed germination (King and Roberts, 1980). In seeds, the outer seed coat or testa is covered by slippery material known as mucilage. Mucilage is produced by the developing seed and deposited in the outer cell layer of the seed coat. It is generated from pectin-rich pellicles on the testa or pericarp layers of one seeded fruits or seed (Huang *et al.,* 2007). Like the seed coat itself, the mucilage is believed to be important for many reasons and it is thought that mucilage plays role in seed germination and dispersal (Windsor *et al*., 2000; Penfield *et al*., 2001). Along with all characteristics, mucilage is also a site of choice for fungal attack because it is a pectin polysaccharide; a compound carbohydrate that may fulfill the nutrition requirement of fungus. Pathogenic fungi may attack on any part of the life cycle; seed survival, germination, pre-emergence growth and post-emergence establishment (Kirkpatrick and Bazzaz, 1979). Much evidences are listed on the role of fungus-induced mortality in seeds (Wagner and Mitschunas, 2008), both seed-borne and soil borne fungi leads to deterioration of seeds from seed bank (Kiewnick, 1964). The objective of present study was to explore the role of mucilage in desiccation and germination of citrus rootstock seeds.

## MATERIALS AND METHODS

Healthy and mature fruits of different citrus rootstocks i.e. Rough lemon (*Citrus jambheri* Lush), Sachtion citrumelo (*Citrus paradisi* x *Poncirus trifoliata*) and Yuma citrange (*Poncirus trifoliata* × *Citrus sinensis*) were obtained from Experimental Fruit Orchard Sq.# 9, Institute of Horticultural Sciences, University of Agriculture, Faisalabad-Pakistan. The fruits were stored at 4±1°C until use.

The method was adopted for seed desiccation studies as described by Khan *et al.* (2003). Fruits were cut into two halves with a sterile knife and seeds were collected over sieves. A lot of 60 seeds (20 seeds replicated 3 times) from each rootstock fruit was employed for assessment of desiccation studies. A second lot of 60 seeds from each rootstock fruit were separated under air laminar flow hood cabinet (Dalton, Japan) as seeds with mucilage. Similarly, the same quantity was washed thoroughly and sterilized with 0.1% mercuric chloride for one minute and finally washed several times in running tap water to treat seeds as without mucilage.

Desiccation (weight loss) percentage of seeds of citrus rootstocks, with mucilage and without mucilage was studied at constant temperatures of 25°C and 35°C in an incubator (ISUZU, Japan). The seeds were weighed daily until constant weight. After 12 days, these seeds with and without mucilage were shifted to growth room at 27±2°C and germination data were recorded upto 60 days.

The fungus was stored at 4±1°C. Twenty seeds (5 seeds replicated 4 times) from each rootstock fruit were employed for assessment of fungus. The identified fungus was inoculated on seeds with mucilage and without mucilage to observe growth pattern of fungus. The inoculated seeds were placed in an incubator at 20±2°C (Neergaard, 1979). Data on fungus growth were collected daily upto 6 days.

The experiment was laid out in complete randomized design (CRD). Data were assessed by analysis of variance (ANOVA) and least significance difference (LSD) test at 5% probability (Steel *et al.*, 1997).

## RESULTS

There was a significant ($P<0.05$) difference in water loss (%) among Sachtion citrumelo and both of the rest citrus rootstocks. At 25°C, maximum water loss was 29.50% in Sachtion citrumelo seeds with mucilage followed by Yuma citrange (24.42%) and Rough lemon (20.80%) (Fig. 1a). The moisture accelerated as the temperature increased from 25°C to 35°C. Maximum water loss (34.39%) was observed in seed with mucilage of Yuma citrange followed by Sachtion citrumelo (32.08%). The lowest water loss (31.25%) was noted in seed of Rough lemon with mucilage (Fig. 1a).

On the other hand, maximum water loss (32.47%) was observed in seeds of Yuma citrange without mucilage followed by Sachtion citrumelo (31.11%) and Rough lemon (31.03%) stored at 25°C (Fig. 1b). The high temperature (35°C) again affected on seed moisture contents without mucilage. Maximum water loss (40.01%) was observed in seeds of Sachtion citrumelo without mucilage followed by Yuma citrange (37.30%) on final day at 35°C (Fig. 1b).

Highly significant ($P<0.05$) difference was found regarding germination and fungal infection among various citrus rootstocks (Fig. 2a, b). Desiccated seeds without mucilage showed maximium (10%) germinability in Rough lemon followed by Yuma citrange (3.33%) and Sachtion citrumelo (3.00%) (Fig. 2b).

During germination test, maximum infection was found on Sachtion citrumelo, followed by Yuma citrange and Rough lemon (Fig. 2a, b).

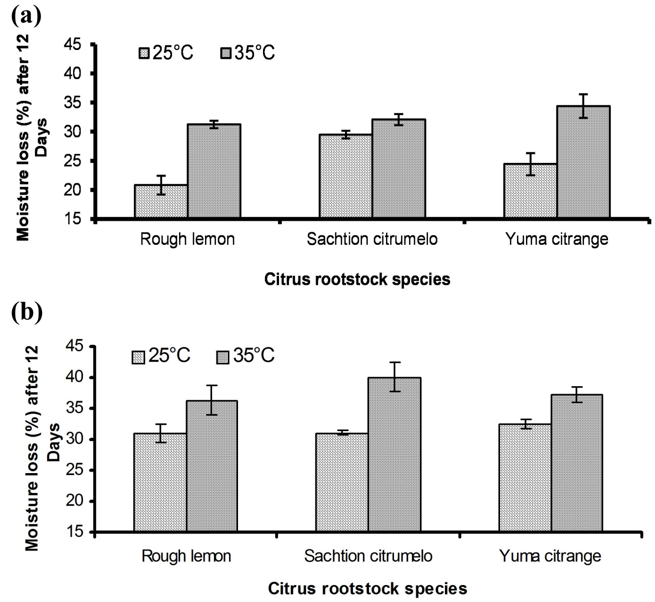



**Figure 1. Percentage moisture loss (Mean±SD) from seeds of Rough lemon, Sachtion citrumelo and Yuma citrange (a) with and (b) without mucilage, MC*=Moisture content at 25°C at 0 day; MC*=Moisture content at 35°C, at 0 day.**

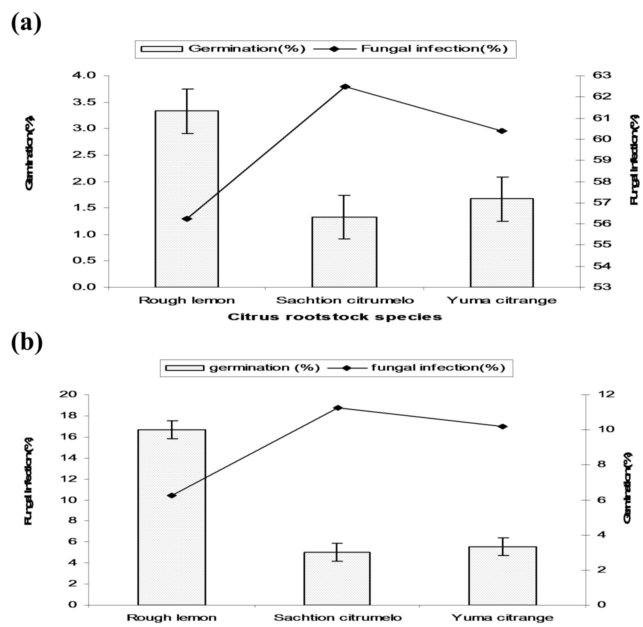
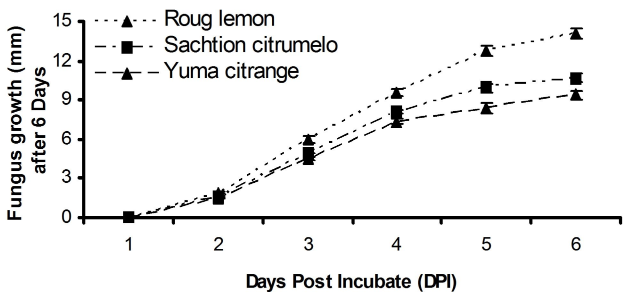



**Figure 2. Percentage germination (Mean±SD) of desiccated seeds of Rough lemon, Sachtion citrumelo and Yuma citrange (a) with and (b) without mucilage with fungal infection observed during germination.**

Maximum fungus growth (14.08 mm) was observed on seeds of Rough lemon with mucilage followed by Sachtion citrumelo (10.65 mm) (Fig. 3a). On the other hand, seeds without mucilage showed maximum fungus growth (5.89 mm) on seeds of Rough lemon and minimum (5.20 mm) in Sachtion citrumelo. However, there was non-significant ($P<0.05$) difference in fungus growth on seeds of all the three rootstocks without mucilage (Fig. 3a, b).

**(a)**

$LSD_{0.05} = 0.2350$




**(b)**

$LSD_{0.05} = 0.2350$

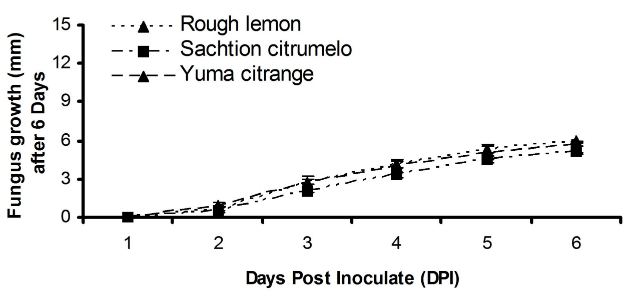



**Figure 3. Diameter of fungus growth (mm) (Mean±SD) on seeds of the Rough lemon, Sachtion citrumelo and Yuma citrange (a) with and (b) without mucilage.**

At the end of the 6th day, seeds of all the three rootstocks without mucilage showed better results against the fungal invasion as compared to that having mucilage.

## DISCUSSION

Desiccation (%) was less in seeds of Rough lemon, Sachtion citrumelo and Yuma citrange with mucilage stored at 25°C or 35°C in respect to those seeds without mucilage on the same storage temperature. Previous reports confirm that this is due to mucilage because it can enlarge the area of contact of seed with substrate, increasing the moisture supply to the seed and retarding loss to the atmosphere. It is reported that high moisture and temperature effect on the seed viability and ultimately leads to deterioration (Doijode, 2002; McDonald, 2004) and desiccation sensitivity of citrus seeds is the major factor in the loss of vigour and causing poor storability (Saipari *et al*., 1998). King *et al*. (1981) reported that citrus are killed by drying because desiccation slows down the germination rate.

Due to more water loss (%) in seeds of Sachtion citrumelo and Yuma citrange with mucilage, less germination was observed, it is because of resistance to the entry of oxygen offered by the layer of mucilage around seeds and the consumption of oxygen by seed coat itself (Bewley and Black, 1985). The results are similar to the findings of Normah *et al*. (1997) and Hong *et al*. (2000) who reported that low moisture content significantly affects the germination. Germination percentage of all the citrus rootstocks without mucilage was higher than that seeds having mucilage. It might be due to infection observed on such seeds having mucilage because more fungal infection was noted on such seeds during germination. Mucilage itself plays a vital role to conserve the moisture and high moisture is favorable for pathogen growth. Reduction in seed germination and seedling mortality by fungal infection was also noted It is also reported in citrus seeds that drying slows down the rate of seed germination (Khan *et al*., 2002). Similarly, *Zizania* seeds water loss declined seed viability and germination (Horne and Kahn, 2000). But according to Hong *et al.*(2005), failure of the dry seeds of *Sterculia foetida* to germinate was not due to desiccation whereas it was due to cracking responsible to fungal infection upon rehydration. Kirkpatrick and Bazzaz (1979) reported that pathogenic fungi may attack at any part of the life cycle: seed survival, germination, survival to maturity and production of seed. Infection may either kill the plant directly or reduce its competitive ability. Overall, the pathogen infection influences the germination capacity of the seed.

More fungal growth rate on the seeds of all the rootstocks having mucilage might be due to the presence of mucilage's because these are the hair like fibrils, which can enlarge the area of contact with substrate, increasing the moisture supply to the seed. So, there is more chance of pathogen infection due to high moisture and mucilage. In other words, mucilage itself, play a vital role to fulfill the nutritional requirement of pathogen. Lilly and Barnet (1951) reported that carbohydrates are major sources of energy and contribute to new protoplasm of the fungus and different organisms have different likings for these compounds.

To wrap up, it is believed that mucilage, desiccation and fungal infection play an important role in citrus seed life. Mucilage has water retention ability which supports the seeds to save from desiccation and increase its germination capacity but due to high moisture supply and sugars, mucilage is the choiced place for fungi growth which leads to seed deterioration.

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