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Effect of scarification on biochemical activities of aged seeds of guava (*Psidium guajava*)

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ABSTRACT

The study was carried out on fresh, one, two and three years aged seeds of guava (*Psidium guajava* L.) L-49 (Sardar) in the laboratory of Department of Seed Science and Technology, CCS HAU, Hisar, during 2018-2019. The seeds were subjected to different seed scarification treatments, *viz.* hot water treatment at 80°C for 1 minute, concentrated sulphuric acid for quick dip and water soaking for 48 hr. The seeds were assessed for peroxidase activity, dehydrogenase activity and electrical conductivity. Hot water treatment at 80°C for 1 minute resulted into maximum peroxidase activity (710.0) and dehydrogenase activity (0.233) with minimum electrical conductivity (11.02). It is concluded that seeds treated with hot water treatment at 80°C for 1 minute showed maximum peroxidase, dehydrogenase activities and minimum electrical conductivity which reflects better seed quality.

Key Words: Seed quality, Scarification, Peroxidase, Dehydrogenase**,** Electrical conductivity

Guava (*Psidium guajava* L.) belonging to family *Myrtaceae,* is fifth most important fruit crop of India (Saxena and Rao, 2017). It is a rich source of vitamin C and fair source of calcium, phosphorous, roughage and is ideal fruit for nutritional security (Bairwa *et al*., 2020). Guava seeds exhibit inconsistent germination with diminished or poor germination under normal conditions. The particular type of dormancy responsible for this has not been thoroughly investigated and understood. Physical dormancy due to hard seed coat is probable cause of poor germination. However, chemical and mechanical scarification can hasten the imbibition of water by making hard seeds permeable and undergo germination. Any deficiency of water during germination strongly limits seed germination (Cavallaro *et al*. 2014; Lamichhane *et al*. 2018). Although scarification method helps to break seed dormancy and improves germination (Maldonado-Arciniegas *et al*., 2018). The gibberellins may also release seed dormancy (Kalyani *et al*., 2014). However, very little information is available on scarification, hence an experiment was conducted.

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MATERIALS AND METHODS

The experiment was conducted on four seed lots, viz. fresh, one, two and three years old seed lots of L-49 (Sardar) at laboratory of Seed science and Technology Department, CCS HAU, Hisar, during 2018-2019.The seeds collected from ripe fruits, were extracted and completely cleaned, dried and stored at room temperature. The seeds were subjected to hot water treatment at 80˚C for 1 minute, quick dip in sulphuric acid and water soaking for 48 hr. The seed quality was assessed through biochemical seed quality parameters, *viz.* peroxidase activity**,** dehydrogenase activity and electrical conductivity test. Peroxidase activity (µmol/gmDw) was determined as per Shannon *et al.* (1966), following oxidation of O-dianisidine in presence of hydrogen peroxide (H_2O_2). The 2.0 ml of acetate buffer (pH 4.5) and 0.1 ml of O-dianisidine solution was added to 0.05 ml of enzyme extract. To initiate the reaction 0.1 ml of 0.2 M hydrogen peroxide was also added.

The observation were recorded at 470 nm wavelength after every 15 second for 1 minute and enzyme unit was expressed as the amount of enzyme required to bring about a change in absorbance of 0.01 per minute. In dehydrogenase activity test (O.D.), the basic principle for topographical tetrazolium test for seed viability is reduction of 2, 3, 5-triphenyl tetrazolium chloride to red formazan by dehydrogenase enzyme in seed embryo. For seeds of similar viability, it is a quantitative method which is used to determine varying dehydrogenase activity and therefore, it is measure of seed vigour. Sample of 2 g seed of in three replications were ground and passed through a 20 mesh screen. The 5 ml of 1% tetrazolium solution was used to absorb 200 mg flour for 3-4 h at 38^{o}C. Then centrifugation was done at 10,000 rpm for 3 minutes and supernatant was discarded.

The 10 ml acetone was used to extract formazan for 16 hr, followed by centrifugation and spectrophotometer was used to determine the absorbance of solution at 480 nm. These observations were expressed in optical density. For estimation of electrical conductivity (µS/cm/sample), three replications of 25 normal seeds were immersed in 75 ml distilled water in 100 ml beakers. Seeds were soaked fully in water and aluminium foil was used to cover the beakers. These samples were put at 25±1^{0}C for duration of 24 hr. Then conductivity meter was used to measure the reading of EC of seed leachates and expressed in µS/cm/seed (AOSA, 1983). The analysis was done with the software OPSTAT available on www.hau.ac.in.

RESULTS AND DISCUSSION

The activities of dehydrogenase test (O D g^{-1} ml^{-1}) and peroxidase (mg $protein^{-1}min^{-1}$) decreased, whereas electrical conductivity of seed leachates increased with advancement of ageing period. The maximum peroxidase activity (710.0) was observed in hot water treatment at 80° C for 1 minute in fresh seed lot. Hot water treatment resulted into maximum peroxidase activity (525.0) among all treatments, followed by water soaking for 48 hr (475.2). Hot water treatment and water soaking of seed for 48 h and control differed significantly with each other and minimum peroxidase activity was observed in seeds quick dipped in concentrated sulphuric acid (35.15). Fresh seed lot resulted into significantly higher peroxidase activity (507.5), followed by one, two and three years aged of seed lots.

Minimum activity (201.9) was observed in three-year old seed lot. Maximum peroxidase activity (507.5) was observed in fresh seed lot when seed treated with hot water at 80 ^{0}C for 1 minute and minimum (17.90) was observed in sulphuric acid in three- year old seed lot. There was decreases in activity of peroxidase in aged seeds (Fig. 1). The general, decrease in enzyme activity in seed lowers the respiratory capacity, which in turn lowers both the energy (ATP) and assimilates supply of germinating seeds. The results are in accordance with Kapoor *et al.* (2011), Radha *et al.* (2014), Tabatabei (2015) and Cakmal *et al.*(2010).

Maximum dehydrogenase activity (0.233) was observed in hot water treatment at 80° C for 1 minute in fresh seed lot (Fig. 1). Hot water treatment resulted into maximum dehydrogenase activity (0.127) among all treatments, followed by water soaking for 48 hr (0.084). Hot water treatment and water soaking of seed for 48 hr and control differed significantly with each other and minimum dehydrogenase activity

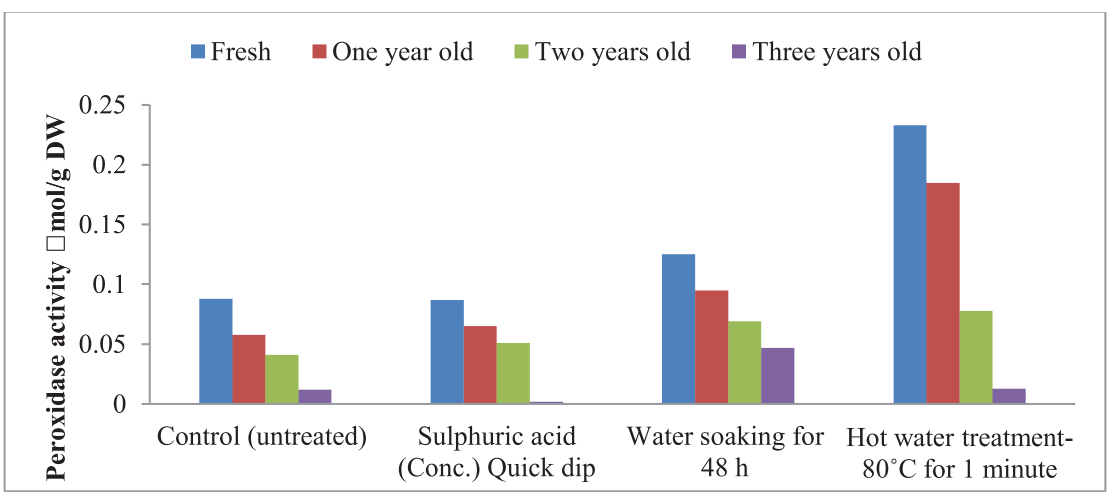

Fig. 1. Effect of scarification treatment and aging on peroxidase activity of different lots of guava seeds

was observed in concentrated sulphuric acid-quick dip (0.05) and control (0.05). Fresh seed lot resulted into significantly higher dehydrogenase activity (0.133), followed by one, two and three year aged. Minimum activity (0.019) was observed in three year old seed lot. Dehydrogenase activity was maximum (0.233) in hot water treatment at 80°C for 1 minute and minimum in concentrated sulphuric acid (0.002).

Maximum electrical conductivity (25.78) was observed in seeds treated with concentrated sulphuric acid had highest amount of seed leachtates and was significantly inferior seed to all others, followed by the control (18.40). Hot water treatment resulted into minimum electrical conductivity (11.02) among all treatments produced very less leachtates was considered good (Fig. 3). Fresh seed lot resulted into significantly very less electrical conductivity (12.83) and produced very less lechates and considered very good, followed by one, two and three -year aged of seed lots. Maximum electrical conductivity was observed in three year seed lot (18.73). Sulphuric acid treatment resulted in maximum electrical conductivity (25.78) and minimum in hot water treatment (11.02). Maximum electrical conductivity was observed in three -year - old seed lots (18.73) and minimum in fresh seed lot (12.82). These results are in accordance with those of Kalsa *et al.*(2011) and Ahmadvand *et al.*(2012).

The activities of catalase, peroxidase ascorbate peroxidase, glutathione reductase and superoxide dismutase decreased with ageing. Seed deterioration can be explained by the findings that ageing of seeds lead to lipid peroxidation that subsequently causes membrane perturbation. Such changes in membrane of aged seeds lead to electrolyte leakage. Enhanced lipid peroxidation

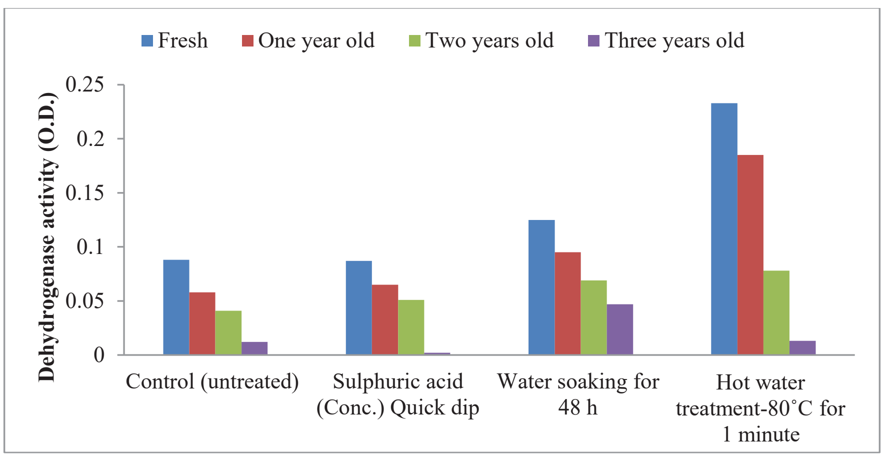

Fig. 2. Effect of scarification treatment and aging on dehydrogenase activity of guava seeds

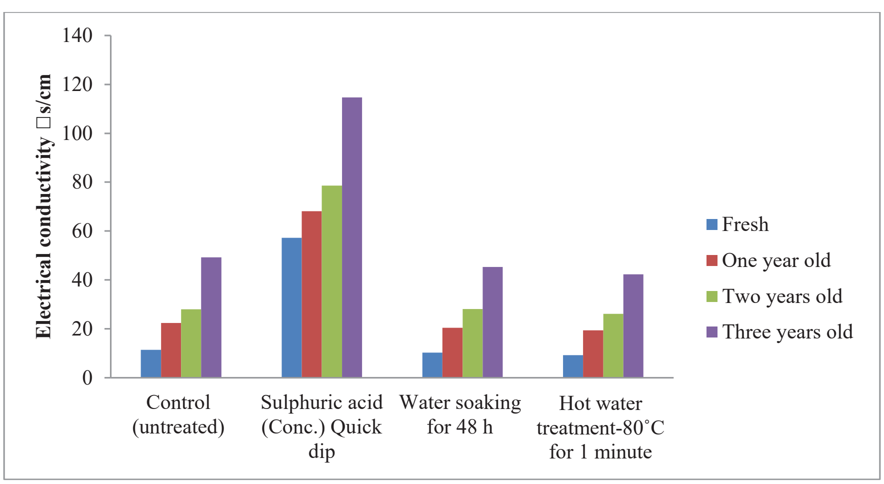

Fig. 3. Effect of different scarification treatment and aging on electrical conductivity of guava seeds

mediated by free radicals and peroxides is one of the probable reasons for seed viability loss during storage. Dehydrogenase and peroxidase activities in aged seeds decreased, while electrical conductivity increased due to harmful changes in their structure during seed ageing. The loss of enzyme activities could be responsible for higher H_2O_2 accumulation in aged seeds.

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