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Studies on seed quality parameters of Rosella (*Hibiscus sabdariffa* Var. *sabdariffa* L.) in relation to seed deterioration under accelerated aging conditions

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Abstract
Roselle (*Hibiscus sabdariffa* Var. *sabdariffa* L.) is an important medicinal plant used in indigenous pharmaceutical industries as a chief source in various synthetic drugs and antibiotics. The present study was carried out to examine the effect of seed deterioration on viability of Roselle seeds by subjecting seeds to accelerated ageing at 45± 2 ^{0}C temperature and 95 (%) relative humidity for 10 days. Various seed physiological parameters (moisture content, germination percentage, seedling vigor index I&II) and biochemical parameter (electrical conductivity) were explored. The results indicated that seed aging had significant effects on seed quality parameters of Roselle with an outcome of decreased speed and percentage of germination and decreased percentage of normal seedlings along with increased electrical conductivity and moisture content progressively with the increment in ageing period (7, 8, 9 and 10 DAA). Prolongation of ageing led to deterioration of both seed germinability and viability indicating its poor storability.

Keywords: Roselle, Accelerated ageing test, Electrical conductivity, Seed germination, Speed of germination

Introduction
Roselle (*Hibiscus sabdariffa* Var. *sabdariffa* L.), a member of the family Malvaceae, locally called as "*Karkade*" in Kannada. It is an important annual crop grown successfully in tropical and subtropical climates (Wilson *et al.*, 2004) [1]. Roselle is known in different countries by various common names including roselle, razelle, sorrel, soursour and queens land jelly plant (Mahadevan and Shivali, 2009) [2]. Roselle may have been domesticated in western Sudan before 4000 BC. It was first recorded in Europe in AD 1576. Sudan is presently the major producer of Roselle (Mohamed *et al.*, 2007) [3].
Economic parts of the Roselle are calyces rich in anthocyanins and protocatechuic acid. The dried calyces contain the flavonoids gossypetine, hibiscetine and sabdaretine. The major pigment formerly reported as hibiscine has been identified as daphniphylline. In China it is used to treat hypertension, pyrexia and liver damage (Odigie *et al.*, 2003) [4]. Recently the sepal extract has been used as an effective treatment against leukemia due to its high content in polyphenols particularly protocatechuic acid (Tseng, 2000) [5]. Roselle seeds are a source of a vegetable oil that is low in cholesterol and rich in other phytosterols and tocopherols, particularly β-sitosterol and γ-tocopherol.
The seeds of Roselle contain mainly alkaloids, saponins and monounsaturated fatty acids. Seed storage is a serious problem in tropical and subtropical countries where high temperature and high relative humidity greatly accelerate seed ageing phenomenon causing consequent deterioration and reduces the viability of seeds. Seed deterioration is inexorable and the best that can be done is to control its rate. Many factors contribute to the predisposition for seed deterioration. These include genetics where certain seeds are inherently longer lived than others. Seed structure can also influence seed deterioration. Simple differences in seed size can mean that smaller seeds with a greater surface area to volume ratio are more exposed to uptake of water that would make them prone to deterioration more than larger seeds.
At the cellular level, seed ageing is associated with various alterations including loss of membrane integrity, reduced energy metabolism, impairment of RNA and protein synthesis,

and DNA degradation (Kumar, 2000) [6]. During storage, a number of physiological and physicochemical changes occur, termed as aging. The rate at which the seed aging process takes place depends on the ability of seed to resist degradation changes and protection mechanisms, which are specific for each plant species [7]. The main external factors causing seed damage during storage are the temperature, relative air humidity and oxygen. Possibility to regulate these factors makes the basis for longer seed storage. With these facts in view a comprehensive study was conducted to estimate the storage potential and deterioration rate of seeds through accelerated ageing.

Material and methods

Seed source

The experiment was conducted in laboratory of the Department of Seed Science and Technology, UAS, GKVK, Bangalore-65 during the period of August 2014 to May 2015. Freshly harvested seeds of Roselle were collected from the "Sanjeevini vatika" an aromatic and medicinal plants division in the Dept. of Horticulture, UAS, GKVK, Bangalore-65. The seeds were cleaned and dried to safe moisture level and graded to uniform size and used to study the different aspects of seed technology. Seeds were surface sterilized using 5 (%) sodium hypochlorite solution for 5 minutes and rinsed thoroughly in distilled water. The seeds were dried at 25 °C for 24 hours in the laboratory.

Accelerated aging treatment

Seeds were aged accelerated at (45 ±1C°) and 95 (%) relative humidity up to 10 days. Seeds were aged in glass desiccators containing distilled water, and spread as a single layer on a metallic net to avoid contact with water. The desiccators were covered and maintained in an incubator at 45±2 ºC for 10 days. Seeds samples were collected at every 1-day interval for seed quality studies. Following the accelerated aging treatment, moisture content was determined and the seeds were air dried at 25 °C until their original moisture content (6.0-6.8%) was restored.

Seed germination (%)

The germination test was conducted in the laboratory by using between paper methods as per ISTA rules (ISTA, 2007) [8]. Three replications of 100 seeds were placed on germination paper and rolled towels were incubated in germination chamber maintained at 25 ± 1 °C and 90 per cent relative humidity. Percentage germination was expressed based on normal seedlings. Seed germination was recognized by emergence of radical and its speed of germination was calculated by using the formula,

$$GSI = \frac{G_1}{T_1} + \frac{G_2}{T_2} + \text{—} + \frac{G_n}{T_n}$$

Where,

G_1, G_2, G_n = Number of seeds germinated

T_1, T_2, T_n = Number of days taken for germination

Seedlings were evaluated for its vigor by calculating its seedling vigor index (SVI) by using the following formula (Abdul-Baki and Anderson, 1973) [9].

SVI-I= Germination percentage x Mean seedling length (cm).

SVI-II= Germination percentage x Mean seedling dry weight (mg)

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

Twenty-five seeds of two replications were taken randomly from each treatment in a beaker. Then the seeds were soaked in 25 ml of distilled water for 24 h at 25±1 °C. The steeped water from soaked seeds was collected and the electrical conductivity (EC) of seed leachate was measured in digital conductivity meter (Model: Systronic conductivity meter 306). After subtracting the EC of the distilled water from the value obtained from the seed leachate, the actual EC due to electrolyte was measured and expressed in μS ppm^{-1}.

Statistical analysis

The experimental data was statistically analyzed by adopting the analysis of variance technique appropriate to design as per the methods outlined by Sundararaj and coworkers (Sundararaj *et al.*, 1972) [10] in computer. Critical differences were calculated at 1 per cent level, where 'F' test was significant. Germination percentages (original values) were transformed into arc sine root transformation. The transformed values were used for statistical analysis.

Results and Discussion

Table 1 represents a summary of the analysis of variance for seed germination characteristics and electrical conductivity test. Seed aging had significant effects on seed quality parameters of Roselle. Seeds which are artificially aged for 10 days showed a significant increase in level of moisture content. It may due to disintegration of cell membranes during accelerating ageing made the seed imbibe more water. The similar results of increase in moisture content during accelerated ageing of *Oryza sativa* seeds were reported by (Kapoor *et al.*, 2011) [11]. Apart from these the accelerated aging showed a significant decline in germination speed and seed germination may be a result of progressive loss of seed viability and vigor, which was evident in the results of this study. These observations that showed a decline in seed vigor were in accordance with earlier works on *Artiplex cordobensis* (Aiazzi *et al.*, 1996) [12].

Seeds which artificially aged for 3 days (3 DAA) had less effect on germination, but seeds aged for 4 days onwards (4,5, 6 and 7 DAA) showed significant decline in germination when compared to fresh seeds. The decrease in germination and increase in abnormal seedlings, dead seeds by accelerated aging may be due to progressive loss of seed viability and vigor, the similar result was obtained by (Ashraf and Habid, 2006) [13] in Ash (*Fraxinus excelsior*) seeds. The speed of germination was decreased gradually for 3 days onwards against fresh seeds. Because ageing increased the length of time to reach 50% germination. This might be due to stronger inhibitory effects during seed ageing (Sara M. and Elena, 2011) [14]. Similarly, reduction in root length, shoot length, mean seedling length, seedling vigor index I, seedling dry weight and seedling vigor index II were noticed in aged (3 DAA to 10 DAA) seeds.

Besides physiological changes, significant changes in biochemical parameters were also observed due to accelerated aging. Seeds aged for one day onwards (2, 3, 4 &10 DAA) showed gradual increase in electrical conductivity. The accelerated ageing resulted in membrane damage as evident from higher solute leakage in aged seeds. Estimation of electrical conductivity was considered as a promising parameter in seed quality studies (Bewely and Black, 1994) [15] compared to fresh seeds. Seed viability loss is often attributed to the loss of integrity of the membrane. In the presence of oxygen, ageing of seed can lead to peroxidative change in polyunsaturated fatty acid (PUFA). This free radical inducing non- enzymatic peroxidation may lead to membrane damages and is likely to cause seed deterioration (Wilson and Mc Donald, 1986) [16].

Conclusion

The results of the present study revealed that, Rosella seeds subjected for accelerated ageing revealed a rapid decline in seed germination and other seed quality parameters. The rapid decline in the germination of this crop seeds indicate the poor storability.

Table 1: Effects of accelerated aging on seed quality parameters of Roselle (*Hibiscus sabdariffa* Var. *sabdariffa* L.) seeds

Accelerated ageing (Days)	Germination (%)	Speed of germination	MSL (cm)	MSD (mg)	SVI-I	SVI-II	Seed Moisture (%)	Electrical Conductivity (μSm^{-1})
0	64.65	1.51	21.8	13.4	1409	866	6.54	139.6
1	61.12	1.39	20.1	12.4	1229	758	7.91	306.5
2	50.34	1.23	15.6	8.94	785	450	9.32	320.0
3	45.00	0.99	9.88	7.66	445	345	11.55	326.0
4	36.87	0.71	7.45	6.46	275	238	13.26	410.0
5	27.04	0.41	5.50	4.05	149	110	17.25	450.3
6	16.41	0.16	1.91	1.02	31	17	19.84	680.0
7	5.6	0.072	0.43	0.22	2	1	22.41	812.0
8	0.00	0.00	0.00	0.00	0.00	0.00	23.64	908.1
9	0.00	0.00	0.00	0.00	0.00	0.00	25.94	972.1
10	0.00	0.00	0.00	0.00	0.00	0.00	28.44	997.0
SEm±	0.44	0.021	0.033	0.170	10.57	5.20	0.19	15.9
CD @(1%)	1.28	0.086	0.123	0.579	32.14	15.7	0.66	45.2
CV (%)	2.54	3.65	2.15	3.37	2.09	2.99	1.87	4.87

MSL: Mean seedling length; MDW: Mean dry weight; SVI-I: Seedling vigour Index-II; SVI-II: Seedling vigour index-I

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