

THE EFFECTS OF SEED TREATMENTS ON GERMINATION AND OTHER SEED QUALITY ATTRIBUTES OF ROSELLA

(*Hibiscus sabdariffa* Var. *sabdariffa* L.)

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Abstract: *Hibiscus sabdariffa* Var. *sabdariffa* L is an important medicinal plant belonging to the family Malvaceae. The propagation of *Hibiscus sabdariffa* is through seeds, which is somewhat difficult which may be due to various germination inhibition factors. In view of the above the present investigation was carried out to enhance the seed quality parameters using physico-bio-chemical treatments and growth regulators (GA₃, Kinetin). Among them most effective treatment was Hot water treatment at 50⁰ C for 5 min followed by 100 ppm of GA₃ for 4 h and 1 % of KNO₃ treatment for 4 h but the effect of 0.2 % of Kinetin at 25 °C, 5 % of Vermiwash and Panchagavya did not show significant difference when compared to the other treatments. Results of the treatments indicate the presence of coat induced and non-deep physiological dormancy in these species. The data's were statistically analyzed by analysis of variance (P<0.01).

Keywords: Roselle, seed germination, GA₃, Kinetin, Hot water treatment, KNO₃, pre-chilling, Vermiwash and Panchagavya.

Introduction

Roselle (*Hibiscus sabdariffa* Var. *sabdariffa* L.), a member of the family Malvaceae, locally called as “Karkade”. It is an important annual crop grown successfully in tropical and subtropical climates (Wilson and Menzel, 2004). Roselle is known in different countries by various common names including roselle, razelle, sorrel, soursour and queens land jelly plant (Mahadevan *et al.*, 2009). 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 (Mohammad *et al.*, 2007).

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). Recently the sepal extract has been used as an effective treatment against leukemia due to its high content in polyphenols particularly protocatechuic acid (Tseng *et al.*, 2000). 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.

Roselle has certain therapeutic properties, the reported benefits of taking it internally in the form of herbal tea include, soothing colds, clearing a blocked nose, clearing mucous, as an astringent, promoting kidney function, aiding digestion, as a general tonic as a diuretic and helping to reduce fever. Taken as a drink made from the calyx, it is a mild diuretic and purgative among many other effects. The drink is said to be a folk remedy for cancer.

In spite of being medicinally important and propagated through the seeds, they have not been cultivated commercially because of its late, erratic and poor germination with substantial loss in seed viability and lack of systematic work for their multiplication. So there is a need to study the problems in their seed germination and seed dormancy in order to uplift the seed quality for better and quick germination.

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.

Viability of seeds: Viability of the collected seeds was determined by using tetrazolium technique and by dissection microscope.

Pretreatments and experimental conditions

Seeds were disinfected by immersing in 0.5 % sodium hypochlorite solution for 2 min followed by rinsing thoroughly with distilled water four times. The sterilized seeds were treated with GA₃ (100 ppm), Kinetin (0.2 %) and KNO₃ (1 %) for 4 hours, Vermiwash and Panchagavya (25 ml/g seeds) for 24 hours, hot water treatment at 50 °C for 5 min, presoaking in cold water for 24 hours and cold stratification (chilling) treatments at 4 °C for 3 to 10 days. Treated seeds were washed thoroughly with distilled water and placed in Petri plates containing moistened Blotter paper. Petri plates were kept at 25±0.5 °C in growth chamber and moistened as needed with distilled water. A set of seeds without pre-sowing treatments were considered as control. Seed germination was recognized by emergence of radical and its speed of germination was calculated by using the formula,

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

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 vigour by calculating its seedling vigour index (SVI) (Abdul-Baki and Anderson, 1973) by using the following formula.

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

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

Protein content of seeds

The protein content of seeds was estimated by using alkaline copper and folin method (Lowry *et al.*, 1951) with crystalline bovine serum albumin as standard curve. Exactly 0.5g of fresh seed material was ground using 1ml of 0.1M potassium phosphate buffer (pH 7.5). The extract was centrifuged at 5000 rpm for 15 minutes. To 0.2ml of protein extract, one ml of alkaline copper solution was added and vortexed. Then 0.2ml of folin reagent was added and the sample mixture was kept in dark. After 30 minutes the volume was made up to 8 ml using distilled water. The absorbance was read at 660nm. A blank was run with no protein extract. The content of soluble protein was determined by reading from standard curve. The protein in the sample was expressed in percentage.

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 *et al.* (1972) 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

The results showed that the properties of speed of germination and germination percentage of Roselle seeds were significantly different ($p < 0.01$) under different treatments (Table 1). *Hibiscus sabdariffa* exhibited significantly higher seed germination and field emergence in Hot water treatment at 50⁰ C for 5 min followed by 100 ppm of GA₃ for 4 h and 0.2 % of KNO₃ for 4 h but the effect of 0.2 % of Kinetin and 5 % of Vermiwash and Panchagavya did not show significant difference when compared to the other treatments and the lower seed germination and field emergence was observed in untreated seeds. The hot water treatment at

50⁰ C for 5 min showed a significantly higher seedling vigour index and speed of germination followed by 0.2 % of KNO₃ and 100 ppm of GA₃ for 4 h. It was found that using 5 % of Vermiwash and Panchagavya resulted in reduced seedling vigour index and speed of germination of garden rue seeds because of higher incidence of disease.

Besides physiological changes, there also some significant changes were observed in biochemical parameters due to the seed treatment. The highest protein content was observed in 100 ppm of GA₃ treated seeds followed by 1 % of KNO₃ treatment for 4 h, 5 % of Vermiwash and Panchagavya treated seeds, but the 0.2 % of Kinetin treated seeds and untreated seeds show a least protein content. The 0.2 % of KNO₃ and hot water treatments maintains the membrane integrity of the *Hibiscus sabdariffa* resulted in least electrical conductivity than other treatments which recorded the higher electrical conductivity.

Table 1: Influence of seed treatments to enhance the seed quality parameters in Rosella (*Hibiscus sabdariffa* Var. *sabdariffa* L.)

Treatments	Germination (%)	Speed of germination	SVI-I	SVI-II	Electrical conductivity (μSppm^{-1})	Protein content (%)	Field emergence (%)
T ₀ : Control	67.33 (53.98)	1.332	1014	621	792.5	9.60	63.0
T ₁ : GA ₃ (100 ppm)	82.33(61.22)	1.611	1502	1086	301.7	17.30	83.7
T ₂ : KNO ₃ (1 %)	80.00 (62.17)	1.667	1504	1230	325.5	13.00	83.0
T ₃ : Kinetin (0.2 %)	72.01(58.14)	1.470	1102	729	745.9	12.45	64.3
T ₄ :Panchagavya (5 %)	73.67 (55.86)	1.402	1159	691	772.6	14.15	73.7
T ₅ : Hot water (50 ⁰ C for 5 min)	82.67 (62.55)	1.611	1585	1280	128.3	13.55	84.7
T ₆ : Cold water	79.67 (61.66)	1.569	1498	1091	233.2	12.30	74.3
T ₇ : Vermiwash (5 %)	71.11 (54.30)	1.370	1072	693	141.1	12.35	72.3
T ₈ : Chilling (5 ⁰ to 6 ⁰ C for 10 d)	74.67 (60.36)	1.523	1357	965	134.3	14.80	76.7
SEm±	0.98	0.029	34.2	24.73	51.25	1.595	1.49
CD (1 %)	2.78	0.062	91.7	85.13	149.6	4.86	4.28
CV (%)	3.54	4.88	3.16	2.34	2.91	4.54	2.08

Discussion

Generally the pre-sowing treatments were reported to enhance seeds germination (Hossain, 2005), Owing to enhance the seed quality, different methods were compared. In this study, seeds were treated with different growth regulators, chemicals, biofertilizers and also pre-chilling and hot water treatment. Chemicals that accumulate in the seed-coat during development and remain in the seed after harvest can act as germination inhibitors. The present study showed the higher seed germination in hot water, pre-chilling and cold water treatments, this may be due to leached out of the inhibitors while soaking in water. The higher efficacy of the hot water treatment may be due to it enhance the germination of hard coated seeds by elevating the water and oxygen permeability of the testa. The similar effects of hot water on seed quality enhancement were reported by Rita *et al.*, (2011) in Kalmegh seeds and Hussain (2014) in *Silybummarianum*. The results reported in the present study also supported by the findings of Hossain (2005), showed that seeds soaking in water improved the germination. Here KNO_3 and GA_3 also play a significant role in enhancing seed germination and other seed quality parameters.

Conclusion

From above finding it may be summarized that the hot water treatment, pre-chilling and KNO_3 treatments can be recommended for the improvement of seed germination in Roselle.

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