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Research Article

Temperature regime, light conditions and sowing depths as determinants of germination potentials of green and red roselle, *Hibiscus sabdariffa* L.

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Abstract: Roselle (*Hibiscus sabdariffa* L.) is a nutraceutical crop that its nutritional and health benefits have been exploited for decades. The plant is not commonly cultivated in South Africa, to improve cultivation of roselle, germination potentials of the plant in the region need to be determined. This study was aimed at assessing the effects of different sowing depths, temperature and light conditions on germination of seeds of green and red cultivars of roselle. The effect of sowing depths on germination of the seeds was determined by sowing at depths 0, 1, 2, 3, 4, and 5 cm, each sowing depth represented a treatment. Also, germination of both cultivars was investigated by exposing the seeds to constant temperature set at 5, 10, 15, 20, 25, 30, 35 and 40°C, each temperature level represented a treatment. Besides, the seeds were exposed to continuous light, continuous darkness and alternating light and darkness each light condition represented a treatment. A one-way analysis of variance was used to analyze the data and means were separated using Fisher's Least Significant Difference. This study revealed that seeds of both cultivars germinate optimally at depth 0 cm and 20ºC in darkness, though red roselle seeds require some light.

Keywords: Germination potentials; green and red roselle; light conditions; sowing depth; temperature regime.

1. Introduction

Roselle has recently received increased demand in many parts of the world due to their multifunctional attributes. Roselle has two basic cultivars: green and red. Red roselle contains anthocyanin resulting in reddish stems, leaves and calyces and pinkish petals, whereas green roselle lacks anthocyanin and thus have green stem and leaves, pale green calyces and pale-yellow petals (McClintock & Tahir, 2004). These two cultivars are consumed as vegetable in salad, soup and sauce; calyces in red cultivar are however on high demand because of their use in beverages and coloring industries.

There are several research findings on the importance of roselle to nutrition and health of people. Roselle is a nutritive plant; 100g of the calyces has the following nutritional composition 100 g of *the calyces* gave: protein (1.9 g), fat (0.1 g), carbohydrates (12.3 g) and fiber (2.3 g) (Amin *et al*., 2008). The whole plant contains high content of vitamin C especially the calyces which are found to be good source of ascorbic acid (14 mg), iron (57 mg) and β-carotene (300 µg) (Salami & Afolayan, 2021). The plants are good sources of antioxidants, which protects against diseases (Chumsri et al., 2008). Utilization of this plant is necessary for improved health of people while propagation of the plant is essential for sustainability.

Propagation of *H. sabdariffa* is mainly through seeds. In order to enhance the cultivation, more good quality seeds are needed (Bamgboye & Adejumo, 2009). However, researches on germination of green and red roselle are scanty. The seeds of different sizes vary in their response to temperatures and soil depths (Karamel & Ozimerzi, 2008). Shruthi (2018) recommended hot

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water treatment, pre-chilling and potassium nitrate ($KN0_3$) treatment to enhance seed germination in roselle while Dressler et al., (2014) reported that *Hibiscus acetocella* does not require any light to germinate. Koduru *et al*., (2006) affirmed that high temperature reduces germination of *Solanum aculeastrum.* Several reports have also indicated that the optimal temperatures for germination and seedling growth depend on species while within the species, optimal temperature differs significantly between genotypes (Buriro *et al.,* 2011; Martins et al., 2000). To promote proper propagation of roselle, it is therefore important to clearly understand their germination requirements. This study aimed at assessing the germination potential of the seeds of both green and red roselle under the influence of different temperatures, light conditions and sowing depths.

2. Materials and Methods

2.1. Seed collection and determination of their weights

Mature seeds of red and green roselle were obtained from Medicinal Plants and Economic Development (MPED) Research Centre, Botany Department, University of Fort Hare. The seeds were sorted out by removing damaged, immature and other materials and later air-dried and stored in a closed plastic bag at room temperature. Three replicates' of 1000 seeds of each type were weighed using Mettler weighing balance. The average weight was then determined.

2.2. Viability test

The sample lots of the seeds of the two cultivars were subjected to viability test using tetrazolium technique. For each type, 50 seeds were soaked in distilled water for 6 hours in three replications for imbibition and ease of sectioning. These were then soaked in colorless 0.1% solution of 2, 3, 5 - triphenyl tetrazolium chloride (TTC) at room temperature (25°C) in the dark. The seeds were later removed from TTC solution, washed with distilled water and soaked in 10 ml of 95% ethyl alcohol to permit direct observation of the embryo.

2.3. Effects of sowing depth on germination

This experiment was conducted in the glass house with temperature range of 24- 28°C. Nursery trays of dimension 65 cm by 33 cm with 200 holes were filled with top soil to the required level. One nursery tray was used for each roselle cultivar and placed beside each other. The effect of depth of sowing on germination of seeds of green and red roselle was determined by sowing a seed each on a hole of nursery tray in a row of 10 at depths 0 (soil surface), 1, 2, 3, 4, and 5 cm under three replications. Each sowing depth represents a treatment; each treatment had 30 holes on which seed emergence is recorded on daily basis. These were watered regularly and put under observation for the period of 14 days (Jimoh et al., 2019).

2.4. Effects of temperature on germination

Incubator manufactured by MRC Laboratory Equipment, Model BOD-150, SN 1000112077C was used to control temperature in this experiment. The effect of temperature on seed germination was investigated by exposing seeds of each roselle type to constant temperatures set at 5, 10, 15, 20, 25, 30, 35 and 40°C. Each temperature level represents a treatment and each treatment was monitored for 14 days starting from 5ºC. For each cultivar, 20 seeds were placed in 9 cm Petri dish containing two pieces of Whatman no. 1 filter paper soaked with distilled water and replicated three times. Thus, 6 petri dishes were monitored in the incubator for a particular temperature, these Petri dishes were displayed in a completely randomized design. The criterion for the germination was the emergence of about 1 mm radicle (Barzgar, 2009).

2.5. Effect of light conditions

The effect of continuous light, continuous darkness and alternating light and darkness (12 h light/12 h dark photoperiod) were investigated under controlled environments. In each experimental setup, 20 seeds were placed in sterilized 9 cm diameter Petri dishes lined with two sheets of filter paper and were moistened on regular basis. Each setup was replicated thrice. Four white fluorescent tubes produced continuous light treatment**.** For continuous darkness treatment, the Petri dishes were covered with three layers of aluminum foil and kept in a dark room (Baskin and Baskin, 2014). The seeds were monitored using a green safety light (Amaral-Baroli & Takaki, 2001)**.** For alternating light and darkness treatment, the Petri dishes were placed in a room with 12 h light/12 h dark photoperiod. Each setup was moistened with distilled water and germination was observed for 14 days. Germination percentages for the seeds of the two roselle types were calculated.

Relative Light Germination (RLG) index is the level of dependence of plants for light. This was calculated for each roselle type using the method of Jankowski-Blaszczuk & Daws (2007) as follows:

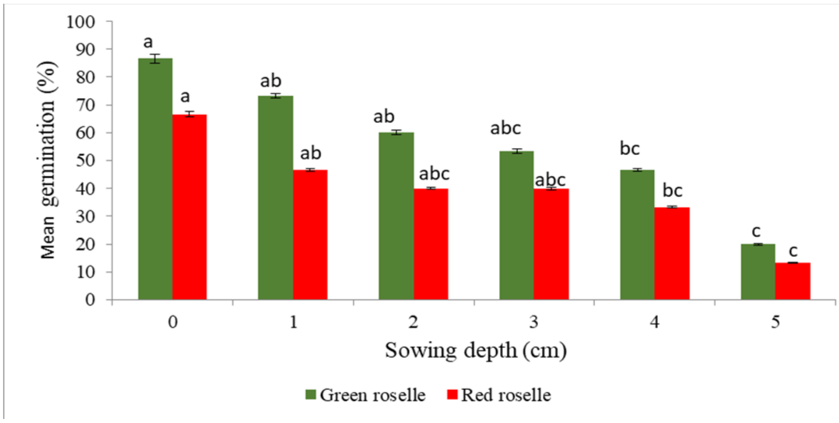

Fig. 1: Effects of sowing depths on germination percentage of green and red roselle.

Means with the same letter are not significantly different at P<0.05.

$$RLG = \frac{\mathrm{Gl}}{\mathrm{Gd} + \mathrm{Gl}}$$

Where Gl= the germination percentage in light,
Gd = the germination percentage in darkness. RLG represents a range of values varying from 0 (germination only in the dark) to 1 (germination only with light). This is relatively unaffected by dormancy (Milberg et al., 2000).
Germination time is a function of how long it takes the seeds to germinate. Mean germination time was calculated as follows

$$\overline{T} = \frac{\sum niti}{\sum ni}$$

where; n_i= number of seeds germinated in the time i corresponding to the ith observation while t_i = time from the start of the experiment to the ith observation (day).
Germination rate is important in monitoring how fast the sown seeds germinate. Germination rate is inversely proportional to germination time and was calculated as follows:

$$\text{Mean germination rate} = \frac{1}{\overline{T}\text{day} - 1}$$

2.6. Statistical Analysis
Data collected were subjected to statistical analysis using MINITAB Release 17. A one-way analysis of variance was used to analyze the data and means were separated using Fisher's Least Significant Difference. All the values were reported as means ± standard deviation of means and the results were considered significant at P< 0.05.

3. Results

3.1. Seed mass and viability

The average weights for 1000 seeds for green and red roselle were 27.36 g and 17.62 g respectively, therefore the average weights for a single seed were 27.4 mg and 17.6 mg for green and red roselle respectively. The embryos of almost all the seeds appeared pinkish in color. Seed viability for green and red types was 91 ± 1.34% and 77 ± 1.17% respectively.

3.2. Effects of sowing depths on percentage and rate of germination

The mean germination percentage decreases with soil depths in both cultivars (figure 1). The highest germination occurred at soil surface: 86.60 ± 0.54% and 66.67 ± 0.88 % for green and red respectively; while the lowest were observed at 5 cm: 20 ± 2.33% and13.33 ± 3.50% for green and red type respectively. At 2 and 3 cm depth the value was the same for the red type 40 ± 1.28%. Generally, the germination percentage for green cultivar was higher than that of red in each sowing depth. There was however no significant difference between mean germination percentages at 1 and 2 cm sowing depths for the green type. Also, for the red cultivar, there was no significant difference between germination percentages at 2 and 3 cm below soil surface.

Figure 2 shows the effects of sowing depths on germination rate of green and red roselle. Green roselle seeds on soil surface had highest germination rate (1.86 day^{-1}), while lowest rate was observed at 5 cm (0.43 day $^{-1}$). The germination rates at 1 and 4 cm deep were the same (1.29 day^{-1}) but higher than those at 2 and 3 cm soil depths (1.14 day^{-1}) which had the same value. There was no significant difference among the rates at 1, 2, 3 and 4 cm sowing depths. In a similar pattern, the red roselle had highest germination rate recorded at soil surface (1.14 day^{-1}) and the lowest at 5 cm deep (0.29 day^{-1}). Just like in green roselle, the rate for red was significantly higher at soil surface and lower at 5 cm than other sowing depths. There was no significant difference between the values at 2 and 4 cm sowing

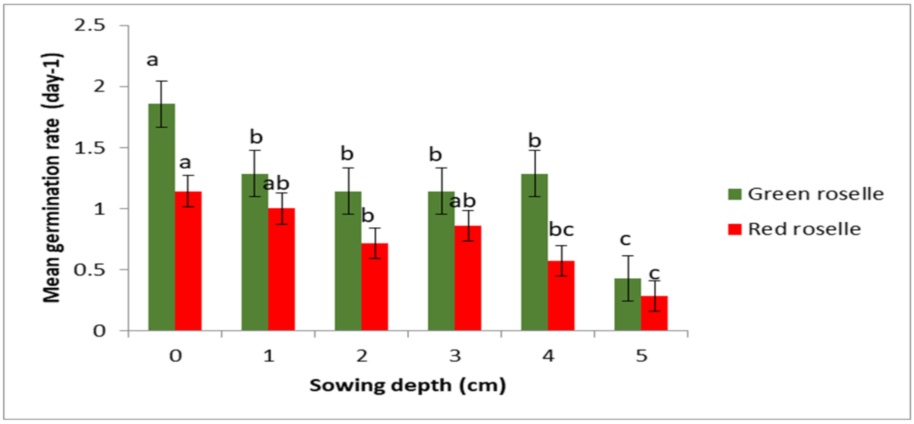

Fig. 2: Effects of sowing depths on germination rate of green and red roselle.

Means with the same letter are not significantly different at P<0.05.

depths. In each of the sowing depths, green roselle had higher germination rate than red roselle.

3.3. Effects of temperature

Germination of seeds occurred at 10ºC to 35ºC in green cultivar and 10ºC to 40ºC in the red Figure 3. Maximum germination was observed at 20ºC for both cultivars with the green having 100 ± 0.0% and red 80 ± 0.00%. It is noteworthy that at 40ºC 13.33 ± 2.31% of the red roselle seeds germinated whereas no germination was observed in the green type at that same temperature. Lowest germination observed at 10ºC: 73.33 ± 2.30% and 26.67 ± 2.29% for green and red roselle respectively. In the green cultivar, the mean value at 20 º C and 35ºC (100 ± 0.00%) was significantly higher than other treatments. There was however no significant difference between germination percentages at 30ºC (93.33 ± 2.31%), 25 º C (93.33 ± 2.31%), 15ºC (86.67 ± 4.62%) and 10ºC (73.33± 2.31) P<0.05. For red type, on the other hand, there was no significant difference in germination percentages at 20, 25, 30 and 35ºC; these were significantly higher than other temperature levels. In addition, mean germination percentage at 10ºC (26.67 ± 2.31%) and 15ºC (33.33 ± 2.31%) were statistically equal but higher than that at 40ºC (13.33 ± 2.31%).

In the green roselle, germination rate of 4.29 day^{-1} at 20ºC and 35ºC were observed to be the highest, while the rate of 2.64 day^{-1} at 10ºC was recorded to be the lowest. The same rate (4.00 day^{-1}) was observed at 30ºC, 25 º C and 15ºC. There was no significant difference among germination rates at 15, 20, 25, 30 and 35 º C. In the red roselle on the other hand, highest rate occurred at 20ºC (3.33 day^{-1}), while the lowest value was recorded at 40ºC (0.57 day^{-1}). The rate at 25ºC and 35ºC were the same (2.86 day^{-1}). There was however, no significant difference in the rates at 20, 25, 30 and 35ºC. (Fig 4).

3.4. Effects of light on germination

Figure 5 shows the effects of light conditions on germination percentage of green and red roselle. Green

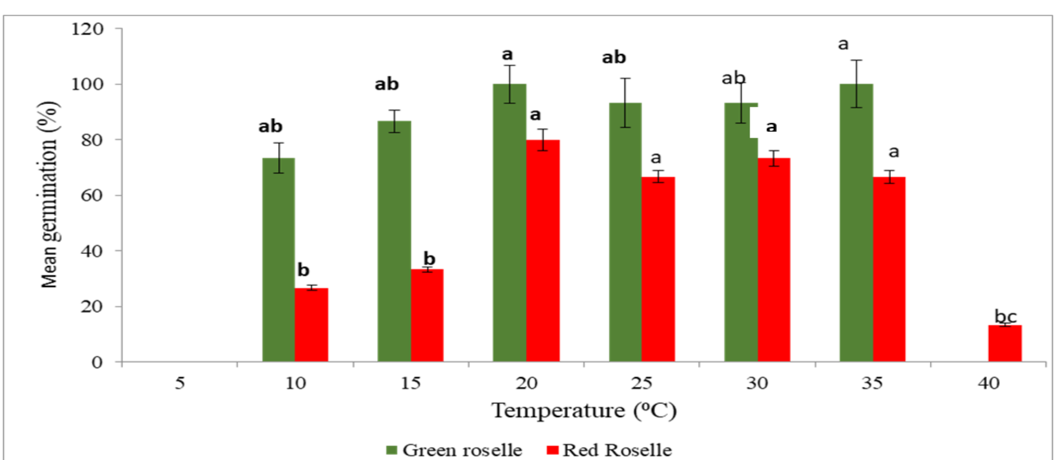

Fig. 3: Effects of temperature on germination percentage of green and red roselle.

Means with the same letter are not significantly different at P<0.05.

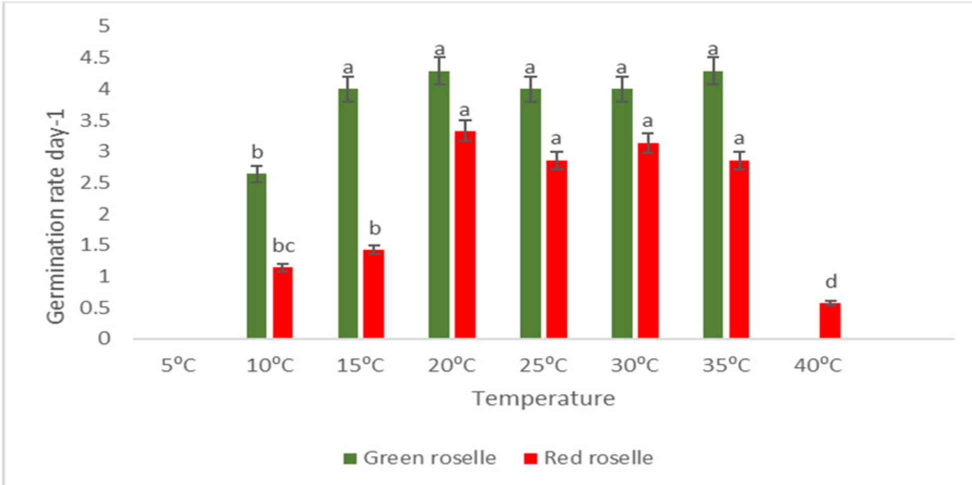

Fig. 4: Effects of temperature on germination rate of green and red roselle.

Means with the same value are not significantly different P<0.05.

roselle had maximum germination (100 ± 0.00%) at both continuous darkness and alternating light and darkness conditions while the value was lower at continuous light (93.33 ± 2.30%). There was no significant difference in mean germination percentage among the three light conditions P<0.05. For red roselle however, highest germination was observed at alternating light and dark (66.67 ± 4.00%). Continuous light had lowest value (53.33 ± 2.30%). Also, there was no significant difference in mean germination percentages among the three light conditions. Generally, the value for green roselle was higher than that of red in all the light conditions.

In green roselle, highest germination rate was observed at alternating light and darkness (8.50 day^{-1}), followed by continuous darkness (4.29 day^{-1}), while continuous light condition had lowest value (4.00 day^{-1}). There was no significant difference between germination rates under continuous light and continuous dark conditions. In red roselle on the other hand, continuous darkness had the highest value (2.86 day^{-1}) followed by alternating darkness and light (2.57 day^{-1}). Lowest rate occurred at continuous light condition (2.29 day^{-1}). However, there was no significant difference in germination rates under alternating dark and light and continuous light conditions P<0.05. Figure 6, table 6. Relative light germination (RLG) index value was 0.48 for green roselle and 0.47 for red roselle.

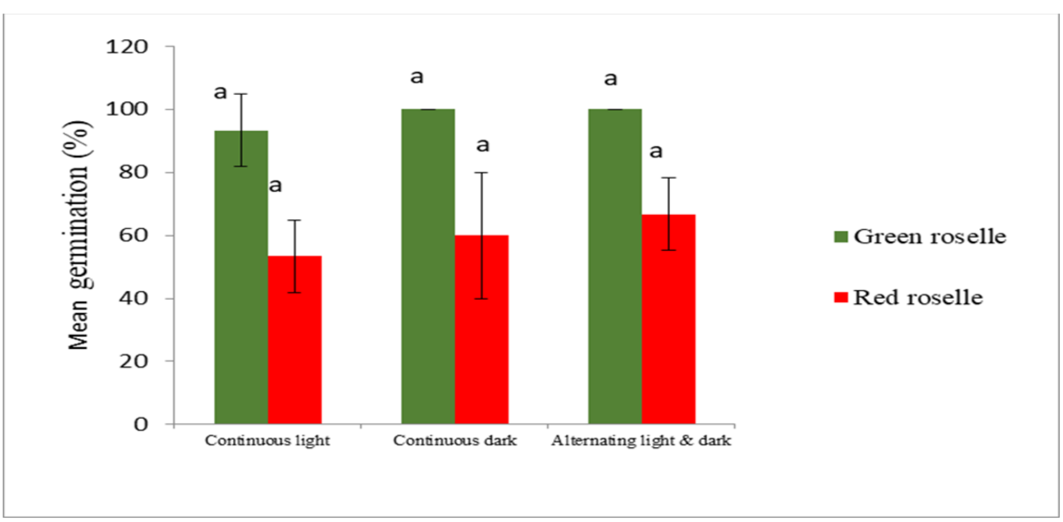

Fig. 5: Effects of light conditions on germination percentage of green and red roselle.

Means with the same letter are not significantly different at P<0.05.

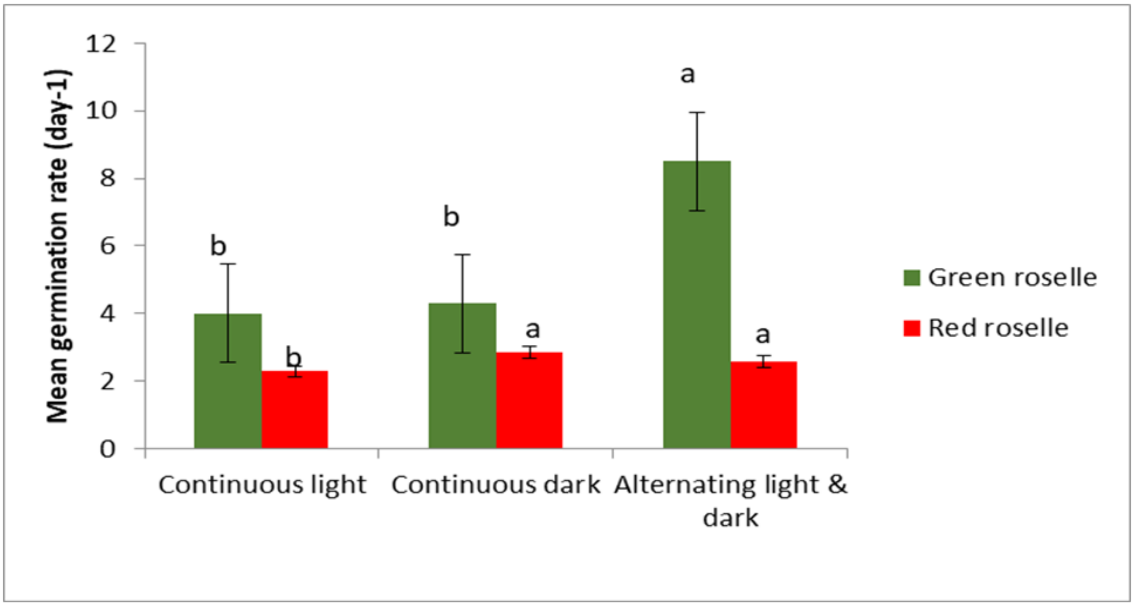

Fig. 6: Effects of light conditions on germination rate of green and red roselle.

Means with the same letter are not significantly different at P<0.05.

4. Discussion

The establishment of crop seedlings requires successful seed germination. The average weight of a single seed of each cultivar was 2 mg, hence roselle seeds have large size (Milberg et al., 2000). High seed weight of the cultivars is an indication of higher food reserve and better seed germination and establishment. High viability of both cultivars suggests that their germination and establishment would be ensured in suitable habitats.

Although for the two cultivars, germination occurred in all the sowing depths, highest germination was observed at the soil surface (0 cm). The report of this study supported the findings of Benvenuti et al., (2001) and Amin *et al.,* (2008) who reported that optimum seed germination occurred at soil surface. But the findings opposed the report of Chachalis et al., (2008) in *Hibiscus trionus* who observed higher seed emergence at the deeper soil depths. Highest germination recorded at soil surface indicates that roselle is better broadcast on the soil surface for successful crop establishment in suitable habitats. The green cultivar which had edge over the red means that it will be more successfully established than red type.

The ability of the seeds of the two cultivars to germinate over a broad temperature range is an indication that both cultivars can get established in habitats with varied temperature range. Germination did not occur at 5ºC and 40ºC in green roselle while in the red it was observed at 40ºC. Lack of germination at the lowest temperature for both roselle reveals that metabolic activity of the seeds was very low and thus hindered germination. Also lack of germination at higher temperature may mean that the temperature destroyed the seed embryo thereby making germination impossible. Finch-Savage and Leubner-Metzger, (2006); Bewley et al., (2012) opined that every species has a base and ceiling temperatures, which represent the limits at which germination can occur. Optimum temperatures for germination of green roselle were 20ºC and 35ºC, while that of red roselle was 20ºC. Since there was no significant difference between 20ºC and 35ºC it may be concluded that optimum temperature for roselle is 20ºC. Thus, for better crop yield, roselle seeds should be raised in an environment with temperature around 20ºC. Red cultivars which germinated at 40ºC suggests that it has higher tolerance level for high temperature than green. This may give red roselle advantage over green when competing in habitats with temperatures above 35ºC. The high temperature tolerance for germination of red type supports the findings of Burino et al., (2011) that within species, different genotypes tolerate temperature differently.

The trend of germination of the two cultivars in different light conditions showed that both types can germinate in all the light conditions though to varying extent. In the green type, optimum germination occurred in dark conditions. Naturally, there is dark condition in the night and light condition during the day; most germination of roselle seeds likely occurred in the night. Thus, short day longer night may favour and fasten the rate of green roselle seeds germination. The fact that complete germination occurred at dark condition suggested that green roselle does not need light for germination. This concurs with the report of Bewley & Black (2012) and Sowunmi & Afolayan, 20 15) who found that seed germination may be inhibited by continuous light and such seed germinate well in the darkness. This also supports the report of Dressler et al., (2014) that *Hibiscus acetocella* does not need light to germinate. Besides, it corroborates with the report of Pearson et al., (2002) who affirmed that seeds, which have seed mass of less than 2 mg, were light sensitive, while larger seeds (> 2mg) such as roselle were not light dependent. In red cultivar, however, higher germination occurred at alternating light and darkness condition. This is the natural light condition obtainable in this part of the world. The seeds of red cultivar required dark and some light to germinate.

Relative light germination (RLG) index value for green roselle (0.48) was higher than that for red (0.47). RLG index values of 0 to < 0.5 refers to the situation where germination in the dark is greater than in the light while RLG values > 0.5 refers to situation where germination in the light is greater than in the dark. Although high germination of red seeds was observed at alternating light and darkness condition, RLG value tends towards darkness (< 0.5). This reveals that red roselle seeds need more dark condition to germinate than light condition. The faster germination rate for red type at dark confirmed this. In this study, germination occurred in both cultivars in all the sowing depths (including 5 cm) though to a varying degree, emergence of seedlings at 5 cm soil depth (where no light occurs) is an indication that roselle does not need light for germination. The reports of Benvenuti *et al*., (2001) which showed that light rarely transmit beyond 4 cm depth in all soil types may confirm this. Hence, germination of roselle be better in longer night and shorter day.

5. Conclusion

This study revealed that sowing roselle seeds on the soil surface (0ºC) at 20ºC is the best for their germination. Though high light intensity is essential for good growth and yield of roselle but green roselle does not need light for germination while red roselle may require some light to germinate.

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