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Germination and Early Growth of *Acacia nubica* Benth. *In vitro*

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Abstract: *Acacia nubica* is well-adapted to harsh conditions and offers valued socio-economic and environmental benefits. This research studied seed germination and seedling growth of *A. nubica in vitro* in response to the application of different concentrations of sodium hypochlorite, sucrose, and Murashige and Skoog (MS) medium. The synchrony, percentage, and rate of germination of *A. nubica* seed were studied. Seedling development was assessed by measuring the shoot, root, and number of nodes. Sodium hypochlorite at 10% produced the maximum germination percentage, while 5% induced a faster and more uniform emergence. Sucrose has various effects on seed emergence and germination depending on the concentrations; 30 g/L elicited the highest germination, while 10 g/L improved seed emergence rate with higher uniformity. The highest seed germination was achieved on ¼-MS medium, although 0-MS strength recorded faster and more synchronized germination. The development of *A. nubica* seedlings was influenced by MS medium strengths and sucrose concentrations. Full MS medium produced the highest shoot length and the highest vigor index with the addition of 20 g/L sucrose and the maximum root length with 50 g/L sucrose. Also, 30 g/L sucrose gave the maximum number of nodes and the maximum root/shoot ratio. However, ½-MS strength yields the highest seedling biomass with 30 g/L sucrose. This is the first successful report on the seed germination and seedling growth of *A. nubica in vitro*.

Keywords: *Acacia nubica*; dormancy; germination; *in vitro*; seedling; synchronization

1. Introduction

Acacia nubica Benth. (*Acacia oerfota* (Forssk.) Schweinf.) Leguminosae family, known in Sudan as Laout or El Ifein, a green-grey or whitish-green multi-stemmed shrub 1-5 m high with basal branching merged into an irregular or obconical crown [1, 2]. It is distributed through the arid and semi-arid zones or short grass savanna woodlands of Africa to Arabia, Persia and India in Asia [3]. In Sudan, known as Laout or El Ifein, *A. nubica* usually occupied dry-hard, bared and exhausted clay fields and rocky-dry slopes under 100–200 mm rainfall of Central and Northern parts [1,2]. It capable to germinate at temperatures ranging from 20 up to 40 °C [4].

Due to withstand of extremely harsh conditions, *A. nubica* is an indispensable component of agro-pastoral systems in dry areas and with exceedingly fundamental environmental roles. Rural peoples within the arid area where *A. nubica* occurs rely on the plant to provide their daily needs of medicines, gums, fuel, fiber, timber, fodder, veterinary practices, and apiculture system [5, 6]. Analysis of the plant parts showed high contents of crude protein, essential minerals, nitrogen degradation and dry matter digestibility with relatively low tannins contents [5,7,8]. In addition, it helps in improving soil fertility through nitrogen fixation and leaf litters as well as stabilizing soils against land degradation and desertification. The miscellaneous services delivered by *A. nubica* and its remarkable adaptability to harsh environments and growth ability in degraded lands make it critical in the semi-arid and savannah regions. However, in Sudan, due to human activities and climate change the natural habitats of *A. nubica* are severely deteriorated. That, *A. nubica* cover was decreased by 9.72% in North Kordofan, and stated to became infrequent

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in Gezira State [9,10]. Reforestation of these deteriorated lands demanded a fast and continuous supply of forest plantlets.

The natural reproduction of *A. nubica* are entirely through seedlings propagation. Seed germination at the establishment phase may be a critical step in determining population distribution [11]. The dynamic of seed germination of *A. nubica* is impede by several factors including hard seed coat that prevents water imbibition, beetles attack, and fungus infection [6]. About 7% of the total number of *A. nubica* seeds collected were being rotten or infested [12]. The water uptake by *A. nubica* seeds is very slow, therefore, seed require pretreatment mostly is scarification with sulfuric acid or boiling water. Moreover, the germination rate of *A. nubica* was reported to be low with maximum germination of 49% during five weeks [12]. Also, 55.5% germination was noted after 3 months from sowing giving *A. nubica* the second lowest value among six studied acacia species [11]. Also, during the early stages of establishment, higher deaths were recorded on *A. nubica* seedlings [6]. Among the six evaluated species, *A. nubica* seedling ranked the least with a mean survival rate of 20% at 190 days of sowing [2].

Plant tissue culture technology provides an alternative to conventional propagation and has the potential to provide high reproduction rates of uniform saplings in a short period of time, as well as the ability of afford stock plants for further multiplication. The first step in establishing *in vitro* plant culture of is to remove all microorganisms by surface sterilization of explants. The type and concentration of disinfectant used should ensure that the explant's viability and regeneration capacity are not affected during the sterilization process [13]. The most commonly used agent for surface sterilization of various plant species is sodium hypochlorite solution [13–15]. Routine *in vitro* propagation is associated with culture in a standard medium, such as Murashige and Skoog (MS) medium [16]. Different MS strengths can be generated by modifying the standard MS medium compositions, such as decreasing macro- and micronutrients. Half-strength MS medium is commonly used due to its low osmotic potential, which allows for easy adjustment of plantlets during in vitro germination and seedling growth. Sugars are important in *in vitro* cultures because they serve as a carbon source, an energy source, and an osmotic agent [17]. Sucrose is preferred over other sugars because it is the primary long-distance transport sugar in many plants [18]. Exogenous sucrose supplies allow plants to perform photosynthesis and respiration *in vitro*. The level of sucrose in the medium had a significant impact on the growth and development of seedlings cultured *in vitro* [19]. Similarly, sucrose was discovered to be essential for the survival of cultured embryos [19] and seed germination [20].

In vitro culture can provide a basis for *A. nubica* plantlets production and possible reintroduction programs. Solitary research on *in vitro* culture of *A. nubica* was done by Al-Khalifah [21], but it offers low-cut data. The present work aims to analyze the seed germination, emergence and early seedling performance of *A. nubica* under *in vitro* conditions.

2. Materials and Methods

2.1. Seed material and pretreatment

Mature seeds of *Acacia nubica* collected from Saata area, West Kordofan, Sudan, were stored in airtight glass bottles in an incubation room under 25±2 °C. Before sowing, the seeds were first cleaned by removing any damaged seeds, soil, or pod particles. Then they were soaked in sulphuric acid for 60 min to facilitate softening of the hard seed coat. Thereafter, treated seeds were washed under running water to remove all residues of sulphuric acid and kept in distilled water to promote imbibition.

2.2. Disinfection of seed explant

The pretreated seeds were surface sterilized, first with ethanol (70%), followed by 3-4 washes in sterilized distilled water. Then sodium hypochlorite (Clorox 0.5% free chlorine) solution was then bring the desired volume of detergent with aid of 2-3 drops of tween 20 and sterilized distilled water. The seeds were shaken in the disinfection mixture

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continuously for 10 min. Surface sterilized seeds were rinsed many times (4-5) with sterilized distilled water to remove residues of detergent. Then seeds were placed in a Petri dish on pre-sterilized soft tissue and left for a few minutes to absorb water of seed surface.

2.3. Medium and culture conditions

MS medium [16] was adjusted to a pH of 5.8 and solidified with 7.0 g/L agar before autoclaving at 121° C (1.1 kg/cm^2) for 15 min. One seed was inoculated per a screw-capped glass tube (20 × 3 cm) for the germination experiment. Cultures were kept in an incubation room at 25±2°C under darkness for 15 days, and seeds that germinated were individually exposed to a 16/8 h (light/dark) photoperiod. Germinated seeds were reserved in culture for further seedling development.

2.4. Experiments

Various experiments were applied to seeds to investigate their effects on the emergence rate, germination uniformity, and seedling development of *A. nubica*. The effect of sodium hypochlorite in different concentrations (5, 10, 15, or 100%) was studied to determine the best concentration for decontamination and the germination of seeds. Surface sterilized seeds were cultured on standard MS medium supplemented with 30 g/L sucrose. To examine the effects of sucrose on germination and growth of seedlings, seeds were inoculated into MS medium with different concentrations (10, 15, 30, 40, and 50 g/L). In another experiment, seeds were cultured on different strengths of MS medium including full-strength MS (1 MS), half-strength MS (½ MS), quarter strength MS (1/4 MS) and medium without MS salts (zero MS). The strengths of MS medium were prepared by reducing the medium contents to appropriate strengths except for sugar which used the concentration chosen as optimal in the sucrose experiment.

2.5. Data collection and analysis

Radicle protrusion to 5 mm is considered germination of seed. Germination was scored at an interval of 3 days for a total of 5 readings, or until the germination of all seeds. Germination and emergence responses were assessed with the germination curve, mean time to germination (MGT), and synchronization index (ZG). For each reading in all treatments, the cumulative germination percentage was calculated and plotted against time. MGT estimated the velocity of germination by measuring the time taken to a specific germination percentage. The synchronization index measures the degree in synchrony of germination among seeds; i.e., quantifying all seeds that germinated simultaneously. The value of the synchronization index would be between 0.0 (no synchrony) – 1.0 (complete synchrony). The higher value of ZG was applicable for uniform emergence. MTG and ZG were calculated as stated by Ranal and Santana [22]. Regarding measuring seedling development, the lengths of shoot and root, number of nodes, and seedling dry weight (DW) were measured, and root/shoot ratio and vigor index were calculated. Each treatment included thirty test tubes, representing three replicates, and repeated twice of total 60 seeds per treatment. Statistical analysis was performed using one-way ANOVA. Separation of treatment means was applied at the significance level of $P \leq 0.05$ using Duncan multiple range test (DMRT).

3. Results

3.1. Effect of sodium hypochlorite on seed germination

All the concentrations of NaOCl produced cultures free of contamination. However, the application of NaOCl significantly affected the percentage and pattern of germination. Higher seed germination percentage of 85% were obtained when seeds were treated with 10% NaOCl, and the lowest value recorded was 40% with 100% NaOCl (Figure 1A). Correspondingly, the germination pattern of *A. nubica* seed varied with the NaOCl concentration (Figure 1A). Thereby, for each concentration, the germination accelerated from the day of emergence to a point where it decelerated (final germination), then remained static

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until the end of the germination process (15 days). The point of deceleration for 5% and 15% NaOCl was day 9, and it was days 12 and 6 for 10% and 100% concentrations, respectively (Figure 1A). Nevertheless, 5% NaOCl exhibited faster seed emergence, as 50% germination was achieved at day four of sowing compared to day five for 10% and 15%, and the concentration of 100% does not reach 50% germination (Figure 1A). The faster emergence of seeds treated with 5% NaOCl was proven by a lower MGT value recorded of 2.43 days (Figure 1B). Also, 5% NaOCl induced the best uniformity germination, as indicated by the maximum synchronization index value of 0.46 (Figure 1C).

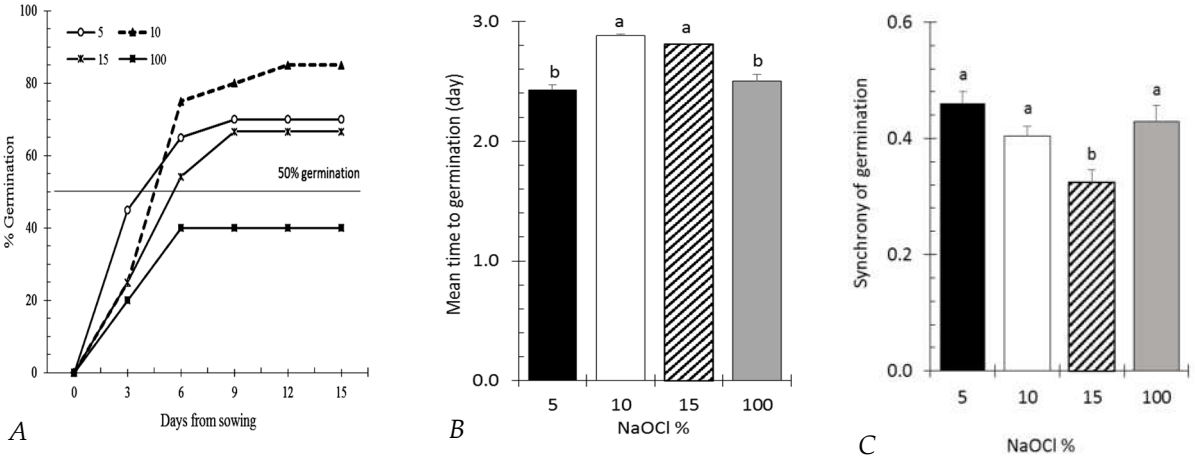

Figure 1. Seed germination of *A. nubica* as effected by sodium hypochlorite (5–100%) after 15 days of culture. *A*; germination curves, *B*; synchrony of germination, *C*; mean time to germination (days). Different letters above columns indicate significant differences at the $P \leq 0.05$ level using DMRT and bars indicate standard error of the mean. MS: Murashige and Skoog basal medium, 0 MS: water agar medium.

3.2. Effect of sucrose on seed germination

In the present study, the different levels of sucrose provided in full-strength MS medium significantly influenced the final germination percentage. The highest germination of seed (85.7%) was obtained on 30 g/L sucrose, and the lowest germination (57.1%) was observed on 50 g/L concentration (Figure 2A). On the other hand, the concentration of 10 g/L improved seed emergence rate as 50% germination reached earlier ≈ 3 days (Figure 2A), with the better uniformity as indicated by a high synchronization index of 0.56 (Figure 2B), and the faster germination with lower MGT to 2.0 days (Figure 2C). The pattern of germination through 15 days of culture was also affected by the concentration of sucrose, as the peak of final germination was reached 12 days for 10 g, 20 g, and 30 g, while it was 9 days for 40 g and 50 g.

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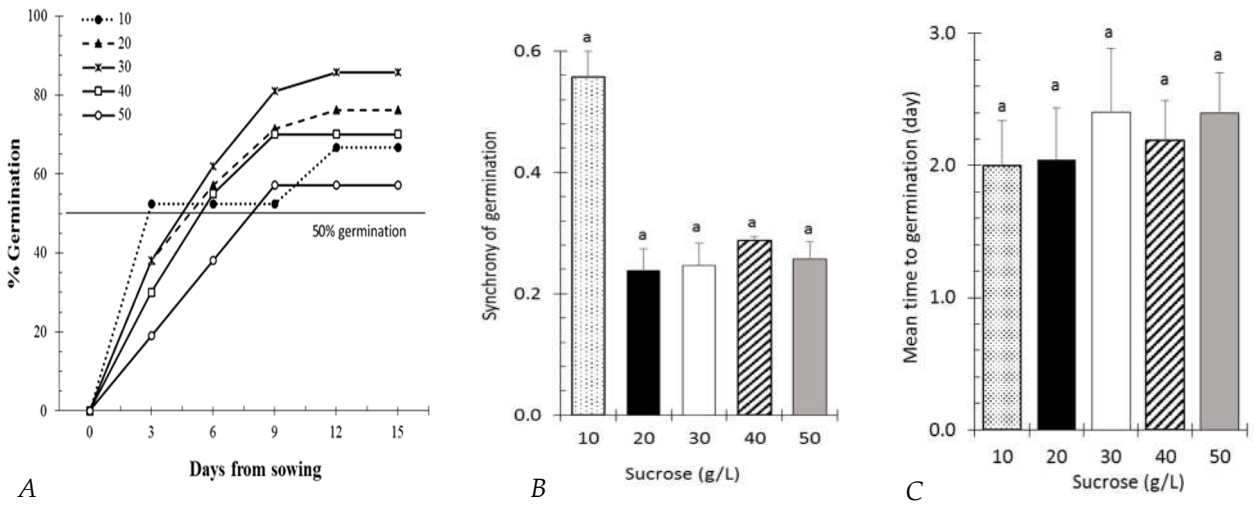

Figure 2. Seed germination of *A. nubica* as effected by sucrose (10-50 g/L) after 15 days of culture. A; final germination, B; synchrony of germination, C; mean time to germination (days). Different letters above columns indicate significant differences at the $P \leq 0.05$ level using DMRT and bars indicate standard error of the mean.

3.3. Effect of MS strength on seed germination

The strength of the MS medium influenced the *in vitro* seed germination of *A. nubica* significantly (Figure 3). The highest percentage of seed germination obtained was 90.5% on ¼ MS medium, while the lowest was 57.1% on the 0-MS treatment (Figure 3A). The level of 50% germination was scored after three days of sowing on all MS strengths except 0-MS, which needed four days. Figure (3A) demonstrated that the MS medium strengths influenced the germination pattern of *A. nubica* seed. In this manner, for each MS strength, the germination accelerated from the day of emergence to a point where it decelerated (final germination), and then the germination entered a stationary platform with no new emergence. The point of deceleration for 0 MS was day six; it was day nine for ¼-MS and full-MS, and day 12 for ½-MS (Figure 3A). The 0-MS strength also recorded more synchronized germination with the highest ZG value (0.39), although it was not significantly different from that obtained on full-MS medium (0.32) (Figure 3B). ¼-MS and ½-MS were almost similar in ZG value. The results on MGT (Figure 3C) exhibited an alike manner to ZG in that seeds sown on 0-MS strength medium germinated in 2.0 days, followed by full MS in 2.08 days, and the maximum was 2.84 days on quarter MS strength medium (no significant differences).

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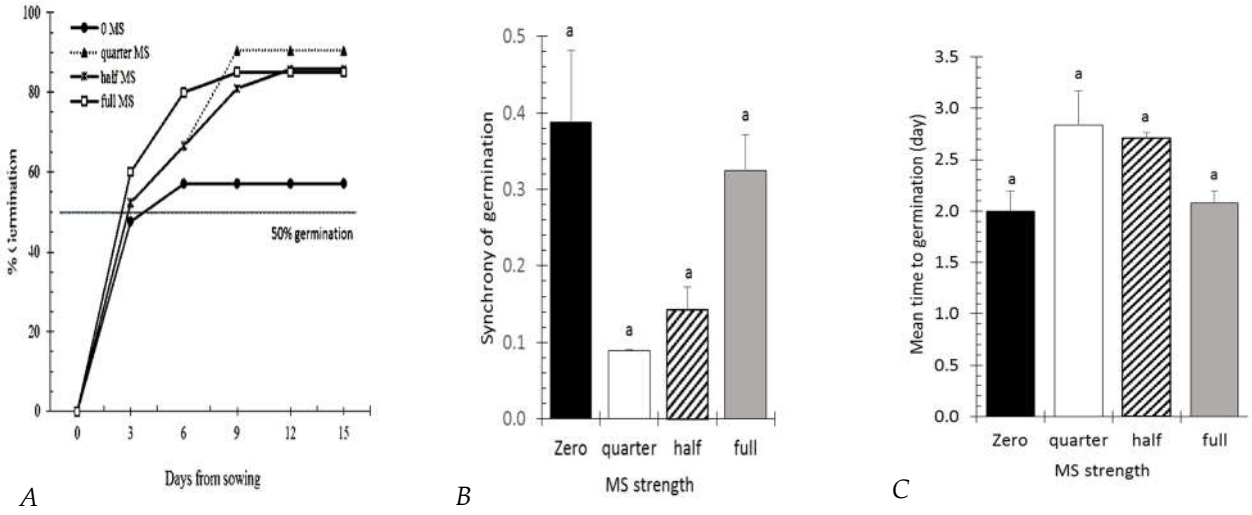

Figure 3. Seed germination of *A. nubica* as effected by MS strengths (zero–full MS) after 15 days of culture. *A*; germination curve, *B*; synchrony of germination, *C*; mean time to germination (days). Different letters above columns indicate significant differences at the $P \leq 0.05$ level using DMRT and bars indicate standard error of the mean. MS: Murashige and Skoog basal medium, 0 MS: water agar medium.

3.4. Effect of sucrose on seedling development

Seeds sown on full MS medium containing 20 g/L sucrose produced seedlings with the highest shoot length of 10.7 cm (Table 1). On the other hand, it appears that increasing the sucrose level enhanced root growth; therefore, the maximum root length (15.58 cm) was reported at 50 g/L. The effects of sucrose on shoot and root growth were significantly different only from the concentration 10 g/L. Sucrose at 20 g/L produced more vigorous seedlings with the highest vigour index (8.03), although not significantly different from that of 30 g/L (Table 1). 30 g/L sucrose had significantly the greatest effect on the number of nodes with the highest value (5.78 nodes).

Table 1. Effect of sucrose concentrations (10-50 g/L) on seedling growth of *A. nubica* after 3 weeks of culture.

Sucrose (g/L)	Shoot length (cm)	Root length (cm)	Vigor index	Number of nodes
10	7.58b (±0.32)	9.64b (±0.82)	5.68b (±0.24)	3.56b (±0.63)
20	10.70a (±0.71)	13.50a (±0.83)	8.03a (±0.53)	5.44a (±0.38)
30	9.08ab (±0.25)	13.56a (±0.76)	7.94a (±0.22)	5.78a (±0.47)
40	8.80b (±0.96)	13.76a (±1.14)	5.50b (±0.60)	4.78ab (±0.28)
50	8.30b (±0.54)	15.58a (±1.7)	5.19b (±0.34)	4.78ab (±0.55)

Values are mean (±standard errors). Mean values within the column followed by the different letter are significantly different at the 0.05% probability level using DMRT. MS – Murashige and Skoog basal medium.

3.5. Effect of MS medium strengths on seedling development

The various strengths of the MS medium influenced the development of *A. nubica* seedlings significantly (Table 2). In general, decreasing the strength of the MS medium negatively affected the measured seedling parameters. In this sequence, full MS medium produced seedlings with the maximum values of shoot length (9.29 cm), root length (13.37 cm), and root/shoot ratio (1.53). However, ½ MS medium produced the maximum seedling biomass (55.4 mg).

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Table 2. Effects of MS strengths on seedling growth of *A. nubica* after 3 weeks of culture.

MS strength	Shoot length (cm)	Root length (cm)	Root/shoot ratio	Seedling DW (mg)
0	4.30c (±0.22)	2.43c (±0.32)	0.58b (±0.08)	36.8b (±2.8)
¼	6.64b (±0.41)	9.00b (±0.67)	1.40a (±0.14)	51.4a (±3.3)
½	9.10a (±0.36)	12.13a (±0.67)	1.41a (±0.08)	55.4a (±4.2)
1	9.29a (±0.67)	13.37a (±0.53)	1.53a (±0.15)	54.9a (±2.4)

Values are mean (±standard errors). Mean values within the column followed by the different letter are significantly different at the 0.05% probability level using DMRT. DW – dry weight, MS – Murashige and Skoog basal medium.

4. Discussion

4.1. In vitro seed germination

In the present study, NaOCl affected the emergence, percentage, and uniformity of *A. nubica* seed germination depending on the concentration (Figure 1). The lower concentration of disinfectant (5%) was the best for emergence and uniformity of germination. Increasing the NaOCl concentration to 10% resulted in a higher final germination percentage. In contrast, 5% NaOCl was the best for *in vitro* seed germination of *A. sieberiana* with the maximum percentage achieved [14]. However, a substantial drop in all germination parameters was observed beyond this level. A similar negative effect of increasing the concentration of NaOCl on seed germination was reported on *Linum usitatissimum* [13], *A. sieberiana* [14], and *Ziziphus spina-christi* [15]. The level of seed dormancy can vary among seeds within a population, causing individuals germination to occur over several seasons. The concentration of 10% appeared to be suitable for eliciting seed germination over a wide term (Figure 1A). On the contrary, the concentration of 100% produced more regular germination because full emergence happened within a narrow period, i.e., six days (Figure 1A). This indicated water imbibition started at different occasions, then germination spread over time and ended on day 15. This arrangement of emergence limits uniformity of germination, spreading the chances of germination inability over several seasons. After studying 48 Acacia species, Burrows *et al.* [23] concluded that the lens part of the testa of pretreated seed delayed water from reaching the embryo, ensuring not all seeds in a population germinate after short rain events. In this process, some seeds of a single species in one treatment imbibe early, while others remain un-imbibed for a while before imbibing. In the next treatments on the seed germination of *A. nubica*, 10% NaOCl was utilized for the disinfection procedure because of the higher final germination achieved.

The present study revealed the effect of sucrose concentrations on the emergence and germination of *A. nubica* seeds (Figure 2). The lower concentration of sucrose (10 g/L) was the best for emergence, rate, and uniformity of germination. The increment in sucrose level enhanced the final germination percentage, with the highest value achieved by the standard sucrose concentration of MS medium (30 g/L). Improved seed germination using 30 g/L sucrose compared to low and high concentrations was also reported on another Acacia species, namely *A. sieberiana* [14], and on other tree species such as *Prunus armeniaca* [24], *Boswellia serrata* [25], and *Drypetes roxburghii* [26]. However, sucrose at 0–25 g/L revealed the same percentage germination of *Olea europaea* [23]. The pattern of germination was also affected by the sucrose level, as the lower and intermediate concentrations (10–30 g/L) revealed a peak of germination at 12 days, compared to 9 days at the higher concentrations (40 and 50 g/L). According to Xu *et al.* [20], intermediate sucrose treatment extended the peak time of germination, whereas higher sucrose concentration delayed the peak time of germination of *Brassica napus* seeds. Normally, seeds germination does not require an exogenous supply of sucrose because the contents of the reserves are abundant for energy supply at this stage. However, the outcome of sucrose existence in a medium might be linked to osmotic pressure consequences (i.e., water availability in the medium). Higher concentrations of sucrose result in a considerable decrease in the medium osmotic potential [17]. According to de Souza *et al.* [27], the germination of *Genipa americana* seeds necessitates the addition of sucrose for the maintenance of osmotic balance in the culture medium. To germinate, seeds must accumulate water potential during imbibition. The

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resulting decrease in water potential reduces the rate and capacity of the emergence and germination processes [28]. In the case of untreated seeds, the hard coat protects the embryo from water availability disturbances.

The different strengths of the MS medium have distinct effects on the studied parameters of seed germination (Figure 3). Quarter-strength MS gave the highest final germination percentage, but with less synchronized and slower emergence. While, the 0-MS treatment has the fastest and highest uniformity of emergence. Similarly, the reduction in MS salts in the cultivation medium was more efficient for the germination potential of *Genipa americana* seeds [27]. On the contrary, full-strength MS was better than other MS strengths for seed germination of *A. sieberiana* [14], *Drypetes roxburghii* [26], and *Psoralea corylifolia* [29]. The strengths of the MS medium also had an effect on the overall pattern of *A. nubica* seed germination (Figure 3). Thus, the peak point of final germination was reached early, at 6 days, by 0 MS medium (which has the lower final germination percentage), and it was reached late, at 12 days, by the other strengths. The patterns of germination depicted by MS strengths are possible indicators for uniformity of emergence. The deceleration point of germination represents the time in which all viable seed was germinated. As a result, the faster the deceleration, the more uniform the germination. The reason behind the high synchronized germination of seeds in the lower and higher MS-strength treatments is probably due to variances in salt concentrations.

In the current study, germination of *A. nubica* seed was achieved at a rate of 90.5% under lab conditions, comparable to the 90% achieved by Abulfatih [5], while only 4% was achieved *in vitro* [21], and only 55.5% [11], and 53% [30] were reported under *ex vitro* conditions. Moreover, the germination rate of *A. nubica* was reported to be low and improved from 43.5% at 20/15 °C to 49% when temperature raised to 30/25 °C [12]. For germination time, the maximum final germination was achieved after 12 days of emergence, compared to 8 days under lab conditions [4], 28 days *in vitro* [21], and 3 months *ex vitro* [11]. *In vitro* seed germination of *A. nubica* was fast, with a mean germination of 2 days compared to 5.8 days [2] and 9.3 days [6]. The seed germination pattern of *A. nubica* typically displayed a point of acceleration from the day of emergence to a point where germination decelerate (final germination), then static through 15 days of germination course. The timing of acceleration, deceleration, and stationary germination were affected by the concentration of treatments. Elhag [2] stated that the germination curve of *A. nubica* usually shows a preliminary delay in the establishment of germination, followed by a quick increase in the number of seeds that germinate, followed by a decrease in the rate of emergence. Al-Ghamdi *et al.* [6] found that *A. nubica* required 7.3 days to first emergence and 13 days for 50% emergence. Physical dormancy resulting from a thick seed coat is an adaptive mechanism for delaying seed emergence and asynchronous germination during growing under dry habitats. Considering the thickness of the seed coat, *A. nubica* ranked sixth out of the seven acacia species studied [4]. This reproductive strategy increases long-term fitness by preventing the mortality of the entire seed cohort in a dynamic seed bank during adverse conditions, provided seeds survive through multiple seasons of waiting for favorable conditions. The seed coat thickness of the *Acacia* species is highly associated with the water availability in their habitat [4].

4.2. Growth and development of A. nubica seedling

A sucrose concentration of 10–50 g/L is generally used for *in vitro* tissue culture since it is also synthesized naturally by the tissue. The various concentrations of sucrose affected considerably the early growth and further development of *A. nubica* (Table 1). The concentration of 20 g/L increased the maximum length of the shoot to 10.7 cm after 3 weeks of culture. Al-Khalifah [21] reported that *in vitro* seedlings of *A. nubica* reached a height of 2.6 cm after 28 days of culture on full MS. At the nursery, shoot lengths of 21.8 cm were reported at the age of 3 months [2] and 32.3 cm at the age of 4 months [6]. In the current study, shoot length was improved with increasing sucrose to an optimal level (20 g/L), but decreased with rising sucrose levels. Similar trends in sucrose concentrations were

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also reported on *Prunus armeniaca* [24] and *Olea europaea* [19]. Shoot length of *A. sieberiana*, on the other hand, increased gradually with increasing sucrose concentration up to 50 g/L [14]. As a carbon source consumed for energy, the presence of sugar in culture is essential for all cellular activities. Despite the fundamental role of sugar in the growth of plant cells, high levels inhibit growth and development [19]. Sucrose had a negative effect on autotrophic growth because it inhibited chlorophyll formation as long as photosynthesis was active [17]. This notion was consistent with the finding of the present study, which showed that the shoot length of *A. nubica* seedlings decreased steadily with the increment in sucrose level (Table 1). Also, this might be attributed to the fact that increasing sugar concentrations in the medium lead to rising the osmotic pressure in the medium, which limits water availability for the plant. At 25 °C, the osmotic potential of sucrose solutions in concentrations ranging from 10 to 50 g/L is between -0.074 and -0.461 MPa [17]. Such a situation employ a drought condition within the culture medium, therefore the plant devotes more energy to root growth, as demonstrated by the fact that root length increased with increasing sucrose concentration (Table 1). The adjustment concerning the necessity of sugars for the growth of plants and their suppressive effects is poorly defined [18].

The *A. nubica* root, in contrast to the shoot, grows with increasing sucrose concentration (Table 1). Alike results were also reported on *Prunus armeniaca* [24], where roots were enhanced with intensifying sucrose concentrations up to 50 g/L. Moreover, although lower concentrations of sucrose have been used (0–25 g/L) for *Olea europaea*, root growth was improved with increasing the concentration [19]. Differently, on *A. sieberiana* [14] and *Brassica napus* [20], the root length was improved with the application of an intermediate level of sucrose, and the high levels inhibited root growth. In the present study, a maximum root length of 15.6 cm was recorded after 3 weeks of culture. At 3 months, *A. nubica* seedlings in nursery conditions exhibited root length up to 23.2 cm [2].

When compared to other concentrations, the 20 g/L sucrose produced seedlings with greater vigor growth of *A. nubica* (Table 1). Nonetheless, 30 g/L yield seedlings with the maximum number of nodes per shoot. Similar results have been reported by Garcia *et al.* [19] on the *in vitro* establishment of *Olea europaea* seedling. The standard concentration of 30 g/L sucrose is commonly recommended in MS medium.

The results on the influences of MS strengths on *A. nubica* seedling development are presented in Table 2. In this experiment, the energy source required for photomixotrophic growth; i.e., sucrose, is equal for all strengths of MS. Therefore, the variations in seedling parameters were results of the basal strength provided in the medium. It has appeared that seedlings grown on full MS medium were well developed and recorded higher values of shoot length, root length, and root/shoot ratio. While the ½-MS medium yielded seedlings with a high dry weight (slightly higher than the full MS). Similarly, full MS medium produced the longest shoot compared to other media on different species seedlings such as *A. sieberiana* [14], *Psoralea corylifolia* [29], and *Taxus baccata* [31]. In contrast, half-strength MS was found better for improving root length than full MS on *A. sieberiana* [14] and *Taxus baccata* [31]. Likewise, zero MS produced the longest shoots of *Grewia tenax* compared to other MS strengths [32].

As presented in Table 2, *in vitro* grown *A. nubica* seedling displayed a root/shoot ratio of a minimum of 0.58 recorded by distilled water free of MS salts and a maximum of 1.53 by full MS strength, i.e., high salt content. A root/shoot ratio of 0.67 was recorded for *A. nubica* grown at the nursery [2]. According to Bebawi and Mohamed [11], because *A. nubica* species are found in relatively drier habitats, when exposed to a short irrigation period, they produce a higher yield of root + shoot dry weight than the other five *Acacia* species found in humid habitats. Generally, trees in arid land have a high root-to-shoot ratio because a lower root-to-shoot ratio causes a high potential for evapotranspiration. In the current study, *A. nubica* yielded a maximum dry weight of 55.4 mg per seedling compared to 6.6 g of *ex vitro* seedlings at 4 months age [11]. A higher proportion of roots can help plants improve the soil's nutrient uptake, while those with a higher proportion of shoots can collect more light energy.

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5. Conclusions

The above results on *in vitro* seed germination of *A. nubica* suggest that for rapid and uniform emergence, 5% sodium hypochlorite was more effective, while in terms of germination percentage, 10% was the best. The sucrose concentration of 10 g/L was sufficient to induce uniform and fast emergence; however, 30 g/L was required to obtain a higher germination percentage. Water agar medium (zero MS strength) was sufficient to induce uniform and rapid emergence, and only the quarter constituents of MS medium are required to get a higher percentage of seed germinated. Full MS medium strength is superior to other strengths in enhancing the growth of *A. nubica* seedlings up to the age of 3 weeks. The modification of sucrose concentrations, on the other hand, has an important interference. That is, 20 g/L is profuse for a longer shoot and vigorous growth of *A. nubica* seedlings, 30 g/L to increase the number of nodes, and 50 g/L for longer roots. The results revealed that the *in vitro* establishment of *A. nubica* could provide a reliable source of seedlings for reforestation projects and for further *in vitro* culture programs.

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