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RESEARCH ARTICLE

Effect of sucrose concentration on micropropagtion of ginger (*Zingiber officinale* Rosc.)

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Abstract

Ginger is the most expensive and popular medical and spicy plant in Libya. However, local cultivation has not been documented. The main objective of this study is to optimize the sucrose concentration in the culture medium to achieve a better response of ginger micropropagation. Rhizome sprouting buds of ginger were sterilized and cultured four weeks on semisolid Murashige and Skoog (MS) medium supplemented with 3 mg/L BAP (6-benzyl- amino-purine). The effect of sucrose concentration was studied by using four different concentrations (30, 60, 90, and 120 g/L) for shoot and root induction. Results showed that sucrose concentration at 30 g/L gave the best results regarding shoot length and number as well as root length. Furthermore, the results demonstrated that root number was not affected by sucrose level in the medium. The percentage reduction of shoot number, shoot length, and root length, respectively, were 12%, 43%, and 29% at 60 g/L sucrose and 53%, 34%, and 10% at 90 g/L sucrose, and 48%, 68%, and 42% at 120 g/L sucrose. Correlation analysis for the relationship of shoot growth parameters and root length illustrated that reverse correlation existed between these parameters and sucrose level. Plantlets were acclimatized before transplanting, and high survival rate (85%) was recorded. The findings of our study significantly add to exiting knowledge regarding the micropropagation of the ginger and may find useful to the large scale production of the ginger plantlets through tissue culture.

Key words: Ginger, effect, sucrose, micropropagation, roots

Introduction

Ginger (*Zingiber officinale*), the spicy and aromatic rhizome, is one of the most commonly used spices and herbal medicine in the world due to its health promoting properties (Nafi *et al.*, 2014), it belongs to the family Zingiberaceae.. The plants are grown as an annual crop, they are rhizomatous, and aromatic herbs often of large size, bearing flowers either terminally on aerial leaf shoots or from ground level.

These are plants of tropical and subtropical regions. Several authors have mentioned different figures for the total number of genera and species, but it is reasonably appropriate to estimate the world record to be at least 51 genera and 1500 species (Newman, 2001). Ginger sexual propagation and breeding cannot be easily attained due to poor flowering and seed set. Therefore, improving seed propagation and breeding is highly desired. The development of female parent of Sorghum for instance has been reported (Maiga *et al.*, 2021). Ginger is usually propagated vegetatively through rhizome, which produces only about 12 lateral buds in a season of 9 months (Kambaska and Santilata, 2009). Moreover, vegetative propagation of ginger has a high risk of spreading infections. Slow propagation rate and the risk of disease transmission by sectioning of the rhizomes have deprived propagation by conventional means. Therefore, plant tissue culture is considered the best alternatives method that may supply many planting materials (Hamirah *et al.*, 2010). The in vitro technique's success largely depends on the aseptic culture establishment, shoot regeneration capacity, rooting, and acclimatization. The main components of plant tissue culture media are minerals salts and sugar as carbon source. Sugar in culture medium is considered the sole carbon source for the growth of cells, buds, shoots, and even plantlets. Sugars enter the metabolic pathways and transformation of energy which are required for growth of cells (George, 1993). Sucrose is the most common carbohydrate found in phloem sap and involved in controlling various development processes (Gibson, 2000). An interesting fact regarding sucrose responsiveness is that a specific concentration of sucrose is needed for plant development. It has been proposed that plants respond to varying sucrose content by undergoing morphological and anatomical variations along with regulating the expression of various genes via a variety of signal transduction pathways (Calamar and Klerk, 2002). The previous studies' varying results indicated that sucrose and plant growth regulators need to be optimized in the culture medium to achieve a better response of the ginger micropropagation. Ginger is the most expensive and popular spicy in Libya, however, its micropropagation has only been studied lately by our team (Estouka *et al.*, 2021), when we established a successful protocol for direct in vitro regeneration of *Zingiber officinale* Rosc. Therefore, the main objective of the study was to determine the optimum concentration of sucrose for shoot multiplication and rooting.

Materials and methods

Plant material and culture conditions

The experiment was carried out at the National Research Center of Biotechnology, Tripoli, Libya in 2021. Rhizomes of *Zingiber officinale* Rosc were imported from China by Mecca Trade Company (Cairo-Egypt). Healthy rhizomes were kept in the dark 15 days for sprouting. The sprouted rhizome buds were collected in beaker and kept under running tap water for 10 minutes prior to sterilization in the laminar airflow cabinet, then were chopped to fragments and surface-sterilized in 70% (v/v) ethanol for 2 minutes. For explants sterilization, 3% of Sodium hypochlorite (NaOCl) were used for 20 minutes of exposure. After rinsing three times with sterile distilled water, the shoots were cut apart from the rhizomes and trimmed to a final size of 1 to 2 cm, each explant was cultured in an individual jar. The explants were cultured in Murashige and Skoog (1962) medium supplemented with 3 mg/L BAP, 30 g/L sucrose and 7 g/L agar. In order to study the effect of sucrose concentration on plantlets growth and development, the medium was modified using different sucrose concentrations (30 (control), 60, 90, and 120 g/L).

The pH of the medium was adjusted to 5.8 prior to autoclaving at 121ºC and 100 kPa for 20 min. The cultures were kept in a growth chamber at a temperature of 25±1ºC with a 16-h photoperiod under an irradiance of 45 µmol/m2/s provided by cool white fluorescent light. After four weeks, plantlets were subjected to two weeks of in vitro acclimatization, then the rooted plantlets were carefully taken out of the jars and the roots were washed under running tap water. Data related to number of shoots per explant, shoot length (cm), root length (cm) and number per shoot were recorded. Plantlets were then placed in small pots containing beat-moss for ex vitro acclimatization. These pots were covered with polythene transparent bags and sprayed with water 2 to 3 times a day to ensure high humidity around plantlets. The plastic bags were punctured and gradually removed to expose the plantlets to the outside environment; greenhouse temperature and relative humidity were about 25±2°C and 70%, respectively. Plantlets were kept in the greenhouse four weeks then the survival rate was determined. Experimental design and data analysis: The design used was a completely randomized design. Eleven replicates per treatment were used; each treatment consists of three explants. Data was analyzed statistically by SAS procedure of the General Linear Model of ANOVA, mean separation was analyzed by Duncan´s multiple range test at 5% level of significance.

Results and discussion

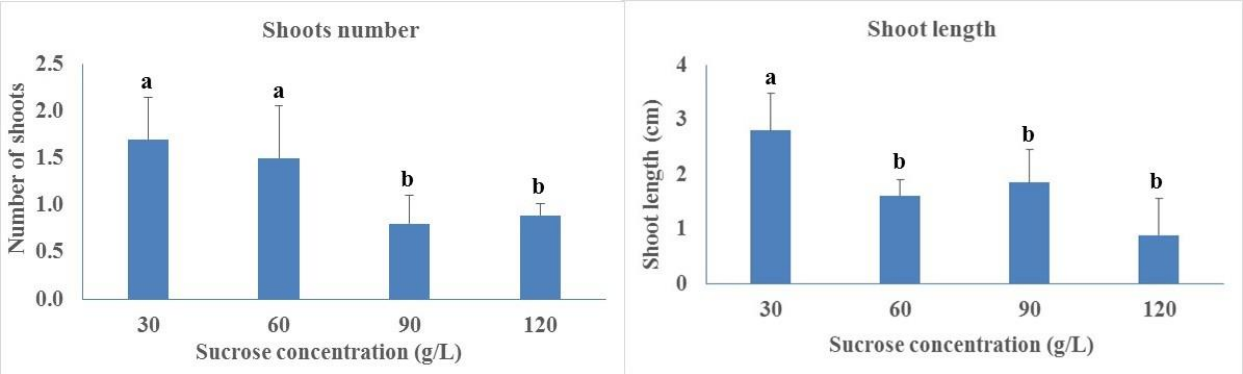
Effects of sucrose level on shoots proliferation

The MS-sucrose concentration directly influenced in vitro ginger explants growth. Different concentrations (30, 60, 90, and 120 g/L) of sucrose in MS medium were used for the growth of rhizome sprouted bud of *Zingiber officinale* Rosc. Increasing sucrose level in the medium more than the normal concentration (30 g/L) of MS medium showed diminution in shoot number, and length at all sucrose levels. This reduction was significant with the exception of shoot number of plantlets grown on MS supplemented with 60 g/L sucrose, where the diminution is not significant compared to control (Figure 1). The percentage reduction of shoot number and shoot length, respectively, were 12%, 43% at 60 g/L sucrose and 53%, 34% at 90 g/L sucrose, and 48%, 68% at 120 g/L sucrose. In tissue culture, explants could not conduct photosynthesis processes as plants that are cultivated on open field, so they require additional carbohydrates. Ramage and Williams (2001) elucidated that the differences in growth regulators concentration in tissue culture cannot control plants development without carbohydrates supplementation. These carbohydrates have an essential role in the process of shoot proliferation and affect shoot growth and survival. The use of sucrose with optimal concentration will ensure the availability of carbon sources for the cell to grow. If the carbon source is sufficient, the cells will form quickly, so that the explant is able to grow shoots (Sitorus *et al.,* 2011). However, high sugar concentrations inhibits plantlets growth due to osmotic stress in the medium (Jo *et al.,* 2009).

Effects of different sucrose level on root induction

Number of roots was not affected by the increase in sucrose concentration of more than 30 g/L in in vitro culture medium (Figure 2). Increasing sucrose level in the medium more than the normal concentration (30 g/L) of MS medium lead to reduction in root length at all sucrose levels. However, this reduction was significant only in plantlets grown on MS medium supplemented with 120 g/L sucrose. The percentage reduction of root length respectively, were 18%, 6%, and 41% at 60, 90, and 120 g/L sucrose concentration in the medium. We expected not to perceive any improvement in root parameters with increasing sucrose since MS complete medium is very rich medium.

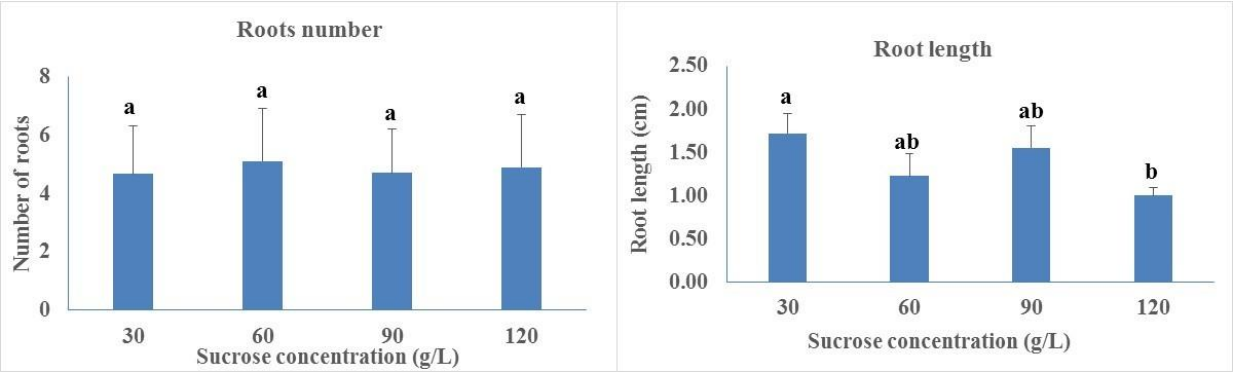
Fig. 1: Effects of different sucrose concentrations (g/L) on shoot proliferation of rhizome bud of Zingiber officinale Rosc after 4 weeks of culture. Values are means ± SE (n = 11), and bars indicate SE. Columns with different letters indicate significant differences at P = 0.05 (Duncans test).



Correlation analysis for the relationship of shoot growth parameters and root length showed that reverse correlation existed between these parameters and sucrose level. In plant tissue culture, photosynthesis is insufficient or could be absent, since growth taking place in unsuitable conditions, therefore, sugars additional to the medium is necessary. The sugar concentration chosen is highly depended on the type and age of growth material; very young explants require a relatively high sugar concentration. In general, the growth and development increase with increased sugar concentration until the optimum is reached, and then decreased at higher concentrations. Effect of sucrose concentration on proliferation of *Zingiber officinale* Rosc was examined by Inden *et al.*, (1988). The authors of the study found that the maximum promotion effect of sucrose on shoot and root formation of rhizome buds was at 40 g/L, shoot and root formation was reduced when sucrose concentration either decreased to 20 g/L or increased to 50 g/L.

Among the sugars, sucrose is commonly used as the main carbon source, since it is the most common carbohydrates in the phloem sap of many plants (Gibson, 2000). Sucrose also supports the maintenance of osmotic potential and the conservation of water in cells. However, high sucrose level in the medium restricts the photosynthetic efficiency of cultured plantlets by reducing chlorophyll levels, key enzymes for photosynthesis and apicultural waxes promoting the formation of structurally and physiologically abnormal stomata (Hazarika, 2006). It has been reported that the initial concentration of sucrose can affect growth and biomass accumulation. In contrast, the development of cultured cells can be retarded by higher amount of sucrose by causing a termination of the cell cycle when nutrients are limited or under osmotic stress (Wu et al., 2006). Many researches on ginger illustrated that using a sucrose concentration of less than 3% suppressed plantlets growth and development. For instance, Zhou *et al.*, (2008) examined three different sucrose concentrations (0%, 1%, 3%), and their results highlighted that as the sucrose concentration decreased, the growth of the plantlets of two ginger cultivars was restrained. Furthermore, Chaidir *et al.*, (2019) tested three sucrose concentrations (20, 40, and 60 g/L) for in vitro ginger micropropagation, and they reported that 40 g/L sucrose was the best concentration and produced optimal growth of ginger shoots. The use of 40 g/L sucrose had been able to meet the needs of carbon as an energy source for explants, so the best shoot growth of ginger explant can be generated.

Fig. 2:Effects of different sucrose concentrations on root induction of rhizome bud of *Zingiber officinale* Rosc after 4 weeks of culture. Values are means ± SE (n = 11), and bars indicate SE. Columns with different letters indicate significant differences at P = 0.05 (Duncans test).



After 2 weeks of in vitro acclimatization, the derived plants were acclimatized for two weeks under ex vitro conditions in the greenhouse, where plantlets were transferred to pots containing peat-moss taking special care not to harm the roots and moistened uniformly at periodic intervals. About 85% of the regenerated plantlets tolerated and survived under ex vitro environment in greenhouse conditions. Some of plantlets were lost owing to less adaptive abilities for photoautotrophic and/or lowing of relative humidity during acclimatization in ex vitro conditions. Many similar observations have been reported in previous studies. For example, Preece and Sutter (1991) reported that plantlets growing in vitro exhibit low photosynthetic capacity, and during acclimatization, there is a necessity for quick transition from the heterotrophic to the photoautotrophic state for endurance. Pospisilova *et al.*, (1999) demonstrated that photosynthetic parameters are very important for the growth of in vitro plantlets during transition period to greenhouse. Hence, transfer of plantlets from in vitro to ex vitro condition has to be carefully handled especially in term of photosynthetic and humidity considerations. Therefore, researchers, in general, focused on these features to reach high survival rate.

In conclusion, sterilized rhizome sprouting buds of ginger (Zingiber officinale Rosc.) were cultured four weeks on semisolid Murashige and Skoog (MS) medium supplemented with 3 mg/L BAP (6-benzyl- amino-purine) and four different sucrose levels (30, 60, 90, and 120 g/L) to study the effect of sucrose concentration on shoot and root induction. The results highlighted that using the normal sucrose concentration (30 g/L) in MS medium for ginger (*Zingiber officinale* Rosc) micropropagation presented the best plantlets growth and development specially shoot length and number as well as root length. In comparison to the optimal concentration of sucrose in media (30g/L), the percentage reduction of shoot number, shoot length, and root length, respectively, were determined to be 12%, 43%, and 29% at 60 g/L sucrose and 53%, 34%, and 10% at 90 g/L sucrose, and 48%, 68%, and 42% at 120 g/L sucrose. These findings illustrate that increasing sugar concentration behind the conventional concentration (30g/L) suppressed shoot proliferation and root induction. Furthermore, in order to justify the results of our study, the plantlets were acclimatized before transplanting and a significant survival rate of 85% was obtained.

In conclusion we propose that further studies need to be taken in account to obtain the optimal components and their combination for higher shoot and root multiplication rate of ginger. It is recommended to assess other *Zingiber officinale* cvs to establish simple and economical protocols for their micropropagation. The studies will further aid the success of field culture and increase production ginger in Libya and other lands of similar conditions.

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