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Influence of Sucrose On Germination and Seedling Growth of Wheat (*Triticum aestivum* L.) In Vitro Condition

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ABSTRACT

A study was carried out to determine the effect of different concentrations of sucrose because of its great importance in supplying plants with their needs of carbon and energy.From this study it was shown that the extent of its effect on the efficiency of the construction process photosynthesis and the consequent increase in the content of starch and soluble proteins in the wheat plants. Furtrhermore, it was found that adding sucrose to the nutrient medium at a concentration of 15% had a very significant effect in improving the content of optical pigments from chlorophyll a (0.72 mg g^{-1} FW), chlorophyll b (0.56 mg g^{-1} FW) and carotenids (1.27 mg g^{-1} FW). It had also a great effect on starch and protein content 147 mg g^{-1} FW and 118 mg g^{-1} FW respectively when measured in seedlings of wheat plants at the age of 21 days compared to the control.

تأثير السكروز على إنبات ونمو شتلات القمح (*Triticum aestivum* L.) فى حالة المختبر

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تأثير السكروز على إنبات ونمو بادرات القمح (*Triticum aestivum* L.) في المختبر. في هذه التجربة ، أخترنا دراسة تأثير تراكيز مختلفة من السكروز لما له من أهمية كبيرة في إمداد النباتات بأحتياجاتها من الكربون والطاقة ، وكذلك لكثرة جدل التوصيات العديدة التي قدمت في هذا المجال ، بما في ذلك تلك الموصى بإضافة السكروز إلى المغذيات وبعضها لم يوصى بذلك. ولهذا الغرض قمنا بهذه الدراسة لمعرفة ما مدى تأثير السكروز على كفاءة عملية البناء الضوئي وما يترتب على ذلك من زيادة في محتوى النشا والبروتينات الذائبة في نبات القمح. ووجدنا أن إضافة السكروز إلى وسط المغذيات بتركيز 15٪ كان له تأثير معنوي جداً في تحسين محتوى الأصباغ الضوئية من كلوروفيل أ(0.72) و ب (0.56) والكاروتينات (1.27) ملجم لكل جرام وزن طازج .كما كان له تأثير معزز على محتوى النشا والبروتين 147و 118 (ملجم لكل جرام وزن طازج) على التوالي عند قياسه في شتلات نباتات القمح عند عمر 21 يومًا مقارنة بالمجموعة الضابطة.

INTRODUCTION

Sucrose is one of the important sugars, and is the major carbon form translocated in higher plants. It has multiple functions such as regulating my process photosynthesis, respiration, developmental processes and acts as a storage compound and helps to maintain the osmotic pressure in the cytosol, especially during stress (Rita, 2005). Sucrose plays an important role during germination and seedling development, where it is transferred to the roots and plant

growth promotion (Eastmond, 2006). Seed germination is a basic factor which contributes for the crop yield. In addition, seed germination stage is the initial and delicate stage in the plant life cycle. It is greatly influenced by any external addition as well as environmental factors (Soltani et al., 2008).

Wheat (*Triticum aestivum* L.) is an important cereal crop used as staple food in many parts of the world and is a moderate salinity tolerance crop. Wheat is an essential food for humans, and it is one of the most important sources of carbohydrates in most parts of the world. It is involved in many food industries and wheat ranks first among other crops (rice, corn and potatoes) in terms of area and production at the global level (MOA, 2013).

Hence, this study was conducted to determine the optimal seed germination by assessing the role of sucrose in germination and seedling development by investigating some of the physiological and biochemical characteristics of wheat tissues.

MATERIALS AND METHODS

Plant Materials

The wheat seeds used in this study were purchased from local market in Al Bayda – Libya. The seeds were identified and authenticated as *Triticum aestivum* by Botanists in Department of Botany, Faculty science, Omar Al-Mukhtar University, Al Bayda – Libya.

Sterilization and germination

Mature seeds of Triticum *aestivum* were sterilized according to Khadija and Rabha (2017). Sterilized seeds were placed on half basal of MS medium (20 ml) with 0.1 g l^{-1}myo-inositol, 5 g l^{-1} agar and deferent conentrations of sucrose (5, 10,15,20, 25, and 30 g l^{-1}) were tested for *in vitro* condition. Culture medium without sucrose as control than the pH of the culture media was adjusted to 5.8. Six seeds were grown in glass culture vessels and all cultures were incubated under a 16/8 h light \dark at a temperature of 25±1Cº.

Estimation of photosynthetic pigment contents

The photosynthetic pigment was extracted from the *Triticum aestivum* plant Leaves (0.2 g) disks using the Hiscox and Israelstam (1979) procedure. Each disc was cut into smaller pieces and placed in a test tube containing 10 mL of dimethyl sulfoxide (DMSO). All samples were incubated at 70ºC for 30 min. Extract was centrifuged at 5,000 rpm for 15 min and absorbance was recorded at 646 and 663 nm for chlorophyll (*a* and *b*) estimation and at 470 nm for carotenoids. Pigment content was calculated according to the following formulae as reported by Lichtenthaler and Wellburn (1983):

Chlorophyll *a* = 12.25 A_{663} – 2.79 A_{646}
Chlorophyll *b*= 21.21 A_{646} – 5.1 A_{663}
Carotenoids = (1000 A_{470} – 1.8 Chl *a* – 85.02 Chl *b*) / 198

Extraction and estimation of starch

Estimation of starch was carried out following by McCready et al., (1950) method. Sample (0.1 g) from dried sugar free pellet following by Angelov et al., (1993) method were suspended in 2.5 ml of distilled water and subsequently 3.5 ml of 52% (v/v) perchloric acid (PCA) was added to the residue after stirring the mixture, the content was centrifuged for 15 min at 4,000 rpm. The supernatant was decanted, collected and the whole procedure was repeated twice. Supernatant of each step was then hydrolysied poured and the total volume was made up to 15 ml with distilled water. After filtration, 1.0 ml of the aliquot of this filtrate was analyzed for starch content following the same procedure as that of total soluble sugars. Quantity of starch was calculated in terms of glucose equivalent. The quantity of starch was expressed mg glucose/g DW.

Estimation of total soluble proteins

The total soluble protein measurement was conducted according to the method described by Hartree (1979).

Statistical analysis

The test of least significant using difference (L.S.D) at the level of 0.05% significance was used to examine differences among treatment means and interactions. Data were statistically analyzed using MSTAT-C software package according to the method described by Freed et al., (1989).

RESULTS AND DISCUSSION

Changes in photosynthetic pigments

The content of photosynthetic pigments (chlorophyll a, b and carotenoids mg g^{-1} FW) in *Triticum aestivum* leaves were significantly higher with different concentrations of sucrose compared to control (**Table** 1). Sucrose-treated plants (15%) caused a high significant increase in photosynthetic pigments compared to untreated plants (72 and 56 mg g^{-1} FW chlorophyll a, b, respectively). The recorded data indicated that the use of sucrose with different concentrations had been successful in improving the content of pigments in plant leaves. The recorded data in **Table** 1 showed that the application of sucrose enhanced the biosynthesis of carotenoid in the treated plants and this increment was greater at plants treated with 15% of sucrose (1.27 mg g^{-1} FW).

Changes in starch contents

The changes of starch contents of *Triticum aestivum* shoots of regenerated plantlets are

shown in **table** 2 . High significant correlation had been showed between the photosynthetic pigments content and carbohydrate fractions (total starch). However, cultured medium amendment with 15 and 20% of sucrose enhanced the synthesis of soluble sugars, which lead to increase in starch content. On the other hand, increasing of sucrose in the culture media up to 30% significantly reduced the tested carbohydrate fractions referred to control.

Changes in total soluble protein

The effect of various concentrations of sucrose on the total soluble protein of *Triticum aestivum* plantlets leaves is represented in **table 2**. The results indicated that the high concentrations of sucrose (25 and 30%) caused high significant decrease in the total soluble proteins content of plantlets shoots below those of untreated ones (28.09 and 31.07 mg g^{-1} FW). On the other hand, the low concentration of sucrose (10 and 15%) caused opposite pattern of change in shoot of Triticum *aestivum* plantlets.

Table 1: Effect of exogenous different consternation of sucrose (%) to MS culture media on photosynthetic pigments chlorophyll *a*, chlorophyll *b* and carotenoids of leaves of *Triticum aestivum* regenerated plantlets at two weeks harvesting time after application.

Parameters / consternation of sucrose (%)	Photosynthetic pigments (mg g^{-1} FW)		
	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
control	0.20e	0.11d	0.35f
5	0.42d	0.28c	0.61d
10	0.25e	0.16d	0.45e
15	0.72a	0.56a	1.27a
20	0.70a	0.49b	1.11b
25	0.66b	0.32c	0.70c
30	0.59c	0.30c	0.65cd
LSD at 5%	0.62	0.064	0.085

Means having the same letters in a column were not significantly different at $p<0.05$

Table 2: Effect of exogenous different consternation of sucrose (%) to MS culture media on starch and total soluble proteins (mg g-1 FW) of leaves of Triticum aestivum regenerated plantlets at two weeks harvesting time after application.

Parameters / consternation of sucrose (%)	starch	proteins
control	72.50c	99.03c
5	62.17d	87.32e
10	55.07e	118.12a
15	147.67a	118.22a
20	101.51b	112.21b
25	72.50c	28.09d
30	60.18d	31.71f
LSD at 5%	0.44	0. 1

Means having the same letters in a column were not significantly different at $p<0.05$

DISCUSSION

Sucrose plays a role as a signaling molecule that regulates a variety of genes operating in the photosynthetic system and the building blocks of many compounds such as amino acids and proteins (Koch, 2008). An increase in the content of photosynthetic pigments leads to an increase in the rate of photosynthesis in plants, which is attributed to the role of sucrose in modifying the plasma membrane (Jang, 1997), and thus increases the absorption of nutrients, as well as improving metabolic activities (Araki, 2001).

The effects of sucrose on germination differ according to the concentration and the genotype of the plants, as low concentrations had a weak effect while medium concentrations succeeded in promoting germination, and on the contrary, high concentrations negatively affected the germination process and its delay, indicating that sucrose promote or prevent healthy germination depending on Focus This is what our study and many other studies confirmed. The 15% concentration of sucrose was significantly better at improving the efficiency of photosynthesis and the content of starch and protein pigments. In contrast, elevated levels of sucrose (25 and 30%) in the nutrient medium impeded hypocotyl elongation in the dark (Jang et al., 1997) and induced light in Arabidopsis seedlings (Dijkwel et al., 2010; Jang et al, 2012). Application of Sucrose medium concentrations (15 and 20 %) of the concentrations used increased the total chlorophyll contents and its fractions in *Brassica juncea* (Goren et al., 2011). Previously Fujii *et al*., 2010 also reported that photosynthetic pigments in *Hibiscus sabdariffa* were increased by sucrose. In addition, Sucrose was protected pigment-protein complexes resulting in decreased degradation of chlorophyll. However, sucrose treatments with high concentration *in vitro Triticum aestivum* plantlets resulted in Significant decrease in chlorophyll *a*, *b* and carotenoids content compared to the control. The main functions of soluble polysaccharides are osmotic regulation and carbohydrate storage and polysaccharides can cause cell membrane and protein stability. This can be done by forming hydrogen bonds between carboxylic protein groups and the polar sugar chain (Koster and Leopold, 1988). It was found that the sucrose content increased in tomato (Solanum lycopersicum) under salinity due to the increase in the activity of sucrose phosphate synthase, hence the efficiency of sugars in protecting plants under physiological stress (Gao et al., 2018). The positive impact of the Sucrose on the production and metabolism of carbohydrates is quite well known in plants (Vardhini et al., 2011 and Agami, 2013). The results presented in this study indicate an increase in the Photosynthetic pigments starch and total soluble proteins content in shoot and root in vitro plantlets under Sucrose application, which might be caused by an enhanced photosynthetic capacity of tissues account by elevated photosynthetic pigments contents. The results presented in this study

indicate an increase in the starch content in shoot *in vitro* plantlets under sucrose application, which might be caused by an enhanced photosynthetic capacity of tissues account by elevated photosynthetic pigments contents. In accordance the previous study performed by Vardhini and Rao (2002) which revealed the ability of BR to increase content of carbohydrates fractions. sucrose treatments cause enhancement in soluble protein levels in *in vitro* plantlet shoot in comparison with non-treated which may be due to sucrose function in accelerating cell division and cell elongation commitments to their specific enzymes synthesis superior levels were recorded in shoots (**table** 2). In accordance with the present study, the profound increase in soluble protein contents reported in many plants under the influence of sucrose application: in *Pisum* and *Raphanus sativus* (Mahesh *et al.*, 2013).

CONCLUSION

We have concluded through our study that it is necessary to add sucrose in medium concentrations because of its great importance in promoting and stimulating the germination process of *Triticum aestivum* plants through large increase in the content of starch and protein and increasing the efficiency of the photosynthesis process. Hence, the plants germination and stimulation enhanced with the addition of sucrose and could be possible way growth for wheat seedlings *in vitro* condition.

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