INFLUENCE OF DIFFERENT CARBON SOURCES ON IN-VITRO ROOT FORMATION OF DATE PALM (*PHOENIX DACTYLIFERA* L.) CV KHANEZI

A.A. Al-Khateeb
Date palm Research Center , King Faisal Univ. .KSA

ABSTRACT

The influence of different carbon sources on root formation of date palm was studied. The carbon source influenced the percentage of root formation, number of roots per plant as well as root length. All roots produced in media supplemented with 90 and 120 g/l of sugar source were comparatively thicker and shorter.

INTRODUCTION

Several factors such as concentration of rooting media, auxin type and concentration affect in-vitro rooting stage (Wang and Charles, 1991). In cultured plant tissues, the normal function of chloroplasts as a source of energy is reduced and a continuous supply of carbohydrates from the medium is therefore necessary. In addition, root initiation and growth are high energy requiring processes that can only occur at the expense of available metabolic substrates, which are mainly carbohydrates (Thorpe, 1982). The establishment of an effective root system on in-vitro is essential for subsequent success during acclimatization to autotrophic conditions.

Although there are data on the effect of different carbon sources on in-vitro rooting of some plants, no data are currently available for date palm (*Phoenix dactylifera* L.) plants. Studies by Pua and Chong (1984) on the influence of carbon source, sorbets, glucose, sucrose and fructose during stages of in-vitro propagation of the apple rootstock *Malus rubosta* Rehd No. 5, demonstrated that sorbitol and sucrose were equally effective for in-vitro rooting. Li and Xu (1992) found that glycerol was better than sucrose as a carbon source in rooting media for Shimeichen orange (*Citrus sinensis*). Okezie et al. (1994) reported that in white yam (*Diosconea rotundata*) plants the whole plantlets were regenerated when only glucose or sucrose served as a carbon source, while roots and stunned shoot buds were produced with fructose, galactose and maltose. Lactose, maltose and raffinose supported only root production. Romano et al. (1995) revealed that sucrose (3%) and glucose (4%) were the best carbon sources during proliferation and rooting

phases of tissue culture of conk oak *(Quercus suber* L.). In the cotton cv. CNPA Precoce, shoot and root growth were generally best with apical buds, glucose and gelrite (Carvalho et al.1997). El-Karzaz et al (1997) found in mulberry (*Morus alba* L.) plant that root formation on in-vitro shoots was most extensive on MS medium supplemented with 3% sucrose. The main objective of the present paper was to study the ability of in-vitro proliferated shoots of date palm cv. "Khanezi" to utilize different carbon sources to promote root formation as well as shoot growth.

MATERIAL AND METHODS

Uniform proliferated shoots (4-5 cm in length) resulted from direct organogenesis (Al-Maarri and Al-Ghamdi, 1995) were transferred to test tubes (25mm x 150mm) filled with 17 ml of one half strength modified MS (Murashige and Skoog basal salt and vitamins) (Murashige and Skoog, 1962) based medium supplemented with 170 mg/l $NaH_2PO_4.2H_2O$, 100 mg/l Inositol, 1 mg/l thiamine, 6.5 g/l purified agar and 0.2 mg/l NAA. The media was further supplemented with different carbon sources, sucrose, fructose, glucose and maltose with different concentrations of 0, 30, 60, 90 and 120 g/l, respectively. Each treatment was represented by 9 replicates in a randomized complete block design with one shoot per each replicate. Rooting response was expressed in terms of percentage rooting, root number, root length, root thickness, root fresh weight and root dry weight per shoot. Other parameters were also taken; shoot length, shoot fresh and dry weight. Root and shoot dry weights were obtained by drying both plant parts in a forced air oven at 75^0C for 72 hours. Root and shoot lengths were determined by measuring the longest root in each shoot and the longest shoot in each culture.

RESULTS AND DISCUSSION

Concentration and interaction of sugars had significant effects on rooting percentage whereas sugar type showed no effects (Table 1). The concentrations of 30, 60, and 90 g/l produced the highest rooting percentage while the 120 g/l concentration resulted in poor rooting percentage.

Fresh root weight was significantly affected by sugar type and concentrations (Table 2). Sucrose and glucose produced the highest amount of root fresh weight under 60 g/l concentration. The lowest root fresh weight was observed at concentration 0 and 120 g/l.

Table 3 showed that type and concentration of sugar had significant effect on root dry weight. Sucrose and glucose produced the highest amount of dry weight whereas fructose and maltose produced the lowest. Concentrations 60 and 90 g/l significantly improved root dry weight. The interaction between types and concentrations of sugars was highly significant. At a higher sugars concentration of (120 g/l), sucrose produced significantly more dry weight than others.

The type of sugar had no significant effect on root number, but sugar concentrations caused a substantial reduction (Table 4). As sugar concentrations increased, the root number considerably decreased. The 60 g/l concentration produced the highest significant root number than others. The interaction between types and concentrations of sugars was highly significant and sugar types caused different responses at different sugar concentrations.

Types and concentrations of sugars significantly affected root length (Table 5). Sucrose produced the longest root. Concentrations of 60 and 90 g/l substantially increased root length. Sugar types, sugar concentrations and their interactions had significant effects on root thickness (Table 6). Sucrose and concentrations of 60 and 90 g/l caused the production of thicker roots. The interactions between types and concentrations of sugars were highly significant and sugar types caused different responses at different sugar concentrations.

Sugar types and concentrations did not exert any influence on shoot fresh weight (Table 7). However, both parameters significantly affected shoot dry weight (Table 8). Sucrose and glucose produced the highest significant dry weight. The concentration of 120 g/l improved the ability of shoots to produce more dry weight. The shoot length was only affected by sugar concentrations (Table 9), whereas sugar types and their interactions provided no significant effects. Concentrations of 30 and 60 g/l produced the longest shoots, respectively. The shoot length was significantly decreased as sugar concentrations increased above 60 g/l.

The results indicated that date palm cv. Khanezi shoots were capable of utilizing, fructose, glucose or maltose as the sole carbon source for vegetative growth as well as for root formation. The carbohydrates, however, differed in their ability to support root formation of date palm. Shoots grown on medium containing sucrose as well as maltose had the highest percentage of root formation, whereas shoots grown on medium

containing glucose and fructose produced the lowest percentage of root formation. Furthermore, shoots grown on sucrose medium had vegetative as well as root growth rates similar to that grown on glucose and were the best among the other sugars. These results are similar to earlier reports for other plants (Pua and Chong, 1985; Li and Xu, 1992; Okezie et. al., 1994; El-Kazzaz et. al., 1997; Carvalho et. al., 1997).

Carbohydrate is known to modify osmotic strength (Thompson and Thorpe, 1987) and high osmotic strength of media often tends to reduce growth (Short et. al., 1987). Our results contradicted the previous concepts. In our study the root fresh and dry weights were increased at an optimum sugar concentration of 60 g/l (Table 1). This might be related to the increase in root number (Table 4).

Media devoid of sugar did not produce roots indicating the importance of sugar in root formation (Table 1). Knowledge over the years on the exact role of carbohydrates on rooting has been meager. Thorpe (1982) indicated that root initiation and growth were high energy requiring processes that could only occur at the expense of available metabolic substrates, which were mainly carbohydrates. In *Pinus bansiana* the accumulation of carbohydrates in the basal region of stem cutting was related to callusing and rooting (Haisig, 1984). Furthermore, in rooting of apple plants, Chong and Pua (1985) concluded that osmotic adjustment regulated by carbohydrate in tissue also influenced the initiation of root primordia

Based on our results, further investigation on the specific role of plant carbohydrates during the process of rooting seems imperative. The investigations may cover and examine the demand for energy or/and the indirect activation of some genes during the rooting process.

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Table 1. Influence of different carbon sources and concentrations on percent of root formation of date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	0.11	0.89	1.00	0.78	1.00	0.76
Glucose	0.00	0.89	1.00	1.00	0.11	0.60
Fructose	0.11	1.00	0.89	0.78	0.33	0.62
Maltose	0.00	0.78	0.89	0.67	1.00	0.67
Mean	0.06	0.89	0.94	0.81	0.61	

LSD 5% source 0.13: conc. 0.14: interaction 0.06

Table 2. Influence of different carbon sources and concentrations on root fresh weight of date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	7	207	748	472	618	410
Glucose	0	390	777	687	29	377
Fructose	16	637	496	305	126	316
Maltose	0	218	297	255	308	228
Mean	6	379	579	430	270	

LSD 5% source , 116 : conc., 129 : interaction , 51

Table 3. Influence of different carbon sources and concentrations on root dry weight of date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	1	15	102	80	127	65
Glucose	0	45	114	115	10	57
Fructose	1	63	64	49	23	40
Maltose	0	30	35	33	46	29
Mean	0.4	38	79	70	51	

LSD 5% source , 17 : conc., 19 : interaction , 1.2

Table 4. Influence of different carbon sources and concentrations on number of roots produced by in-vitro culture of date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	0.1	5.7	9.3	5.7	4.4	5.0
Glucose	0	4.4	8.9	5.0	0.6	3.8
Fructose	0.2	8.0	5.8	3.9	0.8	3.7
Maltose	0	4.3	4.7	3.9	3.6	3.3
Mean	0.1	5.6	7.2	4.6	2.3	

LSD 5% source , 1.3 : conc., 1.5 : interaction , 6.6

Table 5. Influence of different carbon sources and concentrations on root length of in-vitro cultured date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	0.5	4.5	5.6	5.3	5.9	4.3
Glucose	0	4.4	6.0	6.2	0.2	3.4
Fructose	0.2	3.9	5.2	4.5	1.3	3.0
Maltose	0	2.7	5.3	2.6	5.1	3.1
Mean	0.1	3.9	5.5	4.6	3.1	

LSD 5% source , 1.0 : conc., 1.1 : interaction , 3.6

Table 6. Influence of different carbon sources and concentrations on root thickness of in-vitro cultured date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	0.2	1.0	1.0	0.8	1.2	0.9
Glucose	0	0.8	1.0	1.2	0.1	0.6
Fructose	0.1	1.0	0.8	0.8	0.4	0.6
Maltose	0	0.5	0.8	0.6	0.8	0.5
Mean	0.08	0.8	0.9	0.85	0.65	

LSD 5% source , 0.2 : conc., 0.2 : interaction , 0.1

Table 7. Influence of different carbon sources and concentrations on shoot fresh weight of in-vitro cultured date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	502	670	700	695	675	648
Glucose	543	540	696	460	586	565
Fructose	395	590	419	364	512	456
Maltose	434	497	365	376	746	483
Mean	469	574	545	474	630	

LSD 5% source, 168 : conc., 187 : interaction , 112

Table 8. Influence of different carbon sources and concentrations on shoot dry weight of in-vitro cultured date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	48	91	123	148	181	118
Glucose	61	81	117	112	174	109
Fructose	44	77	68	80	138	81
Maltose	44	71	56	66	60	59
Mean	49	80	91	102	138	

LSD 5% source, 23: conc., 26: interaction, 2

Table 9. Influence of different carbon sources and concentrations on shoot length of in-vitro cultured date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	10.2	14	16	11.5	11.8	12.9
Glucose	12.8	12.3	15.2	12.2	8.7	12.2
Fructose	9.6	14.7	10.3	10.4	7.1	10.4
Maltose	9.5	15.2	10.2	11.9	9.4	11.2
Mean	10.5	14.3	12.9	11.5	9.3	

LSD 5% source, 2.1: conc., 2.4 : interaction, 17.4