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GROWTH OF LICORICE (*GLYCYRRHIZA GLABRA* L.) AS AFFECTED BY PROPAGATION MATERIALS

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ABSTRACT

A field experiment was conducted to study licorice growth using seeds and rhizomes. Seeds and rhizome segments with 1, 2, or 3 bud(s) were planted in barrels that had been dug into the field. Measurements of the shoot and underground parts were taken at 6 wk intervals. Results showed that shoot dry weight of plants from all propagation materials increased up to 18 wk after planting, but, except for the 3-bud segment, did not change significantly thereafter. Thirty wk after planting, root and rhizome dry weight of plants grown from 2 or 3-bud segments were significantly higher than plants grown from seeds. Results of HPLC determination, 30 wk after planting indicated that roots and rhizomes of plants grown from rhizome segments contained more glycyrrhizic acid than plants grown from seeds. Furthermore, in all treatments, roots produced more glycyrrhizic acid than rhizomes. Anatomical observations of underground organs showed that both roots and rhizomes were produced by plants grown from 2 or 3-bud segments and by those that were grown from seeds. However, plants that were grown from 1-bud segments did not produce rhizome.

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اثرمواد افزایشی بر رشد شیرین بیان (*Glycyrrhiza glabra* L.)

ناصر باقرانی ترشیز و حسین غدیری

به ترتیب دانشجوی کارشناسی ارشد و دانشیار بخش زراعت و اصلاح نباتات دانشکده کشاورزی دانشگاه شیراز، شیراز، ایران.

چکیده

آزمایشی مزرعه ای برای مطالعه رشد شیرین بیان با استفاده از بذر و ریزوم انجام گرفت. مواد افزایشی (بذر و قطعات ریزوم دارای ۱، ۲ یا ۳ جوانه)در گلدان هایی در گلخانه کاشته شدند. پس از ۶ هفته، گیاهچه های تولید شده در بشکه هایی که درون خاک در مزرعه قرار داده شده بودند، کشت گردیدند. اندازه گیری های اندام های هوایی و بخش های زیر زمینی در فواصل ۶ هفته ای انجام گردید. نتایج نشان داد که وزن خشک اندام های هوایی گیاهان از همه مواد افزایشی تا ۱۸ هفته پس از کاشت افزایش یافت، اما پس از آن، به جز قطعات ۳ جوانه ای ، به طور معنی داری تغییرنکرد. سی هفته پس از کاشت، وزن خشک ریشه و ریزوم گیاهان رشد یافته از قطعات ۲ یا ۳ جوانه ای به طور معنی داری بیشتر از گیاهانی بود که از بذر رشد کرده بودند. نتایج HPLC، ۳۰ هفته پس از کاشت، نشان داد که ریشه ها و ریزوم های گیاهان رشد یافته ازقطعات ریزوم، گلیسریزیک اسید بیشتری نسبت به ریشه ها و ریزوم های گیاهان رشد یافته از بذرداشتند. افزون براین، در همه تیمارها، ریشه ها در مقایسه با ریزوم ها، گلیسریزیک اسید بیشتری تولید کردند. مشاهدات تشریحی اندام های زیر زمینی نشان داد که هم ریشه وهم ریزوم توسط گیاهان رشد یافته از قطعات ۲و۳ جوانه ای و بذر، تولید ریشه و ریزوم نمودند. اما، گیاهانی که از قطعات یک جوانه ای رشد یافته بودند، ریزوم تولید نکردند.

INTRODUCTION

Licorice (*Glycyrrhiza glabra* L.) is an industrial and medicinal plant which forms the basis of products used in many industries including tobacco, confectionary and pharmaceuticals (5). There are numerous reports indicating the presence of glycyrrhizic acid in the subterranean parts of licorice (6, 10, 11, 13, 17). Glycyrrhizic acid is a triterpene glycoside considered to be the main biologically-active principle of licorice (4, 12). The glycyrrhizic acid content varies greatly according to species, cultivation condition, and growth environment.

Some investigators have reported that different propagation methods affect the accumulation of active substances in the underground organs of licorice (11, 14, 18). Omidbaigi and Bernath (13) showed that propagule characteristics (such as length, diameter, age and number of buds) had marked influences on growth, development, and production of licorice plants and on the content of the active substances. These results indicated that rhizomes which were grown from horizontally planted propagules of 21 to 30 cm in length and 10 mm in diameter accumulated the maximum amount of the active substance during a year of vegetative growth.

Pauzner and Badalov (15) studied the possibility of vegetative propagation of licorice by cuttings taken from vertical or horizontal rhizomes. The best results were obtained by using horizontal rhizomes or the upper end of vertical rhizomes. Yaskonis (18) indicated that licorice propagation by rhizomes was much more successful than that by seeds.

The widespread occurrence of licorice in Iran suggests genetic variability within the species for seed production, stand establishment, forage production, and soil conservation characteristics. Before any native plant is evaluated for cultivation or for soil conservation purposes, efficient methods of propagation must be identified. There seems to be a great potential for extension of licorice cultivation in Iran, but the present knowledge in propagation technique is rather limited.

The objectives of this research were: a) to study licorice growth using seeds and rhizome segments with different number of buds under field conditions, and b) to study the effects of the propagation method on the accumulation of glycyrrhizic acid in roots and rhizomes.

MATERIALS AND METHODS

Field Experiment

Field experiment was conducted at the Experiment Station of College of Agriculture, Shiraz University, located in Badjgah, 18 km north of Shiraz, 1810 m above the sea level. In the spring before planting, seeds and rhizome segments were prepared from natural stands of licorice. Rhizome segments with a mean diameter of 1±0.2 cm were used for propagation. These were classified based on the number of buds into 3 classes namely 1, 2 and 3-bud segments with mean weight of 1.7, 4.5, and 8 g and mean length of 2.1, 7, and 11 cm, respectively. All segments were soaked in a 0.5% benomyl solution for 15 min. Seeds were mechanically scarified and then soaked in a 5% benomyl solution for 5 min.

Propagation materials (seeds and rhizome segments) were sown in 3 kg plastic pots in a greenhouse at 30/10°C day/night temperatures and 16 hr photoperiod. After 6 wk, uniform and vigorous seedlings were transplanted into barrels that had been burried in the field. The barrels (85 cm in height and 50 cm in diameter) were filled with 200 kg of a Daneshkadeh clay loam soil (fine, mixed, mesic, Calcixerollic, Xerochrept) composed of approximately 35% sand, 35% silt, 30% clay with 2% organic matter, and a pH of 8. Irrigation was done regularly to maintain the soil at field capacity (FC). Shoot dry weight, leaf area, root dry weight, rhizome dry weight, and number of buds per rhizome were measured at 6 wk intervals. Glycyrrhizic acid percentage in roots and rhizomes was measured only at 30 wk after planting.

Leaf area was measured by a portable leaf area meter (model LI-3000, LAMBDA Instrument Corporation), and dry weights of shoots, roots, and

rhizomes, were measured after putting the samples in an oven at 60°C for 48 hr.

The glycyrrhizic acid content was determined by high performance liquid chromatography (HPLC) method (3). The HPLC used in this study was a Shimadzu model C-R4A, with a Shimadzu LC-9A injector. The column was an EM Laboratories C-18 (25 cm × 4.5 mm). The HPLC mobile phase consisted of methanol and 2.5% acetic acid in the ratio of 37/13 (v/v) at a flow rate of 1.5 ml min^{-1}. The concentration of glycyrrhizic acid was calculated by comparing the peak area of the standard to that of the sample.

The experimental design was split plot in time and the basic design was randomized complete block with three replications. Data were subjected to the analysis of variance.

Anatomical Procedure

In order to indentify the types of underground organs (roots or rhizomes) produced by propagation materials tested, subterranean parts were collected from plants every 6 wk and were fixed in formalin-acetic acid-alcohol (FAA). Sections, 25 μm thick, were cut by a Reichert sliding microtome and stained with carmin-iodine green. Stained sections were then dehydrated and mounted in Canada balsam (16). Slides were studied and photographs were taken by a Wild stereo microscope model MB, equipped with a Leitz automatic camera, and also Zeiss photo microscope II.

RESULTS AND DISCUSSION

Shoot Dry Weight

Maximum licorice shoot dry weight for most sampling dates was obtained from plants that were grown from the 3-bud segments, whereas shoot dry weight of plants grown from seeds was lower than those grown from the 3-bud segments (Table 1). No difference in shoot dry weight was observed between plants grown from seed and 1-bud segments at 6, 12, 18, and 24 wk after planting. In 30th wk after planting, shoot dry weight of 3-

bud segments was approximately three times as much as that produced by seed. Shoot dry weight of plants from all propagation materials increased up to 18th wk after planting, but, except for the 3-bud segments, did not change significantly thereafter. These results are in agreement with McWhorter (9) who stated that the shoot dry weight of Johnsongrass [*Sorghum halepense* (L.) Pers.] plant produced from rhizomes is affected by the weight of rhizomes at planting.

Table 1. Effect of propagation materials on shoot dry weight (g) of licorice.

Weeks after planting	Propagation materials				
	1-bud segment	2-bud segment	3-bud segment	Seed	Mean
6	0.2	0.3	0.4	0.1	0.3
12	1.1	4.1	5.5	2.2	3.2
18	17.2	27.8	26.6	15.6	21.8
24	20.4	30.2	33.7	16.4	25.2
30	24.3	31.2	39.4	13.6	27.1
Mean	12.6	18.7	21.1	9.6	

LSD (0.05) (within columns) = 4.7.

LSD (0.05) (between columns) = 4.8.

Leaf Area

Maximum licorice leaf area for all sampling dates was obtained from plants that were grown from the 3-bud segments (Table 2). Significant differences in leaf area occurred between plants that were grown from the 3-bud segments and seed at 18, 24 and 30 wk after planting. Significant differences in leaf area between plants grown from 1- and 3-bud segments occurred only at 18, 24 and 30 wk after planting. At 30 wk after planting, the leaves of plants grown from seed were abscised due to senescence and their area was significantly decreased as compared to the palnts grown from 1, 2, or 3-bud segments. It appears that the underground parts of plants grown from seed were more active sinks for assimilates as compared to other

plants and this significant reduction in leaf area of the plants grown from seed may be due to the translocation of assimilates to the underground parts.

Table 2. Effect of propagation materials on leaf area (cm^2) of licorice.

Weeks after planting	Propagation materials				
	1-bud segment	2-bud segment	3-bud segment	Seed	Mean
6	5	7	12	6	8
12	52	165	241	134	148
18	948	1134	1259	748	1022
24	1070	1385	1466	1004	1231
30	1222	1374	1578	766	1235
Mean	659	813	911	532	

LSD (0.05) (within columns) = 208.

LSD (0.05) (between columns) = 233.

Root Dry Weight

No difference was observed between root dry weight of plants grown from 2 or 3-bud segments and seed (Table 3). Maximum root dry weight was obtained from plants that were grown from 2 or 3-bud segments at 24th and 30th wk after-planting. These values were approximately 2.5 times greater than plants that were grown from seed. In all treatments, root dry weight increased throughout the growing season (Table 3). The increase in root dry weight was also significant for the plants grown from seed at 30 wk after planting. These results suggested that the root of licorice has a major role as a food reserve, and photosynthates are continuously translocated to the root during the growth of licorice. Since licorice plant is a perennial herbaceous plant, its underground organs (especially roots) operate as a reservoir of photosynthates to supply the essential carbohydrates for the next growing season.

Although either propagation material or time showed significant differences in shoot dry weight, leaf area and root dry weight, the

interactions between propagation material and time were not significantly different. This indicates that the effects are additive.

Table 3. Effect of propagation materials on root dry weight (g) of licorice.

Weeks after planting	Propagation materials				
	1-bud segment	2-bud segment	3-bud segment	Seed	Mean
6	0.01	0.02	0.02	0.02	0.02
12	0.16	1.20	1.10	0.55	0.75
18	4.37	16.73	16.78	9.16	11.76
24	18.95	40.40	50.42	20.49	32.57
30	68.45	113.70	111.69	45.56	84.85
Mean	18.39	34.41	36.00	15.16	

LSD (0.05) (within columns) = 12.28.

LSD (0.05) (between columns) = 11.96.

Rhizome Dry Weight

Anatomical studies of the underground organs indicated that plants obtained from 1-bud segments did not produce any rhizome during the course of the experiment. Rhizomes are the basic propagules by which licorice plants propagate and survive. Plants grown from seeds produced rhizomes 6 wk earlier than plants from 2 and 3-bud segments. At 24 and 30 wk after planting, dry weight of rhizomes obtained from 2 or 3-bud segments was significantly higher than plants grown from seeds (Fig. 1). For each planting material, maximum rhizome dry weight was obtained 30 wk after planting. This indicates that photosynthates are also continuously translocated to the rhizome during the growth of licorice.

Buds per Rhizome

Rhizomes obtained from plants that were grown from 2 or 3-bud segments and seed, produced 27, 28, and 15 buds, respectively, at 24 wk after planting, (Fig. 2). Thirty wk after planting, these numbers increased to 86, 91, and 76, respectively. These values were significantly higher than the number of buds formed 24 wk after planting.

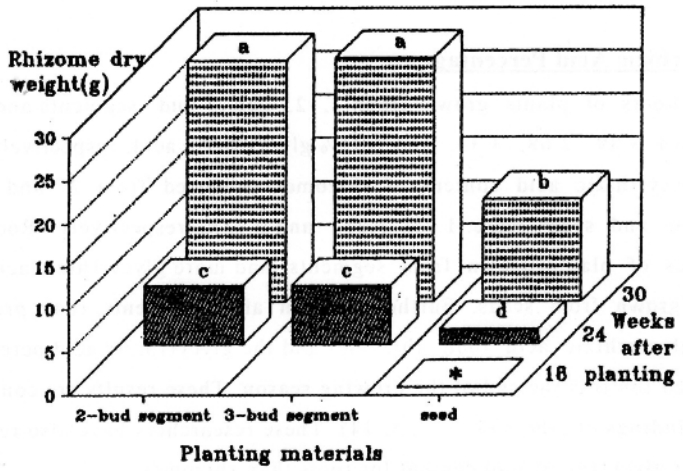

Fig. 1. Effects of propagation materials on rhizome dry weight of licorice during the growth period. (Bar rows with similar letters are not significantly different at 1% probability level according to Duncan's test).

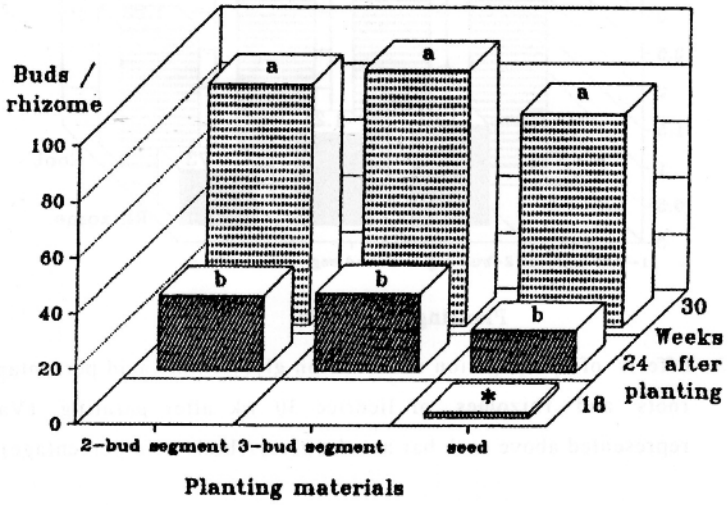

Fig. 2. Effects of propagation materials on buds per rhizome of licorice during the growth period. (Bar rows with similar letters are not significantly different at 1% probability level according to Duncan's test).

Glycyrrhizic Acid Percentage

Roots of plants grown from 1, 2, and 3-bud segments and seeds contained 3.39, 2.68, 3.33, and 1.82% glycyrrhizic acid, respectively (Fig. 3). Glycyrrhizic acid content of rhizomes produced from 2- and 3-bud segments and seeds were 1.31, 1.21, and 0.73%, respectively. Roots and rhizomes of plants grown from segments had more glycyrrhizic acid than plants grown from seeds. Furthermore, in all treatments, roots produced more glycyrrhizic acid than rhizomes and the glycyrrhizic acid percentage increased towards the end of the growing season. These results are consistent with findings of others (1, 2, 7, 8, 14). These researchers have also reported a higher glycyrrhizic acid content for roots than rhizomes.

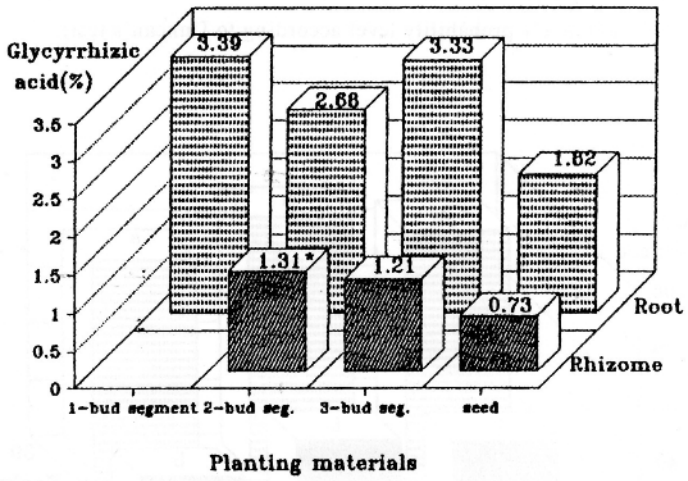

Fig. 3. Effects of propagation materials on glycyrrhizic acid percentage of roots and rhizomes of licorice 30 wk after palnting .(Values represented above each bar are the glycyrrhizic acid percentage).

Irrespective of propagation materials, dry weight of roots was much higher (between 3.8-4.5 times, Table 3 and Fig. 1) than that of rhizomes. Moreover, glycyrrhizic acid content of roots was higher than rhizomes for all

treatments. Therefore, harvesting licorice roots is more efficient for glycyrrhizic acid extraction.

Anatomical Observations

Anatomical differences between roots and rhizomes can help in identifying the underground organs of licorice. Cross section of a root grown from 1-bud segment showed a functional vascular cambium and the formation of secondary vascular system in a polyarch form. There was no pith in the center of the root, but a fine periderm covered its surface. Cross sections of roots grown from 2 or 3-bud segments or from seed showed no anatomical difference as compared to those grown from 1-bud segments.

Fig. 4 shows the cross section of a rhizome grown from a 2-bud segment. A large pith occupies the center of the rhizome and the vascular system has the form of a complete circle. The vascular system of the rhizome is interrupted at the site of attachment of a bud, where the gap parenchyma is produced. A thick periderm covers the surface of the rhizome.

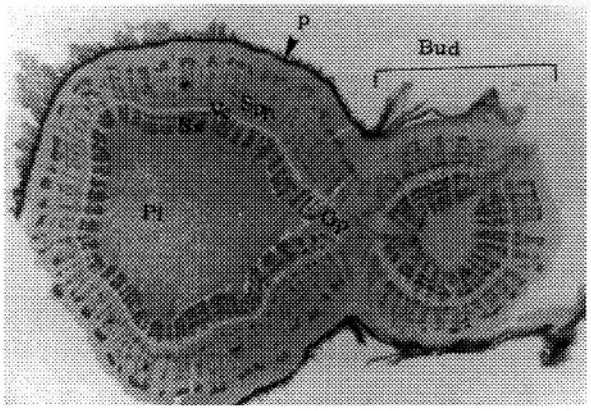

Fig. 4. Cross section of 30-wk old rhizome of licorice plants grown from 2-bud segments, ×13. (Pi) pith, (Gp) gap parenchyma, (Sx) secondary xylem, (Vc) vascular cambium, (Sph) secondary phloem, (P) periderm.

As shown in Fig. 5, the arrangement of the primary vascular system is typical of all the stems or rhizomes, i.e., protoxylem towards the pith and metaxylem in a centrifugal position. Cross section of rhizomes obtained from seeds showed a similar structure to the rhizomes grown from 2 or 3-bud segments. Here again, the initiation of a bud and its attachment to the rhizome could be observed. Plants that were grown from 1-bud segments did not produce any rhizome.

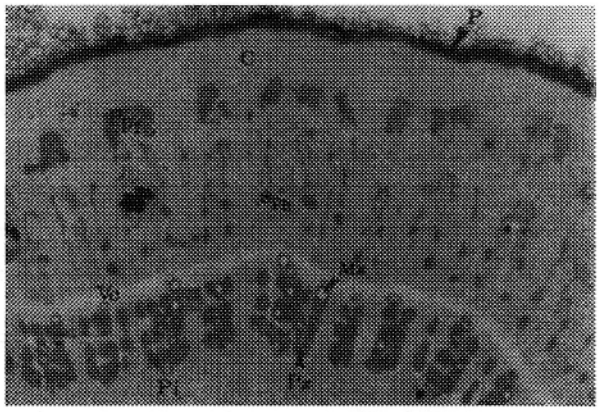

Fig. 5. Cross section of part of 30-wk old rhizome of licorice plants grown from 2-bud segments, ×42. (Pi) pith, (Px) protoxylem, (Mx) metaxylem, (Vc) vascular cambium, (Ph) phloem, (Ppf) periphloem fiber, (C) cortex, (P) periderm.

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