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Full Length Research Paper

The effects of prolonged vegetative reproduction of the two Iranian olive cv. tree cultivars (Dezful Baghmalek and Dezful Safiabad) on morphological traits

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Somaclonal variation of the two Iranian olive cultivars named Dezful Baghmalek (DB) and Dezful Safiabad (DS) during the long-term propagation among 7 subcultures were evaluated. Morphological traits such as leaf length, leaf width, number of leaves on shoot, the length of shoots, internode distance and rooting percentage were measured. The study results showed that DB affected somaclonal variations more than the DS cultivar, especially rooting percentage, but the DS cultivar had a steady behavior, especially rooting percentage, during several subcultures. Although in all the traits that were fluctuating, irregular and unpredictable changes such as the length of shoots were observed, the most significant trait that was studied with almost a similar vibration in the two cultivars were leaf length and width changes measures. Totally, we could not select any specific subculture period for the creation of the maximum satisfied morphological changes, because it was suitable for increasing the internode distance (DB) and leaf length which were in the second and seventh subcultures that were optimized. Consequently, it was suitable for DS in the seventh subculture. For the purpose of accomplishing the proper morphological changes in the length of shoots, number of leaves and enhancement of rooting percentage in DB cultivar and also, internode distance and leaf width increase in DS cultivar, somaclonal variation during several subcultures will be appropriate.

Key words: Olea europaea L., somaclonal variation, Dezful cultivars.

INTRODUCTION

The most likely benefit of somaclonal variation is in plant improvement and it is manifested as cytological abnormalities, frequent qualitative and quantitative phenotypic mutation, sequence change, gene activation and silencing (Shawn et al., 2000). Tissue culture variation has been applied in some cases to confer desirable traits to cultivars including desirable noteworthy morphological traits, disease and insect resistance, acid, salt and chemical tolerance and production of virus-free explants, as well as for increased production of secondary metabolites (Duncan, 1997; Veillenx and Johnson, 1998).

Tissue cultures are always initiated from a number of different explants sources. Cells from these explants dedifferentiate, thereby resulting in totipotent cultures from which plants can be regenerated (Messing and Grossniklaus, 1999). However, primary regenerants (Ro) are often more variable than their progeny (Richards, 1997). Sequential accumulation of mutations over time provides evidence that mutations are occurring during the culture process and not pre-existing in the explants (Shawn et al, 2000). Consequently, transposons and

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Abbreviations: Ro, Primary regenerants; **DB,** Dezful Baghmalek; **DS,** Dezful Safiabad*;* **DKW,** Driver and Kuniyuki; **2-ip,** 2-isopentyl adenine; **2,4-D,** (2,4-dichlorophenoxyacetic acid.

retrotransposons are activated by the culture process (Peschke et al, 1987; Hirochika et al, 1996). Study of somaclonal variation is relevant to applications such as *in vitro* plant propagation and plant transformation. In addition, somaclonal variation is likely a reflection of response to cellular stress and asexual vegetative reproduction and has long been used in some agriculturally important trees like the cultivated olive (Zohary and Spiegel-Roy, 1975). For this purpose, we have studied the changes and morphological traits in Dezful Baghmalek (DB) and Dezful Safiabad (DS) for several subcultures. Although a large number of olive accessions are growing in Iran, there have not been any reports on morphological, cytogenetical and molecular characteristics of these accessions (Noormohammadi et al., 2009; Sheidai et al., 2007). To assess the tissue culture effects on morphological traits, two tree cultivars, DB and DS, within 7 subcultures were studied.

MATERIALS AND METHODS

Two cultivars of Olea europaea L.: DB and DS (Source: The existence of olive trees in Khozestan province in the southwest of Iran) were used in this study. One centimeter of internode sample was cut and cultured after sterilization. For sterilization, single nodes were taken from a matured container that was grown in DB and DS olive cultivars and then ddH_2O and NaOCl, based on the present protocols, were used (Kiani et al., 2005). The DKW medium (Driver and Kuniyuki, 1984), containing 30 gl^{-1} sucrose, 7.0 gl^{-1} agar, 0.1 gl^{-1} inositol and 0.2 mll^{-1} 2-ip, was used for shooting and rooting explants production. The medium was sterilized by autoclaving for 20 min at 121°C in all media containing the pH that was adjusted to 5.8 before autoclaving. Then three to five samples were placed inside the glass bottles and maintained at 25 ± 2°C under a 16/8 h light photoperiodic and under a light intensity of 3000 lux in a germinator. After 30 days, some samples were transferred to a fresh medium and were processed till the seventh subculture for detection of morphological variations. Morphologic characteristics such as number of leaves, length and number of shoots, rooting, internode length in cm, leaf length and width in mm were evaluated in each subculture for 2 cultivars. The experiment was repeated thrice for each treatment used and the morphological data were analyzed by analysis of variance test (ANOVA) followed by the least significant difference test (LSD).

RESULTS AND DISCUSSION

Internode distance and number of nodes

Despite the relative increase of internode distance between DS and DB in several subcultures (Figure 1a), statistical analysis did not show significant difference between internode increase and number of nodes in these cultivars. This outcome is in agreement with the experience of Peyvandi et al. (2009). The most uttermost observed increase in internode distance was 2.16 cm (related to the second DB subculture) and 1.97 cm (related to the seventh DS subculture). Totally, in both olive cv., the number of nodes increases, as a result of an increase in shoot-length during subcultures.

Shoot length

In the tissue culture and existing cytokinin, 2-isopentyl adenine (2-ip), there is a regeneration of explants growth and shoots. In several subcultures, DB was more affected and the highest shoot was seen in the seventh subculture namely 13 cm. The highest shoot DS cultivar was 6 cm, in that it was relevant in the same subculture (Figure 1e). However, it is possible to acquire higher shoots at the end of the subcultures. Also, response to shoot length increases DB cultivar in several subcultures and this is confirmed by the statistical analysis (Table 1).

Leaf length and width

In this study, morphological changes of leaves (such as colour, length, width etc) estimated, with regard to statistical analysis did not show significant variations (Table 1). However, it was compatible to other results (Leva et al., 2000, 1995; Rani et al., 2000). Nevertheless, the best response to length and width changes was related to DS cultivar due to the fact that it contained 7.0 and 2.43 cm leaf length and width, respectively, in the seven subcultures, respectively (Figure 1c). In DB cultivar, the length leaf maximum was 1.7 cm (related to the second subculture) and the width leaf maximum was 0.6 cm (related to the seventh subculture) (Figure 1d). However, it seems that the first and last subcultures were the best subcultures for studying length and width leaf variations. By a steady increase of the leaf length and width during subcultures, it is hoped that the eugenic of these cultivars will be produced in the auxiliary budding cultures by somaclonal variation.

Rooting percentage

We purposely applied the chemical auxin, 2,4-dichlorophenoxyacetic acid (2,4-D) for rooting in media. It was remarkable that no effect was found on the rooting DS cultivar, but the best result was attained in DB cultivar (Figure 1b). We arbitrarily gave number 1 that was equivalent to zero (20%) to no rooting or the least rooting and number 5 that was equivalent to 100% to the most rooting and the basis. Based on this, rooting quantity was 100% in the sixth DB subculture (Figure 2). However, the two cultivars were related, but the reasons for their different effects on these were not obvious. As a result, no report was found on this matter.

Altogether, by measuring the changes of morphological traits and statistical analysis that were presented in Table 1, the study results displayed that the most oscillation in morphological traits occurred in the second (internode distance and increased leaf length: Figure 1a, c and Table 1) and seventh DB subcultures (leaf width, increased stem shoot length and leaf number: Figure 1d, e and Table 1). However, the best desirable changes occurred in

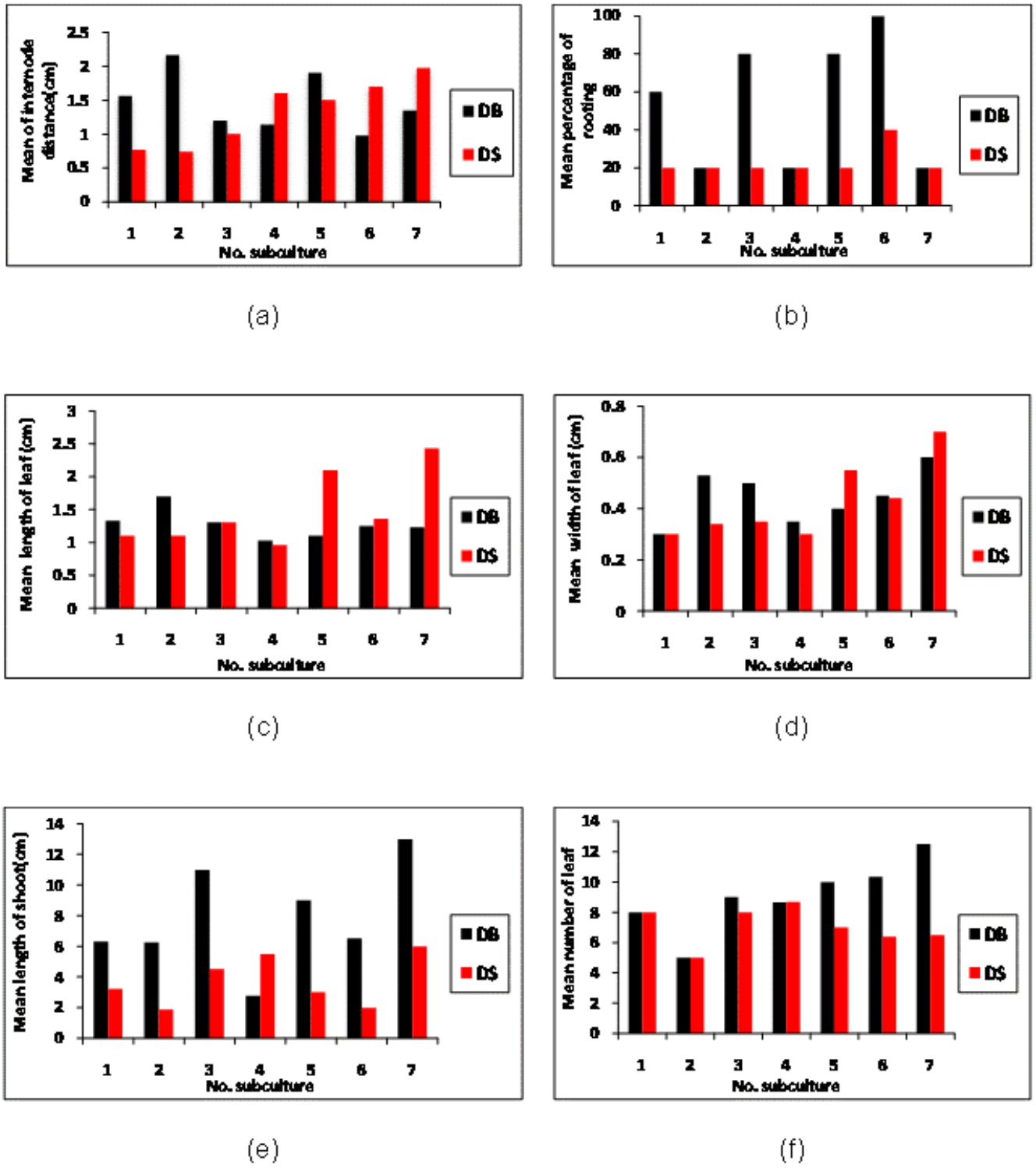

Figure 1. (a) Mean of internode distance during subcultures in the two cultivars; (b) Mean percentage of rooting during subcultures in the two cultivars; (c) Mean length of leaf during subcultures in the two cultivars; (d) Mean width of leaf during subcultures in the two cultivars; (e) Mean length of shoot during subcultures in the two cultivars; (f) Mean number of leaf during subcultures in the two cultivars.

the DS morphological traits that were observed in the seventh subculture (such as increasing of internode distance, leaf length, leaf width and stem shoot length: Figure 1a, c, d, e and Table 1). Therefore, the olive tree

Table 1. Representative mean difference test (LSD) for morphological characters between DS and DB subcultures. The mean differences are significant at the 0.05 level.

Plant part	Parameters		
(I) Leaf length	(J) Leaf length	Mean difference (I-J)	Significance
DB2	DB1	0.70000 (*)	0.002
	DB3	0.40000 (*)	0.043
	DB5	0.60000 (*)	0.005
	DB6	0.46667 (*)	0.21
	DB7	0.46667 (*)	0.21
(I) Leaf width	(J) Leaf width	Mean difference (I-J)	Significance
DB7	DB1	0.26667 (*)	0.002
	DB4	0.25000 (*)	0.003
	DB5	0.20000 (*)	0.012
	DB6	0.16667 (*)	0.030
(I) Internode distance	(J) Internode distance	Mean difference (I-J)	Significance
DB2	DB3	0.90000 (*)	0.15
	DB4	0.80000 (*)	0.028
	DB6	1.2000 (*)	0.002
	DB7	0.76667 (*)	0.034
(I) Shoot length	(J) Shoot length	Mean difference (I-J)	Significance
DB7	DB1	6.6667 (*)	0.000
	DB2	6.8333 (*)	0.000
	DB4	10.1667 (*)	0.000
	DB5	4.0000 (*)	0.006
	DB6	6.16667 (*)	0.000
(I) Leaf number	(J) Leaf number	Mean difference (I-J)	Significance
DB7	DB1	2.6667 (*)	0.0.033
	DB2	7.6667 (*)	0.000
	DB4	4.0000 (*)	0.003
	DB5	2.6667 (*)	0.033
(I) Leaf length	(J) Leaf length	Mean difference (I-J)	Significance
DS7	DS1	1.3667 (*)	0.000
	DS2	1.3333 (*)	0.000
	DS3	1.2667 (*)	0.000
	DS4	1.5000 (*)	0.000
	DS6	1.0667 (*)	0.000
(I) Leaf width	(J) Leaf width	Mean difference (I-J)	Significance
DS7	DS1	0.4000 (*)	0.002
	DS2	0.2667 (*)	0.028
	DS3	0.3333 (*)	0.008
	DS4	0.4000 (*)	0.002
	DS6	0.3000 (*)	0.015
(I) Internode distance	(J) Internode distance	Mean difference (I-J)	Significance
DS7	DS1	1.2000 (*)	0.006
	DS2	1.3400 (*)	0.003
	DS3	0.9667 (*)	0.020
	DS6	1.1000 (*)	0.010
(I) Stem length DS7	(J) Stem length	Mean difference (I-J)	Significance
	DS1	2.8000 (*)	0.001
	DS2	3.8333 (*)	0.000
	DS3	1.5000 (*)	0.044
	DS5	3.0000 (*)	0.001
	DS6	4.2333 (*)	0.000

Table 1. Cont

(I) Leaf number DS2	(J) Leaf number	Mean difference (I-J)	Sig.
	DS1	-3.0000 (*)	0.016
	DS3	-3.0000 (*)	0.016
	DS4	-3.6667 (*)	0.005

DB = Dezful Baghmalek cultivar, DS = Dezful Safiabad cultivar.

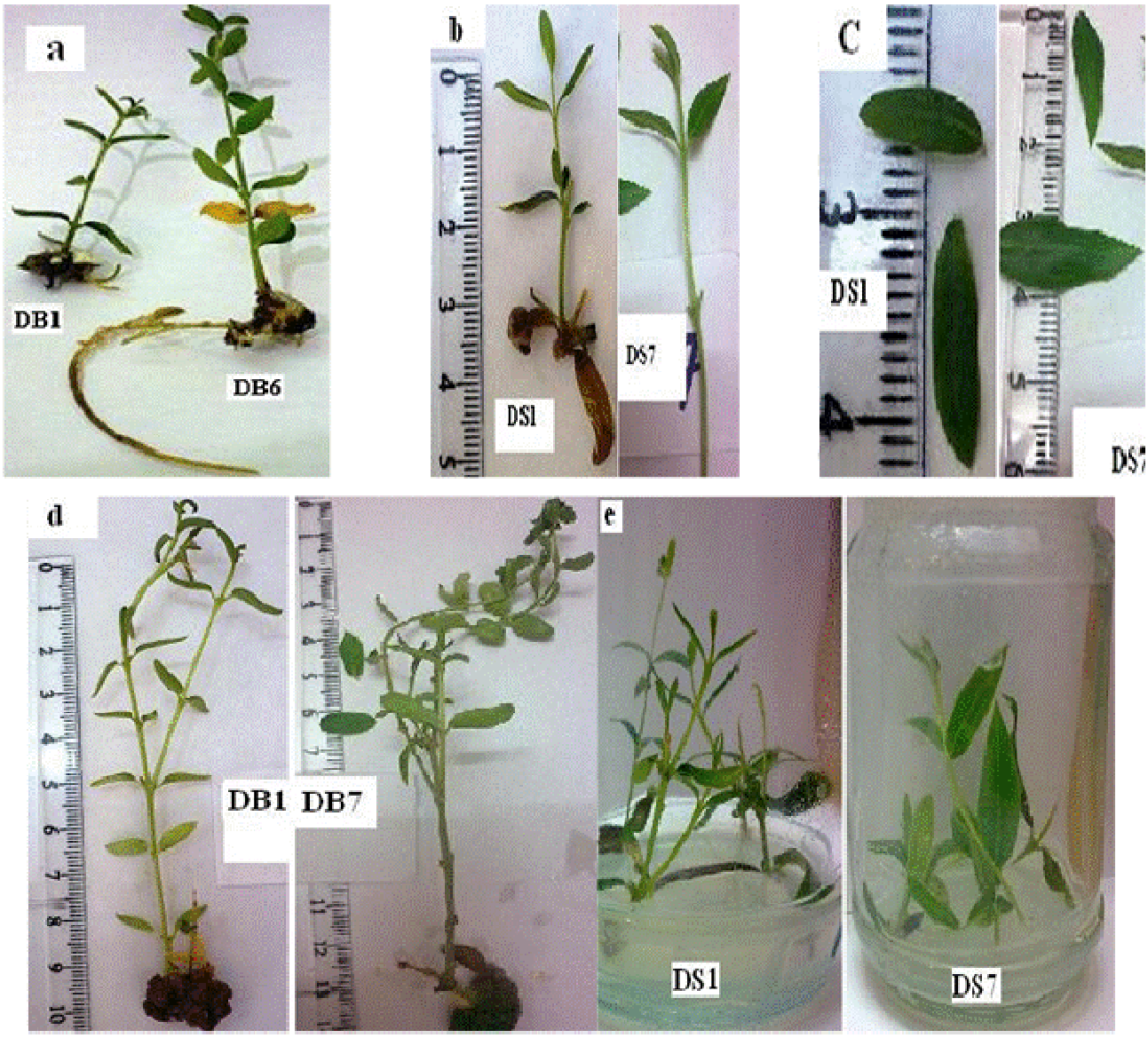

Figure 2. (a) Root formation in the first DB subculture with 20% rooting (left) and sixth DB subculture with 100% rooting (right). (b) Difference of internode distance between the first DS subculture (DS1) and the last DS subculture (DS7). (c) Leaf length and width in the first and last DS subcultures. (d) Total shoot length in the first and last DB (DB1, DB7) subcultures. (e) Number and morphology of leaves in the first and last DS (DS1, DS7) subcultures.

can be an appropriate organism to assess the effect of a long-term vegetative propagation in morphological traits by attending to the study's results, and as a consequence, it is recommended to attain stabilized and suitable

somaclonal variations in olive explants subcultures, which will continue for long-term periods, just like Leva (2009) has shown.

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