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Full Length Research Paper

Study of morphological traits changes in prolonged vegetative reproduction of three olive tree cultivars domesticated (Zard, Roughani and X) in Iran

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Somaclonal variation of three Iranian olive cultivars namely Zard (Z), Roughani (R) and unknown cultivar Iks (X) during long-term propagation were evaluated among 5 subcultures. Morphological traits such as length and width of leaf, number of leaves on shoot, length of shoots, internode distance and rooting percentage were measured. Our results showed that R more than Z and X cultivars affected somaclonal variations especially rooting percentage and root length but Z cultivar had steady behavior especially leaf length and width, root length, leaf number and rooting percentage during several subcultures. Although in all traits fluctuating changes observed but the most significant trait studied with almost similar vibration in three cultivars were internode distance values. Totally we could not select specific subculture period for creation of the maximum satisfied morphological changes because it was suitable increasing of leaf length and width R and X cultivars in second subculture and was suitable for Z in fourth subculture. In order to accomplishment of morphological changes in length of shoots, number of leaves and enhance of rooting percentage in R cultivar and also internode distance in X cultivar somaclonal variation during several subcultures will be appropriate.

Key words: *Olea europaea* L., somaclonal variation, Iranian olive cultivars.

INTRODUCTION

The major likely benefit of somaclonal variation is in plant improvement. 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 morphological traits, disease resistance, insect resistance, acid, salt and chemical tolerance and virus- free explants as well as for increased production of secondary metabolites (Duncan, 1997; Veillenx and Johnson, 1998; Rugini and Pesce, 2006). Tissue cultures are initiated from a number of different explants sources. Cells from these explants dedifferentiate resulting in totipotent cultures from which plants can be regenerated (Messing and Gross-niklaus, 1999). 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) and transposons and retrotransposons are activated by culture process (Peschke et al., 1987; Hirochika et al., 1996). Study of somaclonal variation is relevant to

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applications such as *in vitro* plant propagation and plant transformation. In addition somaclonal variation is likely a reflection of response to cellular stress. Asexual vegetative reproduction has long been used in some agriculturally important trees like the cultivated olive (Zohary and Spiegel-Roy, 1975; Kaeppler et al, 2000; Rugini, 1984). For this purpose, we studied the changes morphological traits in Zard (Z), Roughani (R) and X Iranian olive cultivars for several subcultures. Although a large number of olive accessions are growing in Iran, there have not been reports on morphological, cytogenetical and molecular characteristics of these accessions (Noormohammadi et al., 2009; Sheidai et al., 2007; Peyvandi et al; 2009). To assess the effect of tissue culture on morphological traits, three olive cultivars Zard, Roughani and Iks within 5 subcultures were studied.

MATERIALS AND METHODS

Three cultivars of *Olea europaea* L.: Zard, Roughani and Iks (Source: The olive trees exist in khozestan province in southwest of Iran) were used in this study. One centimeter of internode sample cut and cultured after sterilization. For sterilization, single nodes were taken from mature container grown Z, R and X olive Cultivars and then ddH_2O, NaOCl based on present protocols were used (Kiani Feiz et al., 2005). The DKW medium (Driver and Kuniyuki, 1984) containing 30 g/L sucrose, 7.0 g/L agar, 0.1 g/L Inositol, 0.2 ml/L 2-ip was used for shooting and rooting explants production. The medium was sterilized by autoclaving for 20 min at 121°C. All media containing adjusted pH to 5.8 before autoclaving. Three to five samples were placed into glass bottles and maintained at 25±2°C under a 16/8 h light photoperiodic under a lig ht intensity 3000 lux in a germinator. After 30 days some samples transferred to fresh medium and processed till fifth subculture for detection of morphological variations. Morphologic characteristics such as number of leaves, length and number of shoots, rooting, internode length in cm, length and width leaf in mm were evaluated in each subculture for the 3 cultivars. The experiment was repeated for 3 times for each treatment used and morphological data were analyzed by analysis of variance test (ANOVA) followed by least significant difference test (LSD).

RESULTS AND DISCUSSION

Leaf length and leaf width

In this study morphology changes of leaves (such as color, length, width, etc) estimated and regard to statistical analysis significant variations were seen. It was compatible and challengeable to other results (Leva et al., 2000; Leva et al, 1995a). The best response to length and width changes were related to R and X Cultivars whereat those containing 1.99 and 1.86 cm length leaf and 0.7 and 0.9 cm width leaf in second subculture R and X respectively (Figure 1a and b). In Z Cultivar length leaf maximum were 1.41 cm and width leaf 0.6 cm related to fourth subculture (Figure 1a and b).

Shoot length

In tissue cultures containing cytokinin named 2-ip(2-isopentyl adenine) explants growth and shoots regenerate. In several subcultures, Z and X cultivars was no more affect. The highest shoot was seen in third subculture up to 8.3 cm. The lowest shoot was seen in Zard and X Cultivars during all subcultures (Figure 1c).

Leaf number and Internode distance

The pattern of leaf number was nearly in R and Z cultivars during of subcultures similar and was 12 in each cultivar that related to second and third subcultures respectively. In X cultivar increasing gradual leaf number were seen which the most leaf number was 13.3 in fifth subculture (Figure 1d). Despite of relative increase of internode distance among Z, R and X during Several subcultures to third, we saw then internode distance in three cultivars gradually reduced (Figure 1e).

Rooting percentage

We purposely applied chemical auxin named 2,4-D (2,4-Dichlorophenoxyacetic acid) for rooting in media. It was remarkable that no effect on rooting X and Z cultivars but especially the best result gained in R cultivar (Figure 1f). We arbitrarily gave number 1 that was equivalent from 20% to no rooting or the least rooting and number 5 that was equivalent from 100% to the most rooting and the basis on rooting quantity was 100% in the first and fifth R subcultures. Three cultivars were relative but reasons of different effect on those were not obvious and no found report about this matter.

Altogether, by measuring changes of morphological traits and statistical analysis presented in Table 1 our results displayed that oscillation in morphological traits occurred in all subcultures of any cultivar (Figure 1a to f). The best desirable changes occurred in R cultivar; morphological traits observed in second subculture (such as increasing of leaf length, leaf width and leaf number (Figure 2a, b, d and Table 1).

Finally, the olive tree can be an appropriate organism to assess the effect of long-term vegetative propagation in morphological traits by attention to our results and we recommend it so as to attain stabilized and suitable somaclonal variations in olive explants, thus, subcultures will be continued for long-term periods like to Leva experience (Leva, 2009).

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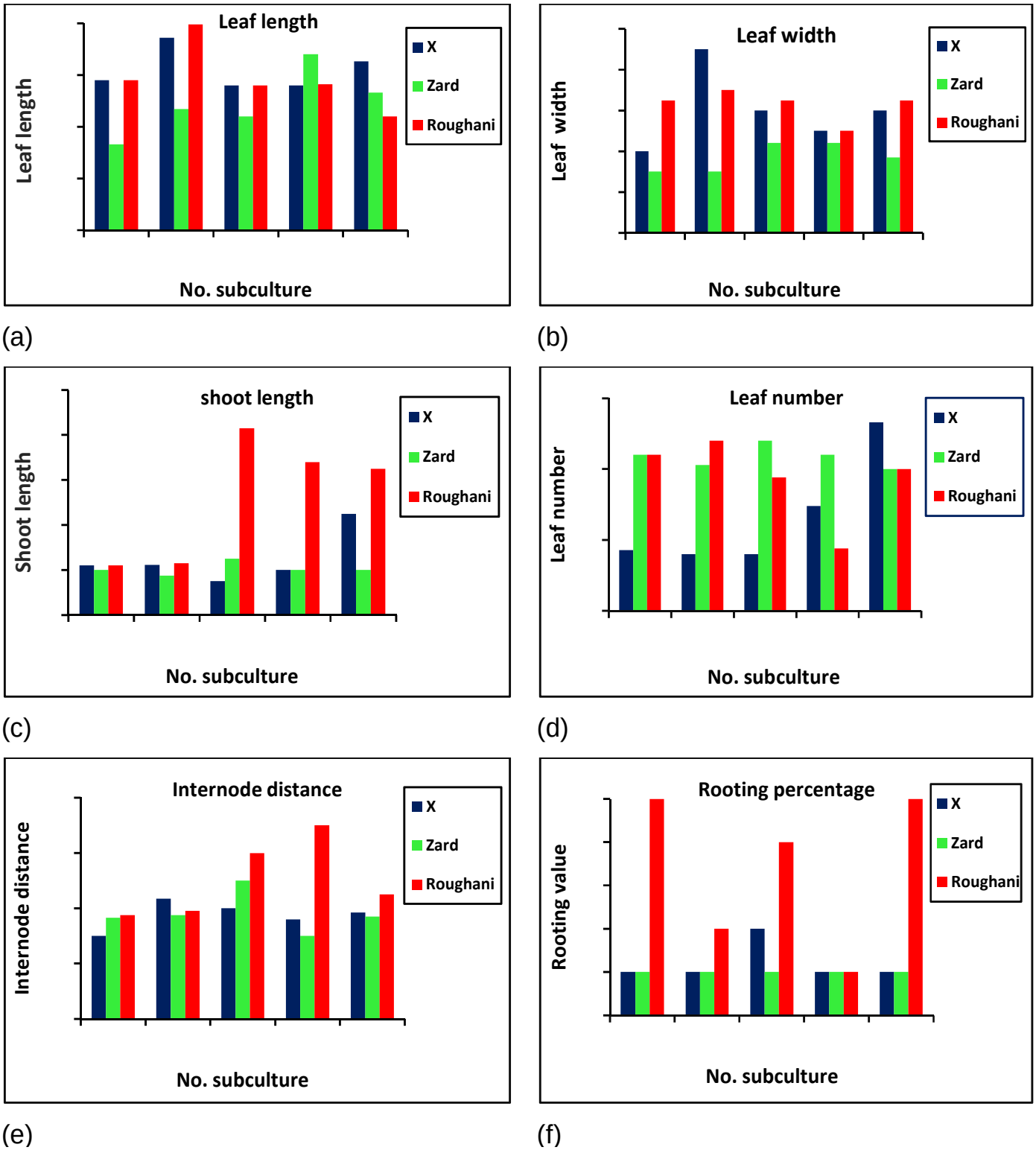

Figure 1. a) Mean length of leaf during subcultures in three cultivars; b) mean width of leaf during subcultures in three cultivars; c) mean length of shoot during subcultures in three cultivars; d) mean number of leaf during subcultures in three Cultivars; e) mean of internode distance during subcultures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

Table 1. Representative mean difference test (LSD) for morphological characters among X, Zard and Roughani subcultures.

	(J) leaf width	Mean difference (I-J)	Sig.
(I) Leaf width X2	X1	0.5000 (*)	0.000
	X3	0.3000 (*)	0.001
	X4	0.40000 (*)	0.000
	X5	0.36667 (*)	0.001
(I) Internode distance	(J) Internode distance	Mean difference (I-J)	Sig.
X2	X1	0.2667 (*)	0.041
	(J) Shoot length	Mean difference (I-J)	Sig.
(I) Shoot length X5	X1	2.3333 (*)	0.000
	X2	2.2833 (*)	0.000
	X3	3.0000 (*)	0.000
	X4	2.5000 (*)	0.000
	(J) leaf number	Mean difference (I-J)	Sig.
(I) Leaf number X5	X1	9.0000 (*)	0.003
	X2	9.3333 (*)	0.002
	X3	9.3333 (*)	0.002
	X4	6.0000 (*)	0.027
(I) Leaf length Z4	(J) leaf length	Mean difference (I-J)	Sig.
	Z1	0.8333 (*)	0.008
	Z3	0.6000 (*)	0.039
(I) Internode distance	(J) internode distance	Mean difference (I-J)	Sig.
Z3	Z4	0.4000 (*)	0.010
	(J) leaf length	Mean difference (I-J)	Sig.
(I) Leaf length R2	R3	0.5833 (*)	0.038
	R4	0.5667 (*)	0.043
	R5	0.9167 (*)	0.004
	(J) leaf width	Mean difference (I-J)	Sig.
(I) Leaf width R4	R1	-0.1667 (*)	0.016
	R2	-0.2167 (*)	0.004
	R3	-0.1500 (*)	0.027
	R5	-0.1500 (*)	0.027
I) shoot length R3	(J) shoot length	Mean difference (I-J)	Sig.
	R1	6.1000 (*)	0.035
	R2	6.0000 (*)	0.038
(I) Internode distance R4	(J) internode distance	Mean difference (I-J)	Sig.
	R1	0.6167 (*)	0.016
	R2	0.6400 (*)	0.013
(I) leaf number	(J) leaf number	Mean difference (I-J)	Sig.
R2	R4	7.6667 (*)	0.032

X= Unknown cultivar, Z= Zard cultivar, R= Roughani cultivar. The mean differences are significant at the 0.05 level.

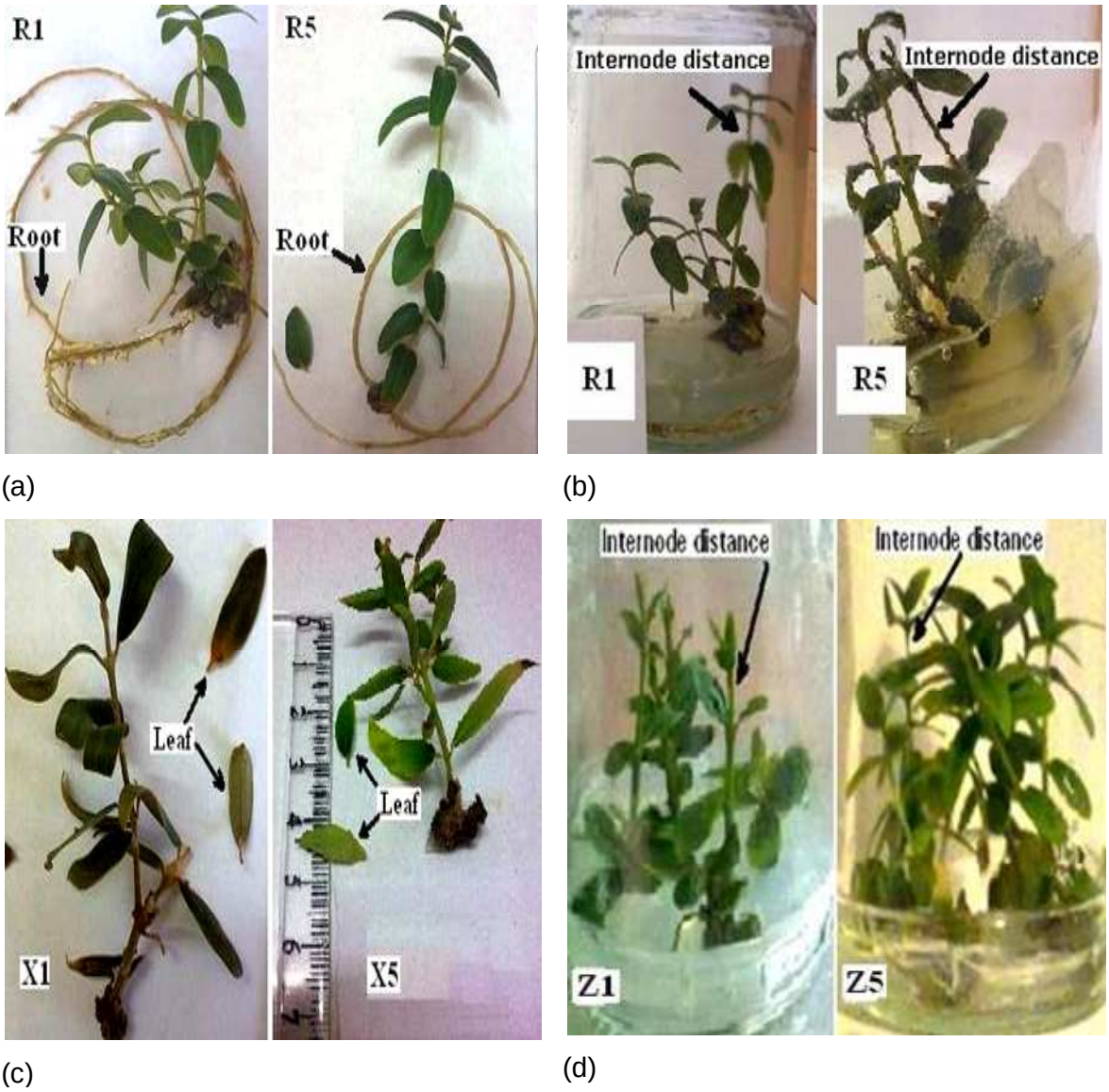

Figure 2. a) Shoot length, leaf morphology and root formation in the first (left) and the fifth (right) R subcultures with 100% rooting; b) difference of internode distance between the first R subculture (left) and the last R subculture (right); c) leaf morphology in the first (left) and the last (right) X subcultures; d) length and width of leaf and internode distance between the first (left) and the last (right) Z subcultures.

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